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PROSPECTS

Manipulation of Cellular Interactions With Biomaterials Toward a Therapeutic Outcome: A Perspective

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Abstract Manipulation of the wound healing process and the manner in which tissues interact with inert biomaterials were both made possible with the discovery of arginine-glycine-aspartic (RGD) acid as a major cell recognition signal in the extracellular matrix. Whether promoting cell adhesion or selectively inhibiting cell-cell aggregation mediated by integrin cell surface receptors, RGD-containing peptides can be rationally designed to incorporate both stability and integrin specificity. Synthetic peptides containing this sequence have been linked to biodegradable biopolymers and introduced for the enhancement of dermal and corneal wound healing. By accelerating the healing reaction using RGD-containing peptides, the quality of regenerated tissue seems to be improved, the extent of fibrosis restricted, and the risk of microbial infection may be reduced. Controlling the degree of fibrosis that often accompanies the healing of wounds and the reaction of tissue to foreign materials can also be achieved by natural antagonists of fibrogenic activity of TGF-beta animal models of kidney fibrosis. These advances in the biotechnology of wound healing and tissue regeneration eventually will have an overall impact on the quality of health care. © 1994 Wiley-Liss, Inc.

Key words: wound healing, fibrosis, microbial invasion, growth factors, extracellular matrix

To successfully repair or replace a failed body part, be it bone, tendon, skin, or any internal organ, physicians are faced with the task of balancing three powerful forces that are, by nature, pitted against one another. These are the processes of wound healing, fibrosis, and microbial invasion. Many tools available to ward off infection compromise wound healing, and delayed wound healing, or inflammation, can exacerbate fibrosis. Moreover, growth factors, such as TGF-β and PDGF, frequently cited as promoters of wound healing, can actually drive fibrosis which in turn can itself impair successful healing. Even though accelerated healing offers the most promise for reducing the risk of infection and the resulting inflammation that can drive scar formation, therapeutic attempts to accelerate the wound healing process have met with relatively little success. Indeed, the successful integration of a prosthetic device into the body could be envisioned as a race against time among these three opposing forces.

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As the number of prosthetic tissue augmentations grows to impact more and more adults throughout the world, we are not only faced with the challenge of inventing novel materials and innovative applications in tissue bioengineering, but also with finding ways to enhance the performance of existing prosthetic devices. Such improvements should reduce the risk of device failure and the potential morbidity and even mortality related to that failure. To accomplish this, we are taking advantage of the emerging synergy between pure mechanical engineering and materials science and the areas of biology and bioengineering.

In most tissues of the body, cells reside within an interwoven network of complex glycoproteins referred to collectively as the extracellular matrix (ECM). During the past decade, a great deal of progress has been made in identifying the individual components of the ECM and in determining their structure and to some extent their function. Each of the ECM glycoproteins has the property of interacting with itself and other ECM components to form the sturdy, yet flexible, three-dimensional structure of the body. However, it is the ability of the cells to specifically recognize and attach to the individual com-

ponents of the ECM through cell surface receptors, along with the capacity of the ECM to bind and regulate the impact of growth factors in the local environment of the cell, that likely holds the keys to understanding the profound influence that the ECM has on processes such as tissue regeneration, development, and even the progression of tumors. As we continue to build our understanding of how cells interact with their natural environment, the interstitial extracellular matrix, it is now feasible to develop rational therapeutic agents that manipulate this cell behavior.

In the mid 1980s it was discovered that many of the ECM proteins carry a sequence of amino acids, arginine-glycine-aspartic acid, RGD [1], to which cells can bind using specific surface receptors [2]. This discovery rapidly led to the identification of a large family of cell surface adhesion receptors, now called integrins [3,4], and the findings that many of these integrins specifically recognize the RGD sequence in one or more of the ECM proteins (Fig. 1). Integrins play a prominent role among the mechanisms involved in cell adhesion and migration, and the ability to synthesize RGD-containing peptides that bind one or more integrins allows manipulation of those cellular events that are mediated by the RGD-binding integrins.

Because the RGD sequence is quite small, it can be easily engineered to accomplish a number of objectives, such as stability, affinity, and specificity. RGD-containing peptides can be stabilized to proteolytic degradation in the body by incorporating into them nonnaturally occurring amino acids or the D-form of an amino acid (such as D-arginine), by substituting the peptide bond with alternate chemical structures, or by

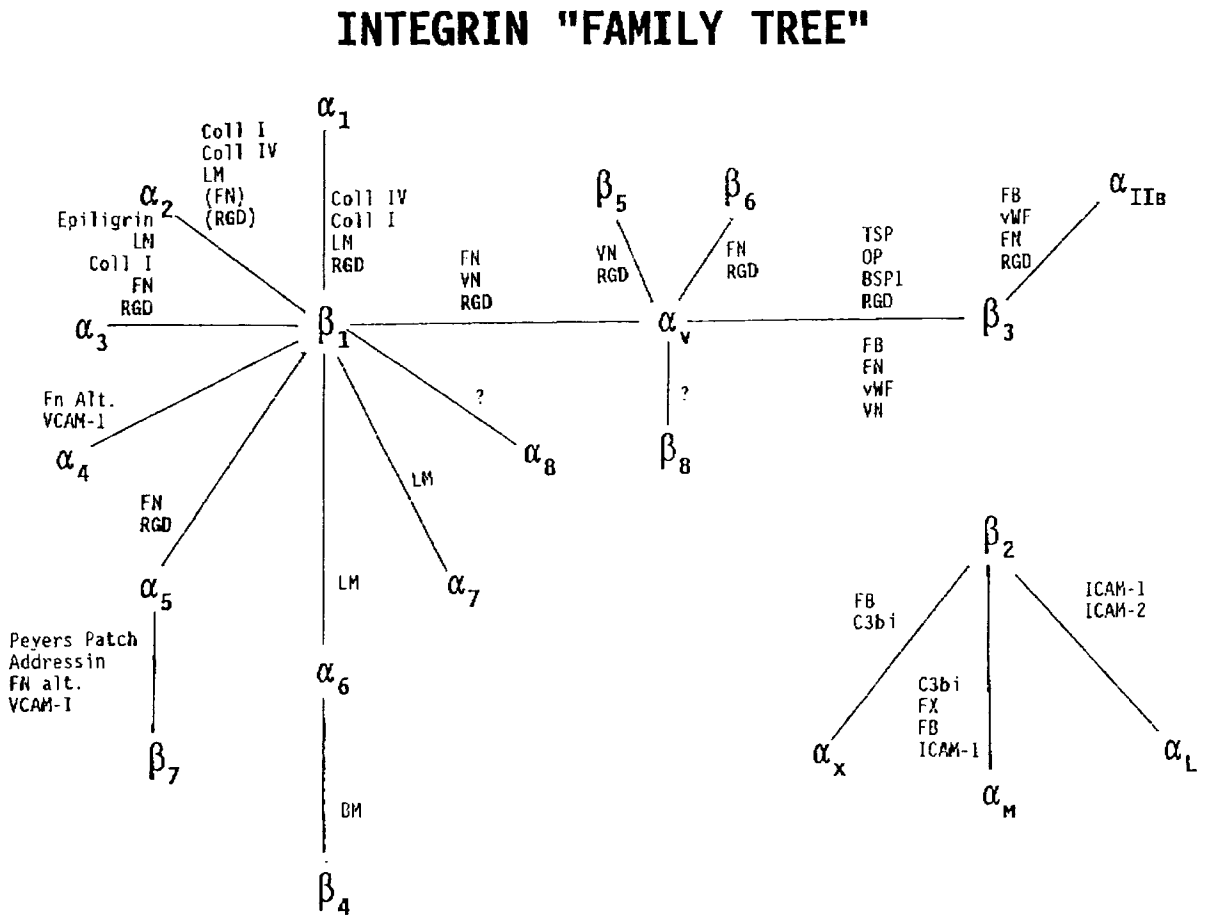

Fig. 1. Diagram of the integrin family showing subunit associations. The known ligand specificites are indicated. Nine separate integrins have been reported to bind the RGD sequence. BSP1, bone sialoprotein I; Coll I, Coll IV, collagens type I and IV; C3bi, the third component of the complement cascade; FB, fibrinogen; FN, fibronectin; Fn Alt., the alternatively spliced region of fibronectin; FX, factor ten; LM, laminin; OP, osteopontin; TSP, thrombospondin; VN, vitronectin; vWF, von Willibrand factor.

blocking the termini with chemical groups or with NH_2-terminal to COO^--terminal cyclization [5,6]. These modifications have also been found to enhance the affinity between the peptide and an integrin by virtue of stabilizing the peptide in an active conformation. Frequently the resulting peptides bind selectively to one RGD-directed integrin over another. Presumably this integrin specificity is due to the differential preference of certain integrins for a characteristic presentation of the RGD sequence [5]. When taken out of the context of a protein, the RGD signal loses both affinity and specificity [1–4,6]; thus, the enhancement of both potency and integrin selectivity becomes important when considering the use of RGD peptides for therapeutic purposes. When the intent is to provide immobilized RGD peptides to promote integrin-mediated cell adhesion or migration, it may be desirable to target integrins on one cell type and not on another. On the other hand, if the goal is to inhibit undesirable adhesion or migration of a particular cell type by blocking integrin-mediated cell/matrix interaction, both affinity and specificity are required to avoid the potential risk of unwanted side effects. The following developmental projects illustrate how these approaches can be used for the therapeutic, rational application of ECM technology.

By linking an 18 amino acid RGD-containing peptide (Fig. 2) to a large biodegradable polymer such as hyaluronic acid or high molecular weight chondroitin sulfate, we have created synthetic ECMs that serve as effective supports for wound healing of the skin or eye, respectively [7]. In chronic or severe wounds of the skin and the eye, the normal ECM may be destroyed to such an extent that there is little remaining to support the migration into the wound area of those cells responsible for wound healing. According to our hypothesis, this requires that fibroblasts must build a provisional matrix from the edge of the wound toward the center to support the subsequent migration of the endothelial and epithelial cells into the wound bed. It is here that timing may become a factor in determining the quality of the regenerating tissue. If scar tissue becomes too dense at the edge of the wound, it could form a barrier through which the trailing cells cannot effectively follow. Alternatively, if the fibroblasts are compromised, as in diabetes, aging, or concomitant medication, for example, the degradative processes in the wound could overcome the ability of fibroblasts to build the provisional matrix. The rationale behind the synthetic ECM approach is to provide a stable provisional matrix that will support the migration of cells deeper into the wound bed where they can repair all parts of the wound simultaneously rather than being limited to the periphery. This synthetic matrix would then be slowly degraded as it is replaced with new tissue. This approach is now being tested in the clinic in wounds of the skin [14].

Of the many complications associated with diabetes, such as neuropathy and nephropathy, one that is frequently encountered is the development of pressure sores on the soles of the feet. For reasons possibly related to the diabetic condition, these sores may become chronic ulcerations that remain open for many months to years, during which time they are at a high risk of infection. Moreover, when these wounds do heal they frequently break down and reopen. We have observed in a prospective, randomized, blinded, multicenter clinical trial that diabetic foot ulcers treated with the hyaluronic acid-based peptide matrix healed significantly faster than wounds treated with the best standard of care in the control group. At the end of 10 weeks of treatment, four times more matrix-treated wounds had completely healed than in the control group. Work to confirm these results in other types of wounds is currently under way. It remains to be seen whether the wounds that healed under the influence of the synthetic provisional matrix differ in quality and integrity from those that heal unassisted.

A similar approach has been taken to treat wounds of the eye, where a number of conditions, such as autoimmune disease or aging, can lead to a reduction in the volume of tears. In some cases, the resulting condition becomes quite severe. As a consequence of inadequate tear film, the epithelial cells lining the surface of the eye, or cornea, become desiccated, lose their

G(dR) (dR) (dR) (dR) (dR) GGG (dR) GDSPASSK

Fig. 2. The 18 amino acid peptide designed to make provisional matrices. A, alanine; D, aspartate; dR, D-arginine; G, glycine; K, lysine; P, proline; S, serine.

microvilli, slough off, or are abraded off by the mechanical action of blinking, leaving the underlying immature cells and ECM exposed [15]. The architecture of this surface is no longer capable of retaining moisture (the function of the microvilli), and the condition continues to deteriorate. Clinical studies have been conducted using a synthetic ECM that consists of an RGD-containing peptide tethered to a high molecular weight chondroitin sulfate molecule. The chondroitin sulfate serves to immobilize the peptide on the surface of the eye where it acts to support the adhesion of the epithelial cells. The aim of this therapy is to support the reconstruction of the normal architecture of the surface of the eye, restoring its ability to retain exogenously added moisture. The results obtained using a method to assess the quality of the ocular surface, rose bengal staining, have been encouraging in early clinical studies comparing treatment of the dry eye condition with this synthetic provisional matrix with a commercially available artificial tear (unpublished data).

Materials such as titanium, teflon, silicone, carbon fiber, dacron, and others, often used to fashion prosthetic devices, were originally identified for medical use because of their exceptional strength and physiologically inert quality. Because of this, tissue integration into these materials (bonding) is often slow and frequently inadequate. Many times, as with silicone and dacron, a significant inflammatory, foreign body response will drive the fibrous encapsulation of the synthetic material. This can create significant adverse effects on the implant. The capsule thus formed is capable of contracting, which results in inadequate contact between the prosthetic device and the surrounding tissue. As a consequence, the space resulting at the material interface can be colonized by adventitious bacteria. The fibrous capsule also may prevent an adequate blood supply from reaching the device, further complicating the condition by making systemic treatment of the infection difficult, and the device may need to be removed and replaced. An inadequate blood supply due to encapsulation can also be a problem for the development of encased pancreatic islet cells as an implant for the treatment of diabetes. The secreted insulin will be inaccessible for distribution to the body. Finally, in a notable example, encapsulation has been a significant problem in compromising the function of silicone breast implants. These problems may be overcome by coating the surface of implanted materials with physiological cell attachment sites that initiate integrin-mediated cell interactions and allow for the natural development of tissue structure at the material-tissue interface.

A synthetic, RGD-containing peptide has been designed that will spontaneously deposit on any material surface in such a way that the RGD sequence is available to interact with cell surface integrins (Fig. 3) [8]. When materials coated with this peptide have been implanted into animals, a much more rapid integration into tissue has been observed, as well as, importantly, a significant, persistent reduction in encapsulation. Currently a number of prosthetic devices are being prepared for clinical studies using the RGD-based peptide coating. These include heart valves, catheters, and vascular grafts. Devices such as these that come into contact with blood pose another set of problems as a result of their degree of thrombogenicity.

Platelets are designed by nature to recognize breaks in the vasculature, bind to the exposed, underlying adhesive ECM proteins, and activate the clotting process. Prosthetic materials that are exposed to blood must be designed to avoid triggering the clotting response, lest they become congested by an aggressive clot or initiate microthrombi that are carried by the blood to capillary beds where they can cause ischemic blockage of the blood supply to tissue. An attempt to modify such devices to promote the adhesion of protective endothelial cells may increase the risk of making them thrombogenic. The peptide shown in Figure 3 appears unique in this respect. While it promotes the adhesion and spreading of platelets, it does not stimulate them to initiate the clotting process (unpublished data). A device coating with this characteristic would allow for safer integration of catheters, heart valves, and vascular grafts and reduce the time during which they are at risk. Another way to control thrombus formation is by providing soluble RGD-containing peptides

Ac-GRGDSPASSKGGGGSRLLLLLLR-NH_2

Fig. 3. Peptide designed to coat prosthetic materials. AC, acetyl; L, leucine; NH_2, amide; others as in Figure 2.

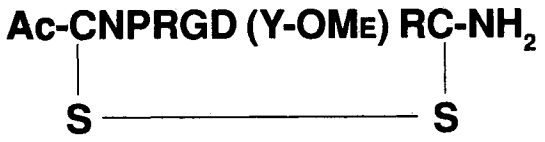

Fig. 4. Peptide designed to specifically inhibit the platelet/fibrinogen interactions that lead to thrombosis. N, asparagine; Y-OMe, o-methyl tyrosine; C, cysteine; S—S, disulfide; others as in Figures 2, 3.

to block the fibrinogen receptor on platelets, thus inhibiting fibrinogen-mediated platelet aggregation [9].

Approaches to thrombosis prevention that involve the inhibition of platelet aggregation carry their own risk—that of compromising the body's ability to control bleeding. Even "minor" bleeding, if allowed to go unchecked, can lead to life-threatening events such as stroke and other forms of internal hemorrhage. An RGD-containing peptide has been designed that, because of its high degree of specificity for fibrinogen receptor, can completely prevent thrombosis without prolonging template bleeding time (Fig. 4) [10,11]. This may prove exceptionally critical in attempting to maintain patency of small diameter, dacron vascular grafts which can leak if clotting is completely inhibited. It has been observed that this specific RGD peptide can completely prevent platelet accumulation onto such grafts without any impact on bleeding time [16]. Another consequence of platelet activation is the release of PDGF and TGF-β which drives the fibrotic process.

In addition to platelet driven fibrosis, chronic or severe injury to tissue can initiate a vicious cycle of TGF-β induction that will drive prolonged accumulation of ECM, scarring that inhibits productive tissue regeneration and can actually compromise organ function [12]. A natural component of the ECM, a proteoglycan called decorin, can act as an inhibitor of the scarring process. Among its many functions, decorin binds and neutralizes the activity of TGF-β [13]. Upon successfully completing the development of decorin as a means of controlling fibrosis, we may then have a handle on both sides of the process of tissue regeneration, enabling us to both promote the healing process and control the body's undesirable responses to tissue injury. Ultimately, we anticipate that this technology will put us into a position to compete and win the race against time and generate successful tissue regeneration.

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