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RPPR Final Report
as of 06-May-2021

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Proposal Number: 64035MS **Agreement Number: W911NF-13-1-0489**
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Title: Four-dimensional Printing: Design, Assembly, and Modeling of Responsive, Temporally Programmable Materials

Begin Performance Period: 01-Oct-2013 **End Performance Period:** 31-Oct-2017
Report Term: 0-Other

Submitted By: Anna Balazs Email: balazs@pitt.edu
Phone: (412) 648-9250

STEM Degrees: **STEM Participants:**

Major Goals: Our goal is to develop unprecedented capabilities in responsive materials via 4D printing: constructing complex objects through 3D direct writing using soft stimuli-responsive inks, which allow us to "program" the temporal behavior of the resulting structures. Hence, we are controlling not only the spatial dimensions of the sample, but also introducing and controlling the time-dependent behavior of the system. In effect, we are patterning in space and time. We will specifically focus on inks that integrate stimuli-responsive gels with stimuli-responsive, functionalized nanofibers, and in this manner, attempt to create composites with exceptional reconfigurability. The gels and fibers will be designed to respond to distinctly different external stimuli or to the same stimulus in a distinctly different manner. Within these composites, the gel will impart flexibility, while the high-modulus nanofibers will provide the necessary strength. The proposed research addresses the critical challenge of creating strong, temporally programmable materials that can effectively self-reconfigure in response to different external cues and thus, exhibit different functionality in different environments.

Accomplishments: (1) We have demonstrated a new approach for fabricating complex shape changing architectures that combines biomimetic 4D printing with a simple theoretical model for predicting printing tool-path planning. Our inspiration is nastic plant movement, wherein plants use a combination of turgor that varies in space and time, and material properties to generate organ movement in tendrils, leaves, flowers, etc. via shape changes on slow and fast time scales.

We have created an artificial analog composed of biomimetic hydrogel composite ink that contains highly anisotropic cellulose fibrils, whose local orientation can be programmably defined in space and time via 4D printing. This allows us encode the printed architectures with localized, anisotropic stiffness and, hence, swelling behavior controlled by the alignment of cellulose fibrils along prescribed printing pathways. When combined with a method of

solving the inverse problem of determining the printing pathway, which we also introduce, we show how to create complex reconfigurable architectures that may find potential application in smart textiles, scaffolds for tissue engineering, drug delivery, soft robotics and beyond.

(2) We carried out a study of hydrogel materials, and the 3D-printable inks derived from them, that can be chemically modified for use as 4D scaffolds in cellular microcultures. We specifically examined the physicochemical outcomes of incorporating poly(2-hydroxyethyl-methacrylate) (pHEMA) homopolymers into printable pre-polymer mixtures that, once cured, require modification with the 30 kDa protein poly-?-lysine (PLL, which absorbs irreversibly) to render them biologically compliant as substrates for model fibroblast cultures. Atomic force microscopy (AFM) mechanics studies and confocal fluorescence microscopy (CFM) absorption kinetics studies were performed for a series of hydrated hydrogel films prepared from pre-polymers with different homopolymer to monomer (Mr) ratios, which demonstrated that the inks with high Mr values yield relatively open-mesh gels due to the lowered entanglement capacity these compositions engender during polymerization. The properties of the hydrogel meshes that result from the ink compositional variations provide complementary routes for modulating the biocompatibility of 4D-printed scaffolds.

(3) We developed a materials chemistry affording unique capacities for the fabrication of multifunctional and temporally dynamic 3D bio-scaffolds. The method uses direct-write assembly to 3D print hydrogel nanocomposites using inks based on the hydroxyethyl methacrylate (HEMA) monomer specifically conjoined with property-enhancing nanoscale inorganic fillers. Hydrogels prepared from HEMA require chemical modification to activate their surfaces towards cellular attachment and growth. The addition of inorganic nanomaterial fillers (examples include the synthetic clay Laponite XLG and nanophase hydroxyapatite), which serve as gelation agents to prepare 3D printable inks, were found to confer robustly promotive materials biocompatibility that do not require adhesion-promotive surface treatments to support cellular attachment and anisotropic growth (e.g. alignment in model murine fibroblast cultures). Transmission electron microscopy (TEM) confirmed that cell growth on these materials yields biomimetic modes of conformal interfacial contact between the fibroblasts and gels and accompanying metabolic activation. The results demonstrate that the hybrid inorganic hydrogel nanocomposites yield 4D printable materials that are both structurally tough and innately facilitating of biological integration.

Training Opportunities: Students supported:
S. Gladman (Harvard University); J. M. McCracken (UIUC); C. M. Daly (UIUC)
Postdoc supported:
Xin Yong (UPitt)

Results Dissemination: Results were disseminated through journal publications.

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Honors and Awards: Awards and Honors (Jennifer A. Lewis)
Nelson W. Taylor Award, Pennsylvania St. University (2017)
Cheetam Lecture, University of California at Santa Barbara (2017)
Vannevar Bush National Security Science and Engineering Faculty Fellow (2016)
Robert B. Sosman Award, American Ceramic Society (2016)
Member, National Academy of Inventors (2015)
Baetjer Lecture, Princeton University (2016)
Interviewed on BBC World News, Newshour (January 30, 2016)
Harvard University - Public Science Lecture (2015)
Dartmouth University - Donoho (Public) Lecture (2015)
Boston University's - Distinguished Lecturer (2015)
• Fast Company's 100 Most Creative People in Business (2015)
• Discover Magazine – Top 100 Science Stories, 3D bioprinting (2015)
• Harvard Public Science Lecture (2015)
• Fellow, World Academy of Ceramics (2014)
• Foreign Policy's 100 Leading Global Thinkers (2014)
• The Executive's Club of Chicago – Panelist (2014), Women on the Cutting Edge:
Trailblazers in Disruptive Technologies and Emergent Scientific Advances
• MIT Technology Review EmTech – Featured Speaker (2014)
• Brumley D. Pritchett Lecture – Georgia Tech (2014)
• Dow Lecture – Northwestern University (2014)
• TechConnect National Innovation Award (2014)
• MIT Technology Review "Top 10 Breakthrough Technologies" (2014) – in recognition of our microscale 3D printing research

Awards and Honors (Ralph G. Nuzzo)
• Appointed Affiliated Professor of Applied Physics and Materials Science and Engineering, California Institute of Technology, Pasadena, CA, 2015.
• Appointed Affiliated Professor of Chemistry, KTH Royal Institute of Technology, Stockholm, Sweden, 2015.
• Appointed Director, Light Materials Interactions in Energy Conversion, Energy Frontier Research Center, California Institute of Technology, Pasadena, CA, 2015.

Awards and Honors (Anna C. Balazs)
*World Technology Award Finalist (in "Materials" category) and Fellow, 2016
*American Physical Society Polymer Physics Prize, 2016
*NSF Distinguished Lectures in Mathematical and Physical Sciences, 2015
*Royal Society of Chemistry S F Boys-A Rahman Award, 2015
*Greater Pittsburgh Women Chemists Committee Award for Excellence in the Chemical Sciences, 2014
*ACS Langmuir Lecture Award, 2014
*Fellow, Materials Research Society, 2014

Awards and Honors (Sydney Gladman, PhD student)
Poster Award, Fall MRS meeting (2014)

Awards and Honors (Joselle M. McCracken, PhD student)
First Place Poster Award, ACS Spring meeting (2015)

Protocol Activity Status:

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Technology Transfer: • Lewis (co-PI) recently founded a startup company, Voxel8 Inc. (www.voxel8.co), which spun out of her lab at Harvard. This company launched its first product – a desktop 3D electronics printer at the Consumer Electronics Show (CES) in January 2015. Fast Company named the product "one of the 9 best ideas" at CES and MIT Technology Review – named Voxel8 "one of the 50 smartest companies" (2015).

• Lewis (co-PI) and her group worked on collaborative research projects with the Additive Manufacturing teams at LLNL (led by Chris Spadaccini) and at AFRL (led by Mike Durstock), which focus on soft sensors / flexible electronics. Lewis is also worked with MIT Lincoln Labs on 3D printing of antennas.

• Lewis (co-PI) has given talks on her 3D printing work at several major companies during the grant period, including Adidas, Dow Chemical, ExxonMobil, Medtronics, Microsoft, Nike, PPG, Saint Gobain, Xerox.

Army Research Lab scientists during the past two years.
• Lewis participated in the ARO Workshop on Heterogeneous Materials (September 2015)

Nuzzo: Interactions with Army Research Lab and other DoD scientists.
• Nuzzo served as an external consultant to the DTRA Basic Science ResearchProgram with a focus on supporting research for Chem/Bio defense capabilities.

PARTICIPANTS:

Participant Type: PD/PI
Participant: Anna Balazs
Person Months Worked: 1.00 **Funding Support:**
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Participant Type: Co PD/PI
Participant: Jennifer Lewis
Person Months Worked: 1.00 **Funding Support:**
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Participant: Ralph Nuzzo
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Article Title: Modeling free radical polymerization using dissipative particle dynamics
Authors: Xin Yong, Olga Kuksenok, Anna C. Balazs
Keywords: computer simulation
Abstract: Understanding the details of free radical polymerization (FRP) in multi-component mixtures and solutions is of great importance for the synthesis of polymeric functional materials. Using the framework of dissipative particle dynamics (DPD), we develop a new computational approach to model FRP that couples the reactions kinetics for the polymerization processes to the dynamics of the complex fluid. We specifically consider two mechanisms of chain termination: disproportionation and combination. We analyze the effects of initiation, propagation, and termination on the polymerization kinetics in three-dimensional bulk polymerization by varying the corresponding reaction probabilities. Our model not only allows us to capture the interplay between hydrodynamics and reaction kinetics, but also provides an effective means to model polymerization in the presence of solid inclusions. We demonstrate that the latter feature by simulating the formation of polymer-clay nanocomposite gels by FRP, wh
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Article Title: Stimuli-responsive behavior of composites integrating thermo-responsive gels with photo-responsive fibers
Authors: Olga Kuksenok, Anna C. Balazs
Keywords: computer simulation
Abstract: Materials that could be reconfigured multiple times into different shapes with the use of different stimuli could dramatically impact manufacturing processes. As a step toward creating such useful, adaptive materials, we use computational modeling to design a composite that integrates a thermo-responsive polymer gel and photo-sensitive fibers. The gel displays a lower critical solution temperature (LCST), and thus, shrinks at elevated temperatures. The elastic fibers are functionalized with spirobenzopyran (SP) chromophores, which become hydrophobic under blue light. If these chromophores are uniformly distributed in this LCST gel (without the embedded fibers), then both light and heat produce the same effect on the sample, causing the gel to undergo a uniform collapse. When the SP-functionalization is confined to fibers that are embedded in the gel, the material displays distinctly different behavior in the presence of light and heat. In particular, samples anchored to a surface bend
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Article Title: Biomimetic 4D printing
Authors: A. Sydney Gladman, Elisabetta A. Matsumoto, Ralph G. Nuzzo, L. Mahadevan, Jennifer A. Lewis
Keywords: 4d printing
Abstract: Shape-morphing systems can be found in many areas, including smart textiles1, autonomous robotics2, biomedical devices3, drug delivery4 and tissue engineering5. The natural analogues of such systems are exemplified by nastic plant motions, where a variety of organs such as tendrils, bracts, leaves and flowers respond to environmental stimuli (such as humidity, light or touch) by varying internal turgor, which leads to dynamic conformations governed by the tissue composition and microstructural anisotropy of cell walls6, 7, 8, 9, 10. Inspired by these botanical systems, we printed composite hydrogel architectures that are encoded with localized, anisotropic swelling behaviour controlled by the alignment of cellulose fibrils along prescribed four-dimensional printing pathways. When combined with a minimal theoretical framework that allows us to solve the inverse problem of designing the alignment patterns for prescribed target shapes, we can programmably fabricate plant-inspired architec
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Article Title: Programming Mechanical and Physicochemical Properties of 3D Hydrogel Cellular Microcultures via Direct Ink Writing
Authors: Joselle M. McCracken, Adina Badea, Mikhail E. Kandel, A. Sydney Gladman, David J. Wetzel, Gabriel F
Keywords: Hydrogel
Abstract: 3D hydrogel scaffolds are widely used in cellular microcultures and tissue engineering. Using direct ink writing, microperiodic poly(2-hydroxyethyl-methacrylate) (pHEMA) scaffolds are created that are then printed, cured, and modified by absorbing 30 kDa protein poly-l-lysine (PLL) to render them biocompliant in model NIH/3T3 fibroblast and MC3T3-E1 preosteoblast cell cultures. Spatial light interference microscopy (SLIM) live cell imaging studies are carried out to quantify cellular motilities for each cell type, substrate, and surface treatment of interest. 3D scaffold mechanics is investigated using atomic force microscopy (AFM), while their absorption kinetics are determined by confocal fluorescence microscopy (CFM) for a series of hydrated hydrogel films prepared from prepolymers with different homopolymer-to-monomer (Mr) ratios. The observations reveal that the inks with higher Mr values yield relatively more open-mesh gels due to a lower degree of entanglement. The biocompatibil
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Partners

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I certify that the information in the report is complete and accurate:
Signature: Anna Balazs
Signature Date: 5/5/21 1:12PM

Recent progress

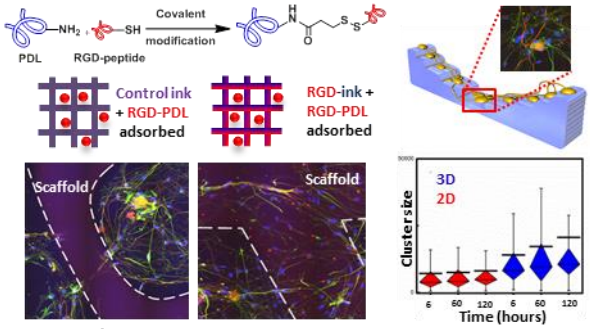
We have continued to develop the materials platforms adoptable within the emerging field of 4D printing. The current work has sought to greatly enhance properties appropriate for manipulating temporal response in chemomechanical contexts—appropriate for actuators and environmentally responsive transformations of 3D shapes—as well as providing new capabilities to control the evolution of dynamic interfaces formed between synthetic soft materials and living systems.

Our work, and that of others in recent years, has developed soft material actuators with increasingly sophisticated capabilities to move, change geometry, and respond to ambient stimuli. In the current grant period we have sought to embody functions of this type within system-level designs that present challenging, and heretofore inaccessible structural/compositional attributes—to develop within 4D printing an ink-centered capability for gradient/grayscale 3D fabrication.

Our initial efforts here focused on material mechanics as the grayscale component to target within a complex, hierarchical soft material structure. Marine life forms e.g. sea jellies, sea stars, sea anemones and coral, exemplify organic life forms that rely on natural hydrogel structures and gradient chemomechanical structural forms made from them in order to productively respond to simple stimuli that include chemical concentration, physical contact, and light.

In the current work, we printed (DIW) 3D ionotropic-origin hydrogel gradients that architecturally mimic Echinoderm and Cnidarian organisms, with particular focus on the tentacle morphology of sea jellies. By tailoring the spatial patterning of ionotropic hydrogels and the valency of their binding agents, we selectively programed the geometry and flexibility of sea jelly-inspired tentacles. When combined with iron oxide (Fe_3O_4)-loaded hydrogel composites, we developed a class of radially-symmetric, 4D-printed soft aquatic actuators (SAAs) that respond to external magnetic fields in programmatic ways that are dictated by their underlying 3D ionotropic hydrogel gradients.

In a separate work, we examined attributes of gradient 4D printed material structures for applications requiring precise capabilities for control of soft material interfaces with living matter. Understanding and controlling the interactions occurring between cells (as prototypical exemplars of living matter) and engineered materials are central challenges towards progress in the development of biomedical devices, bacterial resistant coatings, among other areas of importance to DOD mission capabilities.



In the work carried out in the current grant period, we developed materials for direct ink writing (DIW), an extrusion-based type of 3-4D printing, that embed a custom synthetic protein (RGD-PDL) within the microfilaments of 3D-hydrogel scaffolds to modify these interactions and differentially direct tissue-level organization of complex cell populations *in vitro*. The RGD-PDL was synthesized by modifying poly-D-lysine (PDL) to varying extents with peptides containing the integrin-binding motif Arg-Gly-Asp (RGD). Compositional gradients of the RGD-PDL presented by both patterned and thin-film poly-(2-hydroxyethyl) methacrylate (pHEMA) substrates allow the patterning of cell-growth compliance in a grayscale form. The surface

chemistry-dependent guidance of cell growth on the RGD-PDL-modified pHEMA materials is demonstrated using a model NIH-3T3 fibroblast cell line. The formation of a more complex cellular system — organotypic primary murine dorsal root ganglion (DRG) – in culture was also achieved on these scaffolds, where distinctive forms of cell growth and migration guidance are seen depending on their RGD-PDL content and topography. This experimental platform for the study of physicochemical factors on the formation and the reorganization of organotypic cultures offers useful capabilities for studies in tissue engineering, regenerative medicine, enhanced resistance to biological agents, and diagnostics.