8

Synthetic Extracellular Matrices for Tissue Engineering and Regeneration

Eduardo A. Silva and David J. Mooney
Division of Engineering and Applied Sciences
Harvard University
Cambridge, Massachusetts 02138

The need for replacement tissues or organs requires a tissue supply that cannot be satisfied by the donor supply. The tissue engineering and regeneration field is focused on the development of biological tissue and organ substitutes and may provide functional tissues to restore, maintain, or improve tissue formation. This field is already providing new therapeutic options to bypass the limitations of organ/tissue transplantation and will likely increase in medical importance in the future. This interdisciplinary field accommodates principles of life sciences and engineering and encompasses three major strategies. The first, guided tissue regeneration, relies on synthetic matrices that are conductive to host cells populating a tissue defect site and reforming the lost tissue. The second approach, inductive strategy, involves the delivery of growth factors, typically using drug delivery strategies, which are targeted to specific cell populations in the tissues surrounding the tissue defect. In the third approach, specific cell populations, typically multiplied in culture, are

Current Topics in Developmental Biology, Vol. 64

0070-2153/04 $35.00

directly delivered to the site at which one desires to create a new tissue or organ. In all of these approaches, the knowledge acquired from developmental studies often serves as a template for the tissue engineering approach for a specific tissue or organ. This article overviews the development of synthetic extracellular matrices (ECMs) for use in tissue engineering that aim to mimic functions of the native ECM of developing and regenerating tissues. In addition to the potential therapeutic uses of these materials, they also provide model systems for basic studies that may shed light on developmental processes.

I. Introduction

Advances in medicine over the past several decades have led to significant improvements in the quality and quantity of life. Despite these advances, it is still necessary to develop alternative therapies to treat patients who suffer from the loss or failure of organs and tissues (Langer and Vacanti, 1993). The numbers of individuals willing or able to donate their organs and tissues do not nearly match those that require transplantation therapies. For example, the number of putative candidates for heart transplantation is approximately 16,000, but only 2,202 patients received heart transplants in the United States in the year 2001 (United Network of Organ Sharing, 2002) due to the lack of donor organs. More generally, every 16 minutes, another new patient requires an organ transplantation. The fields of tissue engineering and regenerative medicine are attempting to address this significant number of patients who need a new or improved organ or tissue. In these fields, a variety of life sciences and engineering disciplines is integrated with the goal of promoting and controlling tissue regeneration. The ultimate goal is to develop synthetic constructs that restore and enhance the functions of healthy tissues. Developmental studies provide crucial information about how the interactions of cells with the extracellular matrix (ECM) regulate cell fate and function. In particular, studies regarding how tissues and organs grow during embryogenesis and how they remodel and maintain certain functionalities are prime sources of information for designing synthetic ECMs used in tissue engineering and regeneration. Development signals are mimicked in tissue engineering approaches, using the developmental mechanisms and events as templates to design new synthetic ECMs.

The evolution in the design of synthetic ECMs parallels the development of our knowledge of the ECM. Historically, the ECM was viewed as a structural support for tissues, but it is now clear that the ECM plays a crucial role in controlling cell adhesion, migration, growth, and

differentiation via intracellular signaling pathways. Similarly, the main function of the early synthetic ECMs was to provide mechanical support. Current approaches are more ambitious, as the synthetic ECMs are designed to regulate cell function via presentation of various receptor-binding ligands, in addition to providing structural and mechanical information regulating tissue configuration.

Tissue engineering approaches can be divided into three basic strategies, conductive, inductive, and cell transplantation strategies (Fig. 1), and synthetic ECMs typically play a key role in all three. In conductive approaches, synthetic ECMs are used to maintain a space and passively allow host cell infiltration into the tissue defect site. A different stratagem for new tissue formation involves the delivery of bioactive signaling molecules (e.g., growth

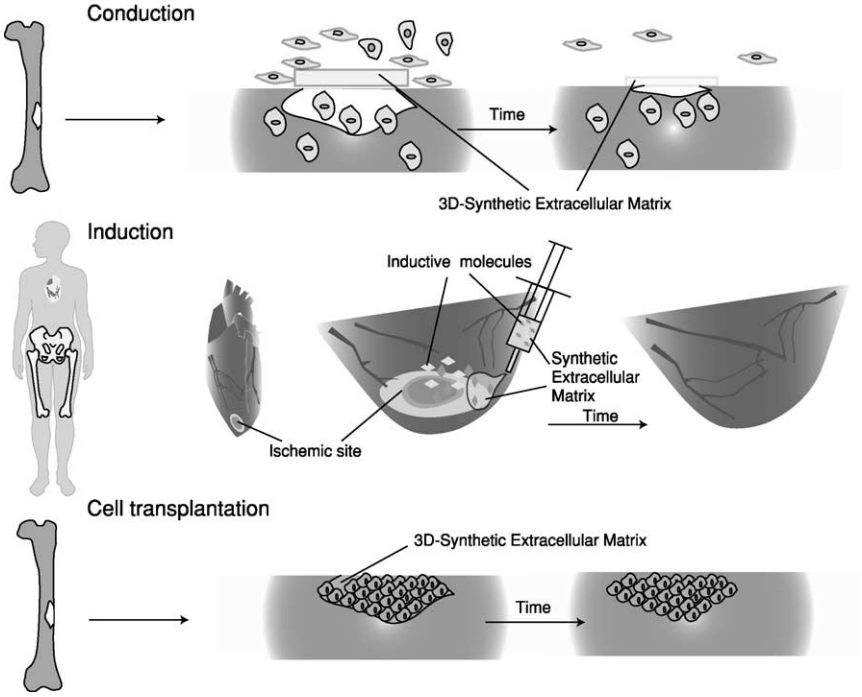

Figure 1 Schematic representation of tissue engineering approaches using synthetic extracellular matrices (ECMs). Conductive approaches exploit the ability of the host cell populations to infiltrate ECMs and guide the tissue regeneration at the defect site. Inductive bioactive molecules may also be delivered from ECMs to target specific cell populations to migrate and direct tissue regeneration. Cell transplantation strategies utilize the delivery of specific cell population cultured *in vitro* to the defect site in order to create new tissues or organs.

factors, adhesive peptides) that bind to target host cell populations and induce them to migrate into the defect site and participate in new tissue formation. In a manner similar to the sequestration and presentation to cells of growth factors by ECM molecules, the synthetic ECMs are often used as both depots and controlled release vehicles for growth factors. Again similar to native ECM molecules, adhesive signals (e.g., cell adhesion peptides) can be incorporated into the matrix to control cell migration from the defect interface with the surrounding tissue. The third strategy involves the transplantation of cells that can participate in tissue formation. The transplanted cells can be derived either from the patient or from a donor, and the multiplication of the cells *in vitro* prior to transplantation allows a small cell supply to be greatly expanded.

In this chapter, we discuss the design parameters for synthetic ECMs used in inductive and cell transplantation strategies of tissue engineering and regeneration. Synthetic EMCs used in conductive approaches are not reviewed, as their design in mainly dictated by materials science principles. A variety of different types and sources of synthetic ECMs is currently used for induction and cell transplantation, but this chapter focuses on polymer-based ECMs. Specific examples of the use of these synthetic ECMs in bone regeneration and therapeutic angiogenesis are provided, as well as a discussion of potential research directions for the future.

A. Design of Synthetic Mimics of the Extracellular Matrix

The ECM of developing tissues regulates development through multiple mechanisms, including the binding of cells to the ECM molecules via specific receptors, conveyance of mechanical signals, and presentation of growth factors and cytokines. These signals vary in a temporally dynamic manner due to the constant remodeling of the ECM. Synthetic mimics of the ECM are currently being designed to incorporate these signaling features, and all except growth factor presentation, which is discussed in the next section, is overviewed in this section.

One function of the ECM in tissue development is providing a foundation for cell attachment, as cell anchorage plays an important role in the regulation of cell growth, differentiation, and apoptosis. The adhesion of cells to native ECM is mediated by specific cell surface receptors, such as integrins that interact with short amino acid sequences presented in the ECM molecules (Hubbell, 1999). This signaling is frequently mimicked in synthetic ECMs by the presentation of cell-binding motifs from the polymers used to fabricate the synthetic ECM. The amino acid sequence arginine-glycine-aspartic acid (RGD), a ubiquitous cell-binding domain derived from fibronectin and laminin, is the peptide most frequently used to promote cellular

attachment to synthetic ECMs (Drury and Mooney, 2003; Hubbell, 1995; Shin *et al*., 2003), and the density of these ligands presented to cells from the material has been shown to regulate the cellular response *in vitro* and *in vivo* (Alsberg *et al*., 2003; Healy *et al*., 1999; Hubbell, 1999; Rezania and Healy, 1999). As different cell populations exhibit varying patterns of integrin expression, it is possible to use distinct peptide sequences in order to bind specific cell types to the synthetic ECM (Table I). For example, the amino acid sequence REDV (notation derived from the amino acid code), derived from fibronectin, has been reported to specifically bind endothelial cells (ECs) (Hubbell *et al*., 1991), and thus may be useful in designing vascular grafts (see Section IV.C).

Several different materials have been used as synthetic ECMs for peptide presentation. An important criterion used to choose an appropriate material is minimal nonspecific cell adhesion to the material, in order to maximize the specificity of cell adhesion via the incorporated peptide. Alginate, a naturally derived polysaccharide, and polyethylene glycol (PEG), a synthetic polymer, both exhibit little native cell adhesion and are widely used as synthetic ECMs for this reason. Different techniques are employed to present peptides from synthetic ECMs solely at the surface or throughout the bulk, and these include absorption and covalent coupling. Surface covalent coupling is a simple way to present peptides with defined orientation and density. For example, carbodiimide chemistry has been successfully used with alginate hydrogels to promote covalent coupling of cell adhesion peptides (Rowley *et al*., 1999). Bulk modification of the material may present advantages as compared to surface modifications, as this approach allows cells throughout the material to contact the peptide. Bulk peptide immobilization in synthetic ECMs is typically achieved by physical, chemical, photochemical, or ionic crosslinking (Shin *et al*., 2003). For example, it has recently been demonstrated that alginate hydrogels covalently coupled with G_4RGD peptide sequence throughout its bulk promote the proliferation of different cell types *in vitro* and *in vivo* (Hirano and Mooney, 2004).

In addition to signaling resulting directly from receptor–ligand binding, the integrin–ECM bonds form a physical link between the cells and the ECM through which mechanical signals may be conveyed to cells. It will likely be critical to the success of tissue engineering approaches of designing synthetic ECMs that allow the passage of stresses and strains from the matrix to the cells in order to direct cellular gene expression and the structure and function of the resultant tissue. For example, the presence of cyclic mechanical strain can be responsible for the maintenance of a differentiated phenotype, cell organization, and development of superior mechanical properties in engineered smooth muscle tissues (Mitchell and Niklason, 2003; Niklason *et al*., 1999; Nikolovski *et al*., 2003). In addition, the ability of synthetic ECMs to accommodate cell-generated forces is important to the ECM's ability to

Table I Representative Cell Adhesion Peptides Used to Modulate Cell Adhesion to Synthetic ECMs

Peptide sequence	Material	Representative reference
RGD	Alginate	Alsberg *et al*., 2003
	PEG	Hubbell, 1999
	PLGA; PLA	Shin *et al*., 2003
REDV	Glass	Hubbell *et al*., 1991; Massia and Hubbell, 1992
YIGSR	Fluorinated ethylene propylene (FEP)	Ranieri *et al*., 1995
	PEG	Dai *et al*., 1994
	Polyethylene terephthalate (PET), PTFE	Massia and Hubbell, 1991
KQAGDV	PEG	Mann *et al*., 2001

control various cellular events, such as adhesion, migration, and differentiation (Ingber, 2003a,b; Ingber *et al*., 1994; Pelham and Wang, 1997; Wang *et al*., 2000). The surface density of binding peptides may be used to control the cellular force balance and switch cells from a differentiated phenotype to a proliferative state (Mooney *et al*., 1992).

Controlling the remodeling of synthetic ECMs is crucial for modulating the development of engineered tissues. Two different approaches for regulating synthetic ECM remodeling can be pursued. One is based on the concept of pre-defining the degradation rate to occur at a specific rate, independent of the environment. This approach has been exploited to demonstrate that increasing the degradation rate of alginate hydrogels leads to an increase in the quality and quantity of regenerated bone formed with cell transplantation (Alsberg *et al*., 2003). This study supports the idea that matching the degradation rate of the material to the rate of tissue formation can improve tissue regeneration. An alternative approach involves allowing the cells themselves to mediate the remodeling process. In this approach, proteolytic degradation sites are built into the matrix to allow cellular enzymes to degrade the material. These materials mimic the substrates for matrix metalloproteinases (MMPs), and when associated with integrin-binding sites can result in an improvement of cell ingrowth *in vivo* (Lutolf *et al*., 2003a). Further, it has been demonstrated that combining RGD peptides and MMP substrates in a matrix can enhance new bone formation (Lutolf *et al*., 2003b).

II. Inductive and Cell Transplantation Strategies

Growth factors have an important role in regulating cell migration, differentiation, and proliferation during development, and the action mechanism of growth factors is typically time and concentration dependent. In therapeutic approaches, growth factors are usually delivered either by systemic administration or by bolus injection. However, these approaches present some disadvantages, such as the low targeting for the specific cell population (systemic administration) and the short half-life of many growth factors in the body. The use of synthetic ECMs to locally deliver either the growth factors or DNA encoding the factors can be used to bypass these disadvantages. Moreover, the polymeric materials used for inductive factor delivery can be simultaneously used for cell transplantation at the desired site in order to provide additional cell populations that can participate in new tissue formation. This section overviews the general principles used with inductive and cell transplantation strategies, and specific applications of these approaches are described in the next sections.

A. Inductive Approaches

Synthetic ECMs can provide growth factors to desired cell populations in a localized and sustainable manner. The growth factors are usually encapsulated into the polymer, preventing their denaturation, and their release can be controlled by the degradation rate of the polymer or by their diffusion through the polymer. Similarly, the synthetic ECMs can be used to locally provide DNA encoding the desired factor in order to transfect cells and increase the local production of the factor.

1. Protein Delivery

The simplest approach to providing a desired growth factor(s) at the site at which one desires tissue regeneration is to directly deliver recombinant versions of the factor. The first, and still dominant, approach involves the injection of solutions containing the factor either into the systemic circulation or directly at the desired site. Supraphysiologic quantities of proteins are typically delivered in an attempt to maintain the required concentration of the factor in the tissue of interest for the desired time frame. However, this approach has significant limitations, including the limited lifetime of many of these proteins in the body and the possibility of side effects at distant sites due to the presence of the factors in undesired tissues.

An alternative approach that more closely mimics the normal process of presentation for many factors in tissue development and regeneration is to locally provide growth factor gradients at a desired site using synthetic ECMs as a carrier. This approach allows physiologically relevant concentrations of the factors to be delivered in a sustainable and localized manner. Polymeric materials are attractive candidates for this approach, as the protein can be slowly and controllably released from the polymer while the polymer protects the incorporated protein from denaturation.

Polymeric delivery vehicles may be either implantable or injectable, and can be fabricated from a variety of synthetic and natural polymers. Implantable materials require invasive procedures in order to be placed at the desired site, while injectable materials may be delivered using minimally invasive technologies. A variety of polymers, including poly(glycolide) (PGA), poly(L-lactide) (PLA), and their copolymer (poly[lactide-co-glycolide]) (PLG) has been widely used for protein delivery (Hubbell, 1999; Richardson *et al*., 2001a; Robey *et al*., 2000; Sheridan *et al*., 2000; Shin *et al*., 2003). These materials are generally considered to be biocompatible and have been used in biomedical applications for over 30 years. These polymers can be formed into a variety of physical structures relevant to drug delivery using several processes (Mikos *et al*., 1994; Mooney *et al*., 1996; Sheridan *et al*., 2000), but a key issue is maintenance of the bioactivity of the encapsulated growth factors. A high-pressure gas foaming process has been developed that allows factor incorporation without the use of organic solvents or high temperatures (both can lead to protein denaturation) (Sheridan *et al*., 2000), and this system can be used for the delivery of growth factors *in vivo* in a bioactive form, resulting in sustainable release that can lead to new tissue formation (Chen and Mooney, 2003).

Both synthetic and naturally derived hydrogel forming materials are used in growth factor delivery due to their ability to be injected. Synthetic materials (e.g., poly[ethyleneoxide] [PEO], poly[vinyl alcohol] [PVA]) are advantageous in that their chemistry and physical properties can be readily controlled (Drury and Mooney, 2003; Sakiyama-Elbert and Hubbell, 2001). Naturally derived gel-forming polymers (e.g., alginate, chitosan) typically exhibit excellent biocompatibility. Some physical properties, such as the degradation rate, can be difficult to control with naturally derived materials. However, covalent modifications can be used to allow control over features such as degradation (Drury and Mooney, 2003).

2. Local Gene Therapy

An alternative inductive approach to direct protein delivery is to instead use gene therapy approaches to locally produce the desired factor(s) from host cell populations at the site. Gene therapy approaches may bypass some

limitations presented by protein formulations, mainly related to the glycosylation of recombinantly produced proteins and protein instability in polymeric delivery vehicles. Viral gene therapy approaches (e.g., adenovirus) can be highly efficient and have been pursued in clinical trials (Epstein *et al.*, 2001). However, there are still are a number of concerns (e.g., immune response to virus) associated with this approach. On the other hand, nonviral approaches that utilize the delivery of plasmid DNA encoding the desired factor may circumvent some of the concerns related to viral strategies (Levy *et al.*, 1998). The main limitation associated with nonviral approaches is the very low transfection efficiency.

Synthetic ECMs may be used to deliver plasmid DNA in order to obtain high expression levels from host cells (Shea *et al.*, 1999). This expands the function of synthetic ECMs beyond the normal functions of native ECMs, but is a natural extension of the concept of using synthetic ECMs to regulate cell gene expression *in vivo*. Polymer-based DNA delivery using various physical forms of the polymer, including microspheres and macroporous matrices (Levy *et al.*, 1998; Murphy and Mooney, 1999), has been pursued. Microspheres can be delivered in a minimally invasive manner. Macroporous matrices, which are typically implanted using an invasive surgical procedure, allow cell invasion and thus serve to bring host cells to the site of DNA release as well as serve as a sustained release device for the plasmid DNA. This approach to produce growth factors locally has led to promising results (Levy *et al.*, 1998; Park and Healy, 2003; Shea *et al.*, 1999). Plasmid DNA released from collagen sponges has led to the transfection of 20–50% of available cells, and porous PLG matrices and PLG microspheres similarly have been used to obtain high levels of local gene expression (Richardson *et al.*, 2001a). Condensation of the plasmid DNA with polycations to form electropositive nanoparticles has been combined with synthetic ECM in a sustained and localized delivery to further enhance gene expression (Huang *et al.*, 2003). Electropositive nanoparticles demonstrate enhanced cellular uptake and can be designed to escape lysosomal degradation (Godbey *et al.*, 2000; Lemkine and Demeneix, 2001).

B. Cell Transplantation

Limitations (e.g., unpredictable and uncontrolled cell type recruitment, time gap between matrix delivery, and tissue development) associated with inductive strategies could be circumvented by directly transplanting the desired cell population to the target site. Cell populations may be delivered without the use of a synthetic ECM (Traggiai *et al.*, 2004), but the use of a synthetic ECM provides an opportunity to both regulate the gene expression of the

transplanted cells and control the structure and function of the tissues formed either *in vitro* or *in vivo*.

The identification of the specific types and sources of cells to be transplanted is critical to the success of this approach. Most often, differentiated cell types related to the target tissue have been used in past work (e.g., dermal fibroblasts were used to engineer dermal tissues), as this provides more certainty regarding the functionality of the transplanted cell population and the possibility that they may have the genetic pre-programming to reassemble appropriately into the desired tissue or organ structure (Alsberg *et al*., 2002; Hubbell, 1995; Oberpenning *et al*., 1999; Uyama *et al*., 2001). The cells may potentially be obtained from autologous, allogenic, or xenogenic sources. Autologous tissues are the most direct source of cells and reduce problems related with compatibility and immune reactions to the transplanted cells. Allogenic cells are typically used when there exists a limited supply of autologous cells and/or banking and when use of a constant cell source is desirable. Xenogeneic cells are attractive when the autologous and allogeneic sources are insufficient to achieve the necessary cell mass. Advances in stem cell research provide encouragement that either embryonic stem cells or adult tissue-derived multipotent cells could be used for tissue repair or formation (Caplan and Bruder, 2001; Passier and Mummery, 2003; Verfaillie, 2002a,b). Stem cell populations offer the possibility of significant expansion in culture from a small starting tissue mass and the ability to form multiple cell types from a single starting source.

Synthetic ECMs are frequently used to deliver cells to the desired tissue site, provide the space for tissue development, and evoke specific cellular responses to modulate tissue architecture and function (Hirano and Mooney, 2004; Parenteau and Hardin-Young, 2002). Their design is based on the criteria discussed earlier, in terms of biological and physical characteristics, and several of the same biodegradable polymers used for protein/plasmid DNA delivery have also been used for cell transplantation. These include synthetic macroporous scaffolds fabricated from collagen and PLG (Ameer *et al*., 2002; Carrier *et al*., 1999; Park *et al*., 2002) and hydrogels formed from synthetic and naturally derived polymers (e.g., alginate) (Dar *et al*., 2002). Due to their structural similarity to the ECM native to many developing tissues (e.g., highly hydrated, space-filling gel), hydrogels are appealing for cell transplantation approaches. Alginate and PGA have been used extensively for new cartilage regeneration via the transplantation of autologous chondrocyte (Fuchs *et al*., 2003; Koch and Gorti, 2002). Combining inductive approaches with cell transplantation may allow the modulation of the fate of transplanted cells and is being pursued to more precisely regulate new tissue development and remodeling (Smith *et al*., 2004).

III. Engineering Vascular Structures

Cardiovascular diseases cause over 15 million deaths in the world each year (Al-Radi *et al.*, 2003). In the western countries, coronary artery disease is a principal cause of mortality despite enormous improvements in prevention and development of therapies for ischemic heart disease. Current treatment approaches consist of pharmacologic agents (anti-anginal medications) or the use of invasive surgical procedures (e.g., coronary artery bypass grafting) aimed at re-establishing perfusion. However, there still remains a major need for new approaches to treat patients who are not candidates for the current approaches. The insufficient and incomplete revascularization associated with residual symptoms of myocardial ischemia, demonstrated by many patients after these procedures, also motivates interest in new therapeutic approaches (Epstein *et al.*, 2001). A promising alternative method to treat heart ischemia involves the delivery of appropriate therapeutic agents that result in activation of the angiogenesis process or a partial activation of the vasculogenic process.

Developmental studies are responsible for many insights that have led to our current understanding of the molecular basis of blood vessel formation (Darland and D'Amore, 2001). The identification of key factors associated with blood vessel formation allows the replication of aspects of this signaling to drive new blood vessel formation in emerging therapies. For example, the identification of signals that recruit or differentiate endothelial cell precursors (Fig. 2) offers opportunities to mimic and maneuver the contributions of these cells to vascular growth.

Blood vessel formation occurs by several mechanisms and occurs with distinct chronological phases. Vasculogenesis refers to the initial development of vascular structures in the embryo by endothelial cell precursors. Angiogenesis, in contrast, refers to new blood vessel formation by sprouting and subsequent stabilization of new vessels from existing vessels (Carmeliet and Conway, 2001; Conway *et al.*, 2001). Both processes are complex, and occur in a stepwise fashion. Despite recent advances in delineating molecular, genetic, and cellular mechanisms of vasculogenesis and angiogenesis, many aspects of these processes are unclear. Tissue engineering systems may provide useful models to study this process while also potentially providing new therapies.

A. Mimicking Vasculogenesis

Vasculogenesis refers to the *in situ* growth of vessels from progenitor endothelial cells (ECs) or angioblasts. Under the influence of specific molecular cues, these cells self-organize and assemble into a primitive network

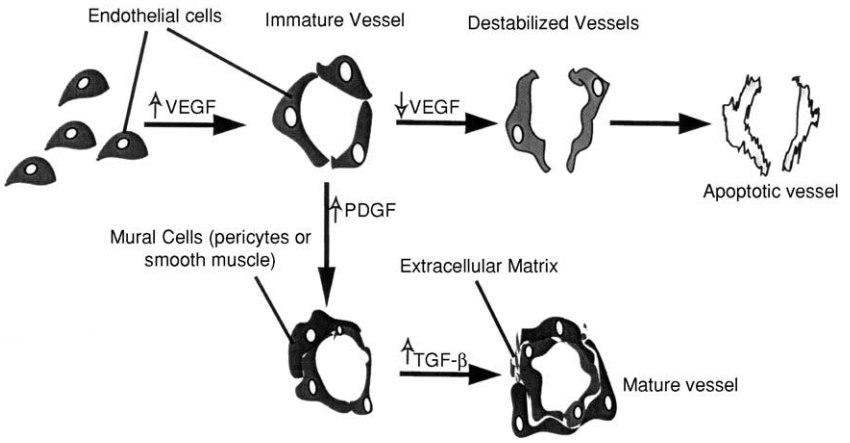

Figure 2 Schematic illustration of the role of certain growth factors in the angiogenesis process. Under the influence of factors such as vascular endothelial growth factor (VEGF), the endothelial cells are recruited and form an immature vessel; upregulation of platelet-derived growth factor (PDGF) may be responsible for the recruitment of mural cells necessary for the maturation of the blood vessel. The absence of VEGF destabilizes the immature vessels, and the endothelial cells are susceptible to apoptosis. The presence of transforming growth factor-β (TGF-β) may be required for the further maturation of blood vessels by the deposition of extracellular matrix.

(Conway *et al*., 2001). There are a number of genes involved in vasculogenesis, including *Hex, Vezf*, and basic helix-loop-helix (bHLH) (Carmeliet, 1999). Some genes that play important roles in this process are, as well, upregulated during blood vessel formation in the adult (e.g., the *vegf* gene) (Luttun and Carmeliet, 2003). Until recently, it was believed that the process of vasculogenesis was restricted to embryonic development, while blood vessel formation in the adult was an exclusive result of angiogenesis, but it was recently demonstrated that processes related to vasculogenesis can occur in the adult. The identification of angioblasts circulating in adults suggests that not only can these cells migrate from vessels in the embryo, but they can also be recruited to form capillaries in the adult (Risau, 1995). The isolation of circulating stem cells in peripheral blood followed by their differentiation by local signals may allow vasculogenesis to be directed in a therapeutic sense to treat tissue ischemia. However, opposite to vessel formation in the embryo, where the activity and proliferation of endothelial cell precursors is high, in adults these cells remain in an extraordinary latent phase. Studies estimate that the interval for the entrance of ECs to the circulation is less than 1000 days (Isner and Takayuki, 1998). One strategy for bypassing the limitation of EC precursors is to instead transplant mature ECs.

A key issue in attempts to mimic vasculogenesis via delivery of mature ECs or precursors is the development of systems that efficiently allow the

delivery of these cells to the desired site and simultaneously provide an environment conductive to the formation of functional vessel networks. Polymeric scaffolds are appealing for this type of cell delivery, as they can deliver cells in an efficient manner and provide the necessary biochemical and mechanical signals to the cells and forming vessels. Three-dimensional porous polymeric scaffolds formed from PLG present high porosity and surface area to readily allow cell introduction and have been used to transplant ECs. Subcutaneously implanted sponges seeded with ECs led to a significant number of new capillaries formed *in vivo* from the transplanted ECs (Nor *et al*., 1999, 2001). Surfaces that specifically allow EC adhesion and discourage adhesion of other cell types could potentially be achieved by the incorporation of specific cell-binding peptides via surface modification (Shin *et al*., 2003) and used in this approach to revascularization.

B. Therapeutic Angiogenesis

Angiogenesis involves a cascade of processes, including EC activation, recruitment, and proliferation followed by interactions with mural cells for the stabilization of the initially immature new vasculature. A number of angiogenic and antiangiogenic signals have been identified, and these factors are presented locally in a well-regulated way to control angiogenesis. Angiogenesis may be described as a process first involving the vasodilation of existing vessels, an event that is mediated by vascular endothelial growth factor (VEGF), accompanied by an increase of the vascular permeability. Associated with these processes is degradation of the ECM, which is necessary for the subsequent EC migration. These processes are regulated by angiopoietin-1 (Ang1) and its antagonist angiopoetin-2 (Ang2). Degradation of ECM is mainly orchestrated by MMPs secreted by the ECs. EC proliferation and migration is followed by the assembly of immature vessels networks. It was reported that monocyte chemotatic protein (MCP)-1 can also provoke EC proliferation (Belperio *et al*., 2003). The immature EC assemblies are susceptible to regression due to EC apoptosis, or maturation. VEGF, Ang1, and Ang2 are key players in all steps of these processes (Holash *et al*., 1999; Kockx and Knaapen, 2000). Finally, the vessels mature to form stable vascular networks via the recruitment of smooth muscle cells (SMC), and this process is regulated by platelet-derived growth factor (PDGF) (Conway *et al*., 2001; Fig. 2).

Therapeutic angiogenesis involves the delivery of various angiogeneic factors to drive new blood vessel formation in an ischemic tissue. Currently, the number of growth factors used in therapeutic angiogenesis is considerable (Table II); they include VEGF, PDGF, and basic fibroblast growth factor (b-FGF). Moreover, VEGF and bFGF might be involved in the

Table II Representative Growth Factors Delivered with Synthetic ECMs to Drive Tissue Regeneration

Growth factor	Synthetic ECM	Regenerated tissue	Representative reference
VEGF	Alginate	Blood vessels	Lee *et al.*, 2003
	PEG	Blood vessels	Hubbell, 1999
	PLG	Blood vessels	Richardson *et al.*, 2001b
PDGF	PLG	Blood vessels	Richardson *et al.*, 2001b
bFGF	Alginate	Blood vessels	Lee *et al.*, 2003
BMP-2	PEG	Bone	Lutolf *et al.*, 2003a
BMP-4	Collagen	Bone	Fang *et al.*, 1996
BMP-7	PGA	Bone	Breitbart *et al.*, 1999

recruitment and differentiation of bone marrow-derived angioblasts (Rossant and Howard, 2002).

Therapeutic angiogenesis can be realized either by using growth factor formulations or by the delivery of genes encoding these proteins. VEGF and b-FGF have been extensively used in phase I/II clinical trials, but no results for phase III trials have been reported to date. Phase I trials typically have reported promising results (Rosengart *et al.*, 1999; Schumacher *et al.*, 1998). However, the results obtained in the larger phase II trials have not shown the expected benefit to patients (Simons *et al.*, 2002). One potential limitation of current approaches in therapeutic angiogenesis is the mode of delivery, which involves the introduction of large doses of the potent angiogenic molecules in solution form. This may not allow sufficient levels of the factors to be present in the target tissue for the necessary time frame and may lead to severe side effects. Synthetic ECMs can potentially be used for the controlled delivery of angiogenic molecules with low systemic exposure. Hydrogels may be particularly useful, as they can supply growth factors using minimally invasive procedures, and several angiogenic molecules, including PDGF, VEGF, and b-FGF, have been incorporated into hydrogels (Lee and Mooney, 2001; Zisch *et al.*, 2003). Growth factor release is typically controlled by diffusion and/or polymer degradation. However, the mechanical environment of the local site may dramatically influence the release rate (Lee *et al.*, 2000). This effect could be exploited to deliver bioactive molecules in locations under mechanical stress (e.g., the heart). VEGF bioactivity is maintained in these systems, and in some cases it seems to be enhanced, perhaps due to monomers associating with VEGF and protecting the protein even after its release (Peters *et al.*, 1998).

Polymeric materials can be also useful for investigating the role of combinations of growth factors in angiogenesis, since a synergy between different

growth factors has been demonstrated in angiogenesis (Pepper *et al*., 1992). For example, a three-dimensional PLG scaffold has been developed that allows the release of sequences of growth factors (Richardson *et al*., 2001b). Release of VEGF followed by PDGF led to both a significant increase in the local blood vessel density and maturation of the newly formed vessels. Simultaneous delivery of the two factors led to little to no revascularization.

C. Large Blood Vessel Engineering

In addition to angiogenesis and vasculogenesis approaches to form new blood vessel networks, there is often a need for immediate replacement of large blood vessels. Autologous veins or arteries are used in cardiac or peripheral bypass surgery. However, many individuals are not candidates for this clinical procedure due to the lack of appropriate blood vessels for use as replacements. In these cases it is necessary to use synthetic vascular grafts, and polymers have been used as a blood vessel substitute (Ratcliffe, 2000). However, these synthetic vessels have a lower patency rate when compared with natural vessels due to their susceptibility to thrombus formation as well as to a lack of mechanical conformity with adjacent native tissues. The failure of synthetic polymers to successfully replace blood vessels is more accentuated in small diameter grafts (<6 mm internal diameter) (Niklason *et al*., 1999).

Cardiovascular tissue engineering strategies have been developed to provide an alternative supply of vessels to replace diseased arteries. Ideally, the three different structures (intima, media, and adventia) present in blood vessels would be mimicked in the tissue-engineered vascular graft and could provide function immediately on implantation. Seeding ECs on the luminal surface of synthetic polymers is one approach to circumvent the thrombogenic events associated with synthetic grafts (Daly *et al*., 2004). However, this strategy is limited by EC detachment from the surface following initiation of blood flow (Daly *et al*., 2004). EC attachment on synthetic ECMs can be increased by surface modifications with adhesion peptides. For example, vascular grafts of expanded polytetrafluoroethylene (ePTFE) layered with fibronectin and RGD peptides led to an increased EC attachment and retention (Ratcliffe, 2000). However, this approach does not address the mismatch in mechanical properties between synthetic and natural vessels. To address this issue, investigators have attempted to engineer complete new vessels. For example, polyglycolic acid scaffolds have been seeded with smooth muscle cells alone or combined with ECs, and maintained under cyclic strain or pulsatile flow for use as cardiovascular graft (Kim *et al*., 1999; Sodian *et al*., 1999). Over time, the polymer undergoes degradation,

resulting in a completely natural new tissue. Application of an appropriate regimen of mechanical stimulation can greatly enhance the mechanical properties of these *ex vivo* engineered vessels and improve their success following implantation (Niklason *et al*., 1999).

IV. Bone Regeneration

More than 800,000 bone grafting procedures are completed each year in the United States (Laurencin *et al*., 1999), and restoring or enhancing the repair of bone is a crucial problem in orthopedics and dentistry. Bone graft procedures often utilize autograft or allograft bone. While autografts are often highly successful, their availability is limited and the bone harvest creates another defect. In contrast, allografts are more available but are susceptible to viral contamination and immune responses by the host. Synthetic bone replacements are widely used, but typically fail over time and may have issues with biocompatibility.

Engineering new bone tissue may bypass the limitations associated with the standard techniques. All three major tissue engineering approaches, osteoconduction, osteoinduction, and cell transplantation strategies, have been investigated as approaches for regenerating bone (Alsberg *et al*., 2001). The following sections briefly review the process of bone formation and the approaches presently under investigation for bone tissue regeneration.

The vertebrate skeleton is composed of craniofacial, axial, and appendicular (i.e., limb skeleton) bone, and the cells that constitute the vertebrate skeleton are derived from three distinct embryonic lineages, cranial neural crest, paraxial mesoderm, and lateral mesoderm. The cranial neural crest cells are responsible for the craniofacial skeleton, paraxial mesoderm (somites) cells give rise to the axial skeleton, and the limb skeleton is derived from lateral plate mesodermal cells (Olsen *et al*., 2000). The bone cell precursors present in these three sections migrate to the precise location of future bones and differentiate into osteoblasts. Several transcription factors regulate the migration events involved in the morphogenesis of bone (Olsen *et al*., 2000; Zelzer and Olsen, 2003). Organogenesis, differentiation of these cells into bone-forming cells called osteoblasts, is known to be controlled by a variety of signals, including the ECM, transcription factors, cytokines, and growth factors (Zelzer and Olsen, 2003). The available knowledge regarding the molecular and cellular basis of bone development during embryo formation has been exploited for therapeutic goals. The signaling molecules and cells responsible for bone morphogenesis and organogenesis are often combined with synthetic matrices mimicking certain functions of the ECM to promote regeneration.

A. Osteoconduction

Osteoconductive approaches to bone regeneration, also called guided tissue or bone regeneration, are used to allow the infiltration of osteoprogenitors from bone marrow to the local defect, while providing temporary mechanical support. The physical properties of the synthetic ECM significantly affect its osteoconduction properties. For example, the pore size distribution, total porosity, and pore interconnectivity can be controlled to regulate cell infiltration. The pore size typically expected to yield successful cell permeation is greater than 10 μm (Wald *et al*., 1993; White *et al*., 1981), as is the connectivity between the pores to obtain space-filling new bone tissue. In certain situations, the material is used to block undesired cells types (i.e., fibroblasts) from accessing the defect site, while allowing cell types that can form bone to migrate into the site from a different location. This more selective cell conduction can be achieved by having the surface in contact with bone cells containing large pores, while the surface in contact with fibroblasts presents a pore size prohibitive for cell conduction. ePTFE has been successfully used as a selective membrane for periodontal guided tissue regeneration (Hermann and Buser, 1996). However, a second surgical procedure is necessary for removal of the ePTFE construct after tissue formation, due to the absence of ePTFE degradation. In order to circumvent this limitation, degradable materials have been developed, including PLA, PLG, and collagen-based materials (Alsberg *et al*., 2001; Christgau *et al*., 2002; Trejo *et al*., 2000). Alginate also has potential in bone conduction strategies (Tonnesen and Karlsen, 2002).

An important requirement for engineered bone constructs in many applications is high mechanical strength and stiffness. Synthetic ECMs formed from polymeric materials often have poor mechanical properties compared to native bone (Orban *et al*., 2002). Ceramic materials have been used for bone regeneration due to their better mechanical and osteoconduction properties. However, they can be too stiff and lead to stress shielding, and they also typically present poor degradation properties (e.g., slow degradation rates) when compared with polymers. One way of bypassing these limations may be to combine the advantageous features of polymers and ceramics. The presence of a bone-like mineral on a polymer scaffold may enhance osteoconduction and mechanical integrity while allowing a readily controlled biodegradation rate (Laurencin *et al*., 1999). The formation of a bone-like mineral film coating on three-dimensional PLG scaffolds has been demonstrated to enhance bone regeneration in cranial defects (Murphy *et al*., 2004).

In spite of the relative successes of osteoconductive strategies in forming new bone in certain situations, this approach exhibits modest control over the molecular mechanisms of bone regeneration. Regeneration may be greatly improved with more active approaches.

B. Osteoinduction

Osteoinductive approaches involve the delivery of inductive signals aimed to provoke the migration, proliferation, and differentiation of desired cell types into a bone defect site in order to actively control bone regeneration. Inductive signals can be delivered both by direct introduction of specific growth factors and by using gene therapy techniques. In both cases, materials are typically used as delivery vehicles for these agents.

A large number of growth factors have been implicated in bone formation and adaptation, but bone morphogenetic proteins (BMPs) appear to be crucially important in the regulation of bone development and repair (Seeherman *et al.*, 2002; Wozney, 2002). BMPs are members of the transforming growth factor-β (TGF-β) superfamily, which is responsible for controlling the phenotype and apoptosis of a variety of cell types, including osteoblasts, chondroblasts, neural cells, and epithelial cells (Wozney, 2002). Recombinant and naturally derived BMPs have been combined with several carrier materials, resulting in enhanced bone regeneration. For example, recombinant human BMP-2 (rhBMP-2) has been associated with sponges of PLG to allow a sustainable and localized delivery of the factor (Howell *et al.*, 1997), and this promotes the migration and proliferation of bone-forming cells into the defect. Vascularization represents a critical step in the formation and remodeling of bone, and a number of studies have demonstrated that angiogenic factor delivery using synthetic ECMs regulates bone regeneration (Uchida *et al.*, 2003). It is possible to use osteoconductive scaffolds to delivery angiogenic factors; this may enhance the effectiveness of the osteoconductive material. For example, mineralized PLG scaffolds capable of localized and sustained VEGF delivery promote new blood vessel formation at bone defect sites and increase bone regeneration (Murphy *et al.*, 2004).

Another approach to osteoinduction involves the immobilization of specific peptides on the surface of polymer materials placed in the defect in order to enhance migration of the desired cell populations into the defect. PEG gels have been adapted to allow cellular invasion by coupling cell adhesion peptides to the polymer chains and providing proteolytic degradation sites for cell-based degradation. Delivery of rhBMP-2 from these gels has led to promising results in bone regeneration (Lutolf *et al.*, 2003b).

Gene therapy approaches can also be applied to provide inductive factors that can drive bone regeneration. In this situation, the synthetic ECM is directly altering gene expression in cells, and plasmid DNA encoding osteogenic proteins is incorporated into implantable polymer constructs for local and sustained delivery. Delivery of plasmid DNA encoding for BMP-4 from natural based hydrogels, such as collagen, has been reported to effectively induce bone regeneration (Fang *et al.*, 1996). The DNA may also be condensed prior to incorporation into three-dimensional PLG scaffolds to

increase transfection levels, and this may further enhance bone regeneration with small doses of DNA (Huang *et al.*, 2003).

C. Cell Transplantation

An alternative approach to osteoconduction/induction strategies for bone regeneration is direct transplantation of desired cell populations into the bone defect. This approach at least partially bypasses the requirement for host cell migration, and may accelerate the process of bone regeneration, and/or make this possible in conditions where insufficient numbers of host cells are available to migrate into the site to regenerate the tissue. The transplanted cells are typically expanded *in vitro* before transplantation, to permit for a large cell supply to be generated from a small initial tissue mass (Langer and Vacanti, 1993). Synthetic ECMs are typically used to deliver bone forming cells (e.g., bone marrow stroma cells [BMSCs]) as they can localize the cells to a desired site, and provide cues regulating bone formation from the transplanted cells and host cells (Alsberg *et al.*, 2001). Combining cell transplantation with inductive approaches may provide a faster or greater extent of bone regeneration, by more closely mimicking the multifactor nature of the normal regenerative process. For example, the transplantation of human BMP-7 gene-enhanced BMSCs on PGA scaffolds into osteochondral knee defects significantly increased bone regeneration as compared to the cells or gene delivery alone (Breitbart *et al.*, 1999).

V. Conclusions and Future Directions

The field of tissue engineering and regeneration has developed in response to the critical lack of tissues and organs available for transplantation. Progress has been rapid, and several tissue engineering therapies are now available for treating patients (e.g., Carticel, Genzyme Corporation; Apligraf, Organogenesis Inc.). The field of tissue engineering has had considerable success to date in the development of small tissue masses. However, a critical challenge is to create large tissue masses and entire organs. Approaches to rapidly promote blood vessel networks will clearly be critical to achieve this goal. Advances in the understanding of development biology will lead to improved approaches to tissue regeneration; on the other hand, tissue engineering systems will provide novel models to study developmental events by controlling the environment signals.

In spite of the tremendous progress to date with approaches focused on single factor or cell type delivery, tissue development is not regulated by a single factor or accomplished by a single cell type. Optimal tissue regeneration may in the future be accomplished by integrating a temporally

synchronized cascade of signals and cell populations to create new tissues. Synthetic ECMs that provide multiple key signals with distinct temporal and spatial availability will be critical to this approach. A combination of cell types may also be useful in engineering organs comprised of multiple tissues types. The use of embryonic or adult stem cells may provide an ideal approach to create these types of tissues structures, as one cell population could lead to multiple tissue types. However, it will be necessary to obtain a better understanding of the biology of these cell populations for their successful use in tissue engineering. Synthetic ECMs may be ideal systems for providing the local cues required to tightly regulate the differentiation of these cell populations. Further, these systems may make ideal model systems for determining the relation between microenvironment signals and stem cell fate.

Acknowledgments

The authors acknowledge funding from the National Institutes of Health. E. A. S. is a student of the Gulbenkian PhD Program in Biomedicine, Portugal and is supported by the Portuguese Foundation for Technology and Science.

References

Al-Radi, O. O., Rao, V., Li, R. K., Yau, T., and Weisel, R. D. (2003). Cardiac cell transplantation: Closer to bedside. *Ann. Thorac. Surg.* **75**(2), S674–S677.

Alsberg, E., Anderson, K. W., Albeiruti, A., Rowley, J. A., and Mooney, D. J. (2002). Engineering growing tissues. *Proc. Natl. Acad. Sci. USA* **99**(19), 12025–12030.

Alsberg, E., Hill, E. E., and Mooney, D. J. (2001). Craniofacial tissue engineering. *Crit. Rev. Oral. Biol. Med.* **12**(1), 64–75.

Alsberg, E., Kong, H. J., Hirano, Y., Smith, M. K., Albeiruti, A., and Mooney, D. J. (2003). Regulating bone formation via controlled scaffold degradation. *J. Dent. Res.* **82**(11), 903–908.

Ameer, G. A., Mahmood, T. A., and Langer, R. (2002). A biodegradable composite scaffold for cell transplantation. *J. Orthop. Res.* **20**(1), 16–19.

Belperio, J. A., Keane, M. P., Burdick, M. D., Lynch, J. P., 3rd, Zisman, D. A., Xue, Y. Y., Li, K., Ardehali, A., Ross, D. J., and Strieter, R. M. (2003). Role of CXCL9/CXCR3 chemokine biology during pathogenesis of acute lung allograft rejection. *J. Immunol.* **171**(9), 4844–4852.

Breitbart, A. S., Grande, D. A., Mason, J. M., Barcia, M., James, T., and Grant, R. T. (1999). Gene-enhanced tissue engineering: Applications for bone healing using cultured periosteal cells transduced retrovirally with the BMP-7 gene. *Ann. Plast. Surg.* **42**(5), 488–495.

Caplan, A. I., and Bruder, S. P. (2001). Mesenchymal stem cells: Building blocks for molecular medicine in the 21st century. *Trends Mol. Med.* **7**(6), 259–264.

Carmeliet, P. (1999). Developmental biology. Controlling the cellular brakes. *Nature* **401**(6754), 657–658.

Carmeliet, P., and Conway, E. M. (2001). Growing better blood vessels. *Nat. Biotechnol.* **19**(11), 1019–1020.

Carrier, R. L., Papadaki, M., Rupnick, M., Schoen, F. J., Bursac, N., Langer, R., Freed, L. E., and Vunjak-Novakovic, G. (1999). Cardiac tissue engineering: Cell seeding, cultivation parameters, and tissue construct characterization. *Biotechnol. Bioeng.* **64**(5), 580–589.

Chen, R. R., and Mooney, D. J. (2003). Polymeric growth factor delivery strategies for tissue engineering. *Pharm. Res.* **20**(8), 1103–1112.

Christgau, M., Bader, N., Felden, A., Gradl, J., Wenzel, A., and Schmalz, G. (2002). Guided tissue regeneration in intrabony defects using an experimental bioresorbable polydioxanon (PDS) membrane. A 24-month split-mouth study. *J. Clin. Periodontol.* **29**(8), 710–723.

Conway, E. M., Collen, D., and Carmeliet, P. (2001). Molecular mechanisms of blood vessel growth. *Cardiovasc. Res.* **49**(3), 507–521.

Dai, W., Belt, J., and Saltzman, W. M. (1994). Cell-binding peptides conjugated to poly(ethylene glycol) promote neural cell aggregation. *Biotechnology (N Y)* **12**(8), 797–801.

Daly, C. D., Campbell, G. R., Walker, P. J., and Campbell, J. H. (2004). *In vivo* engineering of blood vessels. *Front. Biosci.* **9,** 1915–1924.

Dar, A., Shachar, M., Leor, J., and Cohen, S. (2002). Optimization of cardiac cell seeding and distribution in 3D porous alginate scaffolds. *Biotechnol. Bioeng.* **80**(3), 305–312.

Darland, D. C., and D'Amore, P. A. (2001). Cell-cell interactions in vascular development. *Curr. Top. Dev. Biol.* **52,** 107–149.

Drury, J. L., and Mooney, D. J. (2003). Hydrogels for tissue engineering: Scaffold design variables and applications. *Biomaterials* **24**(24), 4337–4351.

Epstein, S. E., Fuchs, S., Zhou, Y. F., Baffour, R., and Kornowski, R. (2001). Therapeutic interventions for enhancing collateral development by administration of growth factors: Basic principles, early results and potential hazards. *Cardiovasc. Res.* **49**(3), 532–542.

Fang, J., Zhu, Y. Y., Smiley, E., Bonadio, J., Rouleau, J. P., Goldstein, S. A., McCauley, L. K., Davidson, B. L., and Roessler, B. J. (1996). Stimulation of new bone formation by direct transfer of osteogenic plasmid genes. *Proc. Natl. Acad. Sci. USA* **93**(12), 5753–5758.

Fuchs, J. R., Hannouche, D., Terada, S., Vacanti, J. P., and Fauza, D. O. (2003). Fetal tracheal augmentation with cartilage engineered from bone marrow-derived mesenchymal progenitor cells. *J. Pediatr. Surg.* **38**(6), 984–987.

Godbey, W. T., Barry, M. A., Saggau, P., Wu, K. K., and Mikos, A. G. (2000). Poly(ethylenimine)-mediated transfection: A new paradigm for gene delivery. *J. Biomed. Mater. Res.* **51**(3), 321–328.

Healy, K. E., Rezania, A., and Stile, R. A. (1999). Designing biomaterials to direct biological responses. *Ann. N Y Acad. Sci.* **875,** 24–35.

Hermann, J. S., and Buser, D. (1996). Guided bone regeneration for dental implants. *Curr. Opin. Periodontol.* **3,** 168–177.

Hirano, Y., and Mooney, D. J. (2004). Peptide and protein presenting materials for tissue engineering. *Adv. Mater.* **16**(1), 17–25.

Holash, J., Maisonpierre, P. C., Compton, D., Boland, P., Alexander, C. R., Zagzag, D., Yancopoulos, G. D., and Wiegand, S. J. (1999). Vessel cooption, regression, and growth in tumors mediated by angiopoietins and VEGF. *Science* **284**(5422), 1994–1998.

Howell, T. H., Fiorellini, J. P., Paquette, D. W., Offenbacher, S., Giannobile, W. V., and Lynch, S. E. (1997). A phase I/II clinical trial to evaluate a combination of recombinant human platelet-derived growth factor-BB and recombinant human insulin-like growth factor-I in patients with periodontal disease. *J. Periodontol.* **68**(12), 1186–1193.

Huang, Y. C., Connell, M., Park, Y., Mooney, D. J., and Rice, K. G. (2003). Fabrication and *in vitro* testing of polymeric delivery system for condensed DNA. *J. Biomed. Mater. Res.* **67A**(4), 1384–1392.

Hubbell, J. A. (1995). Biomaterials in tissue engineering. *Biotechnology (N Y)* **13**(6), 565–576.

Hubbell, J. A. (1999). Bioactive biomaterials. *Curr. Opin. Biotechnol.* **10**(2), 123–129.

Hubbell, J. A., Massia, S. P., Desai, N. P., and Drumheller, P. D. (1991). Endothelial cell-selective materials for tissue engineering in the vascular graft via a new receptor. *Biotechnology (N Y)* **9**(6), 568–572.

Ingber, D. E. (2003). Mechanobiology and diseases of mechanotransduction. *Ann. Med.* **35**(8), 564–577.

Ingber, D. E. (2003). Mechanosensation through integrins: Cells act locally but think globally. *Proc. Natl. Acad. Sci. USA* **100**(4), 1472–1474.

Ingber, D. E., Dike, L., Hansen, L., Karp, S., Liley, H., Maniotis, A., McNamee, H., Mooney, D., Plopper, G., Sims, J., *et al.* (1994). Cellular tensegrity: Exploring how mechanical changes in the cytoskeleton regulate cell growth, migration, and tissue pattern during morphogenesis. *Int. Rev. Cytol.* **150,** 173–224.

Isner, J. M., and Takayuki, A. (1998). Therapeutic angiogenesis. *Front. Biosci.* **3,** e49–e69.

Kim, B. S., Nikolovski, J., Bonadio, J., and Mooney, D. J. (1999). Cyclic mechanical strain regulates the development of engineered smooth muscle tissue. *Nat. Biotechnol.* **17**(10), 979–983.

Koch, R. J., and Gorti, G. K. (2002). Tissue engineering with chondrocytes. *Facial Plast. Surg.* **18**(1), 59–68.

Kockx, M. M., and Knaapen, M. W. (2000). The role of apoptosis in vascular disease. *J. Pathol.* **190**(3), 267–280.

Langer, R., and Vacanti, J. P. (1993). Tissue engineering. *Science* **260**(5110), 920–926.

Laurencin, C. T., Ambrosio, A. M., Borden, M. D., and Cooper, J. A., Jr. (1999). Tissue engineering: Orthopedic applications. *Annu. Rev. Biomed. Eng.* **1,** 19–46.

Lee, K. Y., and Mooney, D. J. (2001). Hydrogels for tissue engineering. *Chem. Rev.* **101**(7), 1869–1879.

Lee, K. Y., Peters, M. C., Anderson, K. W., and Mooney, D. J. (2000). Controlled growth factor release from synthetic extracellular matrices. *Nature* **408**(6815), 998–1000.

Lee, K. Y., Peters, M. C., and Mooney, D. J. (2003). Comparison of vascular endothelial growth factor and basic fibroblast growth factor on angiogenesis in SCID mice. *J. Control Release* **87**(1–3), 49–56.

Lemkine, G. F., and Demeneix, B. A. (2001). Polyethylenimines for *in vivo* gene delivery. *Curr. Opin. Mol. Ther.* **3**(2), 178–182.

Levy, R. J., Goldstein, S. A., and Bonadio, J. (1998). Gene therapy for tissue repair and regeneration. *Adv. Drug Deliv. Rev.* **33**(1–2), 53–69.

Lutolf, M. P., Lauer-Fields, J. L., Schmoekel, H. G., Metters, A. T., Weber, F. E., Fields, G. B., and Hubbell, J. A. (2003). Synthetic matrix metalloproteinase-sensitive hydrogels for the conduction of tissue regeneration: Engineering cell-invasion characteristics. *Proc. Natl. Acad. Sci. USA* **100**(9), 5413–5418.

Lutolf, M. P., Weber, F. E., Schmoekel, H. G., Schense, J. C., Kohler, T., Muller, R., and Hubbell, J. A. (2003). Repair of bone defects using synthetic mimetics of collagenous extracellular matrices. *Nat. Biotechnol.* **21**(5), 513–518.

Luttun, A., and Carmeliet, P. (2003). *De novo* vasculogenesis in the heart. *Cardiovasc. Res.* **58**(2), 378–389.

Mann, B. K., Schmedlen, R. H., and West, J. L. (2001). Tethered-TGF-beta increases extracellular matrix production of vascular smooth muscle cells. *Biomaterials* **22**(5), 439–444.

Massia, S. P., and Hubbell, J. A. (1991). Human endothelial cell interactions with surface-coupled adhesion peptides on a nonadhesive glass substrate and two polymeric biomaterials. *J. Biomed. Mater. Res.* **25**(2), 223–242.

Massia, S. P., and Hubbell, J. A. (1992). Vascular endothelial cell adhesion and spreading promoted by the peptide REDV of the IIICS region of plasma fibronectin is mediated by integrin alpha 4 beta 1. *J. Biol. Chem.* **267**(20), 14019–14026.

Mikos, A. G., Lyman, M. D., Freed, L. E., and Langer, R. (1994). Wetting of poly(L-lactic acid) and poly(DL-lactic-co-glycolic acid) foams for tissue culture. *Biomaterials* **15**(1), 55–58.

Mitchell, S. L., and Niklason, L. E. (2003). Requirements for growing tissue-engineered vascular grafts. *Cardiovasc. Pathol.* **12**(2), 59–64.

Hansen, L. K., Mooney, D. J., Vacanti, J. P., and Ingber, D. E. (1992). Switching from differentiation to growth in hepatocytes: Control by extracellular matrix. *J. Cell Physiol.* **151**(3), 497–505.

Mooney, D. J., Baldwin, D. F., Suh, N. P., Vacanti, J. P., and Langer, R. (1996). Novel approach to fabricate porous sponges of poly(D,L-lactic-co-glycolic acid) without the use of organic solvents. *Biomaterials* **17**(14), 1417–1422.

Murphy, W. L., and Mooney, D. J. (1999). Controlled delivery of inductive proteins, plasmid DNA and cells from tissue engineering matrices. *J. Periodontal. Res.* **34**(7), 413–419.

Murphy, W. L., Simmons, C. A., Kaigler, D., and Mooney, D. J. (2004). Bone Regeneration via a Mineral Substrate and Induced Angiogenesis. *J. Dent. Res.* **83**(3), 204–210.

Niklason, L. E., Gao, J., Abbott, W. M., Hirschi, K. K., Houser, S., Marini, R., and Langer, R. (1999). Functional arteries grown *in vitro*. *Science* **284**(5413), 489–493.

Nikolovski, J., Kim, B. S., and Mooney, D. J. (2003). Cyclic strain inhibits switching of smooth muscle cells to an osteoblast-like phenotype. *FASEB. J.* **17**(3), 455–457.

Nor, J. E., Christensen, J., Mooney, D. J., and Polverini, P. J. (1999). Vascular endothelial growth factor (VEGF)-mediated angiogenesis is associated with enhanced endothelial cell survival and induction of Bcl-2 expression. *Am. J. Pathol.* **154**(2), 375–384.

Nor, J. E., Peters, M. C., Christensen, J. B., Sutorik, M. M., Linn, S., Khan, M. K., Addison, C. L., Mooney, D. J., and Polverini, P. J. (2001). Engineering and characterization of functional human microvessels in immunodeficient mice. *Lab. Invest.* **81**(4), 453–463.

Oberpenning, F., Meng, J., Yoo, J. J., and Atala, A. (1999). *De novo* reconstitution of a functional mammalian urinary bladder by tissue engineering. *Nat. Biotechnol.* **17**(2), 149–155.

Olsen, B. R., Reginato, A. M., *et al.* (2000). Bone development. *Annu. Rev. Cell Dev. Biol.* **16,** 191–220.

Orban, J. M., Marra, K. G., and Hollinger, J. O. (2002). Composition options for tissue-engineered bone. *Tissue Eng.* **8**(4), 529–539.

Parenteau, N. L., and Hardin-Young, J. (2002). The use of cells in reparative medicine. *Ann. N Y Acad. Sci.* **961,** 27–39.

Park, K. I., Teng, Y. D., and Snyder, E. Y. (2002). The injured brain interacts reciprocally with neural stem cells supported by scaffolds to reconstitute lost tissue. *Nat. Biotechnol.* **20**(11), 1111–1117.

Park, S., and Healy, K. E. (2003). Nanoparticulate DNA packaging using terpolymers of poly(lysine-g-(lactide-b-ethylene glycol)). *Bioconjug. Chem.* **14**(2), 311–319.

Passier, R., and Mummery, C. (2003). Origin and use of embryonic and adult stem cells in differentiation and tissue repair. *Cardiovasc. Res.* **58**(2), 324–335.

Pelham, R. J., Jr., and Wang, Y. (1997). Cell locomotion and focal adhesions are regulated by substrate flexibility. *Proc. Natl. Acad. Sci. USA* **94**(25), 13661–13665.

Pepper, M. S., Ferrara, N., Orci, L., and Montesano, R. (1992). Potent synergism between vascular endothelial growth factor and basic fibroblast growth factor in the induction of angiogenesis *in vitro*. *Biochem. Biophys. Res. Commun.* **189**(2), 824–831.

Peters, M. C., Isenberg, B. C., Rowley, J. A., and Mooney, D. J. (1998). Release from alginate enhances the biological activity of vascular endothelial growth factor. *J. Biomater. Sci. Polym. Ed.* **9**(12), 1267–1278.

Ranieri, J. P., Bellamkonda, R., Bekos, E. J., Vargo, T. G., Gardella, J. A., Jr., and Aebischer, P. (1995). Neuronal cell attachment to fluorinated ethylene propylene films with covalently

immobilized laminin oligopeptides YIGSR and IKVAV. II. *J. Biomed. Mater. Res.* **29**(6), 779–785.

Ratcliffe, A. (2000). Tissue engineering of vascular grafts. *Matrix. Biol.* **19**(4), 353–357.

Rezania, A., and Healy, K. E. (1999). Biomimetic peptide surfaces that regulate adhesion, spreading, cytoskeletal organization, and mineralization of the matrix deposited by osteoblast-like cells. *Biotechnol. Prog.* **15**(1), 19–32.

Richardson, T. P., Murphy, W. L., and Mooney, D. J. (2001). Polymeric delivery of proteins and plasmid DNA for tissue engineering and gene therapy. *Crit. Rev. Eukaryot. Gene Expr.* **11**(1–3), 47–58.

Richardson, T. P., Peters, M. C., Ennett, A. B., and Mooney, D. J. (2001). Polymeric system for dual growth factor delivery. *Nat. Biotechnol.* **19**(11), 1029–1034.

Risau, W. (1995). Differentiation of endothelium. *FASEB J.* **9**(10), 926–933.

Robey, T. C., Valimaa, T., Murphy, H. S., Tormala, P., Mooney, D. J., and Weatherly, R. A. (2000). Use of internal bioabsorbable PLGA "finger-type" stents in a rabbit tracheal reconstruction model. *Arch. Otolaryngol. Head Neck Surg.* **126**(8), 985–991.

Rosengart, T. K., Lee, L. Y., Patel, S. R., Kligfield, P. D., Okin, P. M., Hackett, N. R., Isom, O. W., and Crystal, R. G. (1999). Six-month assessment of a phase I trial of angiogenic gene therapy for the treatment of coronary artery disease using direct intramyocardial administration of an adenovirus vector expressing the VEGF121 cDNA. *Ann. Surg.* **230**(4), 466–470; discussion 470–472.

Rossant, J., and Howard, L. (2002). Signaling pathways in vascular development. *Annu. Rev. Cell Dev. Biol.* **18,** 541–573.

Rowley, J. A., Madlambayan, G., and Mooney, D. J. (1999). Alginate hydrogels as synthetic extracellular matrix materials. *Biomaterials* **20**(1), 45–53.

Sakiyama-Elbert, S. E., and Hubbell, J. A. (2001). Functional biomaterials: Design of novel biomaterials. *Ann. Rev. Mater. Res.* **31,** 183–201.

Schumacher, B., Pecher, P., von Specht, B. U., and Stegmann, T. (1998). Induction of neoangiogenesis in ischemic myocardium by human growth factors: First clinical results of a new treatment of coronary heart disease. *Circulation* **97**(7), 645–650.

Seeherman, H., Wozney, J., and Li, R. (2002). Bone morphogenetic protein delivery systems. *Spine* **27**(16 Suppl 1), S16–S23.

Shea, L. D., Smiley, E., Bonadio, J., and Mooney, D. J. (1999). DNA delivery from polymer matrices for tissue engineering. *Nat. Biotechnol.* **17**(6), 551–554.

Sheridan, M. H., Shea, L. D., Peters, M. C., and Mooney, D. J. (2000). Bioabsorbable polymer scaffolds for tissue engineering capable of sustained growth factor delivery. *J. Control Release* **64**(1–3), 91–102.

Shin, H., Jo, S., and Mikos, A. G. (2003). Biomimetic materials for tissue engineering. *Biomaterials* **24**(24), 4353–4364.

Simons, M., Annex, B. H., Laham, R. J., Kleiman, N., Henry, T., Dauerman, H., Udelson, J. E., Gervino, E. V., Pike, M., Whitehouse, M. J., Moon, T., and Chronos, N. A. (2002). Pharmacological treatment of coronary artery disease with recombinant fibroblast growth factor-2: Double-blind, randomized, controlled clinical trial. *Circulation* **105**(7), 788–793.

Smith, M. K., Peters, M. C., Richardson, T. P., Garbern, J. C., and Mooney, D. J. (2004). Locally enhanced angiogenesis promotes transplanted cell survival. *Tissue Eng.* **10**(1–2), 63–71.

Sodian, R., Sperling, J. S., Martin, D. P., Stock, U., Mayer, J. E., Jr., and Vacanti, J. P. (1999). Tissue engineering of a trileaflet heart valve-early *in vitro* experiences with a combined polymer. *Tissue Eng.* **5**(5), 489–494.

Tonnesen, H. H., and Karlsen, J. (2002). Alginate in drug delivery systems. *Drug Dev. Ind. Pharm.* **28**(6), 621–630.

Traggiai, E., Chicha, L., Mazzucchelli, L., Bronz, L., Piffaretti, J. C., Lanzavecchia, A., and Manz, M. G. (2004). Development of a human adaptive immune system in cord blood cell-transplanted mice. *Science* **304**(5667), 104–107.

Trejo, P. M., Weltman, R., and Caffesse, R. (2000). Treatment of intraosseous defects with bioabsorbable barriers alone or in combination with decalcified freeze-dried bone allograft: A randomized clinical trial. *J. Periodontol.* **71**(12), 1852–1861.

Uchida, S., Sakai, A., Kudo, H., Otomo, H., Watanuki, M., Tanaka, M., Nagashima, M., and Nakamura, T. (2003). Vascular endothelial growth factor is expressed along with its receptors during the healing process of bone and bone marrow after drill-hole injury in rats. *Bone* **32**(5), 491–501.

United Network of Organ Sharing, Richmond, VA. http://www.unos.org.

Uyama, S., Kaufmann, P. M., Kneser, U., Fiegel, H. C., Pollok, J. M., Kluth, D., Vacanti, J. P., and Rogiers, X. (2001). Hepatocyte transplantation using biodegradable matrices in ascorbic acid-deficient rats: Comparison with heterotopically transplanted liver grafts. *Transplantation* **71**(9), 1226–1231.

Verfaillie, C. M. (2002). Adult stem cells: Assessing the case for pluripotency. *Trends Cell Biol.* **12**(11), 502–508.

Verfaillie, C. M. (2002). Hematopoietic stem cells for transplantation. *Nat. Immunol.* **3**(4), 314–317.

Wald, H. L., Sarakinos, G., Lyman, M. D., Mikos, A. G., Vacanti, J. P., and Langer, R. (1993). Cell seeding in porous transplantation devices. *Biomaterials* **14**(4), 270–278.

Wang, H. B., Dembo, M., and Wang, Y. L. (2000). Substrate flexibility regulates growth and apoptosis of normal but not transformed cells. *Am. J. Physiol. Cell Physiol.* **279**(5), C1345–C1350.

White, R. A., Hirose, F. M., Sproat, R. W., Lawrence, R. S., and Nelson, R. J. (1981). Histopathologic observations after short-term implantation of two porous elastomers in dogs. *Biomaterials* **2**(3), 171–176.

Wozney, J. M. (2002). Overview of bone morphogenetic proteins. *Spine* **27**(16 Suppl 1), S2–S8.

Zelzer, E., and Olsen, B. R. (2003). The genetic basis for skeletal diseases. *Nature* **423**(6937), 343–348.

Cell-demanded release of VEGF from synthetic, biointeractive cell ingrowth matrices for vascularized tissue growth (2003). Cell-demanded release of VEGF from synthetic, biointeractive cell ingrowth matrices for vascularized tissue growth. *FASEB. J.* **17**(15), 2260–2262.