Biomaterials: Tools and Foundry
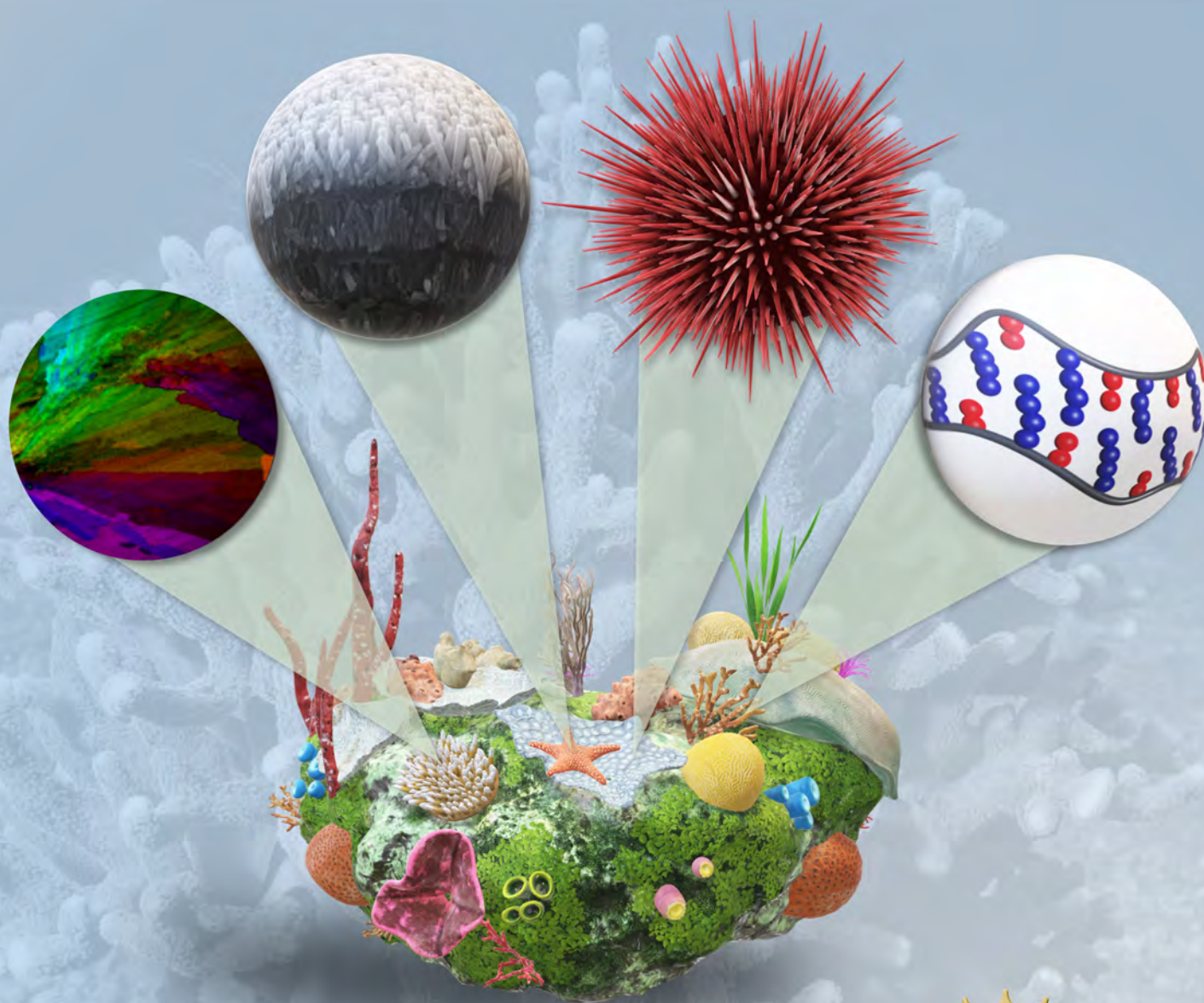## A National Science Foundation Sponsored Workshop

Held at NSF Headquarters
August 2-3, 2016

Biomaterials: Tools and Foundry

A National Science Foundation Sponsored Workshop

Held at NSF Headquarters
Arlington, VA
August 2-3, 2016

WORKSHOP CHAIR

Rigoberto Advincula, Ph.D.
Professor of Macromolecular Science and Engineering, Chemistry, and Biomedical Engineering, Case Western Reserve University

CO-ORGANIZERS

David Dean, Ph.D.	Ohio State University
Pupa Gilbert, Ph.D.	University of Wisconsin
Nicholas Kotov, Ph.D.	University of Michigan
Philip LeDuc, Ph.D.	Carnegie Mellon University
Helen Lu, Ph.D.	Columbia University
Sherine Obare, Ph.D.	Western Michigan University
Marek Urban, Ph.D.	Clemson University

LIST OF INVITED PARTICIPANTS

Debra Auguste, Ph.D.	City University of New York
Joel Brock, Ph.D.	Cornell University
Markus Buehler, Ph.D.	Massachusetts Institute of Technology
Trevor Douglas, Ph.D.	Indiana University
Omolola Eniola-Adefeso, Ph.D.	University of Michigan
Seth Fraden, Ph.D.	Brandeis University
Melissa Grunlan, Ph.D.	Texas A&M University
Anthony Guiseppi-Elie, Ph.D.	Texas A&M University
Neel Joshi, Ph.D.	Harvard University
Joachim Kohn, Ph.D.	Rutgers University
Gang-yu Liu, Ph.D.	University of California at Davis
Treena Livingston, Ph.D.	New Jersey Institute of Technology
Lee Makowski, Ph.D.	Northeastern University
Joanna McKittrick, Ph.D.	University of California, San Diego
Liviu Movileanu, Ph.D.	Syracuse University
Hung Nguyen, Ph.D.	University of California, Irvine
Jonathan Pokorski, Ph.D.	Case Western Reserve University
Debora Rodrigues, Ph.D.	University of Houston
Michael Rubinstein, Ph.D.	University of North Carolina, Chapel Hill
Warren Ruder, Ph.D.	Virginia Institute of Technology

Athanassios Sambanis, Ph.D.	W.M. Keck Foundation
Nicole Steinmetz, Ph.D.	Case Western Reserve University
Matt Tirrell, Ph.D.	University of Chicago
Joyce Wong, Ph.D.	Boston University
Dogic Zvonimir, Ph.D.	Brandeis University

FEDERAL AGENCIES

Joseph Akkara, Ph.D.	National Science Foundation
Sheng Lin-Gibson, Ph.D.	National Institute of Standards and Technology
Tessema Guebre, Ph.D.	National Science Foundation
Daryl Hess, Ph.D.	National Science Foundation
Sean Jones, Ph.D.	National Science Foundation
Kevin Kunkler, M.D.	US Army Medical Research & Materiel Command
Andrew Lovinger, Ph.D.	National Science Foundation
Dinesh Patwardhan, Ph.D.	Food and Drug Administration
Alex Simonian, Ph.D.	National Science Foundation
Leonard Spinu, Ph.D.	National Science Foundation
Paul Sokol Ph.D.	National Science Foundation
Charles Ying Ph.D.	National Science Foundation

SPECIAL ASSISTANT

Brylee Tiu, Ph.D.	Case Western Reserve University

PREFACE

The area of biomaterials is an important branch in the multidisciplinary, interdisciplinary, and transdisciplinary approaches in materials research and technology. There is a need to answer the question "is there anything that can be done to advance biomaterials beyond the current level of effort and creativity?" This inquiry begs another question: "what resources in tools, fabrication methods, instrumentation, and biological or biomedical studies are needed to carry this effort to the next level?" On August 2-3, 2016, we conducted a workshop - to evaluate the status of the field of biological materials science and engineering and identify the most promising directions for the community. By doing this exercise, we intended to identify key driving forces and future directions for mid-scale level tools and instrumentation that may well serve as a blueprint for future funding opportunities. We divided the biomaterials field into several categories:

    A) Biomaterials in Biological Environments
    B) Dynamic and Adaptive Biomaterials Surfaces and Interfaces
    C) Signaling Across Biomaterials Boundaries
    D) Targeted Patterning, Fabrication, and Self-Assembly
    E) Beyond Detection Limits: Characterization, Detection Tools & Diagnostic Methods

The participants drew representatives from several universities and federal agencies. The report summarizes the deliberations of the participants and the conclusions of the workshop. As Workshop Chairman and representing the committee, I would like to first express my appreciation to the National Science Foundation (NSF), Division of Materials Research (DMR) for supporting the workshop and seeking the advice of the biomaterials research community on this important opportunity. I want to acknowledge all of the participants who gave overview lectures and contributed to the discussion sessions and offered constructive feedback in drafting various sections of the report. Sean Liam Jones is a key supporter and provided much guidance in the execution of the workshop project. Tessema Guebre, Alex Simonian, Joseph Akkara, and Charles Ying provided advice and support at critical stages of the process including invitation of participants. I want to express special thanks to the workshop executive committee: David Dean of Ohio State University, Pupa Gilbert of University of Wisconsin, Nicholas Kotov of University of Michigan, Philip LeDuc of Carnegie Mellon University, Helen Lu of Columbia University, Sherine Obare of Western Michigan University, and Marek Urban of Clemson University for their hard work and dedication. Brylee Tiu of Case Western Reserve University served as my special assistant and kept track of the group discussions, prepared materials for website development, and was involved in the preparation of the workshop report. It is my desire that this report will stimulate further discussion and investments in new tools, fabrication methods, and instrumentation in advancing biological materials research.

Rigoberto C. Advincula
Cleveland, OH

Table of Contents

EXECUTIVE SUMMARY

The tools and foundry of the industrial revolution, starting with the 17th century and steady progress through the exponential electronic and information revolutions of the 20th century, set the course for modern day living, improving the overall quality of life and extending lifespan. In the 21st century, the drive for new innovation centers on harnessing natural and biomimetic mechanisms for materials synthesis, and applications impact everyday life. Biomaterials have emerged as a central topic of the bio-inspired research; they are poised to increase the quality of human lives further and stimulate economic development through technological advances. Significant progress in biomaterials can be obtained by fully integrating characterization, synthesis, and theory into a synergistic Biomaterials Foundry.

An important question is "what will it take to get us to that next level for the 21st century? For new and improved biomaterials, their synthesis, and their properties, we must engage and integrate experts in Biology, Chemistry, Physics, as well as Biomedical, Mechanical, Electrical, Chemical, and Materials Engineering.

On August 2-3, 2016 a workshop was organized in Arlington, VA, sponsored by the National Science Foundation aimed at identifying investment opportunities at the mid-scale of funding level in biomaterials. The Chair, Prof. Rigoberto Advincula of Case Western Reserve University, five discussion leaders, and other speakers provided a brief overview of current biomaterials research and technology and brought into focus the tools and foundry that will be needed for leadership in 21st-century research. In contrast to the previous biomaterials workshop held in 2012, the 2016 workshop and report focused on tools and foundry for biomaterials, and specifically on determining top priorities for mid-scale instrumentation investments. The stage was set to define the key scientific questions for biomaterials, to identify the current challenges and opportunities, and to make specific recommendations that will be useful for peers and future funding agencies. Five biomaterials fields and discussion groups were sub-divided as follows:

A) Biomanufacturing: Natural and Synthetic (Leader: Pupa Gilbert)
B) Dynamic and Adaptive Biomaterials Surfaces and Interfaces (Leader: Marek W. Urban)
C) Multiscale Biomaterial Design and Characterization (Leaders: Helen H. Lu and Philip LeDuc)
D) Targeted Patterning, Fabrication, and Self-Assembly (Leaders: David Dean and Sherine Obare)
E) Beyond Detection Limits; Characterization, Detection Tools and Diagnostic Methods (Leaders: Nicholas Kotov and Rigoberto Advincula)

The workshop participants were from diverse backgrounds and came from public and private universities as well as federal agencies. Each had the opportunity to educate their peers or share their views through plenary talks and separate discussion sessions led by discussion leaders, who were also members of the workshop executive committee. At the end of each day, each group reported a summary of their discussion to the rest of the

participants. The executive committee convened after the workshop and continued to meet with the session members for several weeks to synthesize a variety of different points of view into a consistent set of recommendations presented in this report. Several members of the executive committee also visited the national facilities pertinent to the biomaterials work and this report. Based on these discussions, the key recommendations of the committee are presented below.

<u>Summary of Recommendations</u>

The committee unanimously recommends the establishment of a Biomaterials Foundry, synergistically integrating biomaterials synthesis, characterization, data processing, simulations, and theory. Research in the Foundry should emphasize natural, bioinspired, biomimetic, and biocompatible materials essential for the advance of healthcare and technology in the United States and beyond. Together with all workshop participants, we have identified four grand challenges in the field of Biomaterials:

- Elucidating mechanisms of biomaterial and living tissues response to different stimuli from the molecular, nanoscopic, and macroscopic scale.
- Attaining nanometer and nanosecond resolution, in the time and space scales simultaneously, to characterize these responses.
- Understanding the mechanism of formation *in vivo* for hard, soft, and fluid biomaterials and biological tissues, and how to replicate them *in vitro* using advanced multiscale manufacturing and self-assembly.
- Constructing a predictive theoretical and computational description of biomaterials based on extensive data from multiple experimental sources.

With the grand challenges in mind, the committee identified **opportunities** for fundamental research and technology development. In fundamental research, the **high-potential-for-discovery** areas are: (a) molecular level mechanisms governing the formation of natural biominerals, (b) personalized synthetic biomaterials, and (c) nanoscale bio-interfaces. Concomitant technological innovations of great impact are envisioned for biomaterials and must be nurtured. The areas with **high-potential-for-innovation** are (a) precision computationally guided synthesis, (b) self-healing, (c) multi-component 3D architecture, (d) self-organized hierarchical structure inspired by natural tissues and cellular components and (e) multifunctional biomaterials.

Based on the collective knowledge of the committee, past successes of biomaterials were inextricably linked to the advancement of analytical capabilities enabling a better understanding of materials properties, *in situ* compositional analysis, and host-material signaling events. However, <u>many of the existing analytical tools are insufficient</u> to tackle the aforementioned scientific challenges and propel biomaterials research and development to new levels. New discoveries and innovations can be made as cutting-edge techniques and instruments are developed and become available to the widest possible community of researchers in academia and high-tech manufacturing.

The committee identified two categories of mid-size instrumentation as critical components to the future development of biomaterials:

1) Development of unchartered detection capabilities to uncover biochemical phenomena, biomineralization, biodegradation, cell differentiation, *in vivo* metrology, and other molecular and nanoscale phenomena involving biomaterials;

2) Drastic improvement of sensitivity and temporal resolution of the existing instruments making it possible to exploit big data, computational, and systems biology tools.

Both categories will focus on the development of instrumentation that has the highest potential for addressing biomaterials discoveries and innovations. Specific instrumentation needs are listed in Section A.

The above recommendations can be achieved by establishing a comprehensive Biomaterials Foundry with the most advanced and emerging analytical tools and dedicated experienced staff. It is envisioned that the Biomaterials Foundry will have research activities for both external users and in-house scientists. User support can include access to synchrotron methods, full-time expert support in all tools and foundry facilities that enable sample preparation, analysis, and interpretation of results. Computational, data processing, and systems biology tools are envisioned as well. Biological and biomedical cell culture and animal facilities must be readily available to ensure *in vivo* or *ex-situ* studies for harvesting or retrieval methods, which enable rapid feedback on the biological performance of implants, drug delivery agents, imaging techniques, etc. The Foundry will lead to accelerated discoveries and innovations, as assessed by quantitative metrics established by NSF.

It is the desire of the organizers, this committee and the participants of the workshop to make such Foundry a reality and accessible for the research community.

<div align="center">

WORKSHOP PROGRAM

Biomaterials: Tools and Foundry
Nationals Science Foundation (NSF)
Arlington, VA
August 1-3, 2016
http://www.biomatworkshop.org/

</div>

Chair and Organizer

RIGOBERTO ADVINCULA (CWRU)

Executive Committee Members

Biomaterials in Biological Environments:
Session Leader: PUPA GILBERT

Dynamic and Adoptive Biomaterials Surfaces and Interfaces:
Session Leader: MAREK URBAN

Signaling Across Biomaterials Boundaries:
Session Leaders: HELEN LU and PHIL LEDUC

Targeted Patterning, Fabrication, and Self-Assembly:
Session Leaders: DAVID DEAN and SHERINE OBARE

Beyond Detection Limits; Characterization, Detection Tools and Diagnostic Methods:
Session Leaders: NICHOLAS KOTOV and RIGOBERTO ADVINCULA

SCHEDULE

Day 1 – August 1, 2016 (Monday)
Arrival of delegates in Washington, DC area
Executive committee only dinner at 6:00 PM

Day 2 – August 2, 2016 (Tuesday)

8:00 – 8:30 am	Arrival at NSF, Stafford II, room 555, Please get your IDs at Stafford I, main entrance. Breakfast is served
8:30 – 9:00 am	Introduction by Organizers and NSF and Conference Charge *Prof. Rigoberto Advincula (CWRU)* *Dr. Sean Liam Jones (NSF)* *Dr. Clark Cooper (NSF)*

9:00 – 9:40 am	SECTION A: Pupa Gilbert (UW Madison)/ Markus Buehler (MIT)
9:40 – 10:20 am	SECTION B: Matt Tirrell (UC)/Nicole Steinmetz (CWRU)
10:20 – 10:30 am	Break
10:30 – 11:10 am	SECTION C: Joachim Kohn (Rutgers)/ Melissa Grunlan (TAMU)
11:10 – 11:50 am	SECTION D: Nicholas Kotov (UM)/ David Dean (OSU)
11:50 am – 12:30 pm	SECTION E: Michael Rubinstein (UNC)/Gang-Yu Liu (UC Davis)
12:30 – 1:00 pm	Lunch (Working)
1:00 – 3:45 pm	Section: Member Introductions and Day 1- Discussions
3:45 – 4:00 pm	Break
4:00 – 5:00 pm	Section Day 1 Reporting (20 min per section: 10 min-delivery & 10 min open comments)

Day 3 – August 3, 2016 (Wednesday)

8:30 – 8:45 am	Arrival at NSF, Stafford II, room 555, Please get your IDs at Stafford I, Main entrance. Breakfast is served
8:45 – 9:00 am	Introduction by Organizers and NSF and Conference Charge
9:00 – 9:40 am	SECTION A: Trevor Douglas (IU) /Joanna McKittrick (UCSD)
9:40 – 10:20 am	SECTION B: Hung Nguyen (UCI)/ Marek Urban (Clemson)
10:20 – 10:30 am	Break
10:30 – 11:10 am	SECTION C: Joyce Wong (BU)/ Warren Rudder (VTech)
11:10 – 11:50 am	SECTION D: Sherine Obare (WMU)/ Treena Livingston (NJIT)
11:50 am – 12:30 pm	SECTION E: Rigoberto Advincula (CWRU)/ Lee Makowski (NEU)
12:30 – 1:00 pm	Lunch (Working)
1:00 – 2:45 pm	Day 2- Discussions and Writing
2:45 – 3:00 pm	Break
3:00 – 4:00 pm	Section Day 2 Reporting (20 min per section: 10 min-delivery & 10 min open comments)
4:00 – 6:00 pm	Concluding Remarks
6:00 pm	Executive Committee Meeting Dinner

Day 4 – August 4, 2016 (Thursday)

8:30 – 9:00 am	Arrival at NSF, Stafford II, room 545, Breakfast is served
9:00 am – 12:00 pm	Report Writing
12:00 pm – 1:00 pm	Lunch (Working)

BREAKOUT SESSION ASSIGNMENTS

SECTION A: Biomaterials in Biological Environments (Room 555)
Session Leader   PUPA GILBERT (UW-Madison)

Group Members   Trevor Douglas (Indiana U)
Joanna McKittrick (UC San Diego)
Debora Rodrigues (UH)
Markus Buehler (MIT)
Jon Pokorski (CWRU)
Dinesh Patwardhan (FDA)

Details   Natural Biomineralization Mechanisms
Directed- and Self-Assembly in Natural and Synthetic Systems
Biomimetic and Biocompatible Hard Materials and Composites
Theory and Simulation

SECTION B: Dynamic & Adoptive Biomaterials Surfaces & Interfaces (Room 525)

Session Leader   MAREK URBAN (Clemson)

Group Members   Debra Auguste (CUNY)
Nicole Steinmetz (CWRU)
Liviu Movileanu (Syracuse U)
Matt Tirrell (U Chicago)
Neel Joshi (Harvard U)
Hung Nguyen (UCI)
Joe Akkara (NSF)
Paul Sokol (NSF)

Details   Soft Materials
Fouling and Non-fouling Surfaces
Adoptive and Stimuli Responsive Systems
Theory and Simulation

SECTION C: Signaling Across Biomaterials Boundaries (Room 545)

Session Leader   HELEN LU (Columbia) and PHIL LEDUC (CMU)

Group Members   Dogic Zvonimir (Brandeis U)
Joyce Wong (BU)
Melissa Grunlan (TAMU)
Joachim Kohn (Rutgers U)
Warren Ruder (UVT)

Details   Synthetic Biology
Cell Biology and Chemotaxis
Theory and Simulation

SECTION D: Targeted Patterning, Fabrication, and Self-Assembly (Room 565)

Session Leader   DAVID DEAN (OSU) and SHERINE OBARE (WMU)

Group Members   Treena Livingston (NJIT)
Seth Fraden (Brandeis)
Anthony Guiseppi-Elie (TAMU)
Athanassios Sambanis (Keck Foundation)
Omolola Eniola-Adefeso (UMich)

Details   New Fabrication methods and 3-D Printing

Implants and Devices
Patterning
Nanomaterials Self-Assembly
Theory and Simulation

SECTION E: Beyond Detection Limits; Characterization, Detection Tools and Diagnostic Methods (Room 585)

Session Leader	NICK KOTOV (UMich) and RIGOBERTO ADVINCULA (CWRU)
Group Members	Lee Makowski (Northeastern U)
	Michael Rubinstein (UNC)
	Joel Brock (Cornell U)
	Gang-yu Liu (UC Davis)
	Brylee Tiu (CWRU)
	Alex Simonian (NSF)
	Sheng Lin-Gibson (NIST)
Details	Instrumentation and Analytical Methods: Spectroscopy, Microscopy, and Non-traditional methods
	Molecular Imaging
	Diagnostic: In-situ and Real-time Imaging Methods
	Probes, Nanomaterials, and biomolecular signaling
	Theory and Simulation

SECTION 1: Biomanufacturing: Natural & Synthetic

SESSION LEADER

PUPA GILBERT, UNIVERSITY OF WISCONSIN-MADISON

SECTION MEMBERS

MARKUS J. BUEHLER, MASSACHUSETTS INSTITUTE OF TECHNOLOGY

TREVOR DOUGLAS, INDIANA UNIVERSITY BLOOMINGTON

JOANNA M. MCKITTRICK, UNIVERSITY OF CALIFORNIA, SAN DIEGO

DINESH PATWARDHAN, FOOD AND DRUG ADMINISTRATION

JONATHAN K. POKORSKI, CASE WESTERN RESERVE UNIVERSITY

DEBORA F. RODRIGUES, UNIVERSITY OF HOUSTON

1.1 Introduction

Living organisms control chemical reactions with high precision in space and time. They are not homogeneous reaction vessels in which hundreds of individual chemical reactions take place simultaneously; instead, they exhibit extensive internal, heterogeneous, and local structure, determined by well-defined barriers. Such barriers serve to separate an organism from its environment, partition the internal space of complex organisms into organ systems, separate cells from one another in a tissue, and define organelles within a cell. Biological systems use this compartmentalization to great effect in the synthesis, processing, and use of a wide range of biomaterials. We coin the term '*biomanufacturing*' to refer to both natural and synthetic processes. Natural biomanufacturing pertains to how biological organisms synthesize and organize their constituents and synthetic biomanufacturing concerns the methods used to created bioinspired materials and structures.

Figure 1 shows a few examples of naturally biomanufactured materials. Bulk biomaterials produced in eukaryotes include biominerals such as bone, teeth, seashells; protein assemblies such as silk, keratin, collagen; carbohydrate assemblies such as chitin, cellulose, and starches. All these biomaterials exhibit striking structures at the macro-, micro-, and nano-scales. The mechanical properties, in many instances, can be ascribed to the composite nature of materials, increasing stiffness and toughness by incorporating compositional gradients (byssus threads [4], squid beak [5]), or by the elegant use of interfacial interactions between hard and soft materials (nacre [6], silica sponge spicules [7, 8], sea urchin teeth [9]), or by processing the biomaterial after it has been synthesized (silk [10]). This refinement occurs as the components assemble hierarchically from the nano- to the macro-scale. Millions of years of natural selection gave evolutionary advantages to the organisms forming them, thus natural biomaterials provide an ever-increasing inspiration

for the next generation of materials scientists. Multi-scale biomaterial assemblies are found in both eukaryotic and prokaryotic systems. These include the dynamic assembly of tubulin protein fibers, amorphous calcium carbonate crystallizing into diverse sea urchin [11] and mollusk shells structures [12, 13], the assembly of enzymatically active bacterial micro-compartments composed of proteins [14, 15], virus particles assembled from protein subunits such as iridoviruses, which infect insect cells at such high concentrations that they form super-lattices and impart structural color to the host insect [16]. Another astonishing example is provided by sea urchin teeth, which scrape and dig into rock, and self-sharpen with use [9].

Figure 1. (A) The forming part of a sea urchin tooth.[1] (B) Amorphous calcium carbonate (ACC) precursors in forming nacre, mapped with 20-nm resolution.[2] (C) Silk in a spider web.[3] (D) Spider silk β-sheet nanocrystals.[3]
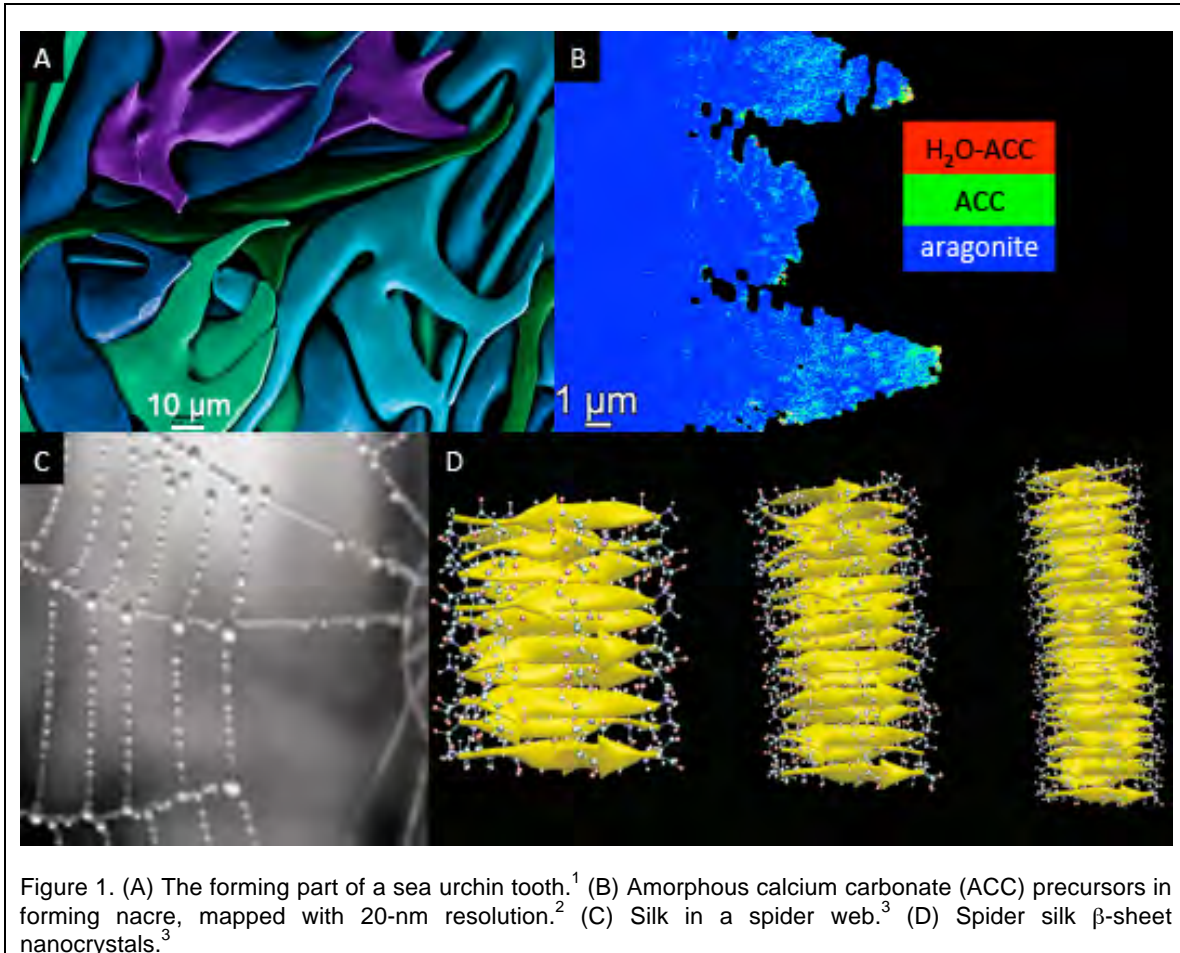
It is clear that we have barely scratched the surface in discovery and understanding of the diversity, structure, and properties of biomaterials produced by living systems. We envision a future when: a) many biomaterials and their properties have been mapped; b) the underlying unique or universal mechanisms for synthesis and processing of biomaterials are understood and harnessed; c) the biochemical pathways that mediate biomaterials formation have been well established; and d) novel, synthetic pathways to fabricate bioinspired materials and structures have been developed. Inspired by this fundamental knowledge base, future materials engineering can use synthetic biology to reprogram cells, tissues, perhaps whole organisms to create designed materials. This level

of biomanufacturing is compatible with materials design, with rapid cell re-engineering, large-scale production, and sustainability.

1.1.1 Biomineralization

The splendid animal diversity we enjoy today is likely a result of biomineral formation, or biomineralization. Before biominerals appeared there were animals, even large ones such as Dickinsonia [17], but they were soft-bodied. A hard skeleton is necessary for fast locomotion in water, in air, and on land, and therefore for effective predator attack or prey escape. Hard biominerals are also functional attack tools, such as fangs, beaks, radular teeth, claws, pinches, as well as effective defense tools, such as mollusk shells, carapaces in crabs, lobsters, or turtles, armors in fish, armadillos, alligators, and spines in hedgehogs or sea urchins. We therefore suggest that the Cambrian explosion of animal diversity [18, 19] was facilitated by the onset of biomineralization, and its resulting prey-predator mechanisms because these accelerate the pace of evolution and thus diversification. In terms of relevance to engineering biomaterials, the ability to design and create hard biomaterials, in conjunction with soft biomaterials, is a critical element to address our emerging needs.

Besides its relevance to the history of life, the mechanisms of biomineral formation are extremely important to discover today, because they will teach us how to build materials faster, better, at ambient temperature and pressure, and without any toxic substances. Calcium carbonate ($CaCO_3$) biominerals, in particular, can teach us how to make crystals grow hundreds of times faster than we currently can grow in the laboratory or industry, and make them much more resistant to fracture [20]. An urgent need is to find a CO_2 capture and sequestration method that is both energy efficient and environmentally friendly. $CaCO_3$ formation inspired by biomineralization is a prime candidate to address this pressing need.
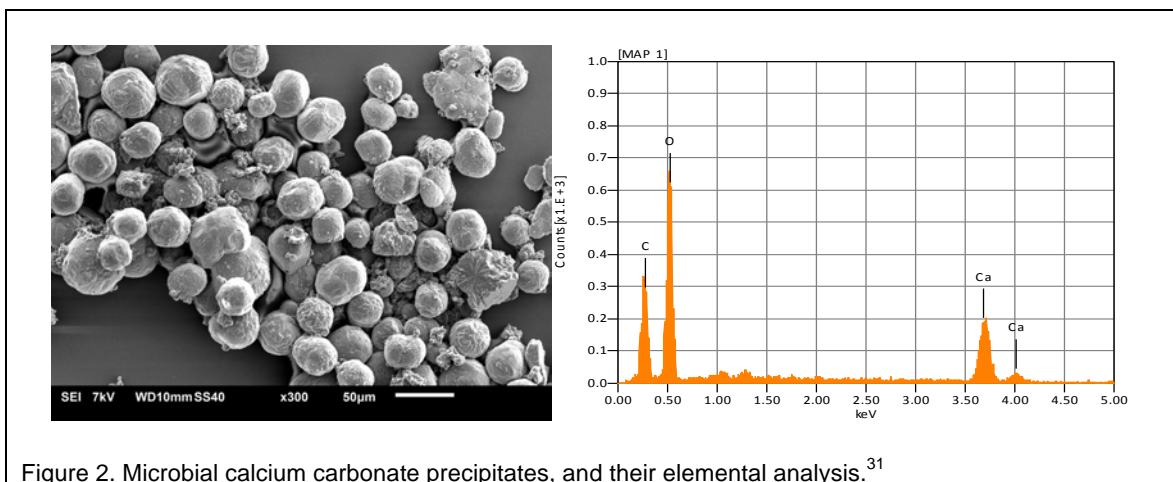
Figure 2. Microbial calcium carbonate precipitates, and their elemental analysis.[31]

1.1.2 Microbial biomineralization

Calcium carbonate biominerals are extremely abundant in nature, being formed by vertebrates, echinoderms (Figure 1A), mollusks (Figure 1B), annelids and many other

phyla. Recently, also bacteria were discovered to induce precipitation of $CaCO_3$, and sequester CO_2 (Figure 2) [21, 22]. This precipitation can be reproduced by bacterial cultures *in vitro*, and is affected by several environmental parameters, but the mechanism by which microbes induce it is still unknown despite extensive studies.[23-30] We regard this as an important area to explore.

1.1.3 Structural biological materials and bioinspired designs

Figure 3. Diagram of the eight most common biological structural design elements.[33]
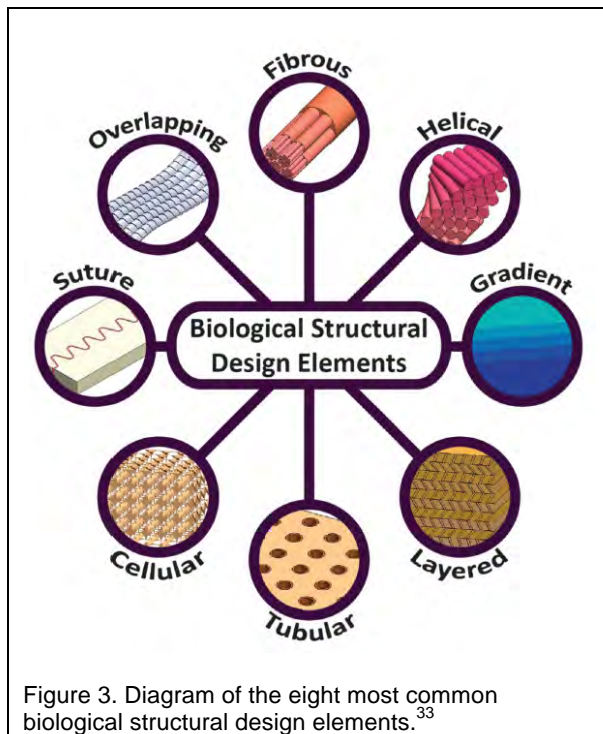
In spite of the estimated seven million animal species living on earth [32], there is remarkable repetition in the structures observed among the diversity of biological materials. This is due to the fact that many different organisms have developed similar solutions to natural challenges such as ambient environmental conditions or predation. Research on biological materials often presents similar solutions because the number of materials available in nature is fairly limited, and therefore complex combinations of materials have to be developed to address specific evolutionary constraints. Eight *structural design elements* have been identified as most common and are shown in Figure 3 [33].

In the emerging field of biological materials science, there is a great need for systematizing these observations and describing the underlying mechanics principles in a unified manner. This is necessary because similar designs are often reported under various names. As an example, the presence of numerous interfaces within a composite introduces a significant property mismatch, which we suggest be named a "layered-lamellar" structure, has previously been described by different names despite the similarity of the structural advantages it provides in all systems. Previous terms were "lamellar" structure in bone [34] and fish scales [35], "brick and mortar" in abalone nacre [36-38], and "laminated" in sea sponge spicules [7].

Structural biological materials such as bone, teeth, spines, and seashells are strong and stiff, yet lightweight. These natural materials have developed structures, forms, and features that fine-tune certain properties (e.g. strength, toughness). The materials are usually anisotropic and formed from relatively weak constituents (biopolymers and minerals) using few molecules and elements. Remarkable stiffness, strength, and toughness are obtained by combining hierarchical structure, nanoscale toughening features (such as lamellar structure and soft-hard materials interfaces in abalone nacre), and a specific morphology (such as elliptical cross-section and internal struts in the

Biomaterials: Tools and Foundry

hollow wing bones of many flying birds). Insights gleaned from studying natural materials, structures developed through millions of years of evolution, provide design templates for the synthesis and fabrication of new materials and structures that have potential use in the medical, aerospace, energy and other fields. An example is shown in Figure 4, using the unusual ability of the seahorse tail to bend and twist, a bioinspired robot tail was fabricated, which has potential application as a catheter or a robotic arm that can probe hostile environments.

(a)   **(b)**
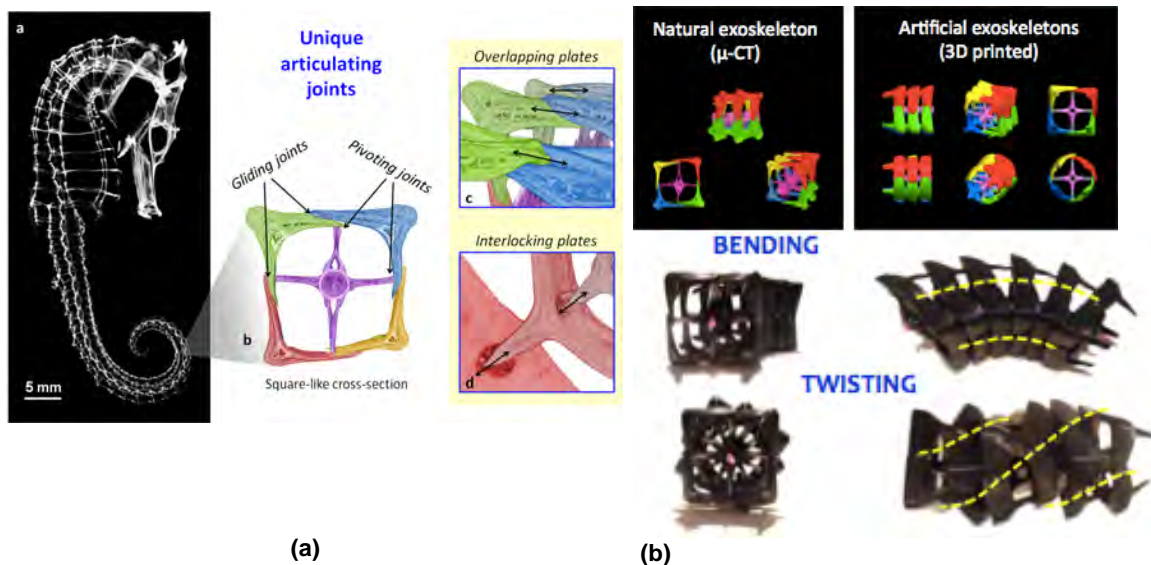
Figure 4. From biology to bioinspiration. **(a)** Structure of a seahorse tail, showing four L-shaped bony plates with joints between them. **(b)** Micro-computed tomography images (artificially colored) and 3D printed segments, showing a bioinspired seahorse robot tail that can bend and twist.[39]

1.1.4 Scalable and Reproducible Manufacturing and Characterization

A limiting factor in biomaterials research is a lack of reproducibility between laboratories at distant sites and the ability to scale the synthesis of new biomaterials (Figure 5). This limitation arises from a combination of several factors that include sourcing of material, experimental design, variability of environmental conditions and a lack of standards for both materials manufacturing and characterization of new biomaterials. A common limiting example is the manufacturing of polymeric nanofibers utilizing electrospinning [40]. Reproducible production of electrospun fibers depends on temperature, humidity, materials sourcing, and other factors [41-43]. Thus, in non-reproducible scenarios it is exceptionally difficult to build on the knowledge of others, because neither biomaterial manufacturing nor characterization is standardized. However, if manufacturing and characterization can be unified across subspecialties within the community, knowledge can be built collectively. Furthermore, should processes become available to synthesize complex biomaterials reliably and at the multi-kilogram scale, batch-to-batch reproducibility would be greater and standardization metrics can be obtained. Finally, as new materials are created, the task of characterizing these novel materials falls on individual laboratories whose practices vary depending upon available resources and internal expertise. To grow the collective knowledge of the field, it is imminent that a set

of characterization tools and techniques be standardized such that materials can be manufactured and compared within the community.

Figure 5. An example of a scalable biomaterial manufacturing process, multilayered coextrusion of polymeric nanofibers. Extrusion has throughputs of kilograms per hour and is a continuous process. (A) Schematic representation of coextrusion. Steps 1-4 represent multiplication elements in the extruder that yield polymer nanofibers. (B) ~1kg of coextruded composite tape; this process yields ~2 kg/hr. (C) Scanning electron micrograph of aligned polymer nanofibers.[44-47]

Another example of scalable manufacturing is the use of micro-computed tomography images and incorporation of these data files into CAD software to produce useful structures and devices. This is illustrated in Figure 6 for the jaw apparatus of the sea urchin, called Aristotle's lantern, as it was already described by Aristotle in his *Historia Animalium* ca. 343 BCE [48]. The teeth in the jaw scrape against much harder rocks for eating kelp and other algae and exhibit an unusual protraction and retraction radial motion [9], making the jaw an inspiration for a sediment sampler.

1.1.5 Synthetic biology

Metabolic pathways in living systems comprise unique chemical transformations that rely on multiple individual catalysts working in concert through reactions, which transform substrates and ultimately drive and sustain life. Rapidly growing understanding, and improved ability to measure changes in the transcriptome, proteome, metabolome, glycome, metalome, microbiome have a great potential to provide new insights into the genetically-encoded machineries for the biosynthesis of novel materials done by model or new organisms. However, the large amounts of data generated by these "omics" techniques are still poorly integrated, and therefore do not yet provide a complete understanding of the operation of cellular synthesis, processes, and functions. Through integrated understanding of the regulation and levels of specific molecules, we will be able to better interpret biological phenotypes and identify biosynthetic pathways, which is especially useful for the synthetic biomanufacturing of materials. Hence, establishing a holistic and integrated view of *all* the molecular changes occurring using high throughput data mining remains a significant challenge.
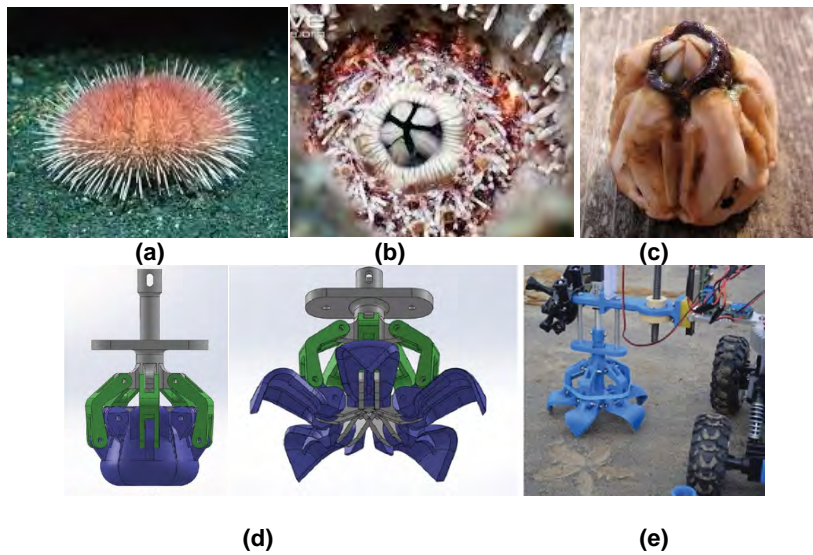
Figure 6. Development of a bioinspired sediment sampler, proposed for the Mars Rover. **(a)** Photograph of a pink sea urchin, **(b)** underside of the urchin, showing the mouth and its five radial teeth, **(c)** photograph of the extracted Aristotle's lantern, **(d)** 3D printed bioinspired gripper based on the biting motion of the sea urchin teeth and **(e)** bioinspired sampler attached to a remotely controlled vehicle being tested on the beach. Adapted from.[49]

Figure 7. Schematic of the assembly of the viral capsid based metabolon nanoreactor. A) Expression of the coat protein gene (CP) and the multi-enzyme fusion with an essential scaffold domain (SP). Capsid formation is facilitated by the interactions of the SP and CP subunits encapsulating the multi-enzyme gene product. A) The three-step reaction catalyzed by the enzymatic pathway with Lactose as the initial substrate showing a cascade reaction, catalyzed by the three sequestered enzymes.[50]

Coordination and regulation of metabolic pathways is not merely temporal, but also spatially controlled by compartmentalization of enzymes into organelles and other sub-cellular structures. Recently, it has been discovered that sub-cellular organization of enzymes into ordered protein cage architectures, such as the carboxysome, is one way in which sequential metabolic enzymes are sequestered and spatially separated from other components of the cell [51, 52]. The encapsulation of enzymes by protein structures, or assembly of enzymes into supramolecular architectures, has been suggested to enhance the efficiency and/or prevent loss of unstable or toxic intermediates that may hinder cellular functions. Mimicking the spatially controlled sequestration of multiple enzymes inside supramolecular protein cage architectures to encapsulate fragments of synthetic metabolic pathways (metabolons) is an exciting direction toward constructing new biomimetic catalytic materials and for studying enzymes in crowded cytoplasm-like environments [50, 53-55] (Figure 7). Biomimetic approaches show significant potential for creating synthetic, metabolon materials by the co-immobilization or encapsulation and stabilization of multi-enzymes that perform coupled cascade of reactions independent of biological origin.

1.1.6 Theory

Theory is a key enabler in driving materials by design. Simulation based approaches, big data, machine learning, and novel statistical methods are critical to further the next generation of the Materials Genome Project, to expand towards complex biomaterials.

Biomaterials: Tools and Foundry
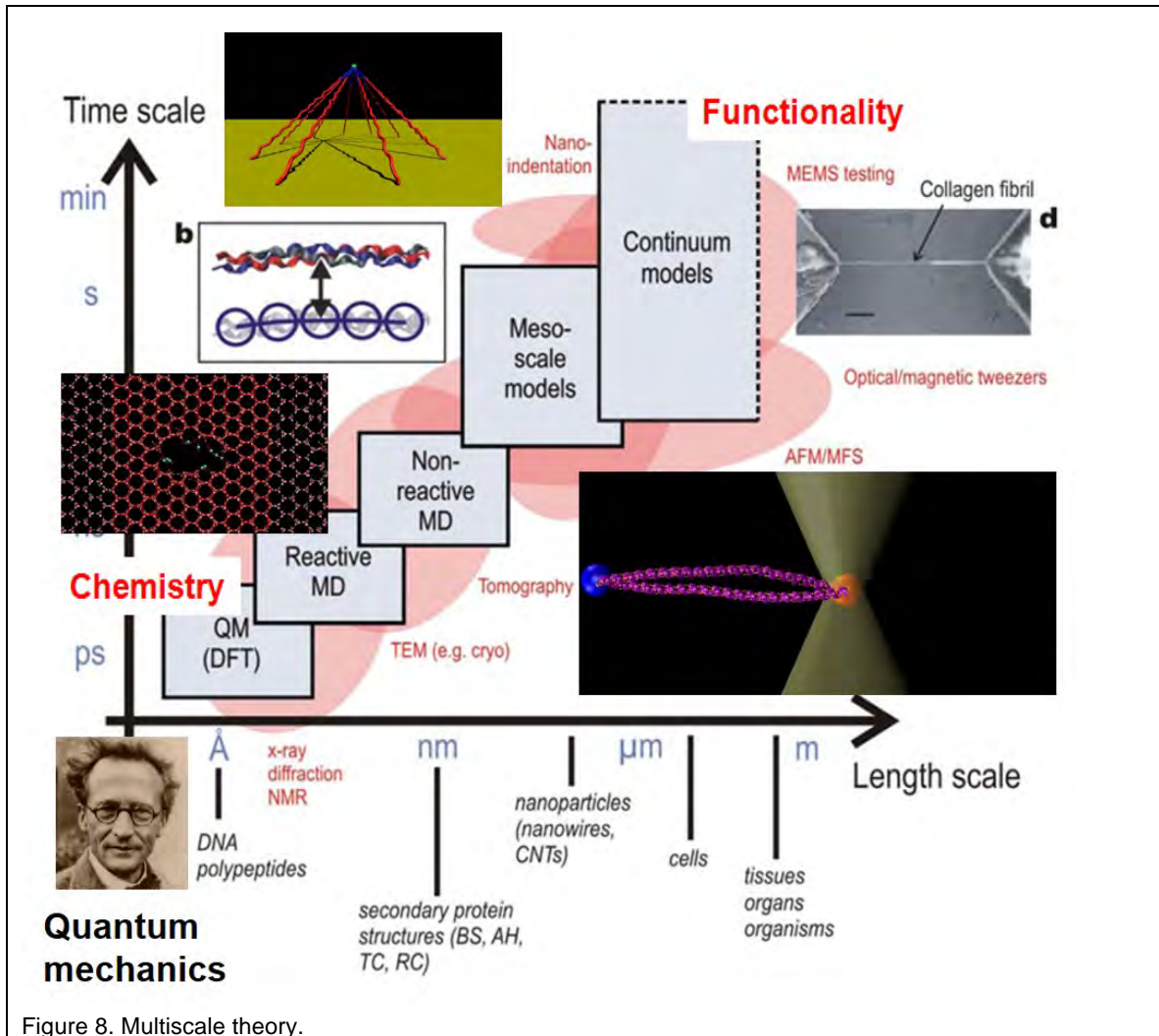
Figure 8. Multiscale theory.

We strongly recommend that the Biomaterials Foundry feature a significant theory effort that includes large-scale, petaflop computing facilities, GPU computing and other hardware accelerated methods, such as the Anton supercomputers that feature hardcoded molecular dynamics of protein models. The span of methods must encompass the scales from electron resolution (e.g. quantum chemistry such as Hartree-Fock or Density Functional Theory), to reactive force fields (e.g. ReaxFF), to classical force fields (e.g. CHARMM, etc.), to coarse-graining (here a variety of tailored methods to be used), to the continuum scale. Such multiscale methods must be validated against multiscale experiments. A key focus must be on modeling processing, as this represents an important step in structure formation in soft and hard tissues, and composites thereof.

The theory component of the Biomaterials Foundry should also feature resources for data analysis from experiment, and the capacity to move experimental data into molecular models. For instance, scarce structural data can be complemented with atomistic resolution by executing structure-finding algorithms based on statistical methods such as Replica Exchange MD.

Finally, inverse problem solving should be a high priority, especially for the materials design process.

The last step in the integration of the methods should encompass an intimate coupling of theory with manufacturing techniques [56-69]; these can range from additive manufacturing, micro- and nanoscale processing (e.g. microfluidics), to subtractive techniques. We propose that the Biomaterials Foundry offers grand challenge milestones for years 2 and 4, and that it tackles key issues such as biomanufactured bone or tooth implants with structural design from the molecular to the multi-cm length scale, that can be incorporated in living organisms with long-term viability. Another such grand challenge is the design of materials feedback loops based on synthetic biological organisms that are designed, co-cultured and nourished to maintain a complex set of materials functions resembling those of living organisms, but built from completely synthetic principles.

1.1.7 A look at the future

Advancing biomaterials to the next level will involve an inter- and trans-disciplinary approach. We identified a set of scientific challenges and opportunities in biomanufacturing, both natural and synthetic, which are detailed below. In this introduction we stress the key relevance to three of the nine "Big Ideas" presented by the NSF Director, Frances A. Córdova in Science, May 10, 2016 [70]. These are:

Understanding the Rules of Life & Predicting Phenotype
- Biomanufacturing, natural and synthetic, engineers materials to interface with living systems, or emulates aspects of living systems by constructing synthetic biological circuits to add autonomous control. Synthetic biology is already capable of designing and testing simple logical circuits; we propose to add the design and implementation of active materials formation and breakdown mechanisms that resemble the complex behavior of living tissues such as bone.
- Manufacturing 'engineered' phenotypes is an ambitious goal of biomanufacturing, which will take the Materials Genome Initiative (MGI) to a new level. Basically, we are proposing MGI 2.0.

Harnessing Data
- Biomanufacturing, both natural and synthetic will generate extensive data from experiments, simulations, synthesis, and processing.
- Biomanufacturing requires a biomaterials database – from making to use – similar to the NASA alloy database.
- Biomanufacturing requires big data analytics for characterization, but also to feed and inform simulation-aided materials design.

Convergence: The biomanufacturing facility is intrinsically convergent.
- Key drivers for innovation will be (1) computer science and algorithms from data mining to synthesis, behavioral predictors based on dynamic sourcing of

data from living organisms, that are fed back into synthetic biologic designs, theoretical chemistry, characterization techniques largely rooted in quantum physical concepts, and others; (2) materials science innovations that include integrating ideas and techniques from basic science through engineering fields, to develop new materials, structures, and devices that are valuable to society; and (3) improving manufacturing strategies to implement 'lab scale' production to bulk materials/structures/devices fabrication.

1.2 Scientific Questions

We have identified the major questions that shall drive research and discovery in the next five years in seven subfields of biomanufacturing. These are listed below.

1.2.1 Biomineralization Scientific Questions

- *What are the stabilizers of metastable amorphous minerals and organic compounds?* For example, biominerals, bioadhesives, and antimicrobials
- *What are the precursors to biomineral formation?* Create a library with hundreds of different biominerals
- *What are the indicators of biomineral formation mechanisms?* For example: nanoparticulate cryo-fracture figure would indicate formation by attachment of nanoparticles, or isotopic signatures may be able to discriminate formation mechanisms.
- *What is the role of proteins and enzymes in biomineral formation?* Protein functions may be elucidated with in vitro testing, but also with genetic manipulation.
- *Do natural environmental conditions affect biomaterials synthesis?* For example ocean acidification, temperature, pressure, ion concentrations.
- *Which aspects of biomineral formation are worth reproducing in biomimetic synthesis*? For example natural biomineral synthesis could inspire bone implants synthesis by 3D printing.

1.2.2 Theory Scientific Questions

The ideal theoretical component in the Biomaterials Foundry would contain:

- Modeling of processes, connecting molecular and microstructural design to materials fabrication:

  o Increased focus on long-time-scale molecular and microstructural simulations both to assess structure and properties, and a capacity to predict a diversity of structures that resemble realistic stochastic variability.
  o Explicit description of process conditions, e.g. gradients in temperature, forces/stress, as assembly of material occurs, at multiple length- and time-scales.

- Autonomous design and manufacturing of biomaterials:

  o Incorporation of materials design aspects in multiscale/multiprocess simulation using synthetic biology circuits.
  o Development of optimization algorithms, and connecting predicted structures to a series of manufacturing techniques.
  o *Can we reprogram living system to do biomanufacturing?* Letting biology do the work, including synthesis and processing.
  o For example: Reprogramming the cell (synthetic biology), use of non-biological precursors for synthesis, creating synthetic biological circuits for control, feedback, and dynamic material reconfiguration

1.2.3 Structural Biological Materials and Bioinspired Designs Scientific Questions

- *What features in structural biological materials are worth duplicating in a bioinspired materials system?* For example: What are the characteristics of the interfaces, gradients, and nano- to macro-scale features that give rise to the enhance materials properties?
- *Are there unifying design principles used across taxa that confer certain materials properties?* For example: What design principles (Figure 3) should be employed to create a flexible but impact resistant material?

1.2.4 Biomaterials Synthesis Scientific Questions

- *How to scalably and reproducibly synthesize or manufacture biomaterials and bioinspired materials?* For example by advanced biomanufacturing design.
- *How can biomaterials be fully characterized in real time, label free, and non-destructively?* This is the Holy Grail in biomanufacturing.
- *How can manufacturing and characterization be standardized?*

1.2.5 Synthetic Biology Scientific Questions

- *What are the design rules for constructing hierarchically assembled materials?* For example, individual vs. collective properties, or dynamic assembly and disassembly.

- *How do we bridge the gap between single particle and ensemble characterization?*

1.2.6 Omics Scientific Questions

- *How to correlate -omics (discovery-based science) with materials properties and synthesis, processes, function?* For example biominerals, protein, carbohydrates, biological glues.
- How to further research and implementation of materiomics

Rapidly growing understanding and improved ability to measure changes in the transcriptome, proteome, metabolome, glycome, metallome, microbiome have a great potential to provide new insights into the genetically encoded machineries for the biosynthesis of novel materials from model organisms or new organisms. However, the large amounts of data generated through these techniques are still too poorly integrated to allow our complete understanding of the operation of cellular synthesis, processes, and functions. Through integrated understanding of the regulation and levels of these classes of molecules, we will be able to better interpret biological phenotypes and identify biosynthetic pathways, especially with respect to the biomanufacturing of materials. Hence, establishing a holistic and integrated view of *all* the molecular changes occurring through high throughput data mining (i.e. bioinformatics) of these "Omics" data remains a significant challenge.

1.2.7 Standardization Scientific Questions

How can manufacturing and characterization be standardized?

There is a universal need for a systematic approach in the selection, characterization and qualification of specific materials that are building blocks of medical devices. This approach can significantly impact the entire medical device ecosystem by potentially decreasing the amount of testing and qualification needed for materials. Currently, when a medical device company changes suppliers or suppliers modify their process for making materials, the device manufacturer often needs to demonstrate that biocompatibility and mechanical performance of a medical device have not been adversely affected, which translates into a regulatory hurdle. A similar requirement exists when a new material is being considered for a new or existing medical device. Improved standardization in the quantification of extractables and leachables, and surface characterization of medical device materials, could help with evaluation of materials changes and the impact on related device properties.

Standardized and/or systematic approaches should help medical device companies, polymer/materials suppliers, and chemical companies, modify processes, which would translate into shortening the time between discovery of a new material and its use in a medical device.

1.3 Opportunities and Challenges

1.3.1 Biomaterials Foundry

There is a tremendous opportunity to invest in a Biomaterials Foundry on US-soil including state-of-the-art spectroscopies and spectromicroscopies, providing **ample access to a synchrotron** and support for users before, during, and after beamtime so the users can design experiments, prepare samples, and make sense of their results. The foundry should include three strong components: natural biomaterials characterization, theory, and manufacturing of synthetic biomaterials.

The challenge is to select for methods that enable at least **20-nm resolution**, as that is the scale of biostructures, both organic (protein and polysaccharide assemblies are typically 30-100 nm in size) and inorganic (mineral nanoparticles are 20-100 nm in size) [71]. These methods are **already available, free of charge,** and continue to evolve and improve at two synchrotrons soft-x-ray light sources in the US: **NSLS II and ALS**, at Brookhaven National Laboratory and Lawrence Berkeley National Laboratory.

Such a Biomaterials Foundry would bring the United States ahead of Germany and Israel, where research on biomaterials currently thrives, in just 1-2 years from the start.

1.3.2 Conceptual opportunities and challenges include:

- Learning how $CaCO_3$ is biomineralized in nature may provide an environmentally friendly CO_2 sequestration pathway and, simultaneously, create feedstocks for high-volume materials such as concrete (concrete production accounts for around 10% of the global CO_2 emission, and concrete is the most consumed material after water).
- Co-localization of all tools in one Biomaterials Foundry, especially theory and experiments, shared and homogeneous expertise.
- Creating a table of all the properties of biomaterials.
- Finding unifying design principles in biological materials that can be applied to any materials synthesis.
- Developing tools to image at high resolution in 3D at a higher speed, with *in situ* capabilities.
- Standardizing materials fabrication and characterization.

1.3.3 Instrumentation opportunities and challenges

Understanding how composite biomaterials are biomanufactured by living organisms, and characterizing their properties is still a great challenge. Recent advances in microscopy, spectroscopy, spectromicroscopy, computed tomography, and nucleic acid sequencing techniques present an opportunity to fully characterize the composites and understand the pathways for their synthesis. In this respect, it is key to acquire or have fast access to existing state-of-the-art tools and methods, and to develop new tools that don't exist yet. These are:

Spectromicroscopy:

- Development of a new beamline and two end stations at a soft-x-ray synchrotron (Berkeley-ALS or Brookhaven-NSLS II), dedicated to biomaterials. The two end stations are: X-ray PhotoEmission Electron spectroMicroscopy (**X-PEEM) with 20 nm** resolution to analyze the surface of **solid biomaterials**, and Scanning Transmission X-ray Microscopy (STXM) **with 20 nm** resolution to analyze **solid thin, liquid, or soft organic biomaterials** in transmission. The two end stations can time-share the same beamline.

- Development of high-resolution PEEM (10 nm) for mineral phase identification in biominerals, and their phase transitions, regulated by polymers. This is a significant challenge, and may exceed the 5-year scope of this report. It took ~15 years in Germany (SMART project) and at the ALS (aberration-corrected PEEM-3) and is still not functional in either place.
- Use of already-existing X-PEEM and STXM at ALS or NSLS II. This option has no cost, but provides only 10-20 days/year of beamtime dedicated to biomaterials, countrywide.
- Development of new, high-throughput PEEM analysis of ink-jet printed nano-samples.
- Low-dose (10 Gy) PEEM and STXM spectromicroscopy for polymer analysis.
- Nano-FTIR (20-nm resolution) at ALS or NSLS II.
- Secondary ion mass spectrometry (SIMS) and nano-SIMS.
- Atom probe tomography.
- Development of real-time analysis of mineral formation in bacterial cultures with Quartz Crystal Microbalance Dissipation (Q-CMD) coupled to a confocal microscope.

Microscopy:

- Scanning electron microscopy (SEM), cryo-SEM, focused ion beam (FIB-SEM), environmental SEM (E-SEM), including instruments equipped with Raman spectroscopy capabilities.
- Transmission electron microscopy (TEM), aberration-corrected TEM (AC-TEM), cryo-TEM tomography, *in situ* TEM.
- State-of-the-art visible light microscopies, including confocal microscopy, super-resolution microscopy, light sheet microscopy.
- X-ray microscopies including computed tomography (CT), micro/nano-CT. Current commercial units have ~150 nm resolution, and are very time consuming and expensive to use.
- Development of higher resolution CT.
- Atomic force microscopy (AFM), *in situ* AFM.
- Atom probe tomography.
- Development of focused ion beam / transmission electron microscopy for high resolution characterization of hierarchical structures.
- Development of improved nano-mechanical testing equipment for *in-situ* testing in electron microscopes.

Spectroscopy:

- X-ray Absorption Near-Edge Structure (XANES) spectroscopy.
- Extended X-ray Absorption Fine Structure (EXAFS) spectroscopy.
- X-ray photoemission spectroscopy (XPS).
- Solid-state nuclear magnetic resonance (NMR) spectroscopy.

- Vibrational spectroscopies include Fourier transform infrared (FTIR) spectroscopy and Raman spectroscopy.
- Gas chromatography–mass spectrometry (GC-MS).
- Liquid chromatography–mass spectrometry (LC-MS).

Scattering and Diffraction:

- Small Angle X-ray Scattering (SAXS) and Wide Angle X-ray Scattering (WAXS).
- Grazing Incidence SAXS (GI-SAXS).
- Micro-X-Ray Diffraction (μXRD).
- Nano-XRD (n-XRD).

Sequencing:

- Sequencing and preparation machines for high-throughput analysis.

Synthesis:

- **Multiscale biomanufacturing capability** must be a core facility of the Biomaterials Foundry. It integrates architectural control from chemical to structural scale through integration of self-assembly, additive techniques, subtractive techniques, etc. – in sum, a range of techniques.
- Development of nano-3D printing: the ability to print out fine features.
- Development of 3D printing with minerals and other hard materials.
- Development of new inks for 3D printing of multiple materials at a finer resolution.
- Development of highly-parallelized synthesis, including multiple assembly lines to allow for parallel processing of manufacturing jobs.

Computing:

- **Supercomputing facility** at the petaflops scale or a fraction thereof, with GPU capability, and perhaps other hardware accelerated methods (e.g. Anton).
- Incorporate a **large-data center** as central repository for experimental, and theoretical protocols, codes, etc.
- **Extensive visualization facilities** that let computational and experimental scientists view data in virtual reality and multiple dimensions, coupled with advanced data analytics to extract design information and mechanisms from complex stochastic data assembled across multiple time- and length-scales. In other words, make things visible to our human eye that occur in a space that cannot be seen; utilize mathematical tools such as category theory to translate.
- An NSF-funded server that can house and store data from large data and image files.

1.3.4 Synthetic Biology and Omics

In addition to instrumentation to allow characterization of the composites and understanding of the mechanisms of synthesis of these composites, the advent of **synthetic biology** opens up the capability to synthetically reproduce some of these biological composites. However, the complete understanding of how biological systems work and biomimetic synthetic biomanufacturing is still in its infancy because the natural biological systems are poorly understood. For instance, how and which metabolites are produced by organisms under different environmental conditions, i.e. temperature, pH, and stress, is still obscure, and a **complete database of all the metabolites** known to be produced by microbes, plants, and animals is not yet available. Compiling such a database of metabolites and linking them to the environmental conditions will provide unprecedented and comprehensive understanding of the optimum natural conditions for the biomanufacturing of biominerals and biocomposites.

Another opportunity is the **integration of the omics**, that is, genomics, metallomics, proteomics and metabolomics data, which would allow deeper understanding of how biological systems function and can be **harnessed for biomanufacturing inspired by nature**. Hence, the understanding of the genetic, physiological, metabolic, and enzymatic diversity of biological systems, as well as how to emulate these *in vitro* or in acellular systems will bridge the gap between characterization and biomimetic synthesis.

A centralized **biomaterials repository** to tabulate/display all or at least the most important properties for all polymers would benefit the entire biomanufacturing community. Such an undertaking is vast and certainly not a good fit for a graduate student time in a laboratory. NIST can perhaps take lead around such an endeavor with help from other agencies, including NSF, DOD, FDA etc.

1.4 Recommendations for NSF:

1.4.1 Biomaterials Foundry

1. Establish a strong US-based Biomaterials Foundry, with focus on key processes such as biomineralization, self-assembly, hierarchical and multiscale, and additive processes. We lag behind Germany and Israel. This foundry should focus on fundamental research on biomaterials formation mechanisms with at least **20-nm imaging resolution**, as that is the scale of biostructures, both organic (protein and polysaccharide assemblies are typically 30-100 nm in size) and inorganic (mineral nanoparticles are 20-100 nm in size) [71]. This Foundry should dedicate 50% of its personnel and instrumentation time to external users, and 50% to in-house research. It should provide state-of-the-art microscopies, spectroscopies, and spectromicroscopies, natural and biomimetic sample preparation facilities, and have ample access to a synchrotron with support for users before, during, and after beamtime so the users can interpret and divulge their results. The foundry should include three strong components: natural biomaterials characterization, theory, and manufacturing of synthetic biomaterials with both organic and inorganic components.

2. Establish a strong program on state-of-the-art characterization of the structures and properties of biological materials to understand the origin of extraordinary properties (e.g. mechanical, optical, electrical).
3. Establish a strong program in the creation of bioinspired materials and structures, based on lessons gleaned from characterization in 2.

In a snapshot, the ideal Biomaterials Foundry will perform the following tasks:

What	How	of What
Having a fundamental understanding of the way organisms make biomaterials gives insight into	Characterization	Natural biomaterials
Computation, modeling, simulation, which drives	Theory	Models and how they are connected to experiment
Manufacturing of synthetic biomaterials	Synthesis	Bioinspired/biomimetic systems

1.4.2 Instrumentation

There are two midscale models that can be followed, with strong preference towards the second. The first includes a significant instrumentation effort at a synchrotron, and a relatively modest investment in other instruments. The second has free and ample access to a synchrotron, but invests on lab-based instruments, personnel, and it is community-building. In detail, these are 1.4.2.1 and 1.4.2.2.

1.4.2.1 Midscale Biomaterials beamline at a synchrotron

A significant investment will enable the development of two high-resolution spectromicroscopes dedicated to biomaterials. It is important that these be dedicated, so the instruments are optimized for diverse biomaterials samples and sample preparation, and the scientists operating them have the background and culture of biomaterials science. Conceptually, this facility will be similar to the GSE-CARS (https://gsecars.uchicago.edu/) at the Advanced Photon Source (APS), which has several beamlines and end stations and is entirely dedicated to geological, soil, and environmental science experiments.

Technically, however, the synchrotron component of the Biomaterials Foundry will be very different from GSE-CARS. It will have to be developed at a soft-x-ray synchrotron, either the Berkeley-ALS or Brookhaven-NSLS II, and will have one beamline and two end stations, to achieve state-of-the-art spectroscopy and microscopy with 20-nm resolution on hard or soft biomaterials.

The beamline to be developed has 100-2000 eV energy range, energy resolution E/ΔE = 8,000 or better, with an Elliptically Polarized Undulator (EPU) as its insertion-device source, and two end stations. One is an X-ray PhotoEmission Electron spectroMicroscope (**X-PEEM) with 20 nm** resolution to analyze the surface of **solid, polished biomaterials**; the other is a Scanning Transmission X-ray Microscope (STXM) **with 20 nm** resolution to analyze **thin solid, liquid, or soft organic biomaterials** in transmission. The two end stations will do time-sharing on the same beamline. We estimate the cost of the EPU, beamline, PEEM, and STXM on the order of **$15 million**. Laboratory equipment to prepare and characterize samples, process data, and staff the beamline design, construction, commissioning, and support the user operation is estimated at **$10 million** in 5 years. Other equipment includes ESEM and cryo-tomography-TEM instruments, 3D printers and other synthesis facilities, and petaflop computing, collectively to cost an additional **$10 million**.

1.4.2.2 Midscale instrumentation at the Biomaterials Foundry

In alternative to developing a new beamline, a Biomaterials Foundry would have extensive, state-of-the-art microscopies and spectroscopies in house, and ample access to spectromicroscopies at a soft-x-ray synchrotron, where ~10% of the available beamtime can be obtained with an Approved Program proposal at the ALS (https://www-als.lbl.gov/index.php/ring-leaders/341-approved-program-proposals.html) or the Block Allocation Group proposal at NSLS II (https://www.bnl.gov/ps/userguide/), which will eventually include other beamlines. In this case the Biomaterials Foundry users and scientists would use already-existing X-PEEM and STXM facilities at ALS or NSLS II. This option has no cost, and will provide considerably more than the ~7 days/year of beamtime dedicated to biomaterials that a single user can obtain. Longer, pre-scheduled periods of beamtime allocated to the Biomaterials Foundry and its users would provide rapid access to PEEM and STXM spectromicroscopies, as well as other attractive synchrotron methods, including SAXS, WAXS, nano-FTIR, nano-x-ray tomography, micro- and nano-x-ray diffraction, etc. This beamtime will enable the experiments planned, as well as new and unforeseen ones brought in by new users, and requiring rapid access.

This approach is extremely attractive because all these techniques already exist and are constantly being upgraded and improved at synchrotrons, are supported by the DOE, and are offered to users at **no cost**. The DOE facilities are excellent at providing equipment, beamtime, and technical support during the beamtime. Before and after beamtime, however, the DOE facilities do not support users at all. There is an immense need for **user support before beamtime**, including designing the most successful experiments, preparing samples for beamtime, and characterizing them with other methods. There is an even greater need for **user support after beamtime**, including data management, storage, analysis, interpretation and conclusions on the significance of the data. With increasingly larger and more complex datasets, the user is usually at a loss converting terabytes of images into meaningful results. A dedicated Biomaterials Foundry and its expert scientists will provide user support at all stages of the experiments, from conception to publication of scientific results on biomaterials science.

A significant investment in expert **personnel**, including theory, characterization, and synthesis is warranted, and is estimated to cost, **$10 million** for 5 years.

An even more significant investment, estimated at **$25 millions**, on biomaterials characterization, synthesis, and computing **instrumentation** should include many of the tools listed in Section 1.3.3.

This **$25 millions** of instrumentation should be housed in the Biomaterials Foundry, which does not need to be located near a synchrotron. In fact locating it on the opposite coast, compared to the synchrotron of choice, or in the Midwest, would serve the largest possible number of biomaterials users nationwide.

With this approach to a Biomaterials Foundry everybody wins: the DOE-supported synchrotrons produce more publications, and the NSF-supported users get to make discoveries at the Foundry and at a synchrotron for no additional cost.

1.5 Concluding remarks

Abudant fundamental scientific questions pertaining to biomaterials (natural and synthetic), including aspects of synthesis, theory, production and omics are yet to be answered. We propose that a Biomaterials Foundry be established by the NSF to include:

- Easy user access to sophisticated tools at the national synchrotron facilities.
- The development of new high-resolution tools to probe materials at the nano-scale and in real time.
- Development of improved and cutting-edge multiscale synthetic biomanufacturing capabilities to create bioinspired materials and structures.
- The creation of a supercomputer facility that incorporates an NSF server (data and images) for the development of a national biomaterials database.

SECTION 2: Dynamic and Adoptive Biomaterials Surfaces and Interfaces

SESSION LEADER

MAREK W. URBAN, CLEMSON UNIVERSITY

SECTION MEMBERS

DEBRA AUGUSTE, CITY UNIVERSITY OF NEW YORK

NEEL JOSHI, HARVARD UNIVERSITY

LIVIU MOVILEANU, SYRACUSE UNIVERSITY

HUNG NGUYEN, UNIVERSITY OF CALIFORNIA-IRVINE

NICOLE F. STEINMETZ, CASE WESTERN RESERVE UNIVERSITY

MATTHEW V. TIRRELL, UNIVERSITY OF CHICAGO

2.1 Introduction

While combining different logic gates and organizing them in particular sequences typically facilitates electronics programming, it seems only natural that combining different chemical or physical motifs in a specific sequence constitute programming in biomaterial interfaces. These motifs and their sequences will determine the specific tasks to be carried out, the order in which they will be executed, and the signaling that will trigger each task. Based on experiments performed over millions of years, nature is the ultimate master of engineering living systems and offers numerous extraordinary examples for the interfacial design and functionality of programmable materials. Examples are countless; in proteins, the coded linear sequence of amino acids is responsible for inter- and intramolecular interactions that govern their behavior and utility, thus dictating their final assembly and specific adaptive functions. The functions of antibodies result from the recognition of specific interfacial shapes achieved by three-dimensional spatial arrangements of amino acids that lead to selective binding to specific antigens capable of recognizing specific foreign objects. But perhaps the most well known programmable biological system is DNA, which acts as remarkable information storage facility, with data written in the sequences of nucleic bases. These are the specific sequences that hold the information for accurate and exact assemblies of conjugated DNA strands, allowing sophisticated molecular tools to be used for replication, regulation, translation, and decoding from one chemical "language" to another. Regardless of whether these events are individual or occur as collective, they rely on programmable functions encoded in structural interfacial features of DNA, and the most critical function is the ability to trigger the execution of instructions encoded in the interfacial boundaries to perform specific tasks.

Interfaces and surfaces are the regions where all key processes between one medium and another take place. These may range from molecular recognition to charge transfer reactions, or polymerization to catalytic reactions, to name just a few. Surfaces and interfaces in biomaterials, which are used synonymously to describe the interfacial boundaries between to condensed phases, are critical not only to the majority of biological functions, but also the interactions of biomaterials with biological systems.

While biological systems exhibit the ability of precise and repetitive placement of its constituents in complex machinery that facilitate living functions, the majority of existing synthetic biomaterials are unable to organize, develop, adapt, and respond changing chemical and physical environments. Since many biological processes occur at the interfacial boundaries, adaptive and stimuli-responsive interfaces capable of interacting with biological systems will be critical in the development of new biomaterials. Their adoptive functions are essential in biological or synthetic environments as they regulate signaling, transport, and delivery across interfacial boundaries. If we understand how biomaterials interfaces can respond to and communicate with biological environments in a controllable manner, many scientific questions will be answered, ultimately leading to technological advances. To gain this fundamental knowledge, it will be essential to control synthetic processes precisely as well as to measure with high precision and resolution dynamic behavior of the interfacial regions.

This section outlines the results of the discussions stimulated by scientific presentations and the input from all participants during the workshop. It is organized into the following sections:
2.2. Scientific Questions, which will be critical when seeking a better understanding of current trends in the adaptive and stimuli-responsive biomaterial interfaces.
2.3. Challenges and Opportunities, which are critical in solving the identified scientific questions during the next five to ten years.
2.4. Recommendations

2.2. Scientific Questions

2.2.1 How do we synthesize biology-inspired sequence-defined biointerfaces with multi-dimensional probes that can interface with multi-modal tools?

Controlled materials synthesis is a key enabler for advances in adaptive and stimuli responsive biomaterials surfaces and interfaces. The last two decades have brought remarkable advances in the controllable synthesis of stimuli-responsive polymers as well as formulated some strategies for the development of living-like programmable polymeric materials. The synthetic capabilities afforded by living polymerizations, perhaps most notably those based on reversible deactivation radical polymerization, have provided access to a plethora of new controlled architecture macromolecules with precision placement of functionality needed for responsive behavior. Also, new polymerization techniques, the rapid development, and growth of new orthogonal chemistry strategies for installing responsive and adaptive moieties via post-polymerization modification have further expanded the toolbox in the preparation of new biomaterial interfaces. Since the precise manipulation of molecular weight, molecular architecture, and functional group placement will yield new biomaterials interfaces with dramatically different properties, there is a need for accurate synthesis by incorporating interfacial functionalities required to detect the

stimulus and to enact a new behavior. It should be realized that when a synthetic biomaterial is in contact with a biological system, there are two surfaces that need to communicate with each other. These interactions many be constructive or destructive. Commonly utilized modes of existing synthetic approaches that lead to modification of biomaterials interfaces are grafting-to, grafting-from, or grafting through. Typical examples are polymer brushes, which have been studied extensively. Another critical component of these interactions is surface topology and there is increasing evidence that a combination of chemistry and surface topologies is responsible for many favorable or unfavorable surface interactions. To understand this interplay new analytical tools are needed that are coupled to the synthesis and integrating computational modeling and materials designs. Non-destructive analytical methods integrated in the synthetic process will allow the simultaneous design and characterization of sequence-defined materials integrating multimodal functions. Such functions may facilitate within the reporting of cellular events, in particular at the biomaterials-cell interface, within biological systems, but also integrate cargo-delivery function to reprogram biology or restore healthy cell function.

2.2.2 What molecular events at the biomaterial-cell interfaces govern interaction/signaling and how do we qualify and quantify these events?

Biomaterials interact with living systems via interfaces defined by surface chemistry, topography, magneto/electric polarization, and mechanics. These interactions govern processes still not yet fully understood. For example, adhesion, biofouling, and immune responses will be critical in the development of biomaterial interfaces. These systems are complicated, and there is a need to understand individual interactions within a complex array of molecules and conditions. The question remains whether studying a single entity describes events of a physiologically relevant milieu. Measurements and models made on single molecules need to translate into macroscopic behavior formulated by many interactions. The situation will become even more complicated in the living systems. Thus, measuring singular events may require parallel measurements of multiple event processes.

Switchability and adaptability of biomaterial interfaces offer many advantages critical to many applications, but their measurements are not trivial. A self-assembled monolayer (SAM) is a single layer of amphiphilic molecules that spontaneously organize themselves on a substrate due to the affinity between the amphiphile and the substrate. One of the methods of creating responsive surfaces is the use of low and high-density self-assembled monolayers (SAM). Synthetic molecules that are often utilized to obtain interfacial responsiveness to an electrical potential, light, pH, and temperature are listed in Table 1.[72] However, the measurements of transient responsiveness at molecular levels are currently not accessible with existing tools.

	Electrical potential	Electromagnetic Radiation	pH	Temperature
Self-assembled monolayers (SAMs)	X		X	X
Azobenzene		X		
Spiropyran		X		X

Polyelectrolyte Brushes			X	X
Rotaxane	X	X	X	
Catenane	X			
DNA monolayers	X		X	
Peptide monolayers	X		X	X

Table 1. Selected categories of interfacial molecular entities responsive to electrical signals, electromagnetic radiation, pH, and temperature.

Although this area had many substantial advances, the development of switchable interfaces with the biological relevance will be critical in the next 10-20 year. The dynamic state of the natural ECM is regulated by a highly complex temporal and spatial coordination of diverse and complex cell–matrix and cell–cell interactions. This progress will be contingent upon our ability to measure these interactions selectively. The switchable biomaterial interfaces are significantly different from traditional synthetic interfaces in the complexity and therefore, the sophistication with which they interact with biological systems must be dynamically controlled and measured. The methodologies used in their analysis may reversibly modulate protein adsorption, which is important for a variety of applications, including biofouling, chromatography, and bioanalytic devices, but more advanced mechano and spectroscopic tools are needed.

2.2.3 How do we measure interfacial dynamics of stimuli-responsiveness across multi length scales?

Living systems and adaptive materials have the capability to morph. Current tools often measure equilibrium stages under specific conditions while practically all interfacial processes are non-equilibria phenomena. The challenge is to detect the events at appropriate time and length scales to capture the dynamics of stimuli-responsiveness. These events may highlight unique, non-equilibrium, transitory states that are yet to be captured outside of simulations. Such capability may leverage thermodynamics and structure-function relationships that underpin material design and properties. Additionally, there lies a limitation to observe phenomena beyond ~1 μm at time scales (on the order of femtoseconds) and without destruction of manipulation of the native environment or sample.

At the interfaces of living systems and adaptive materials conformational changes molecular transport, binding, catalysis, reaction kinetics, occur which, in turn, result in a cascade of events. A simplified example is pH-responsiveness, which relies on protonation of chemical moieties, causing electrostatic repulsion and swelling to minimize entropy. Diffusion of protons (10^{-8} cm^2/s) occurs quickly and thus tools that identify the stress/strain relationships are lacking. In living systems, binding interactions that result in conformational changes at a surface or membrane is transduced via chemical signaling. Understanding these interactions in multicomponent systems will be essential and new tools are necessary. In particular, the tools that can report multiple yet distinct events that can trace and model in real time, and are capable of noninvasively characterizing molecular events at the interfacial regions. Furthermore, these probes should be sensitive to electrical/mechanical/chemical/thermal events and be capable of amplifying signals.

2.2.4 How do we measure heterogeneity in biomaterials interfaces and its effect on functions and responsiveness?

Biological materials are inherently inhomogeneous in structure, which dramatically influences their material properties. For example, many biological systems synthesize structures starting from a relatively narrow range of elements and chemical building blocks (compared to synthetic materials), yet they combine these starting materials to create architectures that are much more complex than could be formed from the individual components by themselves. Thus, structural inhomogeneity can lead to emergent properties like dramatic enhancements in mechanical performance, new optical properties (as in the case of structural color), and non-linear increases in enzyme activity and signaling. Similarly, structural inhomogeneity can lead to differences in the way materials respond to their environments. For example, the specific anisotropic orientation of cellulose fibrils in plants leads to specific environmental stimuli, such as helio- and phototropism (Figure 1).[73] Existing nano-characterization techniques applied to the analysis of natural systems have revealed new design principles that are now fueling the creation of synthetic biomaterials with dynamic environmental responsiveness. However, while these techniques excel at static measurements on molecular ensembles, they largely ignore heterogeneity at single molecule/cell levels and are usually incompatible with dynamic, in situ measurements.

Figure 1. (A) Image of *Pollia condensata* fruit, which exhibits structural color. (B) Optical microscopy images in cross polarisation configuration of the fruit. (C) Color variation of a drying film prepared from a 1.1% suspension of cellulose CNCs, mimicking the fruit's surface structure. Selected reflection spectra during evaporation. The red-to-blue color scale represents the evaporation time. The traces in grey are from the earlier stage of the evaporation process when the suspension has not formed its color yet.[73]

2.2.5 What interfacial molecular entities are responsible for dynamic morphological features?

The chemistry and topography of biomaterial interfaces and surfaces are critical, as these properties impact protein adsorption, cell interaction, and biological responses. Numerous examples have shown that biomaterial-biological system interactions are essential. Although polymeric, ceramic, or metallic materials exhibit different surface properties, ranging from hydrophilic to hydrophobic or hard to soft, and may exhibit similar responses due to non-

specificity of their surfaces, all nature processes employ specific molecular recognitions. To introduce responsive specificity, significant efforts have focused on creating biomaterials with stimuli-responsive interfaces to control interactions with biological systems, a task typically accomplished by decorating surfaces of polymers, ceramics, or metals with signaling molecules.

The central scientific question is how do we determine relationships between structural heterogeneity (e.g., composites, anisotropy, sequence diversity) and responsiveness at biomaterial interfaces in natural and synthetic systems? This question requires further developments and adaptations of the state-of-the-art methodologies and analytical techniques. The majority of the current approaches are not amenable to high-throughput settings for simultaneous acquisitions of mechanical, thermal, electrical, and optical signals at high temporal and spatial resolutions. Detailed characterization strategies should be combined with hybrid multi-dimensional and multi-scale instruments with unprecedented capability for high-speed physical and chemical data and imaging acquisitions as well as automation in a scalable format for a healthy number of repetitions. In this way, various acquired signals would provide a comprehensive knowledge on biomaterial heterogeneity of structure, conformation, flexibility, electrical and thermal conductivity as well as polydispersity. This kind of measurements should enable determinations of both individual single-molecule populations and averages of molecular behaviors in an ensemble. Large data and image acquisitions should be supplemented by further developments of computational approaches for further data processing, analysis, and interpretation.

2.3. Opportunities and Challenges

Even though surface and interfaces between synthetic biomaterials and biosystems have been of scientific interest and technological importance for several decades, their regulatory functions manifested by their adaptability and stimuli-responsiveness are not well understood. Although a significant number of experimental and theoretical approaches have been employed, analytical methods capable of taking molecular events in creating as well as utilizing interfaces are limited, to say the least. While one of the roadblocks is the limited sensitivity and selectivity, inherently low concentration levels of the interfacial regions make quantitative assessments troublesome, in particular, when interfacial processes are dynamic. It is apparent that microscopic or macroscopic measurements or even well-known thermodynamic processes can be addressed, but a large degree of complexity manifested by nonlinearity, heterogeneity, and dynamics of stimuli-responsive biomaterial interfaces prevent us from understanding how many interfacial chemical reactions and physical processes at these critical boundaries regulate many living-like functions. The ability to measure these effects in a dynamic in-situ at the angstrom, nano, micro and greater length scales and the development of the means to rigorously built-in dynamic, stimuli-responsive components into the interfaces will be critical before the field will be able to reach a full potential.

2.3.1 Precision Synthesis at an Interface

One of the primary assets of biological systems is the ability to synthesize complex structural features. Although significant efforts have been made in synthesis, creating ultra-high molecular

weight molecular constructs, especially those, which contain precisely placed responsive components, remain to be a challenge.[74] Current approaches allow precision synthesis using controlled radical polymerization (CRP),[75,76] but are unable of generating high molecular weight biomaterials. In contrast, there is an opportunity to overcome existing approaches using heterogeneous radical polymerization (HRP) capable of producing ultra-high block copolymers[77] as well as controlling copolymer morphologies at nanoscale lengths.

Another research area with tremendous opportunities is the development of synthetic substrates that dynamically regulate biological functions in response to applied stimuli. In essence, the challenge is to mimic the dynamics of the dynamic of biological systems. These surfaces and interfaces should be able to modulate biomolecule functions, cell adhesion, stratification and immobilization and interfacial migration. With proper measuring tools capable of dynamic monitoring and capable of regulating signals between cells or cell-extracellular matrix (ECM) interactions. Cell-ECM interactions are very complex, and to date, no complete molecular-level understanding exists, thus providing an unprecedented opportunity and significant challenge. Systematic studies are critical in tackling these issues, but their success will depend upon the development of new analytical tools capable of monitoring these interactions.

2.3.2 Tools and Analytical Approaches for Dynamic Interfacial Analysis

Interfaces in biomaterials that respond to chemical and biochemical responses may also offer many intriguing possibilities for the development of novel biological sensing tools. For example, responsive bioadhesive surfaces and delivery systems with controlled release capabilities are one of the many examples. One example is the development of the electrochemical DNA sensor based on the alteration of the electron transfer dynamics as a consequence of a structural rearrangement induced by target hybridization.[78] Another example is the cell-based sensors capable of establishing molecular communication between cells and biomaterial surfaces based on enzyme-responsive self-assembled monolayers (SAM) depicted in Figure 2.[79] Sensing devices that utilize bio-censing capabilities represent another area of opportunities for the development of future analytical tools.

On the other hand, biological membranes are vital components of living systems that form the outer boundaries or are internalized within cells. They typically consist of lipid bilayers that control an uptake and communications across, thus acting as a responsive filter. Substrate-supported membranes can be manipulated to tune their architecture and physical properties for optimal immobilization and communication between the membrane itself and supporting surfaces. Since signaling will be critical in interfacing synthetic and biological systems, it is anticipated that using semiconductors as supports may enable the detection of local signals from individual or small numbers of proteins and enzymes. It is anticipated that these signals can be used for detecting interactions and sense responsiveness, thus creating an opportunity for the development of new detecting devices.
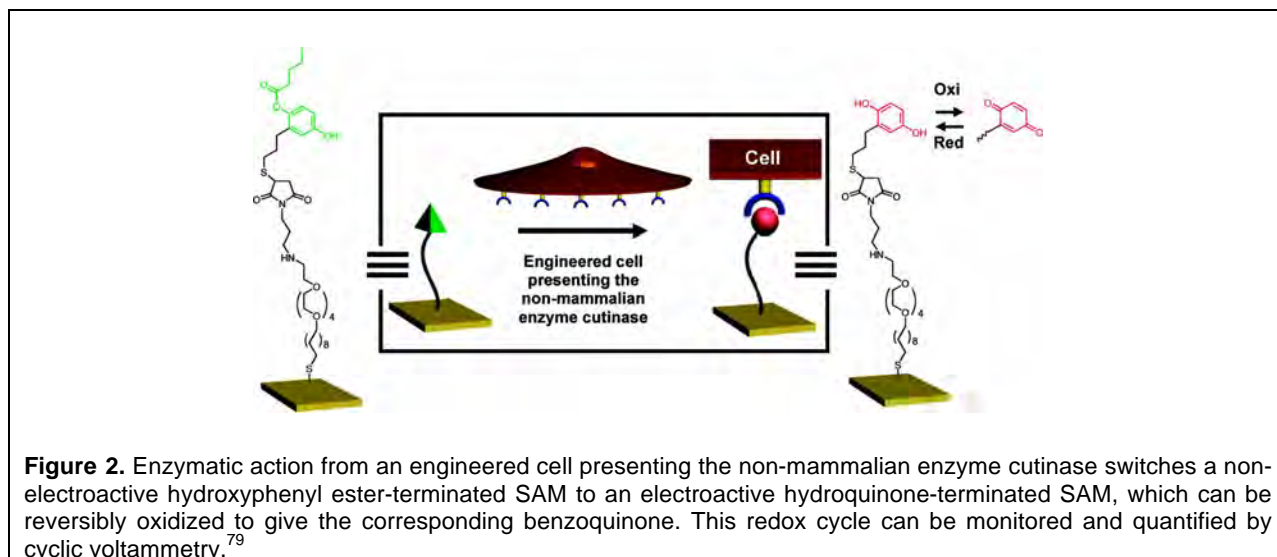
Figure 2. Enzymatic action from an engineered cell presenting the non-mammalian enzyme cutinase switches a non-electroactive hydroxyphenyl ester-terminated SAM to an electroactive hydroquinone-terminated SAM, which can be reversibly oxidized to give the corresponding benzoquinone. This redox cycle can be monitored and quantified by cyclic voltammetry.[79]

2.3.3 Stratification, Heterogeneity, and Responsiveness

While the variation of nature responses to external and internal stimuli involves complex remodeling phenomena of reversible or irreversible processes, one common feature of biological systems is the formation of multi-layered directionally stratified structures that are often compartmentalized. Interactions via signaling and responsiveness within or outside these compartmentalized structures make biological systems unique with an extraordinary ability of healing wounds autonomously. Due to similarities with biological systems the formation of stratified polymer networks has been of significant interest, whereby numerous studies showed the importance of stratification on materials' properties. For example, the presence of bioactive dispersing agents such as phospholipids (PLs), may lead to their stratification near interfacial regions,[80] which is responsible for lowering static and kinetic coefficients of friction as well as the enhanced adhesion. There are other examples, which showed that PLs resulted in the formation of hollow spherical particles or tubules. Controllable release and formation of surface/interfacial localized clusters (SLICs) into phospholipid rafts at the interfacial regions of biomaterials represent another opportunity for the development of controlled morphology developments and interfacial transport. Another opportunity is the formation of stimuli-responsive ion transfer pathways through the phospholipid rafts in the interfacial regions. There are opportunities for capturing for capturing the formation of SLICs and diffusion kinetics of ions through PLs channels.

Conductive materials that alter conductivity based on electronic history are known as memristors. These memory resistors are capable of information retention as well as processing, thus able to create super-fast memory chips with more data at less energy. Employing nontoxic biomaterials as the fundamental building blocks of electronic devices is of growing interest for biocompatible and environmentally compliant electronics. There are opportunities for the exploration of electron transfer processes within biomaterials, which being among the most fundamental processes in biological systems, are essential in measuring biological energy conversion processes at biomaterial interfaces. The development of biomaterials capable of switching resistance between a high resistance state (HRS) and a low resistance state (LRS)

through an applied electrical field will offer a unique opportunity for new detection systems. Based on these principles, the development of highly sensitive and selective detection devices based on biomaterials such as DNA and proteins capable resistive switching characteristics is anticipated.[81] These components could be combined in a single neurotransmitter device in which the conformationally-induced switching properties could be achieved by a tailorable multi-state conductance, appropriate for use as a synaptic substitute in neurotransmitters. These biomaterials may open an opportunity for stimulating metabolism of amines and amino acids in living systems, including neurologic changes, as well as serve as sensing devices allowing monitoring time and temperature dependence, current ratio, and the magnitude of the electric field to initiate resistive switching. One of the challenges in exploring interfacial properties in biomaterials is to capture signaling pathways in a molecular network. One for the proposed approaches is so-called network stratification analysis (NetSA), whereby the whole network can be stratified into function-specific layers representing particular functions, thus converting the network analysis from the gene level to the functional level by integrating expression data.[82]

2.3.4 Interfacial Sensing, Signaling, and Self-Healing

Cell signaling is one of the critical communication paths that occur at the biological interfaces and governs essential activities of living organisms and coordinates their responsiveness and actions. This unique ability of living organisms to respond to external stimuli enables responses by generating immunity repairs as well as normal homeostasis. Typically, synthetic biomaterials do not exhibit these properties. The interfacial regions between biomaterials and biological systems will be critical in interfacial sensing and signaling. Thus, understanding the underlying structure within cell signaling networks in the presence of synthetic biomaterials will be essential in understanding the signal transduction and responsiveness of biomaterials to biological environments. Understanding and measurements of cell and biomaterial communications will require a combination of experimental and theoretical approaches including the development and analysis of models and simulations.

Biomaterials capable of altering their properties in response to chemical, physical, or biological stimuli have been of interest for a number of years, but the last decade has witnessed significant fundamental developments in this area. The ability of a biomaterial to respond to factors such as temperature, pressure, pH, ionic strength, concentration gradients, or electric and magnetic fields at the interfacial boundaries opens the door to a range of unobtainable before applications as well as presents demanding scientific challenges. Responses can be physical or chemical in nature, or both, but perhaps the most intriguing phenomenon is the ability of materials to self-repair broken bonds, which can visually be observed by a naked eye.

Living cells make a number of decisions, including which protein to express or when to divide them, or when to commit suicide. At the interfacial regions, activated genes will express the proteins needed for proliferation and differentiation in a specific synchronized sequence. In addition to these intrinsic cell functions that regulate cell fate, external signals from the surrounding extracellular matrix (ECM) or biomaterials interfaces are essential in biosystems. As small as nanoscale topography changes in biomaterials will impact cell behavior, ranging from changes in cell adhesion, orientation, motility, cytoskeletal condensation, activation of tyrosine kinases, and modulation of intracellular signaling pathways that regulate transcriptional activity

and gene expression.[83] It is believed that not only ~5 nm may impact cell behavior, but also the type of topography (e.g., ridges, steps, grooves, etc.) and even their symmetry (e.g., orthogonal or hexagonal packing of nanopits).[84] Although different approaches and experimental procedures were offered, it is difficult to compare the data even for similar systems because of the lack of analytical tools capable of measuring cell-biomaterials interactions.

Loosely defined in biological systems as a built-in defense mechanism preventing species from losing their living functions, self-healing in Nature are quite complex and involve multi-level transient chemico-physical responses in continually changing bioactive environments at different lengths scales. Self-healing in biomaterials in general, and polymers in particular, is manifested by the ability to regain original properties lost during external damage. If biological systems are the benchmark for self-healing, then a synthetic mimic should be able to continually sense and respond to damages over its lifetime, restoring original chemical and physical features without adverse effects. Damage of macromolecular chains in polymers leads to bond cleavage and/or chain slippage. Thus, in designing polymers with self-healing attributes it will be essential to utilize either reactive chain ends typically represented by free radicals,[86] or incorporate other reactive components capable of repairs upon mechanical damage. Furthermore, the generation of reactive groups upon mechanical

Figure 3. When methyl-α-D-glucopyranoside (MGP) molecules are reacted with hexamethylene diisocyanate trimer (HDI) and polyethylene glycol (PEG) to form crosslinked MGP-polyurethane (PUR) networks, these materials are capable of self-repairing. This process does not occur in the presence of any other gases, but requires atmospheric amounts of CO_2 and H_2O, thus resembling plant's behavior during photosynthesis.[85]

damage is particularly promising when developing biomaterials.[85] For that reason, understanding of the role of coordinated non-covalent supramolecular interactions, including H-bonding, metal-ligand coordination, or π-π stacking will be critical in the design of stimuli-responsive biomaterial interfaces. One example of orchestrated and coordinated self-healing of polyurethane network that contains disaccharides and utilizes carbon dioxide and water to achieve self-healing is depicted in Figure 3.[87]

The developments of new paths to achieve "metabolic-like" self-replicating materials capable of dynamically adapting to environmental changes will be also critical. Defined in biological systems as 'self-cannibalization' or authophagy, metabolism in biomaterials can be viewed as self-healing by replacing 'outdated' degradation products or minute product side reactions. Because neither control nor elimination of side product reactions are trivial, one can envision that combining selected elements of supramolecular networks, covalent bonding, and recently discovered shape memory macromolecular segments will shape paths to the new generation of self-regulating biomaterial interfaces. In order to achieve these goals a better spatial resolution and fast data acquisition tool are needed.

2.3.5 Living Organisms at Biomaterials Interfaces

44

Although the majority of interactions between biological systems and synthetic materials are inherently non-favorable, the formation of biofilms represents unwelcome exception resulting from the attachment of bacteria to a synthetic surface. Being resilient communities, microbial films resist removal by chemical or physical means because their residents, bacterial cells, are capable of adhering to a variety of biotic and abiotic surfaces - and continue to grow biofilms as long as the nutrients become available. These biofilms are responsible for the vast majority of deadly infections as they often become resistant to antibiotics or host defenses. As new approaches are needed to address infections on various fronts, in particular at the interfaces of medical device – biological system, ideally, one would like to create stimuli-responsive attributes, where a surface remains silent unless external stimulus triggers desirable responses. Taking advantage of the ability of bacteriophages to recognize a host bacterium and their ability to kill specific hosts, covalent approaches to attach phages onto polymeric surfaces are needed.[88]

Jet-based technologies are becoming increasingly important as high-throughput and high-resolution methods for the manipulation of biomaterials. Although these methodologies have been utilized in generating scaffolds from biocompatible materials, the use of electrospinning as an alternative platform for tissue engineering become increasingly attractive. However, the main challenges are to identify specific parameters under which viable threads containing living cells can be produced. Although electrospinning is highly promising in depositing active biological threads and scaffolds,[89] the characterization of their interfaces become critical. Such cells can be cultured and initially may show no evidence of cellular damage during the bionanofabrication, but the long-term vitality of interfaces will be essential if these biomaterials are to be used in biomedical technologies.

There are opportunities for the development of two state-of-the-art MD methods: the all-atom continuous constant pH molecular dynamics (CpHMD)[91-94] and the coarse-grained model (BioModi).[95-97] The CpHMD simulation captures conformational dynamics at a range of pH conditions, resulting in the quantitative determination of the pH at the phase transition. The coarse-grained BioModi is capable of simulating large systems containing up to 1000 peptide-based molecules over relatively long time scales.

Figure 4. Predictive pH-dependent nanomaterials transitioning between a cylindrical nanofiber and cylindrical micelle.[90]

These large-scale MD simulations are capable of capturing time-dependent secondary-structure formation, allowing examination of the spontaneous self-assembly process by peptide molecules starting from a random configuration. Carried out in an iterative feedback loop, the modeling studies[90] at multiple spatial and temporal resolutions could guide the *in vitro* and *in vivo* experiments to innovate and expedite the discovery of pH-responsive peptide-based sequences that are capable of forming dynamic nanomaterials (Fig. 4) for biomedical applications.
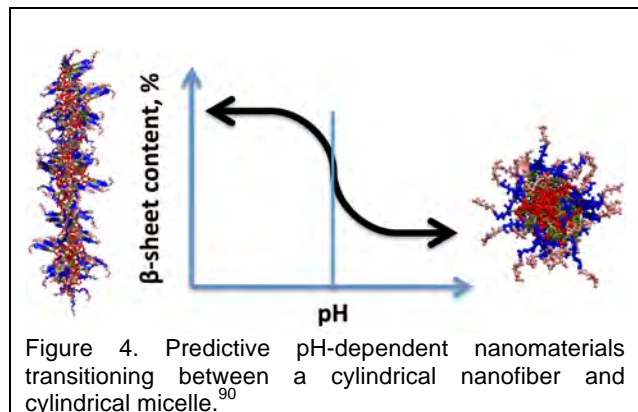
2.4 Recommendations

Mother Nature is probably the best teacher and inspiration of stimuli-responsiveness. Biological systems exhibit an extraordinary ability of healing wounds autonomously; for plants to heal mechanical damages, different substances, such as suberin, tannins, and phenols are activated to prevent further lesions. Similarly, the mechanical damage of the human skin result in the outer flow of blood cells that are arrested by the crosslinked network of fibrin leads to self-repairing. One common feature in these bio-events is the presence of heterogeneous, often multi-layered interfacial morphologies that interact with each other and respond to external or internal stimuli. In recent years, there have been tremendous efforts put into developing synthetic biomaterials that can effectively interact with biological systems. As a matter of fact, at the turn of the 21st century, numerous research articles, symposia, or scientific meetings have focused on this very topic. However, fewer efforts have been made to understand molecular processes leading to stimuli-responsive behavior at biomaterials interfaces. This is primarily related to the fact that low concentration levels, heterogeneity, and dynamics of the interfacial regions are not easily measurable.

2.4.1 Analytical tools coupled to the synthesis and in-situ, noninvasive methodologies
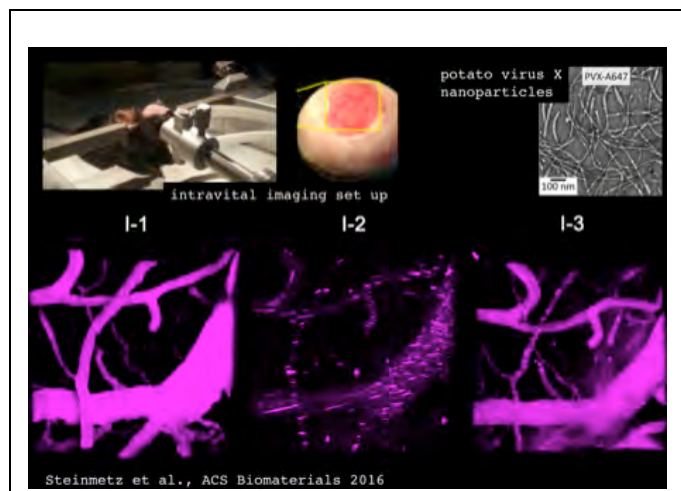
Figure 5. Intravital imaging for real time monitoring of plant virus-based nanocarriers in circulation. C57BL/6-Tg(UBC-GFP) mice with surgically placed cranial windows were used to image CNS vasculature using 2P-LSM following PVX-A647 injections (top panel). Fluorescent-labeled plant virus-based nanoparticles based of potato virus X were intravenously administered as weekly injections. CNS vasculature was imaged continuously for up to 10 min following each injection. Representative image showing a fixed area imaged following successive weekly injections (the nanoparticles are pseudo-colored pink), i.e. injection 1, 2, and 3 at days 1, 7, and 14. While pharmacokinetic measurements revealed little to no changes, intravital imaging highlighted significant changes of the in vivo flow properties of the formulation. Further investigation linked the changes in in vivo fate to innate immune recognition.[98]

Significant advances in biomaterials surfaces and interfaces have been made, but future progress will be limited by the lack of analytical tools capable of selective detection, analysis of larger data sets, and subsequent interpretation. Thus, to advance this critical field, which ultimately will be leveraged by many other areas of studies, new tools are needed. The analytical tools should be coupled to the synthesis and in-situ, non-destructive methodologies will be of particular significance. Mimicking DNA/protein synthesis, where the building blocks are AAs and nts, would be a good starting point. Approaches that have succeeded are cell-free expression systems, but that currently, they are not scalable. Defining the sequence, complex polymer materials of defined sequence and confined spatial situations will be critical to the design and synthesis of new materials. Sensing, single and multiple molecule technologies will be particularly critical to advance biomaterials research. Innovation through new materials design rather than understanding materials-bio interfaces will propel future developments in biomaterials. Many techniques focus on one particular state and static measurements, but what is lacking are limitations attributed to temporal and spatial resolution detectable in a dynamic fashion.

2.4.2 Novel interfacial non-disruptive probes and instruments as tools to investigate multiscale phenomena with spatial and temporal control

There is an increasing need for the development of novel interfacial non-disruptive probes and instruments as tools to investigate multiscale phenomena with spatial and temporal control; an important requirement will be to study events at the interface with minimal disruption of the native state, non- or minimally invasively and in its hydrated state. It is apparent that there is no existing instrument that offers such multimodal capability. A cluster of instruments combined with a selection of multi-functional and multi-modal probes to provide information in the space of mechanical, electrical, thermal, and optical properties at the bio/materials-biology interfaces will be essential in addressing the questions.

Figure 6. Cartoon showing tobacco mosaic virus-based soft matter nanorods in circulation. 'Naked' formulations interact with serum components leading to formation of a protein corona. Artwork by Gabriela Taccir.[99]
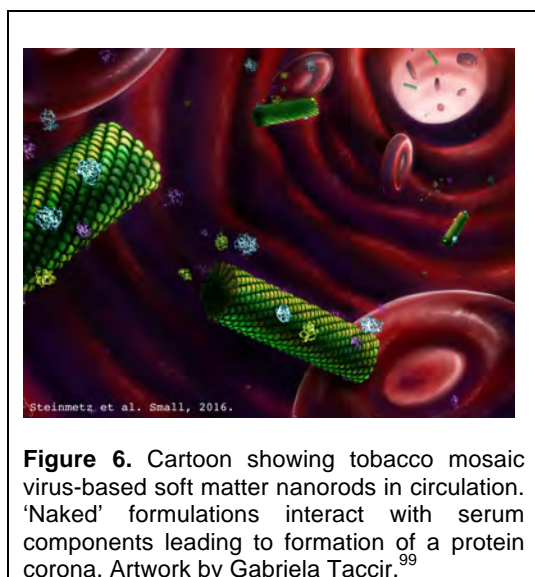
Along similar venues, efforts need to focus on two separate, but integrated thrusts: the development of instrumentation and integration of tools to probe biology, and the biology-inspired tools for sequence-defined synthesis of biomaterials. These tools will formulate one way of envisioning the mimicking of nature's machinery to create functional molecules and assemblies thereof. For example, nucleic acid polymers are synthesized in a sequence-defined manner using nucleotide building blocks and, in turn, these nucleic acids serve as a template for the sequence-defined synthesis of polypeptides and proteins. To date many efforts have focused on symmetrical probes. However, materials in nature are asymmetric, with sequence-defined function. An enzyme, for example, synthesized from a polypeptide chain exhibits domain of binding and catalytic activity. Building on the materials genome initiative, synthetic chemical approaches should aim at mimicking sequence-defined design, yielding multimodal probes enabling to 'react' to a cascade of events providing multimodal signals. The development of analytical tools allowing the measurement of different interactions with sensitivity, specificity, and fast is critical. These may be single user sensing instruments or mid-size tools with built-in multi-probes capable not only to report but also to edit through the integration of editing function making use of the CRISPR/Cas machinery.

2.4.3 Integration of multimodal capabilities combining spectroscopic, electrical, mechanical, and thermal signatures

Intravital imaging has made headways to study molecular events, cellular function, and nanoparticle trafficking using non-invasive approaches. Imaging technology with temporal and spatial resolution has the capability to inform about changing dynamics of a system (Figure 5).[98] For example, the fates of nanoparticles may alter after repeat administration. Blood is not a dilute

solution, and upon contact with the new bathing solution, the interface of the nanoparticle probe may change due to interaction and coating with serum components leading to a protein corona that may affect trafficking and stability (Figure 6).[99] The corona is a function of the innate immune system, which in turn may activate adaptive immunity leading to memory, which may result in distinct clearance rate and trafficking after repeat encounter. So it is clear that intravital imaging, as well as other non-invasive modalities, offer powerful tools to study intricate molecular events and highlight how changes at the nanoparticle interface can impact performance function and biology. Future technologies and instruments should seek to integrate multimodal capabilities not only to report optical signals, but electrical, mechanical and thermal signatures. In addition to the development of multimodal instruments, a key requirement will be the integration of mathematical modeling and computation to integrate complex data function and analysis.

Elucidating the kinetic mechanism of peptide self-assembly as an example of biointerface is feasible via molecular simulations. These processes occur over the μs–ms time scale and the size of resulting nanostructures ranges over the nm-μm length range. These time and size regimes are currently beyond the limits of our most advanced supercomputers and sophisticated algorithms using high-resolution force fields such as atomistic models. To overcome these roadblocks coarse-graining of the peptide system is often employed, which results in a loss of information; moreover, the ability to represent specific interactions, such as hydrogen bonding, is not possible with the loss of atomic detail in certain models. However, such coarse-graining method can tackle the large time and size regimes required in the study of peptide self-assembly. Indeed, coarse-grained approaches could still provide meaningful information about both the self-assembly mechanism of peptide-based systems and the resulting nanostructures.

2.4.4 Employing and development of simulations and models capable of incorporating hydrogen bonding, electrostatic and hydrophobic interactions of long molecular sequences

The MARTINI coarse-grained force field[100] to predict the ability of a dipeptide and tripeptide of any sequence to self-assemble into supramolecular nanostructures has proven to be effective for large-scale molecular dynamics simulation on many monomers of the same sequence solvated in water.[101, 102] The aggregation propensity scores (AP), which were defined as the ratio between the solvent accessible surface area of the peptides in the starting and final states of the simulation) can be calculated from these simulations. Using these results a set of design rules that promote aggregation was validated by comparison with experimental results from the literature. These simulation protocol and scoring method are indeed powerful tools with the ability to predict the propensity of short peptides to self-assemble into supramolecular nanostructures based just on their sequence.

However, the MARTINI force field[1] cannot model hydrogen bonding and requires that the secondary structure of a peptide is predetermined and fixed during the simulation, thus being effective in determining only short peptide sequences. Longer sequences that undergo dynamic hydrogen bonding and secondary structure formation cannot be properly utilized because the volume and packing of the protein backbone and side chains end to excessive collapse without structural restraints in explicit CG water.[103] Therefore, employing and further development other

CG models that are capable of incorporating hydrogen bonding, electrostatic and hydrophobic interactions will be necessary for simulation studies of long peptide sequences.[104, 105]

2.4.5 Combining Mechanical, Imaging, and Spectroscopic Tool into a Comprehensive Midscale Device

Figure 7. Schematic illustration of damage and self-healing process: (a–d) are the images of the damage repair process of modified polyurethane networks. (ref) The self-healing process is represented by a sequence of events A through C at the interfacial regions – an outcome of mechanical damage, creating isolated reactive ends (free radicals or other reactive groups). At the same time, there is a significant mass flow in the damage area due to diminished molecular weights. There is experimental evidence that the repair process will begin longitudinally along the bottom of a scratch depicted as an interactive event connecting reactive n ends, which will be completed when all reacting ends form N pairs. During this process, after a given time there will be a fraction of reactive ends and simple geometrical considerations suggest that reformation cleaved free radicals will require ~20 kcal mol. Remodeling of neighboring chains are the final stages of the process, during which the flow from the side banks of damage-induced surfaces due to liquid-like properties supplies the majority of reactive groups which are consumed resulting in bottom-up self-repair.[106]

Another possibility is the development of tools that may build on atomic force microscopy (AFM) and other spectroscopic tools. For example, by embedding or functionalization of the AFM tip in conjunction with confocal microscopy is one analytical option. While the AFM provides primarily visual images of chemical and physical processes, measurements of at nano- or even smaller scales will be necessary to elucidate the origin of molecular events art biomaterials interfaces entirely. Combining mechanical and chemical imaging capabilities may facilitate in-situ responses at biological interfaces if sufficient spatial resolution is available. For example, internal reflection IR imaging is capable of measuring IR spectra with ~1 μm spatial resolution.[107,86] What is needed is the capability of the same molecular measurements of ~ 1 - 10 nm spatial resolution. Current capabilities of available tools allow measuring of about 100 events in one second, but spatial resolution of not sufficient. Other possibilities are energy transduction tools, which may enable the enhancement of selectivity and sensitivity by conversion of one type of energy to another; for example, conversion of electromagnetic radiation to mechanical or electrical signals.

Minute concentration levels responsible for macroscopic changes make the analysis of interfacial regions challenging, to say the least. New techniques to characterize multi-component structures, e.g. a micelle loaded with cargo or stratification and heterogeneities at the interfacial regions will propel future advances in biomaterials from which other fields of studies will benefit. The characteristic features of new analytical tools should be such that they are sensitive, specific, and fast. This raises the question whether currently available analytical tools are capable of delivering these properties? For most probes, the answer is no. For example, electron spectroscopy for chemical analysis (ESCA) combined with Raman or other molecular probes requires high vacuum conditions. Other combined approaches may have different limitations.

One can envision that this information could serve as an input to computational methods, which in turn, could guide the instrument for more zoomed measurements. How to measure molecular level events responsible for self-healing, which usually occur inside a scratch of damaged biomaterials. Imaging spectroscopic tools (Raman, IR) are capable to a certain extent, but the main obstacle is still limited spatial resolution and the ability to extract molecular information from chemically and physically similar events and environments. The fundamental question is how localized individual reactions at the interfacial regions are capable of generating a cascade of macroscopic responses leading to self-healing (Figure 7.[106])?

2.4.6 Measurements of Weak Interactions in Dynamic Processes

Speed is also critical if dynamic processes at stimuli-responsive interfaces of biomaterials are measured. For example, on average, the fastest IR and/or Raman spectroscopic measurements may acquire as many as hundreds of individual spectra in 1 sec (one scan recorded in 10-2 sec). What is needed is a significantly better special resolution in the order of 1-10 nm, and substantially faster collection data. It should be realized that over the last fifty years the speed of the data collection decreased by several orders of magnitudes (from hours to a few minutes). However, the majority of currently available tools and their average collected scans to enhance the signal-to-noise ratio have not caught up. Thus, the actual, usable speed of the data collection is slow. The same is applicable to nuclear magnetic resonance (NMR) measurements, which is a very powerful chemical tool but requires long acquisition times. Recent advances in dynamic nuclear polarization-nuclear magnetic resonance (DNP-NMR) spectroscopy may enable extended solid-state NMR experiments with unsurpassed sensitivity for new applications in biomolecular research and material science, but the use of microwave irradiation to achieve the transfer polarization from unpaired electron spins to nuclear spins may alter biomaterial interfaces. Photoacoustic measurements in UV or IR regions, which are highly sensitive to the gas phase, exhibit reduced sensitivity in solid state but are highly promising for detection spatially resolved interfacial regions[108, 109] and biomedical applications.[110] Internal reflection IR imaging (IRIRI) is highly sensitive to interfacial regions, but the spatial resolution is in one μm range. Complementary to IR, Raman spectroscopy may offer spatial resolution in the hundreds of nanometers range, and 3D images can be generated via confocal measurements, but the main limitations are fluorescence background and often-undesirable scattering effects. Thus, innovative approaches are needed to enhance special resolution and/or enhance selectivity and sensitivity by cross-fertilizing existing methods or explore new detection methods.

What is needed are minimum or no invasive tools capable of the single and multiple (averaging) scans with the data collection time into the order of pico- or femtosecond per scan and the sensitivity enabling resolving similar nanoscale size molecular entities and events. Current single molecule spectroscopy and imaging techniques do not allow for dynamic measurements. Furthermore, interpretation of the large number of data should be integrated into computational approaches. Although there are libraries of data serving as a useful interpretation tool, new advances in computational approaches should rely on integrating experimental tools with the large data handling computational experiments capable of dynamic guiding an experiment. These capabilities practically do not exist. One can envision an integrated hybrid instrument capable of mechanical, electrical, optical, and spectroscopic measurements integrated into computational facilities that measure in-situ dynamics of interfacial events in biomaterials.

There are also needs for new detection tools that will allow us to explore weak interactions at biomaterials interfaces. For example, can we measure hydrophobic and/or hydrophilic interactions with the satisfactory resolution and speed? Or can we measure van der Waals interactions in the presence of the molecular events at the interfacial regions? It is well known that ligand binding to receptors may trigger a cascade of events that may or may not lead to expected responses. Although fluorescent probes are being utilized, it is recognized that these usually large molecular entities may alter the functions on the synthetic-biological system under investigation. Similarly, AFM measurements have limitations when applied to live cells and ideally, a non-invasive hybrid tool should exhibit non-invasive attributes. Thus, efficient energy conversion detection systems with minimal losses, where one type of energy is converted to another may be of significant importance in the development of new sensitive midsize tools for biomaterial interfaces.

2.4.7 Instrumentation, Tools, Foundry

The creation of new biomaterials has always progressed hand-in-hand with advances in our ability to measure and define transformations in biomacromolecules in a controllable matter. If we can find an efficient way to enhance our knowledge through the development of only a fraction of identified in this report tools, great discoveries and technological payoff will occur. This effort will require the transition from observation to control science. This will require the radical reshaping of current instrumentation tools, particularly midscale tools.

2.4.7.1 Connecting Synthesis, New Detection Tools, and Computational Methods

The objective is to connect typically separated events into one analytical task that will not interrogate matter during synthetic and analytical events and will lead to the developments of new sensing approached in measuring the interactions and molecular modeling/predictions using experimental input.

2.4.7.2 Controlling Synthesis of Biomaterials Interfaces by Analytical Tools

Typically, analytical tools are used to measure synthetic progress and outcomes. Turning things around by controlling synthetic efforts with new sensing devices containing robust chaotic controllers will secure precision and stabilization of synthetic efforts.

2.4.7.3 Development of midscale analytical tools capable of collecting ultra-high spatial resolution data capable of measurements of transient individual events

Midscale instrumentation with a high speed 'non-equilibrium resolution' at nano range spatial resolution and molecular level detection will require combining mechanical, electrical, thermal, and spectroscopic elements into one multi-task instrument. If molecular elements of biomaterials interfaces are initiated in an excited state, they can quickly evolve to a stable state of lower

energy and the nature of this transition will determine the molecular outcomes of interfacial regions.

SECTION 3: Multiscale Biomaterial Design and Characterization

SESSION LEADERS

HELEN H. LU, COLUMBIA UNIVERSITY

PHILIP LEDUC, CARNEGIE-MELLON UNIVERSITY

SECTION MEMBERS

ZVONIMIR DOGIC, BRANDEIS UNIVERSITY

MELISSA GRUNLAN, TEXAS A&M UNIVERSITY

JOACHIM KOHN, RUTGERS UNIVERSITY

WARREN RUDER, VIRGINIA INSTITUTE OF TECHNOLOGY

JOYCE WONG, BOSTON UNIVERSITY

3.1 Introduction

The ability to create new materials that interface with biology has the potential to enable numerous research & development areas that will improve the quality of life across the nation and globe. These new biomaterials would recapitulate nature or interface with biology at multiple scales (molecular, subcellular, cellular, tissue, organ), which will enhance their functionality and utility. The fundamental tenet is in developing new biomaterials toward this goal. A deeper understanding of interfacial interactions between biomaterials and these biological structures at multiple length-scales is required to reveal the essential nature's rules that can then be translated into fundamental design principles to form new functional biomaterials for building tissues and organs.

One grand challenge in this area is to develop biomaterials toward creating life, which is relevant at multiple scales (nano to macro) and encompasses both temporal and spatial considerations. This aligns with goals in many areas including the National Science Foundation's "**10 Big Ideas for Future NSF Investments**", especially in "*Shaping the New Human-Technology Frontier*" and "*Understanding the Rules of Life: Predicting Phenotype*", and "*Mid-scale Research Infrastructure*". We additionally believe that this underscores the need for the Materials Innovation Platforms (MIP). A key component in the MIP platform is to enable researchers to build off each other's work: currently, efforts are largely from individual laboratories, which, without standards, databases, and common sharing practices have limited impact. In addition, we believe that a successful MIP platform would have complementary modeling and experimental platforms that have integrated input and feedback.

In this section, we discuss biomaterials in building life and focus on two main scientific questions: (1) how do we discover material design principles to control desired multiscale

biological response? and (2) how do we exploit nature's rules to design new functional materials? To accomplish this, we describe the Motivation/Significance, Opportunities/Challenges, and Needs/Recommendations are described in these sections below.

3.2 Scientific Questions

3.2.1 How do we discover material design principles to control desired multiscale biological response?

Biomaterials prepared with molecular-level control and whose properties are precisely characterized in a physiologically relevant way have the potential to regulate cellular behavior, enabling the development of superior medical devices and regenerative engineering strategies. However, the understanding of the scientific principles guiding cell-material interactions must be advanced to fully realize the potential of biomaterials. Towards this goal, intelligent biomaterials are necessary; the development of which relies on experimental, theoretical and computational approaches as well as practices to enable consistency and data sharing within the biomaterials community. Intelligent biomaterials can be used to probe how cells interact with and sense materials as well as how cells influence and remodel materials. Spatiotemporal regulation of cellular behavior and material remodeling across different scales is imperative. The design of intelligent biomaterials relies on new synthetic methodologies, comprehensive experimental characterization (including new methods). Theoretical and computational approaches are essential to establish predictive rules for the rational design of intelligent biomaterials and their complex interactions with cells. Finally, it is essential to provide the field with biomaterial controls prepared and characterized with precision and consistency to promote data sharing within the field.

3.2.1.1 Intelligent materials design

Biomaterials prepared with molecular-level control and whose properties are precisely characterized in a physiologically relevant way have the potential to regulate cellular behavior, enabling the development of superior medical devices and regenerative engineering strategies. Towards this goal, it must be thoroughly understood how cells interact with and sense materials[111] as well as how cells influence and remodel materials. Spatiotemporal regulation of cellular behavior and material remodeling across different scales is imperative. Defining cell-material interactions requires new theoretical models, innovative biomaterials designs, and thorough characterization.

3.2.1.2 Theoretical and computational approaches integrated with design

The ability to integrate theoretical and computational approaches in biomaterial design is extremely important. With more complex materials, the number of variables that can be changed when creating new biomaterials is tremendously high. Because of this, the total number of possibilities is way beyond the scope of what can be experimentally tested and thus a rationale-based approach needs to be taken. This is the area where computational design of biomaterials can make tremendous strides. Developing predictive models is an important goal and not just

models that support the data. The predictive models are powerful because then instead of spending years controlling a large number of parameters in developing new biomaterials, one can use predictive computational approaches to determine a set of parameters that would provide good solutions for creating new biomaterials.[112] This approach is complicated though by the biological interactions that are required to be controlled by many cell-material systems.

3.2.1.3 Lab practices to advance material design

Biomaterials can be a powerful agent to manipulate and control cell behavior. Cell phenotype and function can be modulated by many external signals (e.g. mechanical, chemical, electrical, magnetic, light) that can be encoded or embedded in biomaterial designs. While these effects have been known for quite some time, there has been a lack of material design principles that one can use practically with reliable predictive capabilities. Part of the problem is that it is often very difficult to compare results between laboratories, and sometimes even within a laboratory when knowledge is transferred between trainees. This, combined with the inherent materials processing variability and biological variability, makes it difficult to draw broad conclusions that can be translated into theoretical predictions. Addressing these issues would significantly advance the biomaterials field:[113] in particular, large sets of data between multiple laboratories could then exploit methodologies from computer science and big data initiatives with the goal of design rules emerging from this process. Another benefit would be acceleration of commercial translation if one were to have a Handbook of Biomaterial-Cell Interactions similar to how an engineer looks up material properties when they design and build a bridge or building.

3.2.2 How do we exploit nature's rules to design new functional materials?

A significant challenge in biomaterials science consists of exploiting nature's rules to design new functional materials. This approach requires a multistep process consisting of both elucidating the fundamental physics driving this rule set, while also understanding how evolution used these fundamental biophysical phenomena to assemble life. At the same time, as we leverage this rule set, we must ensure that we minimize the over-application and over-engineering of systems using nature's rules. Together, this approach can revolutionize how we approach biomaterials science. In the following sections, we describe the motivation and significance of these different steps, while also outlining key challenges and associated opportunities.

3.2.2.1 Elucidating the Biophysics that Give Rise to Life-Like Functionalities

Using a well-conserved set of biochemical building blocks nature systematically builds hierarchical energy-consuming assemblages with diverse functionalities. One set of such elemental building blocks is microtubules and kinesin molecular motors which use energy from ATP hydrolysis to move linearly along a microtubule track. In one example, these elemental structural elements assemble into long filamentous axonemes. In this assemblage, an entirely new functionality emerges in which linear motion of kinesin motors is transformed into periodic high-frequency beating of cilium that endows cells with swimming motility. At even higher level of hierarchy, beating cilia, can assemble into dense ciliary fields, which exhibit an entirely different functionally, a traveling metachronal waves that clears the human trachea from dust and debris. In an entirely different context, the same elemental units, microtubules and motors, can self-organize into a mitotic spindle, which is a dense liquid-crystalline like droplet that drives the

cell division, thus ensuring the equitable distribution of genetic material amongst the offspring.[114] These are just a few examples of diverse biological structures and functions that nature has achieved from the same set of biochemical building blocks (**Figure 1**).

Figure 1. Far-from-equilibrium structures and dynamical steady-states that can be assembled from a three simple microscopic building, filamentous microtubules, clusters of molecular motors and depleting agent.
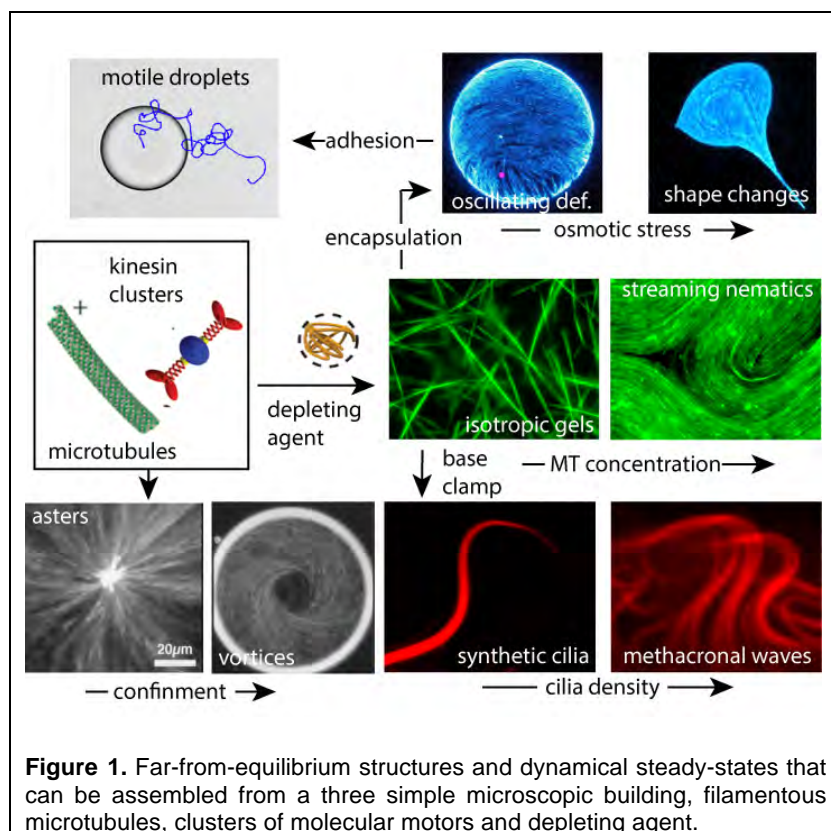
However, the evolutionary pressures have selected only the most optimal and efficient structures and functionalities from a presumably a much wider range of all conceivable structures. A fundamental task is to determine and elucidate this much wider array of all possible structures and functionalities given a specific set of elemental building blocks. In other words the goal is to explore and create life-like materials that could potentially exist but are not currently found in the living word. For example, recent experiments have demonstrated how putting together the same biological building blocks, microtubules and kinesin motors, lead to assembly of active liquid crystals.[115, 116] Such novel sought-after materials, while having no direct biological relevance, have unique functional properties from a material science perspective. Slight alteration to these building blocks lead to assembly of synthetic motile cells, which again are not found in nature but are fundamental from the material science and could lead to new drug delivery vehicles.[117] In other instances, microtubules and motors can be assembled into materials that recapitulate the basic functionalities of biological organism, such as ciliary beating and metachronal waves, yet do it with a significantly reduced number of building blocks.[118]

Besides developing fundamentally new types of materials with life-like functionalities, studying active matter systems is important from fundamental perspective. Theoretical laws developed over the past few centuries, such as theory of elasticity and Navier-Stokes equations, quantitatively describe the emergent properties of conventional materials assembled from passive inanimate molecules and units. However, biology and life is hierarchically assembled from energy-consuming units, and numerous cellular functionalities require continuous input of energy at microscopic scale. Therefore, life and various processes that sustain it, take place far away from equilibrium. Consequently, describing both living organisms and creating a new generation of active life-like materials demands formulation of yet unknown theoretical formalisms that can describe collective properties of materials that are assembled from energy consuming constituent object.[119, 120] In turn developing such theoretical formalism requires

development of simplified experimental model systems that can be systematically tuned and modified and thus used to test theoretical predictions.

3.2.2.2 The Origin of Life and the Evolution of Biomaterial Functionality

The questions of how life originated and how materials evolved their unique capacities are some of the most interesting mysteries in science. Current hypotheses for the origin-of-life often suggest that some combination of chemical elements essential for life (i.e., C, N, O, H, and P) existed in just the right conditions to spark a chain reaction that formed pre-biotic, self-replicating material systems.[114, 119] Out of this "primordial stew" of components, the necessary biomaterials self-assembled (see Figure 2). These pre-biotic cells eventually evolved into living cells. This process of material self-assembly remains critical to the performance of biomaterials and biological components today, billions of years later. The natural bioprocessing of materials, as well as the engineering of biomimetic materials, are both directly related to this continuing evolutionary process.[120] **As a result, inquiries into the origin of life are, in multiple ways, inquiries into biomaterials science**.

Figure 2. A Possible Origin of Life. (A) Essential elements randomly self-assemble into macromolecules, potentially (B) self-catalyzing nucleic acid ribozymes. (C) These different molecules self-assemble into (D) pre-biotic, self-replicating systems similar to artificial cells. Image Source: Ruder Laboratory
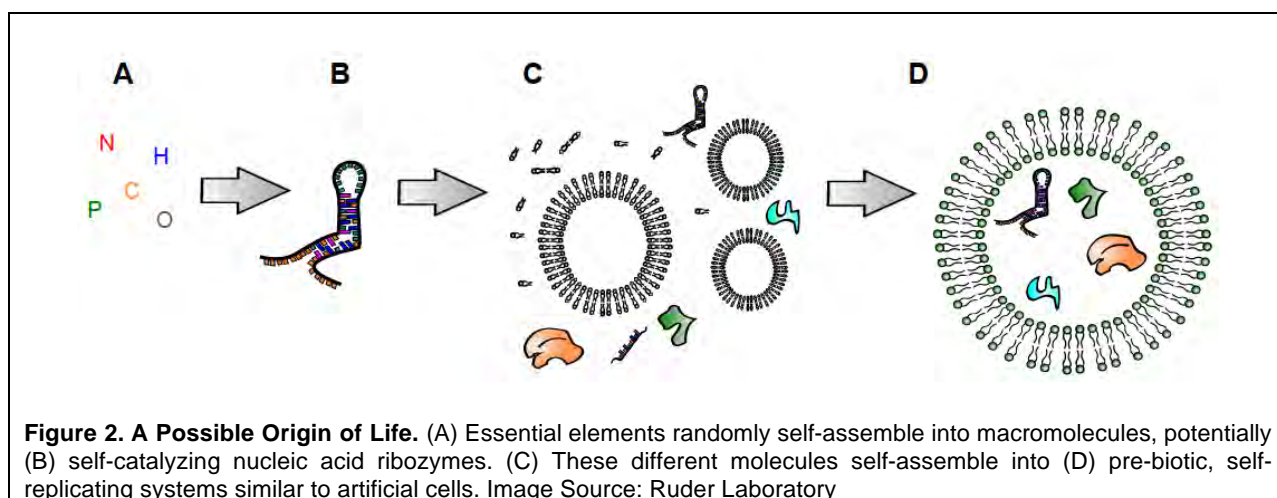
As these materials self-assembled into a living organism, a fundamental question explores what material properties enabled this transition from energy-consuming, self-replicating "intelligent" molecular system of an obviously living system? Beyond origin-of-life questions, a related question focuses on what underlying scientific phenomena enabled the emergence of new material properties during evolution.[114, 119-121]

These questions open the door to key opportunities and associated challenges in understanding these processes. The key opportunity, as well as a challenge to be met in discovering the answer to these fundamental scientific questions, is understanding how new materials might be designed based on the lessons learned during these explorations.[122] By exploiting nature's rules for developing new biomaterials, new functional materials could be enabled.

One of the inherent challenges in studying these systems is a need for tools to provide well-characterized materials for each step of the associated self-assembly processes. In order to

empower a broad community of researchers to work together to tackle these problems, access to a common set of tools is essential. For example, can we generate a set of standard materials and tools for producing the biomacromolecules essential to the origin-of-life of life expression. Along these lines, well-characterized systems for generating materials would allow researchers to have a benchmark against which they could measure properties inherent to these systems. Similarly, as we move to integrate the age of Big Data into biomaterials research, an ability to rapidly produce and sequence the DNA templates for critical biomaterials becomes obvious.

3.2.3 Strategic Biomimicry: How Best to Mimic Nature and Develop Bioinspired Material Design Without Over-Engineering

Biomaterials are designed in many applications, to replace or augment living tissues. To this end, the biomaterial or its assembly into a scaffold is guided by the features of the extracellular matrix (ECM) of interest, aiming to achieve comparable material properties (e.g. mechanical, electrical) or structural organization (e.g. alignment, porosity), in order to recapitulate the native structure-functional relationship of the ECM. Given the complexity of biological tissues, which are often anisotropic and composite in nature, a frontier challenges in biomaterial design is how best to learn from nature. Biomimicry as a field to date has focused largely on designing materials and scaffolds with structural and physical properties approximating those of native ECM, with the properties chosen based on what is currently known or investigated in the field. As such, incorporation of rationalized design and criteria selection in this effort has been limited. Moreover, advances in this area are hindered by the multi-scale and multi-faceted nature of the target biological system, as well as the myriad synergies inherent between different tissues and organ systems, as tissue-tissue synchrony is crucial in providing structural support for internal organs and enabling daily activities. Another challenge is the lack of understanding regarding what are the most important aspects of the tissue formation or regeneration process that must be recapitulated in biomaterial design. Moreover, the apparent tissue properties are developed over time, culminating from prenatal development and postnatal adaptation, coupled with epigenetic changes throughout life. Understanding which stage or stages of this dynamic process should be embodied in the biomaterial remains an uncharted frontier in the field of bio-inspired design.

Clearly, there is a pressing need in **understanding the rules of life** or biology by systematically elucidating the features of the extracellular matrix that is essential for its regeneration, and explore how biomimicry can be strategically applied to avoid over-engineering the biomaterial. This is also an opportunity to identifying unifying parameters governing tissue formation and regeneration. Determining the most relevant parameters for recapitulating native structure-function relationships through strategic biomimicry will reduce the burden for translation and in turn expedite commercialization.[123] One way to tackle this frontier challenge in bioinspired design include systematic studies aimed at prioritizing the most crucial properties of native tissue necessary to recapitulate function. In this process, the biomaterial can be used as a platform to test single or multiple ECM characteristics using relevant *in vitro* and *in vivo* models. These models with well-defined ECM cues can also be standardized and incorporated into the biomaterial foundry (e.g. hydrogels with a series of mechanical properties, fiber meshes with a range of diameters), shared with the community for research and biofunctionality screening. In addition, multi-scale dynamic computational models can be used to simulate the composite nature of the biological tissues, and in particular, help to identify optimal structural features and

related thresholds for recapitulating physiologic and even achieving supra-physiologic levels. Other technologies that can accelerate this area include precision and high-throughput biomanufacturing processes.

3.3 Challenges and Opportunities

Some of the challenges that must be overcome include the development of biomaterial chemistries to enable molecular-level control and reproducibility. These can be bio-orthogonal chemistries as well as chemistries and processing methods that enable biomanufacturing (e.g. bioprinting), high throughput and combinatorial methods. Moreover, biomaterials should have decoupled material properties in order to sort out the relative importance of multiple external stimuli effects. When considering biomaterials that can 'control' cell response, one could exploit advances in materials science and engineering to create dynamic and responsive biomaterials in which one could modulate cellular behavior on-demand. Ideally, the biomaterial properties should be predictively altered via cellular influences. It is also important to have real-time, in situ and/or non-destructive measurement and imaging of cellular processes and material properties over different time and length scales. Importantly, one should develop experimental strategies and theoretical models to assess "local" and "bulk" mechanical properties of polymeric biomaterials with physiologically relevant parameters. Ideally, one should also have methodologies to measure the properties of tissues, which is particularly challenging to achieve in *in vivo* environments.

There are numerous challenges to integrate theoretical and computational approaches with biomaterial design. The ability to scale-up from small scales (i.e. molecular interactions) to macroscale response (i.e. bulk mechanical response) requires a large range of assumptions and coarse graining approaches. To be able to validate that the right assumptions and coarse graining approaches are being implemented, one needs enough experimental data to validate the initial models to then use them in predictive approaches. This is a key critical aspect of developing highly useful computational models with predictive capabilities.

Another challenge that must be overcome to improve reproducibility is to have detailed protocols – as discussed above, it is critical to have input from both experimentalists and theoreticians and computational scientists as to what key parameters need to be specified and/or measured. The biological community has also recognized these challenges, largely due to recent reports in the literature that laboratory reproducibility is a major problem shown in many publications. To this end, there are existing several databases that the biomaterials community can look to emulate: e.g. http://protocolnavigator.org. To incentivize participation in this effort, an idea could be to involve discussions with the FDA early in the process to see how these 'standardization' practices could aid in acceleration of the FDA approval process for translation. In addition, our laboratories are dependent on vendors, so we could develop a 'rating' system where products could have 'ratings' similar to medical-grade ratings that currently exist in materials but for test purposes. The data should also be easily accessible for both experimentalists and theoreticians.

3.4 Recommendations

3.4.1 Funding support for the multiscale biomaterial design and discovery

Frontier Focus areas listed below
- **Intelligent Material Design:** theranostics, stimuli responsive materials and internal feedback
- **Theoretical/Computational approaches integrated with design**
- **Predicative Biomaterial Design & Reproducible Manufacturing**
- **Elucidating the fundamental biophysics governing nature's rules for material science**
- **Using evolution to inform the application of nature's rules for creating biomaterials**
- **Strategic Biomimicry:** how does one minimally mimic nature in biomaterial design without over-engineering?

3.4.2 "Biomaterial Foundry" – Broader Impact in terms of standardize current practices and translate biomaterial expertise to a wider community

- **Biomaterial-omics:** collection of biomaterial building blocks
- **Data Depositories:** reproducible correlations
- **Methods Depositories:** SOP and biomaterial phantoms/testing standards
- **Regional hubs for interdisciplinary Teams (HABO):** collaborated acceleration of biomaterial development and optimization

3.4.3 Development and general adoption of standards (test assays, management system, reference materials, common format for sharing research results, etc), **collaborative effort between NSF, NIST, FDA and others**

3.4.4 Instrumentation, Tools, Foundry Needed

3.4.4.1 Midscale

A national foundry or a network of local hubs that will disseminate knowledge in design, synthesis, expression and purification of diverse building blocks that are required for assembly of the next generation of biomaterials as well as their characterization. Amongst other this foundry would have expertise in chemical synthesis of polymers and other supramolecular structure, as well as design, expression and purification of diverse biochemical proteins. This foundry would follow GMP (good manufacturing practice) such that different laboratories would be able to directly compare results using these biomaterials. Characterization would include molecular weight distribution – this will be important input for the computational studies. The foundry would also provide guidelines on processing of the materials into specified formats that would have to be validated with surface characterization and materials characterization techniques.

3.4.4.2 Others

- **Instrumentation Hub – multiscale characterization/analysis**
  - **Computational tools** (simulations) to connect and predict physical polymer property data with key biological outcomes.

- **Multiscale and Multimodal Tools** for characterization/functional imaging with temporal and spatial resolution, dynamic functionality
- Need for new tools, i.e. tool developments

SECTION 4: Targeted Patterning, Fabrication and Self-Assembly

SESSION LEADERS

SHERINE O. OBARE, WESTERN MICHIGAN UNIVERSITY

DAVID DEAN, THE OHIO STATE UNIVERSITY

SECTION MEMBERS

OMOLOLA ENIOLA-ADEFESO, UNIVERSITY OF MICHIGAN

SETH FRADEN, BRANDEIS UNIVERSITY

ANTHONY GUISEPPI-ELIE, TEXAS A & M UNIVERSITY

TREENA LIVINGSTON, NEW JERSEY INSTITUTE OF TECHNOLOGY

ATHANASSIOS SAMBANIS, W. M. KECK FOUNDATION

4.1 Introduction
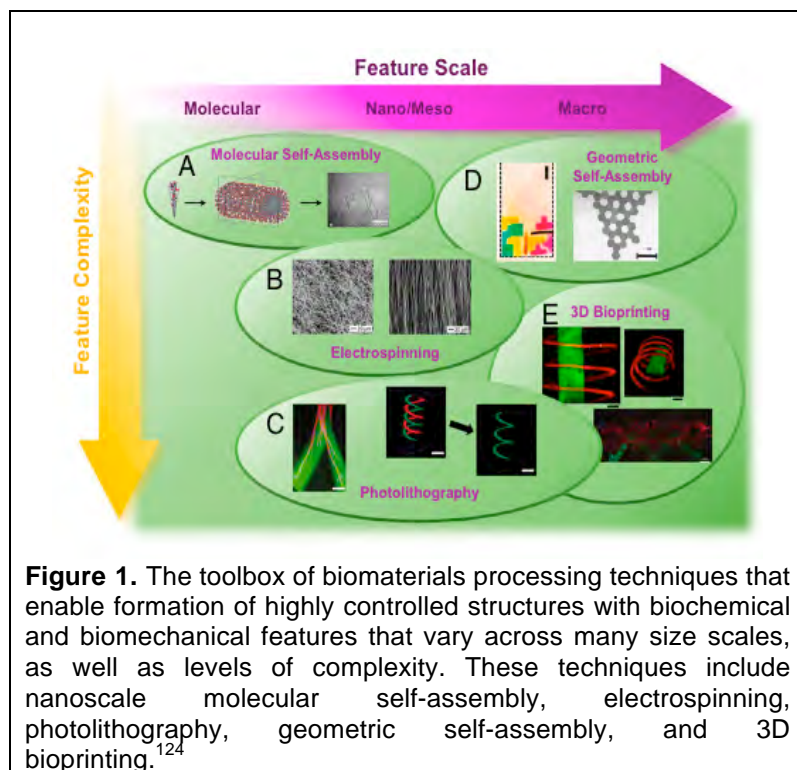
Figure 1. The toolbox of biomaterials processing techniques that enable formation of highly controlled structures with biochemical and biomechanical features that vary across many size scales, as well as levels of complexity. These techniques include nanoscale molecular self-assembly, electrospinning, photolithography, geometric self-assembly, and 3D bioprinting.[124]

The field of biomaterials represents research at the intersection of materials science and the life sciences. Such a wide scope of inquiry encompasses disparate themes ranging from complexity, hierarchy, dynamics and adaptation, healing and morphogenesis, as articulated in the report of the 2012 NSF biomaterials workshop. The impact of progress on these important scientific questions will have impact on health care, energy technology, manufacturing, environmental quality and security. However, the breadth of the scientific questions and the challenge of working on modeling and tracking multi-scale biomaterial synthetic procedures

and a wide range of eventual applications that will arise as a result of breakthroughs makes focus and ranking priorities a challenge.[124]

Furthermore, reproducibility resulting from a lack of standardization is limiting progress in biomaterials research. Addressing standardization, characterization and reproducibility represents opportunities for a Materials Innovation Platform (BioMIP) in Biomaterials.

Multiple fields of research have benefited from adopting model systems in which the field selects several quintessential examples and then deeply investigate that system. The field of neuroscientists chose *C. elegans* and drosophila, cellular biology selected *E. coli* and yeast, soft matter selected polystyrene spheres, and liquid crystals chose the molecules MBBA and PBG. This nar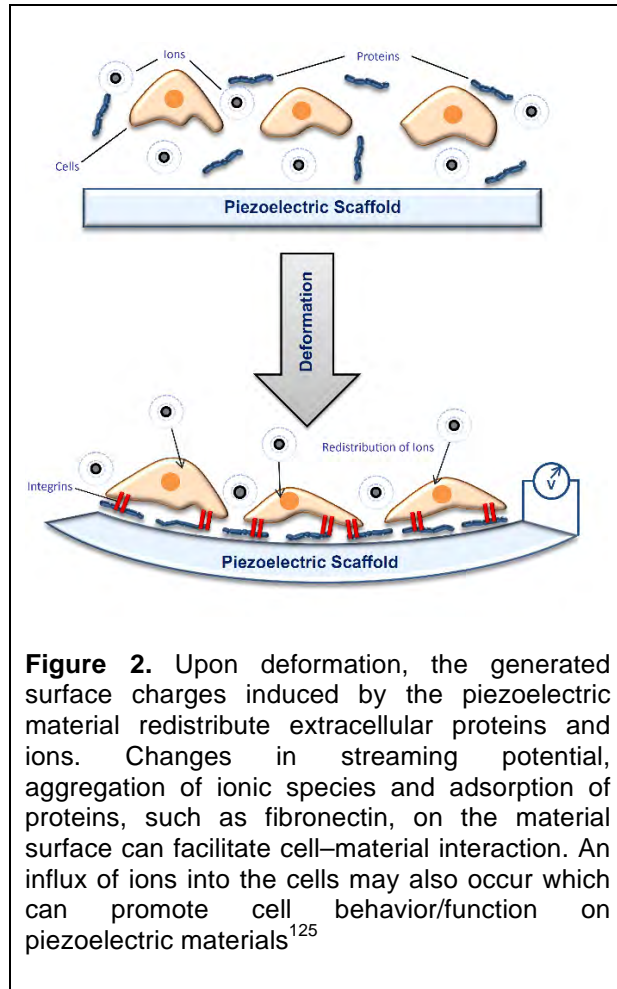rowing of focus allowed the respective fields to perform investigations spanning the full range of scientific questions and led to tremendous progress because by choosing a common system, results could be compared between labs and then leveraged by others.

In fields for which there is a tight focus, the establishment of a repository of data capturing the knowledge is possible. The paradigm for this is the field of structural biology, in which investigators deposit their results in the Protein Data Bank. The challenge for the field of biomaterials is that there is nothing as focused as coordinates of electron density. However, there would be a benefit for a repository of data for several subfields in biomaterials, such as hydrogels, cell-material interactions, protein-material interactions. A well-structured database would be a useful resource immediately for the sharing of information, but also could mined in the future using big data material genome methods. Decisions would need to be made in regards to handling unprotected discoveries. Would there be a process like an FDA master file for vetted procedures and biomaterials that may be held as trade secret or prior to submission within a provisional patent application.

4.2 Scientific Questions

4.2.1 How can smart biomaterials be designed so they can be used for a wide range of tissues?

Improved, clinically relevant biomaterials and encapsulation systems for immunoprotection that do not impact survival of the encapsulated cells, and further allow *in vivo* monitoring, are desirable for next generation smart biomaterials. New biomaterials could offer significant advances toward adult tissue progenitor cells science, bone marrow regeneration, allogenic mesenchymal stem cells heal bone defects, fibrous composites – obtained by electrospinning (polycaprolactone PCL), sheep cortical tissue, and bioinspired materials developed via electrospun gelatin fibers. Understanding how biomaterials respond to various biological models using systems that allow better characterization of the cell/material interface for cytotoxicity studies are needed. As new biomaterials are designed and used it is important to obtain a fundamental understanding of the behavior in complex biological matrices, for example, understanding of the role of charge and water structure – the hydrophobic effect could allow us to fabricate in 2D and 3D forms that contain zwitterion charge.

Figure 2. Upon deformation, the generated surface charges induced by the piezoelectric material redistribute extracellular proteins and ions. Changes in streaming potential, aggregation of ionic species and adsorption of proteins, such as fibronectin, on the material surface can facilitate cell–material interaction. An influx of ions into the cells may also occur which can promote cell behavior/function on piezoelectric materials[125]
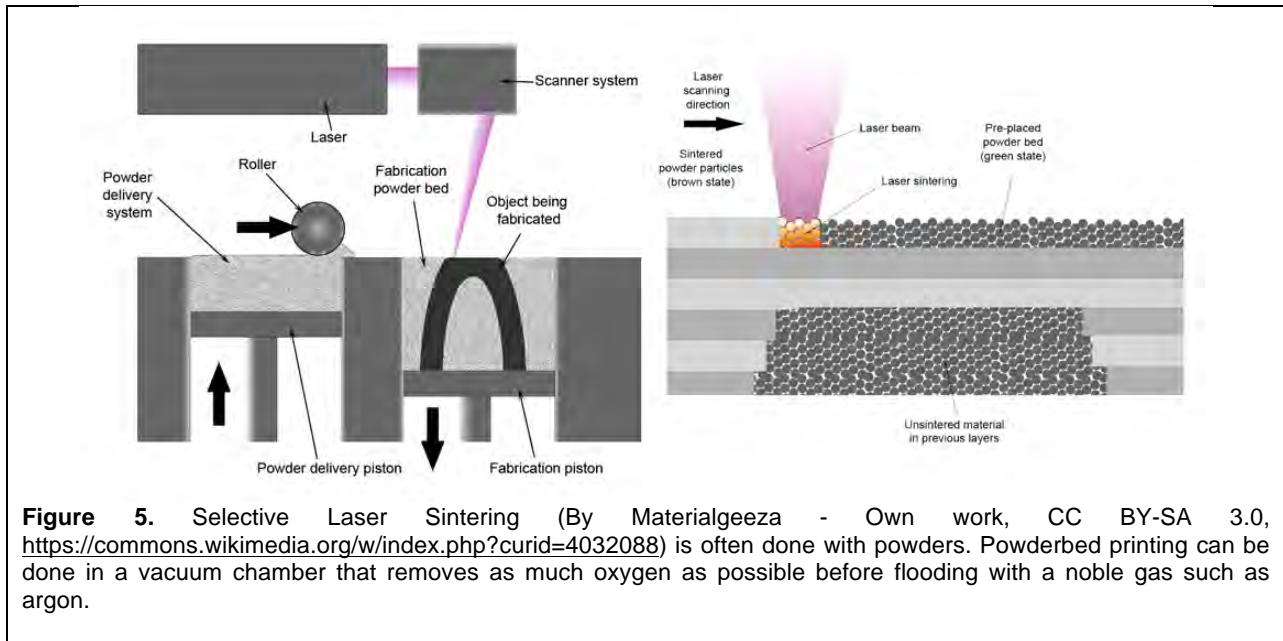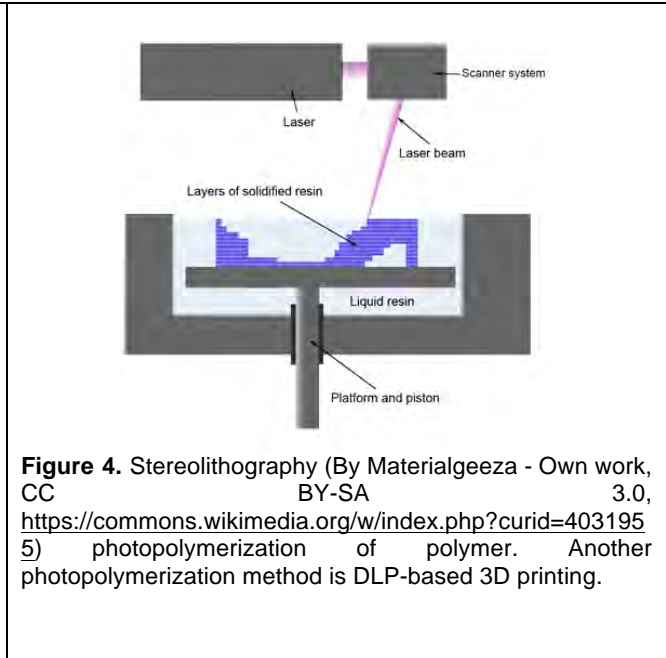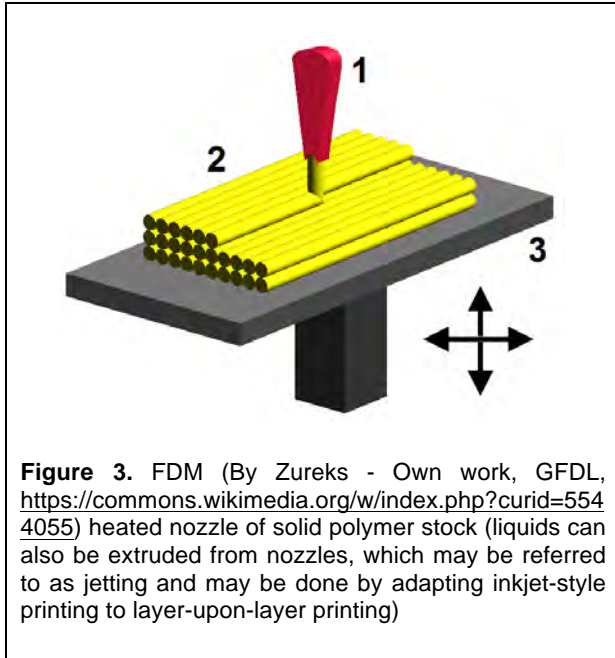
The use of smart materials or stimuli-responsive materials as next generation biomaterials for medical devices is of growing interest due to their ability to change in response to their environment. A largely unstudied smart material for tissue repair and regeneration applications is piezoelectric material. Piezoelectricity can generate an electric potential in response to mechanical deformation and should be used to advance biomaterials research. Extracellular matrix materials, such as collagens and glycosaminoglycans, display piezoelectric activity, i.e. they are capable of converting mechanical strain into electrical output (Figure 2). Piezoelectric materials processed into films or tubes have been shown to enhance bone growth, and enhance cell adhesion and function in bone, nerve, and endothelial cell types.[126-128] Recent studies using non-degradable piezoelectric fibrous scaffolds demonstrate biocompatibility and can stimulate cell differentiation in a variety of cell types.[129-133] The advantage of using piezoelectric materials, unlike conductive materials, is that external electrodes or a power source is not needed to generate electrical activity. The electric fields generated in the material may be caused by cell-matrix interaction and/or physiological movement. Electrical stimulation has been shown to up-regulate gene expression and matrix production and repair of articular cartilage defects.[133] Clinically, electric fields are used for treating bone fractures.[133] Conventional piezoelectric materials that can be used in biological environments are limited.[125] Novel piezoelectric materials with proven biocompatibility and improved material properties for variety of applications are needed. Characterization of piezoelectric materials under biological conditions as well as presenting biological cues at the surface of biomaterials in a stable/amplified manner to achieve the correct cellular response, would lead to novel bioprocesses.[134, 135]

4.2.2 What are the most suitable biomaterials for advanced 3D Printing

The motivation to use additive manufacturing for both inert and resorbable implants (and possibly composites in the future) is increasing rapidly.

However, there are very few commercially available, 3D-printable, and implantable materials. This fact limits scientists and their corporate partners from developing and submitting new medical devices to the FDA. Development and validation of these biomaterials will advance medical device science, especially regenerative medicines. Cell-laden resorbable scaffolds need

to be characterized for porosity, permeability, and resorption—there are few standards in any of these criteria that affect both cell seeding and tissue formation. The most commonly used materials for 3D printing are polymers (e.g., hydrogel [video: https://upload.wikimedia.org/wikipedia/commons/5/5d/Hyperboloid_Print.ogv] and solid curing) resins (e.g., stereolithography in Figure 4) and solid stock polymers (Fused Deposition Modeling [FDM]), metal powders, and ceramic powders (Figure 3).

Figure 3. FDM (By Zureks - Own work, GFDL, https://commons.wikimedia.org/w/index.php?curid=5544055) heated nozzle of solid polymer stock (liquids can also be extruded from nozzles, which may be referred to as jetting and may be done by adapting inkjet-style printing to layer-upon-layer printing)

Figure 4. Stereolithography (By Materialgeeza - Own work, CC BY-SA 3.0, https://commons.wikimedia.org/w/index.php?curid=4031955) photopolymerization of polymer. Another photopolymerization method is DLP-based 3D printing.

Figure 5. Selective Laser Sintering (By Materialgeeza - Own work, CC BY-SA 3.0, https://commons.wikimedia.org/w/index.php?curid=4032088) is often done with powders. Powderbed printing can be done in a vacuum chamber that removes as much oxygen as possible before flooding with a noble gas such as argon.

It is critical that new materials be developed that allow the integration of inert components with healthy tissue, or the temporary bridging of damaged or injured tissues by implanting scaffolds

with the appropriate functional and mechanical properties that are conducive to cell attachment and tissue function. Once materials are invented it is critical to certify that the production process is reliable in terms of the resulting materials' mechanical properties, lack of toxicity, lack of immunogenicity, and if the material is resorbable, its resorption kinetics and by-products should be non-injurious and predictable. Most existing standards for were developed for resorbable or inert polymer devices. These standards need refinement to accommodate new metallic and ceramic materials. These certification criteria will be critical to validating the safety and effectiveness of these materials in order for eventual commercial vendors to consider future use of these materials. Successful materials for 3D printing will need to show homogenous results throughout the build volume. All 3D printing processes also depend on material flow during the printing process. It is important to determine fundamental parameters that control resorption kinetics and also relate how *in vitro* results compare to animal studies and consequently to human studies. Understanding the fundamental factors that make the results different is paramount for advances in biomaterials engineering.

New ceramics are needed for use in coatings of 3D printed devices. Ceramics provide reliable porosity and resorption kinetics for the well-timed release of antibiotics, drugs, or cytokines. Ceramics may also be printed in polymer binders that are later removed or in lower concentrations as minor (by %) constituents of polymer implants. For example, high resolution ceramic printing using polymers as binders is important in dentistry where technology allows materials to be suspended. New polymers are needed to provide guidance of tissue infusion to 3D printed porous structures, as medical devices or device components, and for use as guides for clinical procedures. In many cases a resorbable material must resorb prior to the remodeling of newly generated tissue engineered constructs. Resorbable hydrogel and solid-cured scaffolds are the most likely polymeric devices to be used in cell-based therapies where cells are pre-cultured to produce extracellular matrix material prior to implantation. The availability of certified progenitor cells is another need for this research and eventually its use in the clinic.

Resorbable metals, mostly Mg alloys, and low stiffness metals, such as beta titanium alloys, are needed for traditional and 3D printing-based fabrication (Figure 5). These alloys also provide useful super-elastic and shape memory properties. In the area of bone fixation and joint replacement devices, current Ti-6Al-4V (surgical grade 5), and older lines of alloys, have stiffness high enough to risk stress shielding leading to bone resorption. This high stiffness also risks stress concentration that could lead to device fracture or device pull-out. Furthermore, innovative bioinks (e.g. hydrogel extracellular matrices [ECMs]), printable powders (e.g. polymer, metal, or ceramic), and solid stock polymers (e.g. for FDM printing) would significantly advance the field.

4.2.3 How do we optimize nanoparticles to improve functionality of biomaterials

In recent years, there has been an increased interest in the design and use of materials with nanoscale dimensions for biological processes.[136-139] Such materials can be developed to have unique inherent properties including magnetic, catalytic, biomedical, and electronic, that depends on their size, shape and composition. Designing nanoparticles for biomaterials will need to be developed that can be regarded as safe (e.g. silica that shows some promise). Increased manufacturing and nanoparticles for biological processes require access to materials with well-defined characteristics. Therefore, synthesis is of high importance, particularly for the production

of nanoscale materials with uniformity in size, shape and morphology so that the materials are prepared with well-defined chemical and physical properties. Developing straightforward synthetic procedures for nanoscale materials with well-defined size, shape and morphology and careful characterization.

The impact of nanoparticles on biological species is not well understood but remains a concern due to the increased chemical reactivity of nanoparticles relative to their bulk counterparts. Understanding the stability of nanomaterials in biological systems – i.e. how does shape and structure influence activity with microbial pathogens present, is an important direction. It is crucial to understand what impact nanoparticles have on the activity of biomolecules anchored onto their surfaces especially with regard to the type of surface, and how the overall interaction impact nanoparticle stability as well as biological processes. Often nanoparticles are developed for use in drug delivery. Consequently, it is necessary to determine the key aspects of targeted delivery items that will be important (type, size of the particle), targeting properties and dynamic properties (shear force, rheology of blood). Harnessing cell-particle interactions during drug delivery is an important step in identifying ideal biomaterials.

Many of the current 3D printing or additive manufacturing processes lack nanoscale resolution for printing polymers, such as thermoplastics, for use as biomaterials or tissue engineering scaffolds for a variety of tissues. Nanoscale topography can have a significant effect on cellular/biological response and should be considered in the 3D architecture of biomaterials. Combining processing technologies to be able to print at the nanoscale yet form large micro- to macroscale 3D structures with controlled geometries, such as pore size and porosity, will be of use.

Studies involving nanoparticles that offer signal transduction could be implemented to develop a firm understanding in advanced biomanufacturing (cell/tissues) with regard to scale up *vs*. scale out, differentiating stem-cells, understanding how biomaterials interface with the cells and how the materials interface with the host and behavior of the biomaterial when it is alone *vs*. when it is in the cell and the components around it.

4.2.4 What are the existing challenges in developing biomaterials for tissue engineering and regenerative medicine?

Progress in tissue engineering and regenerative medicine depends strongly on advances in biomaterials science. Constructs to replace injured or diseased tissues, or to promote in vivo tissue regeneration, generally consist of living cells in association with biomaterials. In this context, biomaterials need to provide for appropriate construct architecture and biomechanical properties, support the function of the cells, and properly interface with the host tissue upon implantation. The latter may involve absence of inflammatory or fibrotic response towards the graft, providing a semi-permeable barrier between the construct interior and the surrounding host tissue, or promoting neovascularization around or within the construct.

One type of construct pursued by a number of labs is encapsulated cell systems. A common focus is encapsulated pancreatic islets or other insulin-producing cells for treatment of insulin-dependent diabetes. However, encapsulated cell systems have more general applicability in areas where the graft function is mediated by bioactive molecules secreted by the cells

constitutively or in response to physiologic stimuli. A critical function of the encapsulation material is to provide immune protection of non-autologous grafts from the host by excluding cytotoxic immune cells and macromolecules from reaching the implanted cells. Besides providing immunoprotection, the biomaterial in encapsulation systems should support the function of the encapsulated cells and avoid inflammatory or fibrotic responses from the host.

One biomaterial commonly used in encapsulation devices is a hydrogel consisting of alginate, a complex mixture of polysaccharides obtained from seaweeds, cross-linked with a divalent cation, generally calcium, barium or strontium. The alginate hydrogel is relatively permeable, and to generate a semipermeable barrier, capsules are treated with a polycationic solution, such as poly-L-lysine or poly-L-ornithine. Poly-L-lysine is inflammatory in vivo, so capsules are coated with a final layer of alginate to improve their biocompatibility. Although cell encapsulation in the non-adhesive alginate matrix is adequate for survival and function of pancreatic islets, other cells may require adhesion and spreading in the 3-D environment. In these cases, alginate can be functionalized by adhesive peptides [140] and further manipulated to achieve the desired cell stiffness for cell spreading.[141] The in vivo environment at the transplantation site may be hypoxic and introduction of the graft may exacerbate the level of hypoxia.[142, 143] To further assist graft survival in vivo, investigators are incorporating pro-survival and immunoprotective molecules, such as the CXCL12 chemokine, in the alginate capsules with encouraging results.[144, 145] A common approach for improving the oxygenation of transplanted cells is to encourage the formation of neovasculature proximally to the graft.[146] However, if the vasculature permeates the immune barrier, this would increase the immune recognition of the cells and likely compromise their survival. Another approach that has been proposed for improved graft oxygenation is the use of biomaterials that are hydrolytically activated to generate oxygen.[147] The in vivo location of the capsules can be tracked either by incorporating biomarkers in the encapsulated cells[148, 149] or by labeling with magnetic nanoparticles which are then imaged by nuclear magnetic resonance techniques [148]. Lastly, as cryopreservation is essential for the clinical translation of encapsulated cell systems and tissue engineered constructs in general, cryopreservation methods that preserve the structure and function of both the cells and the biomaterials are pursued [150-152].

4.3 Challenges and Opportunities

The development of flexible, smart materials, specifically piezoelectric or electromechanical materials, is an emerging technology and is being investigated as wearable sensors, robots, and energy harvesting devices. Their use as a smart biomaterial is at an early stage but has great potential due to their ability to stimulate cell and tissue growth. Challenges exist in processing conventional biocompatible piezoelectric materials to achieve improved mechanical properties for both hard and soft tissue applications. In addition, controlling degradation in order to maintain a minimum level of electromechanical stimulus to achieve a beneficial biological response needs to be considered. Opportunities exist in the development of novel chemistries, processing methods to improve properties, and characterization tools that will better understand properties at the nanoscale and at the cell-material interface.

4.3.1 Extracellular Matrix Mimetics

Glycosaminoglycans (GAGs), such as hyaluronan or chondroitin sulfate, are present in many connective tissues. Due to their significant water-binding capacity, GAGs play a biomechanical role in tissues. In addition, studies have demonstrated that the sulfation in GAGs provides beneficial biological properties. Ideally, GAG mimetics need to be designed to have better control on protein interaction and in turn, biological response.

Recent studies have shown that growth factor binding to these molecules is strictly controlled by their pattern and degree of sulfation [153]. It has also been observed that receptor binding of growth factors is regulated by the interactions with sulfated GAGs [154]. Therefore, interest has been in designing novel GAG mimetics or bioactive materials with control over the number and position of sulfate residues in the carbohydrate backbone in order to control growth factor/protein interaction. For GAG mimetics, better understanding of the effect of degree and positioning of functional groups (i.e. sulfate groups) on protein interaction and resulting biological response.

Regenerative engineering facilities are needed to provide innovations in the discovery of biomaterials, controlling biological processes, providing superior bioimaging techniques and facilitating process bioanalytics. We need small polymeric (less than 5 mm blood vessels) vascular grafts that do not occlude. Furthermore, we also need to design and fabricate sensor materials that interface with soft tissue (for example, to measure blood glucose) in a highly sensitive manner. Materials that do not foul are needed. Tools to measure the rate at which fouling occurs and understand the mechanism of fouling – thus the sensors needs to be designed under such environments.

Innovative design of biomaterials for *in vivo* cell delivery that are considered safe, are desired. To make the materials effective, improving the encapsulated systems for improved immune protection, provision of pro-survival signals, enhancement of proximal vascularization without compromising the immune barrier, and synthesizing oxygen producing materials that may be applicable to the clinic, are essential. In addition, understanding the biomechanical properties of these capsules under different preparation conditions and over time *in vitro* and especially *in vivo*.

4.4 Recommendations

An important recommendation to consider for the biomaterials community is the establishment of a foundry for stimuli-responsive biomaterials. Due to the complexity of these biomaterials and their use with cells in the biological environment, improved or new methods for characterizing their electromechanical and/or electrical properties in situ are needed. Conventional methods for characterizing the electrical properties of materials are in dry conditions. In addition, improved methods for synthesis and processing of these materials are needed such that control and tailoring of properties can be achieved at all length scales, from nano to macro-levels, in order to produce functional medical devices. Real time modeling of material properties and geometry – software for design of in situ function (real-time combined) – is needed to advocate for new validation that materials would have use in existing or new applications (i.e., to climb from TRL 1-3 lab-based discovery and development through the "valley of death" towards application). Computational modeling is needed to better understand changes in electromechanical behavior due to chemistry and processing effects at all length scales to better predict the overall behavior of the device. The foundry will further ensure that samples are produced with reproducibility.

Standardization of samples should be in partnerships with national agencies including the National Institutes of Health (NIH) and the National Institute for Standards and Technology (NIST). An added advantage to biomaterials that are standardized is that they will lead to heterogeneity that is a significant advantage for biological studies. It will also help overcome inconsistencies in data produced from different labs. The biomaterials will provide advances as tools in big data. The Foundry will need to be couples with neutron sources for soft matter materials and a synchrotron source for complimentary techniques.

An MIP in biomaterials (BioMIP) would be a hub that would validate synthetic protocols arising from the research community and provide guidance on scaling vetted biomaterials to commercial applications, training on producing the materials, and characterizing the materials. One example of the category of materials handled in the BioMIP would be hydrogels (e.g., PEG, PDMS, agarose and acrylamide) in which material properties such as mechanical, chemical, and electrical would be characterized, as well as biological properties such as cellular interactions including cytotoxicity, resorption, hydrophilicity, permeability/porosity and tissue level interactions including immunogenicity. A second example would be synthetic or purified proteins or peptide sequences for medical device functionalization or active matter research, a new thrust of soft matter in which progress has been stymied by the lack of a common experimental system.[155] A third example would be the characterization (i.e., validation) of academic, government, or commercial cell lines through markers that are directly or indirectly correlated with intended function (e.g., attachment, proliferation, differentiation) in conjunction with biomaterials for use in regenerative medicine and other approaches to therapy.

There is also a need for a foundry on ECM with an emphasis on developing GAG or GAG mimetics as bioactive materials. Synthesis of materials with improved strategies to control the spatial distribution of functional groups (i.e. sulfates) is needed. Advanced tools for characterization of the synthesized compounds, such as nuclear magnetic resonance (NMR) and mass spectrometry (MS) have to be improved. Methods to analyze protein binding to these materials and bioactivity are needed. Computational methods can be used to understand the effect of the degree and pattern of sulfation on protein interaction. Processing methods to incorporate these compounds into structures that can be used for a variety of medical devices all while retaining their bioactive properties are needed. Tools to determine cell function when interacting with these bioactive materials are needed.

It can be very expensive for investigators to contract professional 3D printing vendors and smaller commercial operations to test and optimize printing parameters for new materials. Moreover, it can be expensive to develop highly accurate printing devices in a laboratory setting that is dedicated to biomaterial development. At cost access to these capabilities for research purposes may be transformational to investigator- and student-led materials science research. Similarly, it can be very expensive for investigators to obtain in silico modeling and/or computer aided design and mechanical modeling software. At cost access to a facility where this software exists and/or funding of ImageJ-like initiatives would also be transformational to investigator- and student-led materials science research. Affiliating the foundry with institutes (e.g., industry-led or academic institute) that can provide training, assistance, and/or collaborators with expertise in both of the above activities, which are not always part of material sciences training, could also be transformational.

Equal to the need of a mid-scale characterization facility is a mid-scale fabrication foundry. This could also be a network (with distributed geographical expertise). An ideal foundry will have enough trained professionals to produce biomaterials with a focus on the design, synthesis, and characterization of biomaterials with well-defined standardization. Materials should be designed with reproducibility – designing materials with controlled porosity, geometry, chemical, electrical and mechanical strength. The foundry would support biomaterials nationally by providing access to large-scale manufacturing. Ideally, the foundry would conduct cytotoxicity studies using various cell/tissue models to facilitate understanding of what biomaterials are best suited to specified systems. To be successful in this endeavor, measurements of this nature will need access to certified (for example, disease-based) cells?

In terms of instrumental needs, research should focus on new tools that provide access to new ways of understanding how materials function. For example, instruments are needed to overcome current limitations in 3D printing. Such challenges can be overcome by combining technology to develop printing of structures with micron and sub-micron resolution for a broad class of materials with various characteristics. Similarly, there is a need to increase the speed of two-photon laser scanning microscopy as a polymer printing modality.[156] Current instruments are limited to material properties and geometry thus software for designing *in situ* function is required to further our understanding of the mechanism by which biomaterials interact with biological systems. Instruments that allow us to understand the mechanisms involved in answering biomedical questions relative to biomaterials properties. There is also a need to have access to tools that enable characterization of material-soft tissue (e.g. blood) interactions at high temporal and spatial resolution.

New instruments and techniques for *in situ* measurements that further our understanding of the mechanisms of formation of biomaterials will provide insights into design principles of emerging substances. For example, a combined spectroscopic (nuclear magnetic resonance/mass spectrometry) and atomic resolution imaging, occurring in real-time would be effective. Such instruments will likely require sample holders that allow dual or multiple modes measurements. Furthermore, development of new tools that allow manipulation of biomaterials as they form and leading to understanding mechanisms of formation at the interface of soft tissues will revolutionize biological processes. Therefore, real-time characterization of material properties during fabrication in which imaging is combined with mechanical measurements need to be developed. Researchers need to develop models or simulation techniques correlating parameters that are easy to measure for desired biomaterial properties and monitor the former to guide the fabrication process.

Instruments to characterize processes that provide insights toward understanding fundamental biomaterials properties that define the observed differences in biomaterials responses in multiple biological systems are needed. *In situ* measurements (biological settings) in which microscopic imaging can be combined with electrical measurements.

SECTION 5: Beyond Detection Limits: Characterization, ~~ols~~ Detection Tools and Diagnostic Methods

SESSION LEADERS

NICHOLAS KOTOV, UNIVERSITY OF MICHIGAN

RIGOBERTO ADVINCULA, CASE WESTERN RESERVE UNIVERSITY

SECTION MEMBERS

JOEL BROCK, CORNELL UNIVERSITY

SHENG LIN-GIBSON, NATIONAL INSTITUTE OF STANDARDS AND TECHNOLOGY

GANG-YU LIU, UNIVERSITY OF CALIFORNIA DAVIS

LEE MAKOWSKI, NORTHEASTERN UNIVERSITY

MICHAEL RUBINSTEIN, UNIVERSITY OF NORTH CAROLINA, CHAPEL HILL

5.1 Introduction

Biomaterials display distinct structural and functional characteristics compared with plastics, metals, ceramics, and other materials being used in automotive, electronic, and other industries, and therefore they have additional set of challenges associated with them. They display a wide range of material characteristics from hard to soft, living to non-living, bio-derived to engineered materials. They are also characterized by high degrees of heterogeneity and variability, which often lead to poor reproducibility. In contrast to single function manufactured materials, biomaterials often require multimodal characteristics and functions.

The design requirements of biomaterials generally must rise beyond a single, simple function in order to be attuned to the structural and physiological requirements of the body. Hemoglobin is an oxygen transport molecule. But it is so much more. It is more efficient at transporting oxygen to rapidly metabolizing tissues; it contributes to removing CO_2 from the body. Extracellular matrices (ECM) materials provide support for cells, and contain ligands, which could initiate and direct downstream cellular signaling cascades.

Biomaterials often possess structural hierarchy, complex dynamics, and amazing diversity of biological components based primarily on light elements such as carbon, hydrogen, oxygen, and nitrogen. Structural and dynamic characteristics critical for function may span multiple length and time scales, making their study intrinsically difficult and frequently requiring analytical instrumentation distinct from that used for traditional materials characterizations. Multiple characterization, analytical tools and data sets are needed to enable the design of new

biomaterials, which makes it tremendously difficult to have common data platforms for streamlining of the biomaterials development effort.

It will be a challenge, to create the next generation of analytical methods that will be relevant to lead these efforts. These analytical and characterization methods including spectroscopic, microscopic, and other field-responsive techniques (acoustic, electrochemical, dielelectric, etc.) needs to be carried to the next level and avail of high energy sources such as synchrotron, neutron, spallation, etc. available in national laboratories operated by DOE, NIST, and NSF, to get very high resolution beyond current spatial and temporal limits and molecular probes even with ultrafast lasers. The challenge with live biological species is the need to preserve specimens from irradiation damage. A particular instrumentation direction may be focusing more on hyphenated and tandem techniques that allow simultaneous real time or *in-situ* experiments to understand phenomena and structure at various windows of observation time. Examples include combinations of: optical-electrochemical, spectroscopic- mechanical, temperature-pressure gradient, interfacial-rheological, photochemical-electrical. It should be more common to have 2-in-1 or 3-in-1 types of instrumentation when investigating biological phenomena of an exposed biomaterial. Time and length scale should match the observation window. Increasing resolution from macro- to nano-scale should be matched with live or in-situ imaging and spectroscopy. An important environment is adaptation in aqueous, buffered or physiological conditions.

5.2 Scientific Questions

What new technologies or improvements on existing technologies are needed in order to characterize and measure the structural heterogeneity of biomaterials? and at multi-length scales?

We considered the following questions that clinicians, biomaterials, biomedicine, and nanomaterials communities are facing today:

5.2.1 How can mutually contradictory properties be combined into one material?

For instance, bones while being relatively lightweight also exhibit strength, toughness, stiffness. Moreover, they are structured to allow transport of nutrients and metabolites and are self-healing. Similar requirements need to be met by artificial "bone" or 'bone-mimetic' biomaterials with addition of being manufacture-able. Many biomaterials need to be reconfigurable, adaptive, bioactive and of, course, safe. Addressing these challenges is central to achieving functional integration of biomaterials with living systems to enable repair, regeneration or augmentation of body parts.

5.2.2 How can we functionally engineer the interfaces between biomaterials and organelles, membranes, cells, tissues, organs and microbiotic communities of the human body?

Most problems that biomaterials encounter when implanted or otherwise in contact with living tissue occur at interfaces. For example, most infections including those caused by antibiotic resistant bacteria, occur at the interfaces of the indwelling devices.

Because of this, biointerface design and fabrication represents an area of biomaterials engineering likely to have immediate impact on the design of novel healthcare technologies. While multiple cellular and system responses of the body to external interfaces are critical to biomaterials function, they are, at the same time, difficult to assess. Like most biomaterials, biointerfaces have complex dynamics that span time scales from nanoseconds to years, and dynamic processes across all of these time frames may have large impact on patients.

5.2.3 How can we rapidly and efficiently utilize emerging and established technologies for biomaterials design?

Systems biology, nanomaterials, 3D printing, soft robotics, big data, and others technologies have the potential to support significant improvements in biomaterials design. They represent the growth points in biomaterials, however, there are substantial challenges that must be faced in integrating them into biomaterials design and fabrication. Accelerating their impact is a high priority.

5.2.4 How can we mimic the structural hierarchy intrinsic to so many naturally occurring biomaterials?

Cells build biomaterials molecule-by-molecule, and nanoparticle-by-nanoparticle. Self-organizing phenomena represent an overarching tool that biomaterials utilize to create structural complexity starting with molecular components and ending with functional macroscale systems. We see evidence that subtle geometrical and biochemical properties, such as chirality, propagate their impact through all the dimensions. Synthetic approaches are not (yet) capable of mimicking this process.

5.2.5 How can we mimic the multiscale dynamics of a biological material?

The temporal response of the cellular and tissue components is complex but is critical to understanding function. Biomaterials need to have the ability to match this temporal response. Furthermore, multiple responses need to be integrated across many time and length scales in the context of hierarchical structural organization.

5.2.6 How can we predictively engineer biomaterials with multiple essential properties?

Beyond mechanical properties that often play a central role in conventional industries, biomaterials have engineering specifications that must encompass biocompatibility, dynamic response, flexibility, longevity and resilience to corrosive environments. Determining acceptable limits on these properties may require fundamental research into the response of biological systems to perturbations by materials with different characteristics.
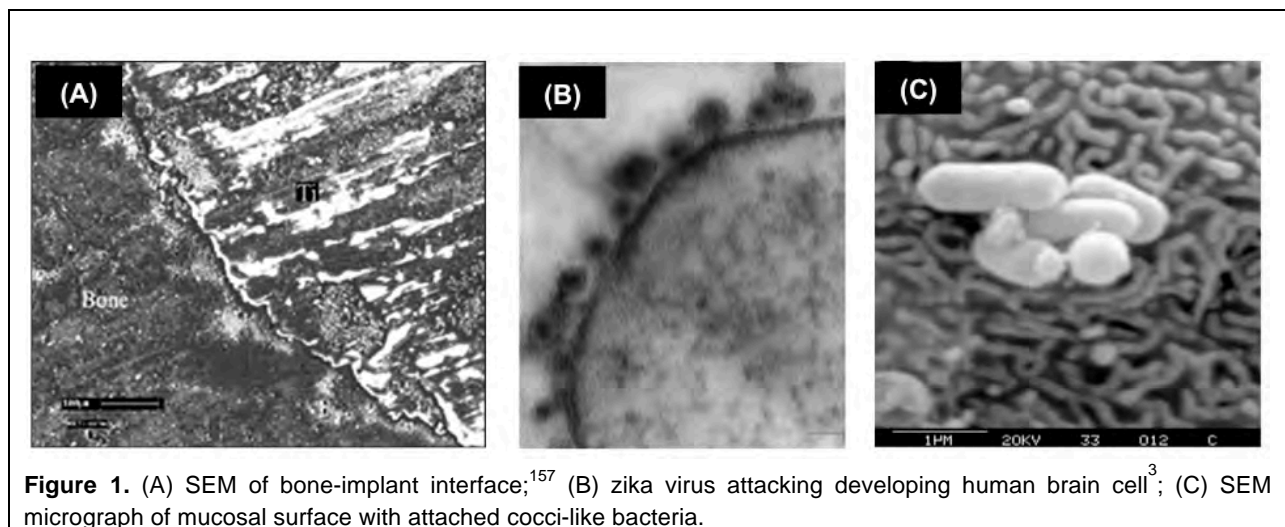
5.2.7 How can a multiscale theory be developed that, in combination with simulations, will generate designs for biological nanocomposites with desired properties?

For example, can we form interpenetrating biopolymer double- or triple-networks that are tough and stiff, but also is adaptive and strong? Could these materials be fashioned to also be bio-regenerating?

5.3 Challenges and Opportunities

Considering the variety of problems facing the field today, the panel identified the following overarching challenges in the area of biomaterials stemming from the current and projected healthcare needs. We attempted to identify the challenges that are descriptive of multiple applications of biomaterials – implantable devices, drug delivery, tissue engineering, nanomedicine, drug discovery, imaging, diagnostics, and others. The following grand challenges represent top priorities.

1. **Predictive Multiscale Biomaterials Engineering**. Given the versatility of the components, properties, and functions, one of the central challenges of biomaterials is their predictive engineering. Convergence of experiment and simulations needs to be achieved for multiaxial/multiparameter materials design. Biomaterials exemplify in the best possible way both the needs and emerging capabilities envisioned by the Materials Genome Initiative (MGI). Eventually, we need to generate a triangle of activities consisting of design, synthesis and characterization coordinated to support development of biomaterials with complex hierarchical structure with desired functionality; and operated in a way that will iterate, validate and ultimately, lead to manufacture of novel materials with targeted properties.

2. **Biointerfaces: hard, soft, mammalian, bacterial, viral.** Engineering of biointerfaces represents an overarching biomaterials challenge that encompasses many of the problems and scientific questions (see above) encountered today (Figure 1).

Figure 1. (A) SEM of bone-implant interface;[157] (B) zika virus attacking developing human brain cell[3]; (C) SEM micrograph of mucosal surface with attached cocci-like bacteria.

3. **"Living" biomaterials.** While some degree of "programming" of biomaterial responses such as controlled drug release or time-dependent resorption of implants[158] is possible, the synthesis of biomaterials that integrate biochemical and cellular responses of the existing library of materials will enable more sophisticated engineering of biomaterials, for instance, providing the capability for self-repair.

4. **Understanding water in biological systems.** Water is an essential component of all living systems, which is required for human health at many levels. Current computational models of water remain inadequate. Based on heuristic rules and approximations, these models fail for many biomaterials and nano to microscale structures with complexity similar to those found in biology.

5. **Imaging of interfaces.** While liquid/air, solid/air, liquid/liquid interfaces have been relatively well studied, biointerfaces are critically important in biomaterial development, but difficult to image and investigate: How does one characterize boundaries within cartilage, layer boundaries at airway (wall/cilia/mucin), implant/environment, intracellular organelles? This challenge demands development of new imaging tools to visualize these interfaces at multiple length scales in order to advance our knowledge in biointerfaces from molecular level, to nanometer and mesoscopic level. Multimodal imaging of biointerfaces combined with computational modeling to incorporate information from different experimental probes and advanced models of water molecules at these interfaces will be key to design of advanced biomaterials.

These challenges are addressable and we anticipate that in the next decade the following breakthroughs in the area of biomaterials are possible. These breakthroughs will universally provide opportunities to develop devices to improve the quality of life for many people.

1. Hierarchical manufacturing of biomaterials with multiple levels of control is likely to become possible. The emergence of high-resolution patterning and 3D printing tools will accelerate this process. Understanding of self-assembly processes to a level that will make possible creation of synthetic processes that mimic biomolecular assembly will support predictive design of these complex hierarchical systems

2. The emergence of super-resolution techniques in optical microscopy and *in-situ* electron microscopic and x-ray imaging techniques creates an opportunity to address the challenges of characterizing biointerfaces in living materials. Real-time visualization towards 1 nm diameter particles or molecular clusters and the possibility of 1 ns temporal resolution and 1 nm spatial resolution in living tissues will generate a wealth of knowledge and design leads for biomaterials engineering. The need for high spatial resolution is essential in characterization of biomaterials, e.g. determining material degradation or tracking of single biomaterial nanoparticles in biofluids in biomimetic environments or *in vivo*.

3. System biology based production of biomaterials will enable facile programmable production of biomaterials that would be difficult to produce otherwise. We

anticipate promising opportunities for integration of both inorganic and biological components for synthesis of biotechnological tools and materials.

4. Detection of single virus particles or bacterial cells will open the path to inexpensive in-home diagnostics**.** This will be enabled through development of new biomaterials capable of simple, inexpensive and fast detection of bioanalytes with high-sensitivity and high-specificity. This challenge has broad impact that the broader community can easily relate to, e.g. paper stripe detection kit of bioanalytes.

5. Integration of relevant modalities in imaging and spectroscopy for acquiring distribution of a broad range of materials properties at multiple length scales, from atom to molecule to cell to organ. While many technologies could attain very high spatial resolution for hard materials, the low contrast and environmental sensitivity of biomaterials present significant challenges for realizing high resolution in biomaterials in relevant environments. This challenge provides researchers with the opportunity to (a) identify which dimensional characterization is necessary for one's scientific pursuit; (b) develop advanced imaging and spectroscopy protocols and controls to acquire reliable and meaningful data; (c) to develop highly integrated and advanced instrumentation that is tailored for biomaterial characterization. Computational modeling presents a promising approach to integrating the results of multiple imaging modes, each of which produces a map of specific materials properties, into a single integrated picture of the biomaterial.

5.4 Research Tools Needed for Biomaterials

Advances in biomaterials will require development of new instruments/approaches capable of meaningful structure-property-function correlation *in situ* and in real time. Past and current practice has a physical and time separation among production, characterization, property and function measurements.   This challenge charges researchers to adopt interdisciplinary approaches and innovation to coherently combine current state-of-the-art methods from individual fields to an integrated approach. Biomaterials tools can be divided as: *1) structural and compositional characterization tools, 2) property – time monitoring tools, 3) integrated function: structure-property-function paradigm deterministic or correlational tools (or sometimes called devices).*   Many analytical tools exist to provide information about composition, structure, dynamics, and function, but:

(**1**) The current resolution and detection limits in dynamics and length scales limit their utility for design of new biomaterials as well as for the efficient utilization of the current ones;

(**2**) Current practices are limited in their capabilities for quantifying the performance of materials and their adaptation for *in vivo* studies

(**3**) Standards for sample preparation, configuration, environment and reproducibility are currently limited preventing us from having better empirical comparison with peers.

Specific Instrumentation that have been used for biomaterials research include: Light optical microscopy, polarized microscopy, fluorescence and confocal microscopy, atomic force microscopy (AFM) – and the many associated surface probe microscopy (SPM) techniques based on field response, FT-IR microscopy, FT-IR spectroscopic imaging with focal plane array

(FPA) detectors, Raman imaging, field enhanced or scanning near-field optical microscopy (SNOM), scanning electron microscopy (SEM), transmission electron microscopy (TEM) and cryo-TEM, non-linear optical (NLO) spectroscopy and imaging, and waveguide imaging. Spectroscopy and diffraction methods include: UV-Vis absorbance, fluorescence, IR (vis, near and mid) with absorption, reflection, transmission, Raman, NMR, X-ray diffraction (SAXS and WAXS) or neutron and synchrotron sources, surface plasmon resonance (SPR) spectroscopy and imaging, dielectric spectroscopy, X-ray photoelectron spectroscopy (XPS). These spectroscopic and microscopic methods can be done in-situ, ex-situ, in real time or simultaneous and tandem methods. The application of controlled temperature, pressure, flow, light exposure, electrical field, magnetic fields, etc. can be used to produced gradient or "ON and OFF" responses. The use of flow and buffered aqueous conditions is always a challenge.

A list of specific biomedical imaging and spectroscopic methods include: Bioluminescence, Fluorescence, Flow Cytometery, Light optical microscopy, In situ cryo-imaging, Magnetic Resonance Imaging (MRI), ultrasound, single photon computed tomography, Positron Emission Tomography (PET), Scintigraphy, X-ray / Computed Tomography (CT).

Two approaches to "designer" measurements have been considered: 1) development of novel techniques beyond the current measurement capabilities and 2) expansion/combinations of existing experimental tools in user-accessible foundries. For example a number of attempts to combine surface plasmon resonance spectroscopy, atomic force microscopy, and electrochemical measurement experiments have been reported (Figure 2).[159] It is also possible to do infra-red spectroscopy and AFM at the nanoscale resolution.[160] But the challenge has always been to correlate with biological and real-time experiments with aqueous (or buffer based) interfaces or live cells, viruses, and bacteria.

There is a need to leverage existing NSF and DOE facilities, infrastructure and expertise for (electronic, magnetic, structural, soft, energy, optically active, chemically active) materials characterization (magnetic lab, synchrotron, e-microscopes, (super) optical imaging, NMR, etc.…) by creating the necessary sample environments for biomaterials. There is a need to formulate a realistic plan (finite time, budget) for making progress in a center or foundry organization.
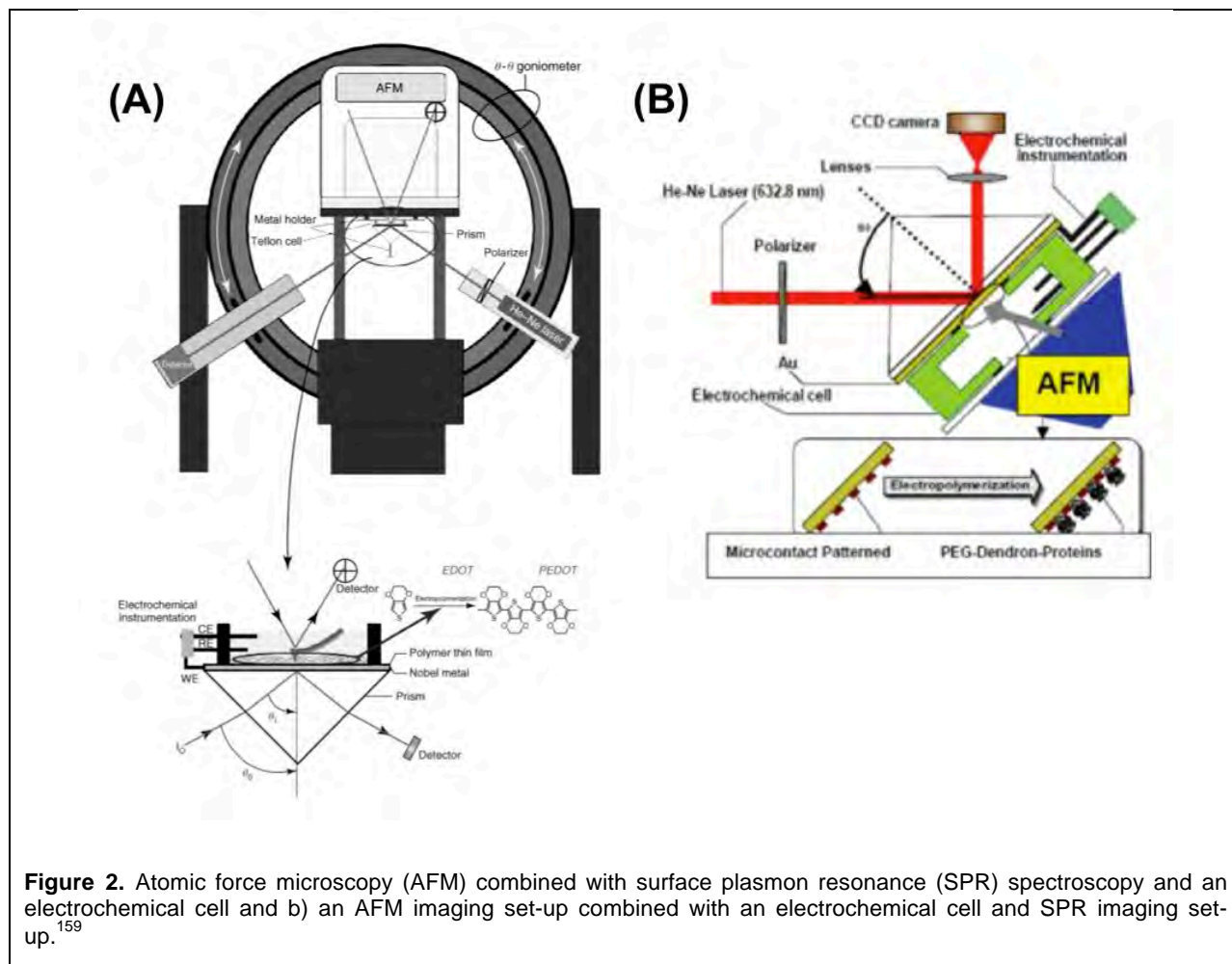
Figure 2. Atomic force microscopy (AFM) combined with surface plasmon resonance (SPR) spectroscopy and an electrochemical cell and b) an AFM imaging set-up combined with an electrochemical cell and SPR imaging set-up.[159]

Additional challenges that were identified by the panel and are deemed important for progress are:

- *In situ* characterization of biomimetic materials in biological environments. Characterization of cellular systems during development/growth/movement on nanostructured substrates.
- Dynamics of materials at multiple time scales spanning from nano-seconds to pico-seconds.
- Facilities designed to readily incorporate iterative cycle of materials design; synthesis and characterization to provide information on further cycles of design.
- Surface characteristics need to be plumbed including adhesive properties; flexibility; permeability; conductivity, charge and other properties amenable to study by surface scattering.
- Heterogeneity in all properties - structural, chemical, mechanical, functional - that occurs in most/all tissues. Mapping differences in properties across these materials is a major challenge, requiring collection and organization of massive quantities of data from multiple experimental probes.

- Long-term stability of tissues/materials within tissues (implants) remains a poorly addressed issue.
- Defining and developing a user base for a mid-scale facility requires staging of capabilities with wide applicability; facile control of materials environment and multi-probe characterization facilities coupled to computational support for integrating all available data.

Utilizing combinatorial changes in genes; environmental conditions provides the capability for generating a huge number of samples; asking many questions; assessing the importance of many variables. To take advantage of this capability, significant development of software including innovative databases will be essential.

5.5 Recommendations

Current understanding of biomaterials function could be limited by shortcomings in characterization and analytical techniques that may be surmounted by integration of existing and/or next generation technologies into capturing real time and in-situ biological phenomena. However, there is a need for structural and operational support to make such a tools and foundry set-up be more useful and accelerate research and innovation.

In a biomaterials tools and foundry set-up our recommendations as a committee include the following:

1. User Support is important. Establishment of a user support or full-time expert support in any tools and foundry facilities that enable sample preparation and mounting and interpretation of results which will enable users to get feedback and adjust their experiments to shorten the experimental methodology development time and capitalize on new dynamic findings. In designing a foundry facility, it should include support and components: natural biomaterials characterization, theory, and manufacturing of synthetic biomaterials with both organic and inorganic components. Biological and biomedical facilities of live organism (bacterial, plant, animal) should be readily available to ensure in vivo or ex-situ translational studies with harvesting or retrieval methodologies to enable a quick feedback on the biological interpretation.

2. Access to expertise in materials synthesis. Expertise in synthesis, fabrication, and assembly is needed which allow for a foundry to match the needs from molecular to macroscopic analytical methods. Organic and inorganic synthesis expertise include the exploration of new synthetic pathways, functionality in molecules, macromolecules, probe molecules, surfactants, de novo polynucleotide and polypeptides, bioconjugation and synthetic biology. Inorganic includes metal, metal oxides, calcite, silicate, chemistry. Nanomaterial foundry will of course contribute towards synthesis and modification of carbon nanomaterials, nanocellulosics, noble metal and inorganic chalcogenide nanocrystals. There has been a lot of effort in developing synthetic biomaterials that can effectively interact with biological systems. Complex materials of defined sequence and confined spatial or environmental situations will be critical to the design and synthesis of new self-assembling and bioinspired materials. All of this will require chemists and materials scientists that will integrate knowledge of biology with scalable methods.

3. Theory and Simulation need to be integrated early with the analytical methods to make clear the first principles in biomaterials function and their related investigative methods. As much as possible thermodynamic and kinetic consideration on the system need to be modeled early or set the basis for hypothesis driven experiments where simulation can allow for some modes of interpreting results based on defined parameters of the measurement methods. In this case, computational modeling and resources should be part of the tools set-up and should be accessible for researchers. In the area of structural biology, supramolecular chemistry, and self-assembly, defining DFT, semi-empirical, and even monte-carlo methods that are appropriate can lead to a more molecular understanding of the phenomena prior to interpreting other orders of hierarchy in structure.

4. Standardization of methods and data reporting. In developing a community of users that use a common language – a Biomaterials Foundry – should initiate the standardization of current practices and methods to enable biomaterial expertise to a wider community. Specific ideas for enabling this common language include: 1) Biomaterial-omics: collection of biomaterial building blocks, 2) Data Depositories: reproducible correlations, and 3) Methods Depositories: SOP and biomaterial phantoms/testing standard. The development and general adoption of standards (test assays, management system, reference materials, common format for sharing research results, etc) can be a collaborative effort between NSF, NIST, FDA and others. It will be interesting to establish Regional hubs for interdisciplinary Teams: collaborated acceleration of biomaterial development and optimization – that will allow expertise to be incubated and nurtured in distribute networks of knowledge and application driven centers.

5. Fabrication Foundry and device development: An ideal foundry will have enough trained professionals to produce biomaterials with a focus on the design, synthesis, and characterization of biomaterials with well-defined standardization. The next level of expertise will be in establishing fabrication and device integration foundries that may even link with commercialization and start-ups. Other than molecular to nanomaterials scale up, there is a need to introduce other advanced fabrication and additive manufacturing processes in biomaterials. The 3D printing for biomaterials: surgery, dental, maxillofacial, bone replacement, etc. are emerging needs for CAD-Design and image or digital design space that makes use of current advances in imaging. This includes interest on utilizing metals, ceramics, oxides, and polymers for replacing bone and dental materials with de novo design features. There is interest on using such facilities for bio-inspired designs that can be found in other organisms of the plant and animal kingdom, which can have new directions for biomaterials development where materials are incorporated with the geometry of the design to come up with new properties or functions. This can be coupled with their biological or biomedical function for new tissue growth or integration.

6. Advances in Instrumentation. We recommend development of instrumentation and methodologies that will support translation of existing approaches into next generation devices. For instance, wireless and implantable devices and sensing units; microrobots, that can bridge *in vivo* and *in-vitro* data. Continuously addressing the limitations of the characterization techniques should be a parallel exercise. The advances in optical, x-ray and electron microscopies coupled with the ever expanding capability to collect, process, manipulate and interpret vast amounts of data presents a new opportunity for researchers to exercise their creativity and analytical acumen.

Facilities for characterization of biomaterials should be "open configuration" and not just black boxes. They should provide facile measurement of multiple properties simultaneously. Capabilities that could potentially be integrated into such a facility would include HT analyses of the responsiveness of materials to mechanical load and many aspects of the microenvironment. Responsiveness as measured over multiple length scales; distortion as measured with multiple spectroscopies; x-ray scattering and including elastic and plastic distortion; re-structuring; re-organization are examples. Development of instrumentation and methodologies to enable simultaneous imaging and measurement with high spatial and temporal resolution, e.g. 1 nm and 1 ns, respectively. While we have tools available to achieve high spatial or high temporal resolution, attaining both is a scientific and technical challenge.

In summary, advancement of the following directions will make a substantial intellectual and practical impact in the field of biomaterials and tools for research:

- Understanding biointerfaces from macro to nanoscale interactions. The devil is in the details at biological interfaces.
- Dramatic improvement in time and spatial scale capabilities of data acquisition tools.
- Multimodal (scattering, spectroscopic, mechanical, electronic, *etc*) and hyphenated data acquisition tools and their integration with computational modeling.
- Theory that can predict measureable properties and/or synthesis routes of bio-materials and their hierarchical self-organization. Validation of the theory and models over multiple spatial and timescales with the right instrumentation.
- Integration of big data approaches into all experimental strategies from the beginning.
- Exploitation of the unique properties of nanomaterials through coordination of simulation, experiments and imaging tools that enable accelerated engineering of systems.
- Modular manufacturing of biomaterials combining both top-down and bottom-up processes and more bioinspired design.

References

1. Gilbert, P. U. P. A.; Killian, C. E., Biomineral Single Crystals. *Science* **2013,** *339* (6119), 510-510.

2. DeVol, R. T.; Sun, C.-Y.; Marcus, M. A.; Coppersmith, S. N.; Myneni, S. C. B.; Gilbert, P. U. P. A., Nanoscale Transforming Mineral Phases in Fresh Nacre. *Journal of the American Chemical Society* **2015,** *137* (41), 13325-13333.

3. Keten, S.; Xu, Z.; Ihle, B.; Buehler, M. J., Nanoconfinement controls stiffness, strength and mechanical toughness of [beta]-sheet crystals in silk. *Nature Materials* **2010,** *9* (4), 359-367.

4. Sun, C.; Waite, J. H., Mapping chemical gradients within and along a fibrous structural tissue, mussel byssal threads. *Journal of Biological Chemistry* **2005,** *280* (47), 39332-39336.

5. Miserez, A.; Schneberk, T.; Sun, C.; Zok, F. W.; Waite, J. H., The transition from stiff to compliant materials in squid beaks. *Science* **2008,** *319* (5871), 1816-1819.

6. Wang, R.; Suo, Z.; Evans, A.; Yao, N.; Aksay, I., Deformation mechanisms in nacre. *Journal of Materials Research* **2001,** *16* (09), 2485-2493.

7. Aizenberg, J.; Weaver, J. C.; Thanawala, M. S.; Sundar, V. C.; Morse, D. E.; Fratzl, P., Skeleton of Euplectella sp.: structural hierarchy from the nanoscale to the macroscale. *Science* **2005,** *309* (5732), 275-278.

8. Miserez, A.; Weaver, J. C.; Thurner, P. J.; Aizenberg, J.; Dauphin, Y.; Fratzl, P.; Morse, D. E.; Zok, F. W., Effects of laminate architecture on fracture resistance of sponge biosilica: lessons from nature. *Advanced Functional Materials* **2008,** *18* (8), 1241-1248.

9. Killian, C. E.; Metzler, R. A.; Gong, Y.; Churchill, T. H.; Olson, I. C.; Trubetskoy, V.; Christensen, M. B.; Fournelle, J. H.; De Carlo, F.; Cohen, S., Self-Sharpening Mechanism of the Sea Urchin Tooth. *Advanced Functional Materials* **2011,** *21* (4), 682-690.

10. Jin, H.-J.; Kaplan, D. L., Mechanism of silk processing in insects and spiders. *Nature* **2003,** *424* (6952), 1057-1061.

11. Gong, Y. U.; Killian, C. E.; Olson, I. C.; Appathurai, N. P.; Amasino, A. L.; Martin, M. C.; Holt, L. J.; Wilt, F. H.; Gilbert, P., Phase transitions in biogenic amorphous calcium carbonate. *Proceedings of the National Academy of Sciences* **2012,** *109* (16), 6088-6093.

12. DeVol, R. T.; Sun, C.-Y.; Marcus, M. A.; Coppersmith, S. N.; Myneni, S. C.; Gilbert, P. U., Nanoscale transforming mineral phases in fresh nacre. *Journal of the American Chemical Society* **2015,** *137* (41), 13325-13333.

13. Nassif, N.; Pinna, N.; Gehrke, N.; Antonietti, M.; Jäger, C.; Cölfen, H., Amorphous layer around aragonite platelets in nacre. *Proceedings of the National Academy of Sciences of the United States of America* **2005,** *102* (36), 12653-12655.

14. Chung, S.; Shin, S.-H.; Bertozzi, C. R.; De Yoreo, J. J., Self-catalyzed growth of S layers via an amorphous-to-crystalline transition limited by folding kinetics. *Proceedings of the National Academy of Sciences* **2010,** *107* (38), 16536-16541.

15. Cheng, S.; Liu, Y.; Crowley, C. S.; Yeates, T. O.; Bobik, T. A., Bacterial microcompartments: their properties and paradoxes. *Bioessays* **2008,** *30* (11-12), 1084-1095.

16.   Williams, T., The iridoviruses. *Advances in Virus Research* **1996,** *46*, 345-412.

17.   Runnegar, B., Oxygen requirements, biology and phylogenetic significance of the late Precambrian worm Dickinsonia, and the evolution of the burrowing habit. *Alcheringa* **1982,** *6* (3), 223-239.

18.   Bowring, S. A.; Grotzinger, J. P.; Isachsen, C. E.; Knoll, A. H.; Pelechaty, S. M.; Kolosov, P., Calibrating rates of Early Cambrian evolution. *Science* **1993,** *261* (5126), 1293-1298.

19.   Knoll, A. H., The early evolution of eukaryotes: a geological perspective. *Science* **1992,** *256* (5057), 622.

20.   Nalla, R. K.; Kinney, J. H.; Ritchie, R. O., Mechanistic fracture criteria for the failure of human cortical bone. *Nature Materials* **2003,** *2* (3), 164-168.

21.   Okyaya, T. O.; Rodrigues, D. F., Biotic and abiotic effects on CO_2 sequestration during microbially-induced calcium carbonate precipitation. *FEMS Microbiology Ecology* **2015,** *91* (3).

22.   Okyaya, T. O.; Nguyen, H. N.; Castro, S. L.; Rodrigues, D. F., CO_2 sequestration by ureolytic microbial consortia through microbially-induced calcite precipitation. *Science Total Environment* **2016,** *accepted*.

23.   Knorre, H.; Krumbein, W., Bacterial calcification. In *Microbial sediments*, Riding, R. E.; Awramik, S. M., Eds. Springer-Verlag: Berlin, Germany, 2000; pp 25-31.

24.   Castanier, S.; Le Metayer-Levrel, S.; Perthuisot, J. P., Ca-Carbonates preciptation and limestone genesis- the microbiologist point of view. *Sedimentary Geology* **1999,** *126*, 9-23.

25.   Douglas, S.; Beveridge, T. J., Mineral formation by bacteria in natural microbial communities. *FEMS Microbiol Ecol* **1998,** *26*, 79-88.

26.   Rivadeneyra, M. A.; Delgado, G.; Soriano, M.; Ramos-Cormenzana, A.; Delgado, R., Precipitation of carbonates by Nesterenkonia halobia in liquid media. *Chemosphere* **2000,** *41* (4), 617-24.

27.   Lam-Schultze, S.; Fortin, D.; Davis, B. S.; Beveridge, T. J., Mineralization of bacterial surfaces. *Chemical Geology* **1996,** *132*, 171-181.

28.   Kawaguchi, T.; Decho, A. W., A laboratory investigation of cyanobacterial extracellular polymeric secretions (EPS) in influencing CaCO3 polymorphism. *Journal of Crystal Growth* **2002,** *240*, 230-235.

29.   Hammes, F.; Verstraete, W., Key roles of pH and calcium metabolism in microbial carbonate precipitation. *Reviews in Environmental Science Biology Biotechnology* **2002,** *1*, 3-7.

30.   McConnaughey, T. A.; Whelan, F. F., Calcification generates protons for nutrient and bicarbonate uptake. *Earth SCience Review* **1997,** *42*, 95-117.

31.   Okyay, T. O.; Rodrigues, D. F., Biotic and abiotic effects on CO2 sequestration during microbially-induced calcium carbonate precipitation. *FEMS microbiology ecology* **2015,** *91* (3), fiv017.

32.   Mora, C.; Tittensor, D. P.; Adl, S.; Simpson, A. G.; Worm, B., How many species are there on Earth and in the ocean? *PLoS Biology* **2011,** *9* (8), e1001127.

33.   Naleway, S. E.; Porter, M. M.; McKittrick, J.; Meyers, M. A., Structural design elements in biological materials: application to bioinspiration. *Advanced Materials* **2015,** *27* (37), 5455-5476.

34.   Weiner, S.; Traub, W.; Wagner, H. D., Lamellar bone: structure–function relations. *Journal of Structural Biology* **1999,** *126* (3), 241-255.

35.   Lin, Y.; Wei, C.; Olevsky, E.; Meyers, M. A., Mechanical properties and the laminate structure of Arapaima gigas scales. *Journal of the mechanical behavior of biomedical materials* **2011,** *4* (7), 1145-1156.

36.   Barthelat, F.; Tang, H.; Zavattieri, P.; Li, C.-M.; Espinosa, H., On the mechanics of mother-of-pearl: a key feature in the material hierarchical structure. *Journal of the Mechanics and Physics of Solids* **2007,** *55* (2), 306-337.

37.   Menig, R.; Meyers, M.; Meyers, M.; Vecchio, K., Quasi-static and dynamic mechanical response of Strombus gigas (conch) shells. *Materials Science and Engineering: A* **2001,** *297* (1), 203-211.

38.   Barthelat, F.; Espinosa, H., An experimental investigation of deformation and fracture of nacre–mother of pearl. *Experimental mechanics* **2007,** *47* (3), 311-324.

39.   Porter, M. M.; Novitskaya, E.; Castro-Ceseña, A. B.; Meyers, M. A.; McKittrick, J., Highly deformable bones: unusual deformation mechanisms of seahorse armor. *Acta Biomaterialia* **2013,** *9* (6), 6763-6770.

40.   Persano, L.; Camposeo, A.; Tekmen, C.; Pisignano, D., Industrial upscaling of electrospinning and applications of polymer nanofibers: a review. *Macromolecular Materials and Engineering* **2013,** *298* (5), 504-520.

41.   Coles, S. R.; Jacobs, D. K.; Meredith, J. O.; Barker, G.; Clark, A. J.; Kirwan, K.; Stanger, J.; Tucker, N., A design of experiments (DoE) approach to material properties optimization of electrospun nanofibers. *Journal of Applied Polymer Science* **2010,** *117* (4), 2251-2257.

42.   Katsogiannis, K. A. G.; Vladisavljević, G. T.; Georgiadou, S., Porous electrospun polycaprolactone fibers: Effect of process parameters. *Journal of Polymer Science Part B: Polymer Physics* **2016,** *54* (18), 1878-1888.

43.   Khanlou, H. M.; Ang, B. C.; Talebian, S.; Afifi, A. M.; Andriyana, A., Electrospinning of polymethyl methacrylate nanofibers: optimization of processing parameters using the Taguchi design of experiments. *Textile Research Journal* **2014**, 0040517514547208.

44.   Kim, S.-E.; Harker, E. C.; De Leon, A. C.; Advincula, R. C.; Pokorski, J. K., Coextruded, Aligned, and Gradient-Modified Poly (ε-caprolactone) Fibers as Platforms for Neural Growth. *Biomacromolecules* **2015,** *16* (3), 860-867.

45.   Kim, S.-E.; Wang, J.; Jordan, A. M.; Korley, L. T.; Baer, E.; Pokorski, J. K., Surface modification of melt extruded poly (ε-caprolactone) nanofibers: toward a new scalable biomaterial scaffold. *ACS Macro Letters* **2014,** *3* (6), 585-589.

46.   Wang, J.; Langhe, D.; Ponting, M.; Wnek, G. E.; Korley, L. T.; Baer, E., Manufacturing of polymer continuous nanofibers using a novel co-extrusion and multiplication technique. *Polymer* **2014,** *55* (2), 673-685.

47.   Kim, S.-E.; Zhang, C.; Advincula, A. A.; Baer, E.; Pokorski, J. K., Protein and Bacterial Antifouling Behavior of Melt-Coextruded Nanofiber Mats. *ACS Applied Materials & Interfaces* **2016,** *8* (14), 8928-8938.

48.   Killian, C. E.; Metzler, R. A.; Gong, Y. T.; Olson, I. C.; Aizenberg, J.; Politi, Y.; Wilt, F. H.; Scholl, A.; Young, A.; Doran, A.; Kunz, M.; Tamura, N.; Coppersmith, S. N.; Gilbert, P. U. P. A., The mechanism of calcite co-orientation in the sea urchin tooth. *Journal of the American Chemical Society* **2009,** *131*, 18404-18409.

49.   Frank, M. B.; Naleway, S. E.; Wirth, T. S.; Jung, J.-Y.; Cheung, C. L.; Loera, F. B.; Medina, S.; Sato, K. N.; Taylor, J. R.; McKittrick, J., A Protocol for Bioinspired Design: A Ground Sampler Based on Sea Urchin Jaws. *Journal of Visualized Experiments* **2016,** (110), e53554-e53554.

50.   Patterson, D. P.; Schwarz, B.; Waters, R. S.; Gedeon, T.; Douglas, T., Encapsulation of an Enzyme Cascade within the Bacteriophage P22 Virus-Like Particle. *Acs Chemical Biology* **2014,** *9* (2), 359-365.

51.   Sutter, M.; Boehringer, D.; Gutmann, S.; Guenther, S.; Prangishvili, D.; Loessner, M. J.; Stetter, K. O.; Weber-Ban, E.; Ban, N., Structural basis of enzyme encapsulation into a bacterial nanocompartment. *Nature Structural & Molecular Biology* **2008,** *15* (9), 939-947.

52.   Tanaka, S.; Sawaya, M. R.; Yeates, T. O., Structure and mechanisms of a protein-based organelle in Escherichia coli. *Science* **2010,** *327* (5961), 81-84.

53.   Jordan, P. C.; Patterson, D. P.; Saboda, K. N.; Edwards, E. J.; Miettinen, H. M.; Basu, G.; Thielges, M. C.; Douglas, T., Self-assembling biomolecular catalysts for hydrogen production. *Nature Chemistry* **2016,** *8* (2), 179-185.

54.   Patterson, D. P.; Prevelige, P. E.; Douglas, T., Nanoreactors by Programmed Enzyme Encapsulation Inside the Capsid of the Bacteriophage P22. *Acs Nano* **2012,** *6* (6), 5000-5009.

55.   Renggli, K.; Baumann, P.; Langowska, K.; Onaca, O.; Bruns, N.; Meier, W., Nanoreactors: Selective and Responsive Nanoreactors (Adv. Funct. Mater. 7/2011). *Advanced Functional Materials* **2011,** *21* (7), 1206-1205.

56.   Qin, Z.; Compton, B. G.; Lewis, J. A.; Buehler, M. J., Structural optimization of 3D-printed synthetic spider webs for high strength. *Nature Communications* **2015,** *6*.

57.   Nair, A. K.; Libonati, F.; Qin, Z.; Dimas, L. S.; Buehler, M. J., Mechanical and interface properties of biominerals: Atomistic to coarse-grained modeling. *Biomineralization Sourcebook: Characterization of Biominerals and Biomimetic Materials* **2014,** *337*.

58.   Nair, A. K.; Gautieri, A.; Chang, S.-W.; Buehler, M. J., Molecular mechanics of mineralized collagen fibrils in bone. *Nature Communications* **2013,** *4*, 1724.

59.   Nair, A. K.; Gautieri, A.; Buehler, M. J., Role of intrafibrillar collagen mineralization in defining the compressive properties of nascent bone. *Biomacromolecules* **2014,** *15* (7), 2494-2500.

60.   Mirzaeifar, R.; Dimas, L. S.; Qin, Z.; Buehler, M. J., Defect-tolerant bioinspired hierarchical composites: simulation and experiment. *ACS Biomaterials Science & Engineering* **2015,** *1* (5), 295-304.

61.   Krishnaji, S. T.; Bratzel, G.; Kinahan, M. E.; Kluge, J. A.; Staii, C.; Wong, J. Y.; Buehler, M. J.; Kaplan, D. L., Sequence–structure–property relationships of recombinant spider silk proteins: integration of biopolymer design, processing, and modeling. *Advanced Functional Materials* **2013,** *23* (2), 241-253.

62.   Giesa, T.; Bratzel, G.; Buehler, M. J., Modeling and Simulation of Hierarchical Protein Materials. *Nano and Cell Mechanics: Fundamentals and Frontiers* **2012**, 389-409.

63.   Dimas, L. S.; Bratzel, G. H.; Eylon, I.; Buehler, M. J., Tough composites inspired by mineralized natural materials: computation, 3D printing, and testing. *Advanced Functional Materials* **2013,** *23* (36), 4629-4638.

64.   Cranford, S. W.; de Boer, J.; van Blitterswijk, C.; Buehler, M. J., Materiomics: An-omics Approach to Biomaterials Research. *Advanced Materials* **2013,** *25* (6), 802-824.

65.   Cranford, S. W.; Buehler, M. J., *Biomateriomics*. Springer Science & Business Media: 2012; Vol. 165.

66.   Buehler, M. J.; Yung, Y. C., How protein materials balance strength, robustness, and adaptability. *HFSP journal* **2010,** *4* (1), 26-40.

67.   Buehler, M. J.; Gao, H., Ultra-large scale simulations of dynamic materials failure. *Handbook of theoretical and computational nanotechnology* **2005,** *10*, 1-41.

68.   Buehler, M. J.; Abraham, F. F.; Gao, H., Hyperelasticity governs dynamic fracture at a critical length scale. *Nature* **2003,** *426* (6963), 141-146.

69.   Buehler, M. J. In *Defining nascent bone by the molecular nanomechanics of mineralized collagen fibrils*, ASME 2009 International Mechanical Engineering Congress and Exposition, American Society of Mechanical Engineers: 2009; pp 795-798.

70.   Mervis, J., NSF director unveils big ideas, with an eye on the next president and Congress. *Science* **2016**.

71.   De Yoreo, J. J.; Gilbert, P. U.; Sommerdijk, N. A.; Penn, R. L.; Whitelam, S.; Joester, D.; Zhang, H.; Rimer, J. D.; Navrotsky, A.; Banfield, J. F., Crystallization by particle attachment in synthetic, biogenic, and geologic environments. *Science* **2015,** *349* (6247), aaa6760.

72.   Urban, M. W., *Stimuli-Responsive Materials: From Molecules to Nature Mimicking Materials Design*. Royal Society of Chemistry: 2016.

73.   Vignolini, S., Bio-inspired photonics: from nature to applications.

74.   Liu, F.; Urban, M. W., Recent advances and challenges in designing stimuli-responsive polymers. *Progress in Polymer Science* **2010,** *35* (1), 3-23.

75.   Kato, M.; Kamigaito, M.; Sawamoto, M.; Higashimura, T., Polymerization of methyl methacrylate with the carbon tetrachloride/dichlorotris-(triphenylphosphine) ruthenium (II)/methylaluminum bis (2, 6-di-tert-butylphenoxide) initiating system: possibility of living radical polymerization. *Macromolecules* **1995,** *28* (5), 1721-1723.

76.   Matyjaszewski, K., *Controlled/living radical polymerization: progress in ATRP, NMP, and RAFT*. ACS Publications: 2000.

77.   Lu, C.; Urban, M. W., One-Step Synthesis of Amphiphilic Ultrahigh Molecular Weight Block Copolymers by Surfactant-Free Heterogeneous Radical Polymerization. *ACS Macro Letters* **2015,** *4* (12), 1317-1320.

78.   Fan, C.; Plaxco, K. W.; Heeger, A. J., Electrochemical interrogation of conformational changes as a reagentless method for the sequence-specific detection of DNA. *Proceedings of the National Academy of Sciences* **2003,** *100* (16), 9134-9137.

79.   Yeo, W. S.; Mrksich, M., Self-Assembled Monolayers That Transduce Enzymatic Activities to Electrical Signals. *Angewandte Chemie International Edition* **2003,** *42* (27), 3121-3124.

80.   Misra, A.; Jarrett, W. L.; Urban, M. W., Fluoromethacrylate-containing colloidal dispersions: Phospholipid-assisted synthesis, particle morphology, and temperature-responsive stratification. *Macromolecules* **2007,** *40* (17), 6190-6198.

81.   Meng, F.; Sana, B.; Li, Y.; Liu, Y.; Lim, S.; Chen, X., Bioengineered tunable memristor based on protein nanocage. *Small* **2014,** *10* (2), 277-283.

82.   Zhang, C.; Wang, J.; Zhang, C.; Liu, J.; Xu, D.; Chen, L., Network stratification analysis for identifying function-specific network layers. *Molecular BioSystems* **2016,** *12* (4), 1232-1240.

83.   Curtis, A.; Wilkinson, C. In *New depths in cell behaviour: reactions of cells to nanotopography*, Biochemical Society Symposium, 1998; pp 15-26.

84.   Price, R.; Haberstroh, K.; Webster, T., Enhanced functions of osteoblasts on nanostructured surfaces of carbon and alumina. *Medical and Biological Engineering and Computing* **2003,** *41* (3), 372-375.

85.   Yang, Y.; Urban, M. W., Self-Healing of Glucose-Modified Polyurethane Networks Facilitated by Damage-Induced Primary Amines. *Polymer Chemistry* **2016**.

86.   Ghosh, B.; Urban, M. W., Self-repairing oxetane-substituted chitosan polyurethane networks. *Science* **2009,** *323* (5920), 1458-1460.

87.   Yang, Y.; Urban, M. W., Self-Repairable Polyurethane Networks by Atmospheric Carbon Dioxide and Water. *Angewandte Chemie International Edition* **2014,** *53* (45), 12142-12147.

88.   Pearson, H. A.; Sahukhal, G. S.; Elasri, M. O.; Urban, M. W., Phage-bacterium war on polymeric surfaces: can surface-anchored bacteriophages eliminate microbial infections? *Biomacromolecules* **2013,** *14* (5), 1257-1261.

89.   Townsend-Nicholson, A.; Jayasinghe, S. N., Cell electrospinning: a unique biotechnique for encapsulating living organisms for generating active biological microthreads/scaffolds. *Biomacromolecules* **2006,** *7* (12), 3364-3369.

90.   Cote, Y.; Fu, I. W.; Dobson, E. T.; Goldberger, J. E.; Nguyen, H. D.; Shen, J. K., Mechanism of the pH-controlled self-assembly of nanofibers from peptide amphiphiles. *The Journal of Physical Chemistry C* **2014,** *118* (29), 16272-16278.

91.   Wallace, J. A.; Shen, J. K., Continuous constant pH molecular dynamics in explicit solvent with pH-based replica exchange. *Journal of chemical theory and computation* **2011,** *7* (8), 2617-2629.

92.   Shi, C.; Wallace, J. A.; Shen, J. K., Thermodynamic coupling of protonation and conformational equilibria in proteins: theory and simulation. *Biophysical journal* **2012,** *102* (7), 1590-1597.

93.   Morrow, B. H.; Wang, Y.; Wallace, J. A.; Koenig, P. H.; Shen, J. K., Simulating pH titration of a single surfactant in ionic and nonionic surfactant micelles. *The Journal of Physical Chemistry B* **2011,** *115* (50), 14980-14990.

94.   Wallace, J. A.; Shen, J. K., Charge-leveling and proper treatment of long-range electrostatics in all-atom molecular dynamics at constant pH. *The Journal of chemical physics* **2012,** *137* (18), 184105.

95.   Fu, I. W.; Markegard, C. B.; Chu, B. K.; Nguyen, H. D., The Role of Electrostatics and Temperature on Morphological Transitions of Hydrogel Nanostructures Self-Assembled by Peptide Amphiphiles Via Molecular Dynamics Simulations. *Advanced healthcare materials* **2013,** *2* (10), 1388-1400.

96.   Fu, I. W.; Markegard, C. B.; Chu, B. K.; Nguyen, H. D., Role of hydrophobicity on self-assembly by peptide amphiphiles via molecular dynamics simulations. *Langmuir* **2014,** *30* (26), 7745-7754.

97.   Fu, I. W.; Markegard, C. B.; Nguyen, H. D., Solvent effects on kinetic mechanisms of self-assembly by peptide amphiphiles via molecular dynamics simulations. *Langmuir* **2014,** *31* (1), 315-324.

98.   Shukla, S.; Dorand, R. D.; Myers, J. T.; Woods, S. E.; Gulati, N. M.; Stewart, P. L.; Commandeur, U.; Huang, A. Y.; Steinmetz, N. F., Multiple Administrations of Viral Nanoparticles Alter in Vivo Behavior   Insights from Intravital Microscopy. *ACS Biomaterials Science & Engineering* **2016,** *2* (5), 829-837.

99.   Pitek, A. S.; Wen, A. M.; Shukla, S.; Steinmetz, N. F., The Protein Corona of Plant Virus Nanoparticles Influences their Dispersion Properties, Cellular Interactions, and In Vivo Fates. *Small* **2016**.

100.   Monticelli, L.; Kandasamy, S. K.; Periole, X.; Larson, R. G.; Tieleman, D. P.; Marrink, S.-J., The MARTINI coarse-grained force field: extension to proteins. *Journal of chemical theory and computation* **2008,** *4* (5), 819-834.

101.   Frederix, P. W.; Ulijn, R. V.; Hunt, N. T.; Tuttle, T., Virtual screening for dipeptide aggregation: toward predictive tools for peptide self-assembly. *The journal of physical chemistry letters* **2011,** *2* (19), 2380-2384.

102.   Frederix, P. W.; Scott, G. G.; Abul-Haija, Y. M.; Kalafatovic, D.; Pappas, C. G.; Javid, N.; Hunt, N. T.; Ulijn, R. V.; Tuttle, T., Exploring the sequence space for (tri-) peptide self-assembly to design and discover new hydrogels. *Nature chemistry* **2015,** *7* (1), 30-37.

103.   Jia, Z.; Chen, J., Necessity of high-resolution for coarse-grained modeling of flexible proteins. *Journal of computational chemistry* **2016**.

104.   Morriss-Andrews, A.; Shea, J.-E., Computational studies of protein aggregation: methods and applications. *Annual review of physical chemistry* **2015,** *66*, 643-666.

105.   Morriss-Andrews, A.; Shea, J.-E., Simulations of protein aggregation: insights from atomistic and coarse-grained models. *The journal of physical chemistry letters* **2014,** *5* (11), 1899-1908.

106.   Yang, Y.; Urban, M. W., Self-healing polymeric materials. *Chemical Society Reviews* **2013,** *42* (17), 7446-7467.

107.   Otts, D. B.; Zhang, P.; Urban, M. W., High fidelity surface chemical imaging at 1000 nm levels: internal reflection IR imaging (IRIRI) approach. *Langmuir* **2002,** *18* (17), 6473-6477.

108.   Urban, M. W., Depth profiling by photoacoustic spectroscopy. *Handbook of vibrational spectroscopy* **2002**.

109.   Zhang, P.; Urban, M. W., Photoacoustic FT-IR depth imaging of polymeric surfaces: overcoming IR diffraction limits. *Langmuir* **2004,** *20* (24), 10691-10699.

110.   Xu, M.; Wang, L. V., Photoacoustic imaging in biomedicine. *Review of scientific instruments* **2006,** *77* (4), 041101.

111.   Hartman, C. D.; Isenberg, B. C.; Chua, S. G.; Wong, J. Y., Vascular smooth muscle cell durotaxis depends on extracellular matrix composition. *Proceedings of the National Academy of Sciences* **2016,** *113* (40), 11190-11195.

112.   Gronau, G.; Krishnaji, S. T.; Kinahan, M. E.; Giesa, T.; Wong, J. Y.; Kaplan, D. L.; Buehler, M. J., A review of combined experimental and computational procedures for assessing biopolymer structure–process–property relationships. *Biomaterials* **2012,** *33* (33), 8240-8255.

113.   Plant, A. L.; Locascio, L. E.; May, W. E.; Gallagher, P. D., Improved reproducibility by assuring confidence in measurements in biomedical research. *Nat Meth* **2014,** *11* (9), 895-898.

114.   RicARdo, A.; Szostak, J. W., Origin of life on earth. *Scientific American* **2009,** *301* (3), 54-61.

115.   DeCamp, S. J.; Redner, G. S.; Baskaran, A.; Hagan, M. F.; Dogic, Z., Orientational order of motile defects in active nematics. *Nat Mater* **2015,** *14* (11), 1110-1115.

116.   Sanchez, T.; Chen, D. T. N.; DeCamp, S. J.; Heymann, M.; Dogic, Z., Spontaneous motion in hierarchically assembled active matter. *Nature* **2012,** *491* (7424), 431-434.

117.   Keber, F. C.; Loiseau, E.; Sanchez, T.; DeCamp, S. J.; Giomi, L.; Bowick, M. J.; Marchetti, M. C.; Dogic, Z.; Bausch, A. R., Topology and dynamics of active nematic vesicles. *Science* **2014,** *345* (6201), 1135-1139.

118.   Sanchez, T.; Welch, D.; Nicastro, D.; Dogic, Z., Cilia-Like Beating of Active Microtubule Bundles. *Science* **2011,** *333* (6041), 456-459.

119.   Szostak, J. W., Origins of life: Systems chemistry on early Earth. *Nature* **2009,** *459* (7244), 171-172.

120.   Webber, M. J.; Appel, E. A.; Meijer, E. W.; Langer, R., Supramolecular biomaterials. *Nat Mater* **2016,** *15* (1), 13-26.

121.   Rice, M. K.; Ruder, W. C., Creating biological nanomaterials using synthetic biology. *Science and Technology of Advanced Materials* **2013,** *15* (1), 014401.

122. Chen, A. Y.; Zhong, C.; Lu, T. K., Engineering Living Functional Materials. *ACS Synthetic Biology* **2015,** *4* (1), 8-11.

123. Qu, D.; Mosher, C. Z.; Boushell, M. K.; Lu, H. H., Engineering Complex Orthopaedic Tissues via Strategic Biomimicry. *Annals of biomedical engineering* **2015,** *43* (3), 697-717.

124. Tibbitt, M. W.; Rodell, C. B.; Burdick, J. A.; Anseth, K. S., Progress in material design for biomedical applications. *Proceedings of the National Academy of Sciences* **2015,** *112* (47), 14444-14451.

125. Haddad, J. B.; Obolensky, A. G.; Shinnick, P., The biologic effects and the therapeutic mechanism of action of electric and electromagnetic field stimulation on bone and cartilage: new findings and a review of earlier work. *The Journal of Alternative and Complementary Medicine* **2007,** *13* (5), 485-490.

126. Fine, E. G.; Valentini, R. F.; Bellamkonda, R.; Aebischer, P., Improved nerve regeneration through piezoelectric vinylidenefluoride-trifluoroethylene copolymer guidance channels. *Biomaterials* **1991,** *12* (8), 775-780.

127. Moreira, P. L.; An, Y. H.; Santos, A. R.; Genari, S. C., In vitro analysis of anionic collagen scaffolds for bone repair. *Journal of Biomedical Materials Research Part B: Applied Biomaterials* **2004,** *71B* (2), 229-237.

128. Bouaziz, A.; Richert, A.; Caprani, A., Vascular endothelial cell responses to different electrically charged poly(vinylidene fluoride) supports under static and oscillating flow conditions. *Biomaterials* **1997,** *18* (2), 107-112.

129. Lee, Y.-S.; Collins, G.; Livingston Arinzeh, T., Neurite extension of primary neurons on electrospun piezoelectric scaffolds. *Acta Biomaterialia* **2011,** *7* (11), 3877-3886.

130. Weber, N.; Lee, Y. S.; Shanmugasundaram, S.; Jaffe, M.; Arinzeh, T. L., Characterization and in vitro cytocompatibility of piezoelectric electrospun scaffolds. *Acta Biomaterialia* **2010,** *6* (9), 3550-3556.

131. Lee, Y.-S.; Arinzeh, T. L., The influence of piezoelectric scaffolds on neural differentiation of human neural stem/progenitor cells. *Tissue Engineering Part A* **2012,** *18* (19-20), 2063-2072.

132. Damaraju, S. M.; Wu, S.; Jaffe, M.; Arinzeh, T. L., Structural changes in PVDF fibers due to electrospinning and its effect on biological function. *Biomedical Materials* **2013,** *8* (4), 045007.

133. Wang, W.; Wang, Z.; Zhang, G.; Clark, C. C.; Brighton, C. T., Up-regulation of chondrocyte matrix genes and products by electric fields. *Clinical Orthopaedics and Related Research* **2004,** *427*, S163-S173.

134. Rajabi, A. H.; Jaffe, M.; Arinzeh, T. L., Piezoelectric materials for tissue regeneration: A review. *Acta Biomaterialia* **2015,** *24*, 12-23.

135. Gama, C. I.; Tully, S. E.; Sotogaku, N.; Clark, P. M.; Rawat, M.; Vaidehi, N.; Goddard, W. A.; Nishi, A.; Hsieh-Wilson, L. C., Sulfation patterns of glycosaminoglycans encode molecular recognition and activity. *Nat Chem Biol* **2006,** *2* (9), 467-473.

136. Dissanayake, N. M.; Current, K. M.; Obare, S. O., Mutagenic effects of Iron oxide nanoparticles on biological cells. *International journal of molecular sciences* **2015,** *16* (10), 23482-23516.

137. Nick, S. T.; Bolandi, A.; Samuels, T. A.; Obare, S. O., Advances in understanding the transformation of engineered nanoparticles in the environment. *Pure and Applied Chemistry* **2014,** *86* (7), 1129-1140.

138. Adams, C. P.; Walker, K. A.; Obare, S. O.; Docherty, K. M., Size-Dependent Antimicrobial Effects of Novel Palladium Nanoparticles. *PLOS ONE* **2014,** *9* (1), e85981.

139. Brown, A. N.; Smith, K.; Samuels, T. A.; Lu, J.; Obare, S. O.; Scott, M. E., Nanoparticles Functionalized with Ampicillin Destroy Multiple-Antibiotic-Resistant Isolates of Pseudomonas aeruginosa and Enterobacter aerogenes and Methicillin-Resistant Staphylococcus aureus. *Applied and Environmental Microbiology* **2012,** *78* (8), 2768-2774.

140. Rowley, J. A.; Mooney, D. J., Alginate type and RGD density control myoblast phenotype. *J Biomed Mater Res* **2002,** *60* (2), 217-23.

141. Goh, F.; Sambanis, A., In vivo noninvasive monitoring of dissolved oxygen concentration within an implanted tissue-engineered pancreatic construct. *Tissue Engineering Part C: Methods* **2011,** *17* (9), 887-894.

142. Chen, T.; Yuan, J.; Duncanson, S.; Hibert, M. L.; Kodish, B. C.; Mylavaganam, G.; Maker, M.; Li, H.; Sremac, M.; Santosuosso, M.; Forbes, B.; Kashiwagi, S.; Cao, J.; Lei, J.; Thomas, M.; Hartono, C.; Sachs, D.; Markmann, J.; Sambanis, A.; Poznansky, M. C., Alginate Encapsulant Incorporating CXCL12 Supports Long-Term Allo- and Xenoislet Transplantation Without Systemic Immune Suppression. *American Journal of Transplantation* **2015,** *15* (3), 618-627.

143. Duncanson, S.; Sambanis, A., Dual factor delivery of CXCL12 and Exendin-4 for improved survival and function of encapsulated beta cells under hypoxic conditions. *Biotechnology and Bioengineering* **2013,** *110* (8), 2292-2300.

144. Phelps, E. A.; Headen, D. M.; Taylor, W. R.; Thulé, P. M.; García, A. J., Vasculogenic bio-synthetic hydrogel for enhancement of pancreatic islet engraftment and function in type 1 diabetes. *Biomaterials* **2013,** *34* (19), 4602-4611.

145. Pedraza, E.; Coronel, M. M.; Fraker, C. A.; Ricordi, C.; Stabler, C. L., Preventing hypoxia-induced cell death in beta cells and islets via hydrolytically activated, oxygen-generating biomaterials. *Proceedings of the National Academy of Sciences* **2012,** *109* (11), 4245-4250.

146. Tiernan, A. R.; Sambanis, A., Bioluminescence tracking of alginate micro-encapsulated cell transplants. *Journal of Tissue Engineering and Regenerative Medicine* **2014**, n/a-n/a.

147. Tiernan, A. R.; Thulé, P. M.; Sambanis, A., Therapeutic Effects of a Non-Beta Cell Bioartificial Pancreas in Diabetic Mice. *Transplantation* **2014,** *98* (5), 507-513.

148. Constantinidis, I.; Grant, S. C.; Simpson, N. E.; Oca-Cossio, J. A.; Sweeney, C. A.; Mao, H.; Blackband, S. J.; Sambanis, A., Use of magnetic nanoparticles to monitor alginate-encapsulated betaTC-tet cells. *Magn Reson Med* **2009,** *61* (2), 282-90.

149.   Ahmad, H. F.; Sambanis, A., Cryopreservation effects on recombinant myoblasts encapsulated in adhesive alginate hydrogels. *Acta Biomaterialia* **2013,** *9* (6), 6814-6822.

150.   Ahmad, H. F.; Sambanis, A., Cryopreservation effects on recombinant myoblasts encapsulated in adhesive alginate hydrogels. *Acta Biomater* **2013,** *9* (6), 6814-22.

151.   Ahmad, H. F.; Simpson, N. E.; Lawson, A. N.; Sambanis, A., Cryopreservation effects on intermediary metabolism in a pancreatic substitute: a (13)C nuclear magnetic resonance study. *Tissue Eng Part A* **2012,** *18* (21-22), 2323-31.

152.   Song, Y. C.; Chen, Z. Z.; Mukherjee, N.; Lightfoot, F. G.; Taylor, M. J.; Brockbank, K. G.; Sambanis, A., Vitrification of tissue engineered pancreatic substitute. *Transplant Proc* **2005,** *37* (1), 253-5.

153.   Gama, C. L.; Tully, S. E.; Sotogaku, N.; Clark, P. M.; Rawat, M.; Vaidehi, N., Sulfation patterns of glycosaminoglycans encode molecular recognition and activity. *Nature Chemical Biology* **2006,** *2* (9), 467-473.

154.   Forsten-Williams, K.; Chu, C. L.; Fannon, M.; Buczek-Thomas, J. A.; Nugent, M. A., Control of growth factor networks by heparan sulfate proteoglycans. *Annals of Biomedical Engineering* **2008,** *36* (12), 2134-2148.

155.   Lin, F.; Yu, J.; Tang, W.; Zheng, J.; Defante, A.; Guo, K.; Wesdemiotis, C.; Becker, M. L., Peptide-Functionalized Oxime Hydrogels with Tunable Mechanical Properties and Gelation Behavior. *Biomacromolecules* **2013,** *14* (10), 3749-3758.

156.   Ovsianikov, A.; Mühleder, S.; Torgersen, J.; Li, Z.; Qin, X.-H.; Van Vlierberghe, S.; Dubruel, P.; Holnthoner, W.; Redl, H.; Liska, R.; Stampfl, J., Laser Photofabrication of Cell-Containing Hydrogel Constructs. *Langmuir* **2014,** *30* (13), 3787-3794.

157.   Pal, T., Fundamentals and history of implant dentistry. *Journal of the International Clinical Dental Research Organization* **2015,** *7* (3), 6-12.

158.   Elkarargy, A., Biological Functionalization of Dental Implants with Fibronectin: A Scanning Electron Microscopic Study. *International Journal of Health Sciences* **2014,** *8* (1), 57-66.

159.   Baba, A.; Kaneko, F.; Advincula, R.; Knoll, W., Electrochemical Surface Plasmon Resonance Methods for Polymer Thin Films. In *Functional Polymer Films*, Wiley-VCH Verlag GmbH & Co. KGaA: 2011; pp 723-744.

160.   Giliberti, V.; Baldassarre, L.; Rosa, A.; de Turris, V.; Ortolani, M.; Calvani, P.; Nucara, A., Protein clustering in chemically stressed HeLa cells studied by infrared nanospectroscopy. *Nanoscale* **2016,** *8* (40), 17560-17567.