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Matrix-bound growth factors in tissue repair

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Summary

Morphogenesis in tissue development and repair is guided by a variety of signals from the extracellular milieu, including growth factors that are sequestered in the extracellular matrix. Bioengineering approaches have been developed to mimic the natural interactions between growth factors and the extracellular matrix, by engineering biomolecules as novel growth factors and as novel matrix components.

Key words: regeneration; growth factor; VEGF; fibrinogen; fibrin; biomaterials

Introduction

The process of development is guided by a complex interplay between cells and their extracellular milieu. This milieu includes morphogenetic cues displayed on the surfaces of other cells and throughout the extracellular matrix. In this sense, the extracellular matrix serves as a reservoir of morphogenetic signals; how this reservoir displays or releases these signals in response to cellular influences determines its role in tissue homeostasis, in development, and in response to injury [1–5].

The extracellular matrix contains several classes of morphogens. First, it contains a number of adhesion molecules that play important roles in enabling traction between both static and migratory cells and the matrix; moreover, these adhesion molecules signal a number of cell differentiation processes, depending on which adhesion receptors are ligated by these molecules and the degree to which the receptors are activated and downstream signalling is thereby triggered. Second, the extracellular matrix displays these signalling and traction-enabling adhesion molecules in the context of a biomechanical environment. The elastic properties of the matrix have been shown to control cell phenotype and differentiation directly, including lineage selection by stem and progenitor cells [6]. Third, the proteoglycan components of the extracellular matrix are known to bind to and modulate the activity of a large number of growth factors [7]. Although these growth factors are commonly called "heparin-binding", they bind more predominantly to heparan sulfate and chondroitin sulfate proteoglycans in the extracellular matrix. This binding is known to both protect the growth factors from proteolytic degradation and more importantly to regulate their bioavailability. In some cases, growth factors that are bound may be active in the bound state, and in other cases the growth factor may have to be released by enzymatic cleavage of the matrix or even of the growth factor itself in order to reach full activity. In either case, growth factors may be synthesised and sequestered in the extracellular matrix for liberation and activity at a much later time, all regulated by the enzymatic demand of cells in the environment.

These complex signalling events in development all play important roles in processes of tissue remodelling and repair. Based on the prevalence of the above-mentioned interactions and phenomena in adult morphogenetic processes, these activities of the extracellular matrix have caught the attention of bioengineers, biomaterials scientists, and pharmaceutical scientists, to harness and, if possible, exploit them. This background forms the topic for the present mini-review (focusing here on the third facet of the extracellular matrix mentioned above). We and others have attempted to exploit and mimic growth factor-matrix interactions in the context of tissue repair and remodelling. In this work, concepts of biomaterial science, bioengineering and protein engineering are combined to develop novel materials as cell invasion matrices on the one hand, and to develop novel engineered variants of growth factors specifically for binding to such matrices. The resulting materials and molecularly engineered growth factors are of interest both in basic cell biology research and as potential therapeutics in tissue repair.

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Biopolymer-bound growth factors

Biopolymers make up a large fraction of the extracellular matrix, including macromolecules such as collagen, elastin and hyaluronic acid, and the glycosaminoglycans heparan sulfate and chondroitin sulfate as components of proteoglycans. Growth factor affinity has been described at least for collagen (eg some of the bone morphogenetic protein family members binding to collagen II and IV [8, 9]) and glycosaminoglycans (eg members of the fibroblast growth factor family, the transforming growth factor β superfamily, and the vascular endothelial growth factor family, to name a few [10]).

One interesting biopolymer found in tissue repair but not in the otherwise healthy organism is fibrin, a crosslinked polymer network formed by polymerisation of fibrinogen. While some of the fibroblast growth factors have been shown to have an affinity for fibrin [11, 12], this provisional matrix material found in tissue repair does not display the same general affinity for growth factors, as do the extracellular matrix molecules not involved in tissue repair. This distinction is interesting, in that the cellular components of the clotting cascade, namely blood platelets, are a source of a number of growth factors, such as platelet-derived growth factor, members of the transforming growth factor β superfamily, and members of the fibroblast growth factor family [13]. Thus, in native fibrin blood clots, the cells in naturally-forming fibrin matrices release growth factors, but the fibrin matrix is incapable of binding and retaining those growth factors as would occur when those factors were released in a more physiological, rather than pathophysiological, extracellular matrix.

Just as growth factors bind to the physiological extracellular matrix, bioengineers have sought to develop surgical biomaterials that would also bind such growth factors. Early work on reversible growth factor binding to biomaterials and matrix materials was based on the affinity of many of these growth factors to heparin. Although the growth factors presumably evolved an interaction with heparan sulfate, heparin displays sufficient chemical similarity to bind to these heparan sulfate proteoglycan-binding sites as well. Since heparin is easier to purify, and thus more commonly used in forming affinity chromatography matrices, the binding sites are commonly referred to as heparin-binding sites. Affinity of growth factors for heparin has been used in controlled release from implants [14]. These systems formed controlled release matrices wherein the dissociation kinetics of the growth factor from the bound heparin controlled the overall release rate of growth factor from the matrix. In spite of the potential power of incorporation of heparin-binding growth factors to matrices in this way, the approach has not led to the development of surgically practical methods.

Given fibrin's involvement in native wound healing and tissue repair, fibrin serves as an interesting target in biomaterial development. Fibrin can be formed by polymerisation of fibrinogen (purified from blood) triggered by exposure to the enzyme thrombin (purified from blood or produced recombinantly) in the presence of calcium and the transglutaminase zymogen factor XIII (purified from blood or produced recombinantly; mentioned further below). Fibrin is very commonly used in a wide variety of surgical procedures as a tissue adhesive and sealant. If methods were to be developed to incorporate bioactive agents within fibrin, such as growth factors, they could be used just as broadly as a cell ingrowth matrix for tissue repair and regeneration. Indeed, this is one of its primary roles in the body, to serve as a provisional extracellular matrix, induce and allow cellular ingrowth, and then be gradually replaced by a more physiological extracellular matrix during healing. Methods for incorporation of growth factors would allow healing-inducing matrices to be readily formed and used in tissue repair and regeneration.

We have developed a facile mechanism by which any number of biomolecules can be grafted within a fibrin scaffold during fibrinogen crosslinking [15], including the growth factor-binding molecule heparin. During fibrinogen coagulation, individual fibrin strands are linked together by the transglutaminase enzyme factor XIIIa. This enzyme is capable of linking many biomolecules into fibrin, and it is this activity that we have harnessed. Specifically, bioactive peptides and proteins have been linked into fibrin by either synthesising them or expressing them as bi-partite fusions, one part being a domain that serves as a substrate for factor XIIIa and the other being the bioactive domain of interest [15]. In order to link heparin into a fibrin matrix, a bi-partite fusion peptide was synthesised, consisting of a factor XIIIa substrate and a heparin-binding peptide, modelled after the heparin-binding domain of the protein antithrombin III. In this way, there exists a covalent bond between fibrin and the peptide, an electrostatic link between the peptide and heparin, and an electrostatic link between the heparin and the bound growth factor. Although two of these interactions are only physiochemical interactions, rather than covalent bonds, the overall effect to bind the growth factor to the fibrin matrix can be very strong [16, 17]. This is accomplished by using the bound heparin in high excess to the incorporated growth factor, which results in a prolongation of release more than 100-fold [16, 17]. Using this approach, we were able to demonstrate that release of a number of nerve growth-promoting growth factors could be accomplished from the heparin-laden fibrin matrix [16, 17]. It was further demonstrated that when neurotrophin-3 was bound within fibrin, regeneration in the sciatic nerve and spinal cord was substantially enhanced [18, 19].

In addition to binding to fibrin via heparin affinity, we have also demonstrated that a number of growth factors can be engineered for direct binding to fibrin. In this approach, the growth factor is engineered as a bi-partite or even a tri-partite fusion protein, eg in the latter mode with a domain on one terminus of the protein that binds to fibrin via factor XIIIa, with the bioactive part of the growth factor as a second domain on the other terminus of the fusion protein, with an intervening domain that can be proteolytically cleaved by cell-associated proteases [20, 21]. All cells in migration activate plasminogen to plasmin and matrix metalloproteinase precursors to active matrix metalloproteinases. These active enzymes normally allow the cell to penetrate the extracellular matrix, but here we have used them to liberate growth factors from their bound state in the fibrin carrier to the free state: the enzymes clip the growth factors via the intervening linker between the fibrin-binding domain and the active growth factor domain. Thus, here we have developed a material carrier, and have furthermore developed a variant form of the bioactive growth factor to be delivered by that carrier.

Can such approaches as are described above actually change the way that growth factors function and are used in medicine? A good example to consider is that of the angiogenic growth factor vascular endothelial growth factor (VEGF). VEGF stimulates existing blood vessels to branch and form new blood vessels. Although it was discovered many years ago, VEGF has not yet been developed into a successful therapeutic. One reason is that high concentrations of the growth factor cause the blood vessels to become highly permeable, resembling a tumour vasculature much more than a normal healthy vasculature. This has made it necessary to devise approaches by which to release, in addition to VEGF, also growth factors that can stabilise and mature the nascent blood vessels, countering this untoward effect of VEGF [21–23]. Release of multiple protein therapeutics is very difficult from a practical perspective, and as such this approach has been limited in its application to clinical medicine.

In the attempt to avoid the hyperpermeability that is associated with high concentrations of VEGF, we explored the use of a fibrin-binding variant of VEGF, with an intervening plasmin-sensitive linker [21–23]. Whereas VEGF simply mixed within fibrin and diffused out within a few hours, the engineered variant form of the VEGF was quantitatively bound within the fibrin and remained there until liberated by active plasmin. Our hope was that the very low concentrations of VEGF that were achieved at any point in time due to cell-triggered release would induce enough angiogenesis to be effective, but would not reach the high concentrations that are inductive of hyperpermeability. In animal studies in which vessel quantity and morphology were measured, this hope was indeed realised. Indeed, more angiogenesis was induced by the fibrin-bound VEGF than by the free, wild-type VEGF that was simply mixed into the fibrin carrier. As to quality, when vessel morphologies that are characteristic of hyperpermeability were quantified, the vessels resulting from the fibrin-bound VEGF were demonstrated to be much less tumour-like than those induced from free, wild-type VEGF.

To summarize, the results above demonstrate the potential power of this approach to biomaterial and growth factor engineering: that a biomaterial can be engineered around a growth factor, and that growth factors can be engineered around a biomaterial carrier, and furthermore that much more than the details can be thereby manipulated. Untoward effects of certain growth factors can be addressed, for example the hyperpermeability associated with high concentrations of the growth factor, which has heretofore limited the therapeutic value of the protein.

Outlook to the future: engineering the biomolecule and the biomatrix

The examples described above provide case studies demonstrating the benefits of peptide and protein engineering in the field of tissue engineering. The recombinant growth factors of interest were either combined with engineered peptides (binding heparin, to permit indirect binding of growth factors) or engineered proteins (binding fibrin, to permit direct binding between fibrin and the growth factors). However, the biomaterial was based on a blood-derived product, fibrin(ogen). The next steps of evolution in such approaches could be to engineer a completely synthetic biomaterial to bind the growth factor, perhaps also engineering the growth factor to bind that biomaterial.

Our group has begun making such explorations. To be effective mimics of extracellular matrix molecules like fibrin(ogen) and collagen, an artificial extracellular matrix analogue would have to be proteolytically degradable, just like the natural extracellular matrix [24–26]. For reasons of surgical convenience, it would be beneficial if the matrix could also be converted *in situ* from liquid precursors into the final gel form [24, 26]. To accomplish these ends, we have developed an approach by which two liquids are mixed, the components of the liquids having counter chemical reactivity. One component contains a water-soluble polymer that reacts with thiol groups, as found on the amino acid cysteine. This polymer, poly(ethylene glycol) multivinylsulfone, will then react with peptides that contain unpaired cysteine residues in them. Thus, if the polymer is mixed with the second component, which contains a peptide containing two

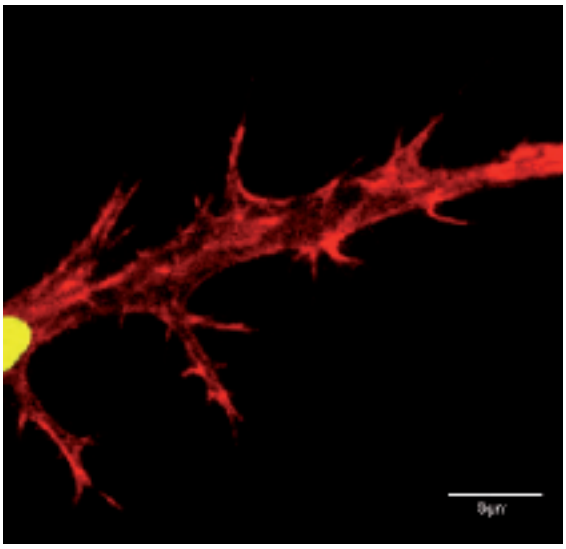

Figure 1

Human dermal fibroblasts were observed to spread in three dimensions in matrix metalloproteinase-sensitive hydrogels in a protease-dependent manner. The spatial fidelity of degradation of the surrounding gel material was very high. Here, small philopodia can be observed, with diameters in the order of 1 µm, penetrating the gel by local, cell-induced proteolysis. (Photo: G. P. Raeber and M. P. Lutolf)

cysteine resides, the final mixture then rapidly converts into a crosslinked hydrogel. To obtain sensitivity of the final mixture to proteases, the peptide can be designed to comprise a sequence that is sensitive to plasmin (as would degrade fibrin) or matrix metalloproteinases (as would degrade fibrin, collagen, elastin, and a number of other extracellular matrix molecules).

In vitro studies with these materials have demonstrated their ability to respond to cell-associated proteolysis by local degradation [27]. In figure 1, a human dermal fibroblast is shown in three-dimensional culture within a matrix metalloproteinase-sensitive gel, and the gel completely surrounds the cell. When originally seeded in the gel, the cell was round and was mixed into the liquid precursor mixture. Solidification occurred, encapsulating the rounded cell within the gel. Over a period of a few hours, the cell begins to spread (in three dimensions), proteolytically degrading a pathway into which it can spread. The high magnification view in figure 1 shows very small philopodia from the cell, order 1 µm in diameter and a few µm long, protruding into the gel. These protrusions did not occur into pores that pre-existed, but rather they occurred by protrusion into pores that came to exist as a result of local cell-induced proteolysis.

Such materials as described above can also be useful in more complicated surgical environments. To explore this, we entrapped the bone-inducing protein bone morphogenetic protein-2 within the gel and implanted these materials within surgical defects made in the skulls of rats [28, 29]. The defects were too large to heal on their own, and it was only in the case of bone morphogenetic protein-containing, proteolytically-sensitive gel implants in which closure of these bone defects was observed.

New classes of therapeutics

When drugs are developed to treat systemic diseases, perhaps relatively little attention is needed to the local environment of their application. However, with drugs to induce tissue regeneration, which are applied and are active locally, the details of their local application may matter. With the former, the drug formulation may include agents that modulate the details of drug stability or potentially even uptake; but with the latter, agents in the drug formulation may fundamentally change the way the biomolecule behaves. A good example was VEGF, presented above, where the free drug induced nonfunctional vessels and the polymer-bound VEGF induced functional and mature vessels. These examples illustrate that the development of biomolecules involved in regeneration into effective drug formulations in tissue engineering requires a new approach, one in which both the drug and the carrier are considered in molecular detail. Indeed, one may think of the "carrier" not merely as something that carries the drug, but as a vector that enables the drug to display its intended biological activity.

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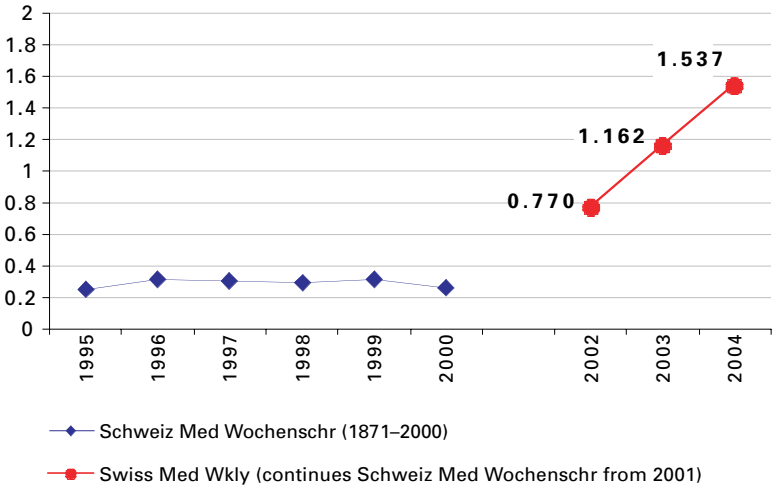

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