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Review

Polymeric Growth Factor Delivery Strategies for Tissue Engineering

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Purpose. Tissue engineering seeks to replace and regrow damaged or diseased tissues and organs from either cells resident in the surrounding tissue or cells transplanted to the tissue site. The purpose of this review is to present the application of polymeric delivery systems for growth factor delivery in tissue engineering.
Methods. Growth factors direct the phenotype of both differentiated and stem cells, and methods used to deliver these molecules include the development of systems to deliver the protein itself, genes encoding the factor, or cells secreting the factor.
Results. Results in animal models and clinical trials indicate that these approaches may be successfully used to promote the regeneration of numerous tissue types.
Conclusions. Controlling the dose, location, and duration of these factors through polymeric delivery strategies will dictate their utility in tissue regeneration.

KEY WORDS: regeneration; drug delivery; gene delivery; cell transplantation.

INTRODUCTION

The rapidly developing field of tissue engineering aims to replace or facilitate the regrowth of damaged or diseased tissue by applying combinations of biomaterials, cells, and bioactive molecules. Tissue engineering seeks to fill the void created by the growing disparity between the number of tissues and organs needed and the numbers available for transplantation (1). Transplantation of tissues from autogeneic (from the host), allogeneic (from the same species), and xenogeneic (from a different species) sources has been a major strategy in tissue repair, but the limited availability of tissue and the issues associated with immunogenicity and disease transmission have fueled the search for a better source for tissue replacement.

Various tissues in the body contain cells competent to regenerate after injury or destruction, but the regenerative capability varies widely with cell type and the circumstances of injury. Actively renewing cells of certain tissues (e.g., skin, bone marrow and intestinal mucosa) are competent for complete regrowth, but this ability of the cells is dependent on a number of factors, such as the size of defect, age of individual, and specific cause of defect. In contrast, cells in other tissues (e.g., heart muscle and nerve) are static and do not typically regenerate lost tissue structure or function. In either case, transplantation of desired cell populations may increase the rate or extent of new tissue formation. In all of these situations, the local presence of growth factors capable of instructing both cells resident in the tissue and transplanted cells will likely be key to the ultimate success of the regenerative process.

Although growth factors clearly play important roles in harnessing and controlling cellular functions in tissue regeneration, the appropriate mode for making these factors available at the desired site remains unclear. Bolus delivery of these molecules is technically simple, but the subsequent distribution of the factors throughout the body and their rapid degradation may lead to undesirable systemic effects and toxicity, and an insufficient local concentration for the required time frame, respectively. The use of polymeric vehicles to locally deliver the factors in various formats provides a method of controlled, localized delivery for the desired time frame. This review focuses on the application of inductive large peptide growth factors through polymeric delivery systems for tissue-engineering therapies for wound healing, bone regeneration, and angiogenesis. Reviews highlighting other tissues (e.g., neural, Ref. 2; skeletal muscle, Ref. 3) are available from other sources. Short overviews of growth factor biology and the design criteria for delivery vehicles these molecules precede descriptions of specific applications.

GROWTH FACTORS

Growth factors are secreted by a wide range of cell types to transmit signals that activate specific developmental programs controlling cell migration, differentiation, and proliferation (Fig. 1). Cell signaling occurs through growth factor binding to its receptor on the cell surface. The signal is transferred through the membrane receptor and amplified, often

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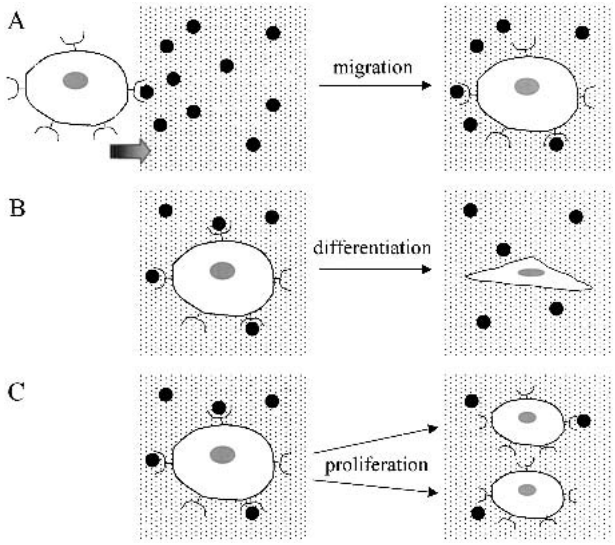

Fig. 1. Growth factor binding to a cell surface receptor can elicit different types of responses to regenerate tissue in a defect site. A, Chemotactic responses to growth factor binding result in cell migration into a defect site. B, Cell differentiation can occur to provide the needed cell population. C, Proliferation of cells within the site fill the tissue defect.

through phosphorylation of secondary messengers within the target cell, to ultimately modify gene expression. Growth factors act in a concentration- and time-dependent manner, often requiring minute quantities to elicit biologic activity, and their action can depend on a variety of factors, including cell location within a tissue structure and cell cycle state. Elucidation of differentiation and proliferation pathways for various tissues has revealed that they are complex events involving a cascade or a network of multiple growth factors (4).

Regulation of growth factor activity occurs on several levels, from controlled gene expression to degradation. Transcriptional activation of growth factors and receptor genes create mRNAs that are unstable, yielding brief synthesis of limited peptide quantities (4). Growth factors are released by cells for immediate signaling or are embedded in the extracellular matrix (ECM) and released as the ECM degrades through controlled proteolysis. Sequestering of growth factors in the ECM allows growth factor stabilization and provides physical cues for cells through compartmentalization or spatial presentation of these factors. Controlled secretion of factors and their release from the ECM is balanced by extracellular degradation. Altogether these processes contribute to the existence of a biologic growth factor delivery system that is responsive and dynamic, changing according to specific cellular requirements and processes.

DESIGN CRITERIA FOR GROWTH FACTOR DELIVERY SYSTEMS

There are several important considerations in the design of a delivery system for growth factors used to regenerate or engineer tissues. First, given the quantity and complex actions of growth factors, it is essential to identify the key growth factor or factors to deliver for a particular tissue application based on an understanding of biologic developmental processes (see Table I for a list of commonly used growth factors and their known activities). The application of growth factors as therapeutic molecules has focused on those that are best characterized and available in large quantities as recombinant proteins.

Second, the mode of factor delivery must target the desired cell population and minimize signal propagation to nontarget tissues and cells. The earliest reported applications of growth factors as therapeutic agents involved intravenous injection, but this delivery method is not localized to the target tissue and is also ineffective because of the growth factors' short half-lives. Although very small quantities (picograms to nanograms) of growth factor are necessary to generate a cellular response, growth factors are rapidly degraded once secreted. The biologic half-lives of platelet-derived growth factor (PDGF), basic fibroblast growth factor (bFGF or FGF-2), and vascular endothelial growth factor (VEGF), for example,

Table I. A List of Growth Factors Commonly Used in Tissue Engineering

Growth factor	Abbreviation	Molecular weight (kDa)	Known activities	Representative supplier of rH growth factor
Epidermal growth factor	EGF	6.2	Proliferation of epithelial, mesenchymal, and fibroblast cells	PeproTech Inc. (Rocky Hill, NJ, USA)
Platelet-derived growth factor	PDGF-AA PDGF-AB PDGF-BB	28.5 25.5 24.3	Proliferation and chemoattractant agent for smooth muscle cells; extracellular matrix synthesis and deposition	PeproTech Inc.
Transforming growth factor-α	TGF-α	5.5	Migration and proliferation of keratinocytes; extracellular matrix synthesis and deposition	PeproTech Inc.
Transforming growth factor-β	TGF-β	25.0	Proliferation and differentiation of bone forming cells; chemoattractant for fibroblasts	PeproTech Inc.
Bone morphogenetic protein	BMP-2 BMP-7	26.0 31.5	Differentiation and migration of bone forming cells	Cell Sciences Inc. (Norwood, MA, USA)
Basic fibroblast growth factor	bFGF/FGF-2	17.2	Proliferation of fibroblasts and initiation of angiogenesis	PeproTech Inc.
Vascular endothelial growth factor	$VEGF_{165}$	38.2	Migration, proliferation, and survival of endothelial cells	PeproTech Inc.

rH, recombinant human.

are 2 (5), 3 (6), and 50 min (7) respectively, when intravenously injected. Direct therapeutic application of growth factors therefore requires substantial systemic doses at levels that can generate undesired effects (8). These issues have motivated the development of controlled delivery systems that allow the sustained and localized delivery of small amounts of these factors to the target cell population and tissue site.

Third, the controlled delivery of factors requires a relatively long-term maintenance of biologic activity within the system. These systems function in many ways as an artificial ECM to stabilize embedded or encapsulated growth factor. This is often challenging because processes used to formulate protein delivery constructs may denature or deactivate the protein. Therefore, methods of fabrication that do not require harsh solvents or high temperatures are often desirable if the protein itself is used as the regenerative agent.

Finally, the release profile of the growth factor from the system should be controlled temporally and spatially to be appropriate for a specific tissue injury or disease. Tissues must frequently be exposed to these factors for relatively long time-frames (e.g., days to weeks) to obtain the desired effects. Spatial localization of the signaling molecule may enable one to control not only the extent of tissue formation, but also the pattern of tissue formation.

A variety of polymeric delivery systems have been designed to meet the design criteria for growth factor delivery (Fig. 2). The factors themselves may be directly incorporated into the polymer (Fig. 3A). Alternatively, plasmid DNA encoding the factor may be immobilized within the polymer, allowing the local production of the factor by cells that take up and express this DNA following implantation of the system at the desired tissue site (Fig. 3B). Finally, the polymer system may be used to transplant cells that secrete the desired factor (Fig. 3C). These differing approaches to factor delivery are described in the following sections and a summary of tissues regenerated using growth factor delivery strategies is provided in Table II.

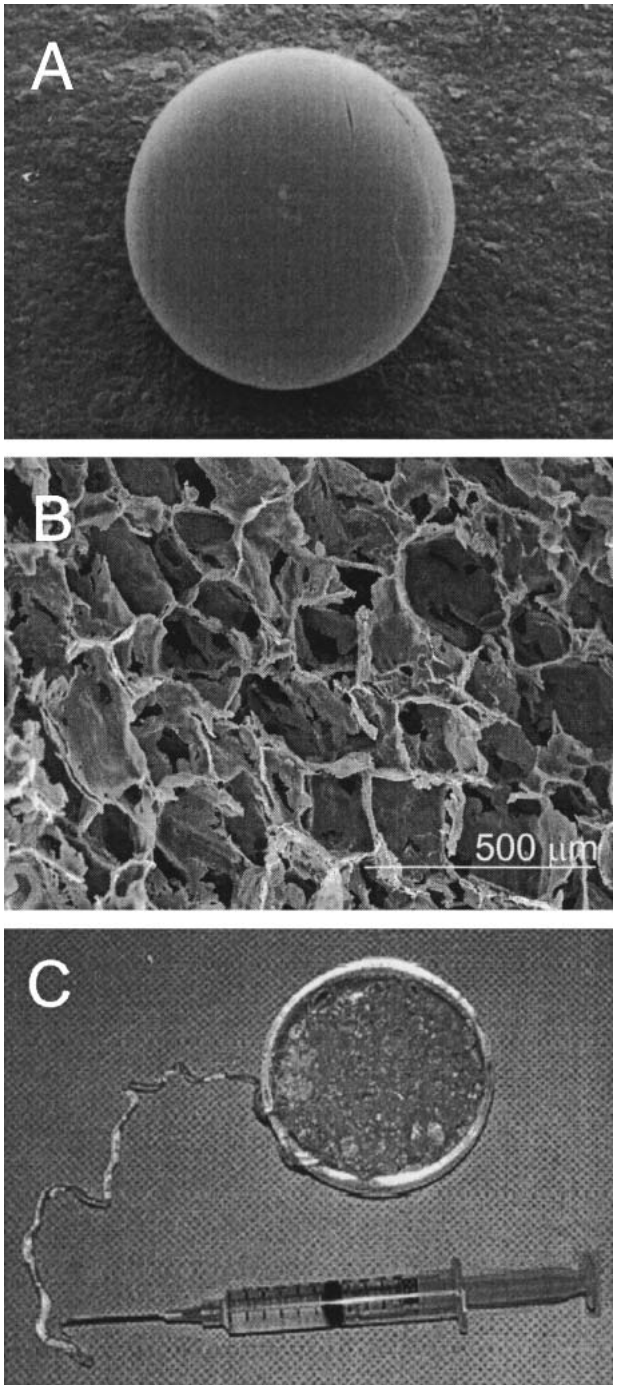

Fig. 2. Polymeric growth factor delivery vehicles may be processed into various forms, including (A) microspheres, (B) porous scaffolds, or (C) injectable gels. A and B are scanning electron microscopy images taken of a typical PLG microsphere and a gas-foamed scaffold respectively (images courtesy of Martin Peters).

PROTEIN DELIVERY SYSTEMS

Polymeric systems can be successfully used to administer small doses of factors at defined dose rates directly to target cells. Polymeric delivery systems composed of various natural and synthetic biocompatible polymers can provide controlled growth factor delivery by differing mechanisms. One technically simple polymer delivery system involved the mixing of growth factors in an albumin gel (9), creating one of the first reported investigations of growth factor delivery in a polymer matrix. Since then, the number and complexity of available constructs has increased to include hydrogels, microspheres, and three-dimensional, porous scaffolds (Fig. 2). The release profiles of molecules from such carriers can be controlled by both factor diffusion and polymer degradation, the dose of the factor loaded in the system, and the composition of the polymer.

Both naturally derived and synthetic polymers have found utility in growth factor delivery. Natural polymers and their derivatives in the form of gels and sponges have been used extensively as delivery vehicles. Collagen in particular is a readily available ECM component that allows cell infiltration and remodeling, making it highly suitable for growth factor delivery. Recombinant forms of collagen may circumvent issues of immunogenicity and disease transfer that can occur with collagen extracted from animal sources. Biodegradable synthetic polymers are also very attractive for tissue engineering applications because of their well-controlled, reproducible chemical and physical properties. Homo- and copolymers of lactide and glycolide have found numerous applications in this field because of their wide use in a number

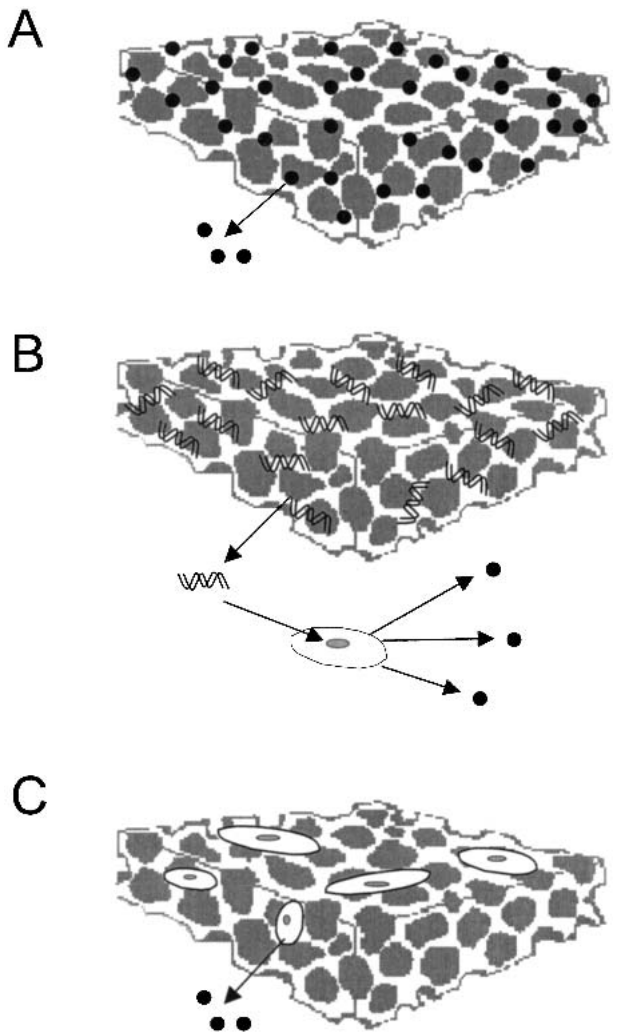

Fig. 3. Three primary polymeric growth factor delivery strategies. A, Growth factors are embedded within the polymer matrix and released. B, Genes encoding a growth factor are embedded within the polymer matrix and released, followed by cellular uptake and expression of the gene to produce growth factor. C, Growth factor is released from cells seeded on the polymer matrix that secrete the factor.

of medical devices (e.g., sutures) and degradation into natural products (lactic and glycolic acid) that enter into metabolic pathways. The physical properties of these polymers can be readily altered by varying the ratio of lactide:glycolide, molecular weight, and crystallinity.

Wound Healing

Skin and wound healing was one of the first areas where the therapeutic potential of growth factors was investigated and the first area with a growth factor based pharmaceutical product. The clinical need for wound healing is vast, ranging from burn victims to patients with diabetes whose natural wound repair mechanisms are impaired. Approximately 2 million people suffer burns each year, with 13,000 requiring skin grafts, and diabetic foot ulcers affect over 800,000 people each year. In this application area, growth factors have the potential to reduce scarring and accelerate the healing process driven by cells already present in tissue adjacent to the wound site.

A number of factors have been identified that mediate skin regeneration, including epidermal growth factor (EGF), transforming growth factor-α and -β (TGF-α, TGF-β), and PDGF (10). Both EGF and TGF-α induce the proliferation and differentiation of various epithelial cells both *in vitro* and *in vivo* (11). PDGF is a mitogen and chemotactic agent of fibroblasts and smooth muscle cells while stimulating macrophages to secrete other growth factors important for various stages of the healing process. PDGF also stimulates the production of several ECM molecules, including collagen and fibronectin (12). TGF-β is also involved in ECM deposition and scarring in the natural progression of wound healing (13). It will likely be critical to deliver these molecules in a localized and controlled manner to achieve the desired biologic response, while avoiding harmful side-effects (14).

Many studies have now documented the utility of localized and sustained delivery of growth factors on wound healing. Natural polymers formulated into viscous gel suspensions (9) or porous sponges (15) have been used to deliver these factors while maintaining growth factor activity (16). Investigations into delivery strategies using such polymeric carriers have resulted in the first Food and Drug Administration-approved growth factor system for tissue regeneration, Regranex, a topically applied gel for the treatment of diabetic foot ulcers. This product is based on PDGF (0.01% recombinant PDGF-BB), delivered in a carboxymethylcellulose-based gel (17). More recently, delivery systems have integrated growth factor release with existing wound healing aids (18,19) to form enhanced wound healing systems. Modifying biomaterials conventionally used for wound repair into growth factor delivery systems may produce systems with significant clinical utility.

Bone Regeneration

Approximately 2500 new cases of primary bone cancer and 13,500 new cases of myeloma are diagnosed in the United States each year. In addition, an estimated 200,000 bone grafts are performed annually in the United States for bone defects incurred from sports injuries or trauma. From these numbers, it is evident that there is a great demand for therapies to enhance bone regeneration. A primary goal of growth factor delivery for bone tissue engineering is to accelerate healing and enhance bone formation. Tissue-engineered bone, whether intended to heal a fracture or to fill a critical-sized defect, needs to not only be mechanically competent but also be vascularized living tissue. Tissue-engineering approaches in this area have focused on osteoinductive or osteoconductive implant strategies. Osteoinduction uses the delivery of growth factors to enable the migration, proliferation, and differentiation of bone-forming cells already present in the body to form new bone. Osteoconductive materials provide an appropriate matrix, in the form of scaffolds that encourage cell infiltration and anchorage for guided osteogenesis to occur. Conductive materials may also reinforce the mechanical stability of a defect site while remodeling occurs.

A large number of factors involved in bone formation and remodeling have been identified, and members of the TGF-β superfamily clearly play an important role. TGF-β is involved in early stages of bone development, as well as bone repair and remodeling after trauma, through its regulation of

Table II. Polymeric Carriers Used to Deliver Various Growth Factors and the Type of Tissue Regenerated

Growth factor	Carrier	Tissue regenerated	Representative ref.
EGF	Gelatin	Dermis	(19)
	PET suture	Tendon	(18)
	PVA sponge	Dermis	(15)
PDGF	Chitosan-PLLA scaffold	Craniofacial bone	(63)
	CMC gel	Dermis	(17)
	Fibrin	Ligament	(64)
	Porous HA	Long bone	(65)
TGF-β	Alginate	Cartilage	(66)
	PLA	Long bone	(67)
	CaP-titanium mesh	Craniofacial bone	(68)
	Polyoxamer; PEO gel	Dermis	(69)
rhBMP-2	Collagen sponge	Long bone	(70)
		Craniofacial bone	(71)
	HA-TCP granules	Spinal bone	(24)
	HA-collagen	Long bone	(72)
	PLA-DX-PEG	Ectopic and hip bone	(73)
rhBMP-7	HA	Spinal bone	(74)
	Collagen-CMC	Spinal bone	(75)
	Porous HA	Craniofacial bone	(76)
bFGF	Chitosan	Dermis	(77)
	Heparin-alginate	Blood vessels	(36)
	EVAc microspheres	Blood vessels	(78)
	Fibrin matrices	Blood vessels	(79)
VEGF	PLG scaffold	Blood vessels	(37)
	PLG scaffold	Blood vessels	(40)
	PLG microspheres	Blood vessels	(80)
	Fibrin mesh	Blood vessels	(81)

Abbreviations: PET, poly (ethylene terepthalate); PVA, polyvinyl alcohol; PLLA, poly(L-lactic acid); CMC, carboxymethylcellulose; HA, hydroxyapatite; PLA, poly(D,L-lactic acid); CaP, calcium phosphate; PEO, poly (ethylene oxide); TCP, tricalcium phosphate; PEG, poly(ethylene glycol); -DX-, -*p*-dioxanone-; EVAc, ethylene vinyl acetate; PLG, poly (lactide-co-glycolide).

the proliferation and differentiation of mesenchymal precursor cells (20). Delivery of TGF-β1 through biodegradable polymer microparticles increased the proliferation and differentiation of marrow stromal cells toward osteoblasts (21), indicating its potential in controlled release systems for bone engineering. Of high interest is the group of bone morphogenetic proteins (BMPs), which are members of the TGF-β superfamily. BMP-2 through BMP-8 are osteogenic proteins that play an important role in embryonic development, generation of the central nervous system, and tissue repair (22). These polypeptides have also been indicated in both endochondral (through a cartilage intermediate) and intramembranous (direct) bone formation. BMP-2, the first BMP to be available in a highly purified recombinant form, has pleiotropic functions, acting primarily as a differentiation factor for bone and cartilage precursor cells toward mature osteocytes. In a variety of preclinical models, BMP-2 has demonstrated the ability to induce bone formation and heal bony defects, in addition to improving the maturation and consolidation of regenerated bone (23). Recombinant human BMP-7 and BMP-2 are among the first growth factor based products available for clinical use to treat patients afflicted with bone diseases.

A large number of studies have been performed to determine appropriate carriers for BMPs. Carriers composed of collagen are popular for bone induction, as it is the major nonmineral component of bone. Recombinant forms of collagen have been used as carriers for BMP delivery for spine fusion in humans (24). Synthetic polymeric systems using poly (lactide-co-glycolide) (PLG) processed into porous scaffolds (25) and capsules (26) have also been used for rhBMP-2 delivery to allow for greater control over the rate of BMP release. Injectable systems of polyethylene glycol hydrogels (27) and PLG microspheres (28) delivering BMP-2 had also promoted osteoblast differentiation and mineralization *in vitro*, and ectopic bone formation *in vivo*.

The utilization of a conductive substrate to deliver an inductive biomolecule may enhance bone regeneration due to the combined effects of the carrier and factor. However, many systems for osteogenic growth factor delivery are not osteoconductive. Osteoconductive materials include hydroxyapatite (HAP), calcium phosphate, and a variety of ceramic biomaterials. Integrated systems are currently being pursued, and a recent example includes the use of mineralized polymeric scaffolds (29) for growth factor delivery. Osteoconductive materials have also been combined with collagen to form porous resorbable BMP delivery systems for spine fusion (30).

In addition to the complexity inherent to regenerating the mineralized tissue component of bone, nerves and blood vessels must also be integrated with this tissue for full functionality. Vascularization in particular, is an essential component of *de novo* bone formation and a high degree of vascularity is present in natural bone. There is recent evidence

showing both bone and blood vessel formation can be triggered in response to the same growth factor, VEGF (31). An extensive network of blood vessels is a general requirement for the formation of any functional large tissues or organs, and this may require the combined delivery of multiple bone inducing and angiogenic factors.

Angiogenesis

New blood vessel formation is a critical requirement in many vascular diseases and in tissue engineering. Coronary artery disease is a major health problem for over 12 million Americans, resulting in 571,000 coronary artery bypass surgeries annually. An increasing number of patients with coronary artery disease are not candidates for revascularization through traditional surgical methods. Therapeutic angiogenesis, the formation of new blood vessels via branching from existing vessels, holds great promise to aid this patient population. In addition, the entire tissue engineering field is limited by the need for vascularity in large tissues and organs for nutrient and waste transport. Strategies to promote new blood vessel networks will be essential in virtually all engineered tissues (32).

Because of the central importance of angiogenesis in disease processes such as cancer and wound healing, the molecular mechanisms underlying angiogenesis have been extensively elucidated. Angiogenesis is the result of a multi-step, sequential process beginning with endothelial cell activation and migration to sprout neovessels and ending with mature vessels surrounded by smooth muscle cells and pericytes (32). Growth factors that play key roles in this process include FGF, VEGF, PDGF, TGF-β, and angiopoietins-1 and 2 (Ang-1 and 2). Among these, VEGF, Ang-1, and Ang-2 are more endothelial cell-specific. bFGF and VEGF are heparin-binding growth factors involved in the initiation of angiogenesis, and promote endothelial cell proliferation and migration. PDGF-BB recruits smooth muscle cells and pericytes which, along with TGF-β, promote ECM deposition to stabilize neovessels.

The identification of growth factors involved in angiogenesis has led to their application in treating coronary artery disease and other diseases involving tissue ischemia. Bolus injections of solutions of growth factors have been investigated as treatments for myocardial ischemia, with promising results in animal models and small-scale clinical trials (33). However, these delivery methods did not appear to have had as significant of an effect in large clinical trials (34). These latter results are likely related to the very short half-lives (minutes to hours) of these factors *in vivo*. This limitation could potentially be overcome by delivering extremely large quantities of these factors in the same fashion, but this may then lead to vessel formation at nontarget sites and elicit harmful side-effects as a result of the presence of these potent drugs throughout the body. Polymeric delivery vehicles have therefore been pursued to allow for localized, controlled delivery of angiogenic factors to alleviate risks and obtain therapeutic benefit. Various types of polymeric materials have been used for controlled release of bFGF and VEGF, including alginate hydrogels, PLG microspheres, and porous PLG scaffolds (35). Controlled release of bFGF encapsulated in heparin-alginate pellets led to significant angiogenesis with low systemic effects in patients undergoing bypass surgery (36), but this approach did not alleviate operative risks. Further information on clinical trials can be found in a recent review on therapeutic angiogenesis (35).

Angiogenesis is also generally required for the integration of engineered tissues with surrounding host tissue. Insufficient blood vessels for nutrient delivery and waste removal can result in the death of transplanted cells and limited tissue regrowth. It has recently been demonstrated that promotion of angiogenesis through the delivery of an angiogenic growth factor enhances the engraftment of transplanted cells, including endothelial cells (37), pancreatic islets (38), and hepatocytes (39).

Whether for tissue engineering or therapeutic applications, new blood vessels formed through the growth factor delivery strategies described in this section are typically small and lack pericyte incorporation. This finding suggests that they may not be functional long-term and may be subject to regression. A greater degree of control of the angiogenic cascade may be required for the formation of functional, stable vascular beds. It has recently been demonstrated that VEGF delivery followed by PDGF resulted in larger, mature vessel structures (40).

GENE DELIVERY AS AN ALTERNATIVE TO PROTEIN DELIVERY

A significant limitation to direct protein delivery approaches is degradation of the molecules when exposed to the *in vivo* environment, and limited stability even when encapsulated into a polymeric delivery vehicle. One approach to potentially bypass these issues is to instead use localized gene therapy to promote the production of the desired growth factor at a specific tissue site. This approach may have great utility in application areas such as therapeutic angiogenesis (41). The stability of DNA in various environmental conditions typically encountered during production and processing is much higher than proteins, and this approach can also lead to a sustained delivery of the factor following single administration of the gene therapy.

To use gene delivery as a growth factor delivery strategy, many complex challenges must be overcome. Low transfection efficiencies, inefficient gene targeting, low gene expression levels, and undesired gene integration into host DNA are all challenges that underlie this growth factor delivery approach. It is also necessary to identify the target cell type most appropriate for gene delivery to elicit the desired regenerative effects.

The challenge of low transfection efficiencies has been addressed with the use of gene guns and viral vectors. Delivery systems using gene guns inject cDNA directly into a site and this approach has been used for delivery of genes encoding PDGF-BB (42) and keratinocyte growth factor-1 (43) for wound repair. However, this strategy depends on the presence of host cells able to take up and express the cDNA at biologically relevant levels. There is also a technical limitation due to the limited penetration depth, and this delivery method may be best suited for surface wound healing. Adenoviral gene delivery has been used to produce active BMP-2 in skeletal muscle (44), and VEGF for therapeutic angiogenesis (45). Concerns regarding the use of adenoviral and lentiviral vehicles are the issues of virus safety, immunogenicity, and long term effects *in vivo*, as demonstrated by

outcomes noted in recent adenoviral gene therapy trials (46). Therefore, polymeric gene delivery vectors are being actively pursued as alternatives to viral vectors.

Polymeric gene delivery vehicles have the potential to effectively and safely deliver growth factor genes to target cells. Direct DNA delivery through a polymeric vehicle (47) may circumvent the issue of immune toxicity, and the use of plasmid DNA addresses the issue of adverse effects resulting from integration with host DNA. Polymeric gene delivery vehicles include collagen (48) and PLGA (47,49). However, DNA delivered in this manner is usually taken up by the cell through nonspecific endocytosis, which can be inefficient and unpredictable, with much of the DNA degraded in lysosomes. Cationic polymer and lipid DNA condensates have also been investigated for nonviral gene delivery (50). The delivery of DNA condensates with polymer scaffolds may greatly enhance transfection efficiency and lead to stable gene expression over a longer period *in vivo* than delivery via other polymeric delivery vectors.

CELL TRANSPLANTATION

A third approach for delivery of growth factors in tissue regeneration involves transplanting cells that synthesize and secrete the desired growth factor. Autologous cells may be used in this approach via the isolation of a small number of differentiated adult cells or stem cells, followed by *in vitro* expansion to produce an appropriate supply (Fig. 4). The cells may naturally secrete or be genetically modified *in vitro* to overexpress the factor, either transiently or permanently (Fig. 4). This approach may be particularly appropriate to deliver growth factors that act by paracrine or juxtacrine mechanisms. Appropriate carriers are required for transplanted cells in order to localize their production of the factors, and polymeric systems similar to those developed for protein delivery have been used. These include collagen (51), peptide modified hydrogels (52), and biodegradable porous scaffolds (37,53).

Transplantation of cells that naturally secrete the desired growth factor is one strategy to deliver biologically active protein. One of the first large-scale applications of cell transplantation arose in wound care, where sheets of cultured keratinocytes and fibroblasts were combined with wound dressing materials to form cultured skin substitutes (54). Although this approach uses transplanted cells to regenerate tissue, cultured skin substitutes may also work via natural secretion of growth factors from the cultured skin layer to the host wound area. Cotransplantation of multiple cell types may mutually induce differentiation and proliferation toward a desired tissue type, through cell mediated growth factor signaling (52). In addition, cells may provide responsive delivery systems able to provide multiple factors. Human mesenchymal stem cells can produce a number of growth factors, including bFGF and VEGF, in response to changing culture conditions (55) and transplantation of these cells may naturally provide the appropriate growth factors needed to the host environment. However, natural growth factor secreting cells often produce the factors at low levels since they may not normally activate the desired cellular processes.

Genetically modified cells are potentially able to provide a stable source of growth factor at a level that is sufficient to elicit a biologic response. For example, cultured dermal fibroblasts have been transduced to express PDGF and the modified cells were transplanted onto PGA scaffolds to promote

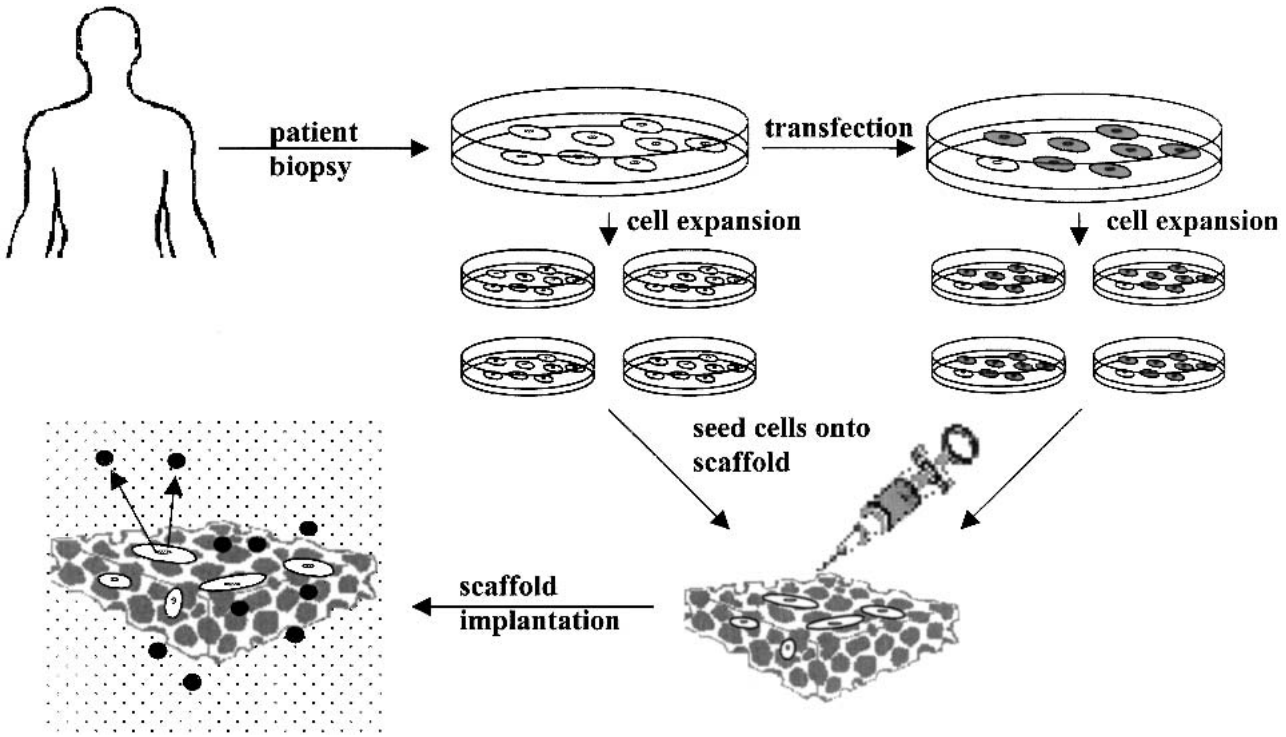

Fig. 4. Schematic of typical cell transplantation strategies. First, cells are harvested from a tissue sample from a patient. The cells are then selected and cultured *in vitro*. Subsequently, cells may be directly expanded to generate a sufficient population or may be genetically modified to produce a desired growth factor before expansion. The cells are then seeded onto the polymer matrix and implanted into the defect site. The unaltered or genetically modified cells secrete growth factor for tissue regeneration.

wound healing (56). For bone tissue engineering, mesenchymal stem cells have been engineered to express BMP-2 (53) or BMP-7 (57) and delivered in a polymeric scaffold for stable production of the proteins. Both endothelial precursors (58) and primary skeletal muscle cells (59) expressing VEGF have been used in therapeutic angiogenesis, resulting in growth factor production for neovascularization. Multiple growth factor delivery may also be achieved through genetic modification; for example, by modifying muscle-derived stem cells to express VEGF and BMP-4 (60) or modifying cultured dermal fibroblasts to express PDGF and VEGF (61). Expression of a non-native growth factor gene can also alter cell fate toward a desired fate, as demonstrated by BMP-7 transduced fibroblasts that were converted into osteoblastic cells (62).

CONCLUSIONS AND FUTURE DIRECTIONS

Growth factor delivery is clearly a therapeutically important approach in tissue engineering and will become increasingly powerful as appropriate delivery parameters and factor specificity are defined. Growth factor therapies show tremendous potential in numerous applications, including wound healing, bone regeneration, and angiogenesis. Sophisticated growth factor delivery systems are a major focus of research in this area, regardless of whether it is the protein itself being delivered, genes expressing the protein, or cells secreting the factor. Strategies to deliver growth factors need to exert control over the complex, integrated networks that define tissue formation, and may need to be responsive to the changing dynamics of the network. Pharmaceutical scientists will play a key role in the future development of growth factor delivery systems used to regenerate damaged or injured tissue.

A limitation of current strategies for growth factor delivery is their focus on the delivery of single growth factors. However, it is clear that often multiple growth factors work in concert in a highly regulated network to promote the regeneration of tissues. Although delivery of a single growth factor may be insufficient to promote complete regeneration of tissue structures, the antagonistic roles of multiple factors may necessitate temporal control over growth factor presentation. The development of polymeric drug delivery systems capable of releasing multiple growth factors with distinct release profiles may be required to address the more complex requirements for regenerating functional tissues. Such a system was recently described (40), and the utility of this system was tested in the context of angiogenesis. The initial delivery of VEGF, followed by a sustained release of PDGF, led to not only the formation but also maturation of blood vessels. Multiple growth factor delivery in an orchestrated sequence may also be useful for cellular de-differentiation toward a proliferative state and subsequent redifferentiation toward a mature cell type. This concept may be crucial to the usage of stem cells in tissue engineering, as it may be necessary to first induce competence toward a fate with exposure to a primary growth factor that subsequently leads to expression of a receptor for a secondary growth factor needed for differentiation and maturation.

The spatial patterning of growth factors may also be a critical issue in tissue regeneration because natural partitioning of biologic signals occurs during embryonic development. Mimicking of biologic patterning may be especially useful to control tissue development processes such as neovascularization, where undirected or uncontrolled growth can lead to pathologic effects, i.e., tumor metastasis. Techniques developed for microarray patterning and photolithography, and micro-electro-mechanical systems may be useful to pattern growth factors to create gradients. This approach may be valuable in the generation of complex networks of temporally and spatially controlled growth factor delivery and to mimic the micro- and nano-topography of natural ECM.

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