In vitro selection technologies to enhance biomaterial functionality

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Abstract

Cells make decisions and fate choices based in part on cues they receive from their external environment. Factors that affect the interpretation of these cues include the soluble proteins that are present at any given time, the cell surface receptors that are available to bind these proteins, and the relative affinities of the soluble proteins for their cognate receptors. Researchers have identified many of the biological motifs responsible for the high-affinity interactions between proteins and their receptors, and subsequently incorporated these motifs into biomaterials to elicit control over cell behavior. Common modes of control include localized sequestration of proteins to improve bioavailability and direct inhibition or activation of a receptor by an immobilized peptide or protein. However, naturally occurring biological motifs often possess promiscuous affinity for multiple proteins and receptors or lack programmable actuation in response to dynamic stimuli, thereby limiting the amount of control they can exert over cellular decisions. These natural motifs only represent a small fraction of the biological diversity that can be assayed by *in vitro* selection strategies, and the discovery of “artificial” motifs with varying affinity, specificity, and functionality could greatly expand the repertoire of engineered biomaterial properties. This minireview provides a brief summary of classical and emerging techniques in peptide phage display and nucleic acid aptamer selections and discusses prospective applications in the areas of cell adhesion, angiogenesis, neural regeneration, and immune modulation.

Keywords: Biomaterial, *in vitro* selection, biomedical engineering, phage, aptamer, peptides

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Introduction

Cells sense and interpret many microenvironmental cues, including soluble effectors, biochemical engagement with a substrate, contact with adjacent cells, and mechanical forces, which dictate their decisions to proliferate, differentiate, migrate, and live or die depending on the collective interpretation of available signals.^{1–4} As such, manipulation of extracellular signaling events is viewed as a promising avenue for therapeutic intervention and has been extensively explored using materials that deliver proteins, materials embedded with cells with or without added proteins, or materials that are intended to recruit endogenous cells and affect their function.⁵ In particular, naturally derived biological motifs have been extensively explored for controlling signaling events in materials constructs. Glycosaminoglycans (GAGs) such as heparin, which reversibly bind proteins in a manner typically governed by electrostatic interactions,⁶ have been used extensively to either sequester endogenous growth factors to potentiate signaling or act as delivery devices when preloaded with

protein prior to implantation.^{7,8} Natural domains from extracellular matrix (ECM) (e.g. fibronectin or vitronectin) or soluble proteins (e.g. fibrin or fibrinogen) also bind a variety of growth factors through non-covalent interactions.^{9–12} These binding domains can be loaded with single or multiple soluble proteins prior to material implantation, and these proteins are then released according to natural dissociation kinetics. These domains can also sequester endogenous growth factors or cytokines after implantation to augment natural repair processes.¹³ However, this methodology is not suitable to manipulate individual signaling pathways because these promiscuous domains will bind and sequester any available protein that is recognized by the motif. As a workaround for this issue, extracellular portions of cell surface receptors or shorter peptides derived from these receptors have been tested for their ability to sequester growth factors with high affinity for the full-length receptor. For example, a peptide mimicking vascular endothelial growth factor receptor type 2 (VEGFR2)¹⁴ has been incorporated into hydrogels that can

subsequently bind or release VEGF in a controlled manner.¹⁵ Conversely, peptides mimicking growth factor epitopes can inhibit binding of the full-length protein to a particular receptor by occupying the binding pocket, and peptides derived from receptors can be “decoys” that prevent growth factors from binding actual cell receptors. For example, a peptide derived from bone morphogenetic protein 2 (BMP2) was found to inhibit binding of full-length BMP2 to particular BMP receptors.¹⁶ In addition, a peptide derived from transforming growth factor- β (TGF- β) receptor type III (TGF β RIII) has been shown to inhibit TGF- β 1 activity *in vitro*¹⁷ and *in vivo*.^{17,18} Similar examples have been demonstrated with a peptide derived from VEGFR2 that inhibits VEGF activity¹⁹ and a peptide derived from tumor necrosis factor receptor 1 (TNFR1) that promotes cell survival in implanted hydrogels by inhibiting TNF α -mediated inflammation.²⁰ Peptide motifs derived from full-length proteins and full-length proteins themselves have also been utilized to activate signaling when tethered to surfaces and have been explored quite extensively for applications in mesenchymal stem cell differentiation.^{21–24} Notably, motifs derived from the same protein can have activating or inhibitory properties on cells depending on how the motif is utilized.^{14,15}

While peptides derived from natural motifs have been generally successful for directing cell behavior, they also possess inherent promiscuity that may limit their ability to explicitly control extracellular signaling. As an example, the entire TGF β superfamily consists of >30 different protein ligands, including the TGF β s, activin/inhibins, BMPs, and growth differentiation factors (GDFs), but these soluble factors bind to only 12 known receptors (7 type-I and 5 type-II), each with varying affinities.²⁵ Therefore, the previously described efforts to inhibit TGF- β signaling using a peptide from TGF β RIII may actually be inhibiting many members of the overall superfamily,¹⁷ and a peptide derived from BMP2 could be inhibiting either BMP or activin receptors.²⁵ Similar growth factor and receptor promiscuity exists within many other protein families, and activation or inhibition of different cascades within these pathways depends on a myriad of variables such as ligand compositions, ligand concentrations, and receptor expression patterns.^{25–27} Furthermore, the mechanisms of action by implanted biomaterials containing GAGs and promiscuous motifs can be confounding in a complex *in vivo* environment, where many different proteins could be temporally interacting with the biomaterial.

Natural biological motifs encompass only a fraction of the available sequence space that can theoretically be generated in a laboratory. *In vitro* selection approaches provide routes to “artificial” biological motifs with tailored affinity and selectivity for proteins, including soluble growth factors and cytokines as well as membrane receptors. When coupled with advances in synthetic chemistry and materials construction, selection strategies have the potential to augment current biomaterial capabilities. In addition, engineered biological motifs could provide a better understanding of biological mechanisms since their mode of action should be highly specific. This minireview focuses primarily on peptide phage display and nucleic acid

aptamer selections by detailing classical and emerging selection techniques and then describing how selected artificial motifs could be applied to biomaterials applications.

Phage display of peptide libraries

Phage display is a laboratory method used to identify high-affinity binders to other molecules. This method involves inserting a library of DNA sequences into a bacteriophage coat gene. Libraries can be random or biased and encode variants of specific proteins or random peptides. This review focuses on peptides because these shorter amino acid sequences are often used in biomaterials applications. The peptides encoded by the library are displayed on the coat of the individual bacteriophage and screened against other macromolecules and DNA sequences.²⁸ Small numbers of binding clones can be identified from libraries of over 10^9 different proteins displayed in phages using a process called biopanning.²⁹ Phage display usually requires 2–4 rounds of affinity selection and amplification, followed by an ELISA assay and sequencing.³⁰ As part of this method, consensus motifs need to be identified. Peptides that share the same consensus sequences can bind to the same target protein through different interaction modes.³⁰ Usually, some consensus sequences can be identified during phage display, which help draw conclusions about the binding of certain peptides to the target protein.³⁰

Phage display has been used to screen for peptides that can alter cell behavior. One application of phage display is to identify peptides that can interrupt receptor–ligand interactions. In one study, phage display was used to identify peptides that inhibit binding of VEGF to the KDR and FLT1 receptors.³¹ The highest affinity peptides were found to bind to target protein–protein interaction sites on VEGF, which inhibited angiogenesis.³¹ Additionally, another group used phage display to identify peptide K237, which binds to KDR with high affinity.³² This interaction disrupted the binding of VEGF and KDR, which was shown to prohibit proliferation of primary human umbilical vein endothelial cells and inhibit fibroblast growth factor 2 (FGF2)-mediated angiogenesis.³² Peptides identified from phage display are also able to deliver proteins from hydrogels and other biomaterials. For example, a study by Lin and Anseth³³ developed a photopolymerized polyethylene glycol (PEG) hydrogel for sustained FGF2 release. A peptide sequence from a previous phage display study was used that interacted directly with FGF2, thus preventing it from binding to the FGF receptors.³⁴ The hydrogel system was able to control the release of FGF2 based on this protein–peptide interaction.³³

Phage display can also isolate peptides used to influence cell behavior by amplifying or replacing native protein function. For example, a classical application of phage display is the selection of peptides that can mediate cell adhesion by engaging integrins.^{35–37} In a more exquisite study, Barker and coworkers³⁸ used phage display to identify peptides that could discriminate force-induced structural states in fibronectin. Other recent examples include the use of phage display to identify peptides that bind to the surface of pluripotent cells, which enable short-term

cell propagation.³⁹ Peptides that selectively amplify a signaling response have been less explored, but examples are beginning to emerge from the literature. Pioneering work was performed by Kiessling and coworkers,⁴⁰ who used phage display against the extracellular domain of TGF- β receptor 1 (TGF β R1) to identify high-affinity peptides that did not interfere with TGF- β binding. When tethered to synthetic surfaces, the peptides could pre-organize transmembrane receptors to potentiate local TGF- β signaling and cause epithelial-to-mesenchymal transitions in cultured cells at picomolar growth factor concentrations.⁴¹ Another study observed similar effects with peptides clustered on a DNA nanostructure.⁴² Thus, peptides that bind to receptors outside the ligand recognition area can potentially direct cell behavior.

Although phage display is a powerful tool for identifying high-affinity peptides, its methods have some limitations. For instance, the limited number of clones identified from phage display does not necessarily lead to the most promising binders.⁴³ This issue stems from the identification of false positives, which can result from phage binding to other components in the screening process such as contaminants in the samples, solid objects including plates and beads, capturing reagents, and any other substances used in the panning process.²⁹ The other reason for false-positive selections is related to propagation rates.²⁹ Certain phages in the phage library may have mutations that lead to faster propagation. These faster-propagating phages have an advantage to stay in the selection pool through different rounds regardless of their affinity to the target protein.²⁹ For example, the HAIYPRH peptide has been identified in the Ph.D.-7 phage library that has accelerated propagation due to a mutation in the gIIp protein.⁴⁴ This peptide has been identified in 13 different phage display experiments.²⁹ Compartmentalization of individual phage clones during the amplification process has been identified as an emerging workaround for this problem.⁴⁵ Phage display can also be a slow and laborious process that only identifies a few clones while missing out on many others due to limitations in traditional Sanger sequencing of the final population. High-throughput sequencing has been proposed as a method to better analyze phage diversity over selection rounds.⁴⁶ Microfluidic technology has also been utilized to shorten the selection process.⁴⁷ Overall, these technological advancements in the selection process should continue to make phage display a useful tool for identifying artificial sequences that can elicit control over cell behavior.

Aptamer selections

Peptides are inherently monomodal – their function is always “on” unless chemical modifications are employed. Their synthesis can also be relatively expensive, and conjugation into biomaterials with site specificity is not necessarily a straightforward task without synthetic chemistry expertise or the use of non-canonical amino acids.⁴⁸ Moreover, peptide release of proteins for delivery applications is controlled exclusively by dissociation kinetics, and peptides identified from a first generation phage library exhibit relatively weak binding affinities to proteins.²⁹

In contrast to peptide affinity reagents, aptamers are single-stranded oligonucleotides selected to bind target molecules. Aptamers can be synthesized at a lower cost than peptides and made with site-specific modifications that more easily facilitate orthogonal chemistry.⁴⁹ They have been utilized for sustained growth factor release in applications similar to GAGs and peptides.^{50–52} Moreover, owing to principles of nucleic acid double-strand formation, aptamers can also be rapidly dissociated from their targets via complementary strand displacement; this “molecular trigger” strategy was pioneered by Wang and coauthors for programmed release of growth factors.^{53–56} Therefore, aptamer binding to a target is modular and can be tuned on demand. These properties make aptamers potentially intriguing alternatives for applications that typically employ peptides.

Aptamers are selected using a process known as systematic evolution of ligands by exponential enrichment (SELEX), which involves screening and selecting single-strand oligonucleotides that bind to targets with high affinity.^{57,58} Starting libraries are typically between 10^{13} and 10^{16} sequences, which is higher than phage display due to the use of chemical synthesis and lack of a transformation step. In each selection round, the library is incubated with a target protein, non-bound sequences are removed, and the enriched pool of oligonucleotides is amplified using PCR. Upon completion of traditional SELEX, prospective aptamers are cloned into a plasmid, amplified in bacteria, and sequenced. Identified sequences are then re-synthesized and binding properties can be analyzed by typical procedures.

Despite many apparent advantages over peptides, aptamers are historically underused for biotechnology and medical applications compared with their amino acid-based counterparts,⁵⁹ which may be due to the methods employed for selection. Though outside the scope of this review, traditional SELEX methods, which often employ nitrocellulose filters or bead-based target immobilization, frequently require 10 or more selection rounds before convergence on consensus targeting sequences.⁶⁰ However, this inefficiency has been countered by recent advancements in selection technologies, which may invigorate the use of aptamers in biomaterials applications.⁶¹ For example, to improve the stringency of selection, microfluidics has been employed. Work pioneered by Soh and coauthors have demonstrated that high-affinity aptamers can be obtained in 1–3 rounds of microfluidic selection.^{62–64} On-chip PCR has further reduced workflow,⁶⁵ although amplification bias in the PCR step can also be a limitation in SELEX. This issue has been combatted using variations in emulsion PCR to avoid nonspecific amplicon addition to the SELEX pool.^{66–68} Incorporation of high-throughput sequencing to the SELEX workflow can also streamline selections by identifying prospective aptamers through relative enrichment but prior to sequence convergence.⁶⁹ The diversity of aptamer structures has also been increased through incorporation of unnatural nucleic acids.^{70,71} However, generation of unnatural aptamers requires skills in organic chemistry, and while many unnatural aptamers are available commercially, their final sequences are

proprietary which limits the widespread use of these reagents. Finally, multivalent aptamer displays on beads have demonstrated great potential for improving selection processes by enhancing avidity compared with single aptamers.^{72,73} Whereas previous limitations in selection technology may have discouraged the use of aptamers in biomaterials, these technological advancements may open new applications as discussed below.

Biomaterial applications

Significant interest has developed over the past several decades towards creating synthetic environments to study and control the behavior of cells *in vitro* and *in vivo*. Popular targets include implanted pluripotent stem cell-derived progeny, resident or implanted multipotent stem cells, and immune cells due to their potential therapeutic implications. Biomaterials that allow for user-directed cell signaling, as well as create an environment in which endogenously recruited or implanted cells can alter or respond to their environment on demand, could represent a significant advancement in therapeutic applications. Below, we highlight some current biomaterials applications focused on manipulating cell adhesion, angiogenesis (growth of new blood vessels), repair of central nervous system (CNS) and peripheral nervous system (PNS) injuries, and directing immune cell function, and make suggestions for how the selection methods described earlier could augment these approaches.

Cell adhesion

Requirements for cell adhesion via integrin expression patterns can change as cells differentiate and mature. For example, a handful of manuscripts describe synthetic constructs with defined peptides that support pluripotent stem cell self-renewal,^{74–76} but surface cues can also influence differentiation propensity.⁷⁷ In human mesenchymal stem cells (hMSCs), available RGD sequences can induce early chondrogenesis but may produce an inhibitory affect if RGD sequences persist later in the native differentiation timeline. This particular issue was combatted by synthesizing an enzymatically cleavable peptide containing both an RGD adhesion sequence as well as a matrix metalloprotease 13 cleavage site that was subsequently crosslinked into a PEG hydrogel;⁷⁸ hMSCs were then able to remove the RGD peptide from their environment as controlled by their native timeline and increase the amount of desirable chondrogenic differentiation compared with a control. Other researchers have employed a different approach by engineering a fibronectin fragment to have greater affinity for the integrin $\alpha 5 \beta 1$, which resulted in enhanced osteogenic differentiation from MSCs.⁷⁹ For user-directed intervention, adhesion can also be controlled by photoactivatable caged peptides,⁸⁰ which can be de-protected at desired time points.

For more complex control over cell adhesion, different peptide epitopes within a biomaterial could be masked by aptamers evolved to bind with moderate to high affinity for each individual peptide using the methods summarized above. Then, by introducing complementary oligonucleotide strands into the biomaterial, the peptides could be

unmasked at defined times, thus sequentially exposing adhesion epitopes (Figure 1(a)). This approach could be especially useful for cell catch-and-release strategies, many of which already employ aptamers and logic-based encryption.^{81–87} If desired, the adhesion epitopes could be blocked again at a later time point by addition of the original aptamer. This complementary strand molecular trigger strategy has been demonstrated for timed growth factor release from hydrogels *in vitro*,^{54,55} and complementary strand technology has been used to target hydrogels *in vivo*,⁸⁸ thereby lending evidence to its suitability. A potential advantage of this aptamer approach over peptide photoactivation would be the amount of diversity in capping ligands that could be achieved with aptamers compared with available permutations in caging chemistry on peptides, as well as the ability to perform cycles of epitope capping and uncapping using complementary strands. However, a potential pitfall could be the long-term stability of an aptamer-based cap. Aptamers with sufficiently slow off-rates for the adhesion peptides would have to be selected, and nuclease-resistant aptamer backbones could be considered for additional stability.⁸⁹

Angiogenesis

An important potential use of biomaterials is to promote the growth of new blood vessels in damaged tissue or within a transplanted construct,^{90,91} either by sequestration or delivery of growth factors or promoting an environment that directs cellular responses. As detailed in the introduction, delivery of VEGF using affinity peptides is one means of promoting angiogenesis. Promiscuous binding of growth factors using GAGs or ECM fragments is another means of facilitating these processes.^{9,92–94} Yet, these approaches offer no avenue for terminating the process on demand, which may be important due to risks of decreased perfusion and hypoxia in hyperbranched vascular networks.⁹⁵ Mooney and coauthors offered an interesting solution to this problem by co-releasing VEGF and an anti-VEGF antibody within scaffolds to generate spatially defined angiogenic and antiangiogenic regions.⁹⁶ However, release rates were dependent on the biodegradable properties of the scaffold. In theory, angiogenesis could be tuned using aptamers that separately bind VEGF and the anti-VEGF antibody, where the rate of VEGF release could be controlled by complementary strand displacement targeting the first aptamer and the overall process could be terminated by strand displacement targeting the second aptamer (Figure 1(b)). Antiangiogenic peptides could also be incorporated within the biomaterial and capped with high-affinity aptamers, and then unmasked at the desired time by strand displacement as another potential route to address this issue. Several aptamers for the angiogenic VEGF-165 isoform have already been identified that inhibit its receptor binding capability^{97–99} and would potentially be suitable for these applications. In principle, on-demand activation and inhibition of any pathway of interest could be accomplished using these molecular trigger strategies (Figure 1(b)).

Figure 1 Directing cell behavior using *in vitro* selected affinity reagents. (a) Adhesion peptides (e.g. RGD sequences) can be conjugated to a synthetic construct to facilitate cell attachment. *In vitro* selection can be used to isolate aptamers with high affinity for these adhesion peptides and their addition to the construct would prevent integrins from binding to the adhesion peptides. Introduction of the aptamer complementary strands would release them from the adhesion peptide, allowing user directed intervention of cell adhesion. (b) High-affinity aptamers can be selected against proteins that activate a signaling cascade (e.g. growth factors or cytokines) and proteins that inhibit a signaling cascade (e.g. endogenous inhibitors or antibodies against receptors). When both activators and inhibitors are bound to a matrix, addition of the complementary strand to the activator-conjugated aptamer will release the activator to initiate signaling. Addition of the complementary strand to the inhibitor-conjugated aptamer will terminate its activity. This process assumes concentrations and distributions of each molecule have been tuned appropriately within the biomaterial. (c) Aptamers or peptides can be selected that inhibit binding of growth factors or cytokines to receptors. If the selection is performed correctly, these inhibitors can be highly specific to receptors even if they are very similar. Incorporation of different aptamers/peptides into biomaterials will selectively inhibit choice receptors, thereby biasing signal transduction depending on which growth factors are available to the cells. Addition of complementary strands could also change the availability of receptor-binding pockets to tune biological responses on demand. Moreover, the biomaterial could be engineered to recruit or release growth factors of interest that would only be able to target these receptors if they were uninhibited

Nervous system repair

Implanted biomaterials have shown great promise for enhancing CNS and PNS tissue repair after injury. A host of growth factors have been delivered from implanted scaffolds via controlled release strategies to enhance tissue regeneration.⁵ Some strategies have relied on release of different factors at varying rates via changes in materials properties.¹⁰⁰ More often, strategies have relied on release of single or multiple factors from affinity-based scaffolds, typically neurotrophic factors, by using heparin-binding protein motifs.^{101–103} Phage display has also been used to isolate peptides with varying affinity for nerve growth factor, which were then tested in a dorsal root ganglia

sprouting model.¹⁰⁴ In addition, combinations of scaffolds and neural stem cells (NSCs) or more restricted neural progenitors have been tested to improve recovery in injury models.^{105–110}

Injured nervous system repair requires coordinated cell responses including differentiation, migration, balances between outgrowth of desired cells and inhibited growth of undesired cells, control of inflammation, and circuit connectivity.^{111,112} Therapeutic delivery efforts are becoming more complex to assist with these intricate events,¹⁰⁹ and more fine-tuned control of cell behavior could provide additional benefits. For example, differential signaling through bone morphogenetic protein receptor 1A (BMPR1A) and

BMPR1B in astrocytes can affect glial scar formation, immune cell infiltration, and axon density in a model of spinal cord injury.¹¹³ A biomaterial containing a peptide or aptamer with selective blocking affinity for one BMPR1 subtype over another, along with an artificial motif for sustained local release of a therapeutic BMP, could potentially improve the repair process. Neuronal axon regeneration can also be inhibited by membrane-bound ligands on neighboring cells or secreted molecules in the microenvironment.¹¹⁴ A biomaterial that could release growth factors to promote repair while simultaneously antagonizing axon growth-inhibitory ligands using tethered peptide or aptamer inhibitors could potentially augment circuit reconnection. Each of these approaches would rely on using the selection techniques summarized earlier to identify peptides or aptamers that could discriminate between structurally related receptors, because off-target activation or inhibition outside of the intended pathway could have undesired outcomes (Figure 1(c)). A more exhaustive literature search could likely identify other neural pathways of interest, and advancements in the biology of nervous system injury and repair will be crucial for identifying new intervention modes.

Immune function

Implanted biomaterials often cause acute inflammation and foreign body responses due to the innate immune response.¹¹⁵ Transition to an adaptive immune response can also lead to chronic inflammation at the implantation site.¹¹⁶ Immune responses can have positive or negative effects on biomaterial function,¹¹⁷ and the physical properties of the material can contribute to these responses.¹¹⁶ However, modulation of signaling using soluble or insoluble cues can also influence immune responses. For instance, systemic intravenous treatment with an antibody targeting integrin $\alpha 4 \beta 1$ after spinal cord injury can reduce leukocyte infiltration and improve functional recovery.¹¹⁸ Because implanted materials are being tested to help correct these injuries, a similar strategy employing a biomaterial-tethered, integrin $\alpha 4 \beta 1$ -specific peptide or aptamer could have similar benefits. Local adenoviral delivery of soluble receptors for interleukin-1 (IL-1) and TNF α reduced leukocyte infiltration in an arthritis model,¹¹⁹ and these soluble receptors could potentially be replaced with peptides or aptamers in implanted constructs. Lentiviral delivery of IL-10 can promote macrophages toward an M2 anti-inflammatory phenotype¹²⁰ or reduce leukocyte infiltration,¹²¹ and an IL-10-specific aptamer or peptide within a biomaterial could prospectively concentrate this cytokine to serve the same purpose. Many more examples for regulating pro- and anti-inflammatory signals have been presented in the literature and are summarized elsewhere.¹¹⁶

More broadly, inflammatory signaling cascades are vast and complicated. More than 40 cytokines have been classified as interleukins and clustered into subfamilies based on sequence homology and receptor chain similarities.¹²² As an example, the IL-1 family consists of 11 family members, including 7 pro-inflammatory cytokines and 4 anti-inflammatory cytokines, which signal through

10 different receptors.¹²³ The ability to regulate these cytokine pathways through specific recruitment of interleukins and transient inhibition of their receptors to bias particular signaling cascades could have profound effects on how cell-free biomaterials affect tissue function or the survival and integration of cell-laden biomaterials after implantation (Figure 1(c)). For receptor inhibition, *in vitro* selection of high-affinity peptides or aptamers may be particularly useful. Cells can naturally shed endogenous receptors to inhibit cytokine function,¹²⁴ and efforts have been made to mimic this process by using soluble, extracellular receptor fragments.¹¹⁹ However, these receptors naturally exhibit relative promiscuity for many related cytokines,¹²² which limits their ability to inhibit specific cytokines. In contrast, aptamers or peptides could be selected to block specific interleukin receptors or bind interleukins to prevent their interaction with desired receptors. This route has already been pioneered with the selection of an IL-6-specific aptamer that blocks binding of IL-6 to the IL-6 receptor.¹²⁵ Aptamers have also been selected that bind the IL-6 receptor,^{126,127} although their ability to interrupt IL-6 binding was not characterized. Selection of aptamers against each interleukin and interleukin receptors could further expand the ability to engineer immune function, and this route to precision extracellular signal engineering could be applied generally to other pathways (Figure 1(c)).

These approaches could also have potential implications for modulating natural and pathological processes that are intimately tied with immune function, such as wound healing and fibrosis. Wound healing involves diverse cellular functions including inflammatory responses, angiogenesis, matrix remodeling, and tissue maturation, and a myriad of growth factors act on multiple cell types to regulate these activities. Deregulation of wound healing can lead to fibrosis, which is a leading cause of death.¹²⁸ Materials have been engineered to promote wound healing by having promiscuous affinity for growth factors,⁹³ and growth factors have been engineered to have extremely high affinity for ECM for the same purpose.¹²⁹ Yet, similar to the arguments put forth for angiogenesis, these routes offer no mechanism to terminate these processes on demand, which could eventually lead to pathology. Modular affinity reagents allowing routes for pathway activation and deactivation could potentially augment the positive outcomes seen in previous studies,^{93,129} while minimizing unwanted side effects.

Emerging tools that can be coupled with selection techniques

While aptamer strand complementarity can be a useful approach for tuning release kinetics and unmasking bioactive epitopes, it also opens possibilities of utilizing logic gate strategies for responsive biomaterials. DNA devices have been developed that contain aptamer motifs, and when these motifs bind their targets, the device releases a therapeutic payload.^{130,131} Similar devices could be incorporated into biomaterials that recognize endogenously recruited cells and release growth factors or cytokines to bias their differentiation status; stability of the devices and their ability to carry out functions in a complex

biological environment will likely be important considerations. Optical control of ligand recognition via photocleavable nucleic acid modifications may also play a future role in aptamer applications.^{132–134} Heterogeneous organization of these functionalities within biomaterials constructs could then direct cell identities and phenotypes in a more robust manner.^{135–137} Aptamer catch-and-release strategies could also be employed to recruit endogenous cells into particular biomaterials locations by recognition of cell-specific surface receptors.^{81–87} It is likely that advancements in synthetic chemistry and 3D printing, coupled with further progress in *in vitro* selection techniques, will lead to exciting new developments in these areas.

Discussion

Engineered affinity reagents offer many potential advantages over naturally occurring biological motifs for biomaterials applications. Whereas affinity reagents derived from endogenous motifs often exhibit promiscuity affinity for many different targets, particularly proteins with similar structures or sequence homology, affinity reagents generated using *in vitro* selection techniques can be screened to possess optimum binding properties for desired versus undesired targets. In this manner, selected affinity reagents can be used to precisely direct cell behavior by manipulating the availability of growth factors and receptors in the extracellular space. Furthermore, aptamers offer emerging opportunities in dynamic actuation, and their relatively inexpensive production and ease of chemical modification may encourage their use. As *in vitro* selection processes continue to be refined to allow faster isolation of affinity reagents with improved specificity that can carry out functions in complex *in vivo* environments, engineered peptides and aptamers could become an exciting addition to the toolbox of biomaterials engineers, chemists, and researchers overall.

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