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14. ABSTRACT
Antimicrobial coatings are important for healthcare, specifically focusing on wound healing and preventive measures of infection and harm from biological agents. Synthetic materials that are capable of serving as antimicrobial coatings have received much attention for their ability to be synthesized in large scale, as opposed to naturally occurring and synthetic polypeptides, that must be extracted and purified from biological sources or require extensive synthetic steps. To overcome this challenge we propose to develop a platform of polyelectrolyte nanoparticles based on a new class of cationic monomers to serve as antimicrobial/biocide coatings. These

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Report Title

Final Report: Synthetic Nanoparticles as Antimicrobial Surface Modifiers

ABSTRACT

Antimicrobial coatings are important for healthcare, specifically focusing on wound healing and preventive measures of infection and harm from biological agents. Synthetic materials that are capable of serving as antimicrobial coatings have received much attention for their ability to be synthesized in large scale, as opposed to naturally occurring and synthetic polypeptides, that must be extracted and purified from biological sources or require extensive synthetic steps. To overcome this challenge we propose to develop a platform of polyelectrolyte nanoparticles based on a new class of cationic monomers to serve as antimicrobial/biocide coatings. These materials have the added benefit of being applicable as smart coatings, thermal photonic lattices, anti-reflective coatings for solar panels, and for various interfacial modifiers. We will focus on systems that are modular in order impact other areas of materials research in future studies.

Enter List of papers submitted or published that acknowledge ARO support from the start of the project to the date of this printing. List the papers, including journal references, in the following categories:

(a) Papers published in peer-reviewed journals (N/A for none)

Received		Paper
06/17/2016	1.00	Kato L. Killops, Spencer D. Brucks, Kourtney L. Rutkowski, Jessica L. Freyer, Yivan Jiang, Erica R. Valdes, Luis M. Campos. Synthesis of Robust Surface-Charged Nanoparticles Based on Cyclopropenium Ions, Macromolecules, (04 2015): 2519. doi: 10.1021/acs.macromol.5b00403
TOTAL:	**1**	

Number of Papers published in peer-reviewed journals:

(b) Papers published in non-peer-reviewed journals (N/A for none)

Received		Paper
TOTAL:		

Number of Papers published in non peer-reviewed journals:

(c) Presentations

Number of Presentations: 0.00

Non Peer-Reviewed Conference Proceeding publications (other than abstracts):

Received Paper

TOTAL:

Number of Non Peer-Reviewed Conference Proceeding publications (other than abstracts):

Peer-Reviewed Conference Proceeding publications (other than abstracts):

Received Paper

TOTAL:

Number of Peer-Reviewed Conference Proceeding publications (other than abstracts):

(d) Manuscripts

Received Paper

TOTAL:

Number of Manuscripts:

Books

Received Book

TOTAL:

Received	Book Chapter

TOTAL:

Patents Submitted

Campos, L. M.; Killops, K. L.; Brucks, S. D.; Freyer, J. L.; Jiang, Y. "Materials for Gene Delivery to Cancer Cell Lines that are Difficult to Transfect." 2015, U. S. Provisional Patent Application, 61/169859

Patents Awarded

Awards

2015 Office of Naval Research Young Investigator Award
2015 Cottrell Scholar Award
2015 POC: Early Excellence feature by the Journal of Physical Organic Chemistry
2014 NSF CAREER Award

Graduate Students

NAME	PERCENT_SUPPORTED	Discipline
Jessica Freyer	0.30	
Spencer Brucks	0.30	
Sebastian Russell	0.30	
FTE Equivalent:	**0.90**	
Total Number:	**3**	

Names of Post Doctorates

NAME	PERCENT_SUPPORTED
FTE Equivalent:	
Total Number:	

Names of Faculty Supported

NAME	PERCENT_SUPPORTED	National Academy Member
Luis M. Campos	0.00	
FTE Equivalent:	**0.00**	
Total Number:	**1**	

Names of Under Graduate students supported

NAME	PERCENT_SUPPORTED

FTE Equivalent:
Total Number:

Student Metrics

This section only applies to graduating undergraduates supported by this agreement in this reporting period

The number of undergraduates funded by this agreement who graduated during this period:	0.00
The number of undergraduates funded by this agreement who graduated during this period with a degree in science, mathematics, engineering, or technology fields:.....	0.00
The number of undergraduates funded by your agreement who graduated during this period and will continue to pursue a graduate or Ph.D. degree in science, mathematics, engineering, or technology fields:.....	0.00
Number of graduating undergraduates who achieved a 3.5 GPA to 4.0 (4.0 max scale):.....	0.00
Number of graduating undergraduates funded by a DoD funded Center of Excellence grant for Education, Research and Engineering:.....	0.00
The number of undergraduates funded by your agreement who graduated during this period and intend to work for the Department of Defense	0.00
The number of undergraduates funded by your agreement who graduated during this period and will receive scholarships or fellowships for further studies in science, mathematics, engineering or technology fields:	0.00

Names of Personnel receiving masters degrees

NAME

Total Number:

Names of personnel receiving PHDs

NAME

Total Number:

Names of other research staff

NAME	PERCENT_SUPPORTED

FTE Equivalent:
Total Number:

Sub Contractors (DD882)

Inventions (DD882)

Scientific Progress

The project deals with the formation of polymer-nucleic acid complexes for gene therapy. The synthesis, conjugation, and delivery of cationic polyplexes based on linear polymers and latex particles to cells are included. This research is applicable to a range of fields including gene delivery, drug delivery, diagnostics, enzyme stabilization, therapeutics, filtration/separation, cosmetics, and imaging, among others.

Brief Description: We studied the synthesis of positively-charged linear homopolymers and block copolymers by living free radical polymerization, and polymer-based particles by emulsion polymerization. The polymers and particles can be conjugated with a wide range of biomolecules, primarily by electrostatic interactions. Additionally, cargo (i.e. drug molecules, contrast agents, dyes, etc.) can be loaded into the interior of the particles prior to polymerization. These conjugated and/or labeled polymers and particles can be delivered to cells to administer their cargo and achieve some therapeutic response.

Background: Cationic nanoparticles are of interest in a diverse range of fields from gene-based therapeutics to cosmetics to drug delivery. The preparation of polymer-based cationic particles can be achieved by a number of different strategies including aggregation and crosslinking of linear polymers, formation and crosslinking of micelles, and polymerization under confinement as in an emulsion.

Cationic polymers and particles have been envisaged as substrates or carriers to deliver a biological or chemical cargo to cells in vivo via complexation to the cargo. Specifically, the development of polymer-based cationic particles has focused on their use as non-viral vectors for gene therapy. Often, a carrier is needed to deliver the genes through the cell's cytoplasm and help protect them from enzymatic degradation. The positive charge exuded by the polymers/particles makes them well-positioned for electrostatic binding to negatively charged polynucleic acids (PNA: DNA, mRNA, siRNA, etc.). Many current technologies containing positively charged groups are derived from pH sensitive protonated or quaternated amines. Therefore, fluctuations in pH may cause the charged group to become deprotonated, affecting the colloidal stability and attachment of the cargo. The delivery of genetic material to cells to regulate genetic expression or interfere with unfavorable cellular processes is highly desirable for next-generation therapeutics. The opportunity for low cost and tunable structures afforded by a polymer-based gene transfection platform is highly advantageous for the advancement and accessibility of genetic therapeutics.

The ability of a substrate to deliver genetic cargo to cells is contingent on the stabilization of the PNA imparted by the particle and the inclusion of targeting moieties to deliver the cargo to the cells of interest. Current non-viral gene transfection agents include cationic lipids (Lipofectamine, iFect), polyamines (poly(L-lysine), PEI, PAMAM), and polysaccharides (chitosan), among others.

Results: During the period of this grant, we studied the synthesis, conjugation, and delivery of cyclopropenium-based polyplexes to cells. A polymerizable form of cyclopropenium has been previously patented.11 A unique feature of this cationic moiety is its relative insensitivity to fluctuations in pH. Specifically, this invention relates to the use of these cationic polymers/nanoparticles to bind to biomolecules of interest and deliver them to cells. Without the particles, the biomolecules of interest could be degraded or rapidly cleared in vivo without ever reaching their target. The particles may also contain drug or dye molecules of interest in their interior, or at the periphery of the particles. Targeting groups, such as peptides, may be incorporated to direct the particles to certain areas in vivo.

Homopolymers, random copolymers, and block copolymers comprising cyclopropenium have been synthesized using controlled reversible-deactivation radical polymerization techniques. The degree of polymerization (i.e. molecular weight) and incorporation of various components can be precisely controlled. Cyclopropenium-based polymers have been shown to bind DNA effectively, as shown by gel electrophoresis studies. The polyplexes formed display a zeta potential near zero and a size of ca. 200 nm by dynamic light scattering. DNA has been shown to bind to cyclopropenium-based polymers at ratios of 1:1 of nucleic acid units to cyclopropenium monomer units.

The styrenic cyclopropenium moiety has been found to act as an amphiphilic stabilizer for oil-in-water emulsions. Thus, it has been used herein to stabilize an emulsion of styrene in water, and with the addition of a radical initiator and heat, polymerization of the stabilized styrene droplets can occur leading to the formation of latex particles comprised of polystyrene and polycyclopropenium. By changing the R groups on the cyclopropenium monomer, different polarities can be achieved. Furthermore, the use of an amphiphilic block copolymer (BCP) containing cyclopropenium groups in one of the blocks can also be used to effectively stabilize the emulsion, and incorporated into stable latex particles.

The as-synthesized particles display a highly positive surface charge, as measured by zeta potential. This positive charge is maintained over a wide range of pH values. The positively charged periphery is then used to bind molecules of interest, such as PNA. Conjugating biomolecules to the particles could have some stabilizing effect that resists cleavage or denaturation. By appending these biomolecules to a polymer-based support, they may be more effectively delivered in vivo. The particle-based platform can be tailored for different applications by incorporating dyes for tracking or peptides for targeting.

As an example, the emulsion polymerization of particles proceeds as follows: the cyclopropenium monomer is weighed out into a vial. To the vial is added styrene monomer, and the two monomers are mixed to dissolve the cyclopropenium monomer. In a separate vial, a water soluble azo initiator, 2,2′-azobis(2-methylpropionamidine) dihydrochloride, is dissolved in a small amount of water. Water is added to the vial containing monomers so that they constitute 10 wt.% of the total weight of the reaction. The initiator solution is added to the monomers, and the vial is vortexed to emulsify. The emulsion is added to a round bottom flask fitted with a condenser and stirbar, and the reaction mixture is stirred and sparged with inert gas for 10 minutes. The vessel is then sealed and heated at 70°C for 6-18h. Particle size is determined by dynamic light scattering and scanning probe microscopy, scanning electron microscopy, or transmission electron microscopy. Electrophoretic potential measurements

were conducted to determine the zeta potential of the particle solution.

As an example of DNA complexation to the particles, the particle solution was simply mixed with DNA at different ratios to match the approximate number of charged groups on the surface of the particle to the number of phosphate units in the DNA backbone. The extent of complexation was determined by gel electrophoresis and DLS/zeta potential measurements.

A distinguishing feature of these polymer-based systems for gene therapy application is their synthetic versatility. The charge exuded by the polymers/nanoparticles can easily be tuned by adding a comonomer at a certain ratio. Furthermore, the hydrophilicity can be altered in several ways, including through the incorporation of comonomers or via the use of different R-substituents on the cyclopropenium monomers themselves. The molecular weight of the synthetic polymers can also be precisely dialed in, which affects their solution aggregation, and thus the final size of the resulting polyplexes with PNAs. The flexibility of these properties can enable the tuning of the transfection ability while maintaining low cytotoxicity. The incorporation of targeting moieties can easily be achieved by incorporating them into the monomer feed or through post-polymerization modification. Additionally, the particle synthesis provides the opportunity to tune the size of the nanostructures simply by changing the incorporation of the cyclopropenium monomer incorporation. We have synthesized cationic particles in the size range of 140 nm all the way down to 30 nm, which could prove useful for targeting different cell types or addressing various biological cargoes. Along these lines, synthesizing the particle in the presence of BCP stabilizers leads to particles displaying a more densely packed core surrounded by chains extending into solution forming a polymer corona. This corona might serve to protect the nucleic acid cargo from degradation or premature release in vivo.

Technology Transfer