Bioadhesive Surfaces for Scaffold Design

In the physiologic environment, most cells are surrounded by a functional, protein-rich extracellular matrix (ECM). This matrix, primarily consisting of fibrillar proteins such as fibronectin and collagen, not only organizes cells into three-dimensional structures but also conveys mechanical and biochemical stimuli that control cell survival, proliferation, and tissue genesis.

To build three-dimensional tissue and organ substitutes, tissue engineers design natural and synthetic polymer scaffolds that mimic this critical ECM. These scaffolds can then be used alone or in combination with cells to guide tissue repair. Because of the central role of this matrix in cellular processes and tissue formation, scaffold design has emerged as one of fundamental research principles guiding the field of tissue engineering.

Early efforts in designing tissue engineering scaffolds have focused on recapitulating the structural and mechanical functions of the ECM, as well as its ability to facilitate mass transport of nutrients and waste. As scaffold architecture becomes increasingly advanced and tissue-specific, researchers are beginning to look at another critical function of the ECM—the ability of its proteins to specifically interact with cells and stimulate certain cellular processes, including tissue formation and cell adhesion.

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Cell adhesion to the ECM is primarily mediated by *integrins*, a widely expressed family of cell surface adhesion receptors consisting of α and β subunits. In addition to anchoring cells and providing tissue structure, integrins transmit intracellular signals that direct cell migration, proliferation, and differentiation, all of which are critical processes for tissue repair and regeneration.

Upon ligand binding, integrins rapidly associate with the actin cytoskeleton and cluster to form focal adhesions, which are discrete complexes of intracellular structural and signaling proteins. These focal adhesion sites function as structural links between the cytoskeleton and ECM. They also activate intracellular signaling pathways that control gene expression and protein activity.

Integrins thus function as the principal mediators of the molecular dialogue between a cell and its ECM environment. They bind specific amino acid sequences in ECM proteins, thereby triggering intracellular signaling events. Many of these integrin-activated signaling cascades are critical for tissue growth, repair, and regeneration.

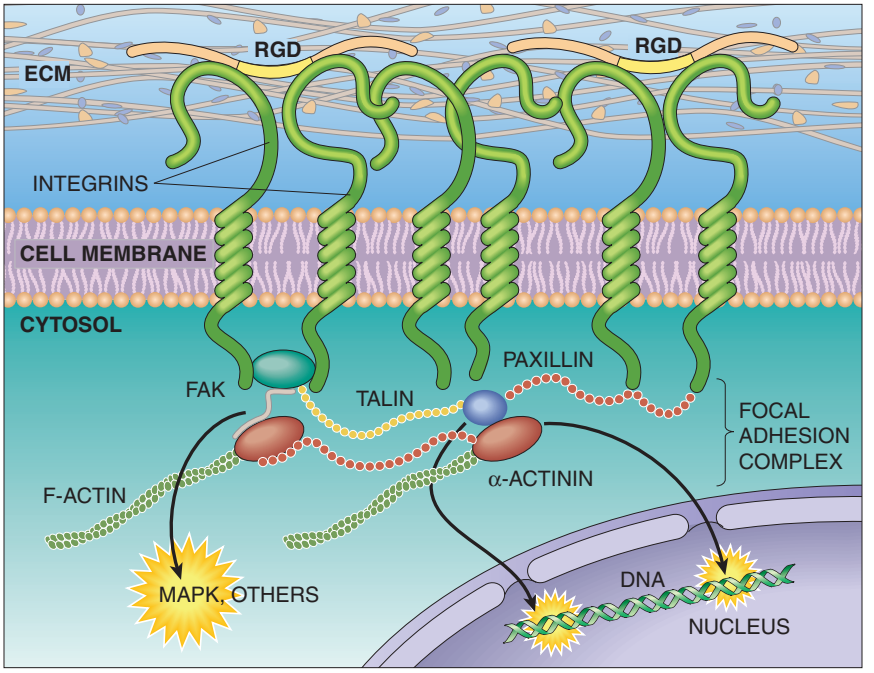
Tissue engineers can guide the cellular response in a scaffold by incorporating discrete integrin-binding motifs derived from ECM components. This biomimetic surface modification strategy has the potential to enhance cell-scaffold interactions and encourage biospecific cell adhesion, differentiation, and tissue formation.

Numerous cell adhesive surfaces have been designed to incorporate the RGD (arginine-glycine-aspartic acid) sequence found in a wide variety of matrix proteins and recognized by a number of integrins, including $\alpha_v\beta_3$, and $\alpha_{IIb}\beta_3$. Non-fouling, nonadhesive materials, such as poly(ethylene glycol) or alginate, are often used as the support matrix to reduce background effects due to nonspecific protein adsorption. Incorporation of these RGD peptides onto tissue-engineering scaffolds results in enhanced cell adhesion and tissue formation in many cases. For example, RGD immobilization improves early bone ingrowth and matrix mineralization in implanted bone tissue-engineering constructs.

Although these results have been promising, several critical factors limit the potential of this approach for tissue repair and regeneration. First, the biological activity of short adhesive peptides, such as RGD, is significantly lower than that of the native protein, due to conformation-dependent effects and the absence of additional functional domains. More significantly, RGD peptides are limited by a lack of specificity for particular integrins and thus allow minimal control over cellular responses.

These limitations underscore the need for engineering surfaces that present more complex, biomimetic peptide formulations that target particular integrin receptors and signaling cascades. Tissue engineers could potentially exploit this integrin specificity to optimize cell function and tissue formation in implanted scaffolds.

One of the most significant limitations of RGD peptides is their inability to bind the putative "fibronectin receptor"— $\alpha_5\beta_1$. This integrin has been implicated in osteoblast and myoblast differentiation, cell cycle progression, and fibronectin matrix assembly. Surfaces that specifically target these processes by engaging the $\alpha_5\beta_1$ integrin could potentially improve tissue regeneration, particularly



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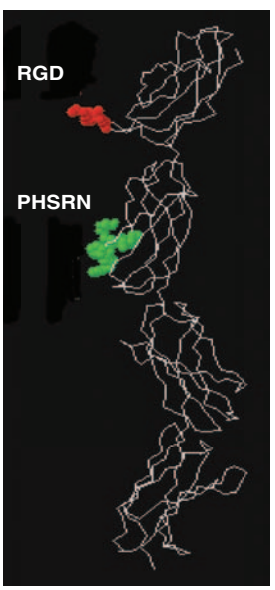
Integrins mediate cell adhesion by attaching at one end to proteins in the ECM and at the other end to the cell's own scaffolding, the actin cytoskeleton. Integrins connect to this cytoskeleton through a highly organized complex of structural and signaling molecules, known as a focal adhesion. Integrins relay messages from the ECM that ultimately affect intracellular processes, such as gene expression and protein regulation.



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Fibronectin fragment spanning the 7th to 10th type III repeats, showing the RGD and synergy sites that comprise the cell-binding domain.

for applications such as bone tissue engineering.

RGD-modified scaffolds have fallen short of this goal because the binding of $\alpha_5\beta_1$ integrin requires both the PHSRN (proline-histidine-serine-arginine-asparagine) motif in the 9th type III repeat and the RGD motif in the 10th type III repeat of fibronectin. In combination, these two domains synergistically bind the integrin to provide stable adhesion and subsequent intracellular signaling events.

In efforts to target the $\alpha_5\beta_1$ integrin, mixtures of RGD and PHSRN peptides, either independently or within a single linear backbone, have been immobilized onto nonfouling support scaffolds. Although these bioadhesive surfaces promote cell adhesion, their specific $\alpha_5\beta_1$-binding activity has not been compared directly to that of native fibronectin.

Due to the acute sensitivity of $\alpha_5\beta_1$ to small perturbations in the orientation and conformation of these domains, reconstitution of the proper binding structure and orientation using synthetic peptides remains a challenge.

As an alternative to these synthetic strategies, we are currently functionalizing nonadhesive surfaces with a recombinant fragment of fibronectin spanning the 7th to 10th type III repeats, which include the PHSRN and RGD binding sites in the correct spatial orientation and conformation. These fibronectin-mimetic surfaces support $\alpha_5\beta_1$-mediated adhesion and focal adhesion assembly at levels comparable to native fibronectin.

In addition to providing enhanced integrin specificity over short RGD peptides, the recombinant fibronectin fragment also offers several advantages over the entire fibronectin molecule, including reduced antigenicity, enhanced cost efficiency, and the elimination of additional binding sites that may have a confounding effect on the desired cell response. Recombinant fragments also allow control over specific biochemical characteristics through site-directed mutagenesis to enhance protein immobilization, orientation, and activity on synthetic polymer scaffolds.

Further studies aimed at elucidating the role of $\alpha_5\beta_1$ in tissue formation and the effects of fragment immobilization on cell function in vivo are necessary to establish the potential of these fibronectin-mimetic bioadhesive surfaces for directing cellular responses.

Relatively little research has concentrated on engineering bioactive surfaces that target non-RGD-binding integrins, primarily due to the lack of known binding sequences, as well as the complex binding interactions associated with other ECM proteins. Nevertheless, signals triggered by non-RGD-binding integrins are essential for differentiation and tissue regeneration in many cell types.

In particular, the collagens constitute a family of abundant ECM molecules that contribute significantly to the integrity and mechanical properties of tissues such as bone, skin, cartilage, and tendon. These structural proteins also play a fundamental role in promoting cell adhesion and mediating intracellular signals critical to tissue function.

Integrin-type collagen receptors, such as $\alpha_1\beta_1$, $\alpha_2\beta_1$, $\alpha_{10}\beta_1$, and $\alpha_{11}\beta_1$, mediate cell adhesion to various collagen types and activate cytoplasmic signal transduction pathways. In particular, integrin $\alpha_2\beta_1$, the primary receptor for type I collagen, is abundantly expressed on platelets, epithelial cells, fibroblasts, osteoblasts, chondrocytes, endothelial cells, and lymphocytes and has been implicated in cell cycle progression and matrix production.

For example, $\alpha_2\beta_1$-mediated osteoblast adhesion to type I collagen controls osteoblast differentiation and matrix mineralization. In the cardiovascular system, the adhesion of blood platelets to collagen via $\alpha_2\beta_1$ mediates the first phase of the hemostatic response to endothelial injury. Integrin $\alpha_2\beta_1$ also regulates the production of matrix metalloproteinase-1 in response to extracellular collagen, which may be critical to the migration of keratinocytes during wound healing.

Recent studies have proposed a hexapeptide sequence, GFOGER (glycine-phenylalanine-hydroxyproline-glycine-glutamic acid-arginine), from type I collagen as a major binding locus for the $\alpha_2\beta_1$ integrin. However, in exploiting the binding properties of this hexapeptide sequence, researchers have had to consider the unique conformation of the native collagen protein and its impact on GFOGER's activity.

Collagen tertiary structure consists of three chains supercoiled in a right-handed helix, yielding a characteristic triple helical coiled-coil. Integrin recognition of the GFOGER sequence is entirely dependent on the presence of this triple helical conformation, underscoring the crucial role of collagen's tertiary structure in $\alpha_2\beta_1$ integrin binding.

We have recently engineered bioadhesive surfaces that specifically target the $\alpha_2\beta_1$ integrin using a stable, triple-helical collagen-mimetic peptide. This bioactive peptide consists of the GFOGER adhesion motif flanked by several GPP (glycine-proline-proline) triplets. The imino acid proline stabilizes a triple helical structure by imparting a high degree of steric restriction on the local twisting of peptide chains. The glycine at every third position is required for close packing at the interface of the three peptide strands.

This triple-helical GFOGER peptide specifically targets the $\alpha_2\beta_1$ integrin receptor, and its cell adhesion activity is comparable to that of type I collagen. For bone tissue-engineering applications, GFOGER-functionalized surfaces support bone cell differentiation and matrix mineralization at greater levels than unmodified scaffold materials, such as polylysine and poly(lactic-coglycolic acid). This triple-helical GFOGER peptide represents a robust and versatile approach to the design of collagen-mimetic bioadhesive surfaces that specifically target the $\alpha_2\beta_1$ integrin.

Biomimetic, integrin-specific adhesive motifs from ECM proteins have emerged as a promising surface modification strategy for tissue-engineering scaffolds. While considerable progress has been achieved using short peptides, such as RGD, that bind a variety of integrin receptors, engineered bioadhesive substrates must also incorporate additional complexities associated with the ECM environment.

These include native secondary and tertiary conformations, additional synergistic binding domains, and integrin specificity. The next generation of scaffold surfaces also might target several integrins using a mixture of biomimetic peptides or exhibit patterned or clustered motifs to mimic compositional variations in the native ECM.

The development of these bioinspired surfaces represents a unique intersection of advances in biochemistry, cell biology, synthetic chemistry, and materials science. Further study of these complex issues involved in mimicking the extracellular environment will ultimately allow tissue engineers to tailor scaffold surfaces for optimal cellular responses, such as growth, migration, or tissue formation.

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