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Thermo-plasmonics in self-organized materials

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14. ABSTRACT Thermoplasmonics deals with the generation and manipulation of nanoscale heating associated with noble metallic nanoparticles. To this end, gold and silver nanoparticles (AuNPs, AgNPs) are unique nanomaterials with the intrinsic capability to generate a nanoscale confined light-triggered thermal effect. This phenomenon is produced under the excitation of a suitable light of a wavelength that matches the localized surface plasmonic resonance frequency of AuNPs/ AgNPs. Liquid crystals (LCs) and hydrogels are temperature-sensitive materials that can detect the host AuNPs/ AgNPs and detect their photo-induced temperature variations. In this perspective, we present and discuss our latest efforts in the field of hydrogel/LCs based thermo-optical components. Moreover, in light of bio-related application, we present the first experimental evidence of the determination of D+ glucose concentration by exploiting AgNPs/hydrogel nanocomposites' properties.						
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“Thermo-plasmonics in self-organized materials”

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Abstract: Thermoplasmonics deals with the generation and manipulation of nanoscale heating associated with noble metallic nanoparticles. To this end, gold and silver nanoparticles (AuNPs, AgNPs) are unique nanomaterials with the intrinsic capability to generate a nanoscale confined light-triggered thermal effect. This phenomenon is produced under the excitation of a suitable light of a wavelength that matches the localized surface plasmonic resonance frequency of AuNPs/ AgNPs. Liquid crystals (LCs) and hydrogels are temperature-sensitive materials that can detect the host AuNPs/ AgNPs and detect their photo-induced temperature variations. In this perspective, we present and discuss our latest efforts in the field of hydrogel/LCs based thermo-optical components. Moreover, in light of bio-related application, we present the first experimental evidence of the determination of D⁺ glucose concentration by exploiting AgNPs/hydrogel nanocomposites' properties. All the results have been adequately described in the publications listed below.

List of Publications and Significant Collaborations that resulted from your AOARD supported project: In standard format showing authors, title, journal, issue, pages, and date, for each category list the following:

- a) papers published in peer-reviewed journals,
 1. Guglielmelli A., Rosa P., Perotto G., Mangino G., Contardi M., Pani R., Calogero A. & De Sio L. - *Biomimetic Gold Nanoparticles-mediated in Vitro Photo-thermal Therapy on Glioblastoma Multiforme* – *Nanomedicine* 2021, 16 (2)
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 3. B. De Angelis, N. Depalo, F. Petronella, C. Quintarelli, M.L. Curri, R. Pani, A. Calogero, F. Locatelli, L. De Sio “Stimuli-responsive nanoparticle-assisted immunotherapy: a new weapon against solid tumours” *J. Mater. Chem. B*, 2020,8, 1823.
 4. L. Pezzi, A. Pane, F. Annesi, M. A. Losso, A. Guglielmelli, C. Umeton, and L. De Sio Antimicrobial Effects of Chemically Functionalized and/or Photo-Heated Nanoparticles *Materials* 2019, 12(7), 1078
 5. F. Annesi, A. Pane, M. A. Losso, A. Guglielmelli, F. Lucente, F. Petronella, T. Placido, R. Comparelli, M. G. Guzzo, M. L. Curri, R. Bartolino and L. De Sio “Thermo-plasmonic killing of Escherichia Coli TG1 bacteria” *Materials* 2019, 12(9), 1530.
 6. L. De Sio, P. F. Lloyd, N. V. Tabiryan, T. Placido, R. Comparelli, M. L. Curri, and T. J. Bunning “Thermo-Plasmonic Activated Reverse-Mode Liquid Crystal Gratings” *ACS Appl. Nano Mater.* 2019, 2 (5), 3315.
 7. L. Pezzi[†], L. De Sio[†], A. Veltri, A. Cunningham, A. De Luca, T. Burgi, C. Umeton, R. Caputo “Plasmon-mediated Discrete Diffraction Behaviour of an Array of Responsive Waveguides” *Nanoscale* 2019, 11, 17931. [†] These authors contributed equally to this work.

8. A. Guglielmelli, S. H. Nemati, A. V. Vasdekis and L. De Sio "Stimuli responsive diffraction gratings in soft-composite materials" Journal of Physics D: Applied Physics 2018 (DOI: 10.1088/1361-6463/aaedf4).
9. G. Palermo, A. Guglielmelli, L. Pezzi, U. Cataldi, L. De Sio, R. Caputo, A. De Luca, T. Bürgi, N. Tabiryan & C. Umeton "A command layer for anisotropic plasmonic photo-thermal effects in liquid crystal" Liquid Crystals 2018 .
10. L. Pezzi, L. De Sio, T. Placido, R. Comparelli and C. Umeton "Photo-induced temperature variations in plasmonic nanoparticles trapped in thermo-sensitive liquid crystals" Journal of Nanoscience and Nanotechnology 18, 6708, 2018.
11. L. De Sio, U. Cataldi, A. Guglielmelli, T. Bürgi, N. Tabiryan, T. J. Bunning "Dynamic optical properties of gold nanoparticles/cholesteric liquid crystal arrays" MRS Communications, 8 (2), 550, 2018
- b) papers published in peer-reviewed conference proceedings,
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- c) papers published in non-peer-reviewed journals and conference proceedings,
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- d) conference presentations without papers,
 1. L. De Sio ""Hot" plasmonic nanomaterials: from agile photonics to nanomedicine, Polish Academy of Sciences (IPPT), 14 May 2018. – **Plenary**
 2. L. De Sio "*Plasmonics powered "hot" multifunctional platforms*" 104th National Congress of the Italian Physical Society, Cosenza and Rende, 17-21 September 2018. – **Plenary**
 3. L. De Sio "Thermo-plasmonic assisted biomedical applications" 6th Conference on Nano- and Micromechanics 3-5 July 2019 - Rzeszów, Poland – **Plenary**
 4. L. De Sio "Thermo-plasmonic triggered hydrogel based optical beam shutter" The 41st PIERS in Rome, Italy 17 - 20 June 2019 – **Invited**
 5. L. De Sio "Thermo-plasmonic triggered light shutter" 17th International Photorefractive Workshop, Sarasota (FL, USA) June 10-14, 2019 – **Invited**
 6. L. De Sio "Thermo-plasmonic triggered optical beam attenuator" 14th Mediterranean Workshop and Topical Meeting Novel Optical Materials and Applications Grand Hotel San Michele, Cetraro - Italy, June 02-08, 2019 – **Invited**
 7. L. De Sio, F. Pierini, N. Tabiryan, T.J. Bunning "Thermo-plasmonic assisted reconfigurable optical components" SPIE Optics + Photonics Digital Forum, 24-28 August 2020 (Virtual Conference) – **Invited**
 8. L. De Sio, F. Pierini, N. Tabiryan, T.J. Bunning "Controlling light with thermo-photonics" Research and Applications of Photonics in Defense - 2020 RAPID, 10-12 August 2020 (Virtual Conference) – **Invited**
- e) manuscripts submitted but not yet published, and
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- f) provide a list any interactions with industry or with Air Force Research Laboratory scientists or significant collaborations that resulted from this work.
 1. Orlando (Florida), December 2017. Meeting with Timothy J. Bunning (AFRL) and Nelson Tabiryan (BEAMCo)
 2. Cetraro (Italy), June 2010 Meeting with Timothy J. Bunning (AFRL)/Nelson Tabiryan/NOMA conference
 3. Online meeting with: T.J. Bunning, Michael Mcconney, Dean Evans, Nicholas Godman; Air Force Research Laboratory

Attachments: Publications a), b), c) and d) listed above if possible.

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Biomimetic keratin gold nanoparticle-mediated *in vitro* photothermal therapy on glioblastoma multiforme

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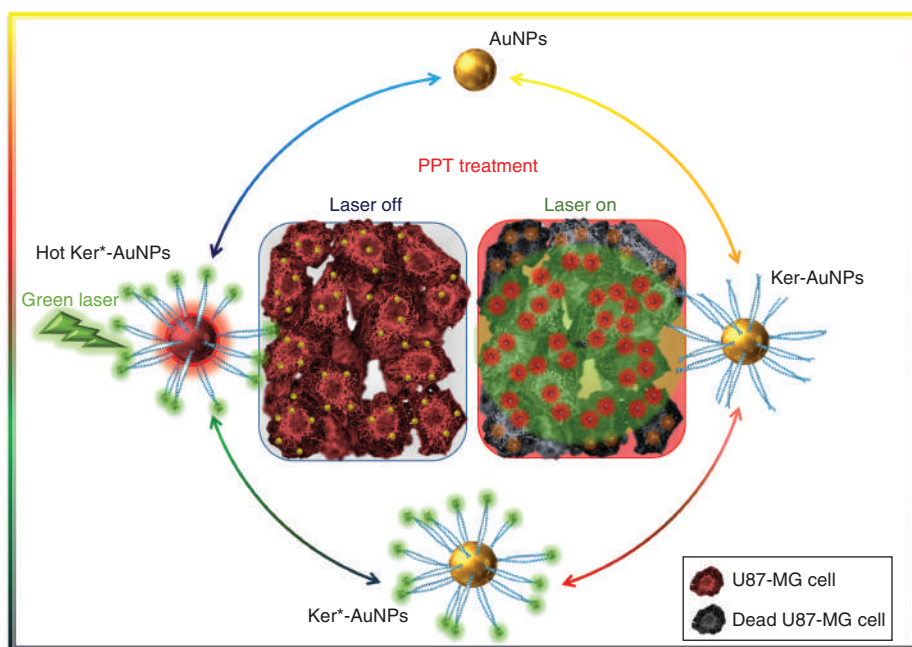
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Aim: To realize and characterize a new generation of keratin-coated gold nanoparticles (Ker-AuNPs) as highly efficient photosensitive nanosized therapeutics for plasmonic photothermal (PPT) therapy. **Materials & methods:** The chemical, physical, morphological and photothermal properties of Ker-AuNPs are investigated using dynamic light scattering, ζ -potential, UV-Visible, Fourier transform infrared spectroscopy, x-ray photoelectron spectroscopy, transmission electron microscopy and high-resolution thermography. *In vitro* experiments are performed on a human glioblastoma cell line (i.e., U87-MG), using viability assays, transmission electron microscopy, fluorescence microscopy, cytometric analyses and PPT experiments. **Results:** Experiments confirm the excellent biocompatibility of Ker-AuNPs, their efficient cellular uptake and localized photothermal heating capabilities. **Conclusion:** The reported structural and functional properties pointed out these Ker-AuNPs as a promising new tool in the field of biocompatible photothermal agents for PPT treatments against cancer-related diseases.

Graphical abstract:



Lay abstract: This work involves the synthesis and application of nanosized keratin-coated gold particles; a protein extracted from wool. Their effect against a specific type of brain cancer, glioblastoma, is investigated. Gold nanoparticles have been the subject of a lot of interest recently due to their ability to convert light energy into heat energy. When the keratin-coated nanoparticles were up taken into cancerous cells and exposed to light from a laser, they heated up and killed the cancer cells. The next step for this research is to conduct more detailed studies in animals, hopefully leading to a new generation of nanosized therapeutics in the fight against cancer.

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Keywords: cancer • gold nanoparticles • keratin • optics • thermoplasmonics

Biomedical applications of plasmonic nanoparticles (NPs), such as gold NPs (AuNPs) and silver NPs, have steadily grown in the past 10 years. In particular, the ability of NPs to convert light into thermal energy (photothermal effect) has been exploited to develop a noninvasive, drug-free therapy defined as plasmonic photothermal (PPT) therapy (PPTT) [1–6]. The PPT effect of NPs is associated with the coherent and dipolar oscillation of the free electrons localized at the metal/dielectric interface, called localized surface plasmon resonance (LSPR) [7]. Indeed, when the NP surface is illuminated, the strong absorption of light, in resonance with the LSPR frequency, induces a significant temperature increase generated by the Joule heating effect, thereby achieving temperatures able to induce apoptosis in cancer cells [8–11]. NP-mediated PPTT has been thoroughly investigated for tumor ablation in animal models, in some cases reaching clinical trial stages [12–14]. This strategy allows the precise localization and controlled heating of the area surrounding the tumor [9,15], producing irreversible cell damage mediated by membrane disruption as well as protein and/or DNA denaturation [16]. The ideal NP formulations for PPT applications have to provide exceptional optical properties (i.e., excellent photothermal conversion efficiency and long-lasting photostabilities), colloidal stability, biocompatibility and a preferential uptake pathway into carcinogenic cells [15]. From this standpoint, AuNPs are suitable candidates for PPT applications because of their chemical and biological stability, small size, strong and tunable LSPR frequency, high level of biocompatibility, and easy surface functionalization and modification [17,18]. Commercially available AuNPs are typically stabilized with cationic surfactants (e.g., cetyltrimethylammonium bromide [CTAB]), which, unfortunately, exhibit a very high intrinsic toxicity toward cells. Indeed, CTAB molecules can be easily desorbed from the AuNPs surface, affecting the biocompatibility of the system [19] by directly interacting with the phospholipid bilayer, causing massive cell membrane disruption [20]. Intrinsic CTAB cytotoxicity can be reduced by utilizing a centrifugation process of AuNP

dispersions, which removes the excess of nonconjugated CTAB [21]. Another commonly used protocol to reduce the CTAB toxicity is based on replacing the CTAB layer with polyethylene glycol [22–25]. Unfortunately, polyethylene glycolated NPs exhibit reduced cellular uptake [26], along with the intrinsic toxicity related to the residual CTAB. Biomaterials and proteins, in particular, are excellent candidates for realizing a new generation of functionalized NPs with improved biocompatibility, biodistribution, stability and half-life [27–29]. For instance, AuNPs capped with endogenous proteins such as bovine serum albumin and human serum albumin showed both surface shielding and functional ligand incorporation [29–33]. To the best of our knowledge, among the different proteins used to functionalize the surface of AuNPs, keratin is still unexplored, and no studies reporting its utilization in the field of PPT-based applications have been found. Keratins are a family of proteins found in several animal tissues such as hair, hooves, skin and claws, providing mechanical reinforcement to tissues. There are several different types of keratins, which all share extensive hydrogen bonds and a high amount of cysteine and disulfide bridges [34]. Keratin has an intrinsic biological activity and biocompatibility [35] and has been studied as a biomaterial platform with applications ranging from wound healing [36,37] to drug delivery [34,38–42]. Water solutions of keratin proteins can be easily obtained and processed in the final material or device in mild conditions. These features are enticing for the use of keratin as a stabilizer (or capping agent) for NPs, coupling the intrinsic biocompatibility of Au and its value as nanotherapeutic with the interesting properties of keratin. A few studies have reported the utilization of keratin as a capping agent for silver NPs, primarily utilized as antimicrobial agents [43–45]. Furthermore, biosynthesis routes for the realization of AuNPs have been previously reported [46,47]. However, these methods are very inefficient in terms of reaction yield and exhibit other drawbacks such as limited size range and broader size distribution. Besides, these methods require long purification steps to separate NPs from their surrounding matrix. Moreover, the AuNPs surface is already in contact with proteins from the bacteria and their extracellular matrix, making the precise control on the AuNPs surface, the aim of this work, significantly more challenging. Here, we report the synthesis, application and characterization of a new generation of keratin-coated AuNPs (Ker-AuNPs) as a highly efficient photosensitive platform for PPT applications. The biomimetic properties of Ker-AuNPs derive from the combination of inorganic AuNPs (synthetic nanomaterials) and a biocompatible coating made of a keratin layer (natural material) [48]. This powerful combination entails the benefits of two worlds: AuNPs are excellent nanosources of heat; and keratin provides a protein-based interface for the AuNP to interact with the biological environment, similar to the naturally occurring protein corona effect [49,50]. We validate the exceptional biocompatibility of Ker-AuNPs and perform PPT experiments on a human cell line (U87-MG) derived from glioblastoma, a brain tumor of glial origin, one of the most aggressive and currently untreatable cancers [51,52]. Thermo-optical experiments are conducted in different environments of increased complexity, from aqueous dispersion to the *in vitro* model represented by the U87-MG human glioblastoma cell line. Morphological, optical, thermo-optical and viability experiments confirm the suitability of Ker-AuNPs. These encouraging results provide the groundwork for the new generation of biocompatible keratin-bonded photothermal agents for PPT treatments.

Materials & methods

Keratin extraction

According to a previously reported protocol, the water solution of keratin was obtained from wool fibers [53]. Olimpia SRL (Treviso, Italy) kindly donated Argentinian sheep wool. Briefly, wool fibers were cleaned using a 1:1 methanol/acetone solution under agitation overnight and rinsed twice with clean solvents then twice with Milli-Q distilled water. Cleaned fibers were dried overnight at room temperature. Keratin was extracted from wool fibers with a water solution containing 7 M urea, 0.05 M sodium dodecyl sulfate and 1.1 M 2-mercaptoethanol. The extraction was performed with 10 g of wool in 180 ml of solution at 60°C for 5 h. Afterward, the solution was filtered with a 50-mesh stainless steel grid and dialyzed against Milli-Q distilled water for 3 days with seven water changes. The resulting solution was centrifuged to remove undissolved fiber residues. The protein concentration was measured by drying a known volume of the solution and weighing the dried residue. When necessary, the solution was concentrated by partial water evaporation.

Synthesis of Ker-AuNPs

AuNPs were synthesized according to the Turkevich method [54], using sodium citrate as a reducing agent. To produce the Ker-AuNPs, AuNPs solution and 1.5 mM Au, was mixed with the keratin solution to have a 1:100 weight ratio between Au and keratin. In carrying out keratin's bioconjugation on AuNPs, an amount of keratin three orders of magnitude higher than what was expected to be conjugated was used. This large excess of keratin was

used to ensure that all the AuNPs binding sites were saturated. Similarly, a water solution of CTAB was added to the AuNPs to allow CTAB to bind to the AuNPs. The synthesis protocols for Ker-AuNPs and CTAB-AuNPs were performed with the same weight/weight ratio to have a similar functionalization. The solutions were gently shaken overnight, then AuNPs were centrifuged twice at $14300 \times g$ and resuspended in MilliQ water to remove unbound keratin or CTAB. To produce the fluorescent Ker-AuNPs (Ker*-AuNPs), keratin was first labeled by using the FluoReporter FITC Protein Labeling Kit (Thermo Fisher, MA, USA) following the manufacturer's instructions. In particular, a buffer solution of NaHCO_3 1 M, pH 9, was prepared. A total of 1955 μl of buffer and 45 μl of keratin solution (44 mg/ml) were mixed to obtain 2 ml of solution with a final keratin concentration of 1 mg/ml. Fluorescein isothiocyanate (FITC) powder was dissolved in 10 μl of dimethyl sulfoxide. The solubilized FITC was added to the buffered keratin solution and mixed under stirrer for 1 h in dark condition at room temperature. The keratin-FITC solution was dialyzed in a dialysis cassette with a molecular weight cut off of 3500 kDa for 3 days against MilliQ water. Ker*-AuNPs were synthesized using the same protocol used for Ker-AuNPs.

AuNPs characterization

Dynamic light scattering & ζ -potential

The hydrodynamic size and ζ -potential of Ker-AuNPs were characterized through dynamic light scattering experiments using a Zeta Sizer NanoZS instrument (Malvern Pananalytical, Malvern, UK). For the hydrodynamic size characterization, three samples were measured. The ζ -potential was measured between -200 and +200 mV, and three measurements were averaged for each sample.

UV-Vis & infrared spectroscopy

UV-Vis absorption spectra were acquired with a UV-Vis light source (CLS 100; Leica, Vienna, Austria) equipped with a spectrometer (USB 2000 + XR1, Ocean Optics, FL, USA). Fourier transform infrared spectroscopy was performed with a Vertex spectrometer (Bruker, MA, USA) in transmission mode using KBr pellets. Keratin and Ker-AuNPs were cast from their respective water solutions on KBr and allowed to dry for 48 h at 50°C. Samples were then finely ground with a mortar and pressed into a pellet. The baseline was acquired using a blank KBr pellet. Spectra were measured from 400 to 4000 cm^{-1} with a resolution of 4 cm^{-1} and the acquisition of 64 scans.

Transmission electron microscopy imaging

Transmission electron microscopy (TEM) images were recorded using a JEOL JEM-1011 (JEOL USA, Inc, MA, USA) apparatus operating at 100 kV. One drop of the sample in its water solution was cast on a Cu grid coated with an ultrathin carbon film that was previously plasma cleaned to increase hydrophilicity. Excess solution was wicked off from the grid's side, and then it was let to dry for at least 24 h before imaging.

X-ray photoelectron spectroscopy

The x-ray photoelectron spectroscopy (XPS) analyses were carried out with a Kratos Axis Ultra DLD spectrometer (Kratos Analytical Ltd, UK) using a monochromatic Al K α source, operated at 20 mA and 15 kV. Samples for XPS were prepared by drop-casting a few microliters of concentrated keratin and Ker-AuNPs solutions onto a freshly cleaved, highly oriented pyrolytic graphite (ZYG grade, NT-MDT) substrate. Survey scan analyses were carried out with an analysis area of 300 $\times$ 700 μm and a pass energy of 160 eV. High-resolution analyses were carried out with the same analysis area and a pass energy of 10 eV. Spectra have been charge corrected to the mainline of the carbon 1s spectrum (due to C-C bonds) set to 285 eV. Spectra were analyzed using CasaXPS software (version 2.3.22).

Thermo-optical setup

Light-triggered photothermal experiments were performed using the thermo-optical setup illustrated in Figure 1. It makes use of a resonant CW green laser (gem532; Laser Quantum, Stockport, UK), operating at 532 nm in the high-absorption band of Ker-AuNPs. The laser beam was directed perpendicularly (from the top) to the air/solution interface, using three mirrors, in the central part of a glass tube.

A high-resolution thermal camera was used to map and identify (side view) both the spatial heating distribution and the temperature profile under top-pumping laser illumination. The dynamic infrared (IR) thermographic analysis was carried out using a FLIR (A655sc) thermal camera (FLIR System, OR, USA) that produces thermal images of 640 $\times$ 480 pixels with an accuracy of $\pm 2^\circ\text{C}$. The thermal camera was suitably equipped with a close-up

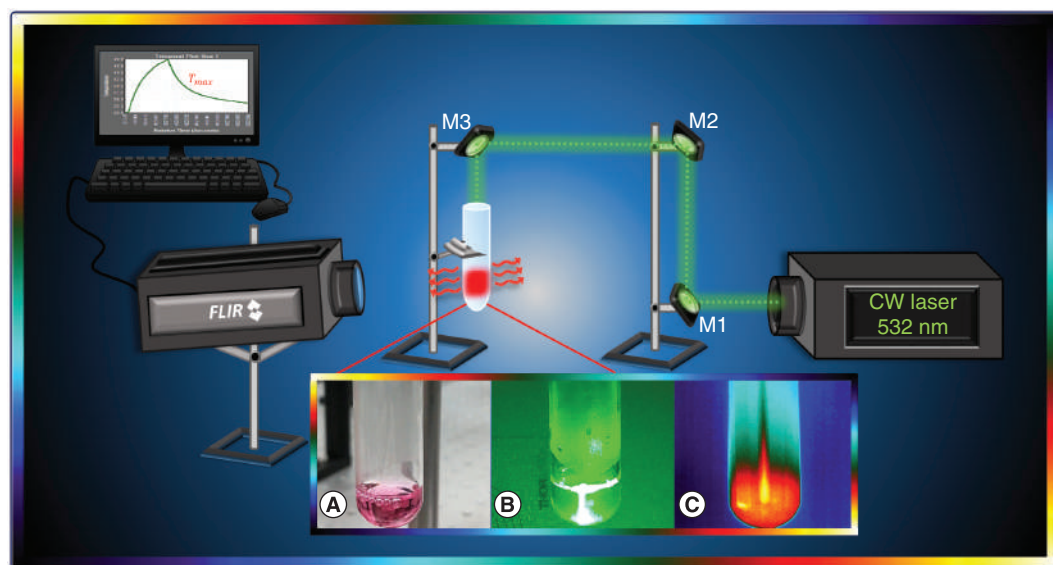


Figure 1. Schematic representation of the thermo-optical setup used for sample characterization. The inset shows a glass tube containing the U87-MG + fluorescent keratin-coated gold nanoparticle sample used to conduct the photothermal experiments, (A) before irradiation, (B) during irradiation, along with (C) the corresponding thermographic view. M1, M2 and M3 are the mirrors.

IR lens characterized by a magnifying factor of $2.9\times$, a spatial resolution (instantaneous field of view) of $50\text{ }\mu\text{m}$, and a reduced working distance. To measure the maximum and mean temperature values reached in the whole sample volume, a circular region (1.76 cm^2) of interest, encompassing the irradiated volume, was selected on thermographic images. The camera works seamlessly with proprietary software (FLIR Research IR Max; FLIR System, OR, USA) that enables recording and processing the camera's thermal data. Camera control parameters were set, such that sample emissivity was 0.89 (glass tube) and camera-sample working distance was 12 cm. Data collection and analysis were based on the careful evaluation of representative IR image sequences acquired during the laser irradiation.

Cell culture

U87-MG glioblastoma multiforme cell line was purchased from CLS Cell Lines Service GmbH (Eppelheim, Germany). It was grown in DMEM supplemented with 10% heat-inactivated fetal bovine serum (FBS; Sigma-Aldrich, MO, USA), 100 IU/ml penicillin G, 100 $\mu\text{g}/\text{ml}$ streptomycin, 1% L-glutamine, 1% nonessential amino acids and 1 mM sodium pyruvate at 37°C in a 5% CO_2 -humidified atmosphere. The medium was changed twice weekly, and the cells were subcultured only when confluent.

MTS assay

U87-MG cells were seeded into 96-well plates (2×10^3 cells/well) and maintained overnight. For comparison, cells were exposed to various concentrations of AuNPs, CTAB-AuNPs and Ker-AuNPs (0–50 μM) for 24, 48 and 72 h, respectively. Viability was measured by MTS–formazan reduction (Promega, WI, USA) by absorbance at 492 nm as previously reported [55]. Results were indicated as percentage of the mean $\pm$ standard deviation of viability versus control. Three independent experiments were performed in quintuplicate, and data were analyzed by one-way ANOVA, and a p-value < 0.05 was considered statistically significant.

Immunofluorescence staining

U87-MG cells (5×10^3) were plated on chamber slides (Nunc Lab-Tek, MA, USA) in DMEM medium supplemented with 10% FBS in presence or not of Ker*-AuNP (50 μM) for 24 h. For immunofluorescence staining, the protocol previously described was followed [56]. Briefly, cells were washed three-times in phosphate-buffered saline (PBS), fixed for 15 min with 4% paraformaldehyde at 4°C and then stained with TRITC-Phalloidin (dilution

1:50, Sigma-Aldrich). Nuclei were counterstained by incubating cells with DAPI for 3 min in the dark. A Nikon Eclipse Ni motorized microscope (Nikon, Tokyo, Japan) was used to acquire the images at 40x magnification.

TEM imaging of U87-MG + Ker*-AuNPs

U87-MG cells incubated with Ker-AuNPs (Ker*-AuNPs) for 24 h were detached and transferred in the Eppendorf tubes for TEM processing. After centrifugation, at $250 \times g$ for 5 min, the cell pellet was fixed with 2.5% glutaraldehyde (SIC, Rome, Italy) in 0.1 M PBS for 2 days at 4°C and then rinsed with PBS. Afterward, samples were postfixed using 2% osmium tetroxide (Agar Scientific, Stansted, UK) for 2 h and rinsed again in PBS [57]. The specimens were dehydrated by exchange with ethanol, immersed in propylene oxide (BDH Italia, Milan, Italy) for solvent substitution, embedded in epoxy resin Embed-812 (SIC). Ultrathin (80–90 nm) sections were obtained using an ultramicrotome (Leica EM UC6; Leica); for the TEM observation, the ultrathin sections were collected on 100-mesh copper grids (Assing, Rome, Italy) stained with a mix of lanthanides solution (Uranyless; Electron Microscopy Sciences, PA, USA) and lead citrate [58]. Imaging was performed using a TEM (Carl Zeiss EM10; Carl Zeiss, NY, USA) with an accelerating 60 kV voltage with a DEBEN XR80 AMT CCD camera (Deben, Melton, UK).

Cytometric analyses

Uptake cytometric assay was performed using FITC-labeled Ker-AuNPs (Ker*-AuNPs). Briefly, 5×10^5 U87-MG cells were incubated in suspension with 50 μM Ker*-AuNPs in 15 ml polypropylene tubes at 37°C in a 5% CO₂-humidified atmosphere. After 2, 4, 6, 24 and 48 h, cells were washed with PBS and resuspended in 500 μl of PBS, 2% FBS. Samples were acquired on a FACsARIA II instrument using FACs DiVa software (v.6.1.1, both by Becton Dickinson, NJ, USA). Biparametric cytotoxic assay was performed by incubating in suspension U87-MG cells (5×10^5) with 50 μM Ker*-AuNPs as already described. Then, cells were washed in PBS, resuspended in PBS, 2% FBS and irradiated (details on the photothermal experiments are reported below). After performing the photothermal experiments, cells were centrifuged at $250 \times g$ for 5 min, resuspended in DMEM supplemented with 10% FBS and grown at 37°C in a 5% CO₂-humidified atmosphere. After 24 h cells were counterstained with propidium iodide (PI 1:100 dilution of a 1 mg/ml ready-to-use solution; Merck, Darmstadt, Germany) and the percentage of PI-positive (PI⁺) cells (dead cells), FITC-positive (FITC⁺) cells (Ker*-AuNPs-loaded cells) and double-positive cells (both dead and Ker*-AuNPs-loaded cells) were evaluated by flow cytometry. In both cases, at least 20,000 events were recorded and analyzed using Flowing software (v2.5.1, Turku Centre for Biotechnology, Turku, Finland).

Trypan blue exclusion assay

U87-MG cells (5×10^5) were incubated in suspension in 15 ml polypropylene tubes with 50 μM Ker-AuNPs at 37°C in a 5% CO₂-humidified atmosphere for 24 h. Then, cells were washed in PBS and resuspended in PBS, 2% FBS and irradiated (details on the photothermal experiments are reported below). After irradiation, cells were centrifuged at $250 \times g$ for 5 min, resuspended in DMEM medium supplemented with 10% FBS and grown 37°C in a 5% CO₂-humidified atmosphere. After 24 h, cells were collected, diluted 1:1 with trypan blue, and cell viability was determined by loading 10 μl of the cell suspension into Countess cell counting chamber slides and read using a Countess automated cell counter (Invitrogen, CA, USA). Results were indicated as percentage of the mean $\pm$ standard deviation of viability versus control. Three independent experiments were performed in triplicate. Student's *t*-test analyzed data, and a *p*-value < 0.05 was considered statistically significant.

Results

Optical & morphological properties of AuNPs

Optical, spectroscopic and morphological analysis of both as-synthesized and Ker-AuNPs was performed to characterize keratin's binding to the AuNPs and its effect on optical and structural properties. Figure 2A shows the absorption spectra of the spherical AuNPs and Ker-AuNPs dispersed in water. A red shift of the LSPR frequency from 522 to 527 nm is observed because of the change of the local refractive index surrounding the AuNPs. A simplified simulation using the Mie theory for LSPR (Supplementary Figure 1) shows that a 5 nm red shift is compatible with an increase of the local refractive index of 0.07 refractive index units. The absorption spectra of Ker-AuNPs dispersed in PBS and in the presence of cells (U87-MG) are reported in (Supplementary Figure 2A). The spectral curves are unchanged. Only a small spectral shift (3 nm) is present, proving the stability of the AuNPs

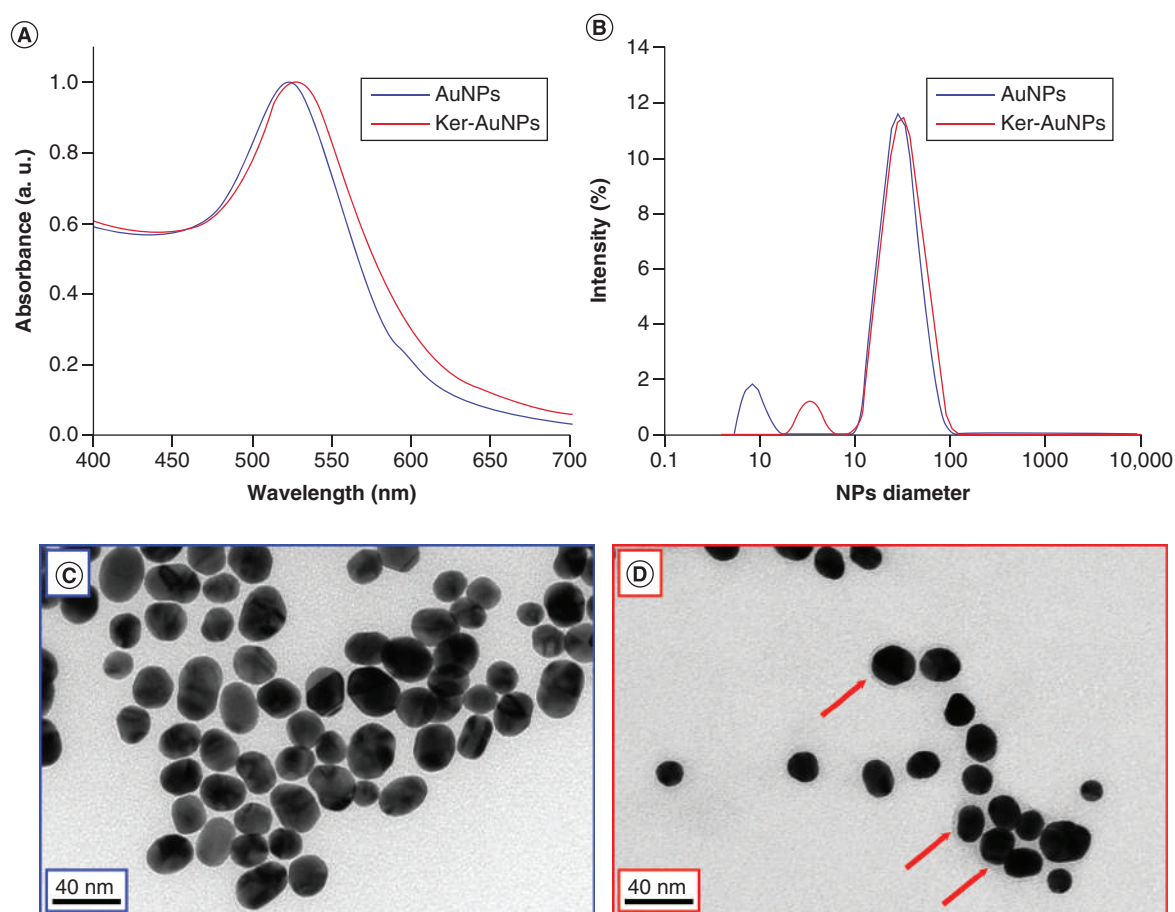


Figure 2. Optical and morphological properties of gold nanoparticles. (A) UV-Vis absorption spectra of AuNPs (blue curve) and Ker-AuNPs (red curve). (B) Dynamic light scattering measurements of AuNPs (blue curve) and Ker-AuNPs (red curve). (C) Transmission electron microscopy images of AuNPs. (D) TEM images of Ker-AuNPs. AuNP: Gold nanoparticle; Ker-AuNP: Keratin-coated AuNP.

and showing the stability/response of Ker-AuNPs to the change of the surrounding medium produced by the presence of U87-MG cells. Besides, absorption spectra at different temperatures (Supplementary Figure 2B) were acquired, evidencing a perfect spectral overlap after all the heating experiments, thus demonstrating the thermal stability of Ker-AuNPs. To further highlight the improved stability provided by keratin, plain AuNPs, CTAB-AuNPs and Ker-AuNPs were dispersed in a physiological water solution (100 mM PBS at pH 7.4) (Supplementary Figure 3B). The plain AuNPs and CTAB-AuNPs showed a drastic color change due to AuNPs aggregation. In contrast, the Ker-AuNPs maintained their original ruby red color thanks to the keratin layer's extraordinary stability present on the AuNPs surface. Figure 2B shows the dynamic light scattering measurements, evidencing a variation of the hydrodynamic diameter from 28.2 (AuNPs, blue curve) to 32.7 nm (Ker-AuNPs, red curve), thus confirming the presence of a thicker (keratin) layer surrounding the AuNPs. Supplementary Figure 3A shows the size distribution of the three different systems (plain AuNPs, CTAB-AuNPs and Ker-AuNPs) used in the experiments. Since they all derive from the same initial batch of AuNPs conjugated with keratin and CTAB, the NP size and distribution are the same. They are not modified by the conjugation protocol, thus ensuring consistency and reproducibility of the experiments. Fourier transform infrared spectroscopy analysis (Supplementary Figure 4) evidences the presence of keratin proteins attached to the AuNPs. The peaks at 1600 and 1450 cm^{-1} are the Amide I and II bands of proteins. The deconvolution of the amide I absorption band, using a previously reported procedure [59,60], shows that when comparing keratin self-assembly on AuNPs and freestanding films, Ker-AuNPs showed a higher percentage of alpha-helices (15% on Ker-AuNP vs 6% on keratin films) and a lower amount of compact beta sheets (23% on Ker-AuNP vs 35% on Keratin films). This different self-assembly reflects a more loosely organized structure of

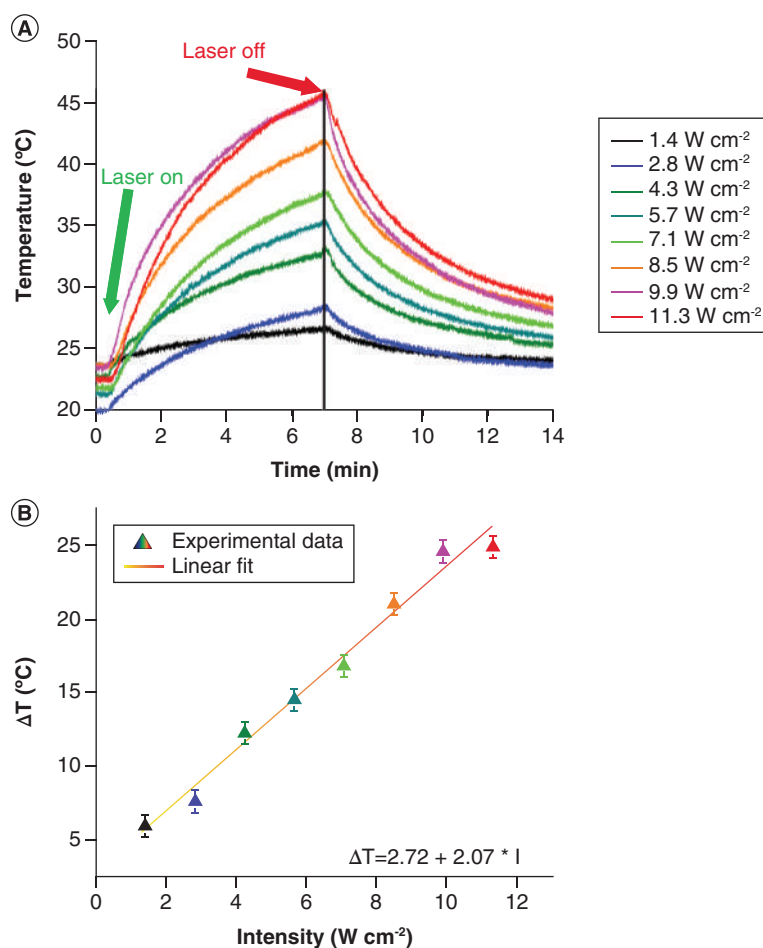


Figure 3. Photo-thermal properties of keratin-coated gold nanoparticles. (A) Time-temperature dependence of keratin-coated gold nanoparticles dispersed in water under pump beam illumination. (B) Linear fit of the temperature variation versus the intensity of the pump beam. The coefficient of determination $R^2 = 0.98$ highlights the goodness of the fit.

keratin on the AuNPs than freestanding films, probably due to the presence of cysteine-Au bonds that prevent more compact self-assembly.

The successful conjugation between the keratin molecules and the AuNPs surface influences the ζ -potential of the AuNPs. Indeed, AuNPs dispersed in water exhibit a ζ -potential of (-42 ± 4) mV, whereas for the Ker-AuNPs (-18 ± 2) mV, confirming the change of the chemical environment surrounding the AuNPs. The ζ -potential of CTAB-AuNPs is $(+33.6 \pm 2)$ mV. TEM characterization performed on both AuNPs (Figure 2C) and Ker-AuNPs (Figure 2D) solutions, besides highlighting the spherical geometry and a moderate size-distribution of the NP populations, confirms the presence of a halo around the AuNPs after the functionalization with keratin (Figure 2D). Similarly, also XPS analysis supports the presence of keratin proteins in the Ker-AuNPs sample. Indeed, the sample shows the presence of nitrogen and sulfur signals typical of the peptide bonds and disulfide bonds of keratin, that are obviously not present in the AuNPs sample. A closer look at the S 2p signal (Supplementary Figure 5) suggests that the interaction between AuNPs and keratin is mediated by the sulfurs, as the Ker-AuNPs show signals that could be assigned to the formation of sulphur-Au bonds.

Photothermal properties of Ker-AuNPs

Photothermal experiments were carried out using the thermo-optical setup sketched in Figure 1. Ker-AuNPs dispersed in water (2.25×10^{-3} M in Au) were exposed at different intensity values of the pump beam (from 1.4 to 11.3 W/cm²) for 7 min. Figure 3A shows that upon illumination (laser ON), there is a gradual and significant temperature increase up to 45°C because of the PPT heating of Ker-AuNPs. The sample starts to cool back down to room temperature as soon as the pump beam is switched off (laser OFF). The temperature profiles reported in Figure 3A were obtained by processing the corresponding thermal images as detailed in the Materials & methods section. Figure 3B evidences a linear correlation between the temperature variation ($\Delta T = T - T_0$, where T_0 is the initial sample temperature when the pump beam is OFF) and the intensity of the pump beam, confirming the

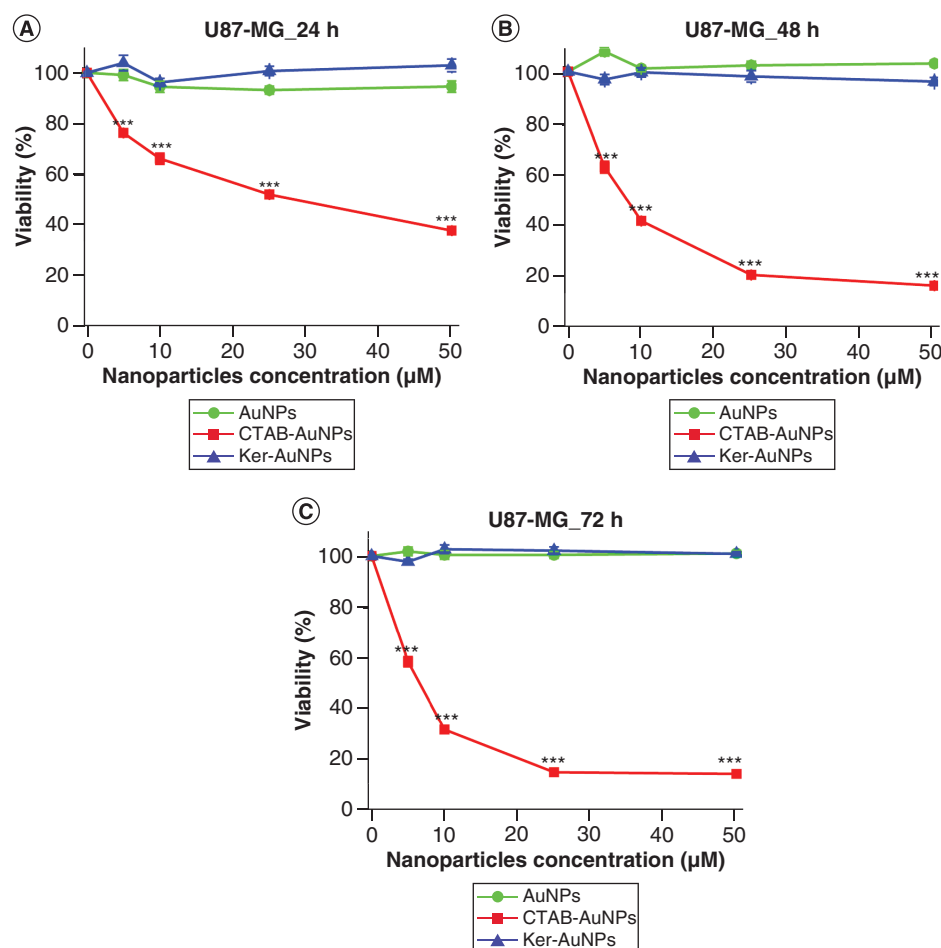


Figure 4. Effects of gold nanoparticles (green curves), cetyltrimethylammonium bromide-coated gold nanoparticles (red curves) and keratin-coated gold nanoparticles (blue curves) on the viability of U87-MG cells. MTS assay shows the viability (expressed as percentage vs control) of U87-MG cells incubated with increasing concentrations (0–50 μM) for 24 h (A), 48 h (B) and 72 h (C). (***) $p < 0.001$.

AuNP: Gold nanoparticle; CTAB-AuNP: Cetyltrimethylammonium bromide-coated gold nanoparticle; Ker-AuNP: Keratin-coated gold nanoparticle.

expected behavior according to theoretical predictions reported previously [61]. Control experiments realized with a water solution of keratin did not show any significant temperature increase (Supplementary Figure 6), supporting that the observed temperature increase (Figure 3A) can be attributed to the photothermal properties of AuNPs.

It is worth pointing out that the light-heat conversion property of the Ker-AuNPs is retained even after performing several illumination cycles (eight), as shown in Figure 3A, hence underlining the excellent photothermal stability of Ker-AuNPs, which is a mandatory requirement for thermal dosimetry and treatment planning in PPT-based applications.

Biocompatibility of Ker-AuNPs

To evaluate the biocompatibility of Ker-AuNPs, a dose-response assay on human glioblastoma cell line U87-MG was performed. Experiments were performed by utilizing AuNPs, CTAB-AuNPs and Ker-AuNPs (see Materials & methods). Figure 4 summarizes the viability results of U87-MG cells incubated with different concentrations (0–50 μM) of AuNPs (green curves), CTAB-AuNPs (red curves) and Ker-AuNPs (blue curves) at different incubation times (A, 24 h; B, 48 h; C, 72 h). As expected, the capping agent plays an important effect on cell viability. Indeed, CTAB-AuNPs were found to be very toxic to cells, causing a reduction in cell viability depending on both concentration and incubation time. In particular, incubation with CTAB-AuNPs at the highest concentration of 50 μM for 72 h reduced U87-MG cell viability down to 20%. Conversely, both Ker-AuNPs and AuNPs were

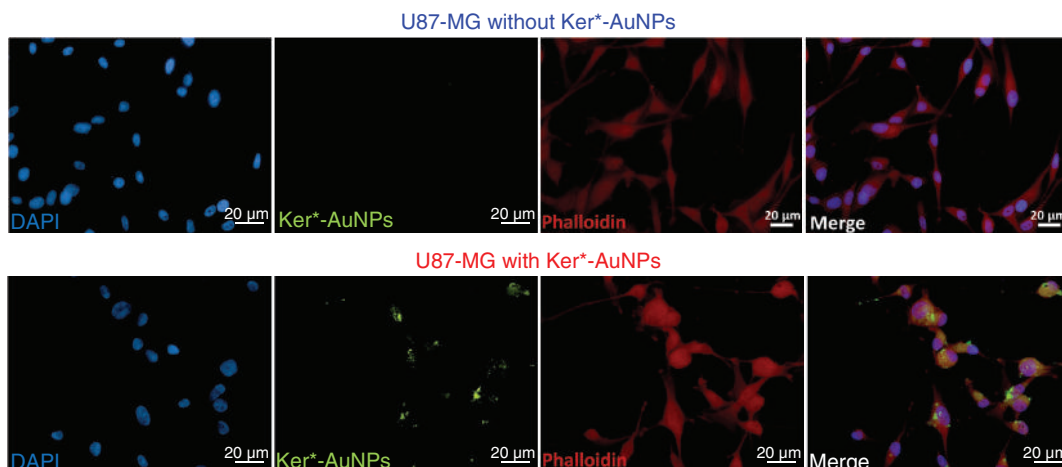


Figure 5. Immunofluorescence staining of U87-MG cells alone and incubated with keratin-coated gold nanoparticles. Photographs show nucleus (DAPI, blue), Ker*-AuNPs (green), the cytoskeleton (Phalloidin, red) and the merged channels (merge) in control cells (without Ker*-AuNPs, upper images) and Ker*-AuNPs-treated cells (lower images).

Ker*-AuNP: Keratin-coated gold nanoparticle.

completely biocompatible, exhibiting viability of about 100% for concentrations up to 50 μM . It is noteworthy that AuNPs, despite their good biocompatibility, are difficult to use in real applications because of the weak stabilizing effect of citrate molecules that is replaced by proteins and other biomolecules as soon as the NPs enter the biological environment [62]. Indeed, plain AuNPs in solution are stabilized by electrostatic repulsion, making them stable only at deficient concentrations. In the viability experiments (Figure 4), larger volumes of plain AuNPs solution were utilized to achieve the same quantities used for Ker-AuNPs (50 μM). Besides, plain AuNPs require a coating to allow their functionalization with fluorescent molecules and are therefore not suitable for detection with fluorescence microscopy and flow cytometry. Conversely, the keratin coating provides improved stability at the higher concentration, a mandatory requirement for performing PPT experiments at physiological pH and salt concentration used for *in vitro* studies. Moreover, the keratin layer's presence easily allows the post functionalization process with other molecules (e.g., fluorescent FITC tag). Ker-AuNPs, on the other hand, possess a very similar biocompatibility profile of AuNPs (see Figure 4C) with a protein coating that provides improved stability in the biological environment, significantly improving their utilization as nanotherapeutic agents.

Because no significant reduction in cell viability was observed using the maximum concentration of 50 μM of Ker-AuNPs, this value was utilized for all the experiments reported below. The utilized concentration value allows from one side, maximizing the photothermal properties while from the other side minimizing the utilized amount of the Ker-AuNPs solution, a condition highly required in PPT-based treatment planning [63].

Interaction between Ker*-AuNP & U87-MG cells

U87-MG cells costained with Phalloidin-Texas Red and DAPI were incubated with and without FITC-labeled Ker-AuNPs (Ker*-AuNPs) for 24 h. Figure 5 shows the fluorescence microscope images of U87-MG cells without (top images) and with (bottom images) the presence of Ker*-AuNPs. It is worth noting that the Ker*-AuNPs green signal colocalizes with both Phalloidin-Texas Red and DAPI signals suggesting that Ker*-AuNPs physically interact with U87-MG cells. Moreover, to quantitatively understand the dynamics of U87-MG cells + Ker*-AuNPs interaction, a cytofluorimetric analysis of U87-MG cells was incubated with 50 μM Ker*-AuNPs for 0, 2, 4, 6, 24 and 48 h was performed. During the first 6 h, the percentage of U87-MG cells loaded with Ker*-AuNPs (shortly defined positivity) is about 3.5% (Supplementary Figure 7B, C & D). Interestingly, as shown in Figure 6, after 24 h of incubation, the positivity raises to 23%, reaching a positivity of about 68% after 48 h, whereas no significant variation was observed at longer incubation times. The kinetics of Ker-AuNPs cellular uptake rate was fitted using a logistic model (Supplementary Figure 7E). These results evidence that the cellular uptake dynamic process is a time-dependent phenomenon governed by several parameters such as NPs size/geometry, cell types and enhanced permeability and retention effect [64–66].

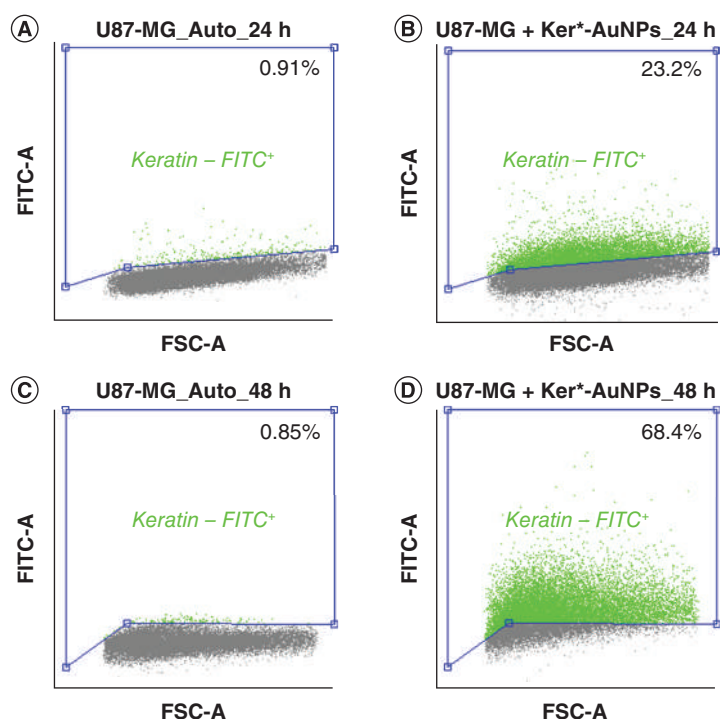


Figure 6. Cytofluorimetric analysis of U87-MG cells incubated with Ker*-AuNPs. Cytofluorimetric analysis showing U87-MG cells positivity (expressed as percentage of Keratin-FITC⁺ cells) to Ker*-AuNPs after 24 h (B) and 48 h (D) of incubation time along with their respective controls (A & C). FITC⁺: Fluorescein isothiocyanate; Ker*-AuNP: Keratin-coated gold nanoparticle.

Ultrastructural analysis of Ker*-AuNPs uptake

Cellular uptake of Ker*-AuNPs and ultrastructural changes were examined by high-resolution TEM analysis. Results reported in Figure 7 confirm the internalization of the Ker*-AuNPs into U87-MG cells. U87-MG cells (Figure 7) exhibit an irregular and great morphological variability, cell surface area and membrane folding exhibited various types of protrusions, including blebs and filopodia. Figure 7A and B evidence the plasma membrane projections surrounding (Figure 7A) and engulfing (Figure 7B) several Ker*-AuNPs before internalization into the cytoplasm. There is evidence of Ker*-AuNPs on the apical surface along with well-packed Ker*-AuNPs internalized in endocytic vesicles with different electron density (Figure 7C & D). Cell structures like nucleus, mitochondria, Golgi apparatus and rough endoplasmic reticulum were visible, and oval or rod-shaped mitochondria with a medium or high electron density matrix were detected (Figure 7A & D).

PPT experiments on U87-MG cells incubated with Ker*-AuNPs

Photothermal experiments, performed by utilizing the thermo-optical setup sketched in Figure 1, were devoted to study the effect of the laser illumination on the viability of the U87-MG cells. Experiments were realized by top-illuminating both U87-MG cells (control sample) and U87-MG cells incubated with Ker*-AuNPs, for 10 min at 11.3 W/cm². Illumination time and intensity values were selected to optimize both photothermal properties and cell viability reduction.

While the illumination of U87-MG cells resulted in a negligible increment of temperature to <2°C (Figure 8A & B, blue curve), U87-MG cells incubated with Ker*-AuNPs once illuminated showed a moderate temperature increase of about 7.5°C (Figure 8C & B, red curve) caused by the PPT effect. The effect of the laser irradiation on cell viability was studied by biparametric cytofluorimetric analysis. This technique aims at detecting the percentage of dead U87-MG cells (incorporating the fluorescent DNA intercalant PI, PI⁺ cells) and cells loaded with Ker*-AuNPs (FITC⁺ cells). U87-MG cells irradiated without Ker*-AuNPs (Figure 8D) showed 23.83% (0.68 + 23.15%) of PI⁺ cells, compared with 40.29% (14.16 + 26.13%) of PI⁺ cells in the presence of Ker*-AuNPs (Figure 8E). These results confirm that the cell viability is minimally affected by laser illumination alone and that the presence of Ker*-AuNPs increases the mortality because of the induced local heating. Noteworthy, among the total percentage of dead cells in the presence of Ker*-AuNPs (Figure 8E), 26.13% are both PI⁺ and FITC⁺ while 14.16% are only PI⁺. The total percentage of FITC⁺ cells obtained after a total incubation time of 48 h (24 h before + 24 h after the laser illumination) is 57.42% (31.29 + 26.13%, Figure 8E), in line with the data obtained after a 48-h incubation without any illumination process (i.e., 68.4%, Figure 6D). A trypan blue exclusion assay (Supplementary Figure

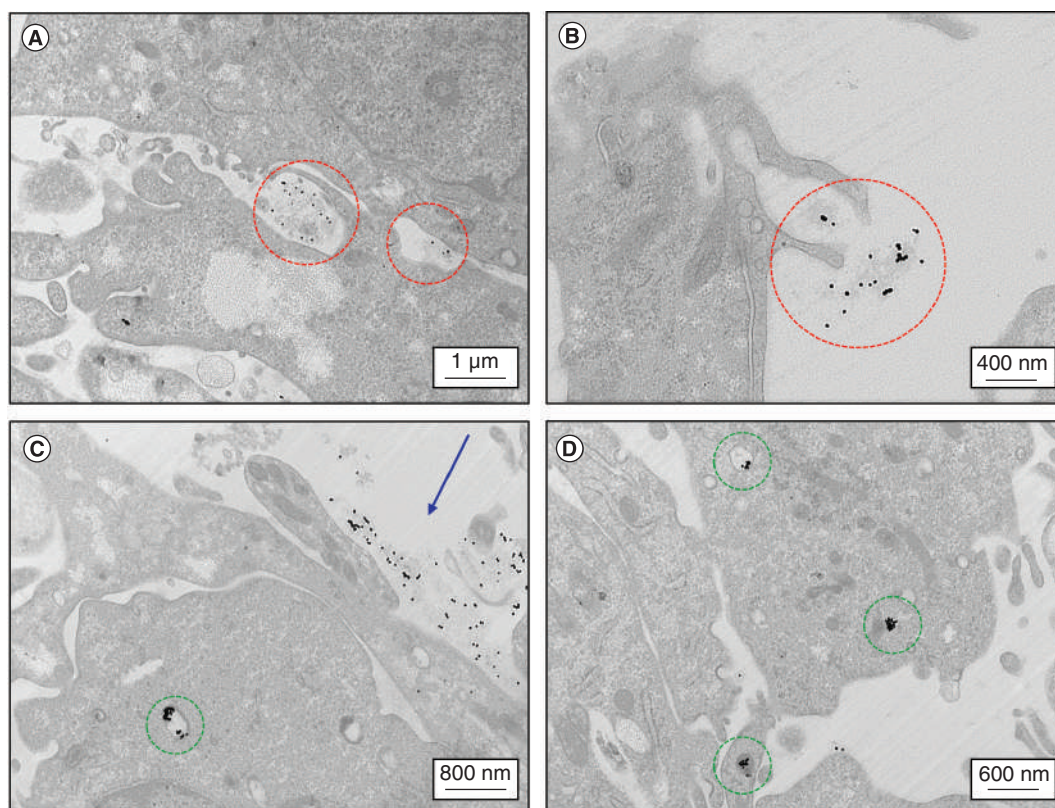


Figure 7. Ultrastructural localization of keratin-coated gold nanoparticles in U87-MG cells. (A & B) Electron dense Ker*-AuNPs were present on the cell surfaces where the plasma membrane projections internalize several Ker*-AuNPs (dotted red circles). **(C & D)** Ker*-AuNPs were found outside the cell along the plasma membrane (blue arrow) as well as internalized inside small dense vesicles (endosomes) (dotted green circles). Ker*-AuNP: Keratin-coated gold nanoparticle.

8) was performed to evaluate the percentage of viability of U87-MG cells incubated for 24 h with Ker*-AuNPs. Percentages of cell viabilities were calculated relative to the control cells (100% viability) to take into account the intrinsic cell death, (t 0) immediately after irradiation, (t 4) at 4 h after irradiation and (t 24) at 24 h after irradiation.

The viability percentage assessed immediately after the experiments did not show any significant variation. After 4 h from the PPT experiments, the percentage of live cells was decreased by about 17%, compared with the control sample.

An even more substantial reduction in the cell viability of about 22% was observed 24 h after irradiation, compared with control samples, comparable with the biparametric cytofluorimetric analysis results. These data highlight that localized photothermal heating produced by Ker*-AuNPs is highly selective and confined, with the capability to suppress (kill) predominantly the U87-MG cells that can uptake Ker*-AuNPs.

Discussion

The photothermal properties of AuNPs have received a lot of attention in the fight against cancer prevalently thanks to a drug-free cancer therapy named PPTT [1,2,4-6,22]. PPTT efficacy compared with other traditional cancer treatments, plays a key role in driving researchers to intensely study new methodologies devoted to improving the PPT-based medical techniques. Among them, the realization of AuNPs with superior optical and bio-related properties is still a compelling solution and yet of great interest. In this framework, we have synthesized, characterized and utilized a new generation of spherical AuNPs layered with keratin (i.e., Ker-AuNPs), thus providing biomimetic properties. The synthesis of Ker-AuNPs is easy and fast, and no limitations for large-scale production are foreseen. Having all the processing carried out in the water, and mild conditions make it easy to combine keratin with AuNPs and preserve keratin's inherent biocompatibility. Moreover, the protocol employed for synthesis

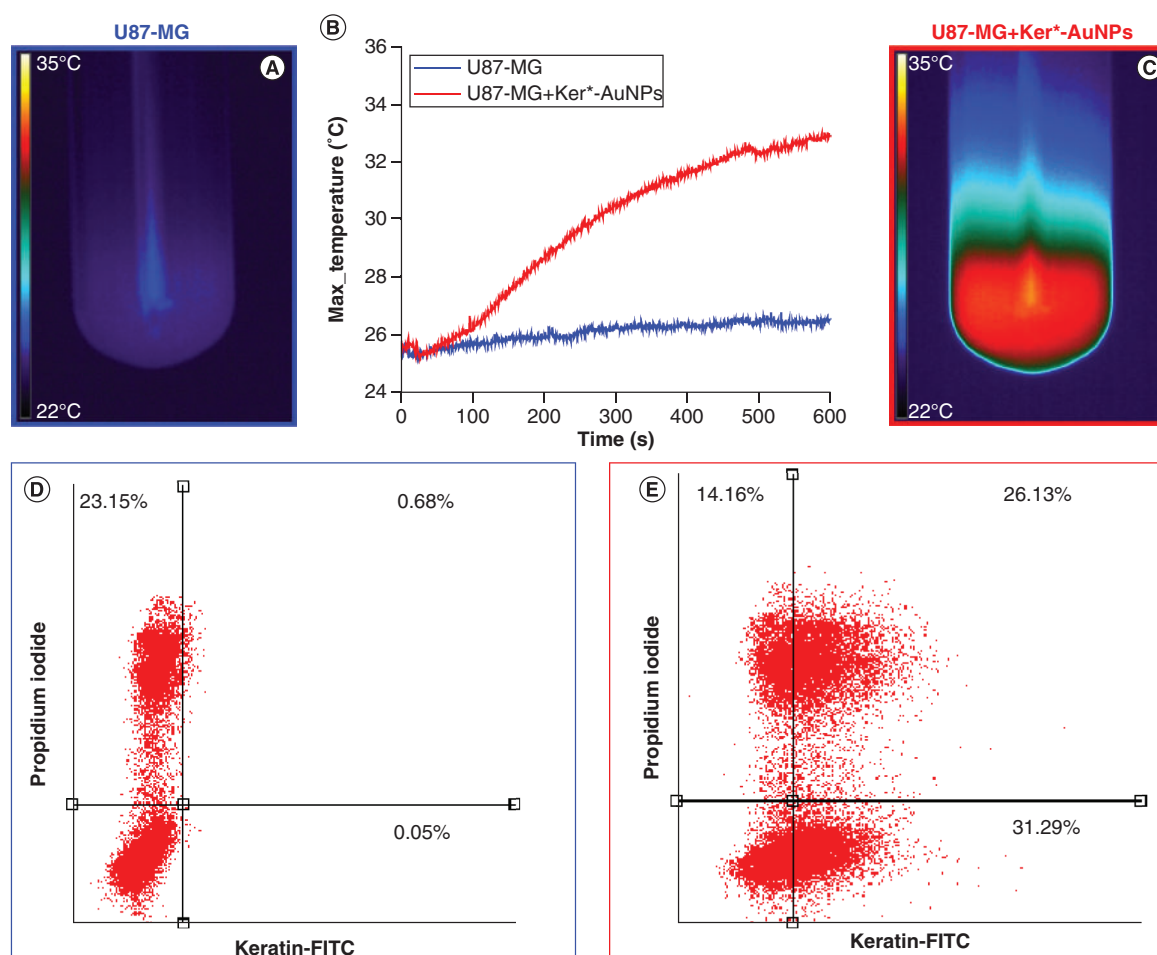


Figure 8. Plasmonic photothermal experiments on U87-MG cells incubated with keratin-coated gold nanoparticles. (A & C) Thermographic images of U87-MG cells alone and incubated with Ker*-AuNPs under laser illumination, (B) along with the corresponding temperature profiles (blue and red curves), (D) biparametric cytofluorimetric analysis of PI staining showing dead U87-MG cells irradiated for 10 min alone and (E) incubated for 24 h with Ker*-AuNPs and then irradiated for 10 min. Dead cells were evaluated 24 h after the irradiation. Ker*-AuNP: Keratin-coated gold nanoparticle; PI: Propidium iodide.

considers a sustainable approach by using a reduced number of components, all nontoxic and nonhazardous. These features make Ker-AuNPs a new generation of AuNPs with superior properties, thus representing a step forward compared with conventional and commercially available AuNPs [20,26]. Ker-AuNPs exhibit elevated colloidal stability, extremely high biocompatibility and excellent photothermal properties. These features have been validated by performing *in vitro* PPT experiments using a glioblastoma cell line (U87-MG). Experimental studies performed with Ker*-AuNPs have evidenced that 68% of the cell population is loaded with Ker*-AuNPs after 48 h of *in vitro* incubation. This evidence can be argued by considering that the cellular uptake's kinetics is influenced by several parameters such as the Ker*-AuNPs size, shape, ζ -potential and surface functionalization [64–66]. Moreover, the cell line's peculiar characteristic along with the membrane structure and the intrinsic cancer cell avidity might play an important role. However, it is important to underlying that 68% of the cell population loaded with Ker*-AuNPs is a great improvement to similar research studies [67]. PPT experiments performed using a high-resolution thermographic optical setup (Figure 1) have shown that the achieved macroscopic temperature increase after 10 min of laser illumination is 35°C (Figure 8B, red curve). Although this value, apparently, is below the minimum threshold to induce cell mortality ($\approx 42^\circ\text{C}$) [63], a reduction of the cell viability of approximately 30% (Figure 8E) has been measured.

This result can be explained by considering that the temperature at the Ker*-AuNPs surface (which are stuck/penetrated on/in the cells membrane) reaches higher values, hence producing a very effective photothermal and/or photo-mechanical damaging [68].

The percentage of cells which turned out to be FITC⁺ ($57.42\% = 26.13\% + 31.29\%$, Figure 8E) is comparable to the percentage of cells interacting with the Ker*-AuNPs after 48 h of incubation (68%, Figure 6), while 26.13% are both PI⁺ and FITC⁺.

The photothermal characterization, performed as a control experiment on U87-MG cells irradiated without Ker*-AuNPs (Figure 8D), evidences a 23.83% of cell mortality. This result can be reasonably explained by considering that, by utilizing the top-pumping illumination geometry (Figure 1), because of the elevated intensity (11.3 W/cm^2), the laser beam gets partially absorbed and scattered by the cells (U87-MG) and their resuspension medium (PBS). For this reason, likely, cells that are at the beginning of the laser beam path might be subject to some heat stress resulting in cell death, although, in the end, the final temperature increase in the whole sample volume is 1.42°C (Figure 8A). In our studies, spherical AuNPs have been used as model particles for PPTT to demonstrate that using a protein-like-corona system can preserve the inherent biocompatibility of AuNPs, facilitating their use as a nanotherapeutic. Ker-AuNPs are stable when dispersed in a physiological environment and when heated at high temperatures upon laser irradiation, owing to the stability of the cysteine-Au bond. The physical interaction given by the keratin coating facilitated both adsorption and internalization on cells, improving the efficacy of the localized heating promoted by the external laser light. TEM analysis reported in Figure 7 shows the interactions between the U87-MG cells and the Ker*-AuNPs. In particular, the blue arrow in Figure 7 C indicates free Ker*-AuNPs near plasma membranes close to the cell's apical surface, thus showing an early endocytosis moment. Figure 7A and B highlights small clusters of Ker*-AuNPs associated with the cell membrane invagination. A careful inspection of Figure 7C (green circle) and Figure 7D evidences the presence of well-packed Ker*-AuNPs in the cytoplasm prevalently located in endosomes, thus presumably representing a later endocytosis moment [69]. Ker*-AuNPs in the last condition are mainly confined inside intact endosomes so every single Ker*-AuNP apparently interacts with the inner membrane (Figure 7C). Based on our findings, only in rare fields, Ker*-AuNPs are free in the cytosol, and thus, the intracellular localization remains almost exclusively endosomal. It is important to point out that the intensity of the laser source (11.3 W/cm^2) has been optimized to maximize the photothermal efficiency of the spherical Ker*-AuNPs *in vitro* experiments. To extend our work to *in vivo*/clinical studies, a new generation of Ker-AuNPs with different shapes such as rods and triangles will be utilized. Indeed, they both possess higher photothermal efficiency [70] and LSPR bands centered in the first biological window [71], where cells and growth media are more optically transparent.

Conclusion

We have reported on a new generation of biomimetic spherical AuNPs capped with keratin proteins (i.e., Ker-AuNPs). They have been studied in terms of morphological, spectroscopic and thermo-optical properties. *In vitro* cell viability assays and PPT experiments, have been conducted by utilizing a glial tumor cell line (U87-MG), representing the most common type of malignant brain tumor among adults. Results have evidenced the extraordinary biocompatibility of Ker-AuNPs, even at the highest concentrations ($50 \mu\text{M}$) and after 72 h of *in vitro* incubation. Moreover, the utilization of Ker*-AuNPs, has allowed a cytofluorimetric analysis to evaluate both cellular uptake and PPT induced cell death. It turns out that 68% of U87-MG cells have been loaded with Ker*-AuNPs, thus generating a reduction in their viability, after performing PPT experiments, of about 30%. A detailed study carried out by a TEM analysis revealed the Ker*-AuNPs cells' internalization mechanism, which turned out to be an endocytosis-based uptake pathway.

Future perspective

Ongoing efforts are devoted to combining spherical Ker-AuNPs with other cancer cell lines such as melanoma, breast and colorectal cancer, and tumor animal models by performing both *in vitro* and *in vivo* experiments. Simultaneously, we are working on a new generation of Ker-AuNPs with different geometries and surface functionalization for improving both photothermal properties and cellular uptake. However, we believe that our results are already promising and can be used to synergistically enhance the efficacy of immunotherapy in the fight against solid tumors [72].

Summary points

- A new generation of biomimetic spherical keratin-coated gold nanoparticles (Ker-AuNPs) has been synthesized.
- An all-optical methodology based on the capability of fluorescent Ker-AuNPs (Ker*-AuNPs) to convert visible radiation into heat for cancer treatment has been reported.
- The *in vitro* efficacy of Ker*-AuNPs has been tested on glioma cell line (U87-MG).
- Viability assay revealed complete biocompatibility of Ker*-AuNPs incubated with U87-MG cells, exhibiting viability of about 100% for concentrations up to 50 μ M.
- Ker*-AuNPs showed good photothermal conversion efficiency and high thermal stability.
- Ultrastructural transmission electron microscopy analysis showed efficient Ker*-AuNPs cells' internalization via an endocytosis-based uptake pathway.
- Photothermal experiments induced a reduction of the cell viability of approximately 30%.

Author contributions

L De Sio and G Perotto conceived the idea and designed the experiments. A Guglielmelli, R Pani and L De Sio conducted the photothermal experiments. P Rosa and A Calogero performed the cell viability experiments. M Contardi, M Prato and A Athanassiou performed the spectroscopic studies. G Mangino realized the FACS characterization. S Miglietta and V Petrozza realized the transmission electron microscopy characterization. G Perotto realized the synthesis and characterization of nanoparticles. A Guglielmelli, G Perotto and L De Sio wrote the paper. L De Sio and G Perotto supervised the project. All authors approved the final version of the manuscript.

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Financial & competing interests disclosure

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Thermoplasmonic-Activated Hydrogel Based Dynamic Light Attenuator

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This work describes the morphological, optical, and thermo-optical properties of a temperature-sensitive hydrogel poly(*N*-isopropylacrylamide-*co*-*N*-isopropylmethacrylamide) [P(NIPAm-*co*-NIPMAM)] film containing a specific amount of gold nanorods (GNRs). The light-induced thermoplasmonic heating of GNRs is used to control the optical scattering of an initially transparent hydrogel film. A hydrated P(NIPAm-*co*-NIPMAM) film is optically clear at room temperature. When heated to temperatures over 37 °C via light irradiation with a resonant source ($\lambda = 810$ nm) to the GNRs, a reversible phase transition from a swollen hydrated state to a shrunken dehydrated state occurs. This phenomenon causes a drastic and reversible change in the optical transparency from a clear to an opaque state. A significant red shift (≈ 30 nm) of the longitudinal band can also be seen due to an increased average refractive index surrounding the GNRs. This change is in agreement with an ad hoc theoretical model which uses a modified Gans theory for ellipsoidal nanoparticles. Morphological analysis of the composite film shows the presence of well-isolated and randomly dispersed GNRs. Thermo-optical experiments demonstrate an all-optically controlled light attenuator (65% contrast ratio) which can be easily integrated in several modern optical applications such as smart windows and light-responsive optical attenuators.

performance parameter for several technological applications ranging from optical fiber communications^[1] to smart windows.^[2] In recent years, polymers and liquid crystal polymer composites have been used as starting materials (instead of glass) because they offer the possibility of light-weight, cost-effective, and energy-efficient light attenuators. To this end, a lot of research interests have been focused on developing smart windows (or optical attenuators), owing to their capability of saving energy by modulating sunlight after being activated/deactivated with external stimuli such as voltage, light or heat. Depending on the nature of the external trigger, different classes of smart windows have been obtained: smart windows based on electrochromism,^[3] photochromism,^[4] mechanochromism,^[5] and thermochromism materials.^[6] Electrochromic and mechanochromic smart windows require extra devices and electricity,^[7] which may bring extra energy consumption. Smart windows based on

photochromism and thermochromism materials certainly represent the ideal candidates as they manage to save energy and source more efficiently.^[8] Unfortunately, the photochromic materials cannot make a response under a cloudy and hot weather while the thermochromic compounds,^[9] such as polymers,^[10] liquid crystals (LCs),^[11a,b,c] polymer-dispersed liquid

1. Introduction

The reduction of light intensity as it travels through a medium can be due to the reflection, absorption, and/or scattering of the incident photons. The attenuation magnitude is a fundamental and important optical property that has been an important

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crystals (PDLCS),^[12a,b] and vanadium dioxide (VO₂)^[13] have the switching temperature much higher than environmental temperature which cause a weak change in colour/transparency. Thus, it is attractive to develop a new kind of smart switching optical devices by integrating simultaneous photo- and thermoresponsive properties. The possibility of using light instead of an electric-field is still a compelling solution because it makes it possible to develop light-sensitive optical components. Within the framework of the development of all-optical components, the driving force is represented by the possibility to use sunlight. Light-triggered optical attenuators have been based on the use of both organic (e.g., dye molecules)^[14] and inorganic (e.g., metallic nanoparticles)^[15] photoconverters. Plasmonic nanoparticles (NPs), such as gold and silver NPs, are excellent materials because they have an intrinsic capability to collect a large amount of visible/near infrared (NIR) light, thanks to a physical phenomenon called localized plasmonic resonance (LPR): the coherent and dipolar oscillation of the bulk free electrons localized at the metallic/dielectric interface.^[16] Under a suitable frequency laser illumination associated with the LPR mechanism, the temperature significantly increases because of strong optical absorption, enabling NPs to act as a localized source of heat.^[17] This remarkable property has led to a new research field called thermoplasmonics,^[18] being studied in various research fields including electronics,^[19] medicine,^[20a,b] solar vapor generation,^[21] and catalysis.^[22] The use of thermoplasmonics coupled to the control of the optical property of the NP-containing media has been studied for the last few years. By combining the photothermal properties of gold NPs with the thermal modulation of LCs properties, we reported on the development of dynamic Bragg mirrors,^[23] Bragg attenuators,^[24] diffraction gratings, and waveplates.^[11c] LC-based optical components, while interesting, suffer many drawbacks such as the need to use an alignment layer or polarizers sometimes undesirable for optical attenuation application. Moreover, the production of LC-based large-area optical devices is always challenging. Temperature-responsive hydrogels have been used extensively in recent years for numerous bio-based applications. Among them, poly(*N*-isopropylacrylamide-*co*-*N*-isopropylmethacrylamide) (P(NIPAm-*co*-NIPMAm)) is a water-based, temperature-sensitive material which has been used for drug-release experiments^[25] due to its capacity to become hydrophobic from hydrophilic above a critical temperature ($\approx 37^\circ\text{C}$).^[25,26a,b,c] The subsequent release of water induces a drastic change in the optical transparency (from clear to opaque) of thin films. Moreover, differently from other classes of hydrogels, the hydrogel family based on poly(*N*-isopropylacrylamide), like P(NIPAm-*co*-NIPMAm), is well known for its chemical and structural stability even at elevated temperatures, thus exhibiting a very long shelf life.^[27] For these reasons, there are several efforts devoted to improving the very low degradability of these materials in order to be more useful for biomedical applications, where the biodegradability is a mandatory requirement.^[28] On the other hand, P(NIPAm-*co*-NIPMAm)-based materials have found applications in other research fields where robustness and mechanical stability are required such as optics,^[29a,b] solar energy,^[30] electronics,^[31] catalysis,^[32] and sensing.^[33] In this manuscript, we report on the development and characterization of a few mm thick, optically clear, and light-responsive film composed of gold NPs

uniformly dispersed in a P(NIPAm-*co*-NIPMAm) film. This film is optically activated/deactivated by exploiting the photothermal properties of gold NPs, enabling the development of an efficient light-triggered and dynamic optical beam attenuator.

2. Preparation of Light Responsive Hydrogel Films

Gold nanorods (GNRs) were selected as plasmonic NPs because they are extremely efficient photoconverters with very high photothermal efficiency (≈ 1).^[34] GNRs exhibit two LPR bands (transversal and longitudinal) centered in the visible and NIR part of the electromagnetic spectrum. The longitudinal LPR band is very sensitive to the refractive index of the surrounding medium.^[35a,b] Citrate-stabilized, water-based, GNRs were synthesized using a slightly modified protocol for the seed-mediated synthesis reported in detail elsewhere.^[36] Figure 1a shows the absorption spectrum of the GNRs solution ($C = 3.2 \times 10^{-9}$ M) with longitudinal (λ_L) and transversal (λ_T) LPR bands centered at 775 nm and 524 nm, respectively. Figure 1b shows the free charge distribution in an elongated NP associated with the LPR mechanism. Figure 1c is a representative high-resolution transmission electron microscope (TEM) image of GNRs. They are highly monodisperse with an average aspect ratio of around 3.6. A hydrogel [P(NIPAm-*co*-NIPMAm)] made of 95 wt% water and 5 wt% of polymer was used. The precursor solution (4 mL) consisted of 231.25 mg of NIPAm (poly(*N*-isopropylacrylamide), from Sigma-Aldrich), 6.25 mg of NIPMAm (*N*-isopropylmethacrylamide, from Sigma-Aldrich), 12.5 mg of BIS (*N,N'*-methylene bisacrylamide, from Roth), and 50.0 mg of Irgacure 2959 UV photoinitiator (from Sigma-Aldrich). The solution, placed in a glass vial and protected from ambient light, was stirred overnight. Subsequently, 1.0 mL of the GNRs solution was added to the polymer precursor solution. In order to fabricate flat discs with an excellent optical quality, Petri dishes (WillCo-dish, GWSt-5030) with 30 mm diameter were treated using an oxidation plasma (by Diener Electronic GmbH-Co. KG, Zepto) for 15 min (Figure 2a). The

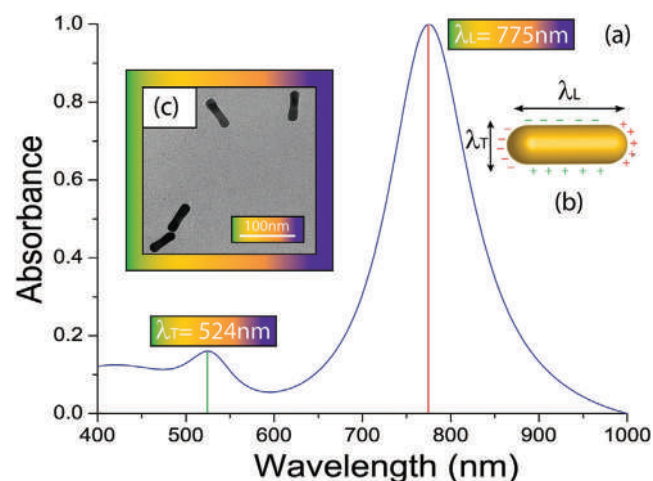


Figure 1. a) Absorption spectrum of a water-based GNRs solution along with b) a schematic representation of the LPR mechanism in a rod-like NP. c) High-resolution TEM image of GNRs.

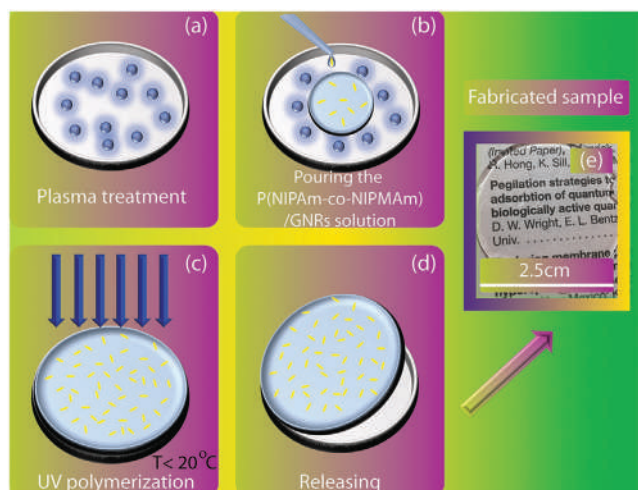


Figure 2. Step-by-step fabrication process of the P(NIPAm-co-NIPMAm)/GNRs sample.

P(NIPAm-co-NIPMAm)/GNRs solution was deoxygenated for 10 min via Argon bubbling, and then 0.5 mL of solution was poured into each Petri dish (Figure 2b). In order to keep the temperature below 20 °C during the photopolymerization process, the Petri dishes were put into an ice bath before illuminating (Figure 2c) the samples from the top for 1 min by UV light (by Dymax Europe GmbH, 5000-PC). The P(NIPAm-co-NIPMAm)/GNR discs were gently detached (Figure 2d) from the Petri dishes and stored in water at room temperature. Figure 2e indicates excellent optical transparency of the sample deposited on a glass substrate (the text behind the sample can be easily read).

3. Experimental Section

The morphology of the P(NIPAm-co-NIPMAm)/GNR samples (Figure 2e) was investigated by a field emission scanning electron microscope (FE-SEM, Nova NanoSEM 450, FEI) operating at an accelerating voltage of 10 kV. Samples were freeze-dried and coated with a 8 nm thick gold layer prior to their characterization, in order to minimize charging. In order to study the GNRs distribution, samples were inspected by a transmission electron microscope (TEM, FEI Talos F200X, 200 kV). Samples were first freeze-dried, and then the lyophilized materials were cut into small pieces, immersed in ethanol, and placed on a copper mesh with an amorphous carbon membrane. The samples were dried in a vacuum for 4 h. Light-triggered experiments were conducted using the thermo-optical setup illustrated in **Figure 3**. This setup uses a CW pump laser (Coherent Inc.) operating at 810 nm (within the LPR of these GNRs [Figure 1a]), a collimated white light source (CLS 100, Leica), a spectrometer (USB 2000, Ocean Optics) for monitoring both spectral response and light attenuation behavior, and a thermal camera (A655sc, FLIR) for mapping and measuring temperature changes in the sample under laser beam illumination. The P(NIPAm-co-NIPMAm)/GNR samples were covered with a glass slide (100 μ m thick) and the gap, controlled via 50 μ m glass microspheres, was infiltrated with water. The water film

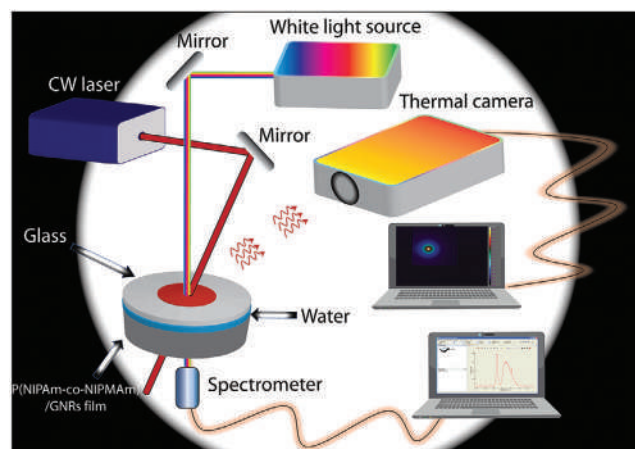


Figure 3. Thermo-optical setup for sample characterization.

acts as a reservoir to keep the samples hydrated, which is a requirement for their functionality as optical components (see below for details).

4. Results

Figure 4a,b shows FE-SEM micrographs indicating a honeycomb-like architecture composed of uniformly distributed pores with an average size of a few microns. The presence of GNRs does not affect the conventional morphology of the P(NIPAm-co-NIPMAm) film as this morphology is similar to previously reported system without GNRs.^[26a] The stability of the P(NIPAm-co-NIPMAm)/GNRs morphology after a prolonged storage time (up to 45 days) in water is reported in Section S1, Supporting Information. **Figure 4c,d** shows TEM images indicating well dispersed and randomly aligned GNRs within the P(NIPAm-co-NIPMAm) matrix. No evidence of

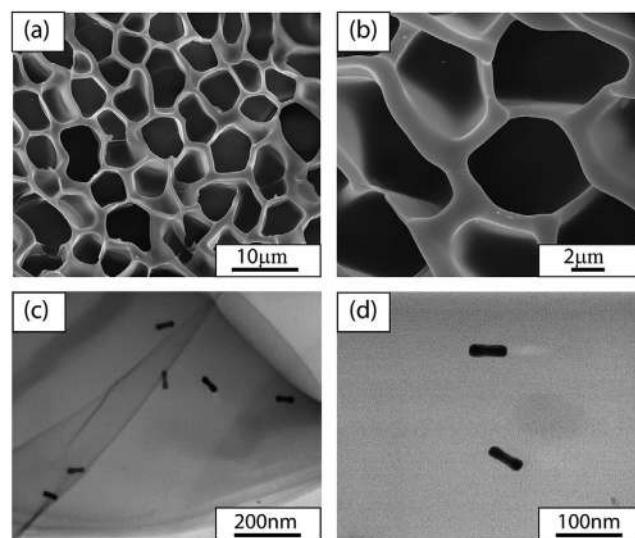


Figure 4. a,b) FE-SEM and c,d) TEM analysis of the P(NIPAm-co-NIPMAm)/GNRs sample for different magnifications.

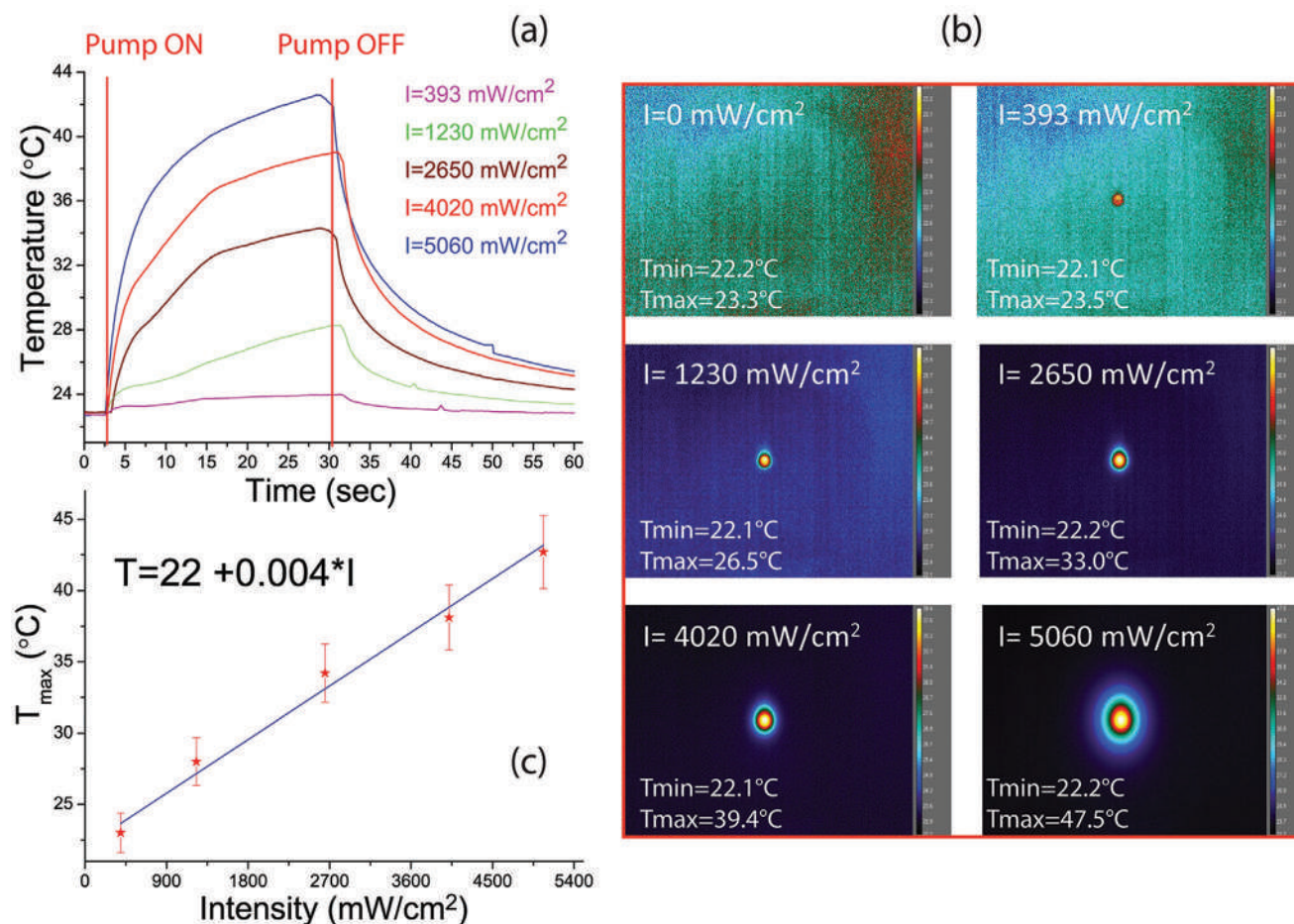


Figure 5. a) Time-temperature dependence of the P(NIPAm-co-NIPMam)/GNRs sample under pump beam illumination along with b) the corresponding thermographic view. c) Linear fit of the maximum temperature versus the intensity of the pump beam. $T_{\min}$ and $T_{\max}$ indicate the maximum and minimum temperature of each color scale reported in (b).

GNRs agglomeration was observed. **Figure 5** reports a thermographic study of the P(NIPAm-co-NIPMam)/GNR samples. Figure 5a shows that there is a significant and gradual temperature increase up to 44 °C upon illumination (Pump ON) at different intensity values of the pump beam (from 393 to 5060 mW cm⁻²) because of the photothermal conversion of GNRs. The sample cools back down to room temperature as soon as the pump beam is turned off (Pump OFF). The curves shown in Figure 5a were obtained by processing the thermal images in Figure 5b with a proprietary software program (FLIR Research IR Max). Figure 5c shows a linear correlation between the maximum temperature (obtained from the curves shown in Figure 5a) and the intensity of the pump laser. This linear behavior is well in agreement with the theoretical model reported in detail elsewhere.^[37] Temperature values (Figure 5c) were obtained by measuring the temperature with the thermal-camera (see thermo-optical setup in Figure 3) on the surface of the glass slide (100 μm thick) covering the P(NIPAm-co-NIPMam)/GNR samples. Samples are quite thin (few millimeters thick), and for this reason we are confident that the difference between the temperature of the surface and that in the bulk of the sample is less than few degrees. The additional experiments (not shown here), performed without GNRs

on similar samples, did not show any significant temperature change, thus confirming the capability of GNRs to convert the incident, resonant, light into heat. In order to understand the influence of the photothermal heating on the spectral position of LPR bands, the spectral response of the samples was investigated. When photoheated to temperatures above 37 °C a significant red shift (≈30 nm) (**Figure 6**, blue and red curves) of the longitudinal band, due to an increase in the average refractive index of the medium surrounding the GNRs (from hydrated $n \approx 1.4$ to dehydrated P(NIPAm-co-NIPMam) $n \approx 1.5$), can be observed. At room temperature with the water reservoir keeping the P(NIPAm-co-NIPMam)/GNRs film hydrated, the refractive index was roughly estimated as an average between the refractive index of the water ($n \approx 1.3$) and that of the P(NIPAm-co-NIPMam)/GNRs composition ($n \approx 1.5$). Upon illumination ($I = 5060 \text{ mW cm}^{-2}$) a phase transition from a swollen hydrated state to a shrunken dehydrated state occurs, producing an increased refractive index (1.5 instead of 1.4). This result is in agreement with the theoretical model which uses a slightly modified Gans theory^[35a] for ellipsoidal NPs (**Figure 6**, magenta and green curves). More details on the theoretical model are offered in Section S2, Supporting Information. It is worth pointing out that because of the low sensitivity

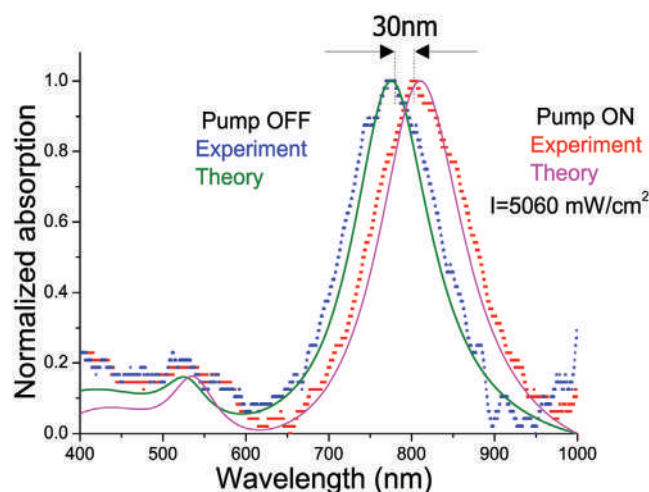


Figure 6. Theoretical (green and magenta) and experimental (blue and red) absorption spectra of the P(NIPAm-co-NIPMAm)/GNRs sample without (green and blue) and upon (magenta and red) pump beam irradiation.

to refractive index variations, the transversal band is red-shifted by only a few nanometers. The ability of these films to work as a dynamic attenuator was tested by probing the samples with a collimated white light source while turning the pump beam ON and OFF on the same probed area. With the pump beam OFF (**Figure 7a**) the sample is optically clear, and all the incident probe light is transmitted producing a visible white spot on the post-mountable view screen. When the pump beam is turned ON ($I = 5060 \text{ mW cm}^{-2}$) there is a drastic change in the optical transparency due to the scattering of the incident probe light (**Figure 7b**). The photothermal-induced phase transition is driven by a morphological change of the P(NIPAm-co-NIPMAm) film which transforms from a straight to a shrunk conformation of the chains at temperatures above 37°C . The photothermal-activated area of the sample is more evident in **Figures 7c** and **d**, where the probe beam and the probe/pump beams were turned OFF, respectively. Note: the photo shown

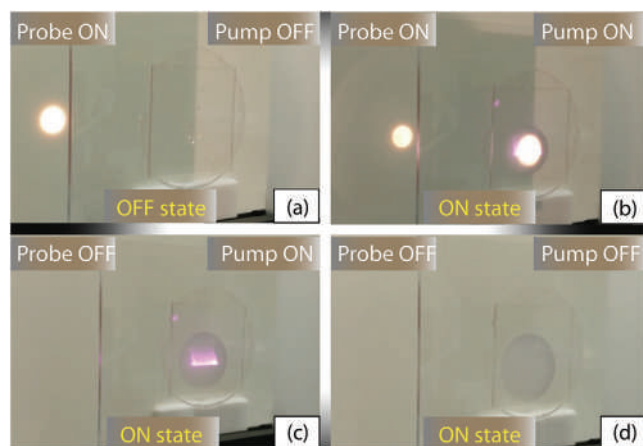


Figure 7. Photos of the P(NIPAm-co-NIPMAm)/GNRs sample in the a) OFF and b–d) ON states for different combinations of the probe and pump beam.

in **Figure 7d** was quickly acquired immediately after the pump beam was turned OFF. Samples show an excellent optical reversibility and start to go back to the initial (transparent) state as soon as the pump beam is turned OFF (see Movie, Supporting Information). **Figure 8a** shows the transmission dynamic response of the sample obtained by measuring the transmitted probe light intensity while turning the pump beam ON ($I = 5060 \text{ mW cm}^{-2}$) and OFF. The contrast ratio (C)—defined as the ratio of the transmitted intensity with the pump beam OFF (**Figure 8c**) to the incident intensity with the pump beam ON (**Figure 8d**)—in percentage, is 65%. Response times (on and off) are 52 s and 55 s, respectively. **Figure 8b** shows the exceptional reversible and repeatable change of the optical response of the sample induced by a sequence of pump beam light pulses (10 cycles). Moreover, it is worth pointing out that the optical response of the P(NIPAm-co-NIPMAm)/GNR samples shows an excellent reversibility even after a long storage time in water. A detailed characterization is reported in Section S1, Supporting Information. Transmission spectra reported in **Figure 8e** acquired by turning ON (red curve) and OFF (blue curve) the pump beam ($I = 5060 \text{ mW cm}^{-2}$) evidence the capability of the sample to attenuate the incident (unpolarized) probe white light in a relatively large spectral range (from 400 to 800 nm) with an average C of about 65%. In addition, as previously discussed (**Figure 6**), there is an evident red-shift ($\approx 30 \text{ nm}$) of the longitudinal plasmon band. Noteworthy, at room temperature the transmission of the sample is higher than 90%, thus confirming its extraordinary optical quality in terms of optical transparency. The spectral properties of the P(NIPAm-co-NIPMAm)/GNR samples are not affected by the prolonged hydration. A detailed characterization of the spectral properties of the samples for different hydration times (up to 45 days) is reported in Section S1, Supporting Information. In addition, in order to confirm the stability of the P(NIPAm-co-NIPMAm) matrix as well as the absence of GNRs reshaping after different cycles of photoswitching experiments, we have performed both wide angle X-ray scattering (WAXS) analysis and TEM characterization. Results reported in Section S1, Supporting Information, do not show any evident variation in the WAXS patterns as well as in TEM morphology, thus confirming that there has been no change in the structure of the P(NIPAm-co-NIPMAm) material and GNRs shape. In addition, a thermogravimetric analysis (TGA) reported in Section S1, Supporting Information, evidences the excellent thermal stability of the P(NIPAm-co-NIPMAm)/GNR samples up to 300°C .

5. Discussions

In the light of light-triggered optical applications, our finding represent a very important step forward in comparison with previously reported results on similar attempts.^[3–8] The proposed P(NIPAm-co-NIPMAm)/GNRs optical attenuator offers flexibility, light responsivity, optical transparency (OFF state), mechanical stability, long-lasting functionality, reversibility, and the last but not the least does not require any electrical power. Moreover, thanks to the presence of an integrated water reservoir, we have faced one of the main issues

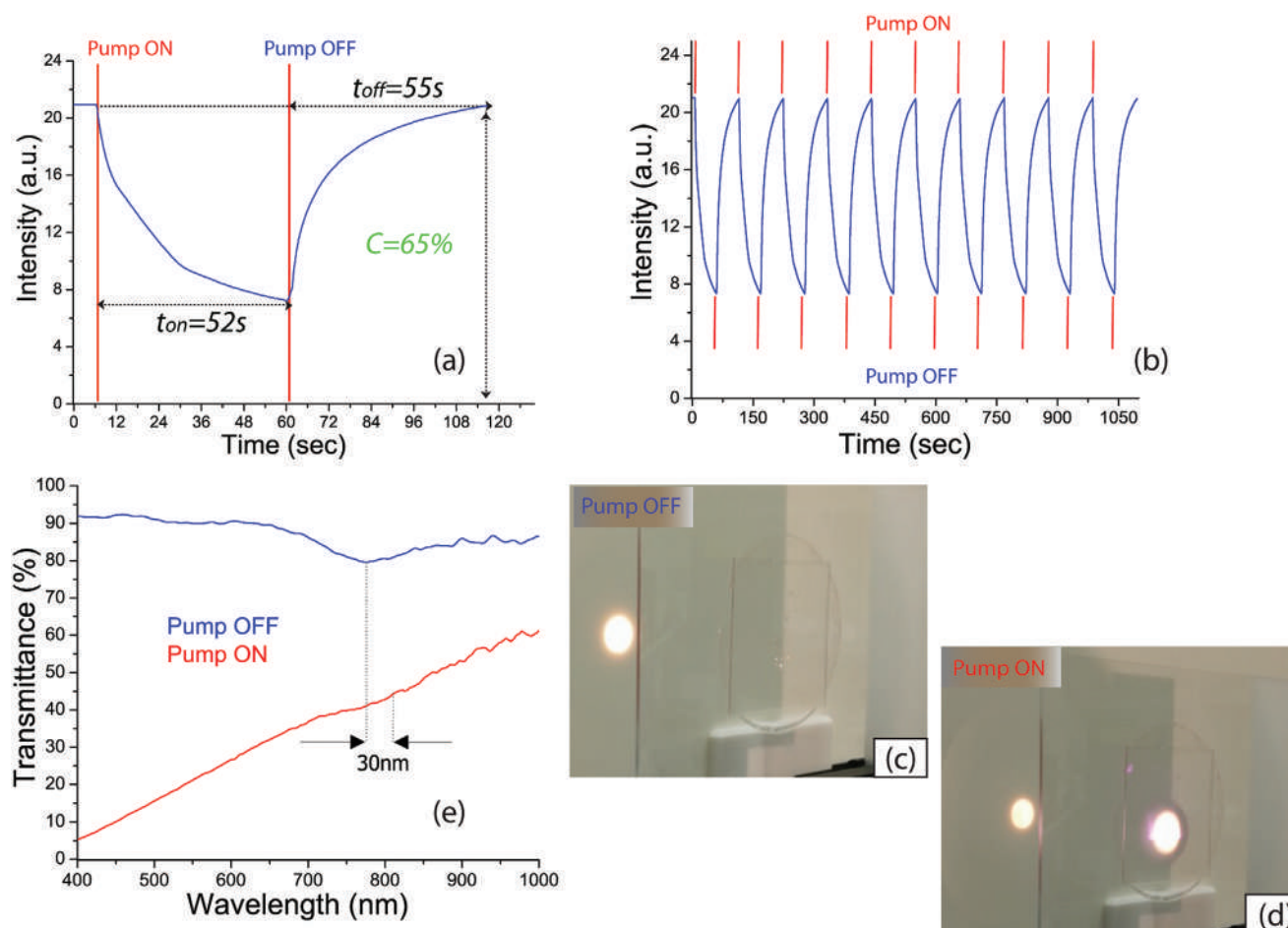


Figure 8. Reversible change of the transmitted intensity a) 1 cycle, b) 10 cycles, induced by turning the pump beam c) OFF and d) ON. e) Transmission spectra of the sample by turning ON (red curve) and OFF (blue curve) the pump beam.

of hydrogel-based materials. Indeed, the water layer keeps the P(NIPAm-co-NIPMAM) film in hydrated state (OFF state) at room temperature while it acts as a well to run out water from the film (ON state) at high temperature (44 °C), thus enabling a long-lasting functionality and reversibility. The optical properties of the proposed thermoplasmonic activated, hydrogel based, dynamic light attenuator can be further improved in terms of spectral sensitivity, attenuation and response times. This can be accomplished by exploiting a new generation of NPs such as nanoplates or metal dielectrics (GNRs encapsulated in a matrix made of titanium dioxide [TiO₂])^[38] with a wider wavelength range in order to maximize the sunlight absorption (e.g., from UV to NIR). Reduction of the response times as well as required power density can be achieved by increasing the NPs concentration. To this end, we are actively working on a new generation of NPs with a chemically modified surface chemistry for improving the dispersibility in P(NIPAm-co-NIPMAM), hence allowing to increase the actual utilized concentrations. As such, this will be a key factor for minimizing the required light intensity for triggering the optical device from a clear to an opaque state, thus enabling the realization of a new generation of low power smart windows.

6. Conclusion

To summarize, we demonstrated the concept of a light-controlled optical beam attenuator by combining GNRs and a responsive hydrogel (P(NIPAm-co-NIPMAM)) material. The morphological, spectral, and thermo-optical properties of thin films were studied. The GNRs are very well dispersed in the polymer yet unaligned. The optical transparency can be modulated using light irradiation resonant to the longitudinal plasmonic resonance of the GNR. Optical contrast of approximately 65% was measured upon irradiation of 5060 mW cm⁻². A theoretical model which uses a modified Gans theory for rod-like particles fits the experimental results very well. Response times (on and off) are symmetric and both falling within the one-min timeframe. We are actively working on a new generation of gold NPs with higher dispersibility in P(NIPAm-co-NIPMAM) in order to increase their concentration in the polymer film. This can allow to further reduce the pump intensity to a value suitable to activate the samples (e.g., down to 0.5 W cm⁻²) making these materials very promising for solar light applications. However, we are confident that our results are already very promising for the development of a new generation of

solar sunlight-triggered smart windows or on-demand optical attenuators.

Supporting Information

Supporting Information is available from the Wiley Online Library or from the author.

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Conflict of Interest

The authors declare no conflict of interest.

Keywords

active plasmonics, gold nanorods, hydrogels, optical attenuators, optical transparency, plasmonic nanoparticles, polymers

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REVIEW

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Stimuli-responsive nanoparticle-assisted immunotherapy: a new weapon against solid tumours

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Although significant improvements in cancer treatment have led to a longer survival period, the death rate of patients with solid tumours has not changed during the last decades. Most researchers are currently concentrating on defining the mechanisms of the different resistance pathways activated by tumour cells; meanwhile, the role of limited drug distribution within tumours has been neglected. The application of nanotechnology in medicine offers unexplored opportunities for realizing a new generation of anticancer therapies that can overcome the physical hindrances that characterize solid tumours. Indeed, surface-engineered nanoparticles (NPs) (both organic and inorganic) have been used as powerful tools in cancer therapy. Particularly, Au NPs have been utilized to develop a new drug-free treatment, photo-thermal therapy (PTT), due to their stimuli-responsive properties. PTT combined with immunotherapy represents a major breakthrough in the fight against malignant solid tumours. In this review, we provide a complete overview of the synergistic approaches based on PTT and immunotherapy, considering the selection, design, and functionalization of the NPs and their thermo-optical properties, moving to *in vivo* studies and finally to clinical trial applications in patients suffering from solid tumours.

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1. Introduction

Cancer is a major cause of death worldwide, with approximately 10 million deaths in 2018. The main origins of cancer death are attributed to malignant solid tumours, such as lung, prostate, colorectal, stomach, liver, breast and cervix cancers.^{1–4} Over the past decade, the development of precision cancer medicine has offered the opportunity to make significant progress in the

management of advanced tumours. To date, in the arena of oncology, in addition to the classic approaches of chemotherapy, radiotherapy and surgery, immunotherapy is starting to play a privileged role. Indeed, conventional medicine exploits chemotherapy, alone or in combination with surgery and radiotherapy, as the main intervention for cancer management. For solid tumours at early stages, surgical resection is the preferable option for treatment of cancer patients. However, surgery is not always possible for several reasons, including the nature of the cancer, tumour stage, body location and underlying pathologies. In these cases, radiotherapy and chemotherapy can be used, before or after surgery, as neoadjuvant or adjuvant treatments, respectively. Unfortunately, neither radiotherapy nor chemotherapy is selective toward cancer cells; also, they may induce severe damage to healthy tissues, primarily because of cytotoxic side effects. Moreover, the systemic administration of traditional chemotherapeutic agents provides dose-dependent antitumour efficacy because of insufficient drug uptake by solid tumours. In the attempt to ensure high drug concentrations in tumour tissues and decrease systemic toxicity, loco-regional delivery of antitumour compounds has been exploited as an alternative drug administration route; however, clinical studies have proved that this approach is mainly significantly efficacious

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in the management of liver cancers.⁵ For patients with solid tumours, a promising alternative and innovative therapeutic option is offered by immunotherapy, which is defined as a therapeutic approach to treating cancer by generating or augmenting an immune response against it. The different immunotherapeutic strategies developed for cancer treatment can mainly be classified as active and passive immunotherapies. Active immunotherapies can be achieved, specifically, by the administration of vaccines containing tumour-specific and/or tumour-associated antigens or, non-specifically, by the administration of cytokines such as IL-2, IL-12 or interferon-alpha (IFN- α), mainly in combinatorial anti-cancer treatments. Passive immunotherapy exploits two different approaches that have emerged as particularly effective over the past decade: immune cell-targeted monoclonal antibody (mAb) therapy and adoptive cellular therapy (ACT).⁶ In the last five years, the immunotherapy approach with engineered T cells expressing a chimeric antigen receptor (CAR) has produced unprecedented results in patients with relapsed/refractory B cell acute lymphoblastic leukemia^{7–9} and refractory large B-cell lymphoma;¹⁰ it shows very exciting results with respect to long-term efficacy, with high complete remission rates and durable remission. However, these results have not been yet obtained in the context of solid tumours. The recent introduction of immune-checkpoint blockade (ICB) therapy has shown remarkable benefit in the treatment of a range of cancer types; conversely, the main drawback of this therapy is toxicity related to inflammatory side effects, often involving the gastrointestinal tract, endocrine glands, skin, and liver.¹¹ Consequently, although immunotherapies have demonstrated therapeutic efficacy in the treatment of hematological malignancies, successful treatment of solid tumours by immunotherapy still represents a challenge. Synergistic effects may be offered by a combination of different immunotherapies that simultaneously involve various phases of the cancer-immunity cycle; this induces multiple targeting mechanisms, which prevents immune escape by tumour cells. Furthermore, multiple approaches can be combined not only with other conventional cancer therapies, including chemotherapy and radiation, but also with modern technologies. From this standpoint, nanotechnology, with the use of NPs, represents a formidable weapon in the fight against cancer. Suitably selected classes of NPs are functional and stimuli-responsive materials that can be used as nanocarriers for different biologically active or therapeutic molecules (*e.g.* antibodies, ligands, drugs) and for improving medical diagnosis and treatments such as imaging, radiotherapy and chemotherapy.¹² The main properties that influence the biological fate of NPs in the body are their composition, hydrodynamic size, shape, coating ligand, surface charge and surface functionalization with specific targeting agents.¹³ Furthermore, in the context of solid tumours, the abnormalities characterizing the tumour microenvironment, such as local deficiency of microcirculation, hypoxia, low pH and pathological pressure gradient, also play roles because they strongly influence the delivery of NPs and hinder their homogeneous distribution inside the tumour. Here, we provide an overview of the use of stimuli-responsive NPs as a high-precision therapeutic tool against solid tumours. As such, we

start by describing the main issues related to the ability of NPs to reach the tumour environment and how these issues can be overcome by suitably engineering the surface of the NPs. In this context, several types of NPs are reported, and we classify them in two main categories: organic and inorganic NPs. In the frame of stimuli-responsive NPs, special attention is devoted to Au NPs. Their unique capability of responding to illumination has been used as a new weapon in the fight against cancer, mainly due to the so-called photo-thermal therapy (PTT). We show how the synergy between PTT and immunotherapy represents a new scenario in the field of light-responsive cancer therapies against malignant solid tumours. Several examples of PPT assisted immunotherapy are presented and a complete examination of the state of the art along with the related clinical trials are reported and discussed.

2. Design of nanoparticles to increase therapeutic efficacy in the solid tumour environment

In vivo ability of NPs to seep into the tumour microenvironment and tissues can only be successfully attained if organic or inorganic nano-therapeutic agents are suitably designed in terms of size, shape, composition, surface charge and functionalization. In particular, after intravenous administration, the NPs adsorb many different plasma proteins, which facilitates opsonization by the mononuclear phagocyte system (MPS). The amount and specificity of these proteins may vary and depend on the characteristics of the NPs. Opsonization is known to play a major role in the clearance of particles because one or more of these proteins may interact with specific receptors on the surface of macrophages and hepatocytes in the liver. Surface coating of NPs with hydrophilic polymers, such as polyethylene glycol (PEG), has been suggested as a useful approach to improve the stability of NPs in the circulatory system and prevent clearance by the reticulo-endothelial system, thus ensuring their long blood circulating time in the body. From a different perspective, cell-membrane camouflaged NPs have been proposed as a new generation of nature-inspired biomimetic delivery systems for cancer therapy; they are characterized by excellent biocompatibility, no immunogenicity and prolonged systemic circulation lifetime.^{14,15} NPs that evade the blood clearance phenomenon exerted by MPS will be able to seep into tumours after several steps, including extravasation across the vascular-endothelial barrier followed by penetration towards the *tumour interstitium* and, finally, internalization by cancer cells.^{16–18} The ability of NPs to reach the tumour mass strongly depends not only on the integrity of the tumour endothelium and the alteration of the vasculature microarchitecture, but also on the composition of the tumour stroma, which mainly consists of connective tissue, blood vessels, stromal cells such as fibroblasts; epithelial, vascular and immune cells; the extracellular matrix (ECM); and extracellular molecules.¹⁹ During tumour progression, the microvasculature of the adjacent host tissues that sustain a growing tumour undergoes significant structural, biochemical and compositional

modifications by switching to an angiogenic state. Elevated levels of pro-angiogenic factors induced by cancer cells, such as vascular endothelial growth factor (VEGF) and platelet-derived growth factor (PDGF), promote angiogenesis and, consequently, the formation of tumour blood vessels, which are characterized by a disorganized morphology and irregularities.²⁰ Proliferation and fenestration of endothelium cells, arteriovenous shunts, pericyte deficiency and abnormal formation of the basement membrane represent the most relevant vascular abnormalities, which determine the enhanced vascular permeability, heterogeneity of tumour blood flow and ineffective drainage within tumour tissues. The enhanced vascular permeability and the improper functionality of the lymphatic system are the two main factors affecting the passive phenomenon called the “enhanced permeability and retention” (EPR) effect, which enables drugs or NPs to reach tumour cells from the bloodstream, across tumour blood vessels, into the tumour interstitial space. Although vascular abnormalities promote the EPR effect, the tumour architecture is also characterized by a very heterogeneous distribution that can inhibit homogeneous accumulation and efficient extravasation of NPs into the tumour microspace.^{21,22} Even when NPs can achieve efficient extravasation by crossing the vascular-endothelial barrier, they still need to pass through the interstitial space before reaching the cancer cells. The tumour interstitium consists of a matrix phase and a fluid phase. The solid ECM is the result of a complex assembly of collagen, glycosaminoglycans, proteoglycans and cancer-associated fibroblasts. The tumour interstitial fluid is composed of a mixture of externalized components, mainly proteins (such as cytokines, growth factors, and metalloproteases) released by tumour cells and their micro-environment. It is also characterized by acidic pH values and low oxygen levels, which can reduce the effectiveness of drug-based anticancer therapy or radiotherapy. Furthermore, the pressure of the tumour interstitial fluid is higher at the center and lower towards the periphery.^{23–25} Consequently, the fluid moves away from the central region of the tumour, which can reduce the ability of NPs to reach the inner tumour mass.²⁶ Several strategies have been proposed to design properly engineered NPs with tailored sizes, shapes and surface functionalization to achieve therapeutic nanovectors that can overcome the described biological barriers (*e.g.*, clearance during circulation, vessel permeability, extravasation) occurring in solid tumours.^{22,27,28}

The ability of NPs to cross the endothelium-vascular barrier and accumulate in the interstitial space by EPR effect-induced extravasation has been successfully demonstrated when NPs with sizes ranging from 10 to 200 nm are administrated. It has also been demonstrated that hydrophilic stealth NPs, which have neutral or anionic surface charges, can diffuse promptly into the deeper tissue matrix.^{29,30} Rod-like and disk-shaped NPs with low aspect ratios (<0.5) have been proven to be able to penetrate into solid tumours more deeply than their spherical counterparts.^{31,32} Following a different approach, it has been widely demonstrated that active targeted NPs, which are surface-functionalized with specific targeting ligands, have more opportunity to penetrate solid tumours and effectively accomplish their therapeutic activity. Namely, targeting the tumour vasculature

and angiogenesis by using peptides modified with the RGD (Arg-Gly-Asp) motif or inhibitors of growth factors, tumour necrosis factors and matrix metalloproteases (MMPs) has been explored to manipulate the tumour vasculature and to enable NPs to cross the vascular-endothelial barrier. The targeting of angiogenesis and tumour vasculature also represents an efficient way to reduce the pressure of tumour interstitial fluid, which is a direct consequence of the heterogeneity in vascular blood flow and dysfunctional lymphatic drainage.¹⁶ After NP extravasation, hypoxia-specific targeting can be explored to enhance the efficacy of chemotherapeutic/immunotherapeutic treatments or radiotherapy. Indeed, the development of intelligent drug delivery nanosystems, which are responsive to specific conditions of the tumour microenvironment (low pH values or elevated expression levels of tumour-associated enzymes) or to external stimuli (ultrasound, light, magnetic field) after their extravasation, can promote the penetration ability of NPs within the ECM of the tumour interstitium and, consequently, the cell permeability of the therapeutic agent; the NPs can then be efficiently internalized by cancer cells.³³

3. Organic and inorganic nano-therapeutic agents for immunotherapy of solid tumours

Several nanostructured carriers have been developed for tumour immunotherapy by exploring different strategies to stimulate immune systems against cancer cells. Targeted NPs have been designed and employed as active and efficient vectors for the delivery of tumour-associated antigens (TAAs) and immunomodulatory molecules to antigen-presenting cells (APCs), namely dendritic cells (DCs), to propagate T-cell activation, expansion and response.³⁴ In some cases, the exploitation of this approach can provide unsatisfactory results owing to poor control of the delivery of specific exogenous antigens to naturally occurring APCs as well as the cluster of differentiation (CDs) expression of inhibitory ligands binding to receptors on T-activated cells, which compromises the immune system response. Furthermore, the tumour microenvironment may influence the activity of natural APCs. Consequently, artificial antigen-presenting cells (aAPCs) have been proposed to mimic different aspects of natural DCs and overcome these limitations.³⁵ In addition, another different strategy for immunotherapy is based on modulation of the tumour microenvironment in favor of effector immunological cells, minimizing unwanted side effects. Interestingly, preliminary preclinical data show that delivering immunomodulatory drugs by NPs significantly reduces the systemic inflammation related to off-target effects of the drugs. NPs can be functionalized with therapeutic compounds to achieve concentrated local drug delivery, with the capability to realize a sustained release dosage, mainly if biodegradable NPs are used.³⁶ The size and shape of the NPs have very important effects on their immune clearance, intra-tissue/tumour dispersion, penetration and retention (Fig. 1). Large NPs tend to be retained in tumour tissue but cannot penetrate deeply into tumours³⁷ because

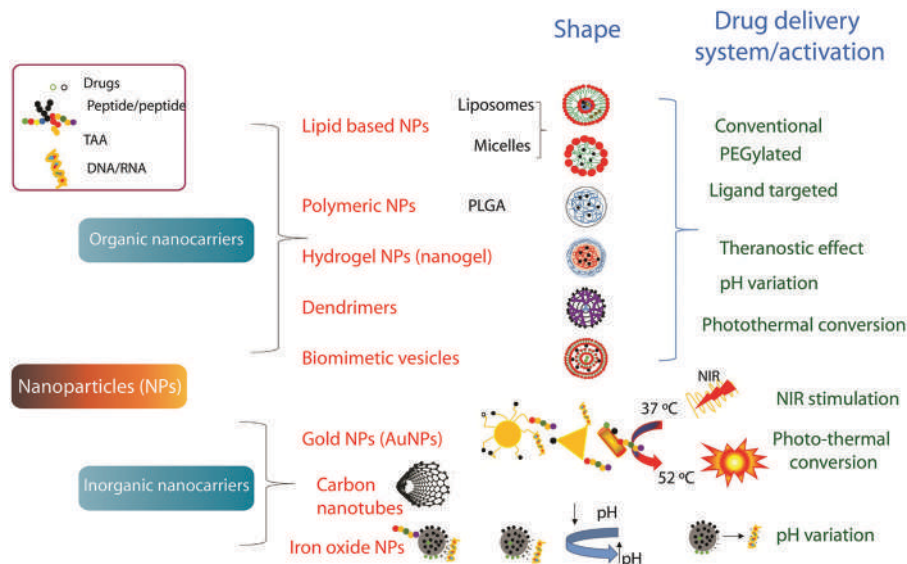


Fig. 1 Scheme of the two main classes of NPs currently explored for cancer immunotherapy and classified as organic and inorganic delivery nanocarriers. Each of these classes is characterized not only by different chemical compositions and shapes, but also by different functionality, therapeutic properties and active mechanisms of stimuli-responsiveness.

the heterogeneous microenvironment and elevated interstitial fluid pressure hinder their penetration. Small NPs possess good penetration ability but are easily cleared and pumped back into the bloodstream.³⁸ In order to maximize the EPR effect in drug delivery to tumour tissues, different strategies of programmed changing of the particle size/charge have been developed, and particles have been fabricated that are responsive to different stimuli, such as pH,^{39,40} temperature variation and magnetic field.⁴¹ Nano-therapeutic agents developed for immunotherapy of solid tumours can be conveniently classified into two main categories: soft organic nanocarriers and solid inorganic NPs (Fig. 1).

3.1 Organic nanoparticles

Organic NPs, formed of organic compounds, are characterized by biodegradability and a high degree of biocompatibility; they include lipid-based (liposomes and micelles) and polymeric NPs as well as hydrogels, supramolecular structures (dendrimers) and biomimetic vesicles (Fig. 1).^{42–45} Among these NPs, lipid and polymer-based nanoformulations are generally characterized by high drug encapsulation efficiency and versatility to deliver mostly hydrophobic drugs, oligonucleotides, immunotherapeutic agents and adjuvants. The different types of liposomal drug delivery systems include conventional, PEGylated, ligand-targeted, theranostic liposome⁴⁶ and pH-sensitive.⁴⁷ Their surface features can be simply modulated by chemistry routes.^{48–52} Several studies have proved that pH-sensitive liposomes represent good candidates for targeted delivery of TAAs and immune-adjuvants to APCs, as well as for targeting the immunosuppressive tumour microenvironment (TME), due to their ability to release their cargo into the cytoplasmic compartments of cells.³⁴ In this context, Yoshizaki *et al.* reported⁵³ that pH-sensitive liposomes entrapping peptides derived from OVA (ovalbumin)-I specific for

major histocompatibility complex (MHC) I on DCs activated cytotoxic T lymphocytes (CTLs) more efficiently than free OVA-I did in mice bearing E.G7-OVA tumours. Liposomes constituted of a pH-sensitive polymer, YSK05, and loaded with cyclic-di-GMP, which is a cyclic dinucleotide that is useful as an adjuvant to stimulate the innate immune system *via* the STING-TBK1-IRF3 pathway, induced interferon (IFN)- β production in APCs, promoting enhanced expression of both CD80 antigen and MHC I as well as higher CTL activity.^{34,54} PEGylated liposomes bearing surface-conjugated agonists, including anti-CD40 and CpG oligonucleotides, have been demonstrated to be efficient as aAPCs; they induced robust anti-tumour response in the B16F10 murine model of melanoma, with minimal systemic off-target inflammatory effects.⁵⁵ In the same B16F10 melanoma model, intratumoural liposome-coupled anti-CD137 + interleukin (IL-2) Fc therapy has cured most established primary tumours while avoiding the lethal inflammatory toxicities caused by equivalent intratumoural doses of soluble immunotherapy.⁵⁶

These results have been confirmed in multiple tumours.⁵⁷ A novel nanoformulation based on liposomes is currently under investigation in phase I clinical trials for breast, ovarian and prostate cancers. Namely, DepoVax™ (DPX-0907) is a liposome-based DC vaccine that consists of a blend of seven tumour-specific epitopes (TAAs), including TNF- α -converting enzyme (TACE/ADAM17), B-cell receptor-specific protein 31 (CDM protein), topoisomerase II α , Abelson homolog 2 (Abl2), epithelial discoidin domain receptor 1 (EDDR1) and γ -catenin. To date, clinical studies have proved the effective activation of cytotoxic T-cells.⁵⁸ Biodegradable polymeric nanocarriers, namely PLGA (poly(lactic-co-glycolic acid))-based NPs, have been tested for the development of aAPCs or vaccines and for targeted delivery of TAAs to DCs. For example, PLGA NPs conjugated to anti-CD40 and loaded with OVA, Pam3Csk4 and

poly(I:C) have been tested *in vivo* for selective delivery to DCs, proving the ability of the NPs to promote enhanced priming of CD8⁺ T cells and to ensure prolonged survival of melanoma tumour-bearing mice.⁵⁹ Furthermore, chitosan (Ch)/poly-(γ -glutamic acid) (γ -PGA) NPs have been reported to modulate macrophages and the inflammatory profile of DCs, impairing their ability to promote cancer cell invasion.⁶⁰ These data suggest that delivery of immunostimulatory molecules by organic nanovectors and scaffolds can rouse the immune system with greater rigor than delivery of these same molecules in solution, leading to improved antitumour therapy.

3.2 Inorganic and carbon-based nanoparticles

Inorganic NPs (metal, semiconductor), and carbon-based nanostructures have also been explored as therapeutic nanotools in cancer treatment, with promising results towards clinical applications.^{12,61–73} Indeed, inorganic NPs can act as multifunctional agents for the treatment of solid tumours due to their unique plasmonic, magnetic, or photoactive properties and their ability to function as selective delivery nanoplateforms of immunotherapeutic agents and/or targeting ligands, which can be linked to their surfaces *via* covalent bonds, capping agent exchange, or physical interactions.^{48,74}

Furthermore, therapeutic compounds and inorganic NPs can be co-embedded into either targeted or non-targeted organic nanocarriers, such as those previously described (lipid or polymer-based NPs), thus obtaining nano-formulations that can protect the therapeutic cargo from degradation and efficiently retain the peculiar properties of the inorganic functional domain.^{50,52,75,76} Several examples have been reported of the use of these NPs in the field of immunotherapy of solid tumours, proving the use of Au NPs as efficient cancer immunotherapy carriers.⁷⁷ Also, magnetic and carbon-based NPs have been exploited for immunotherapy of solid tumours. For example, paramagnetic iron-dextran NPs loaded with MHC-IgG dimers and anti-CD28 antibodies have been tested as efficient aAPCs that can be localized by magnetic targeting, thus inducing expansion of antigen-specific splenocytes in mice and stimulating proliferation of human peripheral blood T cells. Magnetic targeting has also been performed using a fucoidan-dextran-based magnetic nanoformulation conjugated with anti-PD-L1, anti-CD3 and anti-CD28; this is useful for modulation of the immunosuppressive TME.⁷⁸ Carbon nanotubes (NTs) have been explored as aAPCs due to their large surface areas, where high concentrations of antibodies can be achieved. Indeed, unique properties of NTs presenting T-cell stimuli have been obtained by absorbing specific T cell-stimulating antibodies (anti-CD3 and anti-CD28) onto the NT surface, which has been demonstrated to induce activation of lymphocytes.^{79,80} Inorganic NPs can also exhibit inherent therapeutic effects because they can be successfully employed in various therapeutic strategies to induce the death of cancer cells, such as magnetic hyperthermia, PTT and photodynamic therapy (PDT). Regional mild hyperthermia (42 °C to 46 °C) has been revealed to be an effective and selective therapeutic approach that can be used as an adjuvant with chemo-, radio- or immunotherapy for

treatment of solid tumours. Indeed, mild hyperthermia increases the permeability of the tumour vasculature and cell membranes and consequently reduces the interstitial pressure. Due to increased tumour perfusion induced by hyperthermia, blood flow increases throughout the irregular architectures of solid tumours, thus allowing the chemo or immune or radio-delivery agents to overcome some of their biological barriers.⁸¹ Namely, Au, carbon-based and iron oxide NPs (IONPs) are the most commonly used nanostructured platforms for hyperthermia treatments. Although carbon-based nanomaterials, such as NTs and graphene, have revealed very promising heating properties by utilizing the near-infrared (NIR) or radiofrequency (RF) wave regions, their cytotoxicity is still a crucial issue for their translation to clinical management.⁸² Consequently, efforts are currently being carried out to synthesize carbon dots (CDs) with high absorption coefficients in the NIR region because CDs are considered to be zero-dimensional carbon-based nanomaterials and are characterized by low cytotoxicity.⁸³ Similarly, IONPs are characterized by high degrees of biocompatibility, and they can generate heat by application of an alternating magnetic field. Very few limitations (*e.g.* intensity of the applied magnetic field) of penetration depth are encountered when using magnetic NPs.³⁰ Despite more recent progress, certain crucial challenges still need to be addressed before IONP-based hyperthermia therapy achieves standard clinical use for cancer treatment. Indeed, the magnetic field intensity must be carefully controlled when applied in humans because physiological conditions need to be considered. Among the inorganic metal (gold, copper, molybdenum, tungsten and palladium)-based NPs employed for PTT, Au-based nanostructures are non-toxic and present strong light absorption and, consequently, fast and efficient photon energy conversion into heat; thus, they are excellent nanoheaters for PTT.^{84,85} The optical properties of Au NPs can be tailored to absorb light in the NIR region and convert it into heat that can be quickly released in the surrounding tumour tissues. Also, in the case of Au NPs, as with carbon-based nanomaterials, RF waves can be used as a source to promote heat generation. Although RF wave heating is characterized by a high penetration depth, the heating effect is not prominent and the heating mechanism is not yet fully understood; therefore, its application is still in its infancy.^{86,87} Conversely, the use of NIR radiation in cancer PTT is very advantageous because radiation in this spectral range displays high penetration depth (up to 1 to 2 cm) in tissues and low interactions with their main biological components.⁸⁸ The PTT treatment of solid tumours located deeper than 2 cm can be successfully achieved by using proper optical fibers.⁸⁹ Actually, clinical trials are being conducted only on Au NPs-based treatments for PTT;⁹⁰ this highlights that, at present, Au NPs possess higher potential than that offered by other nanomaterials for therapeutic approaches to tumour treatment.

Therefore, Au-based nanostructures represent promising candidates for the design and development of multifunctional nanoformulations that are able to extravasate from tumour vasculature, penetrate the complex architecture of solid tumours and selectively exert multiple therapeutic effects through the combination of PTT with immunotherapy and chemotherapy

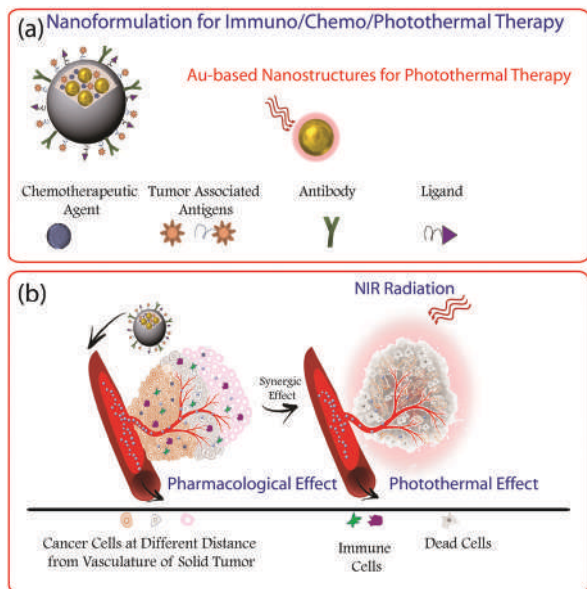


Fig. 2 Schematic of a multifunctional targeted nano-formulation for the co-delivery of Au-based nanostructures and therapeutic agents (a) and its synergistic effect on cancer cells resulting from the combination of pharmacological (immunotherapy/chemotherapy) responses with photo-thermal ablation for efficacious treatment of solid tumours by a multimodal approach (b).

(Fig. 2a and b). On these bases, an overview of the synthesis, properties, surface functionalization and applications of Au NPs as photothermal agents and/or as delivery carriers of chemo or immunotherapeutic compounds will be provided in the following paragraphs of this manuscript.

3.2.1 Au nanoparticles. Au NPs exhibit outstanding chemical-physical characteristics; thus, they are appealing for applications related both to cancer therapy and diagnosis.⁹¹ Indeed, Au NPs are extremely stable and biocompatible, possess low toxicity and, because of their surface chemistry, are promptly processable for functionalization that is specifically suited to achieve optimal interactions with biological systems. When Au NPs are activated with proper electromagnetic radiation, they exhibit a physical phenomenon called localized plasmonic resonance (LPR), which is a collective oscillation of the electrons localized at the metallic/dielectric interface.⁹² The LPR mechanism enables Au NPs to be employed for different applications, such as tuneable filters,⁹³ photo-thermal converters,⁹⁴ imaging⁹⁵ and sensing.⁹⁶ Morphology control represents a powerful tool to tune the aforementioned properties and to improve the interaction of Au NPs with biological substrates. As an example, the dimensions of Au NPs play a crucial role in organ penetration and accumulation⁹⁷ as well as in the ability to bind and activate overexpressed membrane receptors.⁹⁸ Remarkably, for Au NPs in a size regime less than 50 nm, an increase of the uptake kinetics in HeLa cervical cancer cells with increasing NP size has been demonstrated.⁹⁹ Further, Au NPs with a diameter of 15 nm have shown uptake in Sk-BR-3 breast cancer cells up to 2.4 times higher than that of Au NPs 45 nm in size.¹⁰⁰ In addition, shape control is fundamental in defining the optical and physical-chemical

properties of Au NPs.¹⁰¹ Fascinating opportunities in the field of nanomedicine arise from complex geometries. A striking example of the importance of shape control is provided by Au nanorods (Au NRs);¹⁰² these rod-shaped NPs can shift the LPR bands from the visible into the first physiological “biological window”,^{103–106} in the NIR region of the spectrum, namely in the range from 700 nm to 900 nm.¹⁰⁷ Further, the anisotropy of Au NRs enables a longer blood circulation time, with higher amounts of ligand binding sites and increased cellular affinity with respect to Au nanospheres.¹⁰⁸ The shape of Au NPs can also determine the mechanism of light-induced release for therapeutic purposes. As such, the light-induced DNA release from Au nanoshells (Au NSs) is both directly light triggered and induced by heat generation upon Au NP irradiation; meanwhile, only thermally induced DNA release is observed when Au NRs, with comparable spectral properties, are used as a carrier.¹⁰⁹ The great advances in nano-material preparation strategies, the increasing understanding of their synthesis mechanisms, as well as the outstanding progress in their characterization techniques allows precise design of Au NP features that are tailored to specific applications, as reported in excellent reviews.^{110–112} Here, we intend to provide a concise selection of synthetic approaches that have been investigated for the preparation of Au NPs with tuneable properties specifically suited for their application in the field of solid tumour treatments, targeting, and minimally invasive surgery through PTT.

3.2.1.1 Au nanospheres. Spherical Au NPs have been deeply investigated because of their size-dependent optical response and the possibility to modify their surface chemistry with functional molecules with a coverage density that can be controlled by the size of the Au NPs.¹¹³ Among the most investigated synthetic approaches for the preparation of Au nanospheres is the reduction of $\text{HAuCl}_4 \cdot 3\text{H}_2\text{O}$ by ascorbic acid in the presence of citrate, as a stabilizing agent, by which the growth and size dispersion can be controlled. This method, known as Turkevich’s synthesis or Frens’s synthesis, allows the achievement of Au nanospheres in a dimensional range of 16–150 nm as a function of the ratio of the Au precursor to the reducing agent.^{112,114} A narrower size distribution can be obtained, instead, by synthesizing Au nanospheres using a “seed-mediated” technique. In this case, pre-synthesized Au nuclei behave as nucleation centers by allowing the adsorption of surface bulk Au ions, which are subsequently reduced. An improved size distribution can be further accomplished by the slow and controlled addition of a reducing agent, which can suppress additional nucleation.¹¹⁵ In addition to the NP size, a fundamental issue affecting the control of cellular uptake and the absorption of proteins is the surface chemistry of Au NPs.¹¹⁶ Therefore, suitable stabilizing agents that are able to facilitate post-synthesis functionalization and manipulation are essential for reliable applications of Au NPs. The case of oleylamine-capped Au nanospheres is an interesting example because of the interaction between the Au surface and amino groups, which facilitates post-synthesis surface functionalization through a ligand exchange. When the synthesis of Au nanospheres is performed using the “hot injection” technique,

the oleylamine molecules play the multifold role of reducing agent, capping ligand and reaction solvent.¹¹⁷ Using this technique, the size of Au nanospheres can be controlled by adjusting the reaction temperature.¹¹⁸

3.2.1.2 Au nanorods. Excellent properties, including optical response in the first physiological “biological window” and high photon-to-energy conversion ability, are provided by Au NRs. The most common preparation protocol for Au NRs synthesis is the seed-mediated approach, which essentially involves the growth of anisotropic Au NPs promoted by pre-synthesized seeds in the presence of suitable stabilizing agents and selected additives that are able to force the growth of the resulting nanocrystals along a preferential direction. Among the first synthetic “seed-mediated” protocols for the preparation of Au NRs, the protocol proposed by Murphy *et al.* relies on the preparation of citrate-capped penta-twinned Au NPs as seeds.^{110,119} This protocol exhibits the drawback of low Au NR yield; this limitation of this approach has been overcome by the procedure developed by El-Sayed *et al.*, which uses CTAB instead of citrate as a stronger stabilizing agent for the Au seeds. The Au seeds are obtained from the reduction of HAuCl₄ in the presence of CTAB and ice cold NaBH₄ as the reducing agent. The seeds are then added to a Au(I) growth solution that is prepared from the reduction of HAuCl₄ promoted by ascorbic acid as a mild reducing agent in the presence of CTAB. The introduction of AgNO₃ to the reaction environment facilitates the anisotropic growth and affects the resulting aspect ratio.^{110,111,123} Ag⁺ ions play a relevant role in driving the anisotropic growth of Au NRs because they can adsorb on a specific crystallographic facet (110) of the forming Au NRs.¹²⁰

3.2.1.3 Au nanoshells. Despite the unique chemical-physical properties and the excellent photothermal conversion efficiency of Au NRs, their real application in nanomedicine is still limited because of the cytotoxicity of CTAB.¹²⁴ Among the Au-based nanomaterials with photoactivity in biological windows, increasing attention is being devoted to Au nanoshells (Au NSs), which are spherical NPs consisting of a dielectric core which is covered by a thin metallic shell.

This class of Au-based nanomaterials shows peculiar optical properties that can be tuned from the visible to the NIR spectral range. The plasmonic properties of Au NSs are mainly related to the inner and outer dimensions of their metallic shell layers, as described by the “plasmon hybridization” concept.¹²⁵ Au NSs have been investigated for preclinical biocompatibility, toxicity, and biodistribution with the goal to develop injectable devices to be used in the photothermal ablation of tumours.¹²⁶ Several configurations of Au NSs are reported in the literature; the most investigated are SiO₂-Au NSs, consisting of a SiO₂ core surrounded by an Au shell, hollow Au NSs (H-Au NSs), and nanomatryoshkas (NMs), which are defined as a metallic core embedded in an inner dielectric cavity, on top of which a second metallic shell is grown. A typical preparation protocol for SiO₂-Au NSs involves several steps, including the synthesis of the APTES (3-aminopropyl)triethoxysilane)-functionalized SiO₂ core (120 nm in size),

the subsequent surface growth of ultrasmall Au NPs and, after several purification steps, the final reduction promoted by CO or H₂CO to generate a continuous multicrystalline layer upon the coalescence of ultrasmall Au NPs on the SiO₂ core surface.¹²⁵ Recently, a one-step procedure was reported for the synthesis of SiO₂-Au NSs, wherein polyelectrolyte-modified polystyrene spheres (PS) replace SiO₂ as the dielectric core support. The growth of a uniform Au shell is basically achieved through the use of two reducing agents, NaBH₄ and ascorbic acid. The strong reductant is added in a sub-stoichiometric amount so that it promotes the formation of Au nuclei at the PS surface, while the unreduced HAuCl₄ is available for the subsequent shell generation promoted by ascorbic acid.¹²⁷ Conversely, the most effective procedure for the synthesis of H-Au NSs requires the use of a sacrificial core that is intended to be chemically destroyed in the course of or after the production of the Au shell by the so-called “galvanic replacement” reaction. H-Au NSs have been synthesized by growing an Au layer onto Ag colloidal NPs used as a sacrificial core. The progressive reduction of Au NPs occurs concomitantly with the Ag core oxidation *via* the formation of an Ag/Au alloy and the subsequent progressive oxidation of the Ag core.¹²⁸ Cobalt colloidal NPs have been further proposed as a sacrificial template for the synthesis of H-Au NSs through galvanic replacement reactions.^{121,122,129} Alternatively, soft templates can be employed.¹³⁰ Similarly to H-Au NSs, NMs can be produced by the use of SiO₂ as a dielectric spacer between the Au core and the Au shell.¹²⁴ The Au core size and the Au shell thickness, respectively, are able to drive the plasmonic properties of the resulting Au NMs. In comparison with the conventional SiO₂-Au NSs, NMs can achieve the same plasmonic optical response with a reduced size, thus improving the EPR effect. With respect to SiO₂-Au NSs, NMs are characterized by a higher absorption cross section, a higher efficiency of photonic to thermal energy conversion and higher accumulation within large tumours.¹²⁴ The general synthetic scheme for the preparation of NMs consists first of the synthesis of the Au core, followed by growth of the dielectric nanogap and, finally, by the synthesis of the Au shell.¹²⁴ Several materials, such as spherical polydopamine NPs, can be used as an alternative platform to SiO₂.¹³¹

Fig. 3 shows TEM micrographs of Au NPs with different shapes and sizes. In particular, the size of the spherical Au NPs can be tuned according to the reaction temperature; 4 nm Au nanospheres are obtained at 20 °C (a) and 2 nm Au nanospheres are synthesized at 40 °C (b). The anisotropic growth of Au NRs as well as their shape distribution can be driven by the amount of Ag⁺ ions ((c and d)). Hollow Au NPs synthesized through a galvanic replacement reaction are shown in (e and f).

The absorption spectra of the spherical, NR, and NS Au NPs are reported in Fig. 4. It is worth noting that for the spherical NPs, the absorption peak is centered in the visible part of the electromagnetic spectrum (Fig. 4a). Conversely, for the Au NRs and Au NSs, it is possible to tune the main absorption peak in the first biological window (700 to 900 nm) by playing with the aspect ratio (Fig. 4c) *via* the synthesis parameters (Fig. 4e), thus

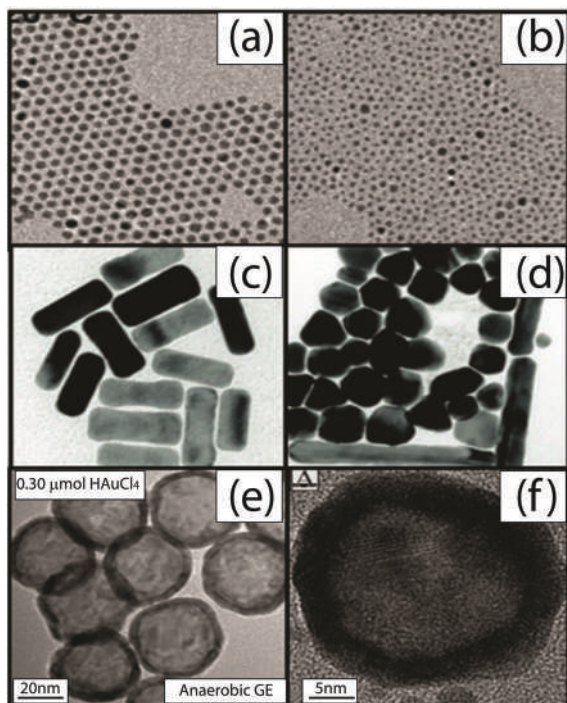


Fig. 3 TEM micrographs of Au NPs with different shapes: spherical (a and b), rod-like (c and d) and hollow (e and f). Reprinted with the permission of ref. 118 (a and b), ref. 120 (c and d) and ref. 121 (e) and ref. 122 (f).

opening the potential of these materials for bio-related applications such as PTT-based cancer therapy.

3.2.2 Surface engineering of Au nanoparticles for the treatment of solid tumours. Due to the outstanding optical and thermo-optical properties of Au NPs, this class of materials is a keystone for extremely promising biophotonic applications that include, but are not limited to, the possibility to reduce the size of tumours with the ultimate goal to achieve complete tumour ablation and limit invasive therapeutic treatments as much as possible.¹³⁵ However, a crucial point is the accomplishment of complete compatibility between the Au NPs and biological tissues. By suitably engineering the surface of Au NPs, it is possible to improve the biocompatibility of the Au NPs and control the interactions between the Au NPs and tumour tissues.¹³⁶ Remarkably, surface modification and engineering opens up additional therapeutic functionalities towards targeting, drug delivery, immunotherapy, gene therapy and photothermal therapy (Fig. 2). The most common chemical procedures for the surface modification of Au NPs are ligand exchange, layer-by-layer ligand assembly and surface coating. The ligand exchange approach can be defined as a post-synthetic procedure to replace the pristine ligand with a new functional capping agent due to the presence of a functional group with an higher affinity to the NP surface than the pristine surface or by exploiting a mass-driven process.¹³⁵ The new capping agent can interact with the surface of the NPs both through covalent attachment and chelation, resulting in an effective interface between the NP core and the selected biological system while simultaneously providing remarkable colloidal

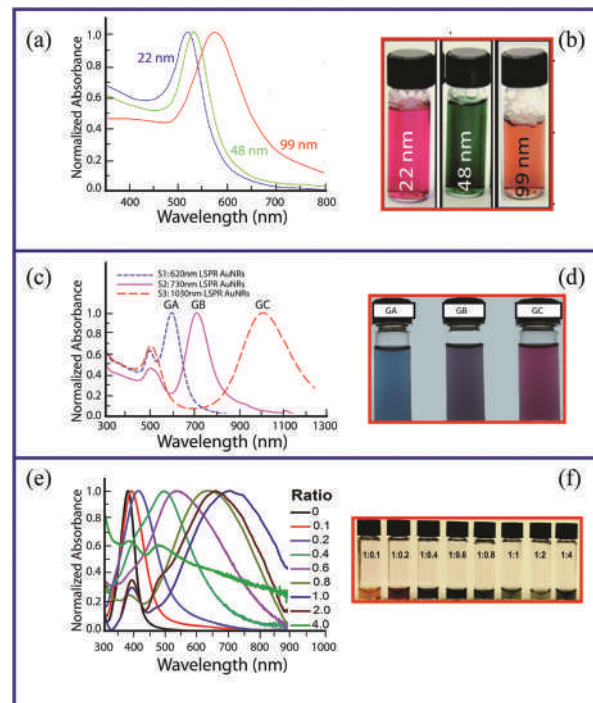


Fig. 4 Normalized absorption spectra of spherical Au NPs with different diameters (a) along with a photograph (b) of the corresponding solutions. Reprinted with the permission of ref. 132. Normalized absorption spectra of Au NRs with different aspect ratios induced by variation of the longitudinal dimension: 45 nm (GA), 49 nm (GB) and 70 nm (GC) (c) along with a representative photograph (d) of the three solutions. Reprinted with the permission of ref. 133. Normalized absorption spectra of Au NPs with different ratios of Ag NPs suspension to a precursor composed of HAuCl_4 and K_2CO_3 (e) along with a photograph of the corresponding solutions (f). Reprinted with the permission of ref. 134.

stability. Layer-by-layer ligand assembly relies on deposition of polyelectrolyte multilayers of opposite charge onto the surface of the Au NPs through electrostatic interactions. This approach, in addition to improving the stability and biocompatibility of Au NPs, is also employed to intercalate small molecules with specific functionality.^{137–140} Lastly, Au NPs can be functionalized epitaxially by growing a suitable shell of a different inorganic material.^{141–144} The functionalization of Au NPs with PEG and its derivatives has been revealed to be extremely useful and effective to improve the biocompatibility and stability of the NPs in the circulatory system, prevent clearance and achieve a longer blood circulation time. PEG, because of its ability to improve the biological compatibility of Au NPs, is also conventionally employed in Au NPs-based architectures with multiple functionalities useful for their bioconjugation with biological molecules. PEGylated Au nanospheres and Au NRs have been further functionalized with specifically designed peptides containing both RGD and nuclear localization signal (NLS) sequences. The RGD motif is able to specifically recognize a certain number of specific integrins that are mainly overexpressed on neovascular endothelial cells.¹⁴⁵ Therefore, the RGD motif plays a very important role by increasing the selective receptor-mediated endocytosis of the NPs

into cancer cells. The peptide iRGD was exploited to coat lipid NPs loaded with the inhibitor PI3K and an agonist of therapeutic T cells, GalCer. The PI3K inhibits immune-suppressive tumour cells, while the GalCer synergistically transforms the tumour microenvironment of solid tumours from suppressive to stimulatory. This formulation resulted in a therapeutic window of 2 weeks, enabling tumour-specific CAR-T cells to home to the lesion, undergo robust expansion, and trigger tumour regression.^{146,147} The NLS sequence promotes the entrance of Au NPs into the nucleus through recognition by importin proteins.¹⁴⁵ CYT-6091 is a nanomedicine based on Au NPs conjugated with PEG and recombinant human tumour necrosis factor (rhTNF); it has been clinically tested for treatment of patients with solid organ cancers.¹⁴⁸ Clinical results have indicated that rhTNF conjugated to Au NPs in the nanoformulation can be administered systemically at doses of rhTNF that had been previously shown to be toxic.¹⁴⁹ In order to increase the intra-tumoural drug penetration, an interesting option that has been evaluated in a preclinical mouse model is the co-administration of junction opener (JO) protein with PEGylated Au NPs. The JO protein has been found to trigger the transient opening of intercellular junctions through binding and cleavage of desmoglein-2 (DSG2) dimers between epithelial cells, increasing the tumour penetration of the Au NPs.¹⁵⁰ Au NSs have been functionalized by means of a “camouflage” strategy that relies on grafting biocompatible Au NSs with macrophage cell membrane (MPCM); this achieves a biomimetic system that can recognize the tumour endothelium, with the further advantage of increasing the *in vivo* blood circulation time and local accumulation at the tumour site. Notably, the biomimetic system preserves the photothermal properties of Au NSs; it has been found to suppress tumour growth with high efficiency and to selectively ablate cancerous cells within the NIR-irradiated surface.¹⁵¹

Au NPs can be also functionalized with RNA aptamers.^{152–155} Namely, nanoporous Au NPs characterized by efficient photothermal properties have been loaded with doxorubicin (DOX), a widely used chemotherapeutic anticancer drug, and incorporated in a copolymer-liposome bilayer (PLB), thus obtaining multi-responsive drug delivery vectors. Furthermore, the resulting

vectors have been surface decorated with RNA aptamer (EpDP3) molecules that (i) behave as targeting agents, being able to bind EpCAM, a biomarker overexpressed on tumour-cell surfaces and (ii) promote cell internalization. The PLB enhances the stability as well as the pH-responsive release of DOX from the surface of the Au NPs. Under NIR irradiation, the release of DOX is thermo-responsive, being mediated by the photothermal response of the Au NPs and by the phase transition of the NIPAM moiety in the PLB layer.¹⁵⁶

4. Photo-thermal properties of Au nanoparticles

Au NPs, due to the LPR mechanism, have the unique capability to generate an extremely high amount of heat at the nanoscale.

Indeed, the nanoconfined electric field associated with the LPR effect is converted to heat, and the NPs can be exploited as a nano-source of heat.

Because of the very low quantum yield of the NPs and their reduced size (10 to 30 nm in diameter), the amount of heat is strictly related to the total absorption rate. From a practical point of view, for the sake of simplicity, the case of a spherical NP with a complex relative permittivity $\epsilon(\omega)$ dispersed in a surrounding (dielectric and isotropic) medium with a real relative permittivity ϵ_m is considered (Fig. 5a). If the wavelength (λ) of an incident light beam is much longer than the NP radius (R), the power absorbed (P) by a single NP is proportional to the intensity (I) of the incident beam through the absorption cross-section (σ_{abs}). It is evident from a theoretical point of view (see ref. 157 for details) that the temperature profile (Fig. 5b) is uniform within the NP diameter ($-R < r < +R$) but strongly decays outside the NP region ($r > R$).

The possibility to confine an elevated amount of heat at the nanoscale represents one of the major applications of NPs in cancer therapy by means of PTT. In order to successfully apply the PTT technique to cancer therapy, the NP absorption must be maximized, while the tissue absorption must be minimized. Indeed, the LPR of the NPs must be kept necessarily close to the first (700 to 900 nm) or to the second (1000 to 1300 nm)

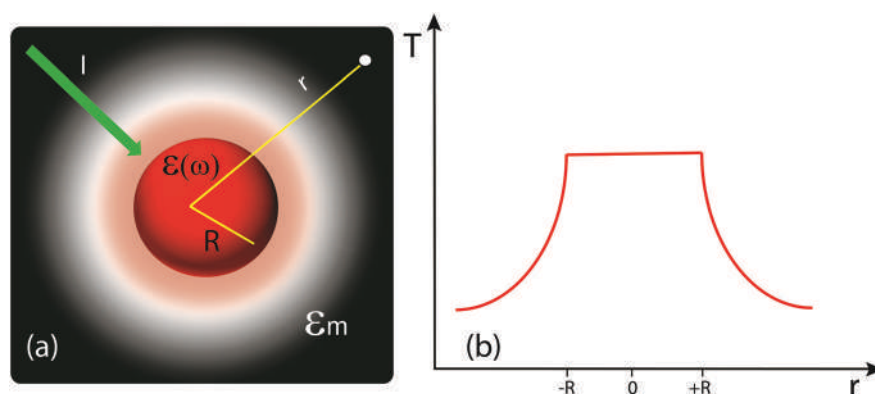


Fig. 5 Schematic of the photo-thermal conversion process in spherical NPs (a) along with its temperature distribution (b).

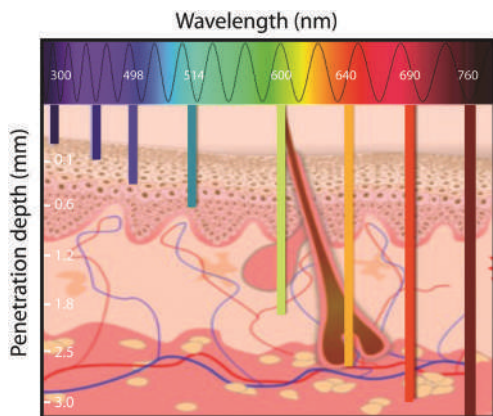


Fig. 6 Propagation of light of different wavelengths in tissues.

biological window, where tissue absorption is low and, thus, the penetration depth of the radiation is high.

Fig. 6 shows the propagation of electromagnetic radiation at different wavelengths in living tissues. It is worth noting that the NIR (≈ 800 nm) exhibits relatively high (more than 3 mm) propagation into tissue; meanwhile, green and blue light is absorbed by the tissue and, thus, converted to heat energy. For this reason, as previously discussed, Au NRs and Au NSs represent NPs with unique features because their LPR can be easily adjusted in the NIR region of the electromagnetic spectrum (Fig. 4c and e) by controlling the aspect ratio of Au NRs or the geometric parameters of Au NSs. In terms of photo-thermal efficiency, μ (defined as the efficiency of transducing incident light to heat), Au NRs have been demonstrated, by means of different techniques,¹⁵⁸ to be better light-to-heat converters ($\mu \approx 1$) than Au NSs due to their smaller size. A simple characterization of the ability of Au NRs to convert light to heat can be obtained by performing photo-thermal experiments by means of a light-assisted thermographic setup. As such, a CW diode laser operating at 810 nm (in the high absorption range of Au NRs) is used as a photo-trigger, while the photo-thermal response is acquired by means of a high-resolution

thermal camera. Fig. 7a shows that upon illumination (≈ 60 s), a significant temperature increase (from 25 °C to 62 °C) is evident, highlighting the strong correlation between the final reached temperature and the intensity of the incident pump beam. The results reported in Fig. 7a were obtained by processing the thermal images shown in Fig. 7b.

5. Interplay between Au nanoparticle-mediated photo-thermal therapy and immunotherapy

The interplay between immunotherapy and PTT is opening a promising field in terms of new opportunities for the development of anti-cancer therapies. Indeed, NPs can be properly designed in order to further optimize and refine the exciting outcomes of the recent developments in immunotherapy. For this purpose, several approaches have been explored, as summarized in Table 1.

An example of a combination approach between immunotherapy and NPs is reported in Fig. 8.^{159,160}

In this regard, Au NPs, when suitably synthesized and engineered, can be strategically exploited to increase the effectiveness of the immunotherapy and to circumvent the limitations in terms of safety and efficacy due to the systemic delivery. Several studies have demonstrated that the photothermal properties of Au NPs not only enable selective tumour ablation but can also induce an immune response. The selectivity arises from the EPR effect and, remarkably, from the possibility to properly functionalize the surface of Au NPs in order to enable biocompatibility and precisely target the tumour site. Au NP-mediated PTT can alter the surrounding tumour environment, thus potentially enhancing the efficacy of secondary therapies, or decrease the required treatment dosages of secondary agents.¹⁶¹ The possibilities arising from the interplay between PTT and immunotherapy have been tested by exploring several morphologies of Au NPs as well as several immunotherapy approaches. An example of the great potential of integration of PTT with

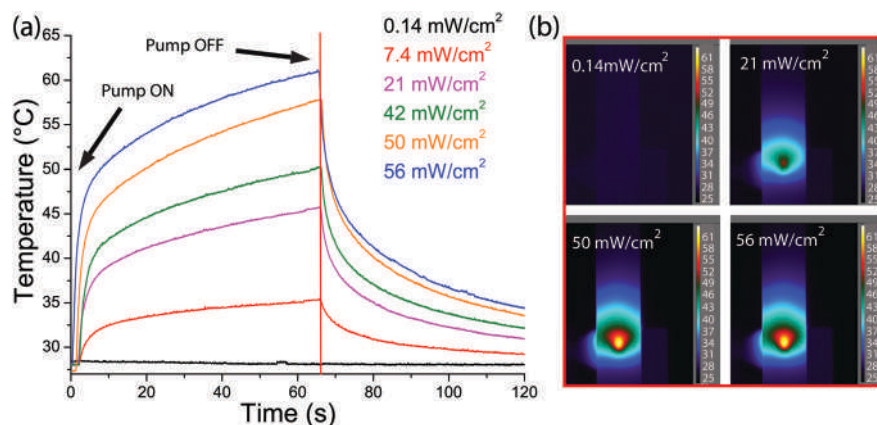


Fig. 7 Time-temperature profiles (a) of a Au NRs aqueous solution (DiagNano™, OD 125, diluted 1 : 35) by turning on (heating process) and off (cooling process) a pump laser beam ($\lambda_{\text{pump}} = 810$ nm) at different intensity levels along with the corresponding thermographic (b) views (FLIR, A655sc) of the sample in the quartz cuvette.

Table 1 Experimental approaches integrating Au NP-induced PTT and immunotherapy

Au NP type	Functionalization	Bio-conjugation/immunotherapy approach	PTT conditions	Main results
Au NRS ¹⁶⁵	Surface modification with PSS polyelectrolyte	Conjugation with the antibody anti-EGFR	630 nm, 10 mW cm ⁻² Irradiation time 3 min	92% of cell mortality for human squamous carcinoma cells (A413)
Au NRS ¹³⁹	m-PEG-SH	Conjugation with DOX, chemotherapy and cytosine-guanosine oligonucleotide (stimulate immune response)	808 nm, 1.5 W cm ⁻²	Efficient internalization, on-demand release of DOX induced by irradiation, delay of tumour growth
Au NRS ¹⁶⁶	PEG and BSA	R837 = immunoadjuvant	1064 nm, 0.85 W cm ⁻² Irradiation 10 min	Death of melanoma cells under laser irradiation, increased immune response (B16-F10 tumour model)
Au NSs ¹⁶⁴	PEG	Imiquimod (immunomodulator through toll-like receptor 7) Transfer of adoptive T cells (ATCT) after PTT	808 nm, 2 W cm ⁻² Irradiation time 3 min	PTT activation of an immune response, tumour growth reduction at distant tumour site, optimized overall tumour control
Au Nanostars ¹⁶⁷	PEG-SH	Immune checkpoint inhibition (anti-PDL1)	808 nm, 0.6 W cm ⁻² Irradiation time 10 min	Induction of effective immunity Survival rate 49%

immunotherapy relies on the use of T cells as carriers for colloidal Au NPs. Spherical NPs with diameters ranging between 40 nm and 45 nm, after PEG functionalization, have been internalized in T cells so that they migrate toward the tumour site. According to this approach, T cells are able to transport the Au NP payload to tumour sites *in vivo* without undergoing any significant alteration in terms of T cell viability, proliferation, or immune function. Remarkably, this approach has been found to promote fourfold increased delivery of Au NPs to tumours with respect to PEGylated Au nanospheres.¹⁶² Another configuration that highlights the potential of Au nanospheres for enhancing immunotherapy effects with PTT relies on their conjugation with an immunostimulant, CpG, namely an oligodeoxynucleotide that has been suitably modified with a triethylene glycol (TEG) spacer (tmCpG-Au NPs). The resulting complex was internalized in macrophage cells, and the antitumoural activity was monitored *in vitro* by analyzing the release of TNF α . As a result, the tmCpG-Au NPs were demonstrated to stimulate a stronger immune response than bare Au NPs or bare tmCpG. Remarkably, the release of TNF α was found to be affected by the size of the Au NPs. *In vivo* experiments suggested that tmCpG-Au NPs can inhibit tumour growth and promote survival compared to the equivalent dose of free CpG.¹⁶³

As previously mentioned, Au NPs with absorption characteristics in the biological windows are regarded as very powerful, valuable and accessible tools for integration of PTT and immunotherapy. Au NRS have been conjugated with an epidermal growth factor receptor antibody (anti-EGFR), resulting in enhanced cellular uptake. The Au NRS-anti-EGFR conjugates were found to induce 92% mortality. This was demonstrated by measuring the markers of apoptosis for human squamous carcinoma cells (A413) upon irradiation with a continuous wave diode laser at 650 nm.¹⁶⁵

Au NRS exhibit the additional advantage of a high load capacity; this has been effectively exploited by loading both DOX and cytosine-guanosine (CpG) oligodeoxynucleotides (ODNs), thus achieving a Au NRS-based complex that can simultaneously merge light-triggered chemotherapy, PTT and immunotherapy. Indeed, DOX can be released by the Au NRS

upon a light stimulus that locally generates heat, while the CpG ODNs can stimulate an immune response. Once the Au NRS-based complex binds to a toll-like receptor (TLR9), it is efficiently internalized by antigen-presenting cells and induces release of various cytokines, which can initiate a cascade of innate and adaptive immune responses to inhibit tumour growth and metastasis. The proposed system increases the accumulation of DOX in the tumour and delays tumour growth due to the beneficial interplay of the photothermal effect, immunotherapy and on-demand chemotherapy, finally leading to long-term tumour-specific immunity.¹⁶⁸ Recently, Au NRS have been functionalized with PEG and BSA and loaded with imiquimod (R837), an immunomodulator through toll-like receptor 7. The resulting nanocomplex was designed to integrate immunotherapy and PTT for the treatment of human melanoma. Under laser irradiation at 1064 nm, the complex revealed ability to ablate tumours and trigger an increase in the immune response that prevents lung metastasis and tumour reoccurrence.¹⁶⁶

However, in the first experimental study exploring the synergy between PTT and adoptive T cell transfer (ATCT), reported by Bear *et al.*, they adoptively transferred pmel T cells specific for the melanoma tumour antigen gp 100 after performing PTT in B16-F10 tumours. For this purpose, the authors selected Au NSs and laser irradiation at 808 nm, 2 W cm⁻² for 3 min; upon treatment of mice with subcutaneous B16-F10 tumour cells, PTT was demonstrated to induce an immune response and a pro-inflammatory cytokine/chemokine profile consistent with the typical wound healing process. Moreover, PTT has been recognized as an initiator of pro-inflammatory processes, resulting in the activation of DCs; this can promote activation of the immune response without inhibiting the growth of distant tumours. However, when PTT is followed by ATCT, significant benefits have been detected because the combination of PTT and ATCT reduced tumour weight, eradicated primary tumours and promoted antitumour activity of adoptively transferred T cells at distant tumour sites (Fig. 8).¹⁶⁴ PTT induced by Au nanostars has been also reported to be

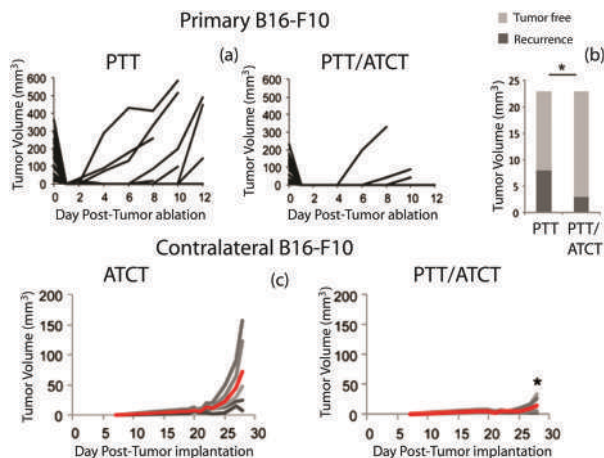


Fig. 8 Growth of primary tumour (a), primary tumour reoccurrence (b) and growth of contralateral tumour B16-F10 (c) in mice treated with PTT alone and PTT followed by adoptive T cell transfer (ATCT). Red tracks represents the mean tumour volume. Experimental data (c) highlighting the beneficial effects of the interplay between PTT and ATCT. Reprinted with the permission of ref. 164.

integrated with an immunotherapy that relies on the use of an immune checkpoint inhibitor, anti-PDL1 antibody. The successful results of this approach have been explained by considering that PTT can promote the release of damage-associated molecular patterns (DAMPs) and heat shock proteins into the surrounding microenvironment. The authors claimed that both the primary tumour and distant tumours were completely cured in one mouse, and no tumours recurred in the following 3 months. Remarkably, no tumour development was identified after performing tumour rechallenge experiments.

6. Penetration of photo-thermal-assisted nanoparticles into solid tumours

The tumour targeting or delivery of NPs is based on the EPR effect. This effect reflects the pathophysiology of solid tumours, including defective vascular architecture, upregulated neo-angiogenesis and excessive production of various vascular mediators.¹⁶⁹ However, looking at the clinical benefits of the three EPR-based Food and Drug Administration (FDA)-approved nanomedicines, including PEGylated liposomal doxorubicin (Doxil/Caelyx), liposomal daunorubicin (DaunoXome), and NP albumin-bound paclitaxel (Abraxane), for the treatment of solid tumours, the results have been found to be only modest.^{23,170,171} Delivery barriers posed by the tumour microenvironment are the main reasons for the modest survival benefits of FDA-approved nanomedicines.²³ In order to increase the intra-tumoural penetration of drugs, a highly relevant option, the co-administration of JO protein with PEGylated Au NPs, can be considered and has already evaluated in a preclinical mouse model. A different approach to locally improve transport of NPs inside the collagen (I) component of tumour tissue is based on the utilization of the photo-thermal properties of the same NPs. Au NRs have been found to be able to alter the collagen (I) matrix of tumours

under NIR stimulation by taking advantage of their capability to convert incident light into heat, thus inducing cell destruction *via* apoptosis and/or necrosis.¹⁷² Au NPs have also been applied for single cancer therapy as a hyperthermia agent^{173,174} and as part of a combinatorial strategy for synergistic killing of tumour cells¹⁷⁵ by direct drug delivery¹⁷⁶ or enhancing the effects of radiotherapy in various cancers (radiosensitizers).^{177–182} Moreover, they are already being used in FDA-approved clinical trials.¹⁴⁸ Several clinical trials based on NP technology are summarized in Table 2.

Currently, several anticancer agents incorporating nano-carriers are under clinical evaluation (Table 2). 14 adult patients with advanced solid tumours have been enrolled in a Phase I clinical trial, proving the safety/feasibility/efficacy of increasing doses of SGT-53 (a liposomal nanocomplex designed for systemic delivery of the wt p53 gene) and/or docetaxel. The nanocomplex SGT-53 has been found to be very well tolerated at each of the tested doses. No maximum tolerated dose (MTD) has been reached, and 95% of the adverse events (AEs) experienced have been of grade 1. 12 out of the 14 patients enrolled in the study were evaluable for tumour response. 3 of these patients achieved RECIST-verified partial responses, with tumour reductions of -47% , -51% , and -79% . 2 more patients reported stable disease, with significant shrinkage of their tumours (-25% and -16%).¹⁸³ The combination of azacitidine and NPs albumin-bound paclitaxel has been evaluated in a Phase I clinical trial. 16 patients with advanced or metastatic solid tumours were treated with azacitidine 75 mg m^{-2} daily for 5 days (priming), followed by weekly administration of nab-paclitaxel 100 mg m^{-2} ; the results show a high grade of tolerability and significant amelioration of patient outcomes in heavily pre-treated cancer patients. Indeed, 13 patients out of 16 were evaluable for response criteria. Clinical activity by RECIST criteria included one complete response (CR) in refractory DLBC lymphoma, two CR in ovarian cancer by CA125, four partial responses (PR) in ovarian and endometrial cancer, four stable diseases (SD) in lung, sarcoma and pancreatic cancer, one unconfirmed PR in breast cancer, and one progression of disease in CLL/SLL.¹⁸⁴ NPs have been also functionalized to deliver cisplatin (NC-6004) and used in combination with gemcitabine in patients with advanced solid tumours.¹⁸⁵ NC-6004 is a novel cisplatin NP developed using micellar technology that enables the generation of NPs with diameters of approximately 30 nm and containing a hydrophilic outer shell composed of PEG; this shell extends the circulation time in the bloodstream by preventing the micelles from being captured by the reticuloendothelial system and enhances tumour-specific accumulation. A total of 22 adult patients were enrolled in treatment with NC-6004. Among the 22 patients, the most common grade III/IV hematologic adverse events were leukopenia (68%) and thrombocytopenia (59%). The MTD was 135 mg m^{-2} . Of the 20 pre-treated patients evaluable for response, half had been previously exposed to a platinum agent. Tumour shrinkage occurred in 11 patients (55%), whereas partial response was observed in 3 cases (15%) and stable disease in 14 (70%). Higher cisplatin equivalent doses were achieved, with no clinically significant neurotoxicity,

Table 2 Examples of Phase I/II and III trials performed utilizing NP-based cancer therapy. Abbreviations: CR, complete response; ORR, objective response rate; PR, partial response; SD, stable disease; AEs, adverse events

Phase	Drug treatment	Disease subgroup	Patient numbers	Median age in years (range)	Response rate
I ¹⁸⁷	Spherical nucleic acid (SNA) Au nanoparticles targeting the oncoprotein Bcl2like12	Recurrent glioblastoma multiforme or gliosarcoma	Estimated enrollment 8	≥ 18	No results posted
I ¹⁸³	Nanocomplex SGT-53 and/or docetaxel	Metastatic, refractory cancer	14 (12/14 were evaluable for tumour response)	58, 5 (33–77)	PR 17% SD 17% (with significant shrinkage but not > 30%) SD 33% (without tumour shrinkage) ORR 61.5% (95% CI, 35–87.95%) CR 23% (95% CI, 0.12–45.88%) PR 38.4% (95% CI, 11.96–64.84%) SD 30.8
I ¹⁸⁴	Azacytidine plus nanoparticle albumin-bound (nab) Paclitaxel	Metastatic solid tumour	16 (13/16 completed at least 3 cycles of the drug and received a scan)	62 (21–83)	Tumour shrinkage 55% PR 15% SD 70%
Ib//II ¹⁸⁶	NC-6004 (nanoparticle cisplatin) plus gemcitabine	Advanced solid tumour	22 (20/22 patients were evaluable for tumour response assessment)	55 (21–69)	No clinical response was reported. Any AEs related to the laser procedure were reported Phase I: SD 58%; PD 25%
I ¹⁸⁸	Nanoshell-directed photothermal therapy	Prostate cancer	22	≥ 18	Phase II: PR 13.5%; SD 32.4%; PD 37.8%
I/II ¹⁸⁹	Pemetrexed plus nab-paclitaxel	Advanced solid tumour	Twelve eligible patients for phase I (10/12 were evaluable for tumour response) 37 patients for the phase II portion	70 (51–76) 63 (45–77)	

ototoxicity, or nephrotoxicity. These data demonstrate the tolerability and promising activity of the NPs formulation based on NC-6004 in combination with gemcitabine.¹⁸⁶

7. Outlook and perspectives

This review clearly demonstrates that nanomaterials have emerged as one of the focal points of modern research that can address all the limitations present in modern medicine, including the fight against cancer. NPs represent the new frontier in oncology because they are able to target massive solid tumours, due to their surface functionalization; penetrate tumours, due to their extremely small size; and finally destroy tumour tissue, due to their stimuli responsiveness. In particular, herein, we have highlighted that Au NPs-assisted PTT combined with immunotherapy offers a powerful interplay and may revolutionize the next generation of high-precision medicine. Although the synergy between PTT and immunotherapy holds great promise, there are still risks involved, such as unwanted immune reaction systems, unknown long-term side effects and risk of the wrong cells being targeted. For these reasons, in order to minimize the associated risks, a polytherapy concept that ingeniously combines the use of PTT, immunotherapy and PDT represents a new route to be further exploited in the near future. PDT is based on the uptake of photosensitizer molecules that, upon excitation with suitable light radiation (UV-blue range), react with oxygen and produce oxidant species that lead to cell death. Several nanomaterials reported in this overview, such as Au NPs,^{190–192} magnetic NPs^{193,194} and carbon-based NPs,^{195,196} can be conveniently used both as photo-thermal and photodynamic agents because of their tailored therapeutic properties; this is useful to provide the most efficient response and the highest safety margin in the treatment of oncological patients. The next generation of cancer treatments ingeniously matching and combining light therapy (PTT and PDT) and immunotherapy hold great potential to successfully provide improved care of oncological patients towards the development of highly efficacious personalized medicine, which is very useful in the fighting against cancer. In this perspective, it has been demonstrated that the simultaneous exploitation of multimodal therapies, namely the synergic combination of hyperthermia with chemotherapeutic activity or cancer immunotherapy, is an effective strategy for the complete elimination of tumour cells, thus providing enhanced therapeutic efficiency, improved specificity and reduced invasiveness compared to single-mode treatments.¹⁹⁷

8. Conclusions

We have reported a comprehensive overview of the application of smart nanomaterials for the realization of novel therapeutic methodologies to fight malignant solid tumours. Recent studies reporting on both organic and inorganic NPs have been reviewed and discussed. In particular, inorganic (metallic) NPs have been demonstrated to possess extraordinary properties for biomedical applications, due to their small size (20 to 30 nm) and prompt

surface processability for their functionalization. In this context, specific attention has been devoted to stimuli-responsive nanomaterials, such as plasmonic NPs. Under illumination with radiation at a suitable wavelength, Au NPs have been found able to generate an extraordinarily high amount of heat at the nanoscale; thus, they are essential functional components towards the realization of PTT as a drug-free medical application. Au NP-based PTT therapy has been used in FDA-approved clinical trials; the outcomes of the Phase I/II and III trials have been reported and critically discussed. In addition, original combinations of PTT and immunotherapy are considered to be promising opportunities in cancer treatment. As such, PTT combined with engineered T cells, expressing a CAR, can function hand-in-hand to trigger an inflammatory process (PTT-triggered necrosis or apoptosis) while activating/improving CAR-T penetration. Overall, this work is intended to review the compelling opportunity offered by engineered nanomaterials and NP-based bioconjugates in the fight against malignant solid tumours, towards the possibility of designing and realizing tailored, minimally invasive, highly selective, and original oncological care treatments.

Conflicts of interest

There are no conflicts to declare.

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Perspective

Antimicrobial Effects of Chemically Functionalized and/or Photo-Heated Nanoparticles

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Abstract: Antibiotic resistance refers to when microorganisms survive and grow in the presence of specific antibiotics, a phenomenon mainly related to the indiscriminate widespread use and abuse of antibiotics. In this framework, thanks to the design and fabrication of original functional nanomaterials, nanotechnology offers a powerful weapon against several diseases such as cancer and pathogenic illness. Smart nanomaterials, such as metallic nanoparticles and semiconductor nanocrystals, enable the realization of novel drug-free medical therapies for fighting against antibiotic-resistant bacteria. In the light of the latest developments, we highlight the outstanding capabilities of several nanotechnology-inspired approaches to kill antibiotic-resistant bacteria. Chemically functionalized silver and titanium dioxide nanoparticles have been employed for their intrinsic toxicity, which enables them to exhibit an antimicrobial activity while, in a different approach, photo-thermal properties of metallic nanoparticles have been theoretically studied and experimentally tested against several temperature sensitive (mesophilic) bacteria. We also show that it is possible to combine a highly localized targeting with a plasmonic-based heating therapy by properly functionalizing nanoparticle surfaces with covalently linked antibodies. As a perspective, the utilization of properly engineered and chemically functionalized nanomaterials opens a new roads for realizing antibiotic free treatments against pathogens and related diseases.

Keywords: antibacterial agents; antibiotic resistance; gold nanoparticles; plasmonic resonance; thermal inactivation

1. Antimicrobial Resistance

Chronic diseases and mortality are consequences of bacterial infections that represent a burden to health and global economics. For their efficiency and effectiveness, antibiotics, exploited as antimicrobial agents, represent a primary treatment method; however, since the first time they were released into the environment, their successful use has been compromised by the development of tolerance and/or resistance. Currently, multidrug resistance (MDR), related to the indiscriminate widespread use and abuse of antibiotics has become, in fact, a serious global health problem, with devastating consequences for patient care [1].

The number of MDR bacterial strains has increased significantly and, although many financial resources have been invested, MDR has become faster than the development of new classes of antibiotic molecules. This problem has not only clinical but also economic implications, as reported in a recent

study [2] where authors estimate the health burden caused in EU and European Economic Area (EEA) in 2015 by antibiotic-resistant bacteria. The study measured the impact of the phenomenon in terms of number of cases, number of attributable deaths and disability-adjusted life years (DALYs), calculated as the sum of “Years of Life Lost” and “Years of Life lived with Disability”. Eight bacterial species frequently isolated from blood or cerebrospinal fluid have been included in this study: *Acinetobacter* spp.; *Enterococcus faecalis* and *faecium*; *Escherichia coli*; *Klebsiella pneumoniae*; *Pseudomonas aeruginosa*; *Staphylococcus aureus* methicillin-resistant (MRSA) and *Streptococcus pneumoniae*. Results showed that 67.9% of DALYs were caused by infections with *E. coli* and *K. pneumoniae* (resistant to cephalosporins of third-generation), *S. aureus* (methicillin-resistant) and *P. aeruginosa* (carbapenem-resistant); bacteria resistant to carbapenems or colistin are responsible for 38.7% of DALYs. Authors showed also that every age is involved in infections, significantly in childhood. In adults, the burden increases with age, suggesting that the overall ageing of population in EU and EEA entails a worsening of the problem. Figure 1a shows the number of attributable deaths, in 2015, as a function of the number of cases in EU and European Economic Area for each bacterial species under analysis. Diameter of bubbles represents DALYs. From data reported in Table 1 of that study [2], we inferred Figure 1b, which shows the association between the number of cases in EU and European Economic Area in 2015 and the percentage of deaths for each antibiotic-resistant bacteria. Figure 1a,b show that the effect on health of antibiotic-resistant bacteria is related not only to virulent infections with large incidences, and therefore large DALYs values, (bubbles diameters in Figure 1a), but also to low incidence infections like carbapenem-resistant *K. pneumoniae*, a case where the percentage of attributable mortality may result very high (see Figure 1b).

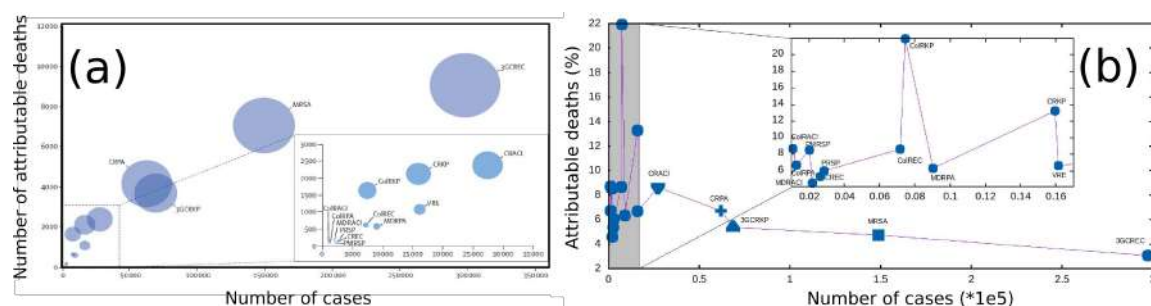


Figure 1. Infections with antibiotic-resistant bacteria, EU and European Economic Area, 2015. (a) The diameter of bubbles represents the number of disability-adjusted life-years [2]. (b) Each blue point represents antibiotic-resistant bacteria in EU and European Economic Area in 2015. Incidence cases are reported on the x-axis, the relative percentage of attributable deaths is reported on the y-axis. Bacteria are the same as in (a). ColRACI = colistin-resistant *Acinetobacter* spp. CRACI = carbapenem-resistant *Acinetobacter* spp. MDRACI = multidrug-resistant *Acinetobacter* spp. VRE = vancomycin-resistant *Enterococcus faecalis* and *Enterococcus faecium*. ColIREC = colistin-resistant *Escherichia coli*. CREC = carbapenem-resistant *E. coli*. 3GCREC = third-generation cephalosporin-resistant *E. coli*. ColRKp = colistin-resistant *Klebsiella pneumoniae*. CRKp = carbapenem-resistant *K. pneumoniae*. 3GCRKp = third-generation cephalosporin-resistant *K. pneumoniae*. ColRPA = colistin-resistant *Pseudomonas aeruginosa*. CRPA = carbapenem-resistant *P. aeruginosa*. MDRPA = multidrug-resistant *P. aeruginosa*. MRSA = methicillin-resistant *Staphylococcus aureus*. PRSP = penicillin-resistant *Streptococcus pneumoniae*. PMRSP = penicillin-resistant and macrolide-resistant *S. pneumoniae*. Reprinted with permission from [2].

In order to understand how MDR develops, we have to recall the functioning mechanism of antibiotics, which interact with their molecular targets to modify or block the normal bacteria functions by exploiting different action. One of the principal antibacterial actions is the inhibition or regulation of the enzymes involved in cell wall biosynthesis, as β -lactams antibiotics and glycopeptides do. Antibiotics can also interfere with cell biochemical reactions (protein synthesis, macrolide, metabolites), such as the folic acid, sulfonamides, and the nucleic acid synthesis do. On the other hand, microorganisms show different alternatives to avoid the action of antibiotics, like the production of

inactivating antibiotic enzymes, such as β -lactamase, alteration of the permeability of the envelope, alteration of the target site, alternative metabolic pathways, biofilm formation [3]. As a matter of fact, antimicrobial resistance to antibiotics is an ancient phenomenon occurring in the environment as a passage of the evolutionary process [4] and represents not only a natural consequence of antibiotic use, but also an intrinsic capacity of bacteria to survive in a hostile environment, due to their genetic plasticity [5]. Furthermore, in addition to their intrinsic and adaptive [6] resistance to a particular antimicrobial agent as a consequence of structural or functional characteristics, and to silent mutations, bacteria have evolved sophisticated mechanisms to acquire, transfer and spread resistance genes. The horizontal transfer of genes (HGT) is mediated, classically, by three main mechanisms [7]: generalized, specialized and lateral transduction (phage mediated) [8]; transformation (transfer of naked DNA); conjugation (transfer by direct cell-to-cell contact) [9]. The emergence of resistance to different types of drug (MDR) in the same bacterial cell allowed to identify mobile genetic elements, transposons and integrons [10], whose special role is to develop and spread MDR among bacteria of clinical relevance.

It is now acknowledged that most of microorganisms grow in the form of biofilms. More than 80% of all microbial infections are biofilm-related, as shown by the group of bacteria that form biofilms called ESKAPE (*Enterococcus faecalis*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *P. aeruginosa* and *Enterobacter* spp.), responsible for high mortality [11]. Biofilms are made of well-structured aggregations of single or multi-species bacteria, which cooperate with each other and their environment, within polymeric matrices produced by cells themselves and attached to biological surfaces or inerts. They have different morphologies depending on the bacterial strains and growth conditions and are responsible for virulent and chronic infections. The classic developmental model of biofilm formation consists of several distinct phases. In a first stage, single cells associate with a solid surface by adhesion; then, in the second stage, they aggregate in micro colonies, usually composed of many types of micro-communities, surrounded by self-produced extracellular polymeric substance (EPS), such as proteins (<1–2%) including enzymes), DNA (<1%), polysaccharides (1–2%) and RNA (<1%). In addition to these components, water (up to 97%) is the major part of biofilms and is responsible for the flow of nutrients inside the biofilm matrix. In the final stage, a detachment from mature biofilm (as planktonic bacteria) in the environment may occur; sometimes, a mechanical stress may also be involved in this process [12].

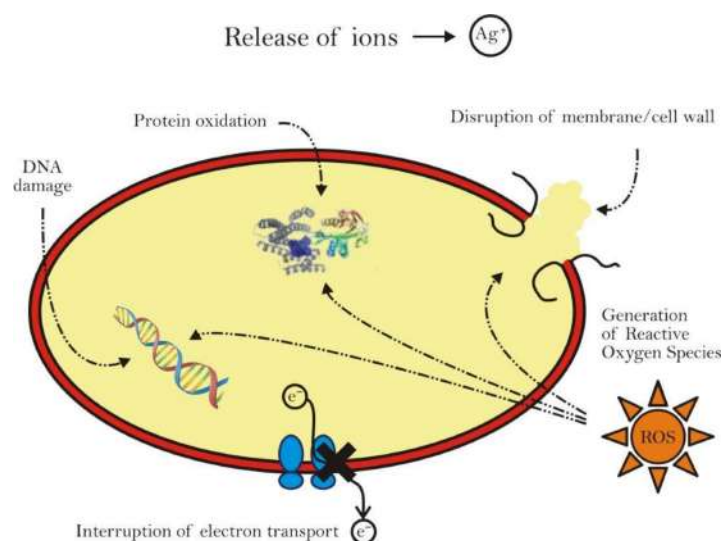
Actually, bacterial biofilms exhibit an enhanced resistance to drug therapies and chemical disinfection, and are more resistant to the immune system. This increased resistance is caused by many factors: polymeric matrices protect cells and, as a result, antimicrobial drugs can hardly penetrate the biofilm; concentration gradients of oxygen and nutrients in the biofilm lead to cell differentiation; metabolic cooperation may cause expression of different antibiotic resistance genes that allow bacteria to resist to the presence of high concentrations [13] of antimicrobial.

2. Nanomaterials as Antibacterial Agents

The term nanomaterials is used to indicate substances made of objects (nanoparticles NPs) where at least one dimension is less than 100 nm. At this scale, they exhibit unique optical, magnetic and electrical properties (different from the bulk material) that have great impact in many fields. The combination of nanotechnology and biology opens new opportunities in biological and nanomedicine applications and offers a potential solution to biological problems such as infection control and instrument sterilization. They may have a strong catalytic activity, high toxicity and can be exploited for novel applications when properties of atomic or bulk materials are unsuitable.

Many nanomaterials exhibit antimicrobial properties and for this reason they may be used to control microbial populations [14–16]. Bacteria are essential to biodiversity on earth, but, as reported in the introduction (Section 1), they are also cause of lethal infections for the human being. They can be exposed to NPs in their natural environment without any adverse effect; for example, the flora

of the skin can be exposed to large quantities of NPs incorporated into topical preparations and cosmetics [17–22].



The formation of reactive oxygen species (ROS) leading to cell membrane, protein and DNA damage. NPs intracellular localization has effects on cell reproduction and development, on DNA and it may interfere with the pumping activity of the protein; moreover it causes an increase in cell permeability and an uncontrolled transport through the cytoplasmic membrane and, ultimately, cell death. Ag^+ leads inhibition of the electron transport.

On the other hand, anti-bacterial NPs have been synthesized from metals, like gold [23], silver [24] copper [25] and iron [26], and from oxides, like cerium oxide [27], zinc oxide [28], titanium dioxide [29] and silicon dioxide [30]. In particular, gold nanoparticles (GNPs) are relatively inert but they become toxic if coated with ligands such as ionic surfactants or antibiotics, which give them antibacterial proprieties [23,31].

As a matter of fact, many studies of bionanotechnology are focused on medical applications, where NPs can be used as anti-bacterials, either independently or in combination with other treatments. A synergistic toxic effect of silver nanoparticles (AgNPs) and antibiotics was observed when tested against *E. coli*. Originally this was reported for amoxicillin [32]; later on, the observation was extended to include enhancement of effects of penicillin, erythromycin, clindamycin and vancomycin [33]; the study reported similar effects against *S. aureus*. Interestingly, both organisms frequently exhibit antibiotic resistance [34]. The effect is not restricted to silver. Zinc oxide NPs increase the efficacy of ciprofloxacin against *E. coli* [28]. Vancomycin, conjugated directly to the surface of GNPs, has more efficient activity compared to vancomycin alone; in this case, the observed synergistic effects occur because of an enhanced reactivity of molecules located at the GNPs surface.

NPs can also have effects in an indirect way, when bacteria must respond to challenging nutrient limitation. Ag and TiO_2 NPs proved to be toxic to bacteria in NPs coated surgical masks [29]; AgNPs are active in water purification filters [35]; Experiment with *E. coli* show that AgNPs can cause cell lysis and cell shrinking [36], with a significant inhibition of the cell growth. A recent hypothesis on the action of silver NPs is that their degree of toxicity is proportional to the release of Ag^+ [24], an antimicrobial cation. AgNPs can also produce free radicals [37], with a diminished activity in presence of an anti-oxidant. Redox-active NPs of iron, or composite NPs containing ferrous iron (Fe_2^+), inhibit *E. coli* growth on solid medium [26]. Also TiO_2 , SiO_2 and ZnO NPs exhibit an oxidative, stress-based, toxic effect [30].

3. Theoretical Description of Induced Temperature Variations

The study of the interaction of light with nanosized objects represents an important issue not only for photonics and plasmonics but also for physics, chemistry, biology and nanomedicine. In application areas, metallic NPs are of high interest because they possess an intrinsic ability to confine light at the nanoscale through the excitation of Localized Plasmonic Resonance (LPR), a phenomenon related to the formation of plasmons, that are oscillations of the free electrons localized at the metal/dielectric interface. Under a resonant light illumination, metallic nanoparticles (MNPs) convert light into heat, thus, becoming nanosources of heat, a phenomenon that opens up an unpredictable number of applications, ranging from photonics to nanomedicine [38,39]. The effect is due to the absorption associated to LPR formation, which triggers a heat generation process; this involves not only absorption of incident photons, but also heat transfer from MNPs to the surrounding medium. In this framework, Gold nanoparticles (GNPs) have gained an important role because their LPR can be tuned from the visible to the near-infrared (NIR) range by simply changing their size, shape, or the dielectric function of the surrounding medium. Moreover, GNPs are bio-compatible and can be easily functionalized with a large variety of molecules, also exhibiting a weak oxidation. Thanks to their properties, GNPs have been widely exploited in cancer therapy [40–44], ophthalmology and dermatology, nanosurgery [45–49], photothermal imaging [50], plasmon-assisted nanochemistry [51], plasmon-assisted optofluidics [52] and sensing [53,54].

Under the action of an impinging, resonant, electric field, due to the LPR onset, a single MNP produces heat by Joule effect (see Figure 2); thus, in order to describe its temperature variation, it is necessary to know its electric response function, that is its polarizability α . This appears in the relation between the dipole moment $\mathbf{p}$ induced in the MNP and the electric field $\mathbf{E}$ of the impinging electromagnetic wave:

$$\mathbf{p} = \alpha \mathbf{E} \quad (1)$$

α depends on shape, structure and nature of the surrounding medium as well as on the metal that the MNP is made of.

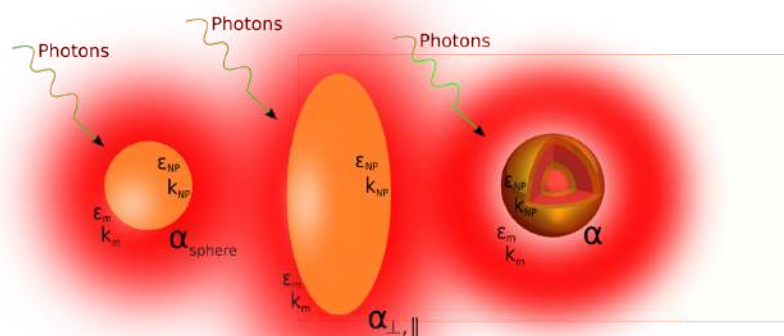


Figure 2. Sphere, ellipse and a complex structure of metal nanoparticles. Temperature variation around the MNP can be described through polarizability α , a parameter that depends on shape, structure and metal that the MNP is made of (dielectric permittivity ϵ_{NP} and thermal conductivity k_{NP}) as well as on the surrounding medium (dielectric permittivity ϵ_m and thermal conductivity k_m).

The conversion efficiency of electromagnetic energy into heat depends on the MNP dimension and, for small nanoparticles, can reach values as high as 100%. In the absence of phase transitions, if α

is known, it is possible to predict the value of temperature around a MNP by solving the heat transfer equation (Equation (2)), which derives from a heat energy balance: the net rate of thermal energy that comes out from the MNP, plus the rate of thermal energy accumulation (internal energy variation in the MNP) must equal the net rate of thermal energy generation:

$$\nabla \cdot (-k(\mathbf{r}) \nabla T(\mathbf{r}, t)) + \rho(\mathbf{r}) c(\mathbf{r}) \frac{\partial T(\mathbf{r}, t)}{\partial t} = Q(\mathbf{r}, t) \quad (2)$$

where $\mathbf{r}$ and t are the spatial and time coordinates, $T(\mathbf{r}, t)$ is the local temperature and parameters $k(\mathbf{r})$, $\rho(\mathbf{r})$, $c(\mathbf{r})$ indicate thermal conductivity, mass density and specific heat, respectively. The function $Q(\mathbf{r}, t)$ represents the source of energy density, coming from the energy dissipation inside the MNP. At the thermal equilibrium, solution of the Equation (2) is obtained by taking into account that heat production takes place at a constant rate per unit time and per unit volume [55]. Calculations, carried out in spherical coordinates, yield a temperature variation at a distance r from the MNP given by:

$$\Delta T = \frac{q}{4\pi K_m r} \quad (3)$$

where K_m is the thermal conductivity of the surrounding medium and the thermal energy generation q is given by

$$q = QV_{NP} = \langle \mathbf{J}(\mathbf{r}, t) \cdot \mathbf{E}(\mathbf{r}, t) \rangle_t V_{NP} = \sigma_{abs} I = K \Im m(\alpha) I \quad (4)$$

where V_{NP} is the total volume of the MNP, σ_{abs} is the absorption cross section of the MNP, K is the wavevector of the impinging light of intensity I , $\langle \mathbf{J}(\mathbf{r}, t) \cdot \mathbf{E}(\mathbf{r}, t) \rangle_t$ is the energy converted into heat due to joule effect into the metal ($\langle \dots \rangle_t$ indicates the time average and $\Im m(\alpha)$ is the imaginary part of α). Equation (4) tells us that, if laser wavelength and intensity and structure polarizability are known, it is possible to calculate thermal energy generation. In order to obtain the polarizability of small MNPs (smaller than the wavelength), it is necessary to solve the Laplace equation by imposing regularity condition in $r = 0$, reconnection to external field in $r \rightarrow \infty$ and tangential and normal continuity of the electric field ($\mathbf{E}$) and electric displacement ($\mathbf{D}$) at the interfaces. For spherical and rod-like shapes (see Figure 2), it is possible to obtain an analytical expression for the polarizability α whereas for complex structures like nano-matryoshkas or onion-like structure it is necessary to resort to a numerical calculation. In spherical coordinates, polarizability α [56] is given by

$$\alpha_{sphere} = 3\epsilon_m V_{NP} \frac{\epsilon_{NP} - \epsilon_m}{\epsilon_{NP} + 2\epsilon_m} \quad (5)$$

where V_{NP} is the total MNP volume, ϵ_{NP} is MNP permittivity, ϵ_m is the host medium permittivity. Equation (5) shows that the resonance of α depends on the dielectric permittivity of the surrounding medium ϵ_m through the Fröhlich condition involving the real part of ϵ_{NP} : $\Re[\epsilon_{NP}] = -2\epsilon_m$.

The polarizability of a metal nanorod can be calculated by using a prolate spheroid coordinate in the dipole approximation [57], which yields $\alpha_{\perp}$ and $\alpha_{\parallel}$:

$$\alpha_{\perp, \parallel} = 3\epsilon_m V_{NP} \frac{\epsilon_{NP} - \epsilon_m}{3\epsilon_m + 3L_{\perp, \parallel}(\epsilon_{NP} - \epsilon_m)} \quad (6)$$

where $\perp$ and $\parallel$ indicates if the direction of light polarization is perpendicular or parallel to the nanorod long axis; $L_{\perp, \parallel}$ are geometrical factors given by:

$$L_{\parallel} = \frac{1 - e^2}{e^2} \left(-1 + \frac{1}{2e} \ln \frac{1 + e}{1 - e} \right) \quad (7)$$

$$L_{\perp} = \frac{1 - L_{\parallel}}{2}$$

e is the eccentricity ($e^2 = 1 - 1/\rho$ where ρ is the aspect ratio of the spheroid ratio between long axis and short axis).

Calculation of the polarizability of nano-matryoshkas like or onion like nanostructures [58], due to the presence of many interfaces, requires to solve an equation system; if the structure symmetry is known, the best way to calculate the polarizability is to use a numerical approach.

In order to provide a possibility of comparison, we report in Figure 3 the absorption cross section σ_{abs} of four MNPs surrounded by water, with the same total volume, but different shapes and/or structures: a gold nano sphere with radius $R_{np} = 10$ nm, a gold nanorod with the same total volume (16 nm and ~ 7.9 nm for long and short axis respectively), a nano-shell made of a silica-core (with radius 7 nm and a gold shell of 3 nm), and a multi-shell structure (onion-like) with total radius 10 nm obtained by starting from a dielectric core and alternating gold/dielectric shells.

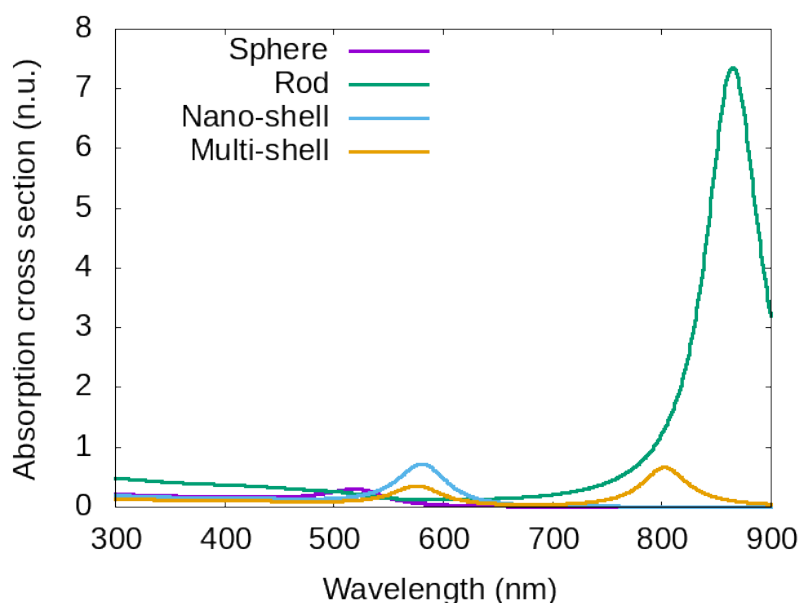


Figure 3. Curves represent the absorption cross section of four MNPs with the same total volume and immersed in water: purple line for a gold nano-sphere with radius 10 nm, green line for a gold nano-rod with long axis 16 nm and short axis ~ 7.9 nm, blue line for a nano-shell made of a silica core (radius 7 nm) and a gold shell of 3 nm, Yellow line for a multi-shell structure obtained starting from a dielectric core and alternating gold/dielectric shell with total radius 10 nm.

Curves of Figure 3 show a plasmonic peak at different wavelength: $\lambda_{peak} = 520$ nm for the sphere, $\lambda_{peak} = 865$ nm for the rod, $\lambda_{peak} = 580$ nm for the nano-shell and two peaks for the multi-shell $\lambda_{peak,1} = 576$ nm and $\lambda_{peak,2} = 803$ nm. The position of the plasmonic peak of the absorption cross section σ_{abs} provides information on the best working wavelength and on the produced heat. Indeed, by starting from the value of σ_{abs} at a particular working wavelength, it is possible to calculate the localized temperature around the MNP (see Figure 2 and Equation (4)). By changing size, metal, structure and surrounding medium (dielectric permittivity ϵ_m and thermal conductivity k_m) it is possible to change the temperature around MNPs [56,59].

It is important to take into account that the effective temperature depends on the number of MNPs acted on by the electromagnetic wave, because each MNP contributes to the heating process through a heat transfer to the surrounding medium. Therefore the temperature will depend on the arrangement (in a line, a surface or a bulk) and on the MNP density [59].

The heating process so far described can be usefully used to obtain an anti-microbial effect, resulting from a temperature variation in mesophilic bacteria as reported in the following Section 4.

4. Anti-Microbial Effect of the Plasmonic Photothermal Heating of Gold Nanoparticles

As already said, anti-bacterial properties of NPs are extensively used in biology. In most cases, the anti-microbial effect of NPs is due to their toxicity, which can be intrinsic (due to the NPs effect directly: Ag or TiO₂) or due to a coating effect (see Section 2). In addition, a new approach is possible, in which no toxicity is involved. If we consider mesophilic bacteria, that grow at a moderate temperature (typically between 20 °C and 45 °C) it is possible to use the thermo-plasmonic effect of MNPs as an antibacterial mean, without triggering any toxic effect. In particular, the heating mechanism of MNPs, described in Section 3, shows how the used metal, shape, structure and surrounding medium [60,61] play the main role in the heating process.

The photothermal-driven approach has been used in [62] where gold nanorods have been exposed to near-infrared radiation and a significant reduction in bacterial cell viability [62] has been observed. It is shown that it was possible to selectively target and destroy a pathogenic Gram-negative bacterium, *Pseudomonas aeruginosa* by using gold nanorods covalently linked to primary antibodies.

A similar bactericidal and antibiofilm activity of gold nanorods (AuNRs) against oral microorganisms, obtained by using plasmonic photothermal therapy (PPTT), is reported in [63]. The efficacy of PPTT was obtained by reaching a temperature as high as 67 °C.

A rapid photothermal bacterial inactivation technique has been developed by Santos [64], by irradiating near-infrared (NIR) light onto bacterial cells (*Escherichia coli*, *Bacillus subtilis*, *Exiguobacterium* sp. AT1B) deposited on surfaces coated with a dense, randomly distributed array of nanoporous gold disks (NPGDs). With the use of cell viability tests and SEM imaging analysis, a complete inactivation of the pathogenic bacteria is confirmed within ~25 s of irradiation of the NPGD substrate.

Gold nanocrosses [65], conjugated to secondary and primary antibodies, completely destroy *P. aeruginosa* and its biofilms upon near-infrared electromagnetic irradiation for 5 min with a $\lambda = 800$ nm laser at low power density (~ 3.0 W cm⁻²). No bacterial activity has been detected after 48 h post irradiation, indicating that the heat generated by the irradiated gold nanocrosses attached to bacteria was effective in eliminating and preventing the re-growth of bacteria. Overall, the conjugated gold nanocrosses allowed an effective photothermal ablation of multidrug-resistant bacteria and their biofilms in a targeted, well localized region, with reduced nonspecific damage to healthy tissue.

By using a LED emission [66] ($\lambda_{peak} = 850$ nm) to irradiate gold nanorods, a 71% of the early biofilm of bacteria elimination has been shown after 5 min of irradiation, a result that shows the potential of this novel antibiofilm technique. All above studies demonstrate that plasmonic generated heat of MNPs offers a novel way to eliminate bacterial biofilms. As a perspective, in future applications this method could also be used to eliminate bacterial contamination during implant surgery or simply could represent a way to obtain solar photothermal disinfection [67].

The possibility to change the wavelength of the plasmonic peak by changing size, shape, structure or surrounding medium makes the photoheating of MNPs a very usefull tool for antibacterial activities (see Section 3). Using equations reported in Section 3 it is possible to take into account if the bacteria are immersed in a water solution (refractive index 1.33) or in a tissue (refractive indices from 1.35 to 1.55). For a nano-shell in a tissue with refractive index 1.55, calculations show a plasmonic peak shift of about 30 nm with respect to the peak position in water and similarly for the peaks of a multi-shell, while, for a nanorod in a tissue, the calculation provides a plasmonic peak shift of more then 100 nm with respect to the peak position in water. This high sensitivity of rods to the surrounding medium is experimentally reported in [68]. Looking at Figure 4 it appears evident that, if bacteria are in a tissue, it is necessary to choose a structure whose plasmonic peak wavelength stands in the bio-window (from 626 nm to 1316 nm), so that the penetration depth results satisfactory. Then, the value of the peak position allows to calculate the local temperature increase (see Section 3, Equation (4)). It is possible to change the absorption (and then the temperature variation) by changing size of the MNPs or their eccentricity (in nanorods) or the ratio between the shell rays in the multi shell (onion-like NPs). In all these cases, it is possible to manage the temperature increase, locally induced around the MNPs.

The desired temperature to completely destroy bacteria depends on the specific bacteria and on the exposition time; in general, for mesophilic bacteria, it is possible to completely destroy bacteria in the range 50–60 °C. It is worth pointing out that, since the reached photo induced temperature depends on all the parameters previously cited (shape, structure, surrounding, ...) and on the NPs density, each specific case requires an “ad hoc” solution.

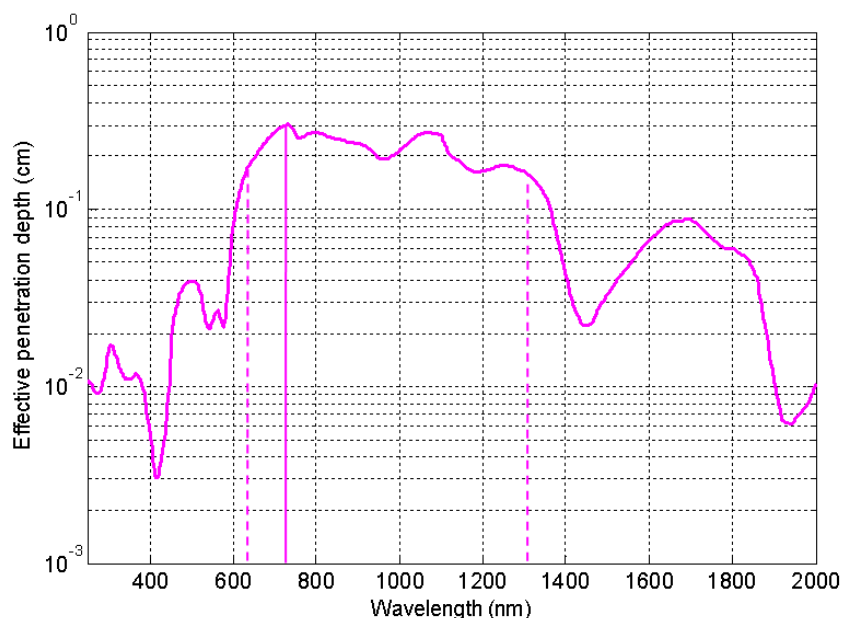


Figure 4. Effective penetration depth in tissue Near-infrared window in biological tissue (626–1316 nm).

5. Discussion

In all previous works, where heat is used as an antimicrobial agent, there is no detailed analysis of the intrinsic toxicity of MNPs and then the percentage of effects due to heat and toxicity remains unknown. Thus, if we want to exclude effects of toxicity, in addition to a coating material that has no toxic activity, it is necessary to properly choose the metal which MNPs are made of, their dimensions and the plasmonic resonant wavelength, by properly choosing shape and/or structure. The main requirement for biological applications is related to the possibility to work with a plasmonic resonant wavelength falling in the bio window (where the human body is transparent) in order to reach all MNPs injected in the body (see Figure 4); this imposes to have the plasmonic resonance in the IR region. This is achievable by using gold nanorods [62,63,66], gold nanocrosses [65], gold nanodisk [64] or more complicated structures like onion-like or nanomatryoshka [58] structures: in all of these differently shaped MNPs it is possible to have a plasmonic resonance (and a following heating process) in the IR region. From a theoretical point of view, it is possible to predict temperature variations around one or more MNPs by means of analytical or numerical analysis. The model can be used to calculate the optimal MNPs concentration needed to obtain the desired effect.

6. Conclusions

In conclusion, we have analyzed the phenomenon of the antibacterial resistance, arguing that the problem of multidrug resistance is related not only to the indiscriminate widespread use and abuse of antibiotic, but also to the intrinsic capacity of bacteria to survive in a hostile environment, as a consequence of their genetic plasticity. In fact, in addition to chromosomal mutations, bacteria have sophisticated mechanisms to acquire, transfer and spread resistance genes. We present a tracking shot of possible solutions to this problem through a nanotechnology-driven approach, showing that metal NPs can be used to obtain an antibacterial effect, both for their toxic action and for an induced photo-thermal

action. We report that the intrinsic toxic action of Ag and TiO₂ or the induced toxicity of a NPs coating with ligands (such as ionic surfactants or antibiotics) are able to exert an antimicrobial activity. We also show that, for mesophilic bacteria, a new approach is possible, in which no toxicity is involved. By using the thermo-plasmonic effect of metal nanoparticles, it is possible to completely destroy bacteria and their biofilms, preventing also their re-growth. Furthermore, it is possible to selectively target and destroy bacteria covalently linking the MNPs to primary antibodies. The importance of MNP shape, structure, metal which they are made of, as well as the nature of the surrounding medium, is reported and analyzed. A usefully implemented theoretical model enables to design those MNPs that prove more suitable to obtain the desired effect.

Author Contributions: L.P., L.D.S. conceived the strategy; L.D.S., C.U. supervision; F.A., M.A.L. wrote the introduction to antimicrobial resistance (Section 1); A.P. wrote the section of Nanomaterials as antibacterial agents (Section 2); L.P. wrote theoretical (Section 3) and photothermal (Section 4) sections and the Discussion and conclusions section; A.G. contributed to write the introduction; all authors contributed to write “Discussion and conclusions” section.

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Conflicts of Interest: The authors declare no conflict of interest.

Abbreviations

The following abbreviations are used in this manuscript:

MDR	multidrug resistance
EEA	European Economic Area
HGT	Horizontal gene transfer
NP	nanoparticle
GNP	gold nanoparticle
MNP	metal nanoparticle
LPR	localized plasmonic resonance
IR	infrared
NIR	near infrared

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Article

Thermo-Plasmonic Killing of *Escherichia coli* TG1 Bacteria

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Abstract: Plasmonic photo-thermal therapy (PPTT) is a minimally invasive, drug-free, therapy based on the properties of noble metal nanoparticles, able to convert a bio-transparent electromagnetic radiation into heat. PPTT has been used against cancer and other diseases. Herein, we demonstrate an antimicrobial methodology based on the properties of gold nanorods (GNRs). Under a resonant laser irradiation GNRs become highly efficient light to heat nano-converters extremely useful for PPTT applications. The concept here is to assess the antimicrobial effect of easy to synthesize, suitably purified, water-dispersible GNRs on *Escherichia coli* bacteria. A control on the GNRs concentration used for the process has been demonstrated critical in order to rule out cytotoxic effects on the cells, and still to be able to generate, under a near infrared illumination, an adequate amount of heat suited to increase the temperature up to ≈ 50 °C in about 5 min. Viability experiments evidenced that the proposed system accomplished a killing efficiency suitable to reducing the *Escherichia coli* population of about 2 log CFU (colony-forming unit).

Keywords: nanomaterials; plasmonics; bacteria disinfection; photothermal effects; optics

1. Introduction

Bacterial adhesion and proliferation represent an extremely critical problem to human and animal health. To date, antibiotics are the best treatment for bacterial infections [1]. They act at different levels by inhibiting DNA replication, translation or synthesis of the cell membrane. On the other hand, resistant strains are the main consequences of their large use. Most pathogen bacteria bind to a surface and form a structured biofilm, where many species interact with each other and get their natural habitat. Biofilms are present in mammals as a result of alterations of different organs such as skin, nose, lung and intestine, as well as on abiotic surfaces, including medical devices, to which living organisms may be critically exposed. Their metabolic and physiological heterogeneity and

their complex interspecies interactions are responsible of the survival of bacterial cells in a hostile environment. Moreover, it is well documented that bacteria biofilms show increased resistance to common disinfection methods and are not easily eradicable with conventional antibiotic therapies [2]. Among the most accredited hypotheses of the increased antibiotic resistance is the presence of an extracellular matrix that acts as cell protection and prevents the penetration of the drugs into deeper layers of the biofilm [3]. Therefore, it is mandatory to identify effective strategies for controlling bacteria growth and the related consequent diseases. Bacterial infections in humans are mainly caused by mesophilic bacteria that find their optimal growth temperature around 37 °C, that is the regular human body temperature. However, moderate temperatures (30–45 °C) have been demonstrated to be still suitable to promote mesophilic bacteria growth while higher temperatures (>45 °C) are known to inactivate bacteria viability or proliferation. Therefore, currently, exposure to high temperature is considered a well-established methodology for disinfecting medical tools and laboratory equipment (e.g., by means of dry ovens) [4]. It is obvious that the same highly efficient approach cannot be simply translated for the treatment of human (or animal) diseases caused by pathogenic bacteria, due to the difficulty to locally increase body temperature upon suitable activation.

Biomedical Applications of Gold Nanoparticles

Nanotechnology offers, due to the design and fabrication of original functional nanomaterials, a powerful weapon against several diseases such as cancer [5–7] and pathogenic illness [8–11]. Particularly, gold nanoparticles (NPs) represent a class of very unique materials, possessing, among other original size dependent properties, the extraordinary intrinsic capability to confine the intense electromagnetic field at the nanoscale. Such a unique feature is enabled due to a physical phenomenon called localized plasmonic resonance (LPR) [12], a coherent and dipolar oscillation of the free electrons localized at the metal/dielectric interface because of the presence of an incident external electromagnetic radiation. Remarkably, the LPR mechanism is associated to a consequent temperature increase, because of the resultant Joule heating effect upon irradiation of a suitable wavelength [13]. Such a feature can pave the way towards the realization of highly efficient nanosized sources of heat [13,14]. Among the different kinds of NPs, colloidal gold nanorods (GNRs), besides the excellent photo-thermal properties, possess unique chemical-physical characteristics related to their specific geometry, that grant them a great potential in a wide range of bio-related applications [15–17]. Indeed, colloidal GNRs are not only intrinsically non-toxic and exhibit a promptly processable surface but are also characterized by an optical response extending to the first biological window (700–900 nm), where light has its maximum depth of penetration in biological tissue. GNRs are characterized by two LPR bands, ascribable to the transverse and longitudinal plasmon absorption, typically positioned in the visible and near infrared (NIR) range, respectively, in spectral position dependent on their aspect ratio [12]. Moreover, GNRs are very efficient light to heat converters with an extremely high (≈ 1) photo-thermal efficiency [14,15,18]. All the aforementioned outstanding properties have represented an incredible breakthrough especially in the fight against cancer through the so-called plasmonic photo-thermal therapy (PPTT) [19,20]. PPTT is a “drug-free” cancer therapy based on the possibility to selectively destroy cancer cells by exploiting the efficient capability of NPs to convert photon energy into heat. In this context, several plasmonic based architectures have been realized with the purpose of photo-thermally inactivate heat-resistant bacteria. Among others, for instance, Norman et al. [21] have used antibodies linked GNRs to selectively destroy gram-negative bacteria. Martinez et al. [22] have reported the minimum inhibitory concentration (MIC) and minimum bactericide concentration (MBC) of GNRs and GNRs combined with PPTT against seven different bacterial strain, observing the ability of GNRs alone to reduce the bacteria population due to the intrinsic toxicity of GNRs (mainly due to the residual free cetyltrimethylammonium bromide (CTAB) employed as a capping agent). In a different approach, Santos et al. [23] have demonstrated a very effective bacterial inactivation technique by depositing bacterial cells onto a random array of nanoporous gold disks. Similarly, Pihl et al. [24] have realized an analogue experiment by using an array of GNRs. In spite of the great potential, such plasmonic

platforms cannot be used as a therapy for in-vivo bacteria inactivation. Similarly, NPs solutions to be delivered through the blood stream present several limitations such as intrinsic toxicity [22].

To overcome these issues, here we propose a simple and effective methodology based on capped GNRs, obtained upon a suitable purification from the excess of CTAB used as a capping agent that aims at investigating the potential hold by GNRs in the development of light assisted antimicrobial therapies. To validate the proposed approach, a strain of *Escherichia coli* (*E. coli*) has been selected as a representative type of bacteria, having a great impact as it causes severe illness in humans since it is found in the lower intestine of warm-blooded organisms. In particular, an effective approach has been designed and realized in order to minimize the concentration of GNRs and, accordingly, of CTAB associated to the nanostructures, and still obtaining dispersible GNRs and a sufficiently high temperature (≈ 50 °C) suited to reduce the *E. coli* population of about 2 log colony-forming unit (CFU). Such a result not only represents an excellent starting point for realizing in vivo light assisted antimicrobial therapies, but also demonstrates to be fully compliant to the typical standard [25] required for antimicrobial treatment in health care facilities. The proposed plasmonic system has been thoroughly investigated highlighting the structure-function relation of their thermo-optical properties and assessing their antimicrobial activity by means of viability assays.

2. Materials and Methods

2.1. Synthesis and Characterization of Gold Nanorods

Cetyltrimethylammonium bromide (CTAB) capped, water-dispersible, GNRs have been synthesized by means of a seed mediated protocol reported in detail elsewhere [26,27]. Shortly here, we reported that the GNRs samples ($C = 3 \times 10^{-9}$ M, determined by utilizing a standard spectroscopic (absorption) technique) were carefully purified from excess of CTAB by several centrifugation cycles in order to reduce the intrinsic cytotoxicity, mainly exhibited by the non-conjugated CTAB. More details on the GNRs purification process and properties are provided in Figure S1 and Table S1 of the Electronic Supplementary Material (ESM) section. Morphological experiments performed with a transmission electron microscopy (TEM, by Jeol JEM-1011 microscope, Jeol, Peabody, MA, USA, operating at 100 kV) indicated that the particle population consisted of GNRs (14 nm $\times$ 70 nm) with an aspect ratio of about 5.1. A representative TEM image is reported in the inset of Figure S1 (ESM section). The UV-Vis absorption spectrum (Agilent, Santa Clara, CA, USA, see Figure S1) of GNRs has evidenced the presence of two distinct bands corresponding to the transverse and longitudinal LPR bands centered at 517 nm and 788 nm, respectively.

2.2. Effect of Gold Nanorods on *Escherichia coli* TG1 Growth

Escherichia coli TG1 (*E. coli*), kindly provided by Prof. Michele Galluccio (University of Calabria), were selected as gram negative bacterium. *E. coli* were grown aerobically at 37 °C with shaking in sterile Luria Bertani (LB) broth (normal rich growth medium: NaCl 5 g/L; yeast extract 5 g/L; trypton 10 g/L). The *E. coli* solution was diluted (1:100 v/v) in 20 mL of fresh LB liquid medium to restart the cell cycle. After 3 h of incubation at 37 °C the cells were synchronized at the log phase of the growth curve, featured with the optical density at 600 nm (OD₆₀₀) of 0.4–0.6. At this OD value the growth rate is constant, and the cells are already dividing and result in being metabolically active. GNRs (5.9 μ L) were added in 195.5 μ L of *E. coli* ($C = 10^6$ CFU/mL) so that the final concentration of GNRs was about 8.76×10^{-11} M. The culture was kept for 6 h at 37 °C. At 1, 2, 3 and 6 h serial dilutions of the culture were made, and 50 μ L of each dilution were cultured onto sterile LB agar (LB broth + agar 16 g/L) and incubated at 37 °C. After 16–18 h, the number of colonies on each plate was determined to get the corresponding concentration of living bacteria. The log N cells were calculated by counting the CFU and using the formula: CFU/mL = (N° of colonies $\times$ dilution factor)/volume of the culture spread. The same assay procedures were used as those described above for the strain cultures without GNRs.

2.3. Photo-Thermal and Morphological Characterization of *Escherichia Coli* TG1/Gold Nanorods Solutions

The thermo-optical setup (Figure 1) used for this purpose was based on a CW diode laser (Coherent Inc. Santa Clara, CA, USA; $I = 6.3 \text{ W/cm}^2$) operating at 810 nm in the high absorption region of GNRs spectrum, corresponding to the longitudinal plasmon band (LPR wavelength is centered at 788 nm). The laser beam was focused by means of a 10 cm focal length lens in the central part of the eppendorf tube (spot size $\approx 2 \text{ mm}$). A high-resolution thermal-camera was used for mapping and identifying (side view) both the heating spatial distribution and the temperature profile under top-pumping laser illumination.

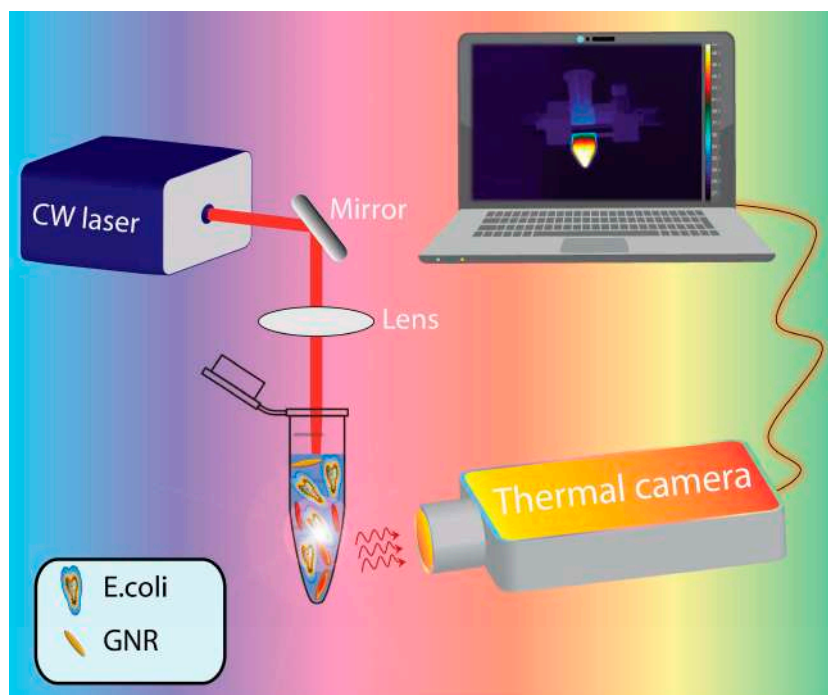


Figure 1. Schematic representation of the thermo-optical setup.

The thermographic analysis was carried out by using a FLIR (A655sc) thermal-camera (FLIR System, Wilsonville, OR, USA) that produces thermal images of $640 \text{ pixels} \times 480 \text{ pixels}$ with an accuracy of $\pm 2^\circ \text{C}$. The camera works seamlessly with proprietary software (FLIR Research IR Max, FLIR System, Wilsonville, OR, USA) that enables recording and processing of the thermal data acquired by the camera. Camera control parameters were set such that sample emissivity was 0.89; camera-sample distance 50 cm and time resolution: 2.67 frame/sec.

Samples for field emission scanning electron microscopy (FE-SEM Zeiss-Sigma, Carl Zeiss Co., Oberkochen, Germany, operating at 10 KV, working distance 3.9 mm) were prepared by dehydrating both *E. coli* and *E. coli*/GNRs solutions on silicon chips, using 70%, 80%, 90%, 95% and 100% ethanol, respectively, for 10 min steps. Subsequently, samples were fixed by using a 3% glutaraldehyde in PBS, pH 7.3 solution and left it to dry overnight. Finally, a thin layer of Au (15 nm) was sputtered just before inserting the samples in the SEM vacuum chamber for reducing the charging effect because of the electrons beam accumulation.

3. Experimental Results

The toxicity of GNRs was investigated by performing viability experiments, with and without GNRs. Figure 2a shows a comparison of the cells viability between *E. coli* (red curve) and *E. coli*/GNRs (blue curve) at different incubation times. In both cases, regular cells growth was evident, thus pointing out that under the tested experimental conditions cells viability and proliferation did not appear

to be significantly affected either by GNRs concentration value and the CTAB molecules present at their surface. A detailed characterization of the *E. coli* viability in the presence of GNRs at different stages of their purification level of residual CTAB is reported in ESM, Figure S2. Thermal treatment experiments were performed in order to investigate the influence of temperature on the cells viability. For this purpose, 500 μL of 1.38×10^7 CFU/mL *E. coli* were deposited on glass slides and temperature was gradually increased from 20 $^{\circ}\text{C}$ to 65 $^{\circ}\text{C}$ by using a hot-plate, without, however, considering the temperature interval from 25 $^{\circ}\text{C}$ to 40 $^{\circ}\text{C}$, where bacteria are expected to grow.

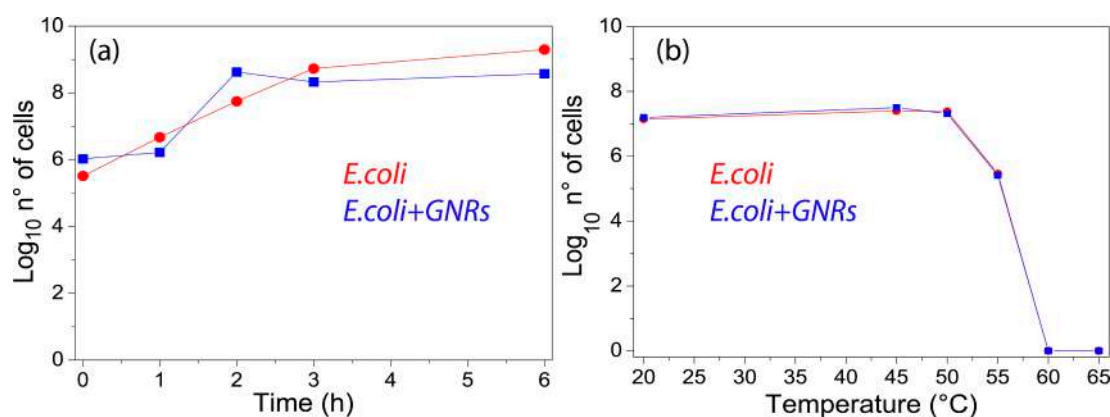


Figure 2. Viability experiments (a) without (red curve) and with (blue curve) gold nanorods (GNRs; $C = 8.76 \times 10^{-11}$ M) at different incubation times. Viability test (b) without (red curve) and with (blue curve) GNRs at different temperatures after 5 min of incubation time.

At each scheduled time interval, a defined volume of *E. coli* solution was withdrawn from the glass slide and a series of dilutions (from 10^{-1} to 10^{-5}) were made by using the LB broth. Subsequently, 50 μL of each diluted sample was cultured out onto sterile LB agar and incubated at 37 $^{\circ}\text{C}$ to investigate cell viability. Figure 2b (red curve) shows the typical curve of temperature-dependent growth for *E. coli*. As the temperature increased, exceeding the optimum growth temperature (≈ 37 $^{\circ}\text{C}$), a decrease in the cell number was observed until their death was noticed at about 60 $^{\circ}\text{C}$, as caused by the rapid denaturation of proteins. The same experiment performed in the presence of GNRs did not show any significant difference, since the two curves almost overlapped (Figure 2b, blue curve). The influences of GNRs on the *E. coli* morphology were investigated by means of a SEM. In Figure 3a, the SEM micrograph of the *E. coli*/GNRs sample showed the typical elongated shape of *E. coli*. The average longitudinal dimension was 1.4 ± 0.4 μm (for 90 counts), this result was compatible with the values found in a similar investigation performed on dehydrated *E. coli* reported in [28]. In Figure 3a isolated GNRs were evident (green circles and red square) placed quite far away from bacteria. A higher magnification observation performed in correspondence of the red squared region, with both “in-lens” and backscattered electrons (BSEs) detectors, in split mode, resulted in the micrographs reported in Figure 3b,c, respectively. Since the BSE signal strongly depends on the average atomic number (Z) of the specimen, the presence of gold ($Z \approx 80$) resulted in a contrast higher than that of the surrounding bacteria ($Z \approx 20$) and therefore the GNRs aggregates (Figure 3a, red square) appeared brighter in Figure 3c. Significantly, no GNR was detected at the bacteria surface, while only few GNRs were detected in the area, without any concomitant relevant alteration of *E. coli* morphology induced by the exposure to the GNRs. The presence of only few GNRs (Figure 3a) could be reasonably accounted by the loss of a large fraction of NPs as a consequence of the multiple washing cycles performed with ethanol in order to completely dry the bacteria.

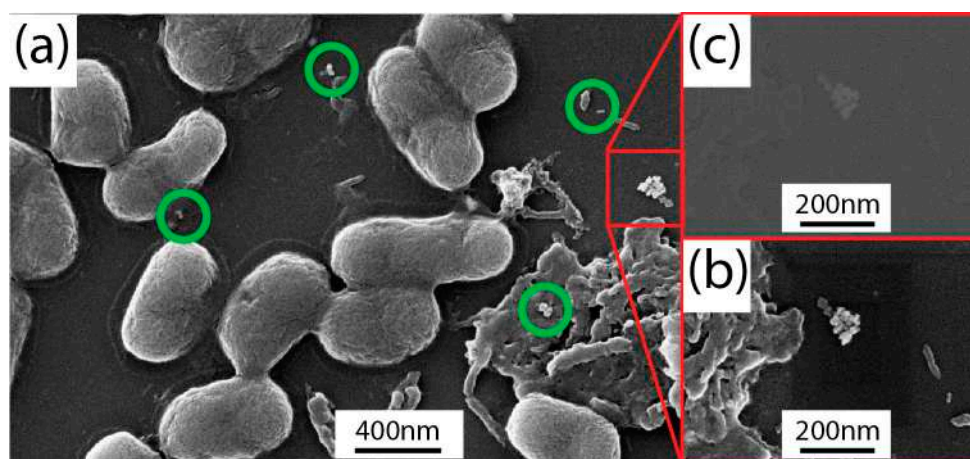


Figure 3. SEM micrograph of the *Escherichia coli*/GNRs solution deposited on a silicon chip (a) along with a higher magnification micrograph acquired with the in-lens (b) and backscattered electron (BSE) (c) detectors, in split operational mode.

Overall the bacterial growth seemed not to be affected by the exposure to GNRs (Figure 2a). Therefore, photothermal experiments were performed to assess whether the GNRs concentration ($C = 8.76 \times 10^{-11}$ M) was able to induce a thermo-plasmonic heating suited to affect the *E. coli* population. Figure 4 shows the time-temperature profiles along with the corresponding thermographic pictures at three increasing illumination times (7.5 min, 15 min and 30 min) for both *E. coli* and *E. coli*/GNRs samples. The temperature plots were obtained by selecting, by means of a control software, an elliptical region of interest (ROI), which includes the center of the illuminated area.

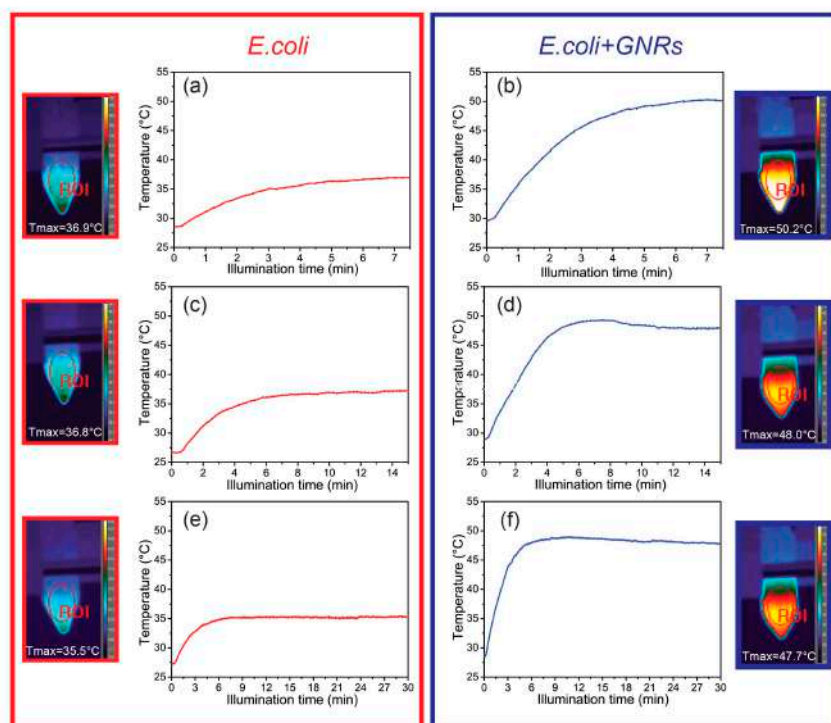


Figure 4. Temperature profiles of the *Escherichia coli* (a), (c), (e) and *E. coli*/GNRs (b), (d), (f) samples for different illumination times along with the corresponding thermographic view of the sample in the eppendorf tube.

Figure 4a,c,e show that upon illumination of the *E. coli* samples, the thermo-camera detected only a relatively low increment of temperature (up to 7–8 °C). Conversely, upon exposure of *E. coli*/GNRs, the absorbed light was efficiently converted into heat because of the plasmonic photo-thermal heating of the GNRs, and the thermo-camera was able to detect a significant temperature increase, as shown in Figure 4b,d,f. Indeed, after 7.5 min, 15 min and 30 min of irradiation, a temperature increase from 20 °C to 50.2 °C, 48.0 °C and 47.7 °C, respectively, were detected that corresponded essentially to the same temperature value, within the experimental variability. The relatively long exposure times (7.5 min, 15 min and 30 min) ensured a uniform distribution of the photo-induced heat (see thermal images reported in Figure 4). Importantly, all the temperature profiles reported in Figure 4 show the same trend. After a rapid temperature increase in the first 5–7 min, a steady state condition was observed. The *E. coli* viability test with and without GNRs, upon laser irradiation, was performed at each scheduled time interval (7.5 min, 15 min and 30 min). Namely, samples obtained upon serial dilutions from each tube were cultured onto LB agar and incubated at 37 °C overnight. Afterwards, the log N cells were calculated by means of the plate count method.

The viability results for both *E. coli* (red column) and *E. coli*/GNRs (blue column) samples (Figure 5a) clearly pointed out that the laser irradiation did not affect the viability of *E. coli* even after 30 min of continuous illumination (Figure 5b (red curve)). Conversely, a significant increase of the killing efficiency (defined as the difference between the bacteria population at $t = 0$ min and $t = 7.5$ min), of about 2 log CFU, was observed in the presence of GNRs as shown in Figure 5b (blue curve). It is worth pointing out that the data reported in Figure 5a were normalized for every experiment to the bacterial population at each specific time interval in order to take into account the intrinsic cells proliferation.

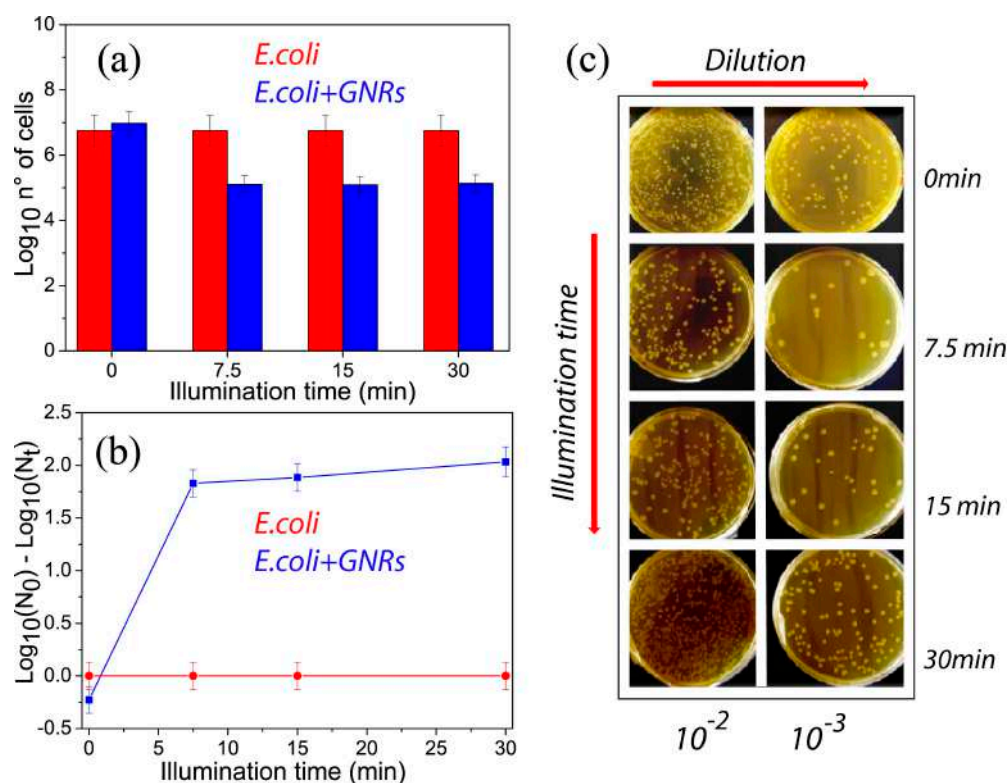


Figure 5. Results of the viability experiments (a) carried out with (blue) and without (red) GNRs at different illumination times along with the corresponding killing efficiency (b). Pictures of the petri dishes (c) containing the *Escherichia coli* /GNRs colonies at increasing illumination times and at different dilution. Data reported in (a) were an average of three independent experiments.

Moreover, it is important to point out that the protocol followed in the experiments for bacteria growth and counting were highly standardized. Indeed, the bacteria population at different illumination times was confirmed to be essentially the same (Figure 5a, red histogram plot), thus highlighting the capability to control the bacteria growth with high reproducibility. This, finally, turns in the same deviation, and hence in the same extent of the error bars for the results reported in Figure 5a and for those reported in Figure 5b.

In order to be able to reliably count the *E. coli* colonies, the samples were diluted with LB broth and the pictures of the plates obtained from the exposed solutions as prepared and after dilution, in the presence of GNRs, is reported in Figure 5c. The plate count method was successfully applied on the cultured diluted samples (Figure 5c, right row), where well-distinguished colonies were present, while the high-density colonies visible in the cultures of the less diluted samples (Figure 5c, left row) resulted in being difficult to be safely counted.

For the sake of simplicity, photos of the petri dishes containing the *E. coli* culture without the GNRs at different illumination times and dilution factors were corresponding to the culture with GNRs at $t = 0$ min. It is important to point out that in order to clarify whether the light driven thermo-plasmonic process explored so far was bacteriostatic or bactericidal, a more careful analysis of the experimental data reported in Figure 5a is required. It turns out that in the presence of GNRs there was a reduction of about 2 log CFU of *E. coli* population (higher than 90% in terms of viability reduction) after 7.5 min of laser illumination. On the other hand, no further reduction in the *E. coli* population was observed after 15 min and 30 min of laser exposure, in the presence of GNRs. This behavior could be explained by considering that after 15 min the bacterial cells had already implemented the molecular response mechanisms to environmental stress such as the plasmonic assisted photo-thermal heating with possible DNA lesions and thus adapted to the new external conditions [28,29]. As a consequence, we can safely state that up to 7.5 min of laser irradiation the light assisted thermo-plasmonic heating resulted in a bactericidal effect, in agreement with the accepted definition of bactericidal property [30]. After 7.5 min of laser illumination, the bacteria population remains constant (Figure 5b, blue curve). This evidence suggests that the conversion of light to heat due to the presence of highly efficient nano-converters (GNRs) ensures a bacteriostatic condition. Furthermore, in order to confirm a long term-effect of the bacteriostatic condition, we performed a comparison between the bacteria population immediately and several hours after the end of the illumination process, being in the meanwhile the solution was incubated at 37 °C (data not shown because were very similar to that reported in Figure 5a). No change in the bacteria population had been measured.

4. Discussion

Currently plasmonic NPs are investigated in several research fields such as electronics [31], photonics [32], agriculture [33], medicine [34] and environmental remediation [35]. As far as their application in medicine, they have successfully demonstrated their potential in cancer therapy and treatment of pathogenic diseases [21,22]. In particular, the use of plasmonic NPs for treatment of pathogenic diseases is a relatively new and promising field, since most of the efforts reported so far indicate several advantages, including the possibility of targeting and delivering. However, a number of unknown side effects, including toxicity, were described. Also, in vivo experiments require the use of colloidal solutions of NPs, suited to be delivered to the targeted sites via blood stream or in situ injections. Here, the study evaluated the possibility to treat *E. coli* by taking advantage of the photo-thermal properties of organic capped GNRs, since they are extremely stable, possess an intrinsic low toxicity and can be promptly functionalized. Indeed, the optical response of the GNRs in the first biological spectral window along with their outstanding photons to thermal-energy conversion property, make them ideal candidates for this purpose [14,15,18]. Similar approaches have been reported based on metal nanostructures [21–23]. However, the intrinsic toxicity of NPs or, alternatively, the use of rigid functional platforms result was not amenable for applications. Therefore, here water dispersible CTAB capped GNRs, synthesized by means of a simple colloidal approach were used.

CTAB, the surfactant used in GNRs synthesis, plays an important role controlling the GNRs size distribution, providing them colloidal stability and preventing NP aggregation. On the other hand, CTAB has been reported to result, above a given concentration, in being very harmful in terms of cells viability [36]. Therefore, the “as prepared” GNRs were purified in order to remove excess of CTAB, that is the fraction of the surfactant molecules that only loosely bind the surface of the GNRs that, however, still result in being sufficiently capped to retain their colloidal stability. Subsequently the work has investigated the suitable conditions, namely the proper concentration of GNRs able to ensure bacterial viability and, at the same time, enable the access to their photo-thermal properties. The results of the comprehensive viability set of experiments performed to this end, have demonstrated that while the CTAB capped GNRs, at the investigated concentration, did not significantly affect the cells viability and proliferation (Figure 2a). GNRs were able to significantly reduce the bacterial viability only upon light irradiation. Such a result represents a significant advance with respect to the current findings [22], that accounts for reporting the ability of GNRs to reduce the bacterial population, relying, however, only on the inherent toxicity of GNRs, without exploiting their ability to photo-thermally induce heating. Here, the photo-thermal experiments have shown that GNRs (not cytotoxic concentration, $C = 8.76 \times 10^{-11}$ M) were able, upon a suitable laser illumination, to increase a temperature of about 20 °C (Figure 4b,d,f). Conversely, under the same laser irradiation (Figure 4a,c,e) in the absence of GNRs, only a relatively low increment of temperature (up to 7–8 °C) had been detected. This variation turned out to be completely harmless in terms of bacterial viability. It is important to mention that bacteria viability experiments were performed at two distinct GNRs concentration ($C = 2.92 \times 10^{-11}$ M and $C = 8.76 \times 10^{-11}$ M), however, although any toxicity effect had been observed for the two concentration values, different photo-thermal properties were detected, resulting in a weaker effect for 2.92×10^{-11} M. Therefore the 8.76×10^{-11} M GNRs concentration were then used in the investigation. The obtained results, highlighting the extraordinary capability of GNRs to efficiently convert a NIR radiation into heat, showed that the dynamic of the system was very fast, as indicated by the time-temperature profiles reported in Figure 4a,c,e. These characteristics appear particularly advantageous for the therapeutic potential of the system, as the temperature of about 50 °C was found to be reached in less than 5 min (Figure 4b,d,f). A set of viability experiments systematically performed on the *E. coli* culture upon laser irradiation (Figure 5b) have demonstrated a photo-thermal killing efficiency suited to reduce 2 log CFU the cell number after 7.5 min. Significantly, the outcome of the viability tests performed on the system that was photo-thermally heated at 50 °C (Figure 5b) resulted in a much higher reduction of the bacteria viability that found for the system heated up to the same temperature (Figure 2b, blue curve). Such a discrepancy could be explained by considering that the photo-thermal heating due to the presence of the highly efficient nano-sources of heat represented by the GNRs, induces a much higher localized temperature variations, while the thermal-camera is able to only detect the temperature variations at the glass/air interface of the eppendorf tube. Moreover, the heat generation at the nanoscale was followed by other secondary mechanisms such as the formation of sound waves and generation of nano-bubbles, which, in their turn, may contribute to the *E. coli* killing. The remarkable achievement of a killing efficiency of 2 log CFU in a short time interval (7.5 min.) could be improved even more, by engineering the GNRs properties in terms of surface chemistry functionalization, aspect ratio and concentration, thus possibly further increasing the temperature to value (e.g., 60 °C) sufficiently high enough to completely kill all the living bacteria (Figure 2b), therefore representing a real breakthrough towards the realization of light assisted antimicrobial technologies.

5. Conclusions

An all-optical methodology based on the capability of GNRs to convert a NIR radiation into heat for an antibacterial application was reported. As a proof-of-concept, *E. coli*, selected as a representative microorganism, was treated with water dispersible, conveniently purified CTAB capped, GNRs, by exposing the *E. coli* culture to GNRs aqueous solution at a suitable, not cytotoxic concentration level. Cell viability assays demonstrated that the GNRs, under the investigated concentration, did not affect

cells viability and proliferation. The SEM analysis on the investigated *E. coli*/GNRs culture, after a dehydration process, clearly accounted for the bacteria morphology, while the backscattered electrons (BSE) micrograph confirmed that the GNRs result was not in contact with bacteria. Photo-thermal experiments carried-out with a laser light ($\lambda = 810$ nm) assisted thermographic setup pointed out that by irradiating the GNRs in the *E. coli*, the temperature could be increased up to 50 °C in about 5 min, and cells viability experiments demonstrated that, under such a laser light irradiation ($I = 6.3$ W/cm²) the presence of GNRs in the *E. coli* culture was able to induce a suppression of living bacteria of about 2 log CFU. A further optimization of the nanoparticles properties in terms of biocompatibility and molecular recognition capability (e.g., using proteins, antibodies, peptides, etc.) is a mandatory step for realizing a new generation of light assisted and on-demand antimicrobial therapies.

Supplementary Materials: The following are available online at <http://www.mdpi.com/1996-1944/12/9/1530/s1>, Figure S1: Normalized spectral response of the samples along with a representative TEM image, Table S1: Zeta-potential of the samples along with the wavelengths of the transverse and longitudinal LPR peaks, Figure S2: Viability experiments without (green curve) and with (red, blue and magenta curves) the three different GNRs solutions at different incubation times.

Author Contributions: Conceptualization, F.A., A.P., R.B., L.D.S.; Methodology, F.A., A.P., L.D.S.; Validation, F.A., A.P., L.D.S.; Investigation, F.A., A.P., M.A.L., F.L., M.G.G., A.G., F.P., R.C.; Resources, T.P., F.P., M.L.C., R.B., L.D.S.; Writing-Original Draft Preparation, M.L.C. and L.D.S.; Writing-Review & Editing, F.A., A.P., L.D.S.; Visualization, F.A., A.P., L.D.S.; Supervision, L.D.S.; Project Administration, F.A., A.P., L.D.S.

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Abbreviations

The following abbreviations are used in this manuscript:

PPTT	Plasmonic photo-thermal therapy
NPs	Gold nanoparticles
PPTT	Plasmonic photo-thermal therapy
GNRs	Gold nanorods
LPR	Localized plasmonic resonance
MIC	Minimum inhibitory concentration
MBC	Minimum bactericide concentration
NIR	Near infrared
<i>E. coli</i>	<i>Escherichia coli</i> TG1

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Thermoplasmonic Activated Reverse-Mode Liquid Crystal Gratings

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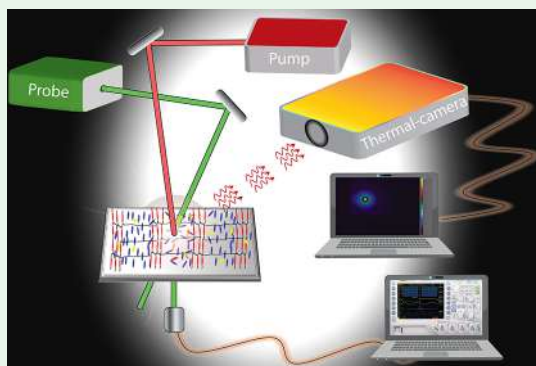
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Supporting Information

ABSTRACT: A new generation of reconfigurable optical components is conceived by bridging the photothermal properties of gold nanoparticles and the thermosensitivity of liquid crystalline materials. As such, gold nanorods (GNRs) heated using light are used to activate efficient hidden diffraction gratings realized in a blend made of a room temperature polymerizable liquid crystal (PLC) and nematic liquid crystal (NLC). Holographic liquid crystal polymer dispersed liquid crystal (HLCPDLC) gratings containing a small percentage of GNRs are fabricated with periodicity of 2.6 μm by means of a conventional UV holographic recording setup. The HLCPDLC structures are characterized using morphological, optical, and thermo-optical techniques. Because of the initial refractive index match between the polymer-rich and LC-rich regions, the “grating” is hidden and the films appear transparent and nondiffractive. Illumination of these GNR-containing structures with a suitable light source (808 nm) induces a local heating due to the plasmonic absorption of the GNRs. This heating induces a refractive index mismatch between the PLC and the NLC as the latter undergoes a phase transition from nematic to isotropic, finally resulting in a transmission diffractive structure activated over a few seconds exhibiting an efficiency of about 70%. The same HLCPDLC samples have also been tested as thermosensitive waveplates, enabling a new, fast methodology for quantifying the photoinduced plasmonic heating with a thermal sensitivity of ~ 0.04 $^{\circ}\text{C}$. Moreover, thermoplasmonic driven waveplates represent a new avenue in the field of light controllable optical phase modulators.

KEYWORDS: diffraction gratings, nanomaterials, gold nanoparticles, plasmonic heating, liquid crystals, polymers, optics



INTRODUCTION

Liquid crystals (LCs) combined with isotropic polymers have been investigated in the past 40 years due to their unique opportunity of creating robust, fast, and switchable photonic structures.^{1,2} Among them, polymer network LCs (PNLCs)³ and polymer dispersed LCs (PDLCs)⁴ have represented the most compelling solutions for realizing composite elements suitable for several photonic applications ranging from sensing⁵ to displays.⁶ Periodic photopolymerization of a LC/monomer mixture through a holographic process has been the enabling technology for realizing films with a periodic distribution of LC droplets. These structures, made of polymeric walls and polymer films containing LC droplets (HPDLCs^{7,8} or POLICRYPS⁹), exhibit dynamic diffraction properties. As formed, both types of gratings exhibit diffraction, the typical

grating coloration in the white-light off state due to the refractive index mismatch between the polymer and the LC rich regions. Power is needed to drive these systems to a clear state. For numerous applications where power consumption is a factor and where a transparent (no diffraction) off-state is required, the alternative or reverse mode is needed. To address this key point, several attempts to realize reverse-mode switchable gratings (nondiffractive in the off state) have been reported^{10–12} with various degrees of success. Very recently,¹³ we demonstrated a new generation of hidden diffraction gratings made in a blend of a room temperature polymerizable

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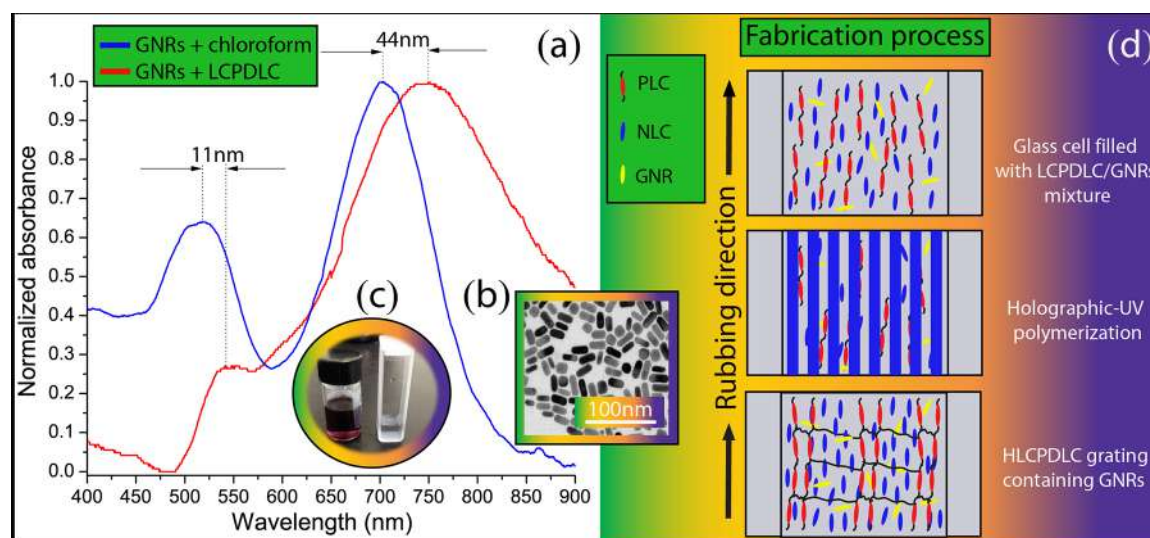


Figure 1. Absorption spectrum (a) of chloroform dispersed GNRs (blue curve) and GNRs dispersed in the LCPDLC mixture (red curve). TEM image (b) of chloroform dispersed GNRs. Photos (c) of the GNRs dispersion in chloroform (left) and LCPDLC mixture (right). Schematic (d) of the fabrication process.

LC (PLC) and a low molecular weight nematic LC (NLC), HLCPDLC (holographic liquid crystal polymer dispersed liquid crystal). HLCPDLCs, due to their intrinsic nature, are transparent in the off state due to the refractive index matching between the as formed PLC and NLC domain regions. Diffraction gratings with very high diffraction efficiency ($\approx 90\%$) are enabled by applying a moderate E -field ($4\text{--}5\text{ V}/\mu\text{m}$) or by increasing the temperature ($\approx 45\text{ }^\circ\text{C}$). The enabler in such a system was the mesogenic monomer which was a LC (fluid) at room temperature (unlike commercial LC monomers which are solid) before polymerization which enabled better physical compatibility during the phase separation process. The ability to control the diffraction of light and in particular the activation/deactivation of HLCPDLC gratings using other mechanisms including optical radiation is of great interest but unexplored. Nanoparticles (NPs) have attracted a great scientific interest in the past years because of their unique size-dependent properties. Historically, NPs have been utilized as nanofillers^{14,15} for the development of conductive polymer composites or to improve the mechanical resistance of polymer based materials. However, plasmonic NPs have the extraordinary capability to convert light to heat by exploiting a phenomenon called the localized plasmonic resonance (LPR).^{16,17} Visible/NIR light can induce the oscillation of bulk free electrons localized at the metallic/dielectric interface of the NPs giving rise to LPR. The resultant joule heating upon irradiation of the appropriate wavelength enables the NPs to behave as highly efficient nanosources of heat. Plasmonic NPs are very useful agents for photothermal applications due to an enhanced absorption cross section ($4\text{--}5$ orders of magnitude larger) compared to conventional photoabsorbing dyes (e.g., rhodamine 6G). Moreover, plasmonic NPs have higher photostability and do not suffer from photobleaching. The photothermal properties of plasmonic NPs have been used in several research field such as medicine,^{18,19} solar energy,²⁰ optofluidics,²¹ and photonics.²² A particular class of very interesting plasmonic NPs is represented by gold nanorods (GNRs). They show two (transverse and longitudinal) LPRs, with peak absorption wavelengths tunable from the visible to NIR depending on the

GNR aspect ratio. The wavelength of the longitudinal LPR presents very high sensitivity to variations in the refractive index of the surrounding medium.²³ GNRs exhibit very high photothermal efficiency ($\mu \approx 1$) as the amplitude of the scattered radiation is almost negligible, and consequently all the absorbed light is converted into heat. In the past years, we have explored the possibility to use liquid crystalline materials for detecting the thermoplasmonic heating.^{24–26} Also LC-based diffraction gratings containing gold NPs have been investigated in recent years.^{27–29} However, the possibility of realizing thermoplasmonic controlled diffraction gratings is definitely a new application and yet unexplored. To this purpose, we show the first experimental evidence of reverse mode diffraction gratings activated by means of a thermoplasmonic mechanism. Particularly, we report the fabrication and characterization of reverse-mode diffraction gratings realized by combining HLCPDLC structures and GNRs. The diffractive properties of HLCPDLC structures can be activated and deactivated by using a low power “resonant” laser beam that, in turn, triggers the local heating of the GNRs. Moreover, the same HLCPDLC structures are employed as thermosensitive waveplates for quantifying and predicting the average photoinduced temperature variations. Both approaches show the possibility of realizing a new generation of optical devices with reduced power consumption.

MATERIALS AND METHODS

The starting reactive mixture was prepared from an LC acrylate monomer PLC-20-14C that exhibits a nematic LC (NLC) phase at room temperature³⁰ and a clearing temperature of $55\text{ }^\circ\text{C}$. The material was photopolymerized with a UV interference pattern (details below) adding 1 wt % of Darocur-4265 (MAPO/ α -hydroxyketone) photoinitiator from Ciba. PLC-20-14C was mixed with the commercially available NLC 6CHBT (4-(*trans*-4'-*n*-hexylcyclohexyl)isothiocyanatobenzene, $T_c = 42.8\text{ }^\circ\text{C}$) in a 40 to 60 ratio by weight. The mixture made of PLC-20-14C + Darocur-4265 + 6CHBT is shortly reported as LCPDLC (liquid crystal polymer dispersed liquid crystal). Cetyltrimethylammonium bromide (CTAB) capped, water dispersible GNRs have been synthesized and subsequently transferred in chloroform in order to obtain a dispersing medium with LCs. The general protocol for seed-mediated synthesis

of GNRs and their transfer from water to chloroform is reported in refs 24 and 31. The absorption spectrum of the GNRs solution exhibits two plasmon bands,^{23,32} a transverse peak at 517 nm, and a longitudinal peak at 704 nm (Figure 1a, blue curve). Morphological experiments (Figure 1b) performed with a transmission electron microscopy (TEM, by Jeol JEM-1011 microscope, Jeol, Peabody, MA, USA, operating at 100 kV) on chloroform dispersed GNRs indicated that the particle population consists mainly of GNRs with 3.9 ± 0.3 aspect ratio. Several homogeneous mixtures were prepared by mixing increasing amount of GNRs (up to 15 wt %) with the LCPDLC composition, although above 10% the order parameter of the LC phase of the LCPDLC composition drops significantly. All the investigated samples containing GNRs in the range 5–10 wt % show excellent optical properties; however the irradiation intensity required for photothermal heating (see below procedures and experimental details) still stays relatively high (from 468 mW/cm² (9 wt %) to 960 mW/cm² (5 wt %)). For sample at GNRs content below 5 wt % even intensities higher than 1.2 W/cm² did not induce suitable temperature variations. For this study, samples with 10 wt % were utilized. The spectral features are almost retained after mixing the GNRs with the LCPDLC composition, although the peak wavelength of the longitudinal band (Figure 1a, red curve) is red-shifted by 44 nm. The same curve appears also broadened with a fwhm of 50 nm. Such features, along with the change in relative intensity of the LPR signals, could be ascribed to the presence of GNRs aggregates, possibly in chains-like geometry,^{33–35} forming in the host medium, and to the change of the characteristics of the surrounding host medium, passing from chloroform to LCPDLC. The wavelength shift can be explained by taking into account that the optical properties of ellipsoidal particles are predicted using the Gans theory³⁶ framework. There is a correlation between the LPR frequency and the refractive index of the medium surrounding the GNRs. The LPR frequency exhibits a red or blue shift for an increasing or decreasing value of the refractive index surrounding the GNRs. For our case, the modification of the refractive index from 1.4 (chloroform) to 1.6 (average refractive index of the LCPDLC composition) induces a noticeable red shift of the longitudinal plasmon band (see Figure 1c). Due to the low sensitivity to refractive index variations, the transverse band is red-shifted by only 11 nm. It is worth pointing out that isotropic gold NPs (e.g., spherical NPs) are not very suited for our experiments for two main reasons: (i) they have a photothermal efficiency lower (≈ 0.5 – 0.6) than that of anisotropic gold nanoparticles (e.g., GNRs); (ii) their effect on the LC phase takes place already at very low concentrations (e.g., above 2–3 wt %). Glass substrates were treated with a polyimide layer and rubbed for planar orientation. A cell with a 20 μ m gap was filled with the LCPDLC/GNRs mixture by capillary action in the dark above the clearing temperature (65 °C), ensuring complete phase transition to the isotropic state. The recording process was performed with a standard UV (He–Cd laser, $\lambda = 325$ nm, $I = 17$ mW/cm²) holographic setup, and the angle (θ) between the two interfering (curing) beams was set to have a periodicity (Λ) of 2.6 μ m. A schematic of the fabrication process is shown in Figure 1d.

LIGHT-INDUCED FORMATION HLCPDLC GRATINGS

Figure 2a and Figure 2b show the sample under ambient lighting conditions at 25 °C (room temperature) and 39 °C (above the nematic to isotropic transition), respectively. The HLCPDLC sample is crystal clear at room temperature (Figure 2a) because of the refractive index matching between the polymer-rich and LC-rich regions both in nematic phase. Conversely, the sample exhibits the typical grating coloration (like a rainbow) when it is heated above the nematic to isotropic transition ($T_c = 39$ °C) of the NLC (Figure 2b) because of the refractive index mismatch of the developed gratings which has polymer-rich and LC-rich regions which are in nematic and isotropic phase, respectively. Note: We have experimentally observed that the presence of GNRs lowers the

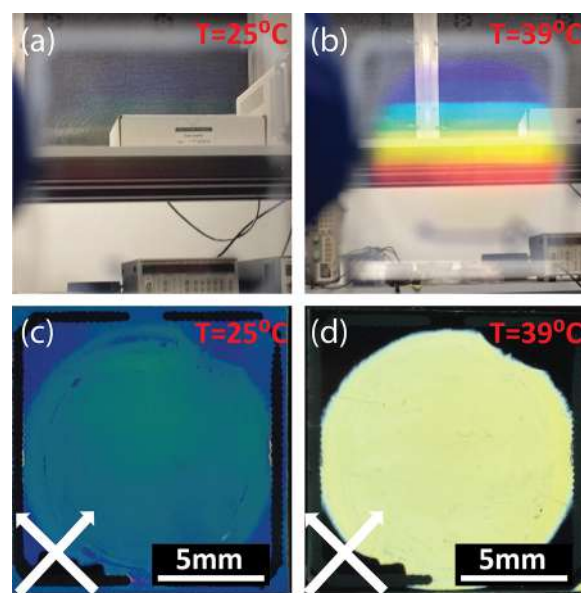


Figure 2. Reverse-mode HLCPDLC grating at 25 °C (a, c) and 40 °C (b, d) under ambient lighting conditions (a, b) and between crossed polarizers (c, d).

transition temperature of the NLC (6CHBT) from 42.8 to 39 °C. Such a phenomenon can be accounted for by an effect very similar to the guest effect observed in dye doped NLC,³⁷ where a dichroic dye added to a NLC film influences the mesophase range by decreasing its clearing point. The photo of the sample corresponding to the high temperature (Figure 2b) was taken immediately after removal from the hot-stage. The HLCPDLC grating area between crossed polarizers at 25 °C is identified by the greenish round area surrounded by the bluish planar aligned (unpolymerized) LCPDLC film (Figure 2c). At high temperature (39 °C, Figure 2d), the HLCPDLC region appears as the round yellow area while the unpolymerized appears black between crossed polarizers. Such a behavior is clear evidence of the average refractive index change of the HLCPDLC area, with a consequent color change (from greenish to yellow), because of the phase transition of the LC rich regions, while the polymer rich regions are not affected by the temperature change because after the polymerization takes place the refractive index of the PLC is temperature insensitive. Conversely, the surrounding HLCPDLC area undergoes a complete phase transition, with a consequent color change from bluish to black, because the nonpolymerized PLC possesses a temperature sensitive refractive index, similar to a conventional NLC. To further investigate the development of the grating structure, the sample was examined with a polarized optical microscope (POM) while the temperature was increased. In the as-formed state (Figure 3a), the HLCPDLC sample appears like a planar aligned NLC cell due to the refractive index matching between the well aligned LC domains and polymer rich regions. As the temperature is increased (Figure 3b–d), the phase separated LC molecules undergo a nematic to isotropic phase transition within the phase separated domains, thereby changing the local refractive index. This local change generates a refractive index mismatch between the LC polymer-rich and LC-rich regions which develops a grating of the periodicity predicted by the holographic writing geometry as shown in Figure 3d (39 °C). To understand the influence of GNRs on the morphology

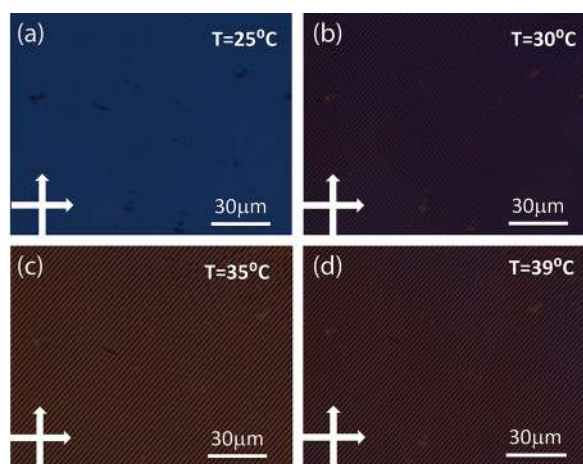


Figure 3. POM view (a–d) of the HLCPDLC sample at increasing temperatures.

of the HLCPDLC gratings, the films were analyzed by means of a low voltage (1 keV) high-resolution scanning electron microscopy (HRSEM). The samples were prepared as for HRSEM analysis by immersing the films in methanol over a 1 h period in order to wash out the LC component. Subsequently, the dried films were fractured in liquid nitrogen to yield an interface representative of the bulk morphology. Figure 4a and Figure 4b are HRSEM micrographs acquired at different level of magnifications. They show the topography of the fractured surface having a well-defined periodic structural variation with a periodicity of 2.6 μm . No evidence of GNRs is noticeable at this level of magnification. A quantitative analysis (see Figure 4c) by means of energy dispersive spectrometry

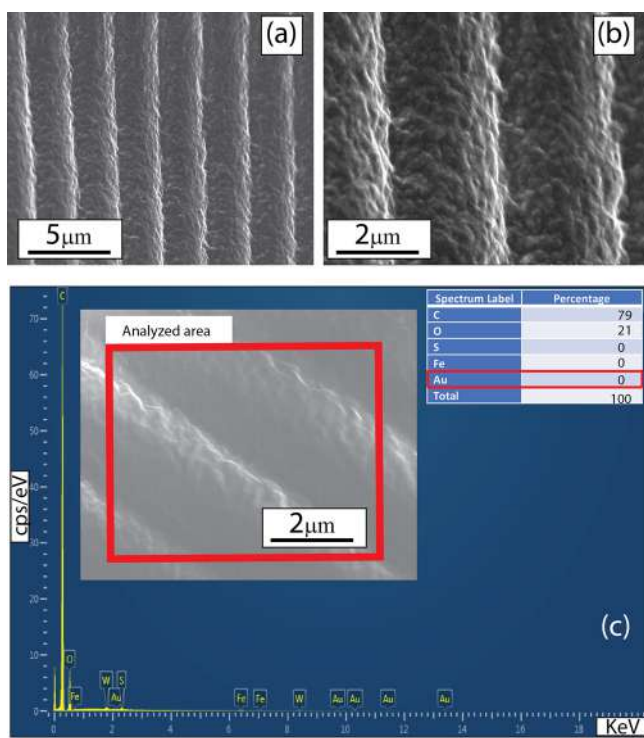


Figure 4. HRSEM analysis of the HLCPDLC film for different magnifications (a, b) along with energy dispersive spectrometry characterization (c).

(EDS) performed on the area evidenced with a red square in the HRSEM micrograph reported in the inset of Figure 4c does not detect the presence of gold (details of the chemical analysis of the investigated area are reported in the table of Figure 4c). Such evidence can be explained by assuming that the heavy GNRs are uniformly distributed across the full periodicity in the bulk of the film, thus nondetectable during the surface topographic scanning performed by the electron-beam. We have performed a polarized spectral analysis of the sample without observing any significant change in the magnitude of the LPR peaks (spectral responses are very similar to the one reported in Figure 1a, red curve). As such, we are confident that GNRs are randomly aligned without possessing any preferential direction. To assess, though indirectly, the presence of GNRs, we have studied the diffractive properties of the HLCPDLC structures by using the all-optical setup sketched in Figure 5a. The setup uses a low power density ($P_{\text{probe}} = 40 \text{ mW}/\text{cm}^2$) CW probe laser, emitting at $\lambda = 532 \text{ nm}$, and a CW pump laser emitting at $\lambda = 808 \text{ nm}$ in the high absorption spectral range of GNRs (Figure 1, red curve). The probe beam is linearly s-polarized and impinges on the sample at the Bragg angle ($\approx 6^\circ$) (both polarization direction and incident angle have been set in order to maximize the diffraction efficiency of the HLCPDLC grating).¹³ The photothermal response of the sample was studied by monitoring the first order (-1th) diffracted intensity temporal profile by means of a photodetector while increasing the pump power density. Figure 5b shows the evolution of the diffraction efficiency (defined as the ratio of the first order transmitted intensity over the total transmitted intensity) for different values of the pump light intensity (from $87 \text{ mW}/\text{cm}^2$ to $500 \text{ mW}/\text{cm}^2$) while the probe power density is kept constant. The irradiation induces electrically driven joule heating with a consequent energy exchange with the surrounding medium. As a consequence, the refractive index contrast of the HLCPDLC structures gradually increased (due to the nematic to isotropic transition of the NLC) with a resulting increasing of the diffraction efficiency (Figure 5b) up to 70%. As soon as the pump beam is turned off, the temperature of the HLCPDLC grating cools down to room temperature and the initial condition (close to zero diffraction efficiency state) is restored (see the Supporting Information Movie 1). For pump power densities between $87 \text{ mW}/\text{cm}^2$ and $370 \text{ mW}/\text{cm}^2$, the dynamics of the diffraction efficiency (Figure 5b) show a fast activation time (tens of ms) before reaching a steady state. Conversely, for pump power density between $370 \text{ mW}/\text{cm}^2$ and $500 \text{ mW}/\text{cm}^2$, a fast activation process (tens of milliseconds) is followed by a slow process (7–10 s). We believe that the second part of the process accounted for the complexity of the system, which approaches a photoinduced phase transition (from nematic to isotropic). Indeed, both response times ($t_{\text{on}} = 10 \text{ s}$ and $t_{\text{off}} = 0.3 \text{ s}$) are relatively slow compared to other activation mechanisms such as electrical or optical field (typical response times are tens of milliseconds). This difference can be explained by considering that the photothermal activation of HLCPDLC gratings is driven by heat transfer from hot GNRs to the surrounding medium. Such a heat transfer typically occurs in a time scale much slower than the characteristics of electrical/optical driven mechanisms. A possible way to improve the thermoresponsivity of the system is to increase the concentration of GNRs dispersed in the HLCPDLC mixture. To this end, an enhanced compatibility between GNRs, in terms of surface chemistry

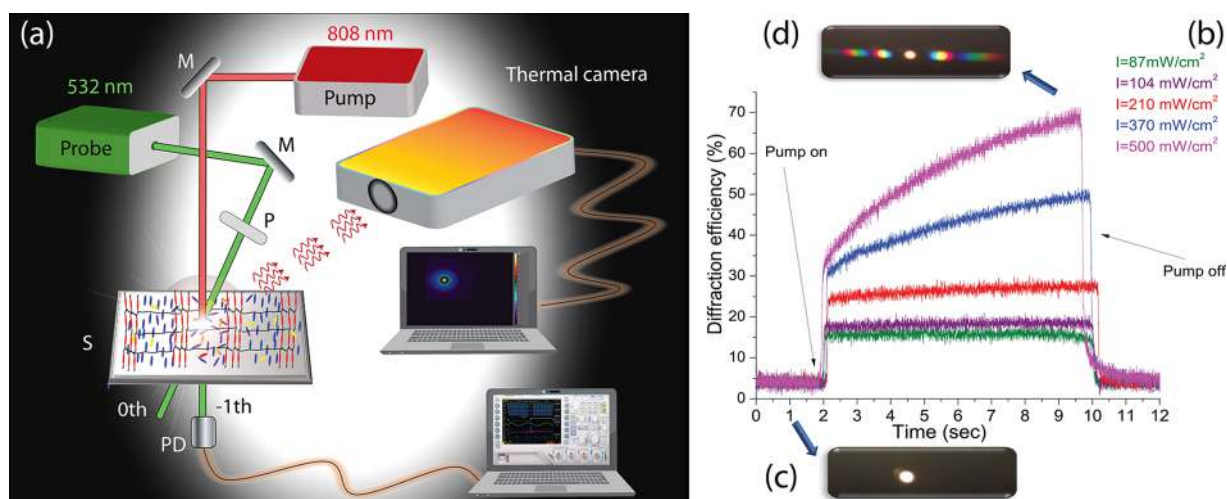


Figure 5. All-optical setup (a) for sample characterization: M, mirror; P, polarizer; S, sample; PD, photodetector. Diffraction efficiency vs time for different values of the pump power density (b). Far field diffraction pattern with the pump beam off (c) and on (d).

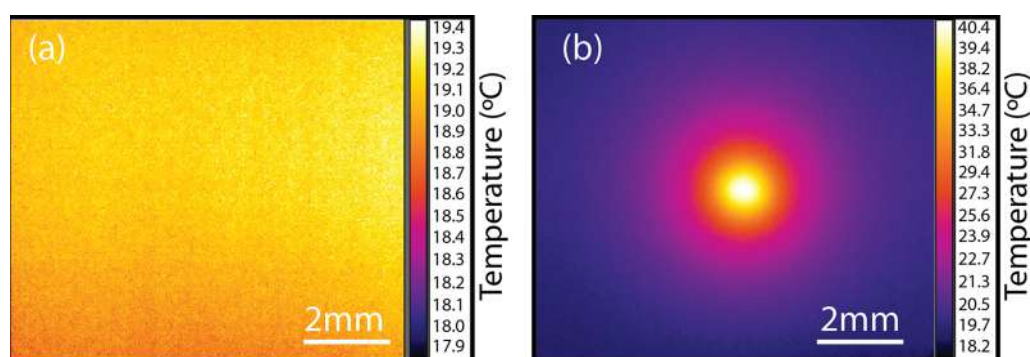


Figure 6. Thermographic analysis of the HLCPDLC samples upon laser illumination ($I = 500 \text{ mW/cm}^2$) without (a) and with (b) GNRs.

and geometry, and LC medium would be required. Photos of the far field diffraction pattern acquired for impinging s-polarized white light with pump beam off and on are reported in Figure 5c and Figure 5d, respectively. Control experiments performed on HLCPDLC gratings realized in the same experimental conditions without GNRs did not show any variation of the diffraction efficiency up to pump power densities approaching 1 W/cm^2 . The result was validated by performing a thermographic analysis (see Figure 5a for details on the thermo-optical setup) of both samples with a high-resolution thermal camera (FLIR A655sc) that produces thermal images of 640×480 pixels with an accuracy of $\pm 2 \text{ }^\circ\text{C}$, as is evident from the thermographic images shown in Figure 6a and Figure 6b. It turns out that without the presence of GNRs (Figure 6a) upon continuous illumination (even at $I = 1 \text{ W/cm}^2$) the thermal camera does not detect any temperature change as confirmed by the uniform color distribution. Conversely, in the presence of GNRs (Figure 6b) the illuminated area ($I = 500 \text{ mW/cm}^2$) turns out to be with a different color from the surrounding background, a distinctive sign of temperature increase. This result clearly indicates that GNRs are highly efficient light to heat converters and the heat generation in HLCPDLC structures containing GNRs is thermoplasmonic driven.

■ PLASMONIC TRIGGERED HLCPDLC WAVEPLATES

In light of all-optical applications, HLCPDLC structures containing GNRs were also examined as sensitive thermal

waveplates for quantifying photoinduced temperature variations using a slightly modified (Figure 7) all-optical setup previously shown (Figure 5a). The all-optical response of the HLCPDLC structures was studied between parallel polarizers with the sample optical axis set at 45° to the polarizer/analyzer axes. Under these conditions, the sample acts as a retardation plate and the transmitted intensity (0th) can be detected by an

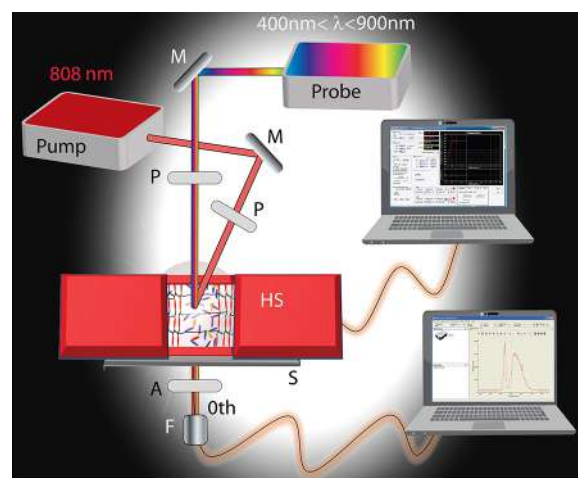


Figure 7. All-optical setup for sample characterization: M, mirror; P, polarizer; A, analyzer; S, sample; HS, hot-stage; F, fiber.

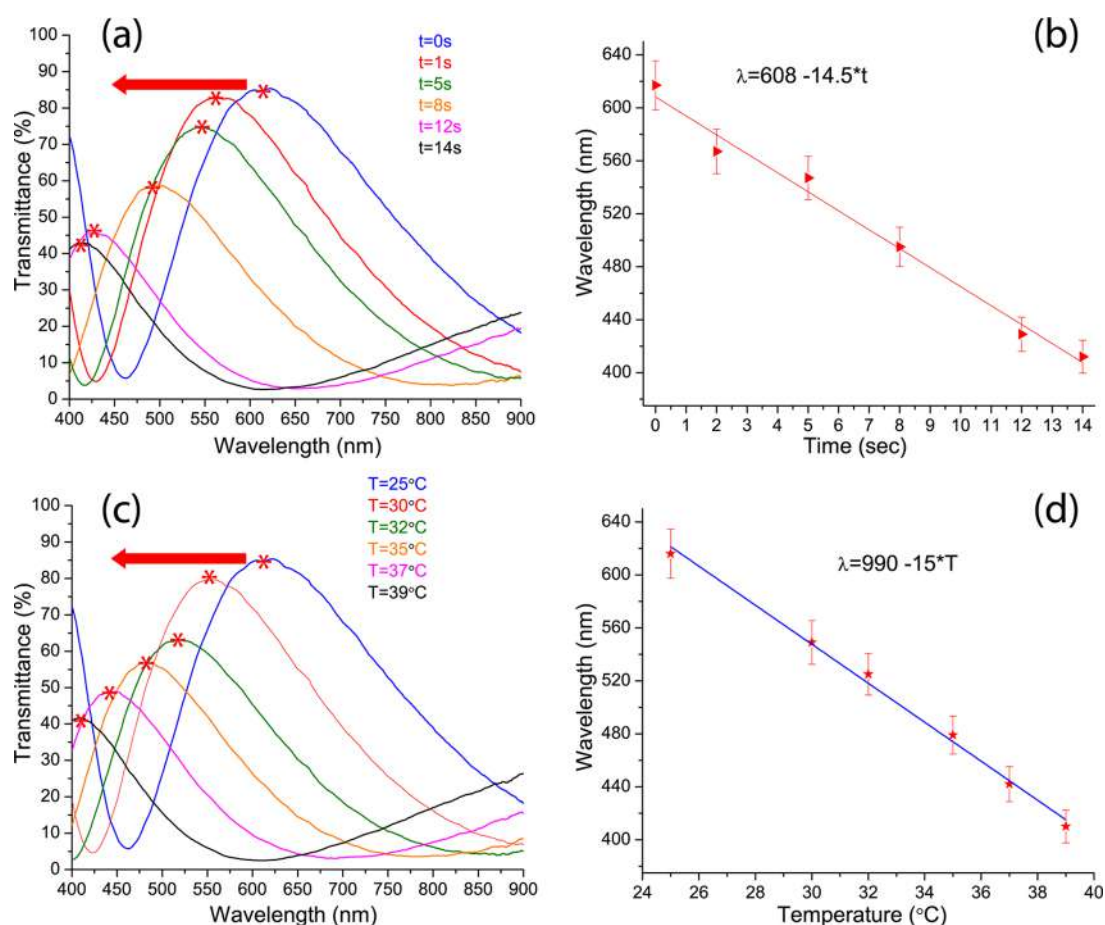


Figure 8. Spectral response of the sample for different values of the illumination time (a) and temperature (c); linear fit of the position of the maximum of the transmitted intensity between parallel polarizers versus illumination time (b) and temperature (d).

optical fiber connected to a spectrometer. Changes in the transmission spectrum for a white light source (400–900 nm) at normal incidence were registered while exiting the same probe area (CW pump laser ($\lambda = 808$ nm)). Figure 8a shows the behavior of the HLCPLC transmission spectrum for different values of illumination time. The HLCPLC film acts as a waveplate and its transmitted intensity, between parallel polarizers, exhibits sinusoidal-like behavior (Figure 8a, blue curve). By optical pumping ($I = 500$ mW/cm²) of the same area at increasing illumination time, a gradual enhancement of the local temperature takes place due to the photoexcitation of GNRs. As a consequence, there is a gradual variation of the optical anisotropy (Δn) of the sample with a consequent linear blue shift (Figure 8b) and attenuation of the transmission maximum (see red stars in Figure 8a). The blue shift can be explained by considering that the maxima in the transmission spectra (red stars, Figure 8a) fulfill the condition

$$\Delta n d = m \lambda \quad (1)$$

where d is the cell thickness, m is an integer ($m = 1$, in the actual case), and λ is the peak wavelength. The reduction of the effective birefringence (Δn) under illumination (Figure 8a), because of the nematic to isotropic transition of the NLC component, requires that eq 1 is continuously fulfilled if λ is gradually reduced (blue shift). Equation 1 can also be used to evaluate the change of Δn under illumination, which turns out to be 0.010. This residual birefringence is due to well aligned PLC that does not exhibit refractive index variation in the

investigated temperature range (20–40 $^\circ\text{C}$). The attenuation of the peak of the transmitted intensity (red stars, Figure 8a) can be easily explained by considering that increasing temperature via plasmonic heating induces the activation of the grating, as reported above, with a consequent reduction of the zero-order transmitted intensity. To validate the effect of the GNRs induced photothermal heating of the HLCPLC waveplate, a control experiment was performed by increasing the sample temperature from 25 to 39 $^\circ\text{C}$ while monitoring the transmission spectrum (Figure 8c). A linear blue shift of the transmission maximum is observed (Figure 8d), which clearly confirms that the behavior reported in Figure 8a is due to a photothermal mechanism. Noteworthy, the two calibration curves reported in Figure 8b and Figure 8d exhibit, within the experimental error, the same linear behavior. Therefore, by using the two linear functions reported in Figure 8b and Figure 8d, it is possible to obtain the following equation:

$$T = 25 + 0.96t \quad (2)$$

Equation 2 can be applied for predicting the average photoinduced temperature (T) of the sample at a given illumination time (t), for a specific value of the pump power intensity ($I = 500$ mW/cm² in the actual case), for specific grating parameters ($\Lambda = 2.6$ μm , $d = 20$ μm), and for a specific GNRs concentration (10 wt %). The thermal sensitivity of the proposed methodology is 0.04 $^\circ\text{C}$.

CONCLUSION

The possibility of controlling light by means of light instead of electric signals has been a challenge for years now. However, all-optical processes require photosensitive materials with high photosensitivity, chemical stability, and cost-effective fabrication methodologies. Because of the development of nanomaterials and in particular gold NPs, the intrinsic capability to convert, with really high efficiency, suitable (resonant) external radiation into heat is allowed. By use of suitably engineered NPs, thermoplasmonics is expected to boost the application of all-optical driven circuits in modern photonics. To this purpose, we have realized a novel generation of thermoplasmonic activated photonic devices. To validate this statement, we have reported a comprehensive study of the photothermal properties of a new generation of hidden gratings realized in a mixture of polymerizable liquid crystal and nematic liquid crystal containing gold nanorods (GNRs). HLCPLC structures, realized through a UV holographic process, were characterized in terms of their morphological, optical, and thermo-optical properties. A thermoplasmonic based mechanism can be identified as responsible for the activation of highly efficient gratings (70%) with a relatively low pump power density (500 mW/cm²). In order to show a great versatility in terms of photonic applications, the same HLCPLC films were investigated as thermosensitive waveplates, enabling a new approach for predicting the photo-induced temperature variation with high sensitivity of 0.04 °C. The possibility of controlling the diffractive properties of reverse-mode gratings by using the thermoplasmonic properties of GNRs along with the proposed methodology for predicting the temperature increase under light illumination opens a new horizon in different research fields, including applications in diffractive sensing and plasmonic-based liquid crystal thermometry. Moreover, the thermoplasmonic triggered HLCPLC structures can be used for implementing a new generation of beam steering devices, optical beam splitters, and phase modulators.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acsanm.9b00843.

Far-field diffraction pattern upon the influence of an external pump beam turned on and off (AVI)

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Notes

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Plasmon-mediated discrete diffraction behaviour of an array of responsive waveguides

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We investigate the discrete diffraction phenomenon in a Polymer-Liquid Crystal-Polymer Slices (POLICRYPS) overlaying a random distribution of gold nanoparticles (AuNPs, plasmonic elements). We study the propagation of a CW green laser beam through the waveguide structure as a function of beam polarization, laser intensity and sample temperature. It turns out that the plasmonic field created at the interface between AuNPs and POLICRYPS waveguides enables and stabilizes the optical field propagation within the responsive nematic liquid crystal channels. The active role of the liquid crystal is pointed out by a polarization, temperature and beam divergence experimental analysis and evidenced by a peculiar trumpet-shaped discrete diffraction pattern. Theoretical simulations confirm that the observed optical behavior is governed by the interaction of the nematic liquid crystal with optical and plasmonic fields.

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1 Introduction

Diffraction of light usually leads to the spatial broadening of its initial intensity profile and can be controlled by modifying materials properties and/or configuration of the medium where the beam propagates. In particular, to control the spatial divergence of a given beam, it is necessary to act on the refractive index of the material. In free space, diffraction in the paraxial limit is always negative. This means that unusual features like diffraction cancellation, or change of its sign to a positive value, are only possible when considering the propagation of light in a linearly coupled array of one dimensional waveguides.¹ In such a discrete medium, diffraction assumes a discrete behaviour, following the same periodicity of the waveguide system and can show a change of sign or can even become zero valued.

Non-linear optical phenomena in waveguide arrays have been experimentally investigated^{1–4} and theoretically interpreted^{1,3–10} by exploiting models that refer to the “Fermi-Pasta-Ulam” approach to one-dimensional dynamical

systems;¹¹ in this framework, the non-linearity is introduced as a perturbation to a primarily linear problem. Numerical models have been implemented by exploiting the Coupled-Mode Theory (CMT),^{5,6} taking into account only nearest-neighbor interactions. By considering the n^{th} waveguide coupled only to the $(n + 1)^{\text{th}}$ and $(n - 1)^{\text{th}}$ adjacent channels, it has been shown that the optical field propagating in the n^{th} waveguide obeys a nonlinear difference-differential equation. The phenomenon is described *via* a ‘coupling constant’, which is proportional to an overlap integral of two adjacent modes;^{2,6} in the continuum (or long-wavelength) approximation, this discrete process is described by the non-linear Schrödinger equation.^{6,12,13} This approach has played an important role in understanding the observed effects, providing fundamental relations between those parameters (like array period and beam incidence angle) that control the discrete diffraction phenomenon.^{1,7,12} In particular, it is possible to predict whether the propagating light beam experiences normal discrete diffraction (the beam spreads while propagating), anomalous discrete diffraction (a diffraction whose sign is opposite to that one usually exhibited in nature and the beam spreads while propagating, but some anomaly is observed) or if diffraction disappears (solitons or discrete solitons formation).^{1–3,14–18} In this paper, we present and discuss the first experimental characterization and theoretical modeling of the electromagnetic wave propagation in a complex waveguide configuration made of a 1D periodic POLICRYPS structure^{19–22} (an acronym for Polymer-Liquid Crystal-Polymer Slices) overlaying a monolayer of randomly distributed gold nanoparticles (AuNPs), deposited on a glass substrate. The POLICRYPS con-

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tains a nematic liquid crystal (NLC) whose director alignment can be perturbed both by the presence of AuNPs and by the electric field of a light beam propagating in the structure.²³ The sample assumes a distinctive behaviour, since the optical response of the structure is modified during the light wave propagation: The presence of a NLC inside the POLICRYPS structure and AuNPs located underneath it makes the entire system extremely responsive to optical^{24,25} and plasmonic^{26–28} fields as well as to temperature variations induced in the sample.

Since the optical behavior of coupled waveguides depends on their geometrical parameters (periodicity, waveguide width and height^{5,12,19,29,30}) and on the refractive index of the medium³⁰ between them, it is of particular interest to use a liquid crystal as such a medium. Indeed, NLC is an active soft-material whose refractive index value can be electro-optically controlled at will, by use of moderate amplitude electric fields (few $\text{V } \mu\text{m}^{-1}$);^{23,24,31,32} in particular, NLC is responsive also to the electric field associated to an electromagnetic wave propagating in the waveguide. This can give rise to the self-confinement of light in solitary waves (spatial solitons) that are coupled to the waveguide itself.³³ Achieving discrete diffraction in an array of waveguides containing NLC is particularly intriguing for the possibility to actively control the phenomenon and hence the optical behavior of an eventual device.³³ Here, we propose a novel system in which the presence of an interface, between the plasmonic elements layer and the POLICRYPS array containing the NLC waveguides, drastically influences the discrete diffraction behavior³⁴ of the system, resulting in unusual optical phenomena. In fact, due to the interplay of optical and plasmonic induced reorientation of the NLC waveguides, the involved system appears different from those ones reported in literature.^{29,30,35,36} In order to model this novel configuration, we have implemented a numerical model which reproduces the beam propagation in a satisfactory quantitative way.

2 Experimental analysis

The system under investigation is a microcomposite structure (POLICRYPS^{19,20,64}) composed of slices of almost pure polymer alternated to films of well aligned NLC. The periodic structure sits on a carpet of randomly-distributed and mono-dispersed Au spherical nanoparticles (20 nm in diameter) (see Fig. 1(a)) creating an active interface able to support plasmon-mediated effects. The sample is fabricated by assembling a glass cell made of a substrate containing the monolayer of Au nanoparticles and another glass slide put at controlled distance from the first one (10 μm) by means of glass microspheres. The conventional mixture used for POLICRYPS fabrication^{37–39} (26 wt% of NLC (BL001, by Merck) + 74 wt% of UV sensitive pre-polymer (NOA61, by Norland)) is then introduced in the cell by capillary flow. The spatially periodic POLICRYPS structure is obtained by irradiating the prepared cell (infiltrated with the pre-polymer mixture) with a curing interference

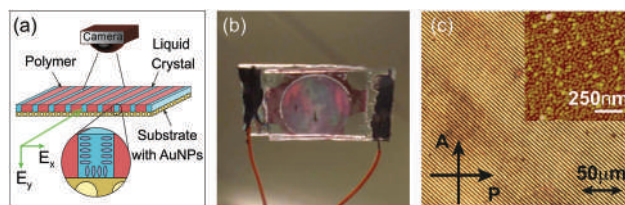


Fig. 1 Sketch, picture, POM view and morphology of investigated sample: (a) sketch of the sample, the impinging light geometry and the acquiring set up, (b) sample picture and (c) its Polarized Optical Microscope (POM) view with Atomic Force Microscope (AFM) morphology of the NP substrate underlying the POLICRYPS structure (inset).

pattern of UV/visible light under suitable experimental and geometrical conditions. The angle between the two interfering curing beams is set to obtain a POLICRYPS with a waveguide periodicity of about 5 μm . The effect of the periodically modulated refractive index profile of the structure^{19,20} can be switched on and off by applying an external electric field of the order of few $\text{V } \mu\text{m}^{-1}$.

Fig. 1(b) is a photograph of the fabricated sample, under ambient lighting conditions, highlighting the typical coloration (see circular spot in the middle) of the produced periodic structure. Its morphology is inspected by a Polarized Optical Microscope (POM). Fig. 1(c) shows the POM view of the sample where a quite regular and uniform structure made of polymeric walls alternated to NLC films can be observed. The inset of Fig. 1(c) shows the atomic force microscopy (AFM) image of the underlying AuNPs layer (AuNPs are monodispersed with an average diameter of about 20 nm). The realized system is investigated by sending a collimated laser beam ($\lambda = 532 \text{ nm}$) at the entrance of one or more of the POLICRYPS channels in a waveguide configuration. As sketched in Fig. 1(a), during propagation in the structure, light scattered by the guides is acquired by a Leica Z16 APO macroscope (10 $\times$ magnification and proper zoom factor) placed on top of the POLICRYPS structure. In order to unveil the complex interweaving of physical processes taking place in our system during light propagation, the active role played by the induced NLC reorientation is highlighted by three different analyses: 1. Polarization dependence; 2. Temperature dependence; 3. Beam divergence analysis.

Polarization dependence

The discrete diffraction behavior of the system has been studied by irradiating few waveguides of the array with green light (wavelength $\lambda = 532 \text{ nm}$). In this analysis, discrete diffraction patterns are acquired while shining linearly polarized light in the waveguide system with two different polarization directions: p- or transverse-magnetic (TM) and s- or transverse-electric (TE) polarization, where transverse is referred to the propagation plane (x - z plane). These two polarization directions are indicated in Fig. 1(a) as E_x and E_y , respectively. The analysis of the discrete diffraction behavior is performed by calculating the propagation length L in the central waveguide.

The propagation length is defined as the distance between two consecutive maxima of light intensity in the central waveguide along the z -direction. It measures the length that light travels in a given waveguide before “jumping” to another one. Propagation of p -polarized light is shown in Fig. 2(a), where an anomalous discrete diffraction is observed, meaning that light is concentrated in two distinct outermost lobes.³ In addition, a peculiar optical feature is observed: L changes during propagation, an effect evidently due to the active role of the NLC during light propagation. The black curve in Fig. 3 represents the propagation length value in the central waveguide of Fig. 2(a) as a function of the number of maxima. In Fig. 3, we see that this value decreases during the propagation, starting from the sample entrance (position 0) to the next jumps (from 1 to 8). Measures presented in Fig. 3 are obtained by converting images reported in Fig. 2 in digital data by using a suitable C++ code; this converts images in a matrix whose entries are values ranging from 0 to 1 where 0 correspond to dark and 1 to light and grays are in between. We then make a cut in the central waveguide (middle of the sample in x -direction) along the z propagation direction thus obtaining a 1D plot of the intensity modulation. Looking at Fig. 2(a) and 3 we observe that L values decrease during light propagation. An explanation of this effect will be given later on in the theoretical paragraph. Different is the physical picture when s -polarized

light is irradiating the guides. In this case, the electric field associated with the impinging wave tends to align the NLC molecules in the direction perpendicular to the sample substrate hence decreasing the refractive index mismatch between NLC and polymer. For this reason, even if the discrete diffraction starts in a way that is similar to the p -polarization case, the discrete pattern rapidly disappears, leaving an unclear light distribution that is probably due to interference effects more than discrete diffraction ones.

Temperature dependence

Once verified that an interesting discrete diffraction behaviour is observed in presence of p -polarized impinging light, the same propagation experiment is studied while performing a temperature characterization of the sample. This is done by heating the sample up to the nematic-isotropic transition temperature of the NLC (~ 60 °C). A temperature increase lowers the NLC extraordinary refractive index value bringing it close to the ordinary one that is, in our case, very close to the polymer one. We acquire the light propagation images at room temperature (Fig. 4(a)) and at 60 °C (Fig. 4(b), nematic-isotropic transition temperature) and a visible change of the discrete diffraction pattern (trumpet-like shape) is observed. As resumed in the red curve of Fig. 3, this is mainly due to a general decrease of the propagation length when increasing temperature. This result is coherent with the observation made in the polarization analysis, where a small refractive index mismatch between NLC and polymer results in shorter propagation lengths. It is also worth noting that, despite at 60 °C the NLC is expected to be in the isotropic state, the discrete diffraction pattern sensibly changes but does not disappear. We suppose that the p -polarized light propagating in the guides excites a strong plasmonic dipolar field in the AuNPs substrate.⁴⁰ This field prevents the NLC thermal fluctuations, thus favoring the director reorientation induced by the optical field.^{24,25,41} The collective excitation of strongly coupled plasmonic particles during energy propagation in waveguides is well known and has been investigated in details in ref. 40 where the efficiency of energy transport along the waveguide, due to surface plasmonic coupling, is studied for different dimensions and shapes of samples. Moreover, it has been

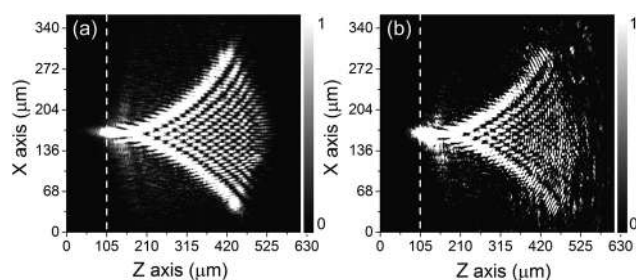


Fig. 2 Top view of light discrete behavior inside the POLICRYPS structure in waveguide configuration: polarization dependence: (a) p -polarized impinging light or transverse-magnetic polarization (TM) (b) s -polarized impinging light or transverse electric polarization (TE).

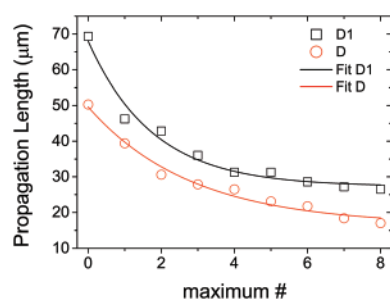


Fig. 3 Black squares (D1) and red circles (D) correspond to the experimental distances between maxima during the propagation of light in p -polarized configuration at room temperature Fig. 2(a) and 4(a) and at the nematic-isotropic transition temperature Fig. 4(b) respectively. Black (Fit D1) and red (Fit D) lines are the corresponding exponential fits.

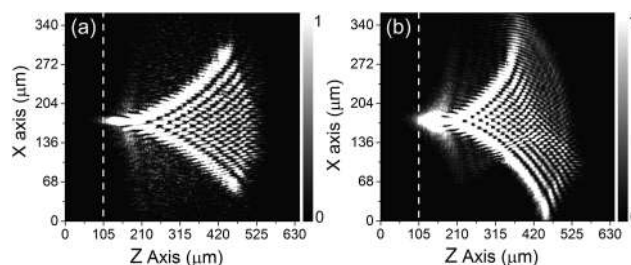


Fig. 4 Top view of light behavior inside the POLICRYPS structure in waveguide configuration in p -polarized configuration: (a) “trumpet” at room temperature in p -polarized light (b) bigger “trumpet” at 60 °C (transition nematic-isotropic temperature).

demonstrated that the near-field coupling between particles distributed in arrays produces a guiding effect of electromagnetic waves at optical frequencies in presence of metal-dielectric interfaces.⁴² These strong distance-dependent interactions lead to a splitting of the plasmon dipolar peak for regular one-dimensional arrays of metal nanoparticles.⁴² Furthermore, in ref. 43, Maier *et al.* experimentally show the coupling of the evanescent wave in a tapered optical fiber to a metal nanoparticle plasmon waveguide. The high efficiency of power transfer into these waveguides solves the coupling problem between conventional optics and plasmonic devices. By repeating the same characterization at higher beam intensities, we observe the same behavior but with longer propagation lengths (images not reported).

Beam divergence analysis

A further interesting information is provided by a beam divergence analysis. Fig. 5(a) shows that light propagating in our samples exhibits anomalous discrete diffraction also in a non-paraxial configuration (when many waveguides are illuminated). In this case, in order to get a detailed insight of the light propagation behavior, the discrete diffraction pattern is acquired for different temperature values ranging from 23 °C to 60 °C (Fig. 5a–f). In Fig. 5, it is possible to observe that, along the propagation direction, the region where the anomalous discrete diffraction takes place reduces with increasing temperature, turning to a normal, non-trumpet shaped, discrete diffraction (shown in ref. 32 and theoretically predicted in the following paragraph (Fig. 7(a) and (b))). It is interesting to note that, as already observed in Fig. 4(b), notwithstanding the increase in the sample temperature, the role of the LC does not vanish: indeed, albeit small, a region of anomalous discrete diffraction is always present. This indicates that, although the temperature increase reduces the average value of the LC refractive index, light induces some reorientational plasmonic effects in the NLC director that preserves the anomalous discrete diffraction behaviour.

The last experimental analysis is performed in a paraxial configuration, in which exclusively one waveguide is excited by

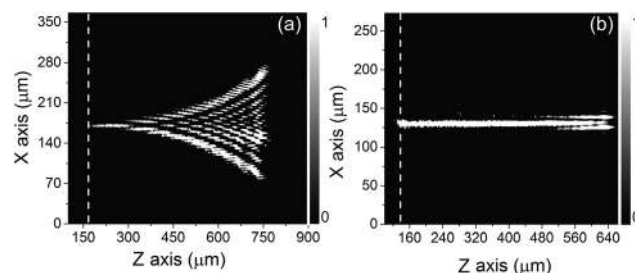


Fig. 6 Top view of light behavior inside the POLICRYPS structure in waveguide configuration at room and nematic–isotropic transition temperature (23 °C, 60 °C) with a convergent beam. (a) At $T_0 = 23$ °C temperature (room temperature). (b) At $T_5 = 60$ °C (nematic–isotropic transition).

the laser beam (Fig. 6). At room temperature (Fig. 6(a)), the “trumpet” shaped pattern is very similar to that reported in Fig. 2(a). Quite different is, on the contrary, the pattern observed at the nematic–isotropic transition temperature, where anomalous discrete diffraction is almost completely suppressed and a soliton-like beam appears (Fig. 6(b)).^{44,45} This confirms that a suitable temperature increase can cancel the periodicity of the refractive index, while light creates its own waveguide through a self-modulation of the refractive index, giving rise to a “nematicon-like” behavior.^{46,47} In our opinion, this behavior is a further demonstration that the plasmonic field stabilizes the waveguiding effect of the NLC (by reducing thermal fluctuations) only in those areas where the impinging light excites a plasmonic response.

3 Theoretical model

In our theoretical analysis, the POLICRYPS structure is modeled as a system with a periodic dielectric permittivity profile (Fig. 8). The light wavevector $\mathbf{k}$ is assumed parallel to the single waveguide of the array (see Fig. 1(a)) while the (p-polarized) electric field is oriented along the x axis in the plane of the structure; this enables modeling the system with a 2D approach. In this geometry, within the LC films of the POLICRYPS structure, light “sees” the extraordinary refractive index of the NLC, whose director alignment is induced by the polymeric boundaries,⁴⁸ *i.e.* perpendicularly to the polymer slices. Thus, in a first theoretical approach we assume a periodic alternation of polymer refractive index and extraordinary index of the NLC. Light modes confined during the electromagnetic propagation are said “guided” waves (or guided modes) while structures supporting guided waves are called waveguides. The numerical implementation of the model takes into account the coupling between several parallel waveguides made of aligned liquid crystal films separated by polymer slices. Exchange of power between guided modes propagating in adjacent waveguides is referred to as directional coupling and is well explained by the coupled mode theory.^{5,49,50} The Floquet-Bloch (FB) analysis predicts that the

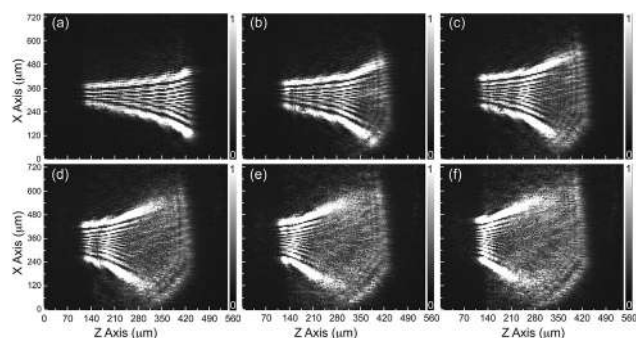


Fig. 5 Top view of light behavior inside the POLICRYPS structure in waveguide configuration at different temperature $T_0 < T_1 < T_2 < T_3 < T_4 < T_5$ with a divergent beam from (a) at $T_0 = 23$ °C temperature (room temperature) to (f) at $T_5 = 60$ °C (nematic–isotropic transition).

propagation-constant spectrum of the array's eigenmodes (the FB waves) is divided into bands, separated by gaps in which no propagating modes are allowed.^{50–52} Light propagation in waveguide configuration is controlled by diffraction that, in free space, is governed by the equation $D_{np} = \frac{\partial^2 k_z}{\partial k_x^2} = \frac{-k^2}{(k^2 - k_x^2)^{3/2}}$ where D_{np} measures the broadening of the beam, (it is the difference in transverse shift between spatial frequency components) and $\mathbf{k}$ is the incident beam wavevector, with k_x , k_z being respectively its x - and z -components. In the paraxial limit ($k_x \approx 0$ and $k_z \approx k$), the previous equation gives $D_p = -1/k$,¹ meaning that D_p does not depend on k_x and is always negative. In case of the wave propagating in a linearly coupled, infinite array of one-dimensional waveguides, the diffraction relation can be derived using the framework of optical equivalence with the continuous model of tightly bound electrons in a one-dimensional atomic lattice.⁵³ The optically coupled mode set of equations for the electric field in the n^{th} waveguide allows Yariv^{5,12,49} to obtain the diffraction relation:

$$D_p = -2Cd^2 \cos k_x d \quad (1)$$

where d is the periodicity of the structure and C is known as coupling parameter; the paraxiality is implicitly assumed in the derivation of expression (1) and is valid as long as $C \ll k$. It is evident from eqn (1) that, when $\frac{\pi}{2} < |k_x d| \leq \pi$, the diffraction coefficient becomes positive, thus enabling light to experience “anomalous” discrete diffraction; this term meaning that most of light is gathered into two distinct outermost lobes and propagates by “jumping” from one waveguide to the external adjacent one. In these conditions, light almost completely disappears in the region with low refractive index (see Fig. 7(a)).³² On the other hand, by illuminating several waveguides, a non-paraxial condition holds, (Fig. 7(b)), which results in a negative (or normal) diffraction, characterized by a light intensity envelope that follows the laser beam Gaussian profile, with non-zero intensity in the regions with low refractive index.

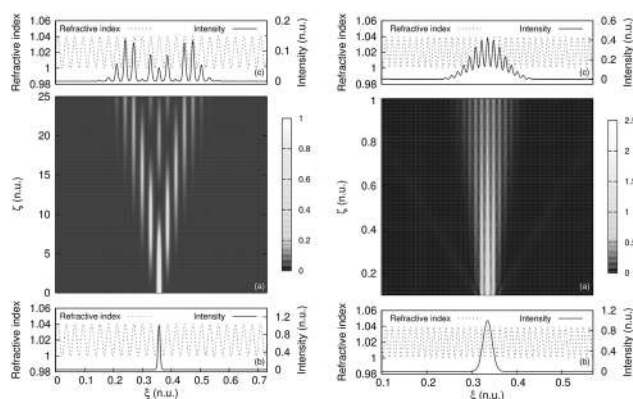


Fig. 7 Anomalous and normal discrete diffraction in a coupled waveguides array.

Furthermore, in the two cases $k_x = \pm \frac{\pi}{2d}$ in eqn (1), diffraction completely disappears, thus enabling soliton formation.^{3,32,45,54}

By fixing the modulation Δn of the structure refractive index that gives rise to the system of adjacent waveguides, and using the coupled mode theory (CMT),⁵ it is possible to show that, in a single waveguide, the power follows a sine-squared behavior (in the z -direction) and the distance between two maxima, in adjacent waveguides, depends on the wavelength λ of the impinging light, on the laser spot size w and on Δn . According to this theory, the light behavior is completely determined when we know the value of the coupling parameter C , whose expression, derived by Yariv,^{5,12,49,50,55} can be extended to subwavelength arrays.⁵⁶ Its expression is

$$C = \frac{2h^2 p e^{(-ps)}}{\beta \left(w_1 + \frac{2}{p} \right) (h^2 + p^2)} \quad (2)$$

where $h = \sqrt{n_1^2 k^2 - \beta^2}$, $p = \sqrt{\beta^2 - n_2^2 k^2}$, n_2 and n_1 are the refractive index of the waveguide and the adjacent medium respectively, $k = \omega/c$, β is the propagation constant, w_1 is the waveguide width, s is the distance between two adjacent waveguides. Using the coupled mode theory⁴⁹ it is possible to obtain the propagation length L simply as:

$$L = \frac{\pi}{2C} \quad (3)$$

Above mentioned models (CMT and FB) are very useful to carry out a discrete diffraction analysis in a structure whose refractive index exhibits a step profile, but they fail to account for key features of a POLICRYPS structure, where the refractive index has a smooth profile, determined by elastic forces and by the molecule anchoring conditions. In Fig. 8, we report the typical dielectric permittivity profile of a usual POLICRYPS structure. In this case, in each waveguide, we have to use a Gaussian transverse profile of the refractive index and a more suitable model is implemented to take account for our experimental conditions.

Description of the electromagnetic field propagation, derived through the Maxwell's spatial wave equation, is inte-

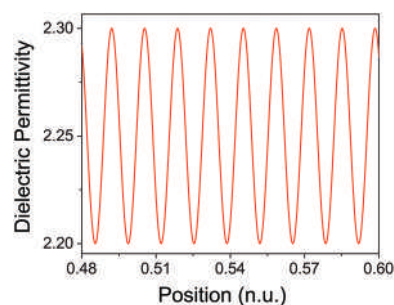


Fig. 8 Dielectric permittivity profile used to carry out the numerical simulation. The guides are made with a dielectric permittivity with Gaussian profile in order to simulate the effective refractive index inside the liquid crystal channels.

grated with the additional terms: $\nabla \left(\mathbf{E} \cdot \frac{\nabla \epsilon}{\epsilon} \right)$, because $\nabla \epsilon / \epsilon$ is not negligible. Thus, the wave equation becomes

$$\nabla^2 \mathbf{E}(\mathbf{r}) + \omega^2 \mu \epsilon(\mathbf{r}) \mathbf{E}(\mathbf{r}) = -\nabla \left(\mathbf{E} \cdot \frac{\nabla \epsilon}{\epsilon} \right) \quad (4)$$

where $\mathbf{E}(\mathbf{r})$ is the electric field at position $\mathbf{r}$, ω is the angular frequency of light, μ is the magnetic permeability of the sample, that can be assumed as equal to that one of the vacuum, $\epsilon(\mathbf{r})$ is the dielectric permittivity of the sample. If z is the wave propagation direction, by using a slab (planar) model, in which no variation exists in the y -direction, and assuming a “slow” envelope variation in z -direction, we can numerically solve eqn (4) by taking into account the ϵ profile shown in Fig. 8 to write the refractive index modulation in the x direction. By considering solutions of the kind $\mathbf{E} = \mathcal{E} e^{i\beta z}$ we obtain, for the amplitude of the electric field:

$$\frac{\partial \mathcal{E}}{\partial \zeta} = \frac{i}{2\beta} \left[\frac{\partial^2 \mathcal{E}}{\partial \xi^2} + \frac{1}{\epsilon} \frac{\partial \epsilon}{\partial \xi} \frac{\partial \mathcal{E}}{\partial \xi} + \frac{\partial}{\partial \xi} \left(\frac{1}{\epsilon} \frac{\partial \epsilon}{\partial \xi} \right) \mathcal{E} + \left(\tilde{k}^2 - \tilde{\beta}^2 \right) \mathcal{E} \right] \quad (5)$$

where we introduced $\xi = x/W$ and $\zeta = z/W$ as the normalized spatial coordinates, W being the normalization length (*i.e.* the typical dimension of phenomenon), $\tilde{\beta} = W\beta$ is the normalized propagation constant and $\tilde{k} = 2\pi nW/\lambda$ is the normalized wavevector. In this equation we neglected the coupling between TE/TM modes because the electric field propagating in the waveguide is linearly polarized and parallel to the transverse coordinate (see ref. 57, page 29)

We find the numerical solution of eqn (5) by imposing the Gaussian profile of refractive index for each waveguide and using the finite differences method for the spatial derivatives. The system shows anomalous discrete diffraction with a propagation length value that is close to the experimental one.

Moreover, we find that terms $\frac{1}{\epsilon} \frac{\partial \epsilon}{\partial \xi} \frac{\partial \mathcal{E}}{\partial \xi}$ and $\frac{\partial}{\partial \xi} \left(\frac{1}{\epsilon} \frac{\partial \epsilon}{\partial \xi} \right) \mathcal{E}$ are, in fact, not negligible, thus confirming the validity of our hypothesis.

To obtain a satisfactory agreement between measured and calculated propagation lengths, we assume an average dielectric permittivity of about $\epsilon_m = 2.25$, with variations $\Delta \epsilon_1 = 0.01$ or $\Delta \epsilon_2 = 0.1$. Results are reported in Fig. 9, where an anomalous discrete diffraction behavior is evident with a longer

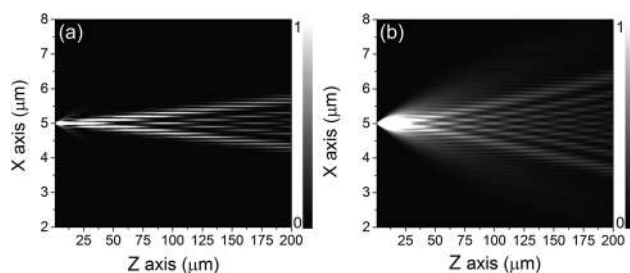


Fig. 9 Numerical result of light intensity propagation in a Gaussian dielectric permittivity profile (like shown in Fig. 8) with average dielectric permittivity $\epsilon_m = 2.25$ and variations (a) $\Delta \epsilon_2 = 0.1$ and (b) $\Delta \epsilon_1 = 0.01$.

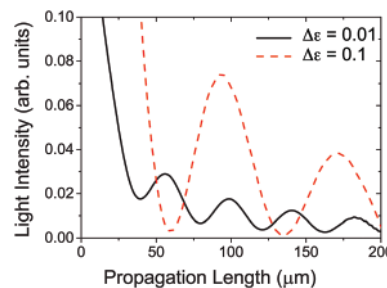


Fig. 10 Cuts in the central waveguide in the Fig. 9(a) (red dashed line) and Fig. 9(b) (black line).

propagation length corresponding to a larger birefringence value. We highlight the difference in the propagation lengths by showing (Fig. 10) cuts of light intensity in the central waveguides of Fig. 9(a) and (b). Differences in the propagation lengths are represented by different distances between maxima. It is well known that a temperature increase leads to a decrease of the extraordinary refractive index,^{58–63} as such, Fig. 10 confirms the experimental result shown in Fig. 3, where lower propagation lengths correspond to higher temperatures hence, lower refractive index mismatch between LCs and polymer. As experimentally observed in Fig. 2, 4 and 6, the decrease of index mismatch, and thus of the propagation length, has a direct effect in the discrete diffraction pattern resulting in a “trumpet” shape. This peculiar pattern cannot be predicted by the actual code because it would require a recursive approach taking into account how local modifications of LCs orientation can influence the light intensity in that specific point. As indicated in Fig. 10, fixed values of the refractive index mismatch between LCs and polymer have been assumed in the simulations.

4 Discussion

In the study of the discrete diffraction taking place when light propagates in a POLICRYPS system of adjacent waveguides, the characterization of the polarization dependence upon wave propagation shows that, in the TM configuration of the impinging light, a discrete propagation with positive (anomalous) diffraction takes place while, in the TE configuration in the initial part of the waveguide, the discrete diffraction pattern shows the same features as in the TM case, but in propagation it becomes mixed up, probably due to some interference effect (Fig. 2). Our interpretation is that this is the result of the interplay between at least two phenomena. Indeed, the electric field direction of s-polarized light (TE mode) tends to align the NLC director in the direction perpendicular to the sample plane thus decreasing the index mismatch between NLC and polymer indices “seen” by the propagating light. Moreover, this field direction allows the propagating beams to interfere with each other thus modifying the discrete behavior during propagation. This hypothesis is reliable

if it is assumed that, in the discrete diffraction, the polarization of light does not vary when the beam jumps from a waveguide to the adjacent one.

In a recent study the appearance of an anomalous discrete diffraction behavior, similar to the one observed here, has been interpreted as due to the presence of polaritons³⁵ (e.g. waves propagating along the surface of separation between metal and dielectric layers²⁶). In our case, we can exclude such interpretation, since these waves can not propagate in the proposed configuration. Indeed, the gold layer underlying the POLICRYPS waveguides is not continuous but made of a carpet of randomly-distributed and mono-dispersed spherical nanoparticles. Thus, it can unlikely support polaritons propagation. Finally, temperature analysis results show that, at higher temperatures, observed propagation lengths are shorter (Fig. 3). In fact, at room temperature, light feels an effective LC refractive index quite close to the extraordinary refractive index of the BL001 NLC.⁶² Then, an increase in temperature lowers the value of this index towards the isotropic value, with a consequent decrease of the birefringence and thus of the propagation lengths. This result is well confirmed by the implemented theoretical model (Fig. 10). However, an accurate observation of experimental results reveals that, at high temperature, when the NLC is expected to be in the isotropic state, the discrete diffraction pattern noticeably decreases but does not completely disappear. Behind this phenomenon, we have supposed a plasmonic contribution that prevents NLC thermal fluctuations thus stabilizing the NLC alignment even in conditions where this should be absent. From the beam divergence analysis (Fig. 5) it can be deduced that light intensity plays a fundamental role: where the beam is more intense (small divergence), discrete propagation is observed. In this case, light intensity affects the LC orientation thus modifying the birefringence of the structure. Theoretical analysis shows that the larger the birefringence of the structure, the longer the propagation length. This is in good agreement with the measured propagation lengths and their dependence on light intensity.

5 Conclusions

The propagation of light in an array of waveguides (Polymer-Liquid Crystal-Polymer Slices, POLICRYPS) overlaying a random distribution of plasmonic nanoparticles has been considered. Experiments evidence an anomalous discrete diffraction behavior in which light jumps from a waveguide to the adjacent one, thus moving into the two outermost lobes. Results support the hypothesis that the anomalous character of discrete diffraction is mediated by plasmonic effects occurring at the interface between the AuNPs layer and the responsive waveguides. Indeed, light propagating in the guides (TM mode) excites a strong plasmonic dipolar field in the nanoparticles that prevents the liquid crystals thermal fluctuations hence preserving the periodical dielectric constant profile of the system during the propagation. The experimental analysis

has been performed by changing the polarization and beam divergence of the impinging light, and the temperature of the sample. It turns out that the light propagation is strongly influenced by both polarization direction, intensity of the optical field of the propagating waves, and temperature variations. In the latter case, the stabilizing role of the plasmonic field on the liquid crystals orientation is particularly evident because the anomalous discrete diffraction pattern partly remains even in case the liquid crystals are expected in isotropic phase (e.g. at temperature above the nematic-isotropic transition point). Starting from the experimental evidence, we implemented a theoretical model able to interpret the active role of the liquid crystals, contained in the POLICRYPS, in their interaction with light contained in the POLICRYPS. This knowledge allows us to control light propagation characteristics in the waveguide (propagation length, kind of diffraction, ...) by acting on polarization, temperature, light intensity and divergence of the impinging light beam. The exchange of power between adjacent waveguides can be exploited in a number of thin-film photonic devices where it is required to control power division, modulation, switching and multi-plexing of optical signals.

Conflicts of interest

There are no conflicts to declare.

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TOPICAL REVIEW

Stimuli responsive diffraction gratings in soft-composite materials

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Topical Review

Stimuli responsive diffraction gratings in soft-composite materials

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Abstract

Diffraction gratings (DGs) are unique optical components with the capability to control and address a travelling light wave because of their micro/nanoscale periodicity. Nowadays, DGs are used in several sophisticated and high-tech applications such as spectrometers, memories, as well as in bioengineering and telecommunications. Advanced micro and nano fabrication processes enable the realization of DGs with excellent morphological and optical properties. However, realizing DGs with on-demand optical parameters is still a challenge because of the absence of reliable and cost-effective smart materials. Herein, we discuss the unique and compelling opportunities obtained by bridging materials with intrinsic flexibility (soft polymers) and re-configurability (liquid crystals (LCs)), as well as emerging fabrication procedures. Further, we present several stimuli responsive DGs used in various research fields such as sensing, biotechnology and solar energy. Lastly, in the light of flexible and reconfigurable DGs applications, we report different examples of light responsive and electro-activated, LCs based, DGs.

Keywords: diffraction gratings, liquid crystals, photolithography, optics

(Some figures may appear in colour only in the online journal)

1. Introduction

Diffraction gratings (DGs) are optical structures able to split the incident radiation in a limited number of directions called diffraction orders [1, 2]. DGs are micrometer/submicrometer range periodic structures exhibiting different profiles (i.e. binary, multilevel, blazed, etc). Micro/nano fabrication processes such as photolithography and interference holography have represented compelling technologies for improving the quality and the performances of DGs. Although nowadays several manufactures provide cost-effective and high quality DGs, most of them have an intrinsic drawback—they lack

of re-configurability. In other words, once the component is fabricated the optical properties (e.g. diffraction efficiency) are fixed. In light of an environmental sustainability effort, there is an urgent need to improve the quality of the materials, to reduce the power consumption (green components) and to minimize the number of unutilized optical components. The possibility to conceive a new generation of DGs with on-demand controllable parameters is still of great interest for applications ranging from photonics to medicine. To this end, flexible polymers (e.g. polydimethylsiloxane (PDMS), hydrogels, etc) [3, 4] have been largely explored for realizing stimuli responsive DGs [5]. On the other hand, liquid crystals

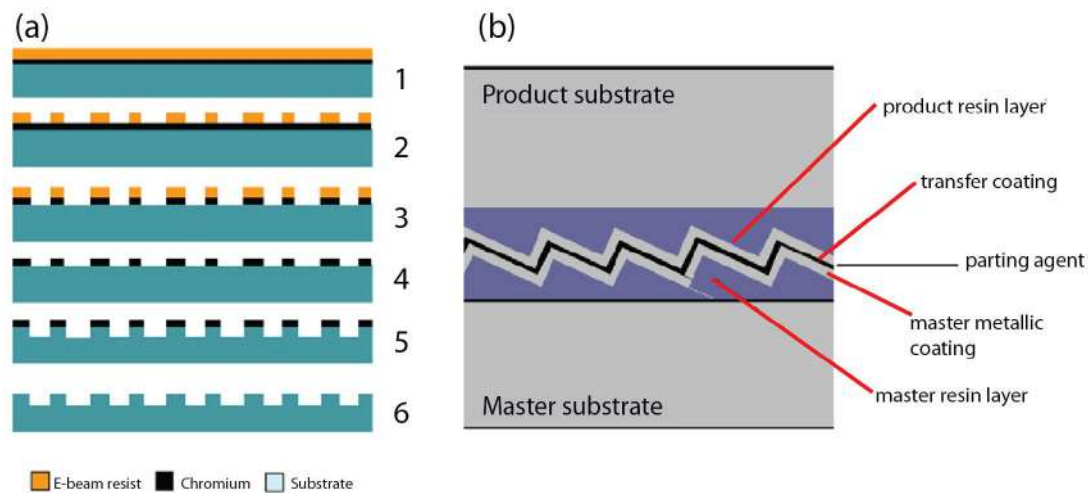


Figure 1. Fabrication steps of DGs made by e-beam lithography (a) and replica molding process (b). [2] © Springer-Verlag Berlin Heidelberg 2012.

(LCs) are smart sensitive materials responsive to several perturbations. Indeed, external perturbations (electrical and optical field, temperature, etc) can cause a significant change in the arrangement of the LC molecules with a consequent variation of the average refractive index (0.1–0.2) [6]. Thanks to this unique possibility, in the last 50 years several LCs based DGs have been realized [7–9]. In the present paper, we report an overview of several one-dimensional (1D) DGs based architectures with controllable geometrical and optical parameters. Particularly, we focus our attention on flexible and LCs based DGs by demonstrating that their utilization allows the realization of agile DGs with on-demand optical properties. Noteworthy, the focus of this overview is devoted to the application of 1D structures, although the fabrication and utilization of more complex and highly sophisticated 2D DGs [10, 11] and/or metasurfaces [12–14] for the same purposes is also of great interest.

This paper is organized as follows: we introduce the fabrication methods and the main optical properties of DGs. Therefore, two classes of stimuli responsive structures are described named *flexible* and *reconfigurable* DGs. In the framework of *flexible* DGs, we focus our attention on PDMS based structures while concerning the second class of DGs (*reconfigurable*), we overview several flexible, LCs based, diffractive elements.

2. DGs

2.1. Fabrication methods

In the past 20 years, several methodologies have been implemented for the realization of high quality DGs [15]. Traditionally a mechanical imprinting technique has been developed for the realization of groove structures on metallic surfaces, the so called ruled gratings [16]. Although they exhibit very high diffraction efficiency, it is very hard to fabricate ruled gratings with very small line spacing. Moreover, they cannot be integrated in high quality spectrometers because of irregular surfaces which cause a lot of stray light.

Another technique used for manufacturing DGs uses an interference pattern for recording high quality structures in photo-sensitive materials. Interference gratings or holographic DGs [17] are made by recording the interference fringes generated at the intersection of two coherent beams of light. In the process of making interference gratings, a beam of light from a laser is divided into two beams which are then recombined by a suitable arrangement of mirrors to produce interference fringes. These fringes are then recorded in a layer of photo-resist which has been coated onto the surface of a suitable blank. Holographic DGs have a periodic modulation of the refractive index without the presence of physical grooves, thus they cause very minimal stray light as their surface is very regular. Typically, the photosensitive material is protected with two transparent substrates (e.g. glass) that make them more scratch and thermal resistant.

Other types of sophisticated DGs can be realized by using the electron beam lithography (EBL) [18]. The standard process flow for the fabrication of this kind of DGs is sketched in figure 1(a). The substrate, usually made of fused silica or glass, is covered with an 80 nm thick chromium layer, on top of which an e-beam resist layer is coated (see figure 1(a) [1]). The microstructure geometry is transferred to the resist trough EBL technique and successive development of the exposed areas (see figure 1(a) [2]). Next, the structured resist layer is used as a mask to transfer the pattern into the chromium layer by means of an RIE process (see figure 1(a) [3]). Consequently, after removing the resist, the patterned chromium layer serves as hard mask for the final deep etching into the substrate (see figure 1(a) [4, 5]). Lastly, the chromium layer is removed from the top of the grating (see figure 1(a) [6]). EBL allows the fabrication of nanometer-sized DGs on working areas up to few mm². However, the technique is quite expensive and does not allow the fabrication of large area DGs, although ongoing efforts are devoted to reduce the average costs in terms of writing time [19], thus enabling the possibility to maximize the working area. Despite these recent advances in minimizing exposure times, the capital requirements for acquiring and maintaining an electron beam lithography system are

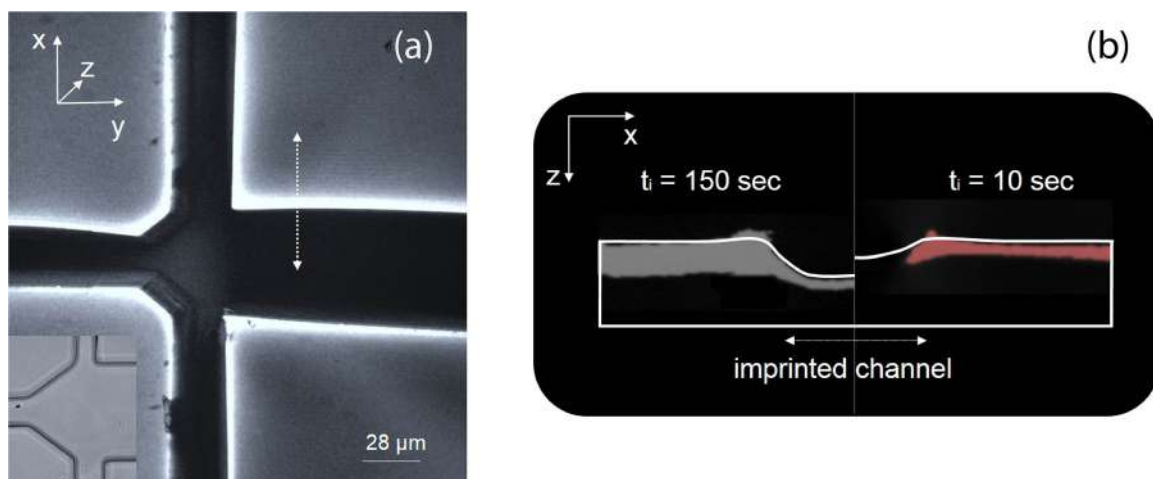


Figure 2. 3D fluorescent image of an imprinted and functionalized microfluidic channel in PMMA; inset shows the bright-field image of the same structure. (b) Comparing the functionalization profile at two immersion durations: 150 s (left—grey) and 10 s (right—red). The solid white lines denote the imprinted and non-imprinted PMMA areas, with the profiles plotted depth-wise in the x - z plane shown in (a). Reprinted from [25], with the permission of AIP Publishing.

substantial. One way for making high quality and cost-effective DGs is based on the replica molding process [20]. This technology is capable of producing thousands of duplicates of master DGs possessing the same quality and performance of the master gratings. The replication process has reduced the cost of a typical DG by a factor of one hundred or more, compared with the cost of acquiring a master grating, as well as greatly increasing their commercial availability. The casting process for the production of a replicated DG is a series of sequential steps. The replication process starts with the selection of a suitable master grating that has the desired specifications (e.g. grooves DGs, blaze DGs, etc). Then a very thin layer of parting (or release) agent is applied to the surface of the master grating in order to facilitate the separation between the delicate master and the replicated DG. After the parting agent is applied, a reflective coating (usually aluminum) is applied to the surface of the master. A substrate is then glued with a layer of resin to the grooved surface of the master grating. A schematic of the ‘sandwich’ formed by the substrate and master glued together is shown in figure 1(b). Recently, a substantially simpler and cost-effective method for fabricating DGs was demonstrated using the solvent immersion imprint lithography (SIIL) [21]. Overall, SIIL can imprint thermoplastic and organic semiconducting polymers in a single, 2' long, processing step with no requirements of dedicated instrumentation. In SIIL, the polymer is immersed in common solvents to generate a soft (gel-like) layer at the polymer-solvent interface. This layer can be readily imprinted [22] using PDMS or Si stamps, as well as bonded to a substrate [23–26]. In the context of DGs, SIIL has been shown to successfully fabricate both 1D and 2D gratings with periods as low as 140 nm [23].

Further, SIIL can be executed not only with pure solvents, but also solutions of chemical moieties dissolved in the solvent itself. In this way, both the solvent and the solute are transported into the polymer, thus softening and functionalizing simultaneously the polymer-solvent interface that can be subsequently imprinted. Importantly, the functionalization profile can be tightly controlled through the immersion

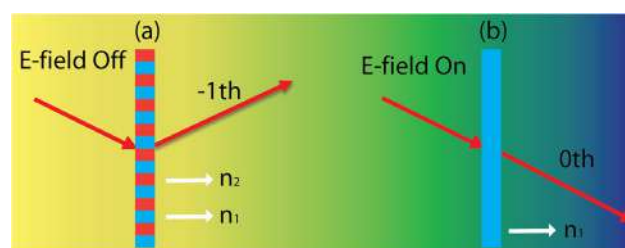


Figure 3. Schematic of the working principle of a phase DG operating in the Bragg regime with the E-field off (a) and on (b). n_1 and n_2 are the refractive indices of two different materials while -1th and 0th are the first and zero diffracted orders respectively.

duration in the solution (see figure 2). This functionalization capability was shown to enable modular and extremely cost-effective O_2 biosensors [27], thus opening up possibilities in reconfigurable DGs by modulating the real or imaginary part of the dielectric constant.

2.2. Optical properties

A main classification of DGs can be made considering whether they operate in the Raman-Nath or in the Bragg regime. Raman-Nath DGs split the incident light beam in several diffracted orders while Bragg DGs divide the impinging light beam in a single diffracted beam (usually the transmitted part is negligible). Gaylord and Moharam [28] have proposed a rigorous difference between the two regimes through the Cook-Klein parameter $Q = 2\pi\lambda d/n\Lambda^2$, where λ is the probe wavelength, d the thickness of the sample, n the average refractive index and Λ the DG periodicity. If $Q \geq 10$, the grating operates in the Bragg regime while if $Q \leq 1$ the grating operates in the Raman-Nath regime. If Q is comprised between 1 and 10, an intermediate regime is noticeable. The correlation between Λ and the angle (θ) at which the light is deflected can be expressed through the grating equation: $m\lambda = \Lambda[\sin(\alpha) \pm \sin(\theta)]$ where m is the order of the principal maxima and α is the angle of the incident

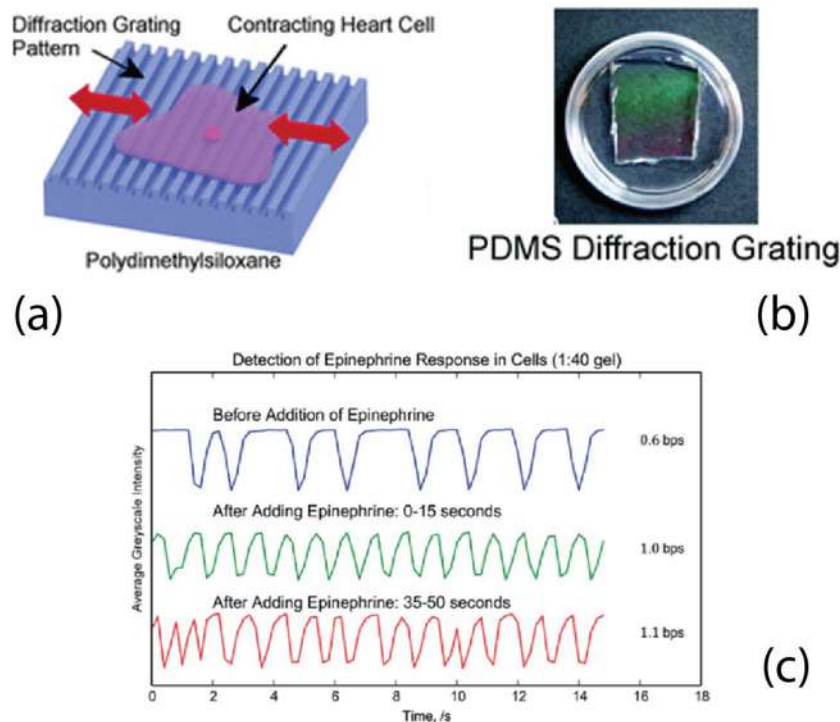


Figure 4. Schematic of a PDMS DG covered with heart cells (a) along with a photo of the realized sample (b). Beating of cardiomyocytes (C) on a PDMS DG before (blue curve) and after (green and red curves) adding epinephrine. Reproduced from [33]. CC BY 3.0.

beam. Another very important parameter useful to quantify the quality of a DG is the ‘diffraction efficiency (η)’, defined as the ratio between the intensity of the selected diffracted beam and the intensity of the incidence beam. The diffraction efficiency $\eta = f(\alpha, \lambda, d, \Delta n)$ is a function of the incidence angle α , wavelength λ , grating depth d , index modulation Δn . Particularly, Δn can be related to the optical phase shift (ϕ) through the equation $\Delta n = \phi / 2\pi L$. By optimizing these parameters (e.g. the phase shift capability), it is possible to obtain grating structures that exhibit high diffraction efficiency.

A phase DG operating in the Bragg regime (only two diffraction orders are present named 0th and -1 th) possess a periodic variation of the index of refraction $\Delta n = n_2 - n_1$. If the DG is partially composed with a reconfigurable material (e.g. n_2 is a LC material), the optical properties can be modulated and a dynamic photonic behavior is induced. If no external E-field is applied the incident radiation is completely diffracted in a single order beam (figure 3(a), -1 th order). Conversely, if the E-field is on, the periodic refractive index modulation is washed out and the incidence light is transmitted (figure 3(b), 0th).

3. Flexible DGs

3.1. Bio-sensing applications

Flexible or stretchable DGs are responsive diffractive structures with the capability to monitor mechanical perturbations such as deformations, oscillations and vibrations. Thanks to the development of optical transparent flexible polymers such as PDMS, in the last few years flexible DGs have been employed for several applications such as sensing, biotechnology and

solar energy [29]. In this framework, Rogers *et al* [30] have realized a photo-thermal detector based on a millimeter thick surface relief PDMS DG. The correlation between the thermal expansion and the diffraction angle has been related to the intensity of the absorbed light. In a different approach, Grzybowski *et al* [31] have utilized a mechanical compression for realizing a diffractive force sensor based on a PDMS grating. To the same end, Hosokawa *et al* [32] have reported on a novel optical methodology for monitoring the local pressure of a microfluidic device by exploiting a deformable DG fabricated via PDMS replica molding. This method is not only desirable for flow characterization of microfluidic devices, but also enables the possibility of producing new types of pressure sensors and pressure-driven optical modulators.

In light of biotechnological applications, Gibbons *et al* [33] have realized a flexible DGs based device fabricated by using a PDMS layer coated with platinum (figure 4(a)). The device (figure 4(b)) is used to monitor the change in cardiac cell beating frequency by measuring the diffraction angle of the PDMS substrate due to the contracting of the cells. To analyze the change in beating rate, epinephrine (also known as adrenalin) was added to the cardiac cell culture with a consequent amplification of the beating rate as shown in (figure 4(c)). Ye and Wang [34] have developed a new approach based on the possibility to use a DGs made of stimuli-responsive hydrogel as transduction medium for glucose detection.

The glucose-sensing DG is made of hydrogel having phenylboronic acid groups, which have glucose binding function (figure 5(a)). Boronic acids are known to bind saccharides with cis-diols structures. As such, the binding induces a shift in the equilibrium by increasing the fraction of borate anions. Consequently, the electrostatic balance in the interior of the

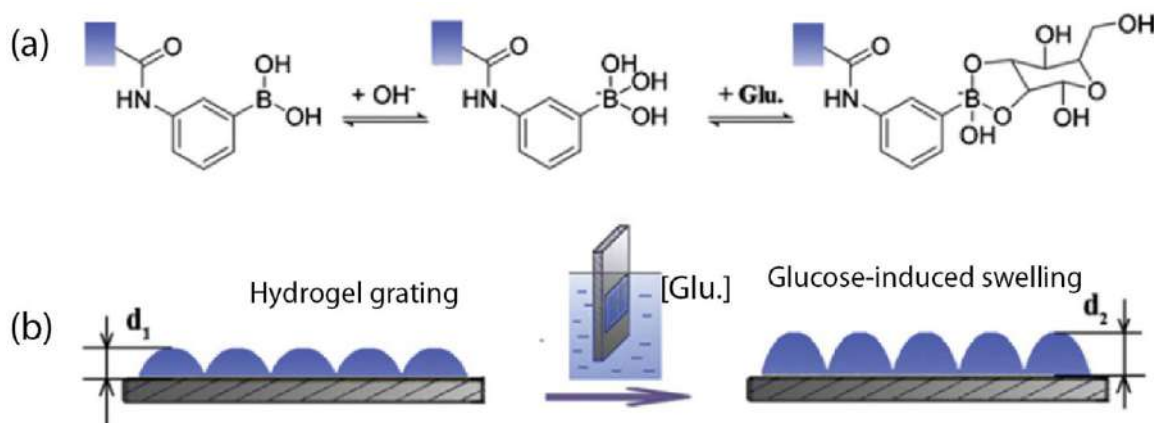


Figure 5. Reaction of phenylboronic acid with glucose (a) along with the response of the hydrogel grating in a glucose solution (b). Reprinted from [34], Copyright 2010, with permission from Elsevier.

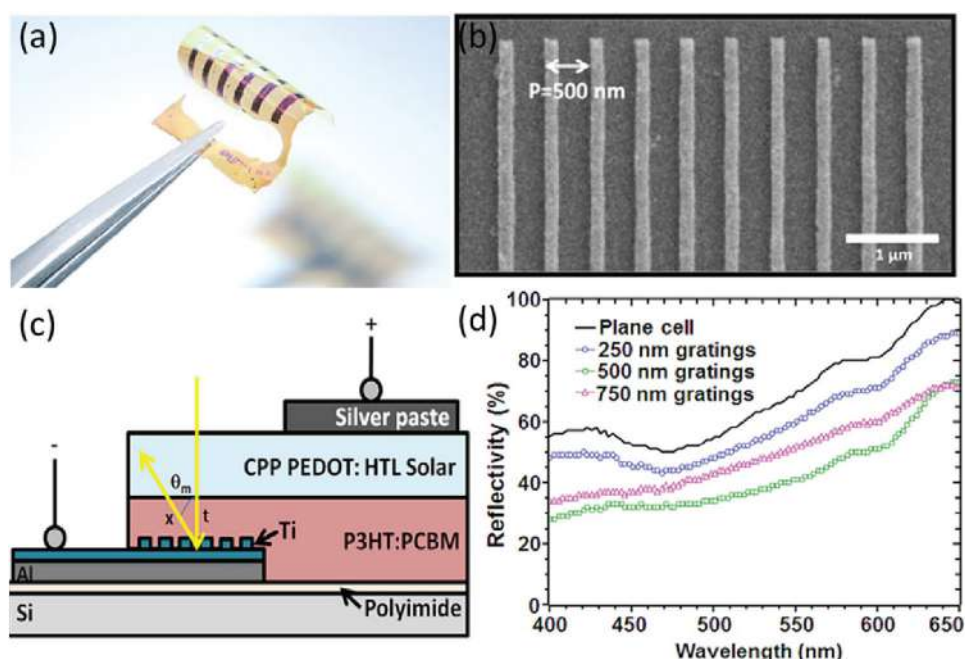


Figure 6. Photo of flexible solar cell after removing it from a silicon substrate. (a) Scanning electron microscope (SEM) image of the realized DG along with a schematic of the capability of a DG to increase the optical absorption length. (c) Reflectivity spectra of the samples (d). Reproduced from [36]. © IOP Publishing Ltd. All rights reserved.

hydrogel DG increases the osmotic pressure inducing a swelling of the hydrogel (figure 5(b)). The depth variation produces an increasing of the diffraction efficiency. The glucose-sensing device can be realized by measuring modulation of the diffraction efficiency of the gratings. The hydrogel gratings show high sensitivity to the glucose in solutions with the detection range from 0.10 to 2.50 mmol l^{-1} . The technique is reversible and the achieved sensitivity is comparable with the ones obtained with other approaches [35]. The hydrogel gratings can be repeatedly used for several times. After each measurement, the gratings can be regenerated simply by washing and soaking in a neutral buffer for hours.

3.2. Solar energy applications

From a different standpoint, flexible DGs have been used for increasing both the light absorption and the charge generation

in the active layer of solar cells. To this purpose, de Oliveira Hansen *et al* [36] have proposed a new method for the fabrication of organic solar cells by utilizing functional light-trapping nanostructures on flexible substrates.

A thin layer of polyimide is spin-coated on a silicon substrate. Subsequently, by means of a photolithography process a high quality DG is realized (see scanning electron microscope (SEM) micrograph in figure 6(b)). After the fabrication steps, the solar cells on polyimide are peeled off from the silicon support substrate, resulting in a flexible device containing a nanostructure useful for light absorption enhancement (figure 6(a)). The incident light is diffracted into the solar cell active layer, increasing its optical path within the layer and thus the absorption of light within the cell (figure 6(c)) with an enhancement of about 23%. The light-trapping effect was experimentally evaluated from reflectivity spectra of a non-structured cell and cells containing gratings with

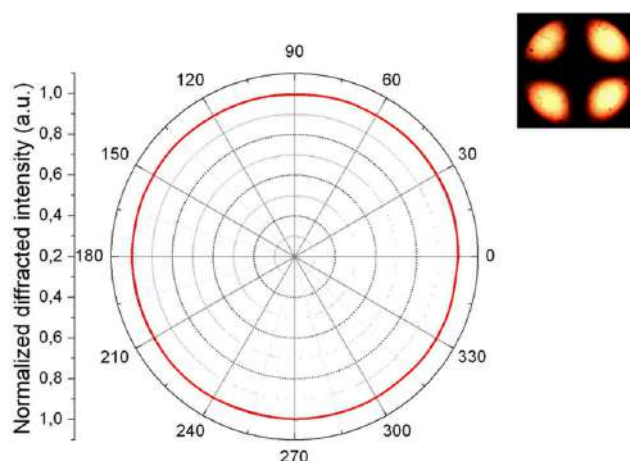


Figure 7. Polar diagram of the first diffracted order as a function of the polarization angle along with the conoscopic characterization of the sample (inset). Reprinted from [49], with the permission of AIP Publishing.

different periodicities. There is a clear correlation between the reflectivity and the pitch of the grating (figure 6(d)), paving the way towards the realization of mechanically actuated (tunable) DGs with varying pitch. Particularly, DGs having 500 nm pitch distance show the lowest reflectivity, enabling the possibility to improve the light trapping. Another interesting example of stretchable DG used for solar cells was proposed by Mahpeykar *et al* [37]. They have demonstrated the first tunable hexagonal diffraction grating by taking advantage of polystyrene (PS) nanospheres as a periodic structure for optical diffusion and PDMS as stretchable substrate for tunability. The innovative aspect of the fabricated device is that the distance between PS nanospheres can be manipulated by means of mechanical strain applied to the elastomer PDMS substrate. It is important to point out that in the light of real applications, flexible DGs can be used several times without changing the optical properties. It is challenging to predict the lifetimes of flexible structures without performing a methodical and systematic study. As such, we cannot offer information on the lifetimes in terms of months/years. All we can say is that the shelf life of a flexible polymer (e.g. hydrogel) is a very important requirement and all the realized components do not perform very well after the expiration date of the raw material is passed. For hydrogels specifically, we do not anticipate that their lifetime will be longer than one day because of its sensitivity to humidity *et al*. In any case, however, these systems are extremely low cost and can be easily cast every time there is a need to be used.

4. Reconfigurable DGs

LCs technologies have played important roles in the realization of reconfigurable optical components for the advantages of light weight, low cost, no moving parts, and low power consumption. Electro-responsive phase grating is one kind of such components that have been studied extensively. As such, there are several papers reporting on switchable DGs such as holographic polymer dispersed liquid crystals

(HPDLC) [38–41] and polymer liquid crystals polymer slices (POLICRYPS) [42–44]. The combination of LCs and flexible DGs has received little attention. Indeed, flexible polymers exhibit an intrinsic hydrophobicity producing a very high contact angle, thus affecting the possibility to organize LCs molecules in a planar or patterned configuration [45]. Moreover, LCs can flow like conventional liquids and for this reason they are subject to the back-flow effect every time the surface is deformed. For the mentioned reasons, LCs alignment on flexible structures is more challenging. As a matter of fact, it is experimentally challenging to organize LC molecules on deformable structures [46–48]. A first demonstration of deformable, LCs based, DGs was reported by De Rosa *et al* [46]. They have demonstrated a reversible tuning of the diffraction efficiency in a HPDLC by mechanical shear deformation. By exploiting a similar approach, Holmstrom *et al* [47] have shown that a compressive mechanical stress can also be used to control the reflection notch of reflective HPDLCs by over 120 nm in the visible spectral range. Although interesting, none of the reported observations deal with flexible platforms or substrates. To address this issue, few years later De Sio *et al* [49] have reported on the realization of a light responsive flexible DG layered with LCs. Indeed, azo-dye LCs have been combined with high resolution PDMS microstructures realized via replica molding. Spontaneously, the LC molecules exhibit a vertical alignment due to the intrinsic capability of PDMS to induce homeotropic alignment of the LC, thus enabling a low diffraction efficiency, polarization insensitive, DG. On the contrary, the surface functionalization of the top cover glass (used to fabricate the sample) produces a hybrid configuration of the LC alignment with a consequent improvement of the diffraction efficiency.

Figure 7 shows the intensity of the first diffracted order (the grating operates in the Raman-Nath regime) as a function of the polarization angle of the impinging probe beam ($\lambda = 633$ nm). The polar diagram remarks a maximum and a minimum of the diffracted intensity due to the variation of the LC birefringence. This observation has been confirmed via conoscopic imaging performed with an optical microscope in Bertrand Lens configuration (inset of figure 7). Pump-probe experiments have been realized by using an all-optical setup made with a CW diode laser (pump) operating at $\lambda = 532$ nm ($P = 0.6$ W cm⁻²) and a He-Ne laser (probe) operating at $\lambda = 633$ nm. When the DG is excited by the pump beam the NLC molecules undergo in a phase transition (trans-cis photoisomerization process) with a consequent variation (decrease) of the diffraction efficiency. As soon as the pump beam is turned off, the disordered NLC molecules relax back (cis-trans photoisomerization process) to the initial configuration, hence restoring the initial diffraction efficiency ($\approx 20\%$).

In a different attempt, De Sio *et al* [48] have improved the performances of the light sensitive flexible DGs by exploiting a double surface functionalization strategy. Indeed, a silicon oxide (SiO_x) evaporation process (thermal evaporation) has been used for modifying the surface chemical properties of PDMS microstructures. SiO_x induced a planar alignment of the LC molecules, thus improving the response time of the DG by approximatively an order of magnitude (figure 8(b))

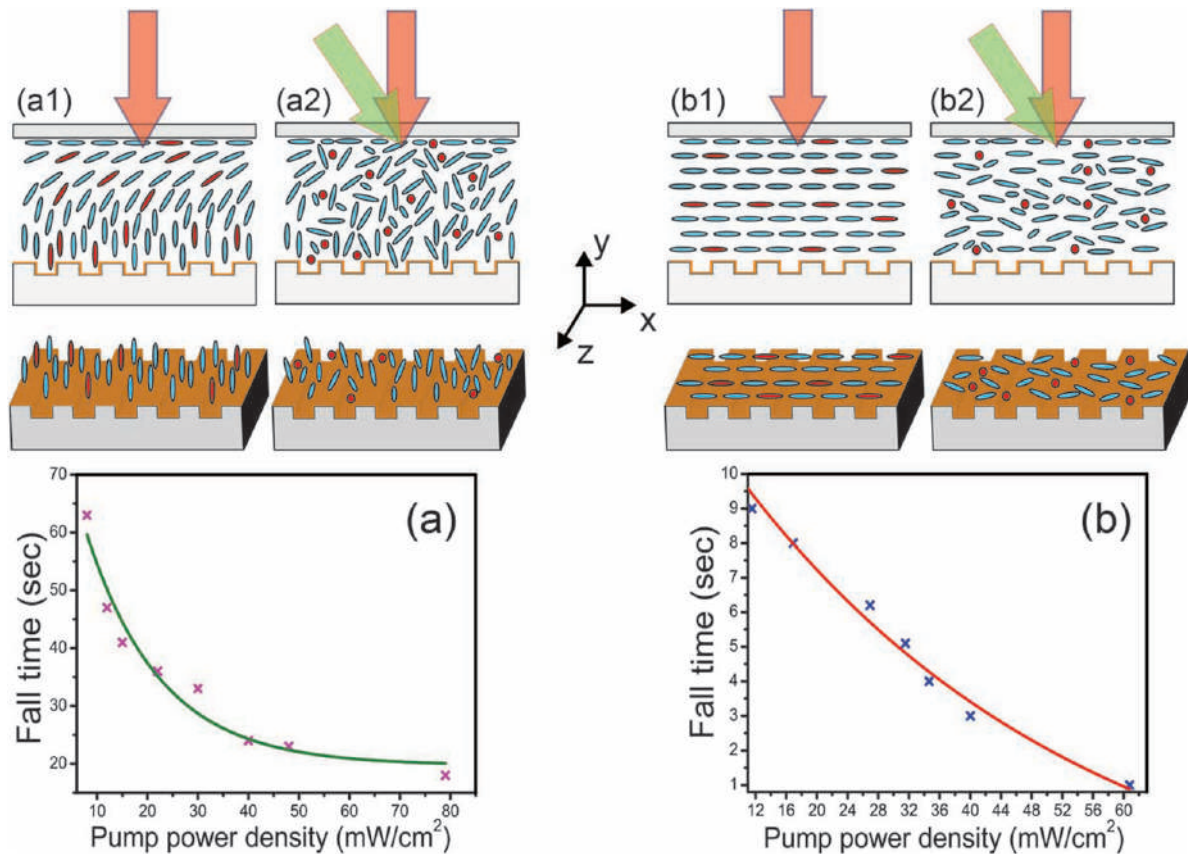


Figure 8. Comparison of the fall times of the structure without (a) and with SiO_x treatment (b) versus the pump power density. (a1), (b1) and (a2), (b2) are the hybrid/planar and hybrid/planar conditions before and after the pump irradiation, respectively. Reprinted with permission from [48], The Optical Society.

compared to the same structure without SiO_x (figure 8(a)). All-optical experiments have been performed by using the approach previously described.

The possibility to control the diffractive properties of LCs based DGs by using an electrical field, instead of a light beam, is of great interest because of the possibility to integrate switchable and flexible structures in modern devices (e.g. flexible displays). Unfortunately, where display oriented applications are concerned, the insulating properties of materials like PDMS, represent strong limitations for realizing electrically switchable devices. In this framework, De Sio *et al* [50] had overcome the intrinsic insulating properties of PDMS by sputtering a thin layer of indium tin oxide (ITO). Figure 9(a) shows the SEM view of the PDMS DG after the ITO deposition. The microchannels appear uniform and no evidence of defects (e.g. crack lines) are visible even at higher level of magnification (figure 9(b)). Subsequently, in order to align the NLC molecules, the surface of the conductive PDMS DG has been functionalized with SiO_x (figure 9(d)) by achieving, one more time, defects free microstructures (figure 9(e)). Lastly, the fabrication of the cell has been realized by sandwiching the NLC (E7, by Merck) between the functionalized DG and a commercial available ITO glass substrate functionalized with SiO_x (figure 9(f)). The realized sample (figure 9(g)) has been inspected between crossed polarizers by placing the optical axis of the sample both at 45° (figure 9(h)) and 0° (figure 9(i)), revealing a uniform and homogeneous alignment of the NLC

film. Electro-optical experiments have shown the possibility to control the diffraction efficiency of the structures by applying a relatively low electric field ($\approx 1\text{--}2 \text{ V } \mu\text{m}^{-1}$).

5. Discussion

DGs with on-demand optical properties can be easily utilized for several applications. Indeed, by applying an external perturbation such as an electrical or optical field it is possible to control the diffraction efficiency (e.g. from 100% to 0%) according to the value required for each specific application. As an example, if the DG is used as a beam splitter the diffraction efficiency can be set to 50% so that the two beams (transmitted and diffracted component) have the same intensity. The same DG can be used as an optical router by setting its initial diffraction efficiency to 100%. Lastly, the same structure can be used as an optical phase modulator by totally erasing the diffractive properties (diffraction efficiency close to 0%) in order to minimize the optical losses because of the presence of the diffraction orders. Spatial light modulators (SLMs) are a wonderful example of LCs based diffractive structures with on-demand optical properties [45]. However, it is very well known that SLMs have several intrinsic drawbacks such as zero-order leakage, passive intergap between pixels, poor spatial resolution, (typically $6 \mu\text{m}$) and pretty high fabrication costs (15–20 K\$). Conversely, it is worth saying that all the DG structures presented and discussed in the present

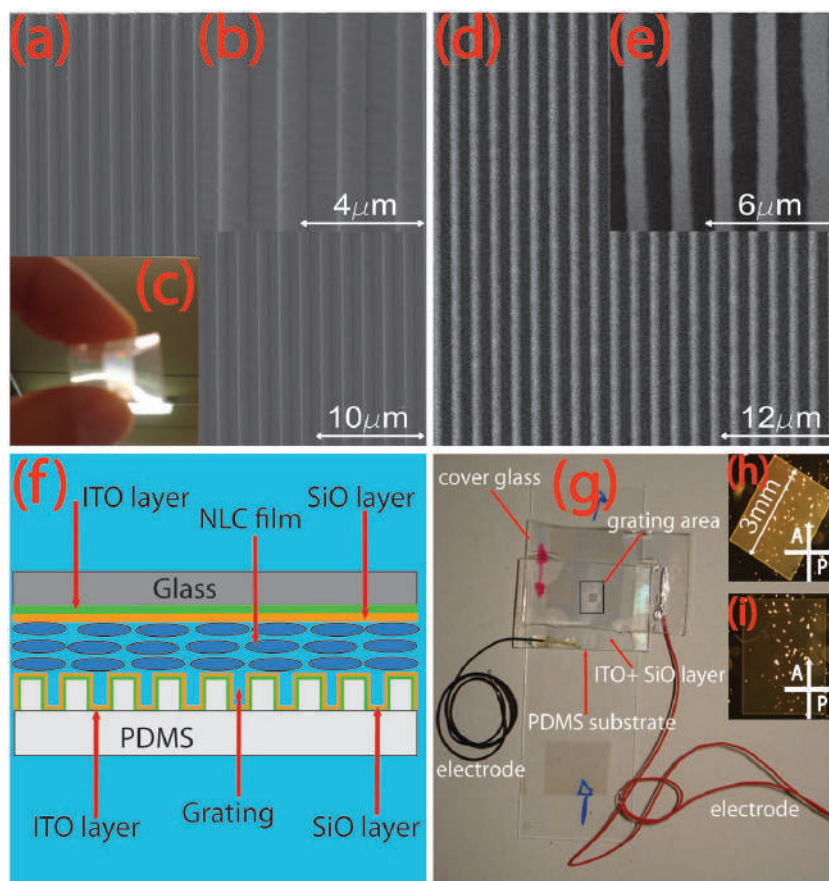


Figure 9. SEM view of the ITO sputtered sample (a) and its high magnification (b) after deformation experiments. (c) SEM view of the sample after evaporating SiO_x on the ITO layer (d) and its high magnification. (e) Sketch of the NLC configuration (f) along with a photo of the realized PDMS cell. (g) Polarized optical microscope view of the grating area containing the aligned NLC molecules with its molecular director axis at 45° (h) and 0° (i) with respect to respect the polarizer/analyzer axis (adapted from [50]).

manuscript are realized by exploiting cost-effective and high-resolution fabrication methods (e.g. lithography, holography, etc) with no limitations in the optical properties. This is important because it allows the realization of high quality and cost-effective optical elements. We believe that this overview is timely because modern technology is dealing with the fabrication and mass-production of flexible and reconfigurable devices such as bendable displays and flexible smartphones. Indeed, smart, lightweight and adaptable devices are in the priority list of several manufacturing companies. As such, the next decade will see the implementation of devices with superior optical properties and having both reconfigurability and flexibility. It is worth pointing out that the marketing of large area devices (e.g. large area displays) possessing both flexibility and reconfigurability is very challenging and currently it is very hard to predict if the technological progress will overcome this limitation.

6. Conclusion

We have reported an overview of a very interesting generation of DGs made with stimuli responsive materials. In order to provide a background on the realization and characterization of conventional DGs, we have introduced the main fabrication processes along with the principal optical and

geometrical parameters. Hence, two types of smart DGs have been considered and, for the sake of simplicity, they have been named *flexible* and *reconfigurable*. Among the *flexible* DGs based devices, we have considered several opportunities such as the possibility to use the diffraction of light for: (i) monitoring the change in cardiac cell beating frequency; (ii) glucose detection; (iii) improving the efficiency of solar cells. In the light of combining flexibility and re-configurability, we have presented in the *reconfigurable* section different examples of LCs based DGs. Particularly, light sensitive LCs enable the control of diffraction of light by using light while a combination of a ITO deposition and SiO_x evaporation enable the possibility to control the diffraction of flexible structures by means of a relatively low electric field. We are aware that the reported overview is not exhaustive but it represents a starting point for understanding the progress made in the last 15 years or so in the field of flexible and stimuli responsive DGs.

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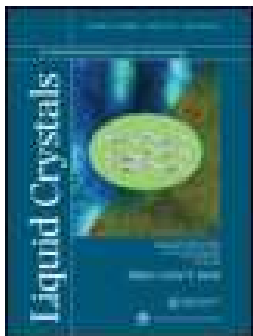
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A command layer for anisotropic plasmonic photo-thermal effects in liquid crystal

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INVITED ARTICLE



A command layer for anisotropic plasmonic photo-thermal effects in liquid crystal

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ABSTRACT

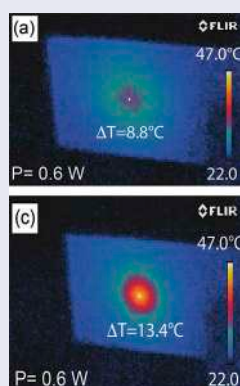
Photo-anisotropic properties of a particular command layer for Liquid Crystals (LCs), based on azobenzene material, are exploited to control the photo-thermal response of a single layer of homogeneously and uniformly distributed Au nanoparticles, immobilised on a glass substrate. Experiments demonstrate that the intrinsic anisotropy of materials can influence the photo-thermal response of plasmonic systems. Indeed, the resonant absorption of radiation by plasmonic subunits is followed by a noticeable increase of their temperature. However, the thermal response observed in presence of a homogeneous and random array of AuNPs directly exposed to air or embedded in ice is typically isotropic; on the contrary, a homogenous, yet thin, coating made of a particular command layer for LCs, deposited on a large-area carpet of AuNPs, influences their thermal response in an anisotropic way. In particular, the temperature increase, induced by pumping with a laser source of resonant wavelength with the plasmonic AuNPs, strongly depends on the alignment direction of the command layer. This effect makes the command layer of particular interest for its capability to drive intriguing optically induced 'thermal-reorientational' effects in a liquid crystal film.

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LC command layer; thermo-plasmonic; optical command; anisotropic thermal conductivity



1. Introduction

Recent development of azobenzene-based photo-anisotropic materials is attracting great attention for their possible use as photo-alignment or command Layers (PALs) for liquid crystals (LCs) and LC Polymers (LCP) [1–8]. While availability of different molecular structures for these materials allows using sources of light in a wide range of wavelengths, their alignment functionality can be related to the optical control of the molecular alignment axis – the axis of photoinduced anisotropy – that, upon illumination, self-organises perpendicularly to the light polarisation direction. The aligning process can be made reversible and

reproducible, thus making these materials a quite flexible tool for realising rewritable optical components [9–11]. The materials also enable high resolution patterning of LCs and LCP, which turns out to be a highly effective resource for the realisation of diffractive waveplates such as cycloidal waveplates for large angle beam deflection [7,12–14], vector vortex waveplates (spiral phase waveplates) with submicron singularity size, and fast waveplate lenses [15–18]. Plasmonic nanoparticles (NPs) are another class of very fascinating materials that exhibit the extraordinary property of confining an electromagnetic radiation at the nanoscale by exploiting a phenomenon called localised plasmonic

resonance (LPR) [19–23]. Among other effects, this mechanism produces a strong temperature increase nearby the NP surface. This peculiar property represents a unique opportunity for realising several photo-thermal-based applications ranging from photonics to medicine [24–27]; noteworthy, the produced photo-heating can be influenced and controlled by varying the optical properties of the surrounding medium (e.g. refractive index) [28–32]. In this framework, the possibility to optically control the intrinsic anisotropy of ultra-thin materials such as a PAL represents an intriguing resource for influencing the photo-thermal response of plasmonic systems as well. Here, we present and discuss experiments based on the possibility to exploit a homogenous, yet thin, coating of a PAL on a large-area carpet of AuNPs (immobilised on a glass substrate) for influencing the photo-thermal response in an anisotropic way. In particular, we investigate the increase of temperature resulting from pumping with a laser source with wavelength resonant with the plasmonic AuNPs, for different orientations of the PAL.

2. Experimental

2.1. Materials and methods

AuNPs have been synthesised by exploiting a slightly modified Turkevich method [33]. The substrate is a glass slide, whose surface has been properly treated with a Piranha solution (a 3:1 mixture of concentrated sulphuric acid and hydrogen peroxide) for 45 min that induces a negatively charged surface. Next, the substrate was rinsed with copious amounts of water and dried under a stream of compressed air. Substrates were functionalised by dipping in a 5% (v/v) solution of N-[3-(trimethoxysilyl) propyl] ethylenediamine in ethanol for 30 min in order to obtain positively charged surfaces; then, the substrates were rinsed with water, dried, and put in an oven at 110°C for a further 40 min. The process of NPs deposition has been realised by dipping the substrates in a solution of colloidal AuNPs for 50 min. The realised samples are characterised by the presence of AuNPs on both sides of the substrate. To evaluate the active role of a single NPs layer, a peel off of the NPs from one side is performed.

By means of a spin coater (2000 rpm @ 30 s), the NPs layer has been covered with a thin film of PAL, made of randomly aligned molecules of azo-dye (PAAD-27 by BeamCo), dissolved in dimethylformamide, which possesses a broad absorption band centred at 415 nm. The elongated molecules of PAAD-27

exhibit the feature that, when acted on by a polarised UV light for a suitable time interval (typically 10 min), they reorient perpendicularly to the light polarisation direction in order to minimise the absorption cross-section. By simply varying the polarisation direction of the impinging UV light, it is therefore possible to reorient the PAL molecules, along with the molecular director of a Nematic LC that the PAL is, possibly, put in contact with [34].

Spectral analysis has been carried out by probing the sample with UV-Vis unpolarised white light (Figure 1).

To evaluate the photo-thermal efficiency of the sample, a CW green laser emitting at $\lambda = 532$ nm, with a spot size of 0.21 cm^2 , is used as pump; measurements are performed at power intervals of 200 mW in the range 0.2–1.2 W, corresponding to intensity variations from 1.0 W cm^{-2} to 6.0 W cm^{-2} .

Thermographic analysis is performed at a small angle with respect to the pump beam (that impinges perpendicularly to the sample) by means of a thermo-camera (E40 by FLIR) characterised by an accuracy of $\pm 1.0^\circ\text{C}$ and a spatial resolution of 2.72 mrad. Control parameters are appropriately set to take into account both environmental and material properties; reflected temperature was set to $T_0 = 20^\circ\text{C}$, emissivity of glass to $\varepsilon = 0.83$ and the working distance between camera and object was adjusted to the allowed minimum value, that is, less than 10 cm. The instrument provides images of 160×120 px, where each pixel represents a temperature value.

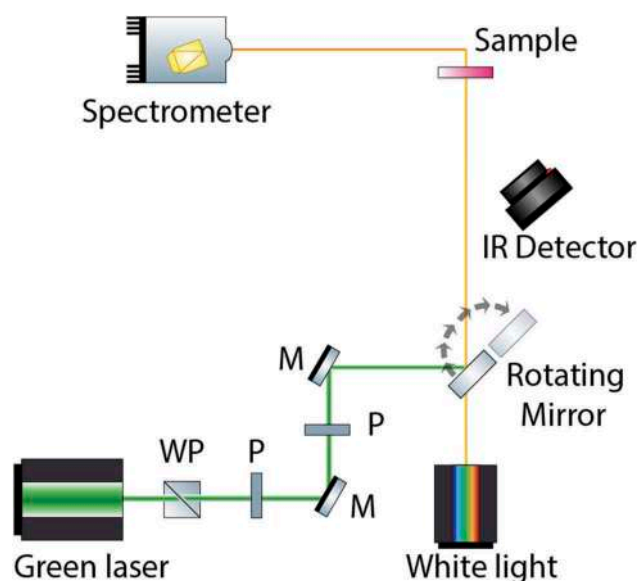


Figure 1. (Colour online) Sketch of the all-optical setup. WP: waveplate, P: polariser, M: mirror.

3. Results and discussion

To investigate the plasmonic photo-thermal behaviour for different orientations of the PAL, the photo-thermal response of a sample of AuNPs before (Sample A) and after (Sample B) the deposition of the PAL has been characterised.

Sample A, with a surface density of $355 \text{ NPs}/\mu\text{m}^2$ and an average height of $(30 \pm 6) \text{ nm}$, reveals a uniform distribution of well-dispersed NPs (see Atomic Force Microscopy [AFM] image in Figure 2(a)).

After the deposition of the PAL by means of spin coating technique (see *Materials and Methods* section), the new sample (Sample B) is characterised by an average NPs height of $(10 \pm 6) \text{ nm}$ (Figure 2(b)).

This indicates a planarisation of the surface due to the presence of the PAL that is interposed between the NPs.

The spectral analysis of sample A shows the typical LPR band of spherical AuNPs in the green region of the spectrum, centred at 533 nm (Figure 3(a), black curve), while sample B (AuNPs + PAL (Figure 3(a), red curve) shows an absorption increase of about 11% and

a red shift of the plasmonic resonance of about 13 nm with respect to sample A.

This result is in agreement with the Mie theory [35], (Figure 3(b)), which provides an analytical solution of Maxwell's equations for the scattering/absorption of an electro-magnetic radiation induced by small and isolated AuNPs. The modification of the spectral response of the sample, in fact, is due to the change in the refractive index n of the medium surrounding the AuNPs: from the air value ($n_a = 1$) to the PAAD-27 value ($n_{PAL} = 1.73$).

In the experiments, the sample is illuminated by a CW green laser in order to excite the LPR of the AuNPs responsible of the photo-thermal effects.

Indeed, it is well known that an AuNP, if excited by a suitable light, delivers heat around it. This effect is related to the damping of the electrons of the conduction band that oscillate in resonance with the electric field of the impinging light. To predict the temperature around an AuNP, it is necessary to solve the heat transfer equation [36], which derives from a balance of heat energy: the net rate of thermal energy that comes out from the AuNP, plus the rate of thermal

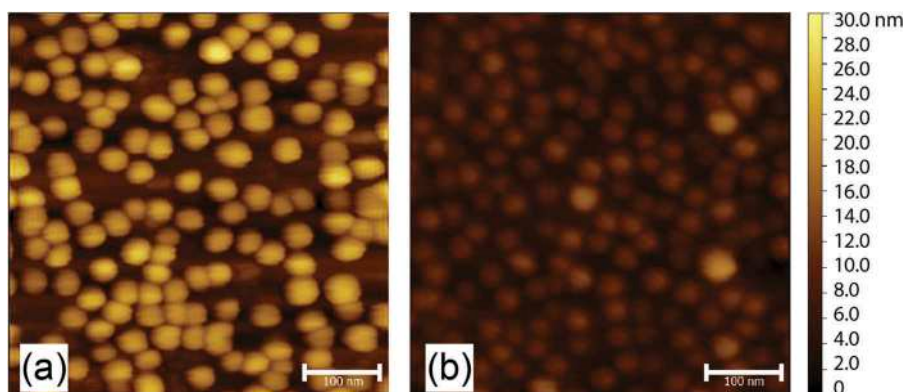


Figure 2. (Colour online) AFM topography of the AuNPs sample (a) before (Sample A) and (b) after (Sample B) the deposition of the PAL.

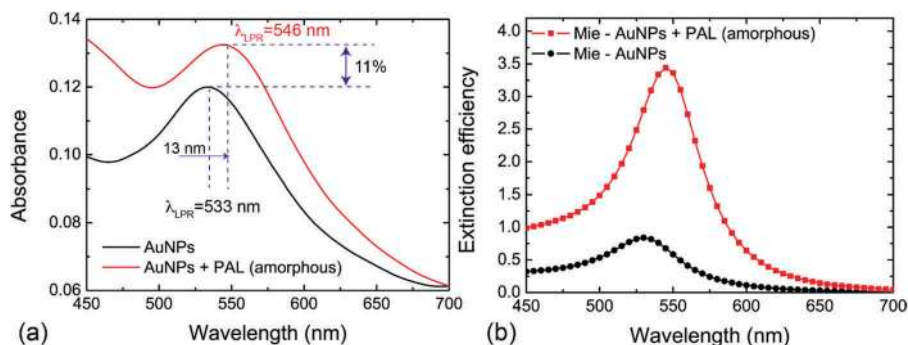


Figure 3. (Colour online) Spectral response (a) and theoretical prediction – Mie theory (b) of the extinction efficiency of the AuNPs sample surrounded by air (black curve), and PAL (red curve).

energy accumulation (internal energy variation in the AuNP) must equal the net rate of thermal energy generation. By solving the heat transfer equation, it is possible to calculate temperature variations produced by the photo-induced plasmonic Joule effect around the AuNPs.

According to the model, the temperature variation at a point r from a single AuNP is calculated as:

$$\Delta T(r) = \frac{QR_{NP}^3}{3rK_H}$$

where Q is the heat production due to Joule effect, R_{NP} is the NP radius, K_H is the thermal conductivity of the host medium. Calculation of Q , which linearly increases with the laser intensity I [30], yield that, in order to obtain a temperature variation of only 1° around a single AuNP with $R_{NP} \approx 20$ nm in water, we need a laser intensity of about 10^5 W/cm² [37]. On the other hand, from the experimental point of view, measures reveal a temperature variation of several degrees with a laser intensity of few W/cm² [30]. To account for this result, we have to consider that, by illuminating a macroscopic surface region of the NPs layer with a laser spot of about 0.225 cm in radius, a huge number of AuNPs are acted on by the light; thus, as a matter of fact, the temperature increase in a given point has to be calculated by adding the contribution of all irradiated NPs as specified in [30]. Once given the density n_{NP} of uniformly distributed NPs and the beam radius w of the resonant impinging light (characterised by an intensity I_0), the expression that allow us to obtain this contribution in the centre of the laser spot is:

$$\Delta T(r=0) = 2\pi w c(\lambda) n_{NP} I_0 = \beta I_0 \quad (1)$$

where $c(\lambda)$ is defined as:

$$c(\lambda) = \frac{V_{NP} \text{Im}(\chi_{NP})}{2\lambda K_H \sqrt{\epsilon_H}} \left| \frac{3\epsilon_H}{2\epsilon_H + \epsilon_{NP}} \right|^2 \quad (2)$$

Here, V_{NP} is the NP volume, K_H is the thermal conductivity of the host medium, χ_{NP} is the NPs dielectric permeability, ϵ_{NP} and ϵ_H are the NPs and host medium dielectric permittivity, respectively.

It is evident that the temperature experiences a resonant enhancement under the condition that $|\epsilon_{NP} + 2\epsilon_H|$ is a minimum, which means $\text{Re}[\epsilon_{NP}(\omega)] = -2\epsilon_H$ [38]. This relationship is called the ‘Fröhlich condition’ and is associated to the dipole surface plasmon of the metal nanoparticle. A good prediction of the temperature variation goes through the determination of the thermal conductivity of the host system, which is made of glass and PAL layer. As we have shown in [30], this is possible through a fit procedure of the experimental temperature values.

In the experiment, when the AuNPs are illuminated by a resonant light, the thermal response of the sample is evaluate by means of a thermographic analysis (see *Material and Methods section*).

By monitoring temperature values of the central pixel of each *hot-spot* of the thermographic images (Figure 4(a-d)), which corresponds to the warmest point, we plot the temperature variation $\Delta T = T - T_0$ versus the impinging Power (T_0 is the sample temperature when the pump beam is off, see Figure 4(e,f)). Measured ΔT values show a linear increase as a function of the pump Power; in particular, the ΔT corresponding to the maximum Power

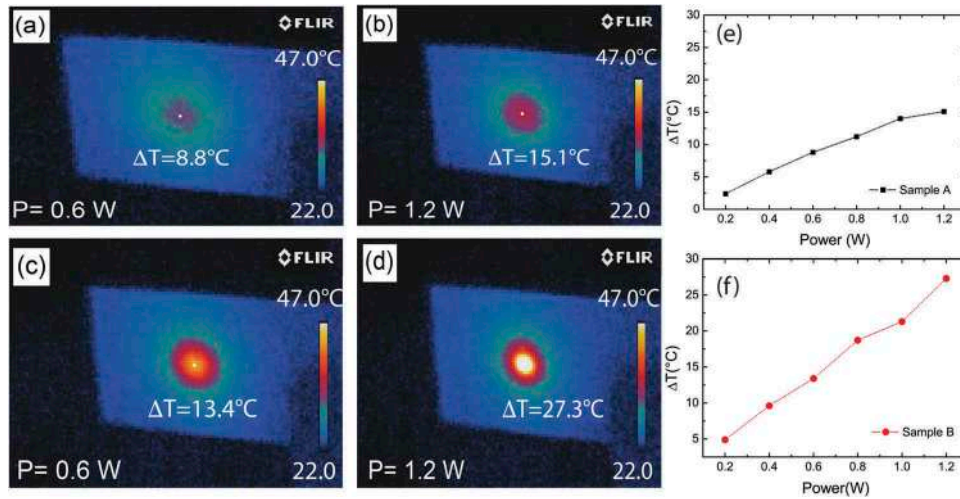


Figure 4. (Colour online) Thermographic images of sample A (a, b) and sample B (c, d), for different values of the pump beam. Temperature variation versus pump beam Power for (e) sample A (black curve) and (f) sample B (red curve).

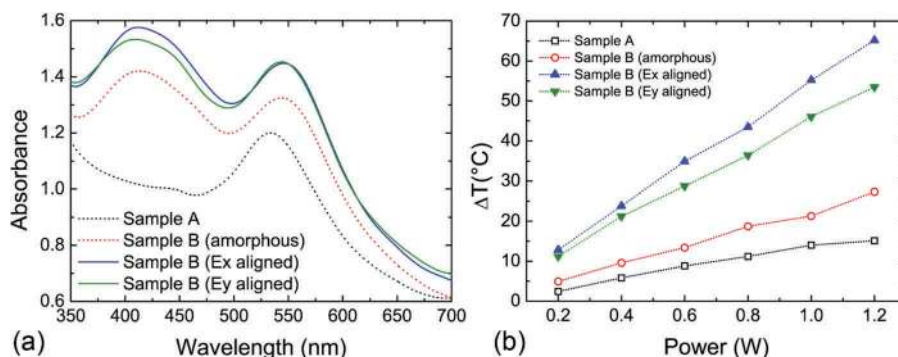


Figure 5. (Colour online) Spectral response of the sample A and B in the case of Ex-polarised aligning blue light (blue curve) and Ey-polarised blue light (green curve) (b). Temperature variations of the samples B for the two different orientation alignments.

(1.2 W) for the sample A is $\Delta T = (15.1 \pm 1.0)^{\circ}\text{C}$, while the ΔT is equal to $(27.3 \pm 1)^{\circ}\text{C}$ in the case of sample B.

Once the photo-thermal response of samples has been estimated, the impact of the different alignment of the PAL is investigated.

After exposing sample B to the uv-polarised light, indicated by ‘Ex aligned’ (*Materials and Methods section*), we repeated the spectral analysis on sample B, observing an increase of the plasmonic band intensity of about 10% and no shift of the position of the LPR peak. By exposing again sample B to the polarised light after rotating of 90° the polariser with respect to the previous one (we indicate by ‘Ey aligned’ the new polarisation direction), we obtain the same spectral response in the whole area of the plasmonic band (Figure 5(a)). The observation that the LPR band does not change for the two different alignments indicates that there are no variations in the refractive index of the PAL since same resonant wavelength means same ‘Fröhlich condition’, that is same dielectric permittivity for the host medium. Furthermore, for the two different Ex and Ey alignments, we observe not only the same resonance wavelength but also the same absorbance value; however, this overlap of the absorption spectrum does not correspond to an overlap of the detected temperature variations. In fact, although the optical response of sample B does not change, its thermal response (Figure 5(b)) results come out to be very different for the two different alignments of the PAL. This can be explained by observing that the system absorbance depends on the polarisability $\alpha = 3 V_{NP} (3\epsilon_{NP} - \epsilon_H) / (\epsilon_{NP} + 2\epsilon_H)$ whose denominator determines the ‘Fröhlich condition’, while temperature variations depend also on the thermal conductivity. Thus, we can argue that the three different PAL alignment conditions (amorphous, Ex aligned and Ey aligned) induce different thermal

conductivities, though they induce the same optical response of the sample. Furthermore, we can say that the ‘Ex aligned’ and ‘Ey aligned’ conditions induce a reversible and repeatable *thermal anisotropy* due to an induced change of the thermal conductivity of the PAL, which depends on the polarisation direction of the resonant impinging light.

As previously specified and detailed in [30], we are able to calculate the thermal conductivity by detecting temperature variations versus laser intensity; therefore, we are able to measure this thermal anisotropy of the PAL by exploiting Equations (1) and (2). The thermal conductivities K_H of the PAL in the three cases are measured as: $0.77 \text{ W}(\text{K m})^{-1}$ for the amorphous PAL, $0.32 \text{ W}(\text{K m})^{-1}$ for the Ex aligned PAL and $0.40 \text{ W}(\text{K m})^{-1}$ for the Ey aligned PAL. These results demonstrate that it is possible to drive the thermal behaviour of PAL material by means of polarised light in order to obtain different thermal responses of a new smart LC device.

4. Conclusions

In this paper, we have studied the photo-thermal properties of a gold NP array layered with a reversible photo-alignment layer (PAL). Samples have been characterised in terms of morphological and optical properties, revealing the presence of randomly aligned, well-separated AuNPs (both before and after the PAL deposition). We have observed a correlation between the photo-induced heat and the optical properties of the PAL material in terms of refractive index and thermal conductivity. Interestingly, the temperature increase can be controlled by varying the orientation of the PAL molecules thanks to the intrinsic variation of their thermal conductivity.

Keeping in mind that the PAL can be used as a command layer for the alignment of LCs, our results

represent a starting point for realising thermo-plasmonic assisted LC devices with reconfigurable thermal conductivity and refractive index.

Disclosure statement

No potential conflict of interest was reported by the authors.

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Dynamic optical properties of gold nanoparticles/cholesteric liquid crystal arrays

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Abstract

A thermoresponsive large-area plasmonic architecture, made from randomly distributed gold nanoparticles (GNPs) located at the substrate interface of a cholesteric liquid crystal (CLC) cell, is fabricated and thoroughly characterized. A photo-thermal heating effect due to the localized plasmonic resonance (LPR) mechanism is generated by pumping the GNP array with a resonant light beam. The photo-induced heat, propagating through the CLC layer, induces a gradual phase transition from the cholesteric to isotropic phase. Both the plasmonic and photonic properties of the system as both the selective reflection properties and frequency of the LPR are modulated.

Introduction

In the last 20 years, plasmonic devices have been extensively investigated both from an experimental and theoretical point of view due to their fascinating and unique optical properties.^[1–3] Several plasmonic-controlled photonic components have been realized including super-high-resolution lenses,^[4,5] subwavelength gratings,^[6] and beam deflectors.^[7] Plasmonic nanoparticles (NPs) have played a key role in this explosion of interest due to their capability of confining electromagnetic radiation at the nanoscale by exploiting a phenomenon called localized plasmonic resonance (LPR).^[8] Irradiation (with suitable frequency) of a plasmonic NP induces the oscillation of free electrons localized at the metal–dielectric interface, which gives rise to a LPR. The frequency (wavelength) of the LPR can be controlled by varying both the size, shape, and the dielectric constant of the surrounding medium.^[9] Plasmonic NPs can also behave as nano-sized sources of heat when pumped sufficiently with resonant radiation due to the Joule effect generated by the photo-induced electrical current.^[10,11] The photo-thermal properties of NPs have been historically considered a drawback because they have contributed to the intrinsic losses of plasmonic-based devices. Numerous efforts for mitigating the losses by exploiting both organics and semiconductors gain media have occurred.^[12–14] Researchers recently have realized that this effect opens the possibility of enabling “drug-free” nano-thermal therapies including

“plasmonic photo-thermal therapy”.^[15] From a fundamental point of view, it is very important to study the heat dissipation from the photo-heated NPs to the surrounding environment. Thermotropic liquid crystals (LCs) are an excellent candidate medium as they are extremely responsive to external perturbations such as electric fields, optical fields, and temperature.^[16] LCs have been exploited as active media for controlling the LPR frequency or for detecting photo-induced temperature variations in several plasmonic architectures.^[17–19] The work here shows the possibility of combining a large area (1 inch²) of randomly distributed gold NPs (GNPs) immobilized on a glass substrate and covered with a cholesteric LC (CLC) as an active overlayer for controlling simultaneously both the photonic and plasmonic properties of the hybrid system.

Materials and method

The GNPs synthesis followed a slightly modified Turkevich method reported elsewhere.^[20,21] A monolayer of GNPs is electrostatically immobilized on a BK7 glass substrate, which had been dipped in a Piranha solution (a 3:1 mixture of concentrated sulfuric acid and hydrogen peroxide) for 30 min. This first process leaves the substrate negatively charged. After washing and drying, the surface was functionalized by dipping it in a 5% (v/v) solution of N-[3-(trimethoxysilyl) propyl] ethylenediamine in ethanol for 30 min in order to obtain a positive surface. Following rinsing and drying, the template was put in

an oven at 120°C for 30 min. The glass substrate at room temperature was successively dipped in a colloidal solution of spherical GNPs for 1 h and 30 min. Subsequently, the same substrate was submitted to a nanochemistry procedure to increase the sizes of nanostructures (one cycle of growth).^[21]

A CLC phase can be obtained by adding to a NLC a small amount of chiral dopant, giving chiral properties to the NLC. High concentrations of chiral dopant can be used to increase the twisting power and to reduce the CLC pitch. In our case, a CLC mixture was prepared by mixing a nematic LC (5CB, 4-cyano-4'-pentylbiphenyl, by Merck, Darmstadt, Germany) with 20 wt.% left-handed chiral dopant [ZLI-811, 4-(1-methylheptyloxy)carbonylphenyl-4-(hexyloxy)benzoate, by Merck, Darmstadt, Germany] obtaining a helix pitch length of about 440 nm. The 20 wt.% of chiral dopant was necessary in order to finely tune (red shift) the CLC reflection band, thus minimizing the overlap between the CLC reflection band and the GNPs absorption peak centered at 600 and 515 nm, respectively (see below). 5CB was utilized due to its low transition temperature ($T_{\text{nematic-iso}} \approx 38^\circ\text{C}$). The transition temperature of the obtained CLC is almost the same as the base 5CB. The photo-alignment layer (PAL, PAAD 72 by BEAMCo, Orlando, Florida)^[22] is a commercial available azo dye dissolved (1 wt.%) in dimethylformamide and possesses a broad absorption spectrum extending into the visible range of the spectrum ($\lambda_{\text{max}} = 430 \text{ nm}$). The thermographic analysis was performed by using a FLIR (A655sc) thermal imaging camera that produces thermal images of 640×480 pixels with an accuracy of $\pm 2^\circ\text{C}$. The camera works seamlessly with a proprietary software (FLIR ResearchIR Max) that enables recording and processing of the thermal data acquired by the camera.

Experimental section

The GNPs substrate exhibits a pinkish tint [Fig. 1(a)] with an absorption peak [Fig. 1(e), red curve] at 515 nm (measured with unpolarized light at normal incidence) due to excitation of the LPR. The substrate was coated with the PAL for inducing planar alignment of the CLC. The PAL was spin-coated onto the NP-coated substrate (3000 rpm for 30 s) inducing a noticeable color change [Fig. 1(c)]. The PAL deposited onto the GNPs monolayer produces a variation of the refractive index surrounding the GNPs [from air ($n = 1$) to PAL ($n = 1.4$)] as evidenced by the red shift (18 nm) of the LPR peak reported in Fig. 1(e) (blue curve) from 515 nm (red curve) to 533 nm (blue curve). The reported red shift of the LPR frequency can be explained in the framework of Mie theory,^[23] which is suited to calculate the optical cross-section for a small and isolated spherical NP. Accordingly, there is a correlation between the LPR frequency and the refractive index of the medium surrounding the GNPs. In particular, the LPR frequency reveals a red or blue shift for increasing or decreasing value of the refractive index surrounding the NPs. In order to confirm that the approximation of small and isolated NPs is valid, the GNPs monolayer was examined by means of an atomic force microscopy (AFM) operating in tapping mode using an Olympus

OML-AC240TS (radius $9 \pm 2 \text{ nm}$) cantilever. Figure 1(b) shows that the GNPs are monodisperse with an average diameter of about 20 nm and a thickness of the GNP array of about 30 nm. The same study performed after the PAL deposition [Fig. 1(d)] shows that the modulation depth is reduced a few nm due to the planarization effect induced by the PAL deposition (the PAL thickness is about 27 nm). The CLC is “sandwiched” between one substrate possessing the treated GNPs/PAL and a second substrate coated with a thin polyimide layer rubbed to induce planar alignment of the CLC molecules (CLC helix axis oriented perpendicular to the glass surfaces, see below). The two substrates are spaced by 10 μm glass microspheres and the CLC is introduced by capillary flow at room temperature. These microspheres are located at the four corners of the cell and they do not affect the optical properties of the sample. Figure 1(f) shows the CLC sample highlighting a reddish color due to its selective reflection. For unpolarized light, the spectral response of the sample [Fig. 1(e), magenta curve] shows a broad dip in the transmission curve due to the overlap of the LPR absorption and CLC reflection centered at 600 nm.

Results and discussion

In order to study independently the photonic and plasmonic properties of our hybrid system (due to the partial overlapping between the LPR absorption and CLC reflection band), polarized spectral characterization of the samples was done. It is widely known that for a CLC layer in a planar configuration, circularly polarized light of the same handedness (right circularly polarized, in the actual case) as the helix and having wavelengths between $n_o P$ and $n_e P$ (n_o and n_e being the ordinary and extraordinary refractive indices of the material, respectively, and P being the pitch) is reflected by the CLC layer [Fig. 2(a), red curve]. This is confirmed by visualizing the far-field back-reflected component [see reddish spot in Fig. 2(c)]. Light of the opposite handedness [Fig. 2(a), blue curve] propagates through the CLC film without being affected (the whitish back-reflected spot shown in Fig. 2(b) is due to the Fresnel reflection of the glass substrates). Both spectra reported in Fig. 2(a) (red and blue curves) have been acquired at normal incidence, while the two far-field back-reflected spots [Figs. 2(b) and 2(c)] have been acquired by slightly tilting the CLC sample. It is worth noting that in the blue curve [Fig. 2(a)], the LPR absorption is visible with a remarkable red shift compared with PAL [Fig. 1(e), blue curve]. On the other hand, the LPR absorption is slightly visible in Fig. 2(a), red curve (see dashed circle) due to the partial overlap between the LPR absorption and CLC reflection band. Indeed, due to the increasing value of the refractive index surrounding the NPs [from the PAL layer to the CLC film ($n_{\text{average}} \sim 1.61$)], the LPR frequency is further red-shifted by $\sim 28 \text{ nm}$ [Fig. 2(a), blue curve]. [Note: the refractive index of the CLC in the cholesteric phase has been calculated as follows: $n_{\text{average}}(\text{cholesteric}) = (n_e + n_o)/2$, where $n_e \approx 1.68$ and $n_o \approx 1.52$ are the extraordinary and ordinary refractive indices of the LC, respectively.] To visualize the overall red shift of the sample, the spectral response of the GNP array [Fig. 2(d),

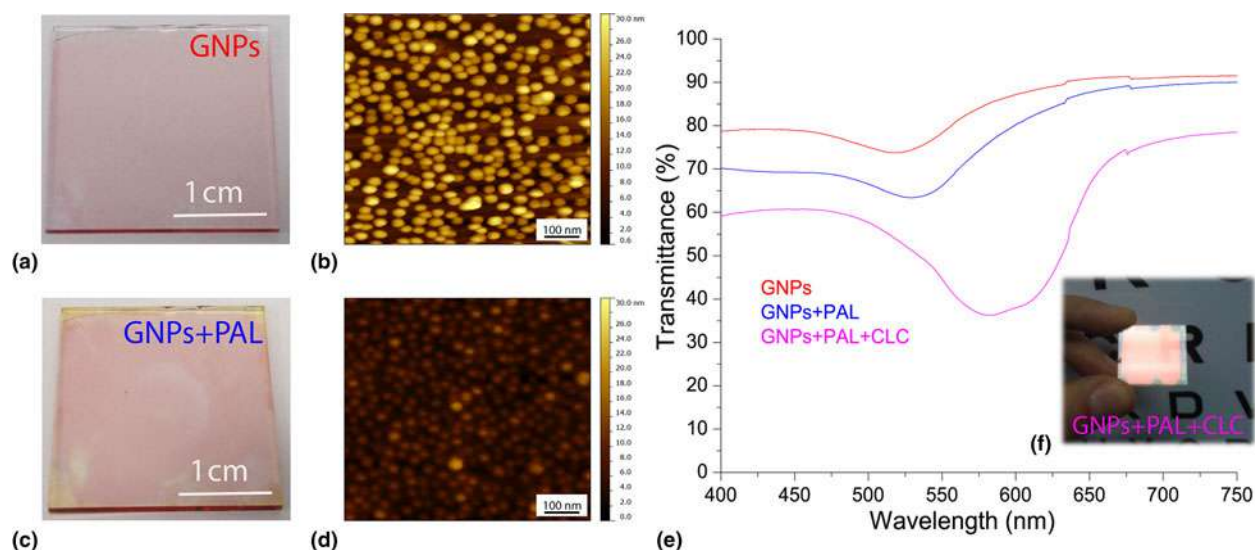


Figure 1. Photo of the NP array immobilized on a glass substrate surrounded by air (a) and PAL (c) along with the corresponding AFM topography (b, d). Spectral response of the sample (e) for unpolarized visible light surrounded by air (red curve), PAL (blue curve), and PAL + CLC (magenta curve) along with the photo of the fabricated sample (f).

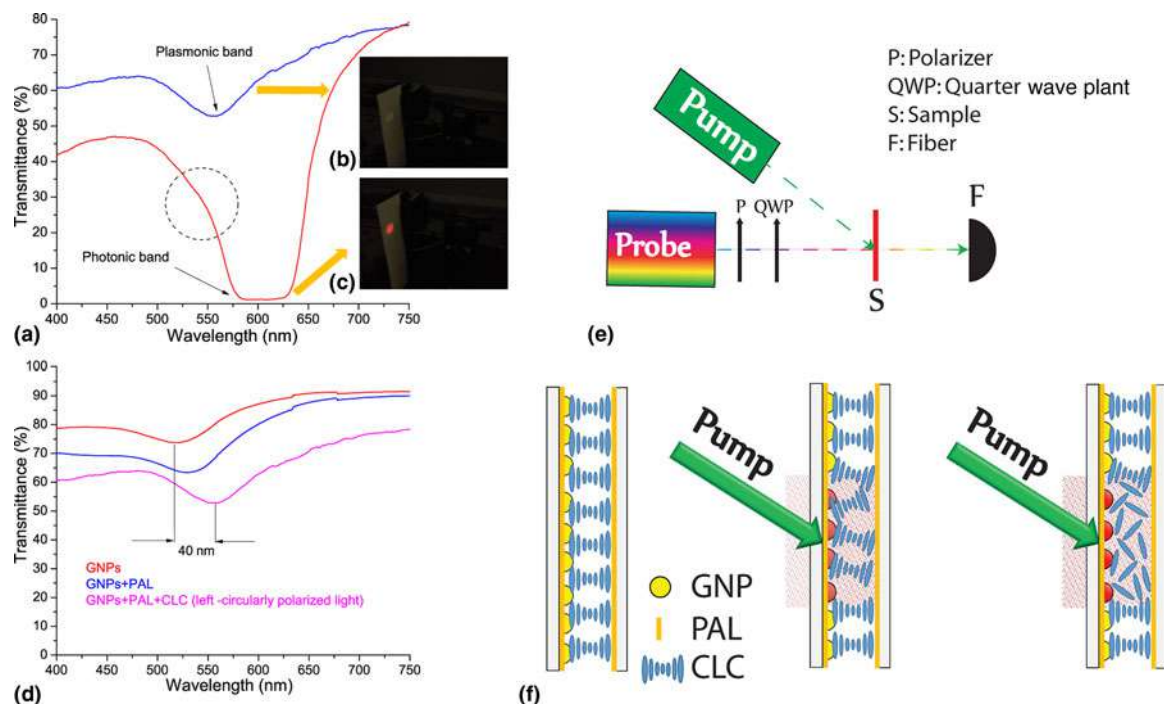


Figure 2. Spectral response of the sample (a) for left (blue curve) and right (red curve) circularly polarized light along with the corresponding photos (b, c) of the back-reflected component. Spectral response of the NP arrays (d) surrounded by different media, air (red curve), PAL (blue curve), PAL + CLC (magenta curve). The magenta curve in (d) has been acquired for the light left circularly polarized. All-optical setup (e) for sample characterization. Schematic of the sample (f) with the pump beam turned off (left) and on (middle and right) at different power level.

red curve], GNP array covered with PAL [Fig. 2(d), blue curve], and GNP array coated with PAL and CLC [Fig. 2(d), magenta curve] is shown. The last one has been acquired for left circularly polarized light in order to remove the presence

of the CLC reflection band. There is a total red shift of the LPR frequency of about 40 nm. The pitch of a CLC film is sensitive to temperature variations allowing a simple probe of average temperature variations induced by LPR-induced heating.

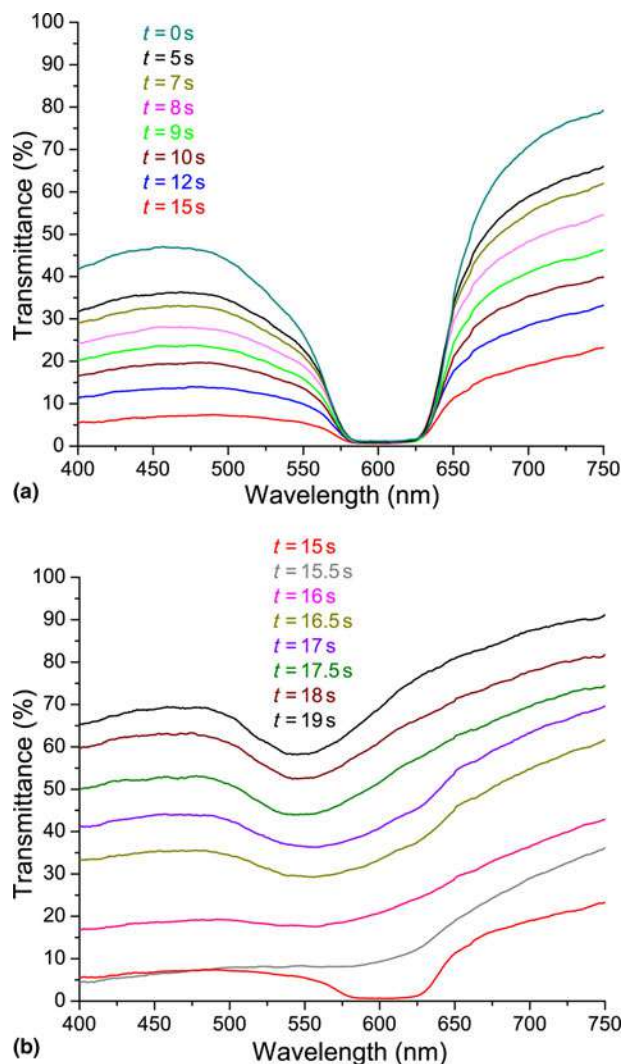


Figure 3. Spectral response of the sample (probe light right circularly polarized) for different values of the illumination time. For the sake of clarity, the spectrum is divided in two different spectra having fast (a) and slow (b) back relaxation.

An all-optical setup reported in Fig. 2(e) was used to probe time/temperature/exposure relationships. This setup utilizes a collimated white source ($400 \text{ nm} < \lambda < 750 \text{ nm}$) for monitoring the spectral transmission properties of the CLC configuration and a CW pump laser emitting at $\lambda = 532 \text{ nm}$ ($P_{\text{pump}} = 60 \text{ mW/cm}^2$) in the high-absorption range of the GNPs surrounded with PAL and CLC [Fig. 2(d), magenta curve]. When the pump beam is off, the CLC is planar aligned as sketched in Fig. 2(f) (left image). In this geometry, for right circularly polarized light, the CLC exhibits a well-defined reflection band [Fig. 3(a), 0s]. By gradually photo-exciting the sample (from 0 to 15 s) from the GNPs substrate side, there is an electric-driven Joule heating of the GNPs and dissipation of that heat into the surrounding CLC medium (the PAL layer does not change its optical properties with temperature). The local heating induces a deformation/distortion of the CLC alignment

[Fig. 2(f), middle image] resulting in attenuation ($\approx 80\%$) and broadening ($\approx 5 \text{ nm}$) of the reflection band [Fig. 3(a)]. The attenuation is due to a large increase in scattered transmitted light, while the broadening is due to a slight chirping of the CLC pitch profile through the thickness of the cell (hot to cold). Continued illumination from 15 to 19 s results in the CLC undergoing a phase transition [Fig. 2(f), right image] restoring [Fig. 3(b)] the broadband transmitted light intensity (the isotropic CLC does not scatter the impinging radiation). Noteworthy, when the CLC is in the isotropic phase, the reflection band is completely erased, while the LPR absorption is clearly visible [Fig. 3(b), black curve]. In order to check the reversibility and the repeatability of the reported effect, we have performed reflection dynamic experiments by using a low-power red probe light ($\lambda = 633 \text{ nm}$, in the middle of the reflection band). By utilizing a photo-detector, we have monitored the reflection component while turning on and off the pump laser beam ($\lambda = 532 \text{ nm}$, $P_{\text{pump}} = 60 \text{ mW/cm}^2$). In the time interval 0–15 s, the CLC configuration is partially deformed [see configuration reported in Fig. 2(f), middle image] due to induced photo-thermal heating (pump beam on). When the pump beam is turned off, the CLC restores to its initial geometry [Fig. 2(f), left image] in $< 1 \text{ s}$. Conversely, in the time interval 15–19 s, since the CLC undergoes a phase transition (and is eliminated) [Fig. 2(f), right image], when the pump beam is turned off, the CLC takes several minutes to relax back to its initial configuration [Fig. 2(f), left image]. We have repeated the same experiments by photo-heating the samples from the glass side observing the same behavior with a longer time interval (the CLC went to the isotropic phase after 26 s). It is important to remark that we have chosen spherical GNPs because they are more stable than other shapes (e.g., nanorods) and easier to prepare. Moreover, we remind that the photo-thermal efficiency (μ) of GNPs can be expressed through the following equation $\mu = \sigma_{\text{abs}}/(\sigma_{\text{abs}} + \sigma_{\text{sca}})$, where σ_{abs} and σ_{sca} are the absorption and scattering cross-sections, respectively. It has been shown by Jain et al.^[24] that if the particle size is about 10–20 nm, σ_{sca} is negligible, thus $\mu \approx 1$. On the other hand, the synthetic path for realizing very small GNPs (e.g., $< 10 \text{ nm}$) is experimentally challenging and time consuming. For this reason, we chose a particle size (20 nm) to maximize the photo-thermal efficiency while ensuring a simple and reliable synthetic protocol. In order to quantify the influence of the CLC refractive index variation on the spectral position of the LPR peak, the transmission curves of the sample when the CLC is in the cholesteric [Fig. 2(d), magenta curve] and isotropic [Fig. 3(b), black curve] phases can be compared. Figure 4(a) shows a blue-shifted ($\approx 15 \text{ nm}$) LPR peak due to the decreasing of the refractive index surrounding the GNPs [from cholesteric ($n_{\text{average}} \sim 1.61$) to isotropic ($n_{\text{average}} \sim 1.57$)], again in agreement with the Mie theory.^[23] Note: the refractive index of the CLC in the isotropic phase has been calculated as follows: $n_{\text{average}}(\text{isotropic}) = (2n_o + n_e)/3$. The photo-induced temperature variations between the two CLC configurations (cholesteric

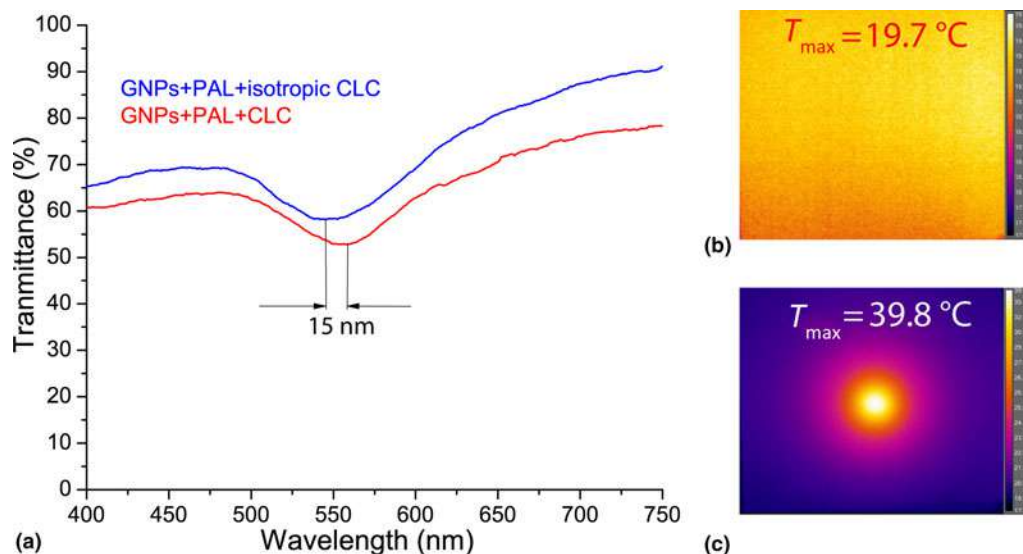


Figure 4. Spectral response (a) of the sample for left (red curve) and right (blue curve) circularly polarized light having the CLC is the cholesteric and isotropic phases, respectively. Thermographic images of the sample (pump laser beam on) acquired at $t=0$ s (b) and $t=20$ s (c).

and isotropic phases) was monitored with a high-resolution thermographic camera. Figure 4(b) shows a uniform temperature distribution ($T \approx 20^\circ\text{C}$) at $t=0$ s, while Fig. 4(c) shows a noticeable temperature increases ($T \approx 40^\circ\text{C}$, under the photo-illuminated area) at $t=20$ s due to photo-induced thermal effect. The control experiment (same experimental conditions) performed on a regular CLC sample did not show any significant temperature variations. This result is an incontrovertible evidence that the photo-induced heat generation can only be associated to the LPR mechanism (plasmonic-assisted photo-thermal heating). Noteworthy, the reported blue shift [Fig. 4(a)] shows the possibility to realize a LC-based plasmonic device having a light controllable plasmonic resonance.

Conclusion and outlook

To summarize, the photo-thermal properties of a GNP array immobilized on a glass substrate and layered with a low-transition temperature CLC was examined. A significant red shift (40 nm) of the LPR frequency due to a local increase of the refractive index (from air to PAL to CLC) was observed. Upon exposure to resonant radiation, local heating of the CLC medium induces changes to the optical properties. The local phase can be transitioned from liquid crystalline to isotropic wherein the expected blue shift (15 nm) of the LPR was observed. The system demonstrates that suitable optical radiation can be utilized to affect both the plasmonic and photonic properties of the system. Ongoing efforts are devoted to improve the photo-induced CLC broadening by using thicker glass cells and materials with higher transition temperature. NPs with different shapes and properties (e.g., gold nanorods) are also under investigation. In the light of plasmonic-based applications, our finding can be used for realizing a large

area, all-optical, plasmonic architectures with adjustable LPR frequency. Moreover, the same system can be integrated in modern photonic devices such as controllable light filters and smart mirrors.

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Author contributions

The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript.

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Photo-Induced Heating in Plasmonic Nanoparticles Trapped in Thermo-Sensitive Liquid Crystals

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Thermo-sensitive liquid crystals may result, for some aspects, good host materials for plasmonic nanoparticles. In particular they are suitable to study and measure the temperature variations produced by photo-induced plasmonic joule effect in the metallic nanoparticles. Combining the properties of liquid crystals and metallic nanoparticles, allows to measure temperature variations in different ways by exploiting the optical properties of thermotropic liquid crystals: In a first attempt, by combining nematic liquid crystals and spherical metallic nanoparticles, we have predicted and measured temperature changes, under a suitable (resonant) optical illumination, by measuring the photo-thermal induced birefringence variation. In a different experiment, we have combined cholesteric liquid crystals and gold nanorods: Light-induced variations of structural colorations exhibited by cholesteric liquid crystals has been used as a new methodology to measure nanoscale heat variation with a very high sensitivity (0.03 K).

Keywords: Plasmonic Nanoparticles, Gold Nanorods, Liquid Crystals.

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Nanophotonics concerns the study of the interaction between light and nanosized objects, and involves many research fields such as physics, chemistry, biology, nanomedicine. Plasmonics is a field of nanophotonics that studies the interactions between an electromagnetic field and free electrons in a metal nanoparticle; indeed, they can be excited by the electric component of light and undergo collective oscillations, that are called plasmons. In this framework, metallic nanoparticles (MNPs) have the intrinsic ability to confine light at the nanoscale through the

excitation of the Localized Plasmonic Resonance (LPR), a phenomenon related to the oscillation of the free electrons localized at the metal/dielectric interface. Under a resonant light illumination, MNPs convert light into heat, thus becoming nanosources of heat and opening up an unpredictable number of applications, ranging from photonics to nanomedicine.^{1,2} This occurs because the light absorption associated to the LPR triggers a heat generation process that involves not only absorption of incident photons, but also heat transfer from the MNPs to the surrounding medium. Gold nanoparticles (GNPs) have gained an important role because their LPR can be tuned from the visible to the near-infrared (NIR) range by simply changing size, shape, or dielectric function of the surrounding medium. Moreover, GNPs are bio-compatible and can be easily functionalized with a large variety of molecules, exhibiting also a weak oxidation. Thanks to these properties, GNPs have been widely exploited in cancer therapy,³⁻⁷ ophthalmology and dermatology, nanosurgery,^{8,9} ophthalmology and dermatology, nanosurgery,¹⁰⁻¹² photothermal imaging,¹³ plasmon-assisted nanochemistry,¹⁴ plasmon-assisted optofluidics¹⁵ and sensing.^{16,17}

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Monitoring nanoscale temperature variations in samples under optical illumination represents, therefore, an important issue not only for nanomedicine but also for photonics and plasmonics. Direct temperature measurements have been made by means of thermal camera analysis¹⁸ with a sensitivity of about (≈ 0.2 K); the technique possesses, however, a limited spatial resolution (10–15 μm), enabling only for a surface measurement of temperature.

So far, approaches, based on a combination of GNPs and phase transitions require, in general, embedding the GNPs in ice¹⁹ or employing suspensions of GNPs in organic solvents;²⁰ these techniques can hardly combine the advantages of reliability, fast readout rate and high-resolution, thus limiting their utility for nanoscale temperature monitoring. In this perspective, an original approach has been presented for monitoring nanoscale temperature variations



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in GNPs under optical illumination, which is based on a combination of gold nanorods (GNRs) and cholesteric liquid crystals (CLCs).^{21,22} Such a combination allows to measure temperature around GNRs, after a given illumination time, with a sensitivity of about 0.03 K. However, the heat transfer mechanism, from the “hot” GNRs to the CLCs remains still mostly unexplored, since modeling GNRs and CLCs, (that can be seen as twisted structures just like in a spring), requires complex theoretical models/numerical simulations. In this paper, a simplified approach to describe the effects of a combination of spherical GNPs and Nematic LCs (NLCs) is presented. By exploiting the symmetry of GNPs and the unidirectional order of NLCs, we have realized a theoretical model along with an experimental investigation to specifically study the heat generation and its transfer mechanism from the photo-heated GNPs to the surrounding medium (NLC).

1. OPTICAL AND PHOTO-THERMAL PROPERTIES

Liquid crystals (LCs) are well-known materials with a broad use in modern devices such as flat panel displays, light modulators, and sensors.^{23,24} Their attractive features include high optic quality and strong nonlinearity.^{25,26} The Giant Optical Nonlinearity (GON) of Nematic Liquid Crystals determines important and useful optical properties of these materials.^{27,28} This nonlinearity is due to the reorientation of the molecular director n , which is the unit vector that describes the mean orientation of the axis of the elongated NLC molecules.^{29–31} As this vector explicitly appears in the terms that describe the interaction with an external electric field, it happens that the reorientation can be also induced by the optical field of the same radiation that experiences the nonlinear propagation. Therefore, LCs are “smart materials” helpful to study the heating process at the nanoscale and they represents a new horizon for nanomaterial based applications.

These materials, exhibiting strong nonlinear response to incident light, play a vital role in the fields of photonics and optoelectronics owing to their potential in building all-optical systems that can switch or modulate light by means

of light. The anisotropic nature of LCs enhances the optical nonlinearity of these materials by up to nine orders of magnitude compared to that of isotropic fluids,^{26,32} making them suitable for building electro-optical devices. In addition, the strong nonlinear response to incident light can also be exploited to control or determine the photo-thermal properties of MNPs.³³

Cholesteric liquid crystals (CLCs) are short pitch (P) chiral materials which organize in layers with no positional ordering of the molecules within each layer, but exhibiting a mesoscopic order, described by a helicoidal orientation of the director axis from layer to layer. Due to the helix periodicity, CLCs behave as one-dimensional photonic bandgap systems where propagation of light of given wavelengths and polarization states is forbidden.³⁴ It is worth noting that the spectral characteristics (width and position) of the photonic bandgap associated with the CLC helix can be controlled by applying an electric field, or changing the system temperature,²⁹ or exposing the sample to a suitable optical radiation.³⁵

Furthermore, CLCs, when used as host materials for MNPs, allow to organise them into 2-D or 3-D arrangements, where self-organization and localized surface plasmon properties tune MNPs properties by exploiting CLCs and vice versa.^{22,36–39} When a light beam of suitable wavelength impinges on a GNP the electrons of its conductive band start to oscillate coherently with the electric field of the impinging light (the LPR). This phenomenon results in a strong absorption that is responsible for the heat production. In the absence of phase transitions, in order to predict the temperature around a GNP it is necessary to solve the heat transfer equation (Eq. 1) which derives from a balance of heat energy: the net rate of thermal energy that comes out from the GNP, plus the rate of thermal energy accumulation (internal energy variation in the GNP) must equal the net rate of thermal energy generation

$$\nabla \cdot (-k(\mathbf{r}) \nabla T(\mathbf{r}, t)) + \rho(\mathbf{r}) c(\mathbf{r}) \frac{\partial T(\mathbf{r}, t)}{\partial t} = Q(\mathbf{r}, t) \quad (1)$$

where $\mathbf{r}$ and t are the spatial and time coordinates, $T(\mathbf{r}, t)$ is the local temperature and parameters $k(\mathbf{r})$, $\rho(\mathbf{r})$, $c(\mathbf{r})$

indicate thermal conductivity, mass density and specific heat, respectively. The function $Q(\mathbf{r}, t)$ represents the energy source coming from the energy dissipation inside GNPs. At the thermal equilibrium the solution of the Eq. (1) is obtained by taking into account a heat production Q at a constant rate per unit time and per unit volume inside the spherical NP (distance $0 \leq r \leq R_{NP}$ and conductivity K_0) while in the region $r > R_{NP}$ the conductivity of the host medium is K_H and no heat production occurs.⁴⁰ Calculations yield a temperature variation

$$\Delta T = \frac{QR_{NP}^3}{3rK_H} \quad (2)$$

where the thermal energy generation Q is

$$Q = \langle \mathbf{J}(\mathbf{r}, t) \cdot \mathbf{E}(\mathbf{r}, t) \rangle_t = \frac{\varepsilon_0 \omega \Im[\chi_{NP}] |\mathbf{E}_{int}|^2}{2} \quad (3)$$

and depends on the electric field $\mathbf{E}_{int}$ inside the GNP and on the imaginary part of dielectric permeability $\Im[\chi_{NP}]$ (that describes energy dissipation of the GNP, or dielectric losses); ω is the angular frequency of light and ε_0 is the dielectric permittivity of vacuum.

The Mie theory⁴¹ provides the correct expression for the electric field $\mathbf{E}_{int}$ inside a spherical GNP. However, in our case the average size of the used GNPs ($r = 10$ nm) is much smaller than the wavelength of the used radiation ($\lambda = 532$ nm), a circumstance that allows us to use the following quasi-static formula:

$$\mathbf{E}_{int} = \frac{3\varepsilon_H}{2\varepsilon_H + \varepsilon_{NP}} \mathbf{E}_0 \quad (4)$$

where ε_{NP} is the dielectric permittivity of the spherical GNP, ε_H the dielectric permittivity of the host medium and $\mathbf{E}_0$ the amplitude of the applied electric field. Then, the resonance of the GNP corresponds to the maximum of the thermal energy production with a temperature variation at a distance r from the NP given by:

$$\Delta T = \frac{V_{NP}}{2k_H \lambda r} \frac{\Im[\chi_{NP}]}{\sqrt{\varepsilon_H}} \left| \frac{3\varepsilon_H}{2\varepsilon_H + \varepsilon_{NP}} \right|^2 I_0 \quad (5)$$

where V_{NP} is the GNP volume, λ the wavelength and I_0 the intensity of the impinging light (gaussian beam).⁴² For instance, Eq. (5) can be used to predict the temperature variation around GNPs in water under optical illumination, where it is possible to assume no refractive index change with temperature¹ variation.

2. SOFT MATTER AT WORK

2.1. Plasmonic Nanoparticles Dispersed in Nematic Liquid Crystals

2.1.1. Theoretical Model

LPRs are characterized both by induced absorption and light scattering,⁴³ processes described in terms of absorption and scattering cross-sections,⁴⁴ whose efficiencies

depend mostly on the morphology of the used nanostructures.^{45,46} In resonance conditions, spherical GNPs dissolved in water are mainly characterized by absorption when they are smaller than 90 nm,⁴⁷ while scattering processes dominate in bigger GNPs.⁴⁴ Being the increase of temperature around nanoparticles due to an absorption enhancement, it is possible to infer that, the smaller the nanoparticle, the more efficient is the heat production; still, being this last effect also proportional to the total metal volume, it can be concluded that, in case of thermal effects induced by a homogeneous distribution of NPs, it is necessary to consider physical phenomena that are much more complex than those related to the plasmonic excitation of a single nanoparticle.³³ At our knowledge, up to date investigations of photo-induced heating are related to systems containing a limited number of NPs distributed in arrays or matrices,^{48,49} or to systems with very complicated geometries, where different phenomena play their own role.⁵⁰ These studies, well known in literature, lack in theoretical accuracy when comparisons with experimental results, obtained in systems with a high number of elements, are considered. It is worth emphasizing that, in order to use gold nanoparticles for photo-thermal bio-applications, a precise prediction of the induced temperature variations in the transition from nano- to macro-scale, and vice versa, represents a fundamental condition. We have both developed a theoretical model and carried out an experimental characterization by considering spherical GNPs dispersed in a well known and commercially available thermotropic NLC (E7, by Merck).⁵¹

In order to study such a complex system, knowledge of the optical properties of the single constituents is required. NLCs are elastic and anisotropic materials that possess both dielectric^{52–56} and thermal conductivity⁵⁷ dispersion. Moreover, their optical properties (e.g., birefringence) can depend on different external parameters, such as wavelength of the impinging radiation, external electric fields, and temperature. In particular, the wavelength and temperature dependence of their refractive indices (both ordinary and extraordinary) are described by the extended Cauchy equations:⁵⁴

$$n_e(\lambda, T) = n_i(\lambda) + G'(\lambda)S(T) \quad (6)$$

$$n_o(\lambda, T) = n_i(\lambda) - \frac{G'(\lambda)S(T)}{2} \quad (7)$$

$$\Delta n(\lambda, T) = \frac{3G'(\lambda)S(T)}{2} \quad (8)$$

where $n_i(\lambda)$ is the refractive index of the isotropic state, n_o and n_e are the ordinary and extraordinary indices of the NLC respectively, $G'(\lambda)$ is a proportionality constant (insensitive to temperature) and $S(T)$ is the order parameter of the NLC. By using typical (experimental) values of the E7 NLC in Eq. (8),⁵⁶ it is possible to obtain the expressions both for n_o and n_e as a function of temperature and wavelength of the impinging radiation.

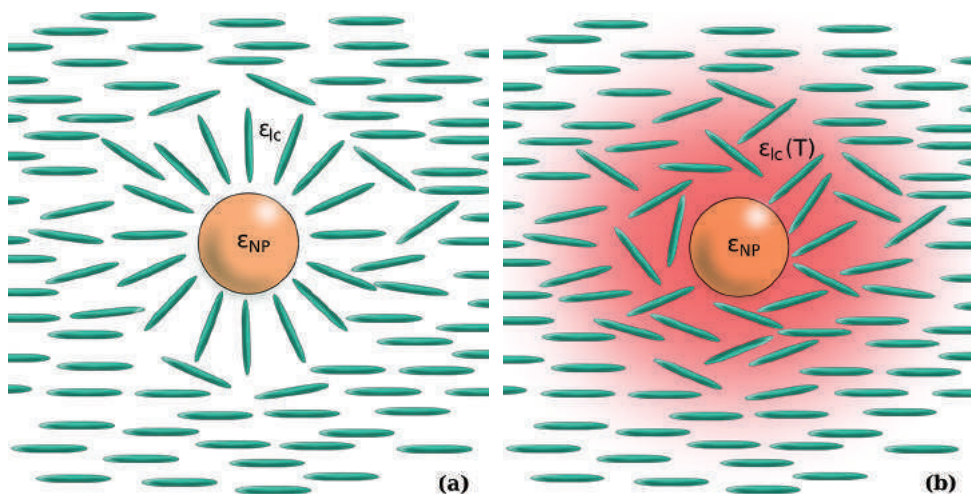


Figure 1. Sketch of the GNP dispersed in NLC: (a) Without heating, (b) with heating.

As for GNPs, an impinging resonant radiation induces an electrical current due to the onset of the LPR; then the Joule heating effect produces a temperature increase in each GNP. Consequently, this cools down by exchanging heat with the surrounding medium (NLC), which undergoes a dielectric permittivity variation, thus affecting the temperature variation of the GNP. We have implemented a numerical simulation in order to estimate both the equilibrium temperature of the system and the variation of the refractive indices of the NLC (surrounding medium). The modeled system is made of a single spherical GNP immersed in NLC (Fig. 1). By fitting the experimental values reported in Ref. [56] it is possible to obtain the Cauchy coefficients.^{53–56} These coefficients provide an equation both for n_e and n_o as functions of T and λ , that can be easily implemented by using our numerical code. The equilibrium temperature of our system has been obtained by using Eq. (5) and performing a do-while loop with Eq. (6) (the loop is closed every time ΔT is less than 0.1 K).

The polarizability α of the particle can be expressed as $\alpha = 3V_{\text{NP}}(\epsilon_{\text{NP}} - \epsilon_{\text{LC}})/(\epsilon_{\text{NP}} + 2\epsilon_{\text{LC}})$,⁴⁴ where ϵ_{NP} is the dielectric permittivity of the GNP, V_{NP} is its volume, and ϵ_{LC} is the permittivity of the NLC. Since α is directly related to the absorption cross section⁴⁴ (C_{abs}) through the equation $C_{\text{abs}} = k\Im[\alpha]$ where k is the thermal conductivity of gold. Figure 2 reports the calculation of the imaginary part of the polarizability as a function of the impinging radiation wavelength in two cases: the blue curve has been obtained by considering that the refractive index of the surrounding medium does not change (e.g., external pump beam Off, no heating); the green curve, represents $\Im(\alpha)$ when the NLC refractive index changes because of the heating (e.g., external pump beam On). In both cases, the two curves exhibit a “quasi-Lorentzian” lineshape which is very similar to the absorption spectrum of monodispersed GNPs (see experimental part for details). The observed blue shift can be explained by taking into account that the resonance condition is fulfilled (Fröhlich condition) if

$\Re[\epsilon_{\text{NP}}(\omega)] = -2\epsilon_{\text{LC}}$.⁵⁸ A modification in the value of the dielectric constant of the host material corresponds, therefore, to a tuning action on the LPR frequency. In our case, due to a heat induced decreasing of ϵ_{LC} , the Fröhlich condition is fulfilled for higher (negative) values of ϵ_{NP} . It is well known⁵⁹ that, in the visible range, the real part of the electric permittivity of GNPs increases with frequency; therefore, fulfillment of the Fröhlich condition takes place for higher values of ω . This yields a blue shift of the plasmonic absorption peak. The slight discontinuity in the curve of Figure 2 is due to a discontinuity in the fitting procedure that reproduces the effects of heating. This highlights how important the active role of liquid crystal is and how interesting the study of this system are.

Figure 3 shows a map of the dielectric anisotropy $\Delta n = n_e - n_o$ versus the impinging light wavelength and intensity. The dark area highlights that a remarkable variation of Δn occurs only nearby the LPR frequency, where the absorption (see Fig. 2) and, therefore, the temperature variation of the system exhibit their maximum. Details of the

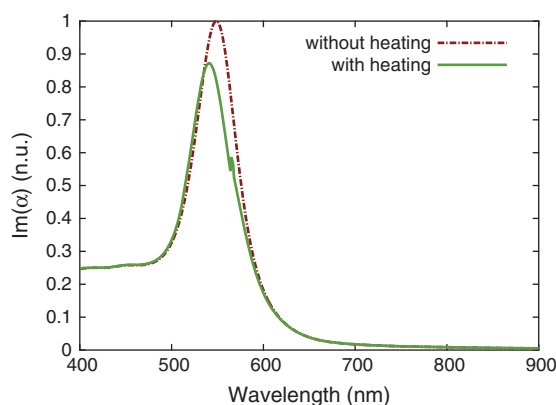


Figure 2. Imaginary part of the polarizability of the system (GNP + NLC) as a function of wavelength of the impinging radiation. Reprinted with permission from [33], L. Pezzi, et al., *PCCP* 17, 20281 (2015). © 2015, RSC.

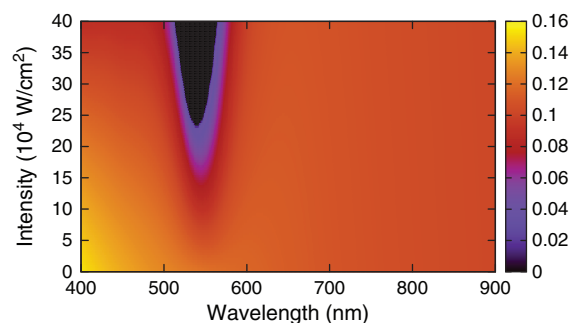


Figure 3. Map of the NLC birefringence as a function of light wavelength and intensity. Reprinted with permission from [33], L. Pezzi, et al., *PCCP* 17, 20281 (2015). © 2015, RSC.

fitting procedure (including parameters) are reported in Ref. [60]. Figure 4 shows a map of the temperature variation as a function of light intensity and wavelength. All above reported theoretical predictions have been validated by means of an experimental analysis reported in the next section.

2.1.2. Experimental Results

Spherical cetyltrimethylammonium chloride (CTAC)-capped GNPs have been synthesized in aqueous solution and subsequently transferred in chloroform, which is a solvent (dispersing medium) also for LCs. The general protocol for seed-mediated synthesis of GNPs and their transfer from water to chloroform is described in details in Ref. [33]. Such an approach makes GNPs stabilized in aqueous solution thanks to a bilayer of CTAC surfactant capping the particles, thus conferring them surface positive charge. GNPs can be successfully extracted into the organic phase by decanoic acid, which electrostatically binds the CTAC bilayer by means of deprotonated carboxylic group in basic pH conditions; the alkyl group, on the other side, makes GNPs dispersible in chloroform.²² The spectral response of GNPs, after the phase transfer in organic medium, has been recorded by using an unpolarised light source in the 400–800 nm range. The spectrum, reported in Figure 5, shows the LPR band, centered at 526 nm, that is typical of a solution of well

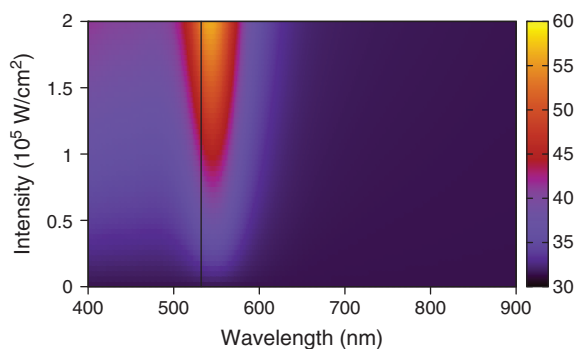


Figure 4. Map of the temperature as a function of light wavelength and intensity.

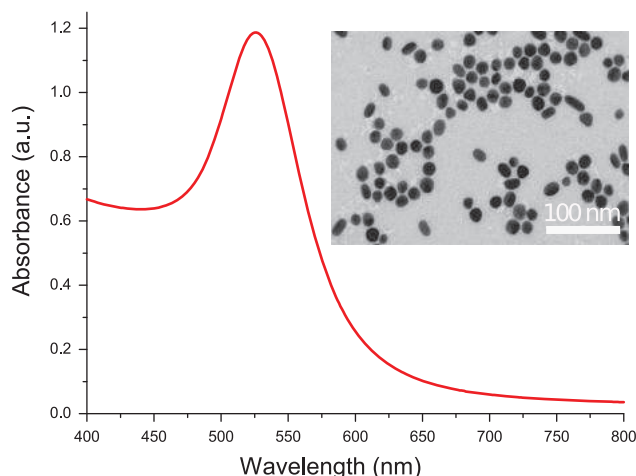


Figure 5. UV-vis absorption spectrum of GNPs dispersed in chloroform and their TEM image (inset). Reprinted with permission from [33], L. Pezzi, et al., *PCCP* 17, 20281 (2015). © 2015, RSC.

mono-dispersed GNPs. Transmission Electron Microscope (TEM) analysis has been performed by a Jeol JEM-1011 microscope operating at 100 kV. The specimens have been prepared by depositing a drop of GNPs dispersion onto a carbon-coated copper grid and then allowing the solvent to evaporate. For a statistical determination of the average GNP size, about 200 objects have been counted. The TEM image of GNPs, shown in Figure 5 (inset), confirms that the particle population consists of GNPs with 17.4 ± 2.2 nm diameter; size and shape remain unchanged after phase transfer from the aqueous to the organic medium. Homogeneous mixtures of GNPs up to 6% in weight in NLC (MDA-00-1444, by Licristal) have been obtained; however, above 4 wt%, the order parameter of the NLC phase is affected by the presence of GNPs as shown by an observed dramatic drop of the birefringence value. On the other hand, for low concentrations, GNPs act like impurities and have the capability to destabilize the LC phase by lowering the transition temperature (from nematic to isotropic) without affecting the nematic phase. In our experiments we have observed that, for a concentration of GNPs as low as 3 wt%, there is a reduction of the transition temperature of about 2 K (from 336 K to 334 K). Thus a 3 wt% concentration of GNPs represents an optimized one for having, simultaneously, no significant change in the LC birefringence and a small variation in the nematic to isotropic transition temperature. For the aforementioned reasons, we have used the mixture with the highest concentration of GNPs (3 wt%) which does not affect considerably the NLC phase. ITO coated glass substrates have been treated with a polyimide layer and rubbed for obtaining a planar orientation of the director of the NLC that will be deposited on it. A cell with a cell gap has been filled with the NLC/GNPs mixture by capillary action at room temperature (about 296 K); the LC is in the nematic phase and its

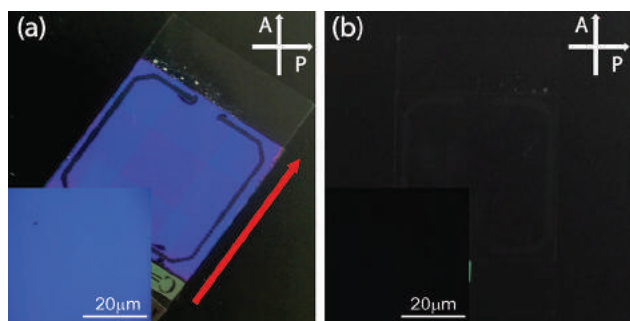


Figure 6. Pictures of the sample between crossed polarizers (a, b) along with their POM view. The director of the NLC is aligned at 45° in (a) and at 0° in (b). The red arrow represents the rubbing direction. Reprinted with permission from [33], L. Pezzi, et al., *PCCP* 17, 20281 (2015). © 2015, RSC.

director is oriented parallel to the substrates along the rubbing direction (see red arrow in Fig. 6(a)). The excellent optical quality of the sample is evident in the polarizing optical photographs shown in Figure 6. The optical contrast between bright (Fig. 6(a)) and dark (Fig. 6(b)) states is higher than 40:1 for white non collimated light, and indicates an excellent NLC alignment. To check the spatial uniformity of this alignment, we have performed optical observations by means of a Polarized Optical Microscope (POM) equipped with a CCD color camera connected to a PC; results are reported in the insets of Figures 6(a), (b). The uniform change in contrast of the whole area (from Figs. 6 (a to b)) suggests the NLC director alignment has not been macroscopically affected by the presence of GNPs, which are well dispersed in the NLC. Indeed, no NLC defects are visible in the POM view of the sample (insets of Figs. 6(a, b)). It is worth mentioning that, due to the low concentration (3 wt%) of GNPs dispersed in the thin (2.7 μm) NLC layer, the spectral analysis of the sample did not show any observable absorption peak (LPR) related to the presence of the GNPs. However, the spectral analysis (not shown here) performed on the vial of GNPs/NLC mixture exhibits the same behavior reported in Figure 2. Experiments devoted to investigate the influence of the local heating induced by a suitable optical radiation (through the GNPs resonance) on the NLC director alignment have been performed by using the all-optical setup reported in Figure 7.

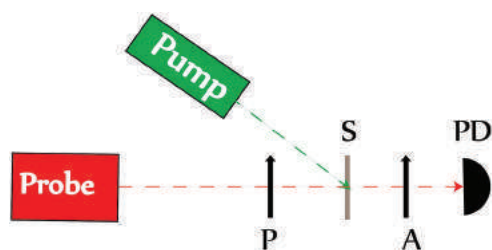


Figure 7. All-optical setup for sample characterization. P: polarizer; S: sample; A: analyzer; PD: photodetector.

The system used a low power density ($P_{\text{probe}} = 0.2 \text{ W/cm}^2$) CW probe laser, emitting at $\lambda = 633 \text{ nm}$ and a CW pump laser emitting at $\lambda = 532 \text{ nm}$, in the high absorption spectral range of GNPs (Fig. 5). For sake of simplicity, an unfocused pump beam, which impinges on the sample with an oval shape of about $(2 \times 3) \text{ mm}^2$, has been used. The photo-thermal response of the sample has been observed between crossed polarizers, with the optical axis of the sample (NLC director orientation) set at 45° with respect to the polarizer/analyzer axes. Under this condition, the sample acts as a retardation plate (introduced phase retardation of 3.8 rad at 532 nm), and the transmitted intensity can be detected by a photo-detector. Figure 8(a) reports the probe intensity transmitted by the system, detected for different values of the pump light intensity (from 0.4 W/cm^2 to 3.1 W/cm^2 , with probe power density ($P_{\text{probe}} = 0.2 \text{ W/cm}^2$) kept constant. By optically pumping the same probed sample area, the photoexcitation

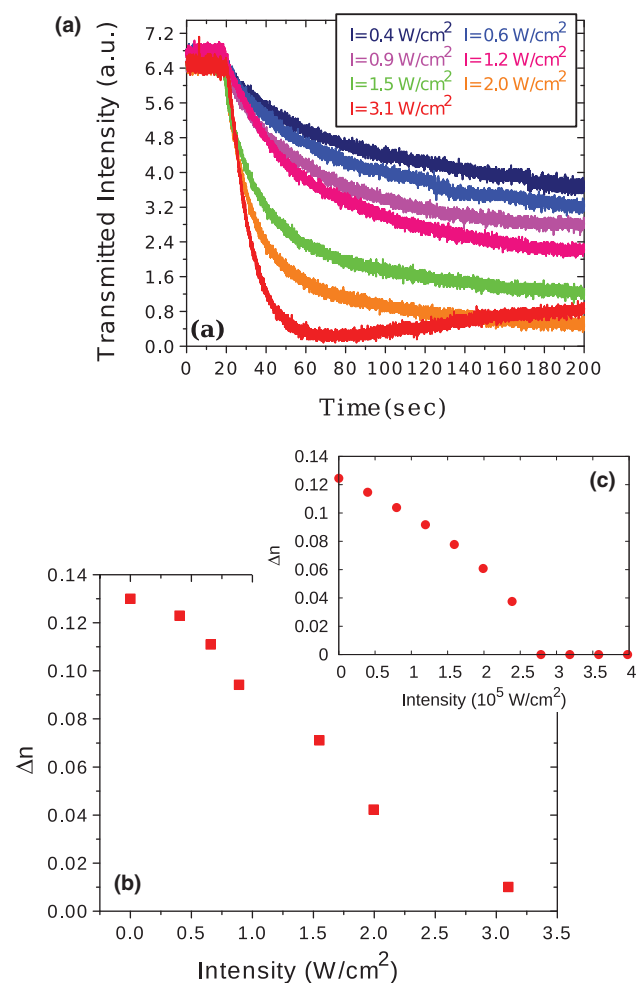


Figure 8. Transmitted intensity versus time for different intensity values of the pump radiation (a). Birefringence of the sample versus the intensity of the pump radiation ($\lambda = 532 \text{ nm}$) (b); calculated birefringence versus the intensity radiation for a specific wavelength ($\lambda = 532 \text{ nm}$) (c). Reprinted with permission from [33], L. Pezzi, et al., *PCCP* 17, 20281 (2015). © 2015, RSC.

of GNPs induces an electric-driven Joule heating, with a consequent energy exchange with the surrounding NLC. Such a local-heating induces a gradual suppression of the transmitted intensity, which is due to an induced reduction of the birefringence (Δn) of the sample. In fact, this has been calculated by using the Jones matrix formalism and exploiting the contrast between the steady state intensity transmitted by the sample with its optical axis at 45° with the crossed polarizers, and the steady state intensity transmitted by the sample with its optical axis at $0^\circ/90^\circ$ with the crossed polarizers.⁶¹ Figure 8(b) shows that Δn values vary from 0.135 down to almost zero. The initial value of Δn is lower than in the pure NLC, where its value is 0.197; this can be explained by taking into account that the NLC order parameter is slightly affected by the presence of GNPs, which may act as impurities, thus locally modifying (at the molecular scale) the NLC molecular order. It is worth noting that pump-probe experiments (not reported here) performed on a “reference” pure NLC cell of the same thickness, show that no variations in the transmitted probe intensity are detected, neither when the sample is acted on by the highest value of the pump beam intensity (3.1 W/cm^2).

We also have performed a control experiment by detecting the transmittance as a function of the sample temperature of both the “reference” pure NLC cell, and our NLC/GNPs cell placed (inside a hot stage) between crossed polarizers, with the optical axis of the sample set at 45° with the polarizer/analyzer axes. In both cases, as the sample temperature was increased, the transmitted intensity decreased towards a minimum which was almost zero. In addition, curves show that, in the reference cell, the pure NLC undergoes transition from nematic to isotropic at $\sim 338 \text{ K}$; on the other hand, in the cell containing NLC and dispersed GNPs, these ones act as a destabilizer perturbation for the NLC, lowering its transition temperature of 10 K , to $\sim 328 \text{ K}$. Figure 8(c) shows the Δn behavior predicted by our theoretical model: both experimental and theoretical results qualitatively exhibit the same behavior, while, from a quantitative point of view there is a discrepancy between the experimental and the theoretical photo-heating intensity needed to induce the observed effect. From curves of Figure 8 it is clear that, starting from 3.1 W/cm^2 , new phenomena take place, that are related to a nonlinear response of the liquid crystal and give rise to effects that are preeminent with respect to the thermal one. At higher enough intensities, the transition of the LC to the isotropic phase takes place and the transmittance becomes zero. Those effects evolve with longer timescales and are out of the interest of the present work.^{62,63}

To further validate our model and verify that the NLC does not play any role in the photo-heating conversion, we have performed a control experiment with the sample made of pure NLC. The comparison has been performed by means of a thermographic analysis (thermocamera

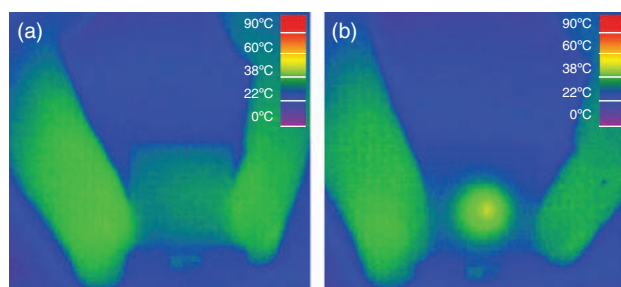


Figure 9. Thermographic analysis of the sample under optical irradiation for the pure NLC (a) and NLC/GNPs (b) cell. Reprinted with permission from [33], L. Pezzi, et al., *PCCP* 17, 20281 (2015). © 2015, RSC.

sensitivity $\approx 0.5 \text{ K}$) both of pure NLC and NLC/GNPs samples, under optical illumination. Experiments have been realized by keeping operator's hand fingers ($T \approx 310 \text{ K}$) close to the sample, in order to have a comparison setpoint: Figure 9(a) shows that the thermocamera does not detect any color change (that is “temperature variation”) for the pure NLC sample ($I = 3.1 \text{ W/cm}^2$; illumination time = 300 s). On the contrary, in the same experimental condition, the thermographic analysis realized on the GNPs/NLC sample (Fig. 9(b)) shows that the surface of the illuminated area has been heated at a temperature of about 333 K (close to the nematic to isotropic transition of the NLC), meanwhile the setpoint is kept at almost the same previous temperature ($T \approx 310 \text{ K}$). This result is an incontrovertible experimental evidence that the photo-heating process is due to the photoexcitation of GNPs, with a consequent heat exchange with the surrounding NLC medium.

Finally, we have studied the dynamics of the system. A switching behavior which turns out to be reversible and repeatable, is observed when a sequence of pump

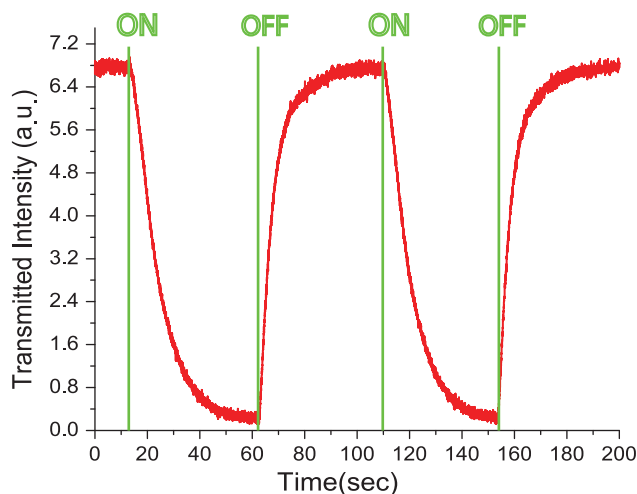


Figure 10. Switching behavior observed by using a periodic sequence of pump beam pulses. ON and OFF refer to the pump beam being allowed to impinge on the sample or not. Reprinted with permission from [33], L. Pezzi, et al., *PCCP* 17, 20281 (2015). © 2015, RSC.

beam pulses, obtained by utilizing an electronic shutter, is allowed to impinge onto the sample, put between crossed polarizers, with its optical axis forming an angle of 45° both with the polarizer and the analyzer axes. Figure 10 shows that, when the pump is switched on ($I = 3.1 \text{ W/cm}^2$), a photoexcitation of GNPs occurs, with a consequent phase transition of the NLC from the nematic to the isotropic state; thus between crossed polarizers the probe light experiences just the isotropic refractive index ($n_{\text{iso}} = 1.56$) of the NLC, and the transmitted intensity drops to zero ($\tau_{\text{off-on}} = 40 \text{ s}$). When the green pump beam is switched off, the NLC cools down and the Isotropic to Nematic transition occurs, thus restoring the sample birefringence, and, therefore, a transmitted light intensity ($\tau_{\text{on-off}} = 20 \text{ s}$).

2.2. Plasmonic Nanoparticles Dispersed in Cholesteric Liquid Crystals: Plasmonic Thermometers

Monitoring temperature variations under optical illumination is an important issue not only for nano-medicine but also for photonics, electronics and plasmonics.⁶⁴ We have already seen that a direct temperature measurement under a suitable optical irradiation in plasmonic nanomaterials can be obtained by means of thermal camera analysis.¹⁸ Despite its high sensitivity ($\sim 0.2 \text{ K}$), the technique possesses a limited spatial resolution ($10\text{--}15 \mu\text{m}$) enabling only surface measurement of the temperature. In a different approach, by means of a scanning thermal microscope (using a modified atomic force microscope to map temperatures), it has

been obtained a very high resolution measurement of the temperature by exploiting a 50 nm tip in contact with the sample.⁶⁵ Imaging techniques that do not require any direct contact with the sample have been recently developed, based on optical methods including Raman spectroscopy, fluorescence intensity or fluorescence time correlation. Baffou et al.⁶⁶ developed a new optical technique which delivers real-time, detailed images based on fluorescence polarization anisotropy. Okabe et al.⁶⁷ reported intracellular mapping of temperature by measuring the excited state lifetime of a water-sensitive methacrylamide (DBD-AA) carried by a small ($8\text{--}9 \text{ nm}$) fluorescent polymeric nanogel. The temperature resolution was $\sim 0.4 \text{ K}$ and calibration was performed in extracts of COS7 (fibroblast-like) cells. The results did not appear to depend on the probe concentration, proteins in the extracts, and viscosity of the medium. As a matter of fact, none of these techniques combines the desired features of reliability, fast readout rate and high-resolution.

Recently, De Sio et al.²² demonstrated the possibility of monitoring nanoscale temperature variations under optical illumination by combining CLCs with Au nanorods. CLCs are thermoresponsive materials and can respond to a wide variety of external stimuli (AC, DC, optical fields and pressure). The helical, macroscopic ordering of the molecules due to molecular chiral interactions between liquid crystal molecules leads to strong nascent coloration caused by structural interference. Small changes to this

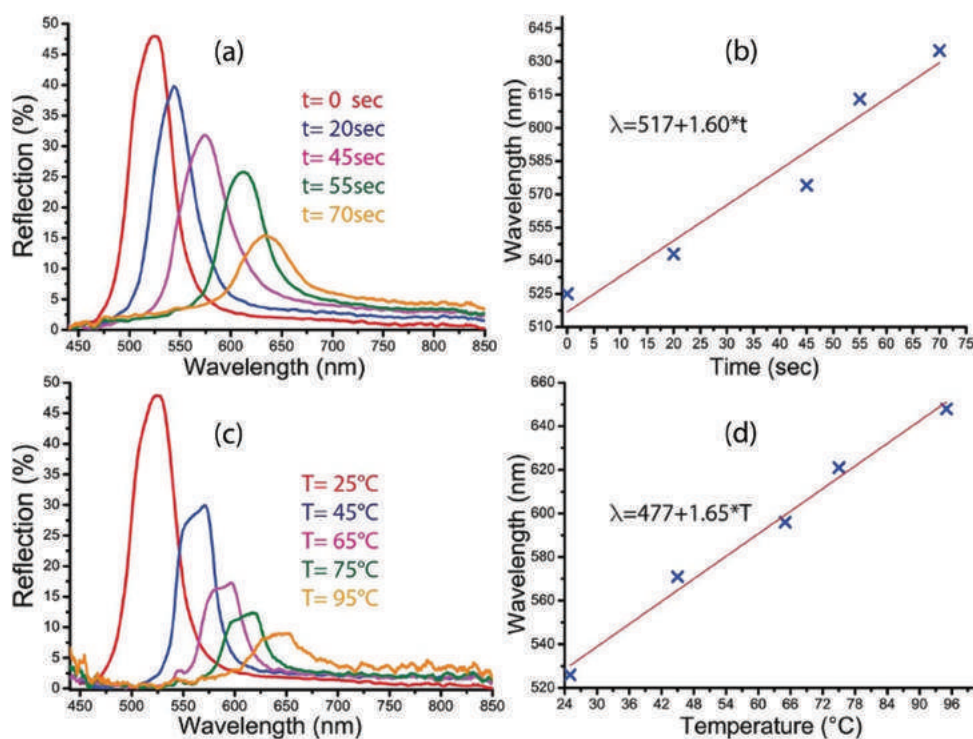


Figure 11. Reflection spectra of the sample for different values of illumination time (a) and temperature (c); linear fit of the position of the center of the reflection band versus illumination time (b) and temperature (d). Reprinted with permission from [22], L. De Sio, et al., *Advanced Optical Materials* 1, 899 (2013). © 2013, Wiley.

mesoscopic helical ordering results in noticeable differences in the local color of the film. CLCs also exhibit the unique feature that they reflect circularly polarized light when it is incident along the helical axis.

In the experiment, cetyltrimethylammonium bromide capped, water dispersible gold nanorods (GNRs) were synthesized and subsequently transferred in chloroform. 100 μm thick glass cells were filled at room temperature by capillary action with a homogeneous solution of GNRs (10 wt%) mixed with a CLCs fluid (helix pitch of about 400 nm). The CLC has been prepared by twisting a NLC (MDA-00-1444, by Licristal) by inclusion of 20% of a chiral agent (ZLI-811, by Licristal), the spectral reflection of the CLC configuration has been analyzed by probing with a white light source while irradiating the thin film with continuous wave near-infrared pump laser emitting at $\lambda = 680\text{ nm}$ ($P_{\text{pump}} = 0.2\text{ W/cm}^2$), which falls in the high absorption range of the GNRs (longitudinal band). Figure 11(a) reports the behavior of the reflection band under illumination with the pump beam, for different exposure times (from 0 sec up to 75 sec). When the pump beam is off, the film acts as a mirror for all the wavelengths within the reflection band of the impinging white light (thus reflection intensity is high). Optically pumping the same sample area photoexcites the GNRs that induces conversion of light to heat, which is subsequently transferred to the CLC fluid. Figure 11(a) shows that keeping the pump power constant and increasing the exposure time results in a gradual increase of the local temperature as indicated by a linear red shift (more than 130 nm, Fig. 11(b)) of the reflection peak wavelength and partial suppression of the reflection band intensity. This is confirmed in a control experiment where the sample temperature was raised from 298 K to 368 K (Fig. 11(c)) and a clear linear red-shift in the peak wavelength was observed. It is worth noting that the two calibration functions reported in Figures 11(b) and (d) exhibit (within the experimental error), the same linear behavior indicating it is possible to measure the temperature around Au nanorods with a sensitivity of about 0.03 K. In the light of nanomedicine application, De Sio et al.⁶⁸ have exploited a combination of plasmonic nanomaterials and deoxyribonucleic acid (DNA) by bioconjugating Au nanorods with DNA via electrostatic interaction. Photo-heating experiments realized through a bio-transparent optical radiation (800 nm) demonstrated that the system represents an accurate temperature sensor able to monitor heating variation at the nanoscale with very high sensitivity (0.02 K).

3. CONCLUSION

We have reported an investigation of the nanoscale heat propagation in a system consisting of photo-excited GNRs surrounded by thermo-sensitive NLC and CLC. A theoretical model, based on the thermal heating equation, has been implemented, which takes into account the asymmetric shape of NLC molecules. Experiments have been

realized by dissolving a suitable amount of GNRs in NLC. The well aligned hybrid system (NLC/GNRs) exhibits a photo-thermal sensitivity, and experimental results show a strong change in the value of the refractive index of the NLC when GNRs are photo-heated by a suitable (resonant) optical radiation. A thermographic comparison between the hybrid system and the pure NLC, carried out in the same experimental conditions, shows that the photo-thermal conversion is only due to the LPR mechanism. In addition, the photo-thermal effect, induced by the presence of a NIR selective plasmonic resonance, can be exploited for controlling the position of the selective reflection exhibited by the CLC configuration. Furthermore, the local heating induces a variation of the surrounding medium refractive index, with a consequent shift of the plasmonic resonance. This synergy between plasmonics and photonics can be profitably used for building up a method for detecting temperature around NPs under optical illumination. Finally, in our opinion, the developed method opens up prospects for realizing, in the future, a nanomedicine tool, useful to avoid invasive treatments (e.g., hypothermic effects) on patients during therapy treatments. In the light of nanomedicine applications, we are considering to extend our experiments to other liquid crystalline materials having different thermo-optical properties. As such, it will be possible to generate several calibration curves (e.g., temperature vs. pump power density) for different materials. The obtained results will be used as a testloop in order to validate the effectiveness of the generalized theoretical model based on the thermal heating equation. Once the theoretical model has been validated for several, well known, materials, it will be possible to predict temperature variations under a suitable optical illumination in different bioenvironments (e.g., DNA, cells, tissues, etc.) containing plasmonic nanoparticles.

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