

# Protein-Engineered Biomaterials: Highly Tunable Tissue Engineering Scaffolds

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A common goal in tissue engineering is to attain the ability to tailor specific cell–scaffold interactions and thereby gain control over cell behavior. The tunable nature of protein-engineered biomaterials enables independent tailoring of a range of biomaterial properties, creating an attractive alternative to synthetic polymeric scaffolds or harvested natural scaffolds. Protein-engineered biomaterials are comprised of modular peptide domains with various functionalities that are encoded into a DNA plasmid, transfected into an organism of choice, and expressed and purified to yield a biopolymer with exact molecular-level sequence specification. Because of the modular design strategy of protein-engineered biomaterials, these scaffolds can be easily modified to enable optimization for specific tissue engineering applications. By including multiple peptide domains with different functionalities in a single, modular biomaterial, the scaffolds can be designed to mimic the diverse properties of the natural extracellular matrix, including cell adhesion, cell signaling, elasticity, and biodegradability. Recently, the field of protein-engineered biomaterials has expanded to include functional modules that are not normally present in the extracellular matrix, thus expanding the scope and functionality of these materials. For example, these modules can include noncanonical amino acids, inorganic-binding domains, and DNA-binding sequences. The modularity, tunability, and sequence specificity of protein-engineered biomaterials make them attractive candidates for use as substrates for a variety of tissue engineering applications.

## Protein-Engineered Biomaterials: Motivation

**A**LTHOUGH THE USE OF BIOMATERIALS has long been a founding principle of tissue engineering, only more recently has the critical importance of tailoring specific cell–matrix interactions been harnessed to control cell behavior. *In vivo* cell–extracellular matrix (ECM) signaling facilitates many important cellular processes such as cell adhesion,<sup>1</sup> migration,<sup>2</sup> apoptosis,<sup>3</sup> proliferation,<sup>4</sup> and differentiation.<sup>5</sup> Cell–ECM signaling is often characterized as bidirectional; while the ECM provides structural support and engages in ECM–cell signaling interactions, cells also provide signals to the ECM that cause regional ECM remodeling and localized degradation,<sup>6</sup> often leading to tissue regeneration. The ideal biomaterial for tissue engineering purposes should thus be able to replicate the dynamic biochemical, structural, and mechanical properties of the naturally occurring ECM, in addition to being both biocompatible and biodegradable. A growing body of literature clearly demonstrates that cells will alter their phenotype in response to specific biomaterial parameters such as material biochemistry,<sup>7</sup> nano-structure,<sup>8</sup> mechanics,<sup>9</sup> and degradation rates.<sup>10</sup> Although several of these parameters may independently modify cell phenotype, a coordinated cell response to all of these material inputs is a result of complex signaling crosstalk. For instance, cell

response to biochemical ligand density can be mediated by the biomaterial mechanical properties.<sup>11</sup> Since the exact parameters of the biomaterial must be optimized depending on the specific tissue application, it is critical to be able to independently tune the multiple properties of the scaffold to give rise to the desired coordinated cell response. Through their modular design, protein-engineered biomaterials enable independent parameter tunability (Fig. 1). These materials are ideal for systematic studies of cell–matrix interactions and for use as optimizable tissue engineering scaffolds.

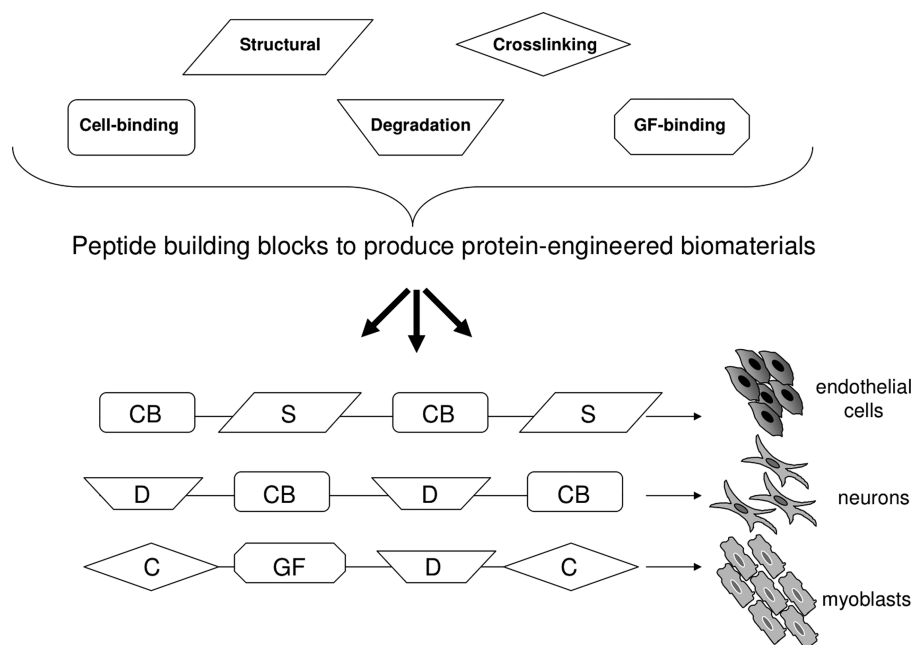
It should come as no surprise that some of the more commonly used biomaterials are harvested from the natural ECM. These materials, such as hyaluronic acid, collagen, and Matrigel, provide appropriate biochemical signaling, but generally do not allow for independent modification of scaffold properties such as mechanics, nanostructure, and degradation rate. Potential limitations of these materials include immunogenicity as well as batch-to-batch differences upon purification. In addition, these materials are generally harvested from animal sources (e.g., Matrigel is derived from the basement membrane of mouse tumors), complicating translation to a clinical setting.<sup>12</sup>

These limitations have led to research that focuses on engineering specific properties into synthetic materials to

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**FIG. 1.** The modular nature of protein-engineered biomaterials. Templated synthesis of protein-engineered biomaterials makes it straightforward to generate families of scaffolds with diverse properties from a few peptide building blocks. These represent examples of various functional peptide building blocks that may be included in protein-engineered biomaterials. These materials mimic many of the essential properties of natural extracellular matrices and can be specifically designed and optimized for various tissue engineering applications such as interactions with endothelial cells, neurons, or myoblasts. CB, cell-binding domain; S, structural domain; C, crosslinking domain; D, degradation domain; GF, growth factor-binding domain.



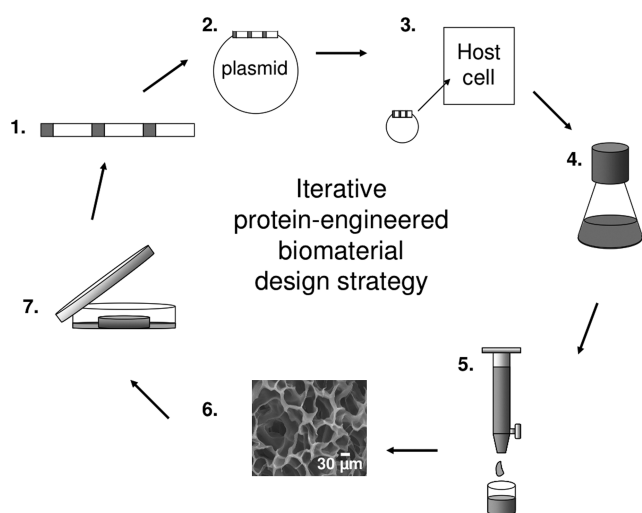
recapitulate the desired properties of natural ECM while circumventing the problems that arise with the use of materials harvested from animal sources. One of the motivations behind the use of synthetic materials is to simplify the independent modification of various biomaterial properties such as material biochemistry and biomechanics. For example, synthetic polymeric systems have been developed, where variations in molecular weight and crosslinking can be used to tune the mechanical properties and/or degradation of the material.<sup>13-15</sup> In addition, these materials often utilize peptides tethered onto the synthetic material to mimic the biochemical properties of the ECM.<sup>13</sup> In these systems, the density of grafted peptide ligands is an important property of the material that can be systematically tailored to alter cell behavior. However, it has been suggested that the peptide-tethering process may alter mechanical properties of the biomaterial, making it difficult to independently tune biochemistry and mechanics in these systems.<sup>16</sup> Therefore, new materials development is underway to create synthetic biomaterials with independently tunable mechanical and biochemical properties. For example, interpenetrating network hydrogels composed of acrylates, poly(ethylene glycol), and acrylamide have been developed as a two-dimensional *in vitro* biomaterial system that is highly tunable.<sup>17</sup> Neural stem cell differentiation on these materials has been shown to be controlled both by alterations in ligand density and

mechanical properties.<sup>11</sup> These *in vitro* cell culture results highlight the importance of developing engineered materials with independently tunable biomaterial properties. Although synthetic materials offer significant tunability, which is difficult to achieve with naturally harvested materials, concerns remain about the potential immunogenicity<sup>18</sup> as well as toxicity of degraded fragments, crosslinkers, and activating agents used to manufacture synthetic biomaterials.<sup>19</sup> An ideal biomaterial would thus combine the tunability of synthetic biomaterials with the biocompatibility and diminished cytotoxicity of natural biomaterials.

An alternative to materials harvested from natural sources and synthetic polymers is the use of protein-engineered biomaterials (Table 1). Protein-engineered biomaterials are made entirely out of recombinant proteins in which amino acids are the monomers of the material. The desired sequence of amino acids is recombinantly encoded into a DNA plasmid, and this genetic message is translated by a host expression system, resulting in the templated synthesis of biomaterials with exact molecular-level precision (Fig. 2). Information from *in vitro* and *in vivo* trials can then be used to re-design the protein-engineered biomaterial for a specific tissue engineering application by modifying the encoded amino acid sequence within the DNA plasmid. Since amino acids are used as the monomers of protein-engineered biomaterials, incorporation of bioactive peptide domains becomes straightforward. Protein-engineered biomaterials have the advantage of being able to combine desirable properties from both natural and synthetic biomaterials, namely, biocompatibility and tunability, respectively. Since the materials are protein based, concerns over toxicity of the bulk material and potential degradation fragments are reduced. At the same time, because the materials are engineered recombinantly, they are synthesized with exact molecular-level precision over the protein sequence, thus affording a degree of control over the structure and function of the protein that natural as well as completely synthetic biomaterials fail to provide. Unlike natural materials,

TABLE 1. COMPARATIVE ADVANTAGES AND CHALLENGES OF DIFFERING CLASSES OF BIOMATERIAL SCAFFOLDS

	Synthetic biomaterials	Natural biomaterials	Protein-engineered biomaterials
Toxicity	Can be high	Low	Low
Tunability	High	Low	High
Yield	High	Low	Low
Bioactivity	Can be low	High	High



**FIG. 2.** Schematic of the iterative protein-engineered biomaterial design strategy. (1) A DNA template for the protein-engineered biomaterial is designed and (2) then encoded into a recombinant plasmid. (3) The plasmid is used to transfect the host cell of choice, which (4) translates the genetic message and expresses the engineered protein. (5) The protein is then purified and (6) processed to fabricate a protein-engineered scaffold. (7) The scaffold is analyzed using *in vitro* and *in vivo* methods, providing information about how to improve the properties of the scaffold, thus prompting biomaterial redesign and modification of the encoding DNA template.

protein-engineered biomaterials represent a completely tunable class of materials and provide batch-to-batch consistency. Unlike synthetic biomaterials, protein-engineered biomaterials yield nontoxic degradation fragments and allow for straightforward, independent manipulation of material properties through a modular genetic design strategy.

### Protein-Engineered Biomaterials: Design and Synthesis Strategies

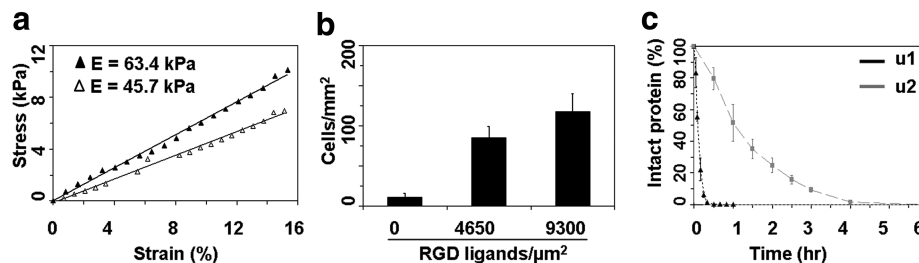
Protein-engineered biomaterials are generally designed to be repeating sequences of modular peptides that can be derived from wild-type<sup>20,21</sup> or engineered sequences.<sup>22,23</sup> Each peptide module is selected to have a specific functionality, and these modules are mixed and matched to create a multifunctional biomaterial (Fig. 1). For example, the creation of an entire family of protein-engineered biomaterials that combines peptide modules with structural, cell-adhesive, and biodegradable properties for spinal cord regeneration was recently reported.<sup>24</sup> Because of the templated synthesis of these protein-based materials from a genetically encoded DNA sequence, the exact location and density of each functional peptide module can be exactly controlled, thereby enabling independent tailoring of multiple material properties. To fully realize the potential of this molecular-level design strategy, an exact DNA template must be created for each engineered biomaterial. Therefore, the versatility of protein-engineered biomaterials is made possible first and foremost through careful control of DNA template design.

The careful design of a successful cloning strategy can enable easy modification of the DNA sequence, where particular DNA cassettes may be ligated into or excised out of the

combinant gene, producing families of protein-engineered biomaterials with tailored properties. Because of the degeneracy of the genetic code, there are multiple ways to template the synthesis of a single engineered protein, and choosing a particular DNA sequence can critically impact protein yield. Although the genetic code is essentially the same for almost all organisms, the frequency with which certain codons are used to specify a particular amino acid differs greatly from organism to organism. Therefore, it is possible to optimize protein yield by choosing the appropriate series of codons for the particular expression system under use. For instance, certain codons for arginine in human cells (e.g., AAG and AGA) are very rarely used in *Escherichia coli*; therefore, expression of a human sequence containing the above codons in *E. coli* will limit protein expression.<sup>25</sup> However, it is generally not sufficient to simply choose the most prevalent codon for each amino acid. There must be a diversity of codon usage to avoid DNA recombination when templating highly repetitive amino acid sequences.<sup>26</sup> In addition, the formation of appropriate or inappropriate DNA structures, proximity or similarity of the DNA codons to any neighboring translational sites, and the presence of appropriate ribosome-binding sites on the mRNA can aid or hamper translation.<sup>27,28</sup> Although codon optimization based on the frequency of codon usage for a particular organism often correlates well with its translational efficiency,<sup>29</sup> it is important to note that different codons may need to be used for recombinant proteins as opposed to highly expressed genomic genes, making codon optimization a nontrivial process.<sup>30–32</sup> Although the process of codon optimization is not yet fully understood, codon bias, tRNA availability, mRNA stability, and mRNA structure are all thought to play an important roles in the gene expression process.<sup>28,33–35</sup> Algorithms are now being developed for specific expression systems, giving rise to high translational efficiency and protein yield.<sup>36,37</sup>

The optimized DNA sequence then needs to be introduced into an appropriate organism for expression. *E. coli* has been the most commonly used expression system because it is relatively inexpensive, grows rapidly, and can be genetically modified to enhance protein expression. In addition to regularly used *E. coli* expression strains, mutant strains have been engineered to include the incorporation of noncanonical amino acids.<sup>38</sup> However, since *E. coli* is a prokaryote without a Golgi apparatus or an endoplasmic reticulum, it cannot incorporate posttranslational modifications such as protein glycosylation into the recombinant protein without extensive organism engineering.<sup>39</sup> Further, overexpression of the protein can lead to inactive, unfolded protein that produces aggregates known as inclusion bodies, which need to be further processed to recover activity. Strains of yeast, such as *Saccharomyces cerevisiae* and *Pichia pastoris*, are also widely used for recombinant protein expression, since yeast is a eukaryotic system with a Golgi apparatus, allowing for a degree of posttranslational modification in the protein.<sup>40</sup> Yeast is also relatively inexpensive and grows quickly compared to other, more complex expression systems. Yeast glycosylation is different from mammalian glycosylation, however, and insect as well as mammalian expression systems have also been used to more closely approximate native posttranslational conditions for engineered recombinant proteins.<sup>41–43</sup> Although higher organisms may be used for protein expression, transfection and cell/organism culture tends to become more

**FIG. 3.** The modular design strategy of protein-engineered biomaterials allows independent tuning of multiple scaffold properties.<sup>24</sup> (a) The elastic modulus of a family of elastin-based scaffolds is independently tailored by altering the stoichiometric ratio of crosslinker to reactive amino acid side chains. (b) The cell adhesive properties of this same family of elastin-based scaffolds are independently tailored by varying the density of cell-adhesion ligands (an extended RGD peptide sequence). (c) The biodegradation rate of this same family of scaffolds is independently tailored by modifying three residues in the primary amino acid sequence to tune the protease-substrate reactivity.



complex and more expensive. In general, the more complex expression system used, the more complex the growth and media conditions, the less effective the cell transfection process, and the longer it takes for recombinant protein expression. The complexity of the protein being engineered as well as the importance of the secondary and tertiary structure of the recombinantly engineered protein will dictate the best expression system to be used. Many protein-engineered biomaterials have successfully used *E. coli* as their expression system for modular proteins that are comprised of functional peptides with simple domain folds.<sup>44,45</sup>

Thus, the design and synthesis of protein-engineered biomaterials come with a specific set of challenges and tasks. For each protein-engineered material, a flexible cloning strategy must be designed, a recombinant gene must be synthesized to achieve translational efficiency, and synthesis and purification protocols using an appropriate expression system must be developed to produce a high yield of engineered protein.<sup>37</sup>

### Protein-Engineered Biomaterials: Diversity of Functional Peptide Modules

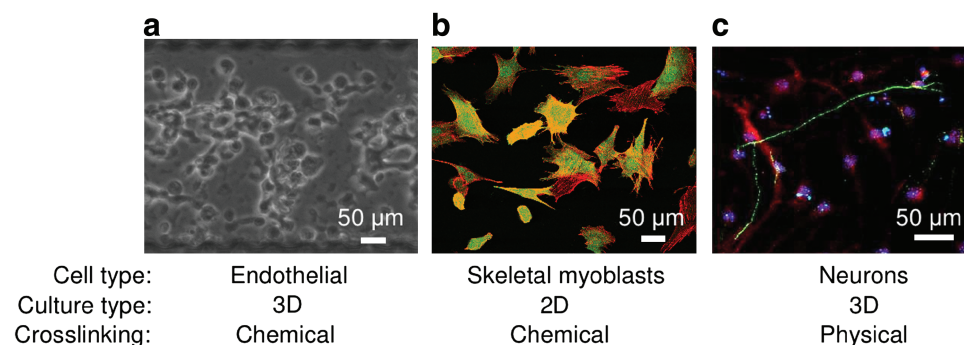
An enormous range of peptide modules are available for incorporation into protein-engineered biomaterials, and these modules can be derived from natural wild-type sequences,<sup>20</sup> computationally derived sequences,<sup>23</sup> or sequences selected through high-throughput screening methods such as phage display.<sup>22</sup>

For tissue engineering applications, the types of modules that are incorporated into the protein-engineered biomaterials are often chosen to enable specific tuning of the structural, mechanical, biochemical, and biodegradation properties of the scaffold (Fig. 3). Often the first design parameter to be chosen is the peptide module that will provide structural integrity to the biomaterial. Although a few examples of novel structural peptides have been reported,<sup>46,47</sup> the majority of structural peptides used to date have been based on repeating structural sequences commonly found in nature. For example, recombinant elastin-derived biomaterials<sup>48,49</sup> and spider-silk-derived biomaterials<sup>50</sup> both utilize motifs that in nature are well known to provide structural integrity. Often, these modules can be induced to associate through physical crosslinks to form a bulk biomaterial.<sup>51</sup> In these systems, the mechanics of the material often arises as a consequence of hierarchical self-assembly such as the for-

mation of fibrillar structures.<sup>52</sup> The resulting hierarchical structure and, hence, the mechanical properties may be tuned both through variations in the primary amino acid sequence as well as variations in assembly conditions.

In cases where physical crosslinking is not possible or not sufficient to create a material of desired stiffness, the structural modules can be further modified by incorporating amino acid residues that facilitate protein crosslinking using chemical crosslinkers (such as bi- and trifunctional isocyanates, N-hydroxysuccinimide esters, and hydroxymethylphosphines)<sup>24,53,54</sup> or enzymatic crosslinkers (such as various transglutaminases).<sup>55,56</sup> Covalent crosslinking can provide direct control over the mechanical properties of the material; through tuning of either the concentration of potential crosslinking sites in the protein or the extent of the crosslinking reaction, the relative stiffness of the protein-based biomaterial can be changed independently of its other bioactive properties.<sup>57</sup> In choosing the appropriate crosslinker, it is important to consider both potential crosslinker cytotoxicity as well as the potential for crosslinking to interfere with the bioactivity of other peptide modules. A variety of crosslinking agents and physical crosslinking mechanisms have been successfully used in protein-based biomaterials for multiple cell types, both in two-dimensional and three-dimensional cell culture (Fig. 4).<sup>58-60</sup>

Once the structural backbone of the biomaterial has been chosen, additional peptide modules can be incorporated into the protein-engineered biomaterial to optimize the scaffold for a particular tissue engineering application. Many of these modules have been previously grafted onto the surface of synthetic biomaterials and are now beginning to be directly incorporated into protein-engineered biomaterials as part of the primary amino acid sequence. These peptide modules can provide a broad range of functionality including cell adhesion, growth factor delivery, and cell signaling (Table 2). For example, the active domains of Jagged and Delta 1 (part of the Notch signaling pathway) have been designed into an elastin-based biomaterial to initiate specific signal transduction pathways.<sup>61</sup> Similarly, cell-binding motifs have been widely explored in protein-based materials.<sup>21,62</sup> Other potential sequences of interest include those that can tether growth factors<sup>63</sup> or interact with cell-cell adhesion receptors.<sup>64</sup> It should be noted that tuning the crosslinker density in hydrogel systems may change the porosity and/or swelling of the scaffold. Therefore, when attempting to independently modify the biomechanical and bioactive prop-



**FIG. 4.** Protein-engineered biomaterials are compatible with a range of cell types in both two-dimensional (2D) and three-dimensional (3D) culture systems. (a) Phase contrast image of human umbilical vein endothelial cells encapsulated within a 3D chemically crosslinked protein scaffold (A. Shamloo and S.C. Heilshorn, unpublished data, 2009). (b) Fluorescent image of green-

fluorescent-protein-labeled C2C12 mouse skeletal myoblasts grown on a 2D chemically crosslinked protein scaffold (D. Sengupta and S.C. Heilshorn, unpublished data, 2009). (c) Fluorescent image of mouse neural progenitor cells differentiated into neuronal and glial cells while encapsulated within a 3D physically crosslinked protein scaffold.<sup>82</sup> Color images available online at [www.liebertonline.com/ten](http://www.liebertonline.com/ten).

erties of a material, it is important to theoretically estimate or experimentally quantify the number of potential ligands available to each cell within the scaffold.

An additional advantage of protein-based biomaterials is the ability to encode for localized protease degradation sites. This biomaterial feature allows for selective, localized remodeling of the biomaterial in response to proteases secreted by cells as opposed to hydrolytic degradation. This strategy was first developed to enable degradation of synthetic biomaterials in response to matrix metalloproteinases, which are widely secreted during tissue remodeling.<sup>13</sup> Because differ-

ent cell types secrete a signature of different proteases, and protease secretion may be upregulated during differentiation, migration, and proliferation, it may be possible to trigger biomaterial degradation in response to specific cellular events. Therefore, the biomaterial proteolytic susceptibility can be optimized for specific tissue engineering applications that require adaptive biomaterials to dynamically respond to the changing needs of the implant site.<sup>14</sup> Employing this strategy, protein-based biomaterials have been designed with sequences that degrade in response to tissue plasminogen activator and urokinase plasminogen

TABLE 2. REPRESENTATIVE PEPTIDES USED IN MODULAR PROTEIN-ENGINEERED BIOMATERIALS

Functionality	Peptide	Source protein	Reference
Heparin-binding domains	FAKLAARLYRKA	Antithrombin III	83
Cell-matrix interaction domains	RGD	Multiple extracellular matrix proteins	21, 48, 50, 60, 62
	REDV	Fibronectin	49, 79
	IKVAV	Laminin	84
	YIGSR	Laminin	62
Cell-cell adhesion domains	GRALARGEANF	Neural cell adhesion molecule	62
	DWVIPPISCPENEKGPFPKNLVQIKSNRDK	E-cadherin	64
Structural domains	GGRPSDSYGAPGGGN	Resilin	85
	VPGXG	Elastin	21, 44, 45, 49, 77
	GAGAGS	Silk fibroin	44, 52, 74
Crosslinking domains	SKGPG and VPGQG	Tissue transglutaminase binding domain	56
Signaling domains	CDEHYYGEGCSVFCRPR	hDelta 1 (Notch signaling pathway)	61
Inorganic binding domains	R5 peptide	Silaffin	66
	RHTDGLRRIAAR	Tra1 copper-binding protein	65
Degradation domains	Variable	Matrix Metallo-proteinase cleavage site	86
	GTAR, TSHR, DRIR	Tissue plasminogen activator cleavage sites	62
	DNRR, FFSR, SILR	Urokinase plasminogen activator cleavage sites	24, 87

activator, proteases secreted by the tips of extending neurites.<sup>24,62</sup> By simply altering the primary amino acid sequence of the proteolytic module, the enzyme specificity and, hence, the degradation reaction kinetics can be tuned. These protein-based biomaterials enable the formation of scaffolds with tailored degradation rates spanning two orders of magnitude despite having identical initial mechanical properties and greater than 97% sequence homology.

Although the peptide modules discussed above have led to the development of an interesting array of biomaterials, they represent only a small subset of potential peptide modules that may be included in protein-engineered biomaterials. There is a tremendous opportunity to broaden the scope of peptide modules included in protein-based biomaterials, which in turn will expand the range of diverse properties and functionalities that biomaterials can provide.

### **Protein-Engineered Biomaterials: Opportunities and Challenges**

The field of biomaterials has seen tremendous growth in the past decade, and protein engineering has clearly emerged as a powerful tool to study cell–matrix interactions and produce further insights into tissue engineering. The field of protein-engineered biomaterials is still in its relative infancy, producing many possibilities and also presenting novel scientific challenges. First, as discussed above, protein design, synthesis, expression, and purification must be optimized for the specific protein-engineered biomaterial being produced. Achieving optimized yields of these materials for commercial applications will require new insights into microbiology and the development of new biotechnological processing.

Second, the number of peptide modules that have been used to produce protein-engineered biomaterials has been rather limited to date. Although clever use of these peptide modules has resulted in a wide array of biomaterials with interesting functionalities, the diversity of peptide modules available throughout the proteome represents an enormous opportunity to broaden the range of functions that protein-engineered biomaterials can achieve. The lengths of most peptide modules used in the field thus far have been relatively short (~5–50 amino acids); longer, more complex peptide modules may be incorporated into future biomaterial designs. Although increased peptide module complexity may provide enhanced bioactivity, it must be kept in mind that these bulkier peptide modules may require specialized processing to achieve accurate protein folding. Further, the scope of protein-engineered biomaterials can be further expanded to produce nontraditional, protein-based materials using substances outside the amino acid lexicon. These materials can include composite materials that combine proteins with inorganic substances or proteins that contain chemical moieties beyond the 20 canonical amino acids, and are discussed in more detail below. Common challenges for all tissue engineering scaffolds include developing strategies to fabricate three-dimensional hierarchical materials that mimic the structural complexity of the natural ECM and designing materials that can elicit a specific, desired immune response (including negligible immunogenicity). In both of these areas, the field of protein-engineered biomaterials offers novel opportunities to exploit the precision of recombi-

nant molecular biology to address these critical challenges. Each of these opportunities are discussed in detail below.

First, the range of bioactive domains may be increased to produce hybrid materials that expand the biomaterial chemistry beyond the 20 amino acids traditionally used in protein-engineering. For example, an inorganic–protein nanostructure has been created using an engineered DNA-binding protein that contains a copper-binding sequence.<sup>65</sup> The DNA-binding activity of the protein is retained, resulting in a nanoengineered DNA–protein–copper nanocomposite. Nanocomposites have also been engineered using domains from a spider-silk protein fused to a silaffin protein domain that binds silica to produce protein–silica nanoparticles.<sup>66</sup> In addition, noncanonical amino acids may be incorporated into the overall design to modify specific properties of the protein and introduce new chemical functionality into the material. Examples include fluorinated,<sup>67</sup> photoactive,<sup>68</sup> and unsaturated<sup>69</sup> amino acids that have been included to enhance protein stability,<sup>70</sup> enable photopatterning,<sup>71</sup> or allow orthogonal chemical tethering,<sup>72</sup> respectively. Depending on the incorporation strategy, noncanonical amino acids may be incorporated in a residue- or site-specific fashion.<sup>73</sup> Site-specific modifications allow orthogonal chemistry to be performed at specific protein sites and may enable systematic investigation of individual cell–protein interactions that are sequence dependent. Residue replacement can be used to control protein biochemistry, to perform orthogonal chemical reactions, and to improve protein stability at a macroscopic level.

In addition to advancements in the design of the primary amino acid sequence, changes can also be made to the way the protein-engineered biomaterials are processed and presented to cells. Since the natural ECM is a hierarchically structured composite material, the introduction of micro- and nanostructure into protein-engineered biomaterials can aid the organization of cultured tissue. The addition of micro- or nanostructure can come about through processing methods, such as electrospinning,<sup>74</sup> micro- or nanomolding,<sup>75,76</sup> photopatterning,<sup>71</sup> or through self-assembly mechanisms.<sup>77</sup> For example, nanoscale hierarchical protein assemblies have been designed using engineered helical protein sequences that may be incorporated into subsequent biomaterial design.<sup>78</sup> In another example, self-assembly into nanofibrils of several centimeters in length has been reported using recombinant spider silk sequences.<sup>52</sup> Therefore, as tissue engineering applications continue to require more complex scaffolds, protein-engineered biomaterials offer new opportunities to design scaffolds that self-assemble into specific hierarchical structures.

Like all biomaterials (both synthetic and harvested), any potential immunogenic responses must be carefully regulated. Protein-engineered biomaterials have the ability to trigger an innate immune response, including nonspecific capsule formation around an implanted protein-engineered material and the triggering of inflammation due to the presence of pyrogenic agents such as endotoxins. Multiple methods exist to efficiently remove most endotoxin from purified protein, and as recombinant protein pharmaceuticals become more widely marketed, this technology is sure to improve.<sup>79,80</sup> In addition, protein-engineered biomaterials may also trigger an acquired immune response, since engineered proteins may act as antigens to stimulate antibody produc-

tion. The protein-engineering community thus stands to learn from the pharmaceutical and vaccine industries, which have substantial experience in managing the immunogenic potential of fusion peptide sequences.<sup>81</sup> An encouragement is that the molecular-level design strategy inherent to protein engineering opens the possibility of screening multiple sequences with similar biomaterial functionality for altered immunogenic potential; this type of optimization cannot be achieved with biomaterials harvested from natural sources that often suffer from immunogenicity.<sup>12</sup> In addition, peptide sequences derived from human proteins can be exclusively used to minimize the potential for immune response.

In conclusion, protein-engineered biomaterials represent a valuable approach to creating new tissue engineering scaffolds. This strategy of biomaterial synthesis provides molecular-level material design, enabling straightforward and independent control over an array of biomaterial properties. These materials are inherently bioresorbable, since they are comprised of amino acids, and the modular design strategy allows easy modification of the material for individual cell types and specific tissue engineering applications. Because of their exquisite tunability, these materials also present a platform for systematic study of fundamental questions about cell–microenvironment interactions. The modularity and versatility of protein-engineered biomaterials make them ideal candidates for translatable biomaterials research and offer the opportunity to design new generations of biomaterials with exciting new functionalities.

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### Disclosure Statement

The authors declare no conflicts of interest.

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