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Recombinant protein blends: silk beyond natural design

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Recombinant DNA technology and new material concepts are shaping future directions in biomaterial science for the design and production of the next-generation biomaterial platforms. Aside from conventionally used synthetic polymers, numerous natural biopolymers (e.g., silk, elastin, collagen, gelatin, alginate, cellulose, keratin, chitin, polyhydroxyalkanoates) have been investigated for properties and manipulation via bioengineering. Genetic engineering provides a path to increase structural and functional complexity of these biopolymers, and thereby expand the catalog of available biomaterials beyond that which exists in nature. In addition, the integration of experimental approaches with computational modeling to analyze sequence–structure–function relationships is starting to have an impact in the field by establishing predictive frameworks for determining material properties. Herein, we review advances in recombinant DNA-mediated protein production and functionalization approaches, with a focus on hybrids or combinations of proteins; recombinant protein blends or 'recombinamers'. We highlight the potential biomedical applications of fibrous protein recombinamers, such as Silk-Elastin Like Polypeptides (SELPs) and Silk-Bacterial Collagens (SBCs). We also discuss the possibility for the rationale design of fibrous proteins to build smart, stimuli-responsive biomaterials for diverse applications. We underline current limitations with production systems for these proteins and discuss the main trends in systems/synthetic biology that may improve recombinant fibrous protein design and production.

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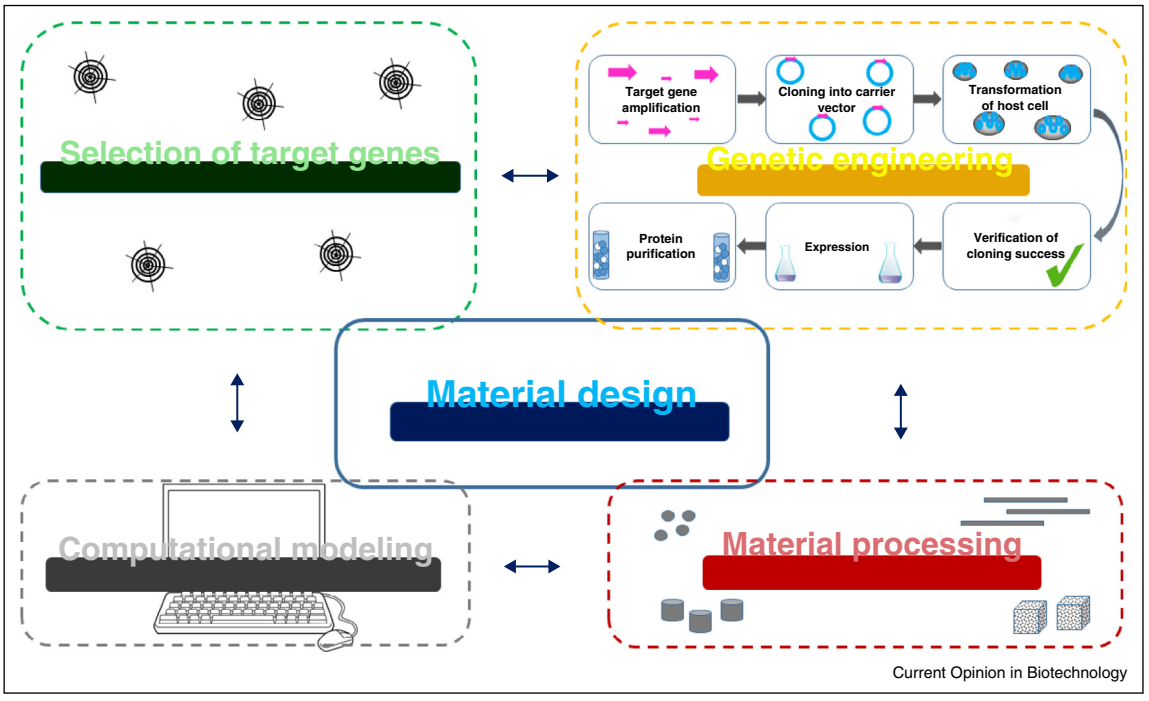
Introduction

Growing needs for biomedical materials are pushing the limits of biomaterial designs. The primary choices for materials in biomedicine have been metals, ceramics and synthetic polymers, mostly petroleum derived, due to superior mechanical properties, relatively low cost, ease of processing, and availability. These materials are being gradually replaced by degradable synthetic polymers and by biodegradable natural biopolymers (e.g., silk, elastin, collagen, gelatin, alginate, cellulose, keratin, chitin, polyhydroxyalkanoates). This transition follows trends in biomedical materials from static to multifunctional dynamic systems to better address tissue and organ repair, drug and gene delivery, nanotechnology-based imaging, diagnostic platforms and related needs [1,2]. Natural biopolymers offer design flexibility since the composition, structure and degradability can be tailored. Genetic engineering has been used to optimize material properties for specific applications via the incorporation of natural or artificial genes encoding the protein of interest into plasmid DNA. Subsequently this plasmid-gene construct is introduced in a host, most often *Escherichia coli* [3•]. The expression of the artificial gene results in the production of the desired recombinant protein, which is ultimately extracted from the host and purified (Figure 1). Genetic engineering has been utilized to produce recombinant fibrous proteins [3•,4–7], with spider silks as one area of focus due to the remarkable material properties (e.g., high tensile strength, elasticity) [8,9]. Collagen fibers are also an outstanding group of fibrous proteins, supporting physiological functions throughout the human body on the basis of combinations of sequence chemistry, different combinations of chains and complex structural hierarchy to achieve both biological and mechanical properties [10]. The list of proteins with superior mechanical performance includes resilin and elastin as well [11••].

Producing biopolymers via genetic engineering provides a route to tune properties on several levels: (i) incorporation of non-natural amino acids, (ii) selection of specific domains and their combinatorial design, (iii) functionalization of the sequence, (iv) hybrid designs, (v) production of dynamic stimuli responsive systems (Figure 2). Aside from expanding the set of available materials, recombinant DNA approaches provide insight into sequence–structure–function relationships. By applying a 'bottom-up' strategy, complex protein systems can be distilled into specific core functional domains to determine patterns by which structure influences function. These core domains can be combined in different ways to build rationally designed biomaterials. Likewise, recombinant DNA-mediated protein blends can be designed to harbor multiple biopolymer components, otherwise not found together in nature. For instance, Silk-Elastin-Like Polypeptides (SELPs) consist of repeating silk and elastin domains to integrate the physical and biological properties of silk

Figure 1



Integrated modeling and experimental approaches in biomaterials designs. Recombinant DNA-derived fibrous protein production with key steps shown such as cloning, expression of designed genetic construct and target protein purification can be combined with the control of processing parameters and computational modeling to develop new platforms for biomaterials.

and elastin [12–15]. Collagen-silk chimeric proteins represent another example of such hybrid systems [16]. Numerous, silk-based recombinant hybrid proteins have been investigated to bring new properties or functions to silk. Constructs such as silk–collagen, silk–laminin, silk–reflectin, elastin–resilin–collagen [16,17,18•] have been studied and demonstrate the vast combinatorial design options provided by genetic engineering. The above examples only focus on structural protein building blocks to generate these chimeric fibrous proteins. Chimeric fibrous proteins with new features on the basis of biological or physical recognition have also been generated, including cellular recognition through RGD domains, hydroxyapatite binding domains for bone regeneration and others, but these will not be reviewed here.

In addition to sequence alterations, control of processing parameters allows for another level of material design. However, to take the advantage of advances in biotechnology and processing, precise control of molecular mechanisms that govern protein folding is required. Recent computational approaches have proven useful in advancing this understanding. Some of the key domains in silks responsible for mechanical properties have been identified, as well as the influence of hydration level, solvents, ion content, pH and protein concentration [19]. Studies of fibrous proteins using replica exchange molecular dynamics have yielded results to compare with experimental structure identification methods [19]. Synergistic approaches on the basis of the integration of processing, experimental and modeling data offer a new discovery path toward material designs (Figure 1).

Herein, we review the advances in recombinant DNA-mediated fibrous protein production and functionalization of biomaterials to address medical needs, with a special focus on hybrid structural protein systems. We discuss the possibility of the rationale design of fibrous proteins to generate smart stimuli-responsive biomaterials for controlled release and tissue engineering applications. Also, we highlight current challenges of recombinant DNA-mediated protein production and pinpoint some of the strategies that might be undertaken to overcome these limitations.

Recombinant protein biomaterial hybrids and biomedical applications

Some of the current trends in biomaterial science are orientated toward biomaterials that mimic the biological and mechanical properties of natural tissues. Proteins are able to confer mechanical properties to tissues and organs, such as elasticity (elastin, resilin) and strength (collagen, silk). Biocompatibility and cellular invasion, proliferation and differentiation are some of the requisites for biomed-