MATRIX METALLOPROTEINASES AND THE MODULATON OF TISSUE RESPONSE TO ARTIFICIAL MATRICES

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ABSTRACT

Matrix metalloproteinases (MMPs) have been implicated in many physiologic and pathologic situations of extracellular matrix remodeling and turnover. Induced and released by cells involved in the inflammatory response, MMPs modify the composition of the extracellular matrix and play an important role in the regulation of cell migration and attachment in the events of tissue regeneration and angiogenesis.

Thus, these proteolytic enzymes are located at a vital crossroad for tissue engineering, creating as much opportunities as problems. Amongst these, the biological response of the organism to artificial matrices often used as support blocks for bioengineering surges as primordial. The reactive production of MMPs can play a role in modulating the success of interventions in the field of vascular tissue engineering

A potential problem involving MMPs concerns the inflammatory response surrounding these matrices, which can decrease a successful incorporation of engineered tissue components. We will discuss these phenomena in terms of matrix composition and biophysical constraints applied on the matrices. Namely, physical forces applied on the tissue and its biophysical response to these stresses can modulate the intensity of MMP production surrounding the matrix.

On the other hand, vascular tissue engineering can take advantage of the positive effects of MMPs upon the capacity of the extracellular matrix to promote cell adhesion/migration, and thus angiogenesis. In this regard, we will discuss the potential brought by the generation of novel synthetic biopolymeric matrices sensitive to controlled degradation by MMPs, which can serve as appropriate building blocks to promote and direct tissue regeneration.

INTRODUCTION

Tissue engineering is an emerging interdisciplinary field in biomedical engineering and aims at regenerating new biological tissue for replacing damaged organs. Tissue engineering generally requires an artificial extracellular matrix for tissue regeneration, because the existence of a cellular scaffold is an absolute requirement for cell proliferation and differentiation resulting in tissue regeneration. This requirement, as well as adequate cell adhesion onto the matrix surface, makes the use of biological materials (either natural or synthetic) interesting for purposes of tissue engineering. This is particularly important in the field of vascular tissue engineering, which faces either the intricate process of angiogenesis (for vascularization of solid organ implants) or the multilayer process of arteriogenesis (for the construction of vascular conducts).

This artificial extracellular matrix should disappear progressively through absorption into the body when the new tissue is regenerated. This fact leads to the reasoning that the biomaterials used for scaffolding should be biodegradable, either spontaneously or through the action of enzymes produced by invading and colonizing cells during the process of tissue regeneration. Amongst these enzymes, proteases play a primordial twofold role: (i) in degrading the scaffold, when it is produced from proteins (e.g., collagen or fibrin) or appropriately designed biomimetic materials; (ii) in maturing the proteins forming the extracellular matrix and which had been progressively secreted and deposited during the process of biomaterial replacement by an endogenous extracellular matrix. In these settings, the most relevant family of proteases are the matrix metalloproteinases (MMPs) , with a multitude of functions both at extracellular matrix degradation and also as a mediator of cellular responses and tissue maturation.

In this review we will discuss basic aspects of matrix metalloproteinase biochemistry and biology, and complement these with current concepts involving the body response to artificial matrices and how MMPs can be involved in the adequate replacement of the artificial scaffold by a mature extracellular matrix and tissue.

MATRIX METALLOPROTEINASES

Matrix metalloproteinases, also called matrixins, are a family of over 20 zinc-containing endopeptidases that are capable of degrading various components of the ECM[1]. All are produced as latent pro-enzymes containing a pro-domain (see below) that must be proteolytically processed to be activated.

MMPs have been subdivided into at least five groups based on their structure and/or substrate specificities:

i) The simplest structural subclass of MMPs is the matrilysins, which consist of a signal peptide, propeptide domain, and catalytic domain with the zinc-binding site[2,3].

ii) The collagenases, which in addition to the minimal domain structure also contain a simple hemopexin-like domain connected to the catalytic domain via a proline-rich hinge region, degrade the native helix of types I, II, III, and other fibrillar collagens[2,4].
iii) The stromelysins have similar structural domains as the collagenases, but like the matrilysins, have a broad substrate specificity and degrade many ECM proteins, including proteoglycans, fibronectin, and laminin[2,5].
iv) The gelatinases contain an additional region of three fibronectin type II repeats within their catalytic domains and show a preference for denatured collagens (gelatin) and also degrade types IV, V, VII, and X native collagens, fibronectin, and laminin[2-4].
v) The membrane-type MMPs (MT-MMPs). These MMPs are bound to the cell surface through a C-terminal transmembrane domain or glycosylphosphatidylinositol anchor[6,7] and degrade gelatin, fibronectin, and aggrecan as well as other ECM substrates[2,3].

Other MMPs include metalloelastase (MMP-12) and enamelysin (MMP-20), which have specialized functions in macrophage-mediated proteolysis/migration and in tooth enamel formation, respectively[7,8], and MMP-19, MMP-23, and epilysin (MMP-28)[2].

The pro-domain of all MMPs contains a conserved cysteine residue, called the "cysteine switch", whose sulfhydryl group coordinates with the zinc ion in the catalytic site to maintain latency [1]. Disruption of this cysteine-zinc binding by physical or chemical means is the first step in activation of the MMPs[5]. The hemopexin (PEX) domain, which forms a four-bladed propeller structure made of b-sheets, along with an appropriate hinge region allows collagenolytic MMPs to unwind/distort the triple helix of fibrillar collagens so the catalytic domain can cleave them[9]. The PEX domain is also necessary for MMP binding to a number of other proteins, including integrins, cell surface receptors, and tissue inhibitors[5,10-13]. The fibronectin-like domain of the gelatinases is important for binding to gelatin[9]. All of the MT-MMPs, stromelysin-3 (MMP-11), MMP-23, and epilysin (MMP-28) also have a furin recognition sequence between their propeptide and catalytic domains, allowing cleavage/activation by furin convertase enzymes in the Golgi apparatus[4,6].

Except for the MMPs activated intracellularly by furin proteases, the other MMPs are secreted as inactive zymogens and must be activated in the extracellular space by proteolytic cleavage of the N-terminal propeptide domain[1,6]. Plasmin, generated from plasminogen through the action of urokinase-type plasminogen activator (uPA), can initiate an MMP activation cascade, activating proMMP-1 and proMMP-3[4,15,16]. Activated MMP-3 can in turn activate proMMP-1 and proMMP-9[15,16]. MT1-MMP activates

proMMP-2 as well as proMMP-13 at the cell surface[17,18]. Activated MMP-2 and MMP-13 can both in turn activate proMMP-9[19,20].

MMP activity is additionally regulated by endogenous inhibitors, primarily the tissue inhibitors of metalloproteinases (TIMPs). There are 4 TIMPs, TIMP-1 to -4, which each consist of an N-terminal domain responsible for their MMP inhibitory activity and a C-terminal domain[21]. Although all of the TIMPs bind tightly to most MMPs, they have differential inhibitory activity against different MMPs. For example, TIMP-2 and TIMP-3, but not TIMP-1, are efficient inhibitors of the MT-MMPs[21]. TIMP--2 has a specialized role in the activation of proMMP-2 by MT1-MMP. The N-terminal domain of TIMP-2 forms an inhibitory complex with the active site of MT1-MMP, while the C-terminal domain interacts with the PEX domain of MMP-2. A second TIMP-2-free MT1-MMP molecule is recruited to the complex and cleaves off the pro-domain of MMP-2[21]. TIMPs form other non-inhibitory complexes *via* their C-terminal domains: TIMP-1 forms a complex with the PEX domain of MMP-9, while TIMP-3 complexes with both MMP-2 and MMP-9 and binds tightly to the extracellular matrix[4,21]. TIMPs also have other biological activities that are independent of their MMP inhibitory activity. With regard to angiogenesis, TIMP-2, but not TIMP-1, inhibits bFGF induced endothelial cell proliferation[22]. In vascular smooth muscle cells, overexpression of TIMP-2 inhibits proliferation, while TIMP-3 induces apoptosis[23].

While the TIMPs are the primary tissue inhibitors of MMPs, in the plasma, the general protease inhibitor, α2-macroglobulin, is the predominant MMP inhibitor[10,24]. The angiogenesis inhibitor TSP-1 has been shown to inhibit the activation of proMMP-2 and proMMP-9, while TSP-2 complexes with MMP-2 to enhance clearance by scavenger receptor-mediated endocytosis[24]. Another protein with MMP inhibitory activity is RECK (REversion-inducing Cysteine-rich protein with Kazal motifs), a glycosylphosphatidylinositol membrane-anchored glycoprotein widely expressed in human tissues and involved in the regulation of angiogenesis[10].

MMPs are also regulated at the transcriptional and post-transcriptional levels. Expression of most of the MMPs is low in normal tissues and is strongly up-regulated when the remodeling of the extracellular matrix is required[25]. Expression can be induced by cytokines, growth factors, chemical agents (such as tumor promoters), physical stress, activated oncogenes, and interactions with matrix components[1,25]. Promoter regions of inducible MMPs (MMP-1, -3, -7, -9, -10, -12, and -13) contain multiple cis-acting elements including AP-1, PEA3, Sp1, and NF-κB binding sites[25]. Stabilization of MMP-1 and MMP-3 mRNAs has been demonstrated following activation of p38α mitogen-activated protein kinase[26]. MMP-9 levels have been shown to be regulated at the level of translation efficiency[27], while translational repression can regulate protein levels of human MMP-13[28]. Post-translational

modifications of MMP-9 include glycosylation[29] and covalent linkage to neutrophil gelatinase-B-associated lipocalin in neutrophils[14] or to chondroitin sulfate proteoglycans in macrophages[30]. In endothelial cells and inflammatory cells, MMPs are stored intracellularly in secretory vesicles and so can be rapidly released upon stimulation[4,14,31].

MMP clearance and catabolism is mediated by the low density lipoprotein receptor-related protein, which is also responsible for clearance of TSP-2-bound MMP-2 and α2-macroglobulin-MMP complexes[10,24,32-34].

Regulation of extracellular signalling by MMPs

Although MMPs can cleave virtually all structural ECM molecules, they can also cleave several circulating, cell surface and pericellular proteins, which enables them to regulate cell behavior in numerous ways. These mechanisms include the alteration of cell-matrix and cell-cell interactions; the release, activation, or inactivation of autocrine or paracrine signaling molecules; and the potential activation or inactivation of cell surface receptors.

The degradation of the extracellular matrix can be viewed as merely disrupting and remodeling structural barriers, thereby permitting cellular invasion to take place. However, extracellular matrices are not just passive cellular scaffolds; they influence cell behavior by sequestering signaling molecules, such as growth factors and growth factor-binding proteins, and by acting as contextual ligands for cellular adhesion receptors, such as integrins, that transduce signals to the cell interior[35]. Extracellular matrices regulate such basic processes as cell shape, movement, growth, differentiation, and survival by controlling cell adhesion and the cytoskeletal machinery[36]. By extension, MMPs also influence these same processes by altering the composition and structural organization of the ECM, thereby altering matrix-derived signals. Additionally, since most cells must adhere to a natural or provisional matrix to survive, MMP-mediated disruption of subcellular matrices can induce apoptosis in anchorage-dependent cells and plays an important role in normal physiologic cell[37]. Moreover, proteolytic ECM remodeling results in the release of modular breakdown products with biologic activity. For example, the MMP-dependent cleavage of native fibrillar collagen exposes otherwise cryptic RGD sites that can then be ligated by αvβ3 integrin, and this interaction promotes the survival and growth of melanoma cells[38]. In addition, the cleavage of intact laminin-5 by MMP-2 generates a γ2-chain fragment that induces epithelial cell motility by exposing an inaccessible cryptic site[39].

Matrix molecules also act as binding reservoirs for various growth factors and cytokines that are released once the ECM molecules are degraded. For example, the small collagen-associated proteoglycan decorin acts as a depot for TGF-β, and its degradation by various MMPs makes the otherwise sequestered TGF-β available to carry out its biologic functions[40]. One such function is to in-

hibit the expression of several MMP genes. Thus MMP-mediated activation and release of TGF-β may act as a negative feed-back mechanism to limit MMP expression and further TGF-β release. Likewise, MMP-cleaved collagen can feed back through discoidin domain receptor-2 to suppress collagenase expression and further collagen degradation, whereas intact collagen has the opposite effect[41].

MMP activity is responsible for modulating the activity of growth factors and cytokines, either directly or by controlling the concentration of its soluble binding proteins. MMP degradation of insulin-like growth factor (IGF) binding proteins releases active IGFs, while MMP-2 and MMP-9 can proteolytically activate latent TGF-vx1 and TGF-β2[2,5]. MMP-3 and MMP-7 have been shown to cleave the membrane-bound precursor of heparin-binding EGF, releasing active EGF, while TNF-á is released from the cell surface by MMP-1, -3, and -7[5]. MMP-9 is also described to cleave the pro-inflammatory and proangiogenic cytokine IL-8, which increases its activity tenfold, as well as degrading and inactivating the angiogenesis inhibitor platelet factor-4[14].

THE BIOLOGICAL RESPONSE TO ARTIFICIAL MATRICES

Tissue repair and wound healing are complex processes that involve inflammation, granulation and tissue remodeling. Injury initiates a complex series of events that involves interactions of multiple cell types, various cytokines, growth factors, their mediators and the extra-cellular matrix proteins. This physiological response is mimicked as a reaction towards an artificial implant, around which a wound healing-like inflammatory response is mounted.

The response towards the implantation of an artificial matrix is comparable to tissue trauma, which in turn induces a physiological healing reaction, consisting of the associated components of inflammation and repair, which represent a spectrum of interdependent mechanisms, in which inflammatory mediators act in a concerted fashion to initiate and control cellular response[42]. Ultimately, the repair process involves regeneration of injured cell populations, angiogenesis (sprouting of new microvascular blood vessels from the pre-existing vasculature)[43], matrix synthesis and remodeling[44], and is followed in its latter stages by stringent control of the repairing and regenerating cell populations by apoptosis[45,46].

Thus, the physiological response of repair will consist of a complex sequence of strictly controlled reactions, involving altered gene expression in cells of the circulating blood (granulocytes, platelets, monocytes, lymphocytes), resident inflammatory cells (e.g. macrophages, mast cells) and the microvasculature adjacent to the injury process. The microvascular endothelium, particularly, plays a special role in the regulation of the inflammatory reaction[47,48]. In addition, a cascade-like activation of blood plasma mediator systems (kinins, complement, coagulation and fibrinolytic systems) takes place in cooperation with the above-named cellular components. All these events must be taken into consideration

in any in vitro models of tissue response to biomaterials[49].

It is becoming increasingly apparent that success in the field of tissue integration of biomaterials depends on the ability to mimic the beneficial physiological response of repair following injury and to control the negative reaction of inflammation. This is particularly important in situations involving implantation of three-dimensional (3-D) matrices colonized by the patient's own cells. As described above, all forms of implantation involve some degree of tissue injury, which will initiate two principal reactions, namely inflammation and the related response of wound healing.

Taken together, this information supports a multi-step cellular recruitment model, in which a sequence of different physiological and pathological stimuli upregulates a spectrum of MMP production by infiltrating cells. The production of these proteolytic enzymes is essential both for cell migration (essential for adequate cell colonization of the scaffold) and extracellular matrix remodeling (leading to improved collagen organization), which are essential for the integration of the biomaterial implants. In this regard, the production of MMP-9 (but not MMP-2) by the initial infiltrating inflammatory cells has been shown to be essential for adequate incorporation of biopolymeric vascular constructs in a murine model, producing not only a more physiological response in terms of cell recruitment and angiogenesis, but also a more efficient process of collagen maturation[50,51].

However, many biomaterial applications lead to an exaggerated healing reaction in the form of fibrosis. It is still unclear which type and extent of inflammation favors optimal biocompatibility and how precisely physicochemical properties of the biomaterials induce specific biological responses.

A characteristic of implants that can regulate the biomaterial potential for incorporation is, besides its molecular composition, the implant microstructure. The microscale organization can regulate cell surface adhesion and infiltration, supporting the progressive replacement of the biodegradable scaffold by remodeled tissue. In these situations, the rugosity and porosity of the implant are primordial. Rugosity facilitates the interaction of cells with the surface of the implant, and is particularly important in vascular tissue engineering as a promoter of endothelial cell attachment[52,53]. The porosity of the scaffold (which by definition incorporates the concept of surface rugosity) relates to the existence, within the biopolymeric structure of the implant, of a network of pores and channels of different diameter that allow the deposition of a provisional extracellular matrix (e.g., composed of fibrin), invasion and population of the scaffold by inflammatory and parenchymal cells, followed by replacement of the matrix by a mature endogenous matrix. Our own results (Rosário *et al*, unpublished results) show that the porosity of the scaffold relates directly with the invasion of the implant by MMP-producing inflammatory cells; in the case where the diameter of the pores do not allow cellular invasion of the artificial scaffold the inflammatory response builds up around it and negatively interferes with the incorporation of the implant in the organism (Figure 1).

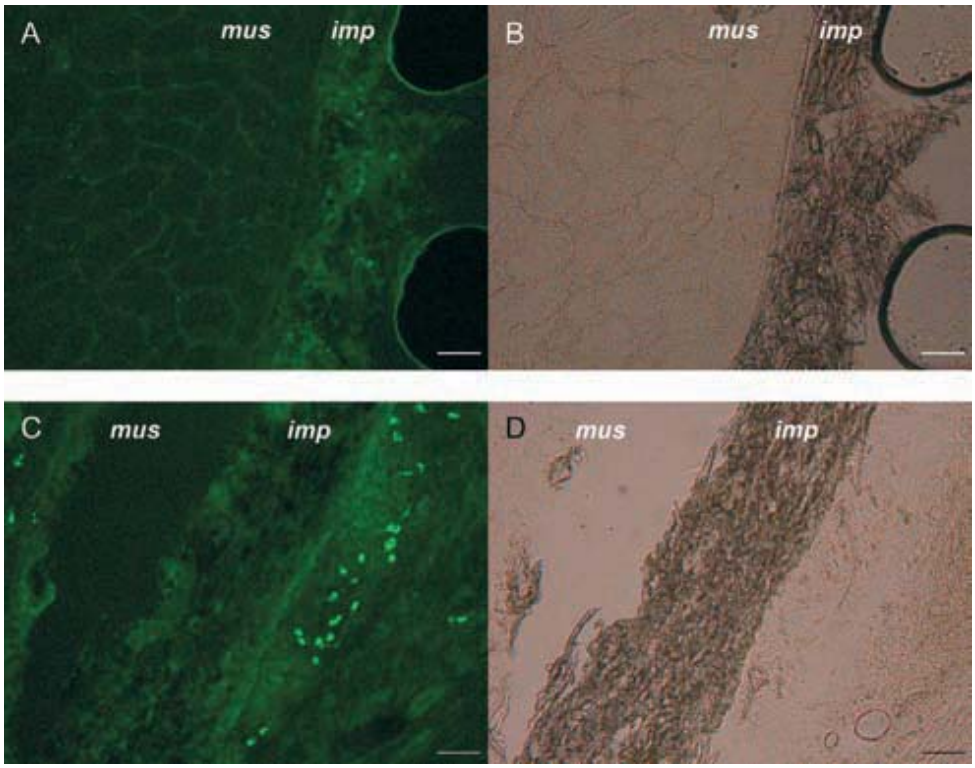

Fig. 1 – In a rat model, a nylon implant was surgically placed subcutaneously in the abdomen, adherent to the muscle wall. The nylon implant had a mesh thickness of 0.6 mm (**a** and **b**) or 30 μm (**c** and **d**). The full thickness of the abdominal wall was collected and analyzed 7 days later. Panels **a** and **c** represent detection of gelatinase (MMP-2 and MMP-9) activity by *in situ* zymography, while **b** and **d** are the equivalent transilumination image. ***imp***, implant; ***mus***, skeletal muscle of the abdominal wall; the bar represents 50 μm.
The implant with a mesh thickness of 30 μm permitted infiltration of gelatinase-containing cells, and simultaneously maintained a good apposition with the underlying striated muscle. On the other hand, the implant with a 0.6 μm mesh dimension does not present evidence of cellularization with gelatinase-containing inflammatory cells, which are clustered at its periphery; of note, in this case, is the fact that the implant detaches from the underlying muscle tissue, suggesting that its incorporation is not as complete as in the former situation.

BIOPOLYMERIC MATRICES: CARVING THE WAY FOR TISSUE RESTITUTION

As described above, the extracellular matrix modulates tissue dynamics through their ability to locally bind, store and release soluble bioactive effectors such as growth factors[54]. The binding of growth factors to ECM molecules raises their local concentration to levels appropriate for signaling, localizes their morphogenetic activity, protects them from enzymatic degradation and in some cases may increase their biological activity by optimizing receptor-ligand interactions. As growth factors are required in only very small concentrations to elicit a biological response, the main focus in designing synthetic matrices for growth factor presentation has been to control local growth factor concentration.

Several strategies to engineer growth factor release from biomaterials have been presented over the past years and some initial success has been reported in animal models for the regeneration of bone and skin as well as the induction of vascularization, as reviewed elsewhere[55-57]. As many cellular processes involved in morphogenesis require a complex network of several signaling pathways and usually more than one growth factor, recent research efforts have focused on schemes for sequential delivery of multiple growth factors[58]. The use of biological feedback mechanisms in growth factor delivery has also been explored[59]. In this case, a growth factor is bound to the matrix and released upon cellular demand through cell-mediated localized proteolytic cleavage from the matrix[60,61]; this approach substantially mimics the mechanism by which these factors are released *in vivo* from stores in the natural ECM by invading cells in tissue repair.

Thus, we will describe next a number of biomimetic materials which have been found, in different experimental settings, to produce an appropriate scaffold for vascular tissue engineering. In particular, we will discuss the potential brought by the generation of novel synthetic biopolymeric matrices sensitive to controlled degradation by MMPs, which can serve as appropriate building blocks to promote and direct tissue regeneration.

Natural Matrices

Alginate Alginate is an anionic polysaccharide composed of mannuronic acid and glucuronic acid and is produced from the marine brown algae. When complexed with divalent cations alginate forms a hydrocolloid gel[62]. Because gelation occurs in an aqueous environment, alginate microspheres are a potential system to entrap either angiogenic growth factor-producing cells[63] or angiogenic molecules such as bFGF and VEGF[64-66]. However, two major drawbacks of alginate as a tissue engineering scaffold have been described described: stimulation of inflammatory cells and poor bioresorbability have been reported[67,68], while on the other hand the release of trapped growth factors (like VEGF and bFGF) was described to undergo an initial non-controlled burst release[66]. To overcome this latter problem, heparinization of the alginate scaffold has been attempted with some positive results[69-73].

Agarose Commercial heparin–agarose beads have been employed as carriers for local release of bFGF in a pig model of myocardial infarction. In this model, administration of heparin agarose beads loaded with bFGF into the infarct border area significantly enhanced the number of arterioles around the implantation site. However, this route of administration was found to be less effective than direct injection of soluble bFGF and heparin[74].

Hyaluronic acid (HA) and chitosan Both HA and chitosan have been used in composite biomaterials for tissue engineering applications. HA is a nonsulfated, linear glycosaminoglycan consisting of repeating disaccharide units of glucuronic acid–*N*-acetyl-glucosamine. HA inhibits endothelial proliferation, migration and assembly. In contrast, HA degradation products with 3–10 disaccharide units exert proang-

iogenic activities[75]. Chitosan, a linear glycosaminoglycan composed of *N*-acetyl-glucosamine units, is produced by the partial deacetylation of chitin has been tested for the sustained release of bFGF[76]. Preparations of chitosan–albumin microspheres and chitosan–albumin fibers have been used for release of aFGF and shown to stimulate neovascularization in rats[77]. Chitosan cross-linked collagen scaffolds have been successfully used towards the growth of endothelialized human skin equivalents[78].

Fibrin Fibrin is the major constituent of the blood clot and represents the immediate response to vessel injury and tissue damage. Thus, it serves as a natural provisional material platform for new cell ingrowth to be replaced by viable tissue during wound regeneration. The clots form by physical polymerization of the fibrin monomers, which is formed by proteolytic cleavage of protecting peptide domains from fibrinogen, and this loose physical network is further enzymatically cross-linked into a dense network barrier that prevents blood leakage.

While new tissue is forming, fibrin gradually becomes degraded by plasmin or MMPs produced in the pericellular milieu of cells invading and replacing the matrix[55,56]. In regard to ischemic tissue, fibrin serves as temporary platform for the gradual development of granulation tissue that is characterized by high degree of vascularity[57]. Experiments using growth factor delivery from fibrin demonstrated enhanced perfusion of ischemic rat myocardium in response to treatment with low doses of aFGF and bFGF[82,83] suggesting that fibrin acts as an efficient vehicle for local release. Binding of bFGF to fibrin was shown to potentiate its proliferative capacity for endothelial cell growth and protect it from proteolytic inactivation[84,85]. In a recent clinical trial for critical limb ischemia caused by bilateral occlusion of the superficial femoral artery, the intramuscular administration of VEGF contained in a fibrin scaffold produced a significant increase in the patient's limb blood supply and exercise tolerance[86].

However, the release kinetics of growth factors from fibrin gels (70–100% of the growth factor initially added after 24 hours) indicates an uncontrolled burst release. As reported for VEGF, when angiogenic growth factors are released too rapidly or at uncontrolled levels from the scaffold, the activity of the growth factor may become detrimental to healing[87].

To prevent the burst release of bioactive factors incorporated within fibrin, new techniques that protect the growth factor from rapid clearance by coupling its release to local proteolytic activity at the cellular surface invading the matrix during healing are been developed[88]. It is also possible to alter the invasion characteristics of the network by incorporating exogenous peptide or protein adhesion ligands, which become either covalently linked to the fibrin network by factor XIII[89], or by providing affinity sites for binding of heparin-binding growth factors[90] such as VEGF. In both methodologies, the release of growth factors will depend predominantly on its cleavage from the fibrin matrix by cell-associated enzymatic activity, i.e., MMPs, plasmin or heparinases, which localize at or near the sur-

faces of cells infiltrating and remodeling the matrix.

Collagen and gelatin Biomaterial prepared from a collagen or gelatin scaffold have been employed as carriers for angiogenic molecules[91]. Functionally important qualities such as adhesiveness for cells and proteolytic degradability are retained in gelatin. Collagen I constitutes the major protein component of natural extracellular matrix and has an important role in the conduit of tissue repair. Like fibrin, collagen I matrices can be produced as highly porous interconnecting networks with good properties as a platform for cell adhesion and migration. For this reason, collagen I has been widely utilized as a tissue engineering scaffold to grow replacements of skin, bone and also blood vessels[92,93].

However, collagen matrices exhibit very poor loading capacities for growth factors. Consequently, several methodologies have been explored to improve the capture of angiogenic growth factors in the collagen matrix during loading, as well as to improve retention and control over the release after application. Similarly to fibrin, these include both the covalent attachment of heparin or glycosaminoglycans to collagen, which bind and retain angiogenic growth factors with affinity for heparin, and the modification of network structure to couple the rate of growth factor (such as VEGF and bFGF) release to the rate of bulk matrix degradation[94-96].

Synthetic materials

The clinical demand for synthetic replacements of biological matrices for drug delivery and tissue engineering applications has promoted the development of novel classes of synthetic polymers that are capable to imitate the base functions of natural healing matrices described above.

Two basic criteria to be met by synthetic tissue engineering matrices are their complete degradation and resorption in the body, and the ability to temporarily act as mechanical support to grow cells into a three-dimensional organization. Simultaneously, they should be formed from precursor molecules that have been shown to be safe and nontoxic in prior applications in humans.

Like biological matrices for wound repair such as fibrin or ECM, the synthetic tissue substitutes should provide a highly porous internal architecture, which is required for sufficient cell seeding density in vitro, for blood vessel invasion to occur in vivo and for oxygen and nutrients to be supplied to cells. Overall, these matrices should allow the possibility to be used for controlled drug delivery and balanced tissue growth tissue.

Polymers of poly(lactide-co-glycolide) Prefabricated solid scaffolds have been formed from polyesters of lactide and glycolide (PLGA) and constitute a family of materials with distinct mechanical strength and degradation properties that vary by the ratios of lactide and glycolide in the polymers and their degree of polymerization. These polyesters are insoluble in water but degrade by hydrolytic attack of the ester bond to become resorbed as lactic and glycolic acids. The release from the scaffold is passive and dependent on the material, which makes the rates of release of growth factors

incorporated within PLGA to be tightly coupled to the hydrolysis rate of the scaffold. Recently, new variants of PLGA scaffolds with interesting properties for blood vessel growth have been introduced, in which simultaneous delivery of VEGF and PDGF, each at a different rate and dose from a single PLGA vehicle, were used to complement both growth factor activities. PLGA scaffolds, when implanted, nonspecifically adsorb a complex layer of proteins from body fluids, and it is this intervening layer of adsorbed protein that is responsible for the cell adhesive character of PLGA implants.

Preclinical and clinical evidence have indicated that the delivery of a single regulator of angiogenesis such as VEGF may not be sufficient to produce mature and stable vascular networks. Complementary promotion of vessel maturation may be achieved by the simultaneous delivery of growth factors such as angiopoietin 1 or PDGF, which promotes blood vessel stabilization by recruitment of smooth muscle cells to the endothelial layer of nascent vasculature. The delivery of VEGF and PDGF from a PLGA scaffold as the mean to both induce new vessels (VEGF) and to ensure their maturation into stable vessel wrapped with smooth muscle cells (PDGF) was studied[97]. Achieving a distinct pattern of release of each growth factor from the matrix, with faster release of VEGF by surface erosion and slower release of PDGF linked to bulk degradation, a higher proportion of mature blood vessels were obtained, compared to VEGF alone.

Polyethyleneglycol matrices An alternative approach is the use of elastic hydrogel matrices. These are composed of water-soluble polyethyleneglycol (PEG) polymers. They are intrinsically nonadhesive, and this property has been used experimentally to prevent cell and tissue adhesion models[98-103].

For applications in the field of tissue engineering, PEG hydrogels have been developed with increasing biological functionality, starting out from biologically inactive PEG gels to PEG matrices that share important functional characteristics of natural wound healing matrices such a fibrin or ECM and therefore represent promising candidate matrices for tissue engineering applications.

In these applications, the PEG polymers are linked by short bioactive oligopeptide domains. These hydrogels provide interactive properties through the incorporation of molecular signals via the oligopeptide domains that permit direct and biologically specific interactions between cells and material. For example, these materials can be engineered with biological functionalities that make them sensitive to degradation by local proteolytic activity specifically associated with cell migration and tissue remodeling. In this way, both the degradation and replacement of the PEG peptide hydrogels is controlled by the healing progress of the responding tissue and is linked to the liberation of incorporated growth factor.

These sites of interaction are typically combinations of small synthetic cell adhesive peptide domains[104,105] and peptide domains for cleavage by targeted proteases[106,107] and singular or multiple growth factors supplied in the matrix[108]. By design of the peptide sequences that cross-link the PEG matrix and functionalize the network, these

hydrogels can be prepared with functionalities of natural ECM, e.g., the ability to mediate adhesion and traction to invading cells and the ability to respond to proteolytic degradation by enzymes, such as MMPs or plasmin, produced at the leading edge of migrating cells[80]. These two pericellular protease activities are particularly important in matrix remodeling by endothelial cells during angiogenic processes in wound healing and directly mediate cell invasion and microvascular vessel formation. Due to their design, this class of proteolytically degradable synthetics may become very useful in diverse healing applications. For example, by selection of the cross-linking peptide sequence, selective cleavage by proteases as well as rate of matrix resorption may be controlled.

In these functionalized PEG hydrogels the processes of cellularization and tissue formation occur biospecifically, with the material signaling the cell by its tethered adhesion ligands and growth factors, and the cell signaling the material to locally degrade or release its growth factor, all modulated via cell-associated protease activity. Although the PEG peptide hydrogel networks consist of more than 90% water, their mesh size is in the range of few nanometers, much smaller than cell processes[109]. As such, cell migration through these materials is totally dependent on local proteolytic breakdown of the matrix. For this reason, the activation of cellular proteolytic programs is an essential aspect for tissue repair via such matrix types and may be stimulated by the additional supply of growth factor proteins.

Cell-demanded delivery of VEGF from MMP-sensitive PEG peptide hydrogels have been investigated in experimental settings of vascular tissue engineering[59]. VEGF was covalently conjugated into the PEG hydrogel network, providing retention of the growth factor in the matrix until release of VEGF by means of plasmin or MMPs released from cells invading the hydrogel. It was found that the functionality of the matrix-conjugated VEGF was preserved and is critical for in vitro endothelial cell survival and migration within the matrix environment. Interestingly, while the biomaterial-potentiated angiogenic process builds up, endogenous VEGF is released as the angiogenic process progresses, establishing a positive feed-back loop. However, all this angiogenic process occurrs in the presence of a tightly regulated release of bioactive VEGF locally, assuring the formation of a native-like blood vessel network at the tissue/hydrogel interface, which is consistent with remodeling of the biomaterial implant into a mature and vascularized tissue.

CONCLUSION

Biomaterials play central roles in the strategies for vascular tissue engineering as designable biophysical and biochemical scaffolds that direct cellular behavior and function. Such materials should provide a provisional three-dimensional support to interact biomolecularly with cells to control their function, guiding the spatially and temporally complex multicellular processes of tissue formation and regeneration. In this regard, the expression of MMPs by surrounding and invading cellular populations plays an important

role, and helps to design a framework around which the incorporation and degradation of the artificial scaffold proceeds, while at the same time restoration of function might occur. The guidance provided by biomaterials, supported by the cellular programme of metalloproteinase expression by populating cells, may facilitate restoration of structure and function of damaged or dysfunctional tissues, both in cell-based therapies, such as those where carriers deliver transplanted cells or matrices induce morphogenesis in bioengineered tissues constructed *ex vivo*, and in acellular therapies, such as those where materials induce ingrowth and differentiation of cells from healthy residual tissues *in situ*.

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