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Review

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.

1. Introduction

1.1. 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 co-opt 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 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. (i) Directed evolution is most easily applied to the

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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. (ii) 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. (iii) 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. (iv) Directed evolution techniques can pair evolvable biomolecules with non-natural components or substrates, such as carbon nanotubes and metallic surfaces.

1.2. Current challenges in biomaterials development

Biomaterials are most commonly recognized as scaffolds potentially able to perform useful functions such as (i) promoting cell attachment, survival, proliferation and differentiation while possessing minimum toxicity in the original and biodegraded forms; (ii) allowing the transport or delivery of gases, nutrients and growth factors; and (iii) 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-physiological chemical and biological 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 by 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

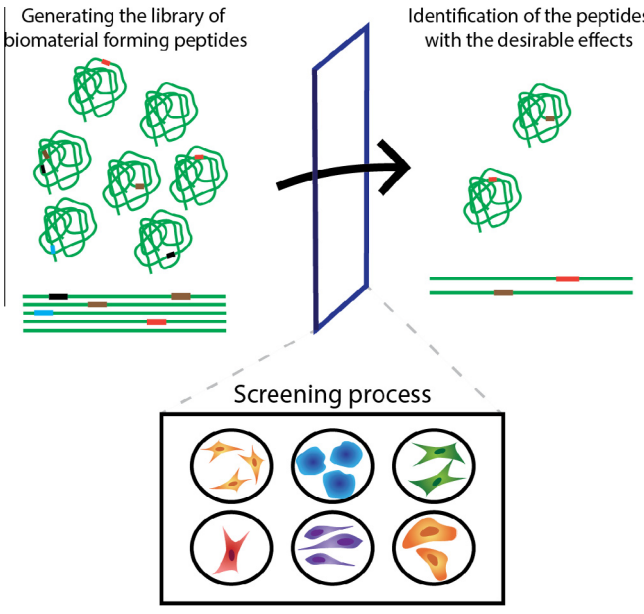

Fig. 1. Directed evolution processes for biological and synthetic materials.