Progress in material design for biomedical applications

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Biomaterials that interface with biological systems are used to deliver drugs safely and efficiently; to prevent, detect, and treat disease; to assist the body as it heals; and to engineer functional tissues outside of the body for organ replacement. The field has evolved beyond selecting materials that were originally designed for other applications with a primary focus on properties that enabled restoration of function and mitigation of acute pathology. Biomaterials are now designed rationally with controlled structure and dynamic functionality to integrate with biological complexity and perform tailored, high-level functions in the body. The transition has been from permissive to promoting biomaterials that are no longer bioinert but bioactive. This perspective surveys recent developments in the field of polymeric and soft biomaterials with a specific emphasis on advances in nano- to macroscale control, static to dynamic functionality, and biocomplex materials.

biomaterials | soft materials | feature control | dynamics | biocomplex

Biomaterials have been used to augment tissue function and treat diseases or injuries for thousands of years—whether selecting coral or wood for dental implants or fabric for sutures, implant materials historically originated by evaluating potential materials in our surroundings that could be used for a specific biomedical application. Many times, this selection process simply involved consideration of the mechanical properties of the material to restore basic function at the implant site; typically, the materials themselves were never originally designed to interface with living tissues. Today, this is no longer the case, as we now have an advanced toolbox of synthetic and processing techniques to rationally create, design, and process materials with specific properties in mind. These advancements have come hand in hand with the integration of theory with experiments, materials chemistry and biology with engineering, and basic science with application. As highlighted by the announcement of the Materials Genome Initiative (1), biomaterial science is often the stealth technology that enables breakthroughs in medical devices that improve health care and save lives.

In fact, the last few decades of research have led to the emergence of numerous biomaterial options, along with an increasing sophistication in the ability to tune and manipulate complex physical and biological properties. Such advances in biomaterial science have not only driven and enabled new medical products, but have served as new tools for investigation of important biological questions. The modern biomaterial evolution initiated

with the design of materials—including hard materials like metals and ceramics—that focused on outcomes such as mechanical properties and biocompatibility. This approach led to the clinical implementation of numerous materials for biomedical applications, such as joint replacement, pacemakers, and orthodontics. The contemporary age of biomaterials has advanced with a further focus on surface functionality, where materials are now smarter and interface with their environment such as by incorporating bioactive signals to achieve multifunctional design. These strategies are leading to progress and improvements in fields ranging from medical devices, to drug delivery, and to regenerative medicine.

As one example, vascular stents have been widely used to open blocked vessels and restore blood flow to ischemic tissues, and the design of these stents has significantly evolved with time. With the development of Nitinol, a metal alloy of nickel and titanium with unique shape memory and superelastic properties, stent design has improved to be implanted with simpler, minimally-invasive procedures and to maintain function for longer periods of time. Next-generation stents transitioned from passive mechanical devices to those that actively regulate the biological interface by integrating biodegradable polymer coatings that locally elute drugs to limit restenosis and resulting stent failure. These advances enhanced both the functionality and efficacy of stent technology for clinical use. Similarly, the coating of traditional metal orthopedic implants with bioactive ceramics

improved clinical outcomes by facilitating osseointegration with bony tissue, and after the discovery of bone morphogenetic proteins and their recombinant production, spinal fusion surgeries benefited from material delivery systems that enabled their local presentation (e.g., INFUSE). Collectively, these examples demonstrate how material design can be used to present biological signals that result in new medical devices and implants with superior clinical performance. In fact, a recent report estimated the 2012 global biomaterial market at $44.0 billion and forecasted a 15% compounded annual growth rate between 2012 and 2017, reaching $88.4 billion by 2017 (2).

This perspective focuses primarily on recent developments in polymers and soft materials, due to the large technological growth in these systems since the 1990s. This review is organized to highlight some of the major advances and modern thinking in biomaterial design, such as the ability to manipulate and control biomaterial properties at multiple length scales, introduce dynamic behavior into biomaterials, and capture biocomplexity and additive functionalities.

Although some examples address surface modifications of biomaterials to promote integration, many of the advances that are discussed focus on bulk modification of materials and especially how this influences the stability and function of encapsulated molecules and cells. We then conclude with a forward-looking perspective about the current challenges and future directions for designing the next generation of biomaterials.

From Molecular to Macroscopic

Biomaterials fabrication has evolved across all size scales—from molecular to macroscopic—to impart biochemical and biophysical cues into cell culture platforms for regenerative medicine, to achieve optimal outcomes in drug delivery systems, and to improve in vivo success of medical implants. Our increased understanding of native tissue architecture and cell–material interactions, as well as the development of processing methods and chemical syntheses has driven the design of new materials. This section will highlight advances that have been made in the development of a toolbox of synthetic approaches and fabrication techniques that afford defined structures over a range of biologically relevant length scales.

From Molecular Organization to Nano-structure. An increased understanding of biological structures, with a focus on their biochemical composition and organization, has provided insight into the manner by which molecular structure and chemistry impart properties into biological systems. Covalent bonds endow stability (e.g., peptide bonds), whereas secondary structures confer material resilience (e.g., resilin, elastin). Peptide synthesis recombinant protein production and evolution via phage display have become invaluable tools to recapitulate similar functionalities in synthetic biomaterial analogs. Likewise, synthetic approaches (e.g., bio-orthogonal chemistry) have evolved to enable the fabrication and functionalization of biomaterials (e.g., hydrogels) that capture aspects of native biological structures (3). Collectively, these techniques have allowed the production of biomaterials with unique capacities, including postmodification of cell culture matrices and to cross-link implantable materials.

Covalent chemistries have dominated the biomaterials field since its conception. However, the emergence of supramolecular chemistry has begun to enhance our understanding of biology and capacity for creating precise, physiologically structured materials. Nobel Laureate Jean-Marie Lehn insightfully described supramolecular interactions as "chemistry beyond the molecule," (4) because they enable dynamic macromolecular interactions, as well as the self-organization necessary to form higher-order structure in proteins and tissues (5). In the body, supramolecular presentation of bio-signals is exemplified by native extracellular matrix (ECM) interactions, including receptor–ECM interactions and heparin-binding proteins. As such, biomolecule presentation through supramolecular interactions has emerged as a means of controllable delivery (6), including through cyclodextrin-mediated sequestration of small molecules (7) or biomimetic electrostatic protein-matrix interactions (8). Beyond the capacity for single molecule–matrix interactions, the general ECM structure itself is largely the result of self-assembly (e.g., fibrillar structure of collagen) and can be recapitulated, in part, by well-designed synthetic analogs. These higher-order motifs are exemplified by self-assembling nanostructures from peptide amphiphiles (9, 10) (Fig. 1A), although many alternative means of biologically inspired supramolecular materials have been explored, and their implications toward cell behavior were recently reviewed (11). In addition to such methods of self-assembly, nanoparticulate-hydrogel composites are an emergent means of introducing a wide array of functional behaviors (e.g., toughness and thermal or electrical conductivity). The development and use of such nanostructured, functional composites has likewise been a topic of recent review (12).

Building at the Mesoscale. Although self-assembly processes based on molecular design have achieved vast success in recapitulating certain aspects of the biological nanostructure, they face notable challenges. Among these are relative homogeneity at larger scales (resulting from thermodynamically controlled assembly) and physiologically low mechanical properties (owing to the underlying weak intermolecular forces). To address these aspects at the nano- and mesoscale, more active processing methods have been used to impart defined structure. Notably, electrospinning (Fig. 1B) of naturally derived or synthetic materials has become a dominant technique to mimic the nanofibrous nature of ECM (13). The functional importance of such microstructural organization cannot be discounted, as it enables mechanical anisotropy (14) and therefore holds great promise for formation of biomedical implants including vascular grafts (15) and orthopedic connective tissues (16). Toward formation of porous architectures, other processes such as phase separation, leaching, and directional freezing have also emerged as versatile methods to process biomaterials that permit cell and tissue infiltration (17, 18).

The aforementioned methods allow realization of bicontinuous structures at the nano- and microscale, yet they often display limited capacity toward generating complex topographical, mechanical, or biomolecular presentation. For modulation of these aspects, postprocessing of larger scaffolds, such as by light-mediated reactions (Fig. 1C), has become instrumental toward spatiotemporal control of biochemical signals on hydrogel surfaces or within 3D hydrogels through either single photon (19) or multiphoton
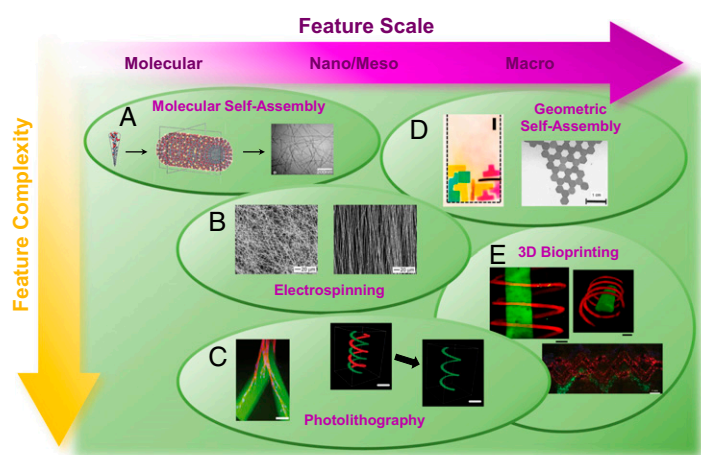
Fig. 1. The toolbox of biomaterials processing techniques that enable formation of highly controlled structures with biochemical and biomechanical features that vary across many size scales, as well as levels of complexity. These techniques include nanoscale molecular self-assembly, electrospinning, photolithography, geometric self-assembly, and 3D bioprinting. Images reproduced from refs. (A, *Left* and *Center*) 10; (A, *Right*) 9, with permission from AAAS; (B) 112; (C, *Left*) 113; (C, *Center* and *Right*) 114; (D, *Left*) 29, with permission from Macmillan Publishers LTD: *Nature Communications*; (D, *Right*) 27, with permission from AAAS; (E, *Top Left* and *Right*) 32; and (E, *Bottom*) 31.

(20) irradiation methods. Building on these advances, selective photopolymerization (21), addition reactions (22), and degradation mechanisms (23) have enabled extension of photopatterning methodologies toward 2D and 3D presentation of spatially or temporally varying mechanical properties.

Macroscopic Materials and Their Subassemblies. Ultimately, material design for biomedical applications must achieve the capacity for preparation at the tissue scale with both structure and mechanical properties suitable for in vivo implantation, preferably with necessary tissue interfacing to achieve functionality. Methods employed in industrial processes, such as injection molding, have been used to achieve macrostructure control in biomaterials. These approaches have enabled recreation of complex structures at the macroscale with utility toward application in craniofacial (24) and meniscal (25) implants. In some cases, the biological interaction with these materials has been mediated by biomolecule presentation within the scaffold, such as sequestration of heparin and, correspondingly, endogenous BMP-2 to enhance bone formation (26).

Toward their utility in tissue engineering applications, material assemblies often require advanced structural flexibility to recapitulate the inhomogeneity of tissue structures (e.g., spatial presentation of cell and matrix components). To achieve this, appropriate molecular-, nano-, and meso-scale signals may be engineered into macroscale structures through either modification of bulk hydrogels (top-down) or directed component assembly (bottom-up) approaches. A powerful means of achieving controlled signal presentation within a homogenous scaffold is photolithography (vide supra), which embodies the top-down methodology.

Alternatively, two primary means of bottom-up approaches have emerged to create tissue-scale structures with nonhomogenous cell and material compositions. First, pioneering work by the Whitesides group (27) has demonstrated means by which materials may be precast into microgel components with the desired composition and allowed to passively self-assemble (Fig. 1D) through hydrophobic or capillary forces (28). Owing to the thermodynamic control of assembly in these systems, repeatable geometric structures may be achieved over large length scales. Toward increasing the complexity of allowed structures, such tools as field-driven input have also been used (29). As an alternative to this self-assembled approach, techniques like 3D printing (Fig. 1E)—the direct spatially controlled deposition of materials,

with or without included cells or signals—have emerged to introduce material structure at the macroscale. Within only the last few years, these methods have been extended to include processes, such as sacrificial printing to enable perfusion and viability within a secondarily cast hydrogel (30), layer-by-layer printing of pluronics or other thermogels (31), and methods to directly write complex structures in 3D (32). Looking forward, it is expected that further inclusion of smaller scale subassemblies, such as nanostructured materials and microscale patterning, will aid in furthering success of these approaches.

From Static to Dynamic

Beyond control of material structure from the molecular to the macroscale, biomaterials are also evolving from a traditional, pre-engineered static design to those that have dynamic properties. Historically, biomaterials were intended to provide consistent functions, such as mechanical support (e.g., orthopedic implants) or optical properties (e.g., contact lenses). This approach has led to the successful design of numerous clinically used biomaterials; yet, advances in material design and polymer chemistry have recently allowed us to incorporate dynamic features into biomaterials. This approach ranges from the design of materials that are degradable, to eliminate permanent implantation or a second surgery for implant retrieval, to those that have stimuli-responsive properties, where various chemical and biological signals can trigger changes in biomaterial properties or release drugs on-demand.

Incorporating Degradation into Biomaterials. Biodegradable materials are those that transition from an initial, stable structure into soluble products that can be resorbed and processed by the body. Examples of such system have been around for numerous years, with biodegradable sutures perhaps being the most common (33). Original resorbable sutures consisted of materials such as catgut that degraded via inherent biological mechanisms, but these were later engineered from synthetic and hydrolytically degradable polymers [e.g., poly(α-hydroxy esters)]. Other examples of biodegradable materials used in the clinic include biodegradable films that limit undesired adhesions after surgical procedures and degradable fixation devices (e.g., screws and plates) in orthopedics (34). Important considerations in the design of biodegradable materials are the rate of degradation and ensuring that the degradation products are nontoxic when released.

Biodegradable materials have been applied widely to biomedical applications to provide

temporal control over material presentation, including toward the engineering of tissues or the release of drugs and growth factors (35). For tissue engineering, the material may temporarily provide a 3D structure or "scaffold" for the growing tissue, whereas degradable materials for drug delivery are engineered to protect and then release molecules at desired rates. Hydrogels are one such class of biomaterials that have been designed with degradable linkers, for example, through the introduction of hydrolytically or enzymatically cleavable bonds into the cross-links. Degradable hydrogels have been synthesized from a range of materials, including synthetic polymers [e.g., poly(ethylene glycol) (36), poly(vinyl alcohol) (37), and poly(propylene fumarates) (38)] and biologically derived polymers [e.g., hyaluronic acid (39)]. Toward tissue engineering or wound healing applications, it is important that the hydrogel remains intact long enough for delivered or recruited cells to secrete their extracellular matrix but not persist too long as to impede tissue formation. For example, hydrogels have been optimized for cartilage tissue engineering by tuning the degradation rate to control matrix production and distribution by encapsulated chondrocytes (40). Likewise, for delivery of entrapped biomolecules, hydrogel degradation is primarily used to alter the diffusion and kinetics of molecule release, which subsequently controls their spatiotemporal presentation to local cells and tissues (41). Often times, these biological signals are designed to act as morphogens and influence tissue formation and healing (42).

As a complement to hydrolysis, which often occurs at pre-engineered rates throughout the bulk of a material, biomaterials have also been engineered to degrade via proteases (Fig. 2A), more similar to how tissues are remodeled in the body. In pioneering studies by Hubbell and colleagues, peptides were incorporated into hydrogel cross-links that cleave through cell-produced proteases (43, 44), such as matrix metalloproteinases (MMPs), elastases, and plasmin (45, 46). With this protease-mediated degradation and the addition of cell adhesive signals, these biomimetic hydrogels were remodeled by cells (47) and could be tuned for specific applications, such as the regeneration of bone and vascular structures (43, 48, 49). In some examples, only growth factors were embedded into the matrices, and their release occurred in a "cell-demanded" fashion (50). This approach can also be harnessed to control the delivery of molecules to treat diseases where protease activity is altered, such as rheumatoid arthritis (51), cancer (52), and after myocardial infarction
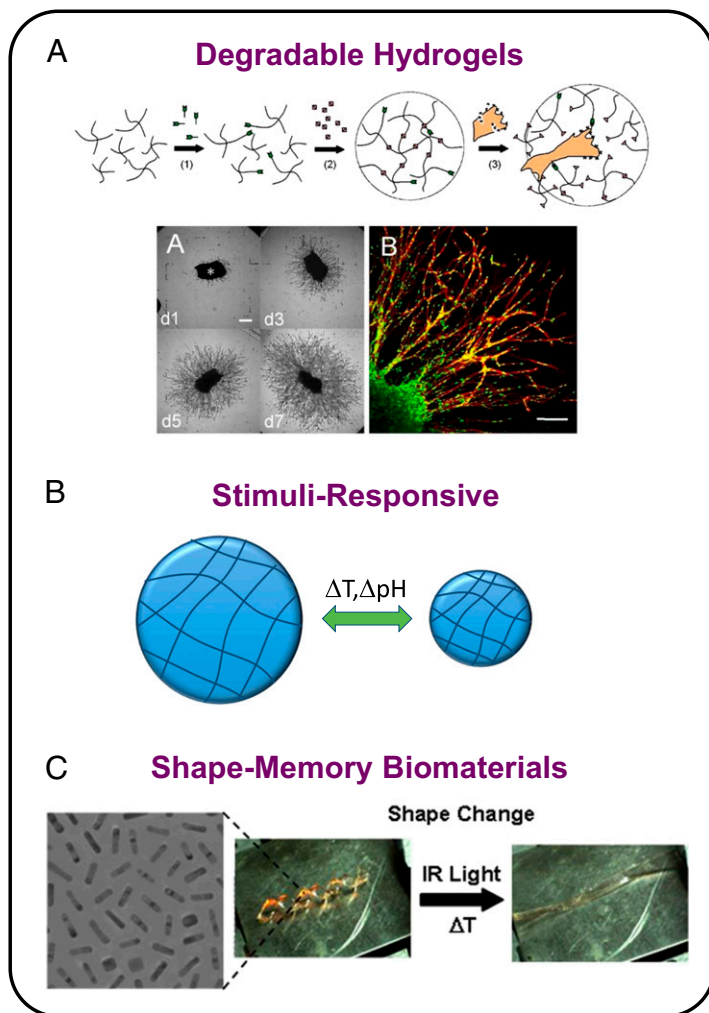
Fig. 2. Dynamic biomaterials that are based on polymer degradation such as through (*A*) protease sensitive peptide crosslinkers to enable cell invasion, (*B*) stimuli-responsive properties (e.g., local changes in temperature or pH) for the release of therapeutics, or (*C*) temperature induced shape-memory changes such as shown where near-infrared light heats embedded gold nano rods to trigger change from temporary coiled shape to permanent uncoiled shape. Images reproduced from refs. (*A*) 115 and (*C*) 116.

(53); here, the drug release rate and dose are controlled through a feedback mechanism (i.e., elevated protease activity releases more drug more quickly). The application of biologically controlled, adaptive degradation has been reviewed with regard to hydrogels and corresponding cell material response and drug release (54, 55).

Although these examples have focused on hydrogels, there are many other instances where degradation is used to control the dynamic properties of biomaterials. As one highlight, drug delivery reservoirs have been incorporated into synthetic devices, where they are covered by a thin biomaterial film [e.g., poly(lactic-coglycolic acid) (PLGA)] (56). Subsequent drug release is mediated through degradation of the films, where the timing is dependent on the biodegradable polymer design. Release profiles can be pulsatile, and efficacy has been shown for the delivery of chemotherapeutics from device for targeting tumors (57). Furthermore, stents have been designed to incorporate various drugs through biodegradable coatings and reservoirs (vide supra), where drugs (e.g., paclitaxel) are passively released through polymer degradation at predetermined concentrations and rates to locally influence tissue response (e.g., suppress unwanted scarring or restenosis) (58).

Stimuli-Responsive Biomaterials. Beyond degradation, biomaterials have been designed to respond to a range of environmental stimuli that may involve signals such as changes in temperature, ionic strength, light exposure, mechanical stress, magnetic fields, or pH (59). These stimuli may initiate from the local biomaterial environment (e.g., after implantation) or be introduced as an external "trigger" (i.e., active systems) (Fig. 2*B*). Biologically responsive mechanisms include enzyme catalysis (60), competitive ligand-receptor binding (61), and nanometer-scale protein motions (62), where material properties and therapeutic release are altered based on the biological environment. Important examples in this area are the release of insulin in response to glucose catalysis (63) or biochemically triggered growth factor release (64, 65), where the disease stimuli controls drug delivery. Materials are also designed so that the presence of specific proteins can disassemble nanoparticles, opening up disease-triggered therapeutics and diagnostics (66). Hydrogels with pH responsive swelling changes provide advantages for the oral delivery of therapeutics, where biomaterials are stable in the stomach and then release drugs in the intestines (67).

As active systems, biomaterials are being designed with dynamic properties that introduce temporal signals to cells, toward the engineering of tissues, the expansion of stem cells, or to understand complex cellular processes. One common dynamic hydrogel system includes those fabricated from poly (*N*-isopropyl acrylamide), which transition from a swollen to a collapsed hydrogel when processed through its lower critical solution temperature (LCST). Changes in volume, mechanics, and optical transparency occur when the material transitions through its LCST, and these changes have been exploited to release cells and cell sheets for tissue engineering (68). Another stimulus of particular interest is light, due to the allowed spatiotemporal control. Anseth and colleagues introduced light as a trigger for the cleavage of crosslinks (e.g., containing *o*-nitro benzyl groups) in hydrogels (23) for the release of tethered signals or to probe how dynamic mechanical properties influence the phenotype of valvular interstitial cells (69). Light has also been used to stiffen materials, where light introduces new cross-links that can alter material mechanics at a user-defined time (70). Beyond light, dynamic properties may be introduced in ionically cross-linked gels, by the addition of multivalent cations (71) or in physically associated DNA-based gels through the introduction of cDNA (72). All of these systems have been used to probe cell behavior in response to dynamic environments.

Actively controlling biomaterials once implanted in the body is more challenging, particularly to introduce the stimuli to materials that are implanted deep within tissues. Light penetration can be attenuated at many depths and wavelengths; however, there are numerous examples where light has been used to either form materials (73) or alter

their properties when implanted (74). Ultrasound is another trigger that can be introduced to disrupt polymer structure and release therapeutics (75, 76). As described with the biodegradable reservoirs above, a similar system has been developed with electrochemically activated microchips with release through the dissolution of a gold membrane (77). Although this system is nonpolymeric, it constitutes an important example of stimuli-responsive properties for controlled release in implanted materials.

As a subset of responsive biomaterials, shape-memory materials exhibit changes in geometry based on triggers such as temperature or light (78, 79). In brief, these materials are fixed into a temporary shape (usually under stress) and then transition into a relaxed permanent shape following an external or environmental trigger (Fig. 2C). Such a dynamic process may lead to the next generation of minimally invasive implantable constructs, capable of altering their geometry once implanted (80). As mentioned above, shape memory alloys (e.g., Nitinol) were developed many years ago and have found commercial application, but the last few decades have led to an increase in the number of polymeric systems available for biomedical applications, along with those that under multiple transitions allow for sequential geometric changes. These polymers can be processed from a range of covalently and physically cross-linked polymers and copolymers, including from biodegradable polymers (78, 79), and have the potential to be designed for degradation, elution of drugs, or even signaling to local cells for improved wound healing.

From Bioinert to Biocomplex

Building on advances in dynamic and responsive biomaterials, another recent direction in soft biomaterials is the design of systems that engage with, respond to, and integrate into the biological landscape. Such systems extend beyond passive biological function (bioinert), and researchers seek to engineer materials that actively interface with biologically complex environments (biocomplex). Discoveries in the biological sciences have revealed how information is processed and exchanged in the body, exposing new routes toward engineering material–tissue interactions. For example, the language of the genetic code presents novel therapeutics; the critical role of the ECM informs tissue engineering and regenerative medicine; the genetic basis of many diseases (e.g., cancer, Marfan syndrome) transforms the way patients are treated; the communication networks of the immune system inform

vaccinations and cancer therapy; and the discovery of the microbiome restructures the way we think about bacteria.

Toward fueling advances in medicine, these basic scientific discoveries are essential in the design of future biomaterials. For example, biocomplex materials have the potential to perceive malignant dysfunction and respond by releasing therapeutics to restore homeostasis; alternative systems could mimic critical aspects of the ECM to direct tissue morphogenesis ex vivo. Often, the biggest challenge is reducing the biological complexity into essential elements (e.g., rate limiting steps, critical signaling factors) that enable a synthetic material to perform a desired task. In this manner, biomaterials scientists are leveraging biologic understanding to design materials that are structurally simple, yet functionally complex to communicate with, react to, and synergize with biology to address clinical needs. This section articulates the concept of biocomplex materials through the following examples.

Materials to Deliver Therapeutics: Manipulating Cells from the Inside. Bioinert micro- and nanocarriers that achieve long circulation times in the blood have transformed parenteral administration of small-molecule drugs (81, 82). Potent macromolecular biotherapeutics (e.g., antibodies, recombinant proteins, and nucleic acids) have been identified that treat a variety of diseases at the molecular scale; however, because of their nature, they present unique challenges for delivery. For example, translation of these therapeutics requires carriers that not only circulate for extended periods of time, but also shield the sensitive molecular cargo from degradation in the bloodstream, target specific cells or tissues, and release cargo at the appropriate site of action. Additionally, the design of biocomplex nanocarriers that address these challenges must be balanced by the need for structural simplicity that enables reproducible manufacturing.

Some of the most clinically relevant biotherapeutics, whose efficacy hinges on the design of biocomplex delivery systems, are nucleic acids (NAs). NA-based therapies, such as RNA interference [e.g., microRNA (miRNA) or short interfering RNA (siRNA)] draw inspiration from native mechanisms and regulation in the transcription and translation of genetic material into protein. RNA interference is a native avenue for posttranscriptional silencing of gene expression, whereby miRNA or siRNA selectively prevent protein synthesis (83). In addition, exogenous messenger RNA (mRNA) can induce the production of specific proteins to

up-regulate protein expression (84). Because NA-based therapies alter intracellular machinery, their efficiency relies on cytoplasmic delivery. Moreover, these biomolecules are particularly sensitive to in vivo conditions, exhibiting very short half-lives before biochemical decomposition is observed. Therefore, successful translation requires a delivery vehicle that offers protection from clearance or nuclease degradation, site-specific targeting, passage across the cellular membrane, and endosomal or lysosomal escape (85, 86).

Toward this end, biocomplex polymeric and lipid-based nanoparticle formulations have been developed to deliver NAs intravenously, some of which have progressed to clinical trials (Fig. 3A) (87). Specific advances have focused on stable nucleic acid lipid particles (SNALPs) and ionizable lipids that package NAs and increase transport across the cell membrane (85, 86). The majority of NA nanocarriers unintentionally accumulate in the liver, and to overcome this issue, lipid structures have been recently developed that allow for selective passive targeting of heart, lung, and vascular endothelial tissues (88). Targeting to tumor cells has been achieved by functionalizing the surface of delivery vehicles with ligands that bind specifically to proteins that are selectively expressed on the surface of tumor cells (89). A major challenge that remains in the clinical use of NA therapeutics is their escape from endosomes or lysosomes into the cytoplasm. Sahay et al. identified NPC1 as a critical protein in the trafficking of lipid nanoparticles that can be exploited in the design of materials that better escape the endosome (90).

Materials to Present Matrix Cues: Signaling Cells from the Outside. The fields of tissue engineering and regenerative medicine rely on the proliferation and maintenance of human cells outside of the body. Seminal culture scaffolds have been designed to permit cell survival and proliferation but are inherently passive. Although these bioinert scaffolds provided a route to maintain and culture cells, recent improvements in scaffold design integrate biofunctional aspects of the native signaling landscape (91). In vivo, cells integrate a complex array of signals from the extracellular environment that synergize with the genetic code to instruct cell function, such as proliferation, phenotype, and differentiation. As discussed, ECM cues include both biophysical and biochemical signals that vary on multiple length and time scales. Toward investigating these cell–material interactions, the development of high-throughput screening methodologies is
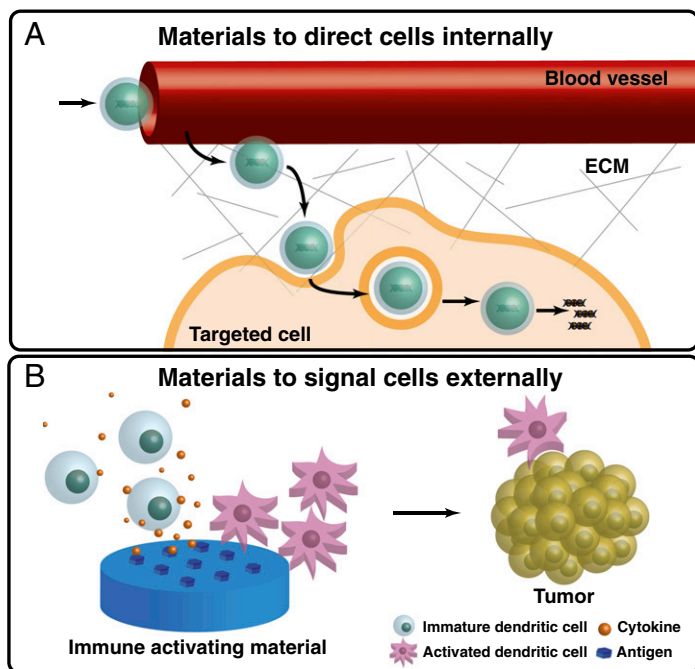
Fig. 3. Biocomplex biomaterials interact with and direct cells both internally and externally. For example, advanced drug delivery vehicles (*A*) introduce exogenous nucleic acid content to up- or down-regulate protein expression by transporting sensitive biomolecules through the circulation, actively targeting specific cells, and releasing the therapeutics into the cytoplasm (86). In addition, biocomplex materials are designed to present external signals to cells (*B*), either those that are delivered with the matrix or those that are recruited exogenously. These biomaterial niches can be loaded with multiple cues, presented in concert or sequentially, to communicate with, recruit or signal to cells locally. For example, immune activating materials cooperate with native biological signaling to recruit naïve immune cells to a site in the body, activate them with target antigens, and equip them to target specific cells or tissues, such as malignant tumor cells (100).

essential, as it enables the study of such aspects as single-cell processes and rapid creation of complex microenvironments through microfabrication (92, 93). Additional signals are introduced by neighboring cells via cell-cell contacts or secreted cytokines and growth factors. Biocomplex 3D culture matrices seek to recapitulate critical ECM cues and cell–cell signaling events through spatiotemporal control over matrix mechanics and ligand presentation (94). Toward this goal, both static and temporally controlled presentation of adhesive ligands has been exploited to bias chondrogenic differentiation of hMSCs (23, 95). In addition, dynamic control over substrate modulus has been leveraged to reveal a mechanism of "mechanical memory," bias differentiation of mesenchymal stem cells, and mature cardiomyocytes (21, 96–98). To better understand these complex multiscale signaling processes, approaches such as "organs-on-a-chip" have emerged and become a means of rapidly studying processes such as tissue growth and drug development (99); however, these fabrication methods have largely relied on a limited set of materials used for soft lithography and have yet to leverage biocomplex materials to their full potential.

In another approach to communicate with cells, researchers have developed biocomplex materials that exploit the language of the immune system to treat and detect disease (Fig. 3*B*) (100). These strategies use an understanding of how the immune systems senses a foreign substance, arms itself for attack, and carries out the attack. Mooney and coworkers demonstrated implantable devices that interact with the immune system to suppress tumor growth (100); specifically, chemotactic factors were released locally to recruit dendritic cells, which were then activated by local presentation of tumor antigens, which then instructed the immune system to target cancer cells. Further, a suite of synthetic vaccines have been developed similarly that communicate with immune cells to increase immunogenicity and, ultimately, vaccine efficacy (101).

Materials for Tissue Integration: Coordinating a Series of Multicellular Events. Beyond intracellular and extracellular signaling, biocomplex materials have been designed to orchestrate multicellular events. For example, Miller et al. used 3D printing of sacrificial sugar networks embedded within hydrogels to fabricate vascularized

neo-tissues (30). Culver et al. used laser writing of adhesive peptides to instruct multicellular organization for the fabrication of vascular networks within hydrogels (102). Further, gradient biomaterials that present biochemical ligands in a spatially defined fashion have been used to recapitulate osteochondral and osteotendinous interfaces (103, 104).

Despite these in vitro advances, a major hurdle in the clinical utility of implantable biomaterials (including joint replacements, smart drug delivery materials, and cell carriers) is nonspecific protein adsorption and the accompanying foreign body response (FBR) (105). No implanted material is truly bioinert; proteins rapidly adsorb to the surface of biomaterials in the body with random orientations and configurations (105, 106). Early, this proteinaceous layer facilitates neutrophil adhesion and activation (105, 106). With time, macrophages fuse to form foreign body giant cells that attack the implant surface while recruiting fibroblasts, which deposit ECM and form a dense, fibrotic capsule that isolates the implant from the surrounding tissue (107). As a more clear picture of implant rejection has developed, the community has begun to present an array of biocomplex materials that mitigate the FBR and assist integration with resident tissue. For example, Jiang and colleagues developed Zwitterionic hydrogels that demonstrate ultra-low protein fouling (108), and the surface chemistry and nanotopography of implant surfaces have been designed to limit macrophage activation (105). Ratner and colleagues further showed that implant porosity can be exploited to tune the FBR and tissue integration (109). Although these advances demonstrate that biocomplex materials can assist in the organization of multiscale tissues, clinical translation remains hindered by an incomplete understanding of which critical signals to present and integrate within biocomplex scaffolds.

Moving into the Future
There are thousands of different types of medical devices, diagnostic kits, and pharmaceutical formulations that exist today as a result of advances in biomaterial science and engineering. The polymers and soft biomaterials used are diverse in their origin, classification, and properties, and many products integrate multiple components that are carefully selected for their performance and function. However, as we look toward the future, the design of soft biomaterials is unifying around concepts that include hierarchy, complexity, dynamics and adaptation,

and healing (110). To realize this potential, better experimental methods and modeling tools are needed so that we can understand how to synthesize and engineer advanced biomaterials systems. Although modern chemistry allows the synthesis of polymers with controlled molecular weights, defined sequences, and integrated biological functionality, biomaterial systems depend on how these structural elements assemble and interact at complex biological interfaces, as this hierarchy ultimately dictates performance and function.

The biomaterials that are native to our body (e.g., the ECM) are profound in their ability to remodel, adapt, and store and retrieve information; this is critical during processes such as development, growth, and wound healing. Although biomaterials do not need to mimic all aspects of the complexities of a living organism (111), understanding the fundamentals of these processes unlocks future opportunities in rational design of biomaterials. Contemporary research topics include the development of healable materials, drug delivery systems that interact with and deliver their contents in response to signals from cells, active materials that promote healing of tissues that could not otherwise occur, medical devices that integrate seamlessly with tissues at the implant site, and stealth nanosystems that serve as sentinels to monitor and treat disease. Many of these developments occur and will continue to revolve around multidisciplinary institutes and environments that eliminate barriers and bring together chemists, biologists, engineers, and clinicians that can bridge the academic-industrial divide and engage researchers on a global scale.

Key to all of these advances will be synthetic tools that allow control of biomaterials from the molecular to the macro, for patterning and dynamically revealing biological functionalities, and for engineering biocomplex materials with enhanced properties, desired stability, and loaded with the biological signals delivered in the right context, locality, and time. Importantly, all of this must be achieved in a manner that allows manufacturing at large scales and overcomes any regulatory challenges with the translation of new materials. Biological complexity demands better tools to characterize changes in material properties in situ, from molecular level features of degradation to structural changes and functional properties. The body is a dynamic environment, so biomaterials constantly experience changes that alter performance, and this highlights the profound need for methods to allow tracking of biomaterials in physiologically complex niches and/or improved in vitro assays that allow better prediction of in vivo performance. Finally, to facilitate the discovery process, methods to screen and model the broad and diverse experimental space is critical. Clearly, rational material design will remain an important and leading approach of the community, but combinatorial and high-throughput strategies that are complemented by biological assays and analyses will allow mining of huge datasets to evolve new hypotheses for improved biomaterial design.

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