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Learning from nature – novel synthetic biology approaches for biomaterial design

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Abstract

Many biomaterials constructed today are complex chemical structures that incorporate biologically active components derived from nature, but the field can still be said to be in its infancy. The need for materials that bring sophisticated properties of structure, dynamics, and function to medical and non-medical applications will only grow. Increasing appreciation of the functionality of biological systems has caused biomaterials researchers to consider nature for design inspiration, and many examples exist of the use of biomolecular motifs. Yet, evolution, nature's only engine for the creation of new designs, has been largely ignored by the biomaterials community. Molecular evolution is an emerging tool that enables one to apply nature's engineering principles to non-natural situations using variation and selection. The purpose of this review is to highlight the most recent advances in the use of molecular evolution in synthetic biology applications for biomaterial engineering, and to discuss some of the areas in which this approach may be successfully applied in the future.

Keywords

synthetic biology; biomaterials; directed evolution; tissue engineering; scaffolds; virus particles; virus-like particles

1. Introduction

Evolution in the Laboratory

Natural selection is nature's way of developing new abilities in response to a changing environment. Enabled by better understanding of its mechanisms and by new analytical tools, scientists have started to bring the power of this process into the laboratory for the development of molecular function. The most straightforward approach has been to coopt biological mechanisms for the production of candidate molecules, and “screen” those candidates by chemical methods, usually their ability to bind to a target. Such methods are

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now routine in many laboratories, and are typified by phage display for polypeptides [1] and SELEX (Systematic Evolution of Ligands by Exponential Enrichment) for polynucleotides [2]. Many variations of these techniques have been developed.

Nature's preferred method is somewhat different: true *selection* couples the generation and performance of new candidate molecules with the reproduction of the organism producing them. This type of structure-survival relationship can be far more complex than simple binding, and therefore is more difficult for the laboratory scientist to direct. But, as living systems prove, selection is enormously more powerful in the development of complex, information-rich function. It is our contention that the development of smart materials can and will be revolutionized by these types of evolutionary techniques.

The discovery of new materials by directed evolution is different from traditional materials science in one fundamental respect: it places the greatest burden not on the creation of candidate materials, but rather on the testing of their properties. A single investigator can generate proteins and nucleic acids in astonishing numbers, each differing from all the others in the identity of one or more components of these linear polymers. To take advantage of this synthetic power requires the identification of those members of an evolutionary "library" that have the desired properties. This is by no means a trivial exercise: the success of evolutionary materials discovery, as with all combinatorial methods, requires great attention to the candidate library preparation as well as screening or selection part of the operation. (Readers are referred to two books describing different tools and approaches for candidate library creation and selection [3, 4] as well as two reviews [5, 6] outlining some recent progress in the field.)

For this reason, directed evolution techniques are particularly well suited for biomaterial design and optimization for four reasons. (1) Directed evolution is most easily applied to the phenomenon of binding: "winners" are selected away from "losers" by virtue of enhanced binding to the target, and repeated cycles of mutation and selection may be used to enhance binding kinetics or thermodynamics as desired. Since the construction of materials involves the self-assembly of component pieces, the evolution of specific binding properties can provide a unique advantage. (2) The molecules subjected to directed evolution – polypeptides and polynucleotides – are nanometers in size and highly diverse in structure and dynamics, providing unparalleled diversity in properties. (3) Biological molecules are inherently prone to self-assembly, so that many examples and functional units exist to imitate and coopt. Collagen, hair, and silk are examples of naturally occurring structural materials derived from the self-assembly of relatively simple molecular building blocks. (4) Directed evolution techniques can pair evolvable biomolecules with non-natural components or substrates, such as carbon nanotubes and metallic surfaces.

Current challenges in biomaterials development

Biomaterials are most commonly recognized as scaffolds potentially able to perform useful functions such as (a) promoting cell attachment, survival, proliferation, and differentiation while possessing minimum toxicity in the original and bio-degraded form; (b) allowing the transport or delivery of gases, nutrients, and growth factors; and (c) offering sufficient structural support while being degradable at appropriate rates for tissue regeneration. Readers are directed to detailed reviews describing different biomaterial scaffold properties [7-10]. It is probably safe to assume that the best scaffold for the tissue engineering would be the extracellular matrix (ECM) of the target tissue in its native conformation. Therefore, decellularized organs that retain the ECM [11] present the most common natural scaffold architecture used today, having been incorporated in materials used in heart [12], lung [13], liver [14], bone [15] and blood vessels [16]. At the same time, decellularized organs have a number of shortcomings that have limited their use in biomaterial applications, including

long processing times (increasing the costs of production), limitations on sourcing tissues, and potential immunogenicity. Also, decellularization typically involves exposure to non-physiologic chemical and biologic agents such as detergents, enzymes and physical forces that cause disruption of the associated ECM, potentially stripping the natural scaffold of its inherent bioactivity [11]. Less expensive bioactive materials can be constructed by modifying traditional “bioinert” materials to mimic physicochemical properties of natural materials [17]. Natural ECM materials such as collagen and fibrin gels, or recombinant peptides [18, 19] or proteins that mimic natural ECM materials [20, 21], have been used in this way. Hybrid approaches that combine the best qualities of synthetic materials with biologically active peptides are also the subject of investigation by a number of groups [22-27].

Each of these approaches has their own advantages and limitations. Modification of bioinert materials allows for finer control over material properties however, recapitulating every physicochemical property of a natural material is nearly impossible. Natural materials such as collagen gels are attractive because of their inherent bioactivity, but the complexity and heterogeneity of these materials can cause unpredictable cellular responses. Furthermore, these natural materials can lack the mechanical strength required for certain applications. Peptides or protein fragments that mimic natural ECM materials can form materials by themselves or can be incorporated into other scaffolds to impart biological activity [18, 19, 28-30]. Biological responses to peptides or protein fragments tend to be more predictable than responses to natural ECM material, but such reductionist approaches often cannot achieve the complexity in interactions and stimuli required to achieve a desired response [10]. The use of selection has the potential to overcome the limitations of these current approaches by specifically identifying material components and scaffolds that meet a set of desired criteria.

In this review we will focus on methods that harness the power of natural selection to produce new types of biomaterials and on natural building blocks particularly suitable for these approaches. The first section focuses primarily on the scaffolds that are produced with the use of the synthetic capacities of living cells. The second part of the review focuses on viruses and virus-like particles as synthetic scaffolds. The three key steps in molecular evolution – randomization, selection and amplification – ideally make for fast-paced development on the laboratory bench. When applied to biomaterials, “replication” can also mean “manufacturing,” adding further to the speed of the process from discovery to application (Fig.1).

2. Protein scaffolds and their selection

A growing body of data has demonstrated that cellular phenotype can be tightly linked to biomaterial parameters such as material mechanics, biochemistry, nanostructure and degradation rate. Protein-based biomaterials are capable of imparting rich biochemical information to direct cell fate, in addition to providing structural support, therefore development of such materials has seen tremendous growth [8, 20, 21]. Historically, protein-based scaffolds are obtained in three different ways: decellularization of existing tissues [11, 14], precipitation of natural protein based fibers [31], or creation and use of recombinant proteins or peptides [32], often with additional modifications. In this section we focus on the design of novel engineered biomaterials that are coded by natural amino acid sequences. The notable advances at the intersection of synthetic biology and biomaterial engineering that are discussed here convey some of the promise of the coming era in which biomolecular engineers will be able to precisely formulate properties of biomaterials to serve specific function.

The availability of gene sequences and modern techniques of molecular cloning and protein expression has led to the widespread use of recombinant proteins in place of natural ones for biomaterials design. Recombinant protein engineering offers many advantages, including (a) significant increases in protein yield and batch-to-batch consistency over that which can be achieved by extraction of a native protein from animal tissues, (b) the ability to use human amino acid sequences, therefore avoiding adverse immunological responses, and (c) the ability to modify scaffold design by manipulation of the sequence of coded genes in combination with detailed knowledge of the protein structure and function, or by combinatorial trial-and-error. Recombinant proteins were described almost four decades ago, with insulin [21], collagen [33], and elastin [34] being among the most significant. Recombinant silk-like and elastomeric proteins became available only in the beginning of this century [35, 36]. Rational design modifications have been made to a multitude of matrix proteins, including fibronectin, for a diverse range of applications. Our group has utilized this approach to create protein fragments with specific point mutations within the integrin binding region of fibronectin (FnIII9-10) that enhance integrin-driven cell attachment for the specific cellular phenotypes [28, 37].

Overall, however, the rational design of novel proteins is not yet a general solution to materials problems. Detailed knowledge of protein structure is often unavailable, and structural proteins often exhibit low solubility and poor process characteristics. In such circumstances, the knowledge of a structure may not be a good predictor of how a particular mutation will affect the function of the protein. There is reason for some optimism on this front, however, as theory and simulation grow in their ability to predict protein structure and function. For example, some of the principles defining how primary sequence of the protein determines tertiary conformation have been recently demystified [38].

Increasingly diverse protein-based materials can be created by fusing multiple protein domains. These protein-engineered biomaterials are typically designed to be repeating sequences from different naturally occurring protein scaffolds [39], computationally derived sequences [40], or sequences selected through high-throughput screening methods such as phage display [41] or peptide arrays [42]. This can potentially lead to the achievement of highly desirable properties such as biocompatibility, biodegradability, and information density. One particularly interesting example from Martino *et al.* featured the fusion of growth factor domains VEGF-A, BMP-2 and PDGF-BB with the fibronectin cell binding domains [43]. The approach led to significant enhancement of the regenerative effects of the growth factors *in vivo* for skin and bone repair. The close proximity of these two domains was found to enhance tissue regeneration through synergistic signaling of the integrin and the growth factor receptor [43].

Since the basic blocks for protein-based biomaterials are naturally occurring amino acids, concerns over toxicity of the bulk material and degradation products are low. Genetic elements responsible for the formation of different types of biomaterials in natural hosts are sometimes not known and may be identified through sequencing of the host genome and gene mutagenesis. Then, using genetic engineering techniques the identified genes can be transferred to the expression host of interest. Subsequently, the recombinant proteins production can be tuned to be batch-to-batch consistent and also cost-effective. Because this approach combines the elements of design with the power of natural selection through the incorporation of genetic libraries [44], it can be superior to the use of proteins from natural sources or synthetic polymers. For example, Banta S. *et al.* [44] reported the design of artificial multidomain proteins composed of two associative leucine zipper end-blocks and a random coil midblock to build hydrogels with desirable self-assembly properties. These domains fold into the amphiphilic α -helices, and the resulting hydrophobic interactions drive association of the proteins into oligomeric clusters [45]. Also, self-assembly of the

leucine zipper domains of the AC10A protein leads to a network, in which oligomer bundles serve as junction points [46]. Due to the introduced variation, the hydrophilic midblock lacks a regular secondary structure and prevents precipitation of the chain under conditions that favor leucine zipper aggregation. Introduction of the variable domain is the first one to our knowledge that has been reported in the field of biomaterials [46]. This study is not a typical directed evolution study since just one selection cycle has been utilized to pick winners. Nevertheless, it opens the road to the use of selection for the purpose of the biomaterial construction.

Natural protein evolution is thought to occur with the guidance of functional rather than structural influences. By this reasoning, non-native amino acid sequences are beginning to be explored to create scaffolds that are often otherwise unavailable. In one example, high throughput screening was used to identify various non-native sequences recognized by a protease for a degradation [47, 48]. Incorporation of these protease sensitive domains into elastin-like polypeptide yielded a family of cell adhesive polypeptides with tunable degradation rates spanning two orders of magnitude despite 97% sequence homology to the original sequences [49].

Proper selection of such libraries against the immune system of the host may eliminate the potential immunogenicity problem. Some other advantages of this technological approach include the easy protein purification of uniform and predictable quality and quantity. For tissue engineering applications, well-defined natural scaffolds can be modified via directed evolution that represents the next logical step in tunable biomaterial design.

In summary, recombinant protein engineering at the molecular (DNA) level is a useful technique for generating large amounts of protein polymers with versatile control over sequence specificity. Furthermore, the field of synthetic biology will likely provide numerous additional tools for development of increasingly advanced recombinant protein production strategies. In the next section, we will elaborate on the application of synthetic biology in design of non-natural, synthetic polymeric biomaterials.

3. The power of selection and synthetic polymeric scaffolds

Unlike most man-made materials, materials used in living systems are frequently multifunctional and dynamic, and are built using ‘bottom-up’ fabrication methods [50]. Both the materials themselves and the biophysical processes involved in their formation are inspiring the design and synthesis of new types of synthetic material that are potentially useful in a wide range of medical and non-medical applications [51-54]. One of the driving forces behind the use of the synthetic materials is to simplify independent changes to the biochemical and biomechanical properties. For example, the degree of crosslinking in polymer systems can be easily modified to create materials with specific desired mechanical properties [55, 56]. Independently from mechanical properties, these materials can be feathered with biologically active peptides that mimic the biochemical properties of the ECM. This widening of biomaterials applications demands an intellectual shift in how such synthetic materials are created and should include the identification of synthetic biology as a useful tool for biomaterials scientists. Distinct aspects of the use of synthetic biology approaches in the biomaterials field and the potential impact on medicine and other industries are our focus here.

The concept of “synthetic biology” is extremely broad and encompasses all aspects of designing biological systems, from modifying natural materials to creating complex synthetic materials or processes that are based on a naturally occurring counterpart. While naturally occurring protein-based materials have numerous inherent advantageous properties, such as appropriate size, specificity and affinity for their specific application,

they are often difficult and expensive to manufacture and lack stability. Artificial components, such as polymers, offer a unique alternative as they are often easy to manufacture, low-cost and display high stability over a range of conditions. Advances in the field of nanotechnology have opened many doors for the creation of completely synthetic materials that recapitulate the features and functions of naturally occurring materials. One particularly promising method to recapitulate the molecular recognition feature of naturally occurring antibodies is through the technique of molecular imprinting. This technique involves the polymerization of monomers in the presence of a desired molecular target, which results in the formation of complementary binding sites. Molecular imprinting has been described using bulk materials, films, beads, membranes nanofibers, soluble micro/nano-gels, nanoparticles and dendrimers [57-65]. Recent and particularly promising examples have been provided by Shea and coworkers in the creation of “plastic antibodies” through solid phase synthesis or polymerization, and with the use of the evolutionary approach [66]. Molecularly imprinted polymers can be stored for years at room temperature, easily sterilized through autoclaving or UV irradiation, produced at low cost, and remain stable at temperatures in excess of 100 °C. In contrast, naturally occurring antibodies are typically only stable for 6-12 months, must be stored frozen, cannot be easily sterilized, cost between \$1-1000/mg and denature at approximately 70 degrees C. The ability of “plastic” antibodies to recognize ligands *in vitro* have been demonstrated in numerous studies and recently the ability of molecularly imprinted polymers to successfully target ligands *in vivo* has been described [67, 68]. These examples highlight the promise of such synthetic materials to effectively target *in vivo* and could be used for a multitude of imaging and or therapeutic purposes.

The creation of biological mimics from synthetic components is also an active field with many potential applications. Another avenue of exploration that seeks to marry biology with synthetic components is the synthesis of polymeric materials through the use of biological processes, such as DNA translation [69]. DNA translation allows for the production of complex biopolymers from a nucleic acid template in a highly controllable manner, while synthetic polymer polymerization is difficult to control at the molecular level. Though the development of molecular imprinting has allowed for more precise control over the nanostructure of polymeric materials, control over the precise polymer length, sequence and molecular weight distribution have remained a difficult endeavor. Attempts to utilize DNA templates for the creation of sequence defined synthetic polymers has recently been described as a method to circumvent these limitations. Niu *et al.* described the development of an enzyme-free DNA-templated translation system that through peptide nucleic acid adapters recognizes a specific DNA sequence and binds specific polymer building blocks, allowing for precise control over polymer synthesis [70]. This method successfully utilized PEG, alpha-(D)-peptides and beta-peptides as building blocks for polymer chain synthesis. The use of DNA-templated synthesis to create polymeric materials that closely resemble natural nucleic acids, modified DNA, peptide nucleic acid, threoses nucleic acid, hexitol nucleic acid, and non-natural peptides have also been described [71-78]. This technique could allow for the generation of a number of polymer libraries that could be rapidly screened for biocompatibility, mechanical properties as well as utility for a particular application. Furthermore, this approach could allow for the selection of biomaterial features at the length scale of individual molecules and cells (tens of nanometers to tens of micrometers). Material properties at this length scale, such as surface topology, have significant effects on how cells perceive, interact and ingest the material, which affects the efficacy of materials used as drug carriers or vehicles targeting specific cells and tissues in the body [79-82].

In summary, bottom-up approaches have a number of advantages for the creation of novel synthetic materials relative to traditional polymeric synthesis methods, including control of

materials properties from the molecular to the bulk level. The ability to specifically select for properties at a variety of scales provides biomaterials scientists with a powerful method for creating materials with precise control over their effects on cellular phenotype.

4. Materials based on Viruses and Virus-Like Particles

Proteins and peptides have naturally received a great deal of attention as components of higher-order materials for medicinal applications [83]. Here we highlight the special case of viruses and virus-like building blocks in this role, represented schematically in Figure 2. Whether or not they bear a lipid coating, viruses and related structures contain a shell made from multiple copies of a limited number of coat protein polypeptides, often intimately associated with packaged nucleic acid. Ranging in diameter from approximately 15 nm (for the iron-storage protein ferritin) [84] to more than 1 micron [85], these scaffolds provide extraordinary examples of the power of self-assembly in the construction of supramolecular materials [86]. Virus particles are also sufficiently stable to be building blocks for larger architectures, with predictable mechanical properties related to their three-dimensional structure [87]. They can be produced in quantity, either as natural virions or expressed by standard methods in the non-replicative form of *virus-like particles*, containing only protein and nucleic acid, and lacking genomic or protein elements required for infectivity. Lastly, viruses evolve rapidly *in vivo*, including changing residues and structural motifs of their capsids in response to adaptation by their host organisms [88]. Viral coat proteins are therefore more tolerant of mutational change than many other proteins. This combination of efficiency in production and plasticity in sequence makes viruses and VLPs building blocks of great promise for biomaterials development. We refer the reader to two excellent recent reviews of this field by Wang [89] and Harris [90]; here we highlight important cases and update these reports with some recent examples. Note that the use of viruses and VLPs as functional entities themselves (such as for vaccines, drug delivery, or gene delivery) is extensive and not considered here; we focus on their use as building blocks in higher-order biomaterial structures.

From a materials perspective, viruses come in two main flavors: rod-shaped (filamentous) structures such as tobacco mosaic virus (TMV) and spherical (usually icosahedral) capsids such as cowpea mosaic virus (CPMV). Many viruses are, of course, more topologically complex than these, featuring desymmetrizing components usually associated with recognition and attachment to host cells or delivery of packaged polynucleotide, but simpler highly-symmetric variants have so far been the building blocks of choice. The past several years have seen much more activity with filamentous virus structures than icosahedral ones in materials applications.

Virus scaffold construction

A good deal of recent effort has gone into variations on the assembly of the rod-shaped virus-like particle architecture. In its simplest incarnation, TMV virus-like particles were stabilized by the introduction of one cysteine point mutation in the capsid protein (T103C), resulting in much greater resistance to pH variation and reductive denaturation [91]. The cell-free expression technology of Schwartz was also used to control intra-particle disulfide formation [92].

Manipulation of the packaged polynucleotide offers other opportunities for the control of particle assembly. Thus, Yi and coworkers prepared TMV virus-like particles of well-defined length using synthetic mRNA as a template [93]. A similar bottom-up approach for the assembly of two-dimensional virus-like particles arrays was described by Azucena, *et al.* in which RNA was first attached to silica or poly(dimethylsiloxane) surfaces, followed by addition of purified subunit protein of TMV [94]. In this case, rod-shaped structures were

formed identical in width but different in length compared to natural TMV virions, even though RNA of the same length as the TMV genome was used. The conversion of brush-motif polynucleotide to nucleoprotein coatings offers the opportunity to change surface properties dramatically and in a modular manner.

The fundamental factors affecting viral assembly have long been a subject of study by both molecular modelers and engineers. Zlotnick, *et al.* recently provided a simple but powerful conceptual framework for the polynucleotide-guided assembly of virions [95]. A set of three physical parameters (capsid protein association to nucleic acid, protein-protein interaction energetics, and the work required to package nucleic acid) were evaluated in the context of well-characterized virus examples to provide insights into the combinations that allow assembly of higher order structures. The interplay between these factors was evident in the assembly of a novel trifunctional peptide-polymer building block around viral DNA to make artificial virus-like structures, developed by Stupp and coworkers [96].

Two-dimensional capsid assemblies

When placed at an air-liquid or liquid-liquid interface, Wang, Russell, and coworkers found CPMV[97] and TMV particles [98] to self-assemble into large-scale two-dimensional arrays in order to minimize interfacial energy. Nanoparticle self-assembly at interfaces has long been known, but the use of viruses and VLPs allow for modulation of the phenomenon by genetic- and chemically-controlled change in particle surface charge and hydrophobicity. An excellent example was provided several years ago by the groups of Belcher and Hammond [99]. In this work, rod-shaped M13 viruses were used to report on the interfacial properties and dynamics of layer-by-layer assemblies of charged polyelectrolyte materials. This application highlights the monodisperse nature of virus-based building blocks: in many respects, their properties can be more controllable and reliable in their composition than any non-biological nanostructure. The self-aggregation behavior of viral nanoparticles at interfaces was later manipulated by extrusion to give larger-scale, continuous films of ordered nanoparticles, as demonstrated by Chung with the filamentous M13 capsid [100]. In both of these studies, the genetic mutability of the phage was used to introduce functional cell-binding peptides, rather than to change the assembly properties or the resulting material structure.

Recent work by Wang, Lee, and colleagues suggests that interfacial assembly is not necessary for forming higher-order structures [101]. Polysaccharides were used to modulate the osmotic pressure-related “depletion interaction” between tobacco mosaic virus particles. Again, the long, thin shape of these virions served to maximize a physical interaction, resulting in micron-scale structures with structures controlled by the nature of the depletion agent. These assemblies take the form of gel-like materials at the macroscale that are able to undergo temperature- and hydration-dependent sol-gel transitions.

Surfaces for cell growth and differentiation

In recent years, a wide variety of techniques have been used to establish interesting connections between the micro- and nanoscale topographical features of surfaces and the adherence, growth, and proliferation of cells on those surfaces. Such studies are important in understanding and controlling the properties of cells in applications such as wound healing, tissue regeneration, and implantation of medical devices. Virus particles represent interesting building blocks for such surfaces since their monodisperse and repetitive structures allow for precise control of topology, assuming that they can be self-assembled in a coherent manner.

Wang and workers have employed three methods for the coverage of surfaces with well-aligned rod-shaped particles for this purpose. In a simple “convective” technique, a high concentration of M13 or TMV virus was physically pushed across a chemically-prepared substrate such as glass, creating a dense monolayer of aligned particles that can be crosslinked in place [102]. The nanostructural features of the resulting surface are controlled by the molecular structure of the rod-shaped particle, in principle allowing for the control of materials properties at the genomic level. Fibroblasts were found to respond to the oriented nature of the surface, exhibiting ordered growth and deposition of ordered extracellular matrices [103].

The aforementioned deposition of particles from the air-water interface has also proven fruitful. A combination of surface and interfacial interactions were found to come into play in a study of TMV self-assembly from evaporating solutions placed inside glass capillary tubes, also by the Wang laboratory [104]. Different patterns of mono- and multi-layered particles were observed depending on parameters such as particle concentration and solution ionic strength. Furthermore, the growth and differentiation of smooth muscle cells cultured inside virus-coated tubes varied with the deposition pattern of the underlying rod-shaped nanoparticles. A related method for the spontaneous formation of different patterns of ordered M13 phage particles on two-dimensional templates was recently published by Lee and coworkers, and the resulting surfaces shown to have interesting optical, biomineralization, and cell-binding properties [100].

Most recently, the simple pressure-induced flowing of solutions through capillary tubes of varying surface composition has been reported to provide aligned surface monolayers of a variety of different rod-shaped particles, including gold nanoparticles and several virus-derived building blocks [105]. Myoblast cells were observed to consistently adhere, grow, and differentiate on the protein particle-based substrates.

Mao and coworkers have started to take fuller advantage of the genetic tenability of phage particles in the context of mammalian cell growth. Deposition of M13 variants displaying different cell-binding peptides resulted in nanostructured films in which controlled surface morphology and chemical signaling could be used to influence the adhesion, growth, and differentiation of mesenchymal stem cells [106]. The resulting observations continue a march toward the development of substrate materials that mirror the complexity and multifunctional nature of natural surfaces from which cells take important cues that determine their fate.

Electronic and photochemical materials

The use of filamentous phage as guides or components of nanowires [107, 108] continues to be a popular application of virus structures in materials science. A common and powerful theme is the installation of peptide sequences and the tendency of rod-shaped phage to adopt collinear aggregates, both of which can help to induce the templated deposition of the desired substance. Examples include metallic silver [109], palladium (with no added reducing agent, characterized by small-angle x-ray scattering) [110], and silicon oxide (by sol-gel synthesis to make millimeter-length fibers) [111]. Gold and iron oxide nanoparticles were also arrayed on TMV by simple charge complementation, the surface electrostatic properties of the scaffold being modulated simply by changing the solution pH, whereas the metal and metal oxide nanoparticles were relatively insensitive to this parameter [112]. Photoresponsive M13 particles were created by Mao and coworkers by the simple reaction of engineered surface tyrosine residues with aromatic diazonium salts [113]. While ensemble materials were not explored, the photochemistry of the attached azo dyes was found to be influenced by the unique functional group density of the protein scaffold.

In addition to these primary reports, two useful reviews to the field have recently appeared. An overview of mineralization and metal deposition techniques for nanomaterials was provided by Faramarzi and Sadighi [114], and Adamcik and Mezzenga contributed a long-overdue assessment of protein-based fiber structures from a polymer physics perspective [115]. The authors of the latter paper discussed the formation, intermolecular forces, structures, and properties of such nanowires, with concepts and insights applicable to virus-based systems.

The most sophisticated use of virus components in electronic materials has been achieved by the Belcher-Hammond team at MIT. These investigators used the high aspect ratio of the M13 particle (approximately 900 nm long and 7 nm in diameter) to excellent effect in the layer-by-layer construction of dye-sensitized solar cells [116]. Embedding the bionanoparticle in a polyelectrolyte matrix, virus-templated growth of semiconducting TiO₂, and bake-out of the organic/protein scaffold resulted in a porous, ordered photoanode material and strong device performance. Incorporation of gold nanoparticles to give added plasmon-derived light harvesting ability highlights the great potential inherent in the controlled chemistry and assembly provided by virus particles [117].

Other applications

While mineralization has been perhaps the most popular materials-focused use of virus capsids [118], and the immunogenicity of virus particles represents an important application in biomedical science [119], a novel combination of these two features was recently described by Tang, Qin, and colleagues [120]. In order to improve the stability and shelf life of the icosahedral enterovirus type 71, a particle used for attenuated live virus vaccines, the virion was genetically modified to display a peptide motif known to nucleate the deposition of calcium phosphide (CaP) mineral. The key biological features of the particle – infectivity, genetic stability upon repeated passage, and immunogenicity – were all unchanged, while the modified structure was imbued with the ability to nucleate the formation of a CaP shell around itself. The resulting biomineralized particles were shown to be both significantly more stable and more immunogenic than the non-mineralized precursors, the latter presumably because CaP can be degraded under physiological conditions to reveal the antigenic polypeptide shell. The Finn group has similarly observed dramatically enhanced thermal stabilities of organic polymer-coated particles [121], but immunogenicity has not been tested. A review of efforts to encase biomolecular scaffolds in inorganic material shells, in which virus particles play a strong role, has recently appeared [122].

As described above and in more extensive reviews elsewhere [89, 90], it is clear that viruses can be unique scaffolds for the construction of functional materials. So far, however, the chemical, structural, and mechanical properties of viruses have claimed the lion's share of attention. Little advantage has been taken of the genetic mutability of such building blocks, other than the relatively simple installation of cysteine or short peptide units for additional covalent and noncovalent connection capabilities. Because the field hasn't yet taken advantage of the full power of evolution hinted at in early contributions [123, 124], the development of virus-based materials can be said to still be in its infancy. It is our conviction that the marriage of methods for natural selection with functional tests of materials properties and performance – still on the horizon for all but a few such functions – has the power to revolutionize the pace and scope of materials discovery and optimization.

5. Conclusion: Evolving Biomaterials

Synthetic biology is the design and construction of biological systems, which inevitably gains its greatest inspiration from the mechanisms by which life generates and manages functional information. As a consequence, the field of synthetic biology is quite diverse,

ranging from the creation of gene circuits to build specific responsiveness into synthetic organisms to the use of evolution in the creation of new molecular species with optimized function. These natural tools for the creation of dynamic and adaptive systems have also been applied to synthetic biology approaches to new and useful biomaterials. The emphasis thus far has been on technological approaches, including molecular biomimicry, directed evolution, and natural self-assembly. It is obvious that we have only scratched the surface with respect to the complexity of systems (e.g. VLPs) being explored. However, it is our opinion that the next levels of complexity and function will require the further use of evolution as the driving force. Few have explored methods in evolution-based design for biomaterials and even fewer have fully capitalized on its potential.

Of the general steps in a molecular evolution approach, the creation of candidate libraries is critical but relatively straightforward (as in peptide design and display for enhanced binding) or at least conceptually simple if laborious (as in the synthesis of synthetic polymer libraries). We have highlighted recent work employing DNA-templated synthesis that could, along with other advances, dramatically improve the generation of materials diversity. The second step, selection of desired function, is much more complex, because one would like to emulate nature's approach of functional selection, rather than molecular screening. However, as in the evolution of functional small molecules, the development of more high-throughput systems for the analysis of complex functional outcomes can be a powerful enabler of screening-based approaches. The final step in directed evolution is modification, iteration, and replication. If the field can overcome the obvious challenges in this completion of the evolutionary cycle, what should emerge are unfathomable biomolecular and polymeric species whose creation has been intimately linked to function. Since functional behavior is likely to involve many interactions of weak or transient nature, this evolution-driven approach should lead to new insights that could not have been designed *a priori*, and new appreciations of structure-function relationships in complex materials.

While the field of biomaterials began with the goal of creating bioinert structures such as titanium and polyethylene oxide polymers, it is clear that “smart” materials – those that dynamically interact with (and perhaps adapt to) surrounding tissue and cellular processes – have many more potential applications than traditional ones. Nature, the master architect of biomaterials, uses evolution to produce matchless responsive systems. If natural biomaterials are the inspiration for our targets in this field, natural mechanisms can be an effective inspiration for their creation. As shown by the above discussion of the building blocks and principles that can be used to produce biomaterials in the laboratory, if any endeavor can be said to have “only scratched the surface,” it is this one.

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Generating the library of
biomaterial forming peptides

Identification of the peptides
with the desirable effects

Screening process

Fig.1.
Directed evolution processes for biological and synthetic materials.

Fig. 2.

Current and anticipated development of evolutionary biomaterials based on viruses and virus-like particles. Genetically-programmable rod-shaped and icosahedral particles can be self-assembled in various ways into different surface and three-dimensional morphologies (step A). Interactions of these materials with cells leads to controlled adhesion, growth, and proliferation (step B). The eventual goal is to guide the development of functioning higher-order structures such as organs by programmed cell differentiation (step C). At each stage, it may be possible to create feedback mechanisms to allow the selection of desired properties from the directed evolution of the building blocks (black arrows).