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Cytokine Delivery and Tissue Engineering

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Abstract

Tissue engineering has been applied to various tissues, and particularly significant progress has been made in the areas of skin, cartilage, and bone regeneration. Inclusion of bioactive factors into the synthetic scaffolds has been suggested as one of the possible tissue engineering strategies. The growth factors are polypeptides that transmit signals to modulate cellular activities. They have short half-lives, for example, platelet-derived growth factor (PDGF), isolated from platelets, has a half life of less than 2 minutes when injected intravenously. Extended biological activity and the controlled release of growth factor are achieved by incorporating growth factor into the polymeric device. This review will focus on growth factor delivery for tissue engineering. Particular examples will be given whereby growth factors are delivered from a tissue-engineered device to facilitate wound healing and tissue repair.

Key Words: Cytokines, peptide delivery, vehicle

INTRODUCTION

Critical limitations in traditional therapies call for new tissue and organ replacement strategies.[1] The emerging field of tissue engineering is concerned with the development of devices that restore, maintain or modify tissue structure and function.[2] Tissue engineering has been applied to various tissues, and particularly significant progress has been made in the areas of skin,[3] cartilage,[4] and bone regeneration.[5] The inclusion of bioactive factors (e.g. growth factors) into synthetic scaffolds has been suggested as one of the possible tissue engineering strategies.

Growth factors are polypeptides that transmit signals to modulate cellular activities. The term cytokine is generally reserved to describe factors associated with cells involved in immune system.[6] Growth factors can either stimulate or inhibit cellular proliferation, differentiation, migration and gene expression.[7] In a concentration dependent manner, growth factors can also act in an opposing manner and up- or down-regulate the synthesis of receptors.[6] Growth factors usually exist as inactive or partially active precursors that require proteolytic activation and may need to bind to extracellular matrix molecules for activation or stabilization.[6] Growth factors initiate their action by binding to specific receptors on the surfaces of target cells. Depending on the proximity of their locations to the target sites, growth factors may be classified as endocrine (target cell is distant), paracrine (target cell is nearby), autocrine (target cell is the same cell that secreted the growth factor), juxtacrine (target cell is apposed to growth factor/receptor complex) or intracrine (growth factor/receptor complex is internalized) (Fig. 1).[7-9] Hundreds of growth factors have been identified, characterized, and classified into at least 20 families and superfamilies on the basis of structural homologies (Table 1).[10-13] Prolonged biological activity and the controlled release of growth factor may be obtained by incorporating a growth factor into a polymeric device.

In tissue engineered devices, there are two different potential delivery systems. Growth factors can be incorporated directly into the scaffold[14,15] or introduced after fabrication.[16,17] Another way of delivering growth factors is via the co-transplantation of either natural growth factor-secreting cells or genetically engineered cells within the device.[18] Specific growth factors, released from a delivery device or from transplanted cells, aid the induction of host parenchymal cell infiltration and improve engraftment of co-delivered cells for more efficient tissue regeneration.[19]

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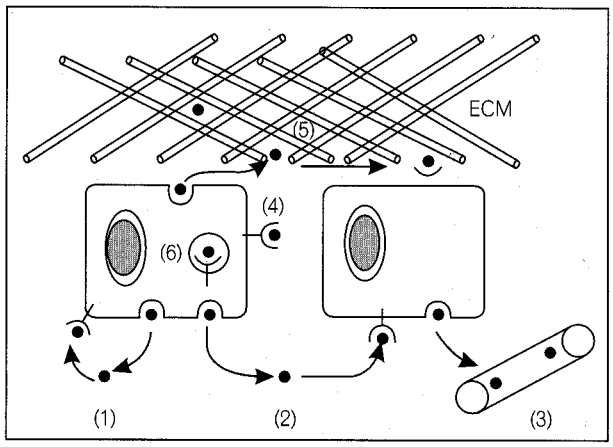

Fig. 1. Various ways in which growth factors exhibit their activities. Growth factor (black dots) are produced by the cells and can act within the cell or in vicinal or remote cells to modulate their activities by reacting with specific receptors. (1) Autocrine; (2) Paracrine; (3) Endocrine; (4) Juxtacrine; (5) Extracelluar matrix mediated; (6) Intracrine.

Local delivery of growth factors can induce cell proliferation, chemotaxis, differentiation, and matrix synthesis, and thus, exhibits potential for regenerative therapeutics. The basic requirements of the tissue engineering scaffold include, degradability, biocompatibility, surface area/volume ratio, mechanical integrity and vascular and neural infiltration.[1] Materials used as tissue engineering scaffolds must be degraded over a predictable and controllable time scale, to enable the synchronization of material degradation and tissue formation. Delivery systems for growth factors often take advantage of the known controllable degradation of synthetic polymer scaffolds, to release quantities of drug over an extended time scale. Another key material requirement is a large surface area/volume ratio to support cell adhesion, and facilitate nutrient transport. Porous material promotes cell activity by extending the substrate area for growth

Table 1. Principal Source and Activity of Growth Factor

Factor	Principal source	Primary activity	Remark
PDGF	Platelets, endothelial cells, placenta	Promotes proliferation of connective tissue, glial and smooth muscle cells	Two different protein chains form 3 distinct dimer forms; AA, AB and BB
EGF	Platelets, endothelial cells, placenta	Promotes proliferation of mesenchymal, glial and epithelial cells	
TGF-α	Common in transformed cells	May be important for normal Wound healing	Related to EGF
FGF	Wide range of cells; protein is associated with the ECM	Promotes proliferation of many cells; inhibits some stem cells; induces mesoderm to form in early embryos	At least 19 family members, 4 distinct receptors
NGF	Tissues that are innervated by neuron	Promotes neurite outgrowth and neural cell survival	Several related proteins first identified as proto-oncogenes; trkA (trackA), trkB, trkC
Erythro-poietin	Kidney	Promotes proliferation and differentiation of erythrocytes	
TGF-β	Activated TH1 cells (T-helper) and natural killer (NK) cells	Anti-inflammatory (suppresses cytokine production and class II MHC expression), promotes wound healing, inhibits macrophage and lymphocyte proliferation	At least 100 different family members
IGF-I	Primarily liver	Promotes proliferation of many cell types	Related to IGF-II and proinsulin, also called Somatomedin C
IGF-II	Variety of cells	Promotes proliferation of many cell types primarily of fetal origin	Related to IGF-I and proinsulin

and proliferation while also allowing for optimal diffusion of nutrients between cells in the scaffold and the surrounding tissue. Vascularization expedites mass transport, which is essential in the region of a developing tissue. Mechanical integrity of the scaffold material is necessary to resist contractile cellular forces, which can cause collapse of a 3-dimensional scaffold structure during tissue growth.[20]

This review will focus on growth factor delivery for tissue engineering. Particular examples will be given in which growth factors are delivered from a tissue-engineered device to facilitate wound healing and tissue repair.

BONE MORPHOGENETIC PROTEIN

Urist believed that osteoinduction by a dematerialized bone matrix (DBM),[21] was caused by a contained factor, which he named bone morphogenetic protein (BMP).[22] Wozney et al. later isolated this protein, which directs cartilage and bone formation. The amino acid sequence of protein obtained from a highly purified preparation has been identified, and the expression of the recombinant human proteins has been obtained. Three proteins in total were demonstrated, BMP-1, BMP-2A and BMP-3.[23]

BMP-4 was originally identified as a factor purified from demineralized bone that can trigger ectopic bone formation at non-skeletal sites *in vivo*.[24] Later, more BMPs were identified, and currently the list stands at least 15. BMPs initiate, promote and maintain chondrogenesis and osteogenesis.[25] BMP-2,3 (Osteogenin),4,5,6 and 7 (Osteogenic protein-1) have osteoinductive potential[23,26-30] and when delivered with a carrier substance these recombinant BMPs have been demonstrated to be effective at healing intermediate sized bone defects in a variety of animal models including rat[31] and rabbit.[36]

Recombinant human BMP-2 (rhBMP-2) when implanted subcutaneously in rat inactive DBM carrier induced cartilage and some new bone.[32] When the

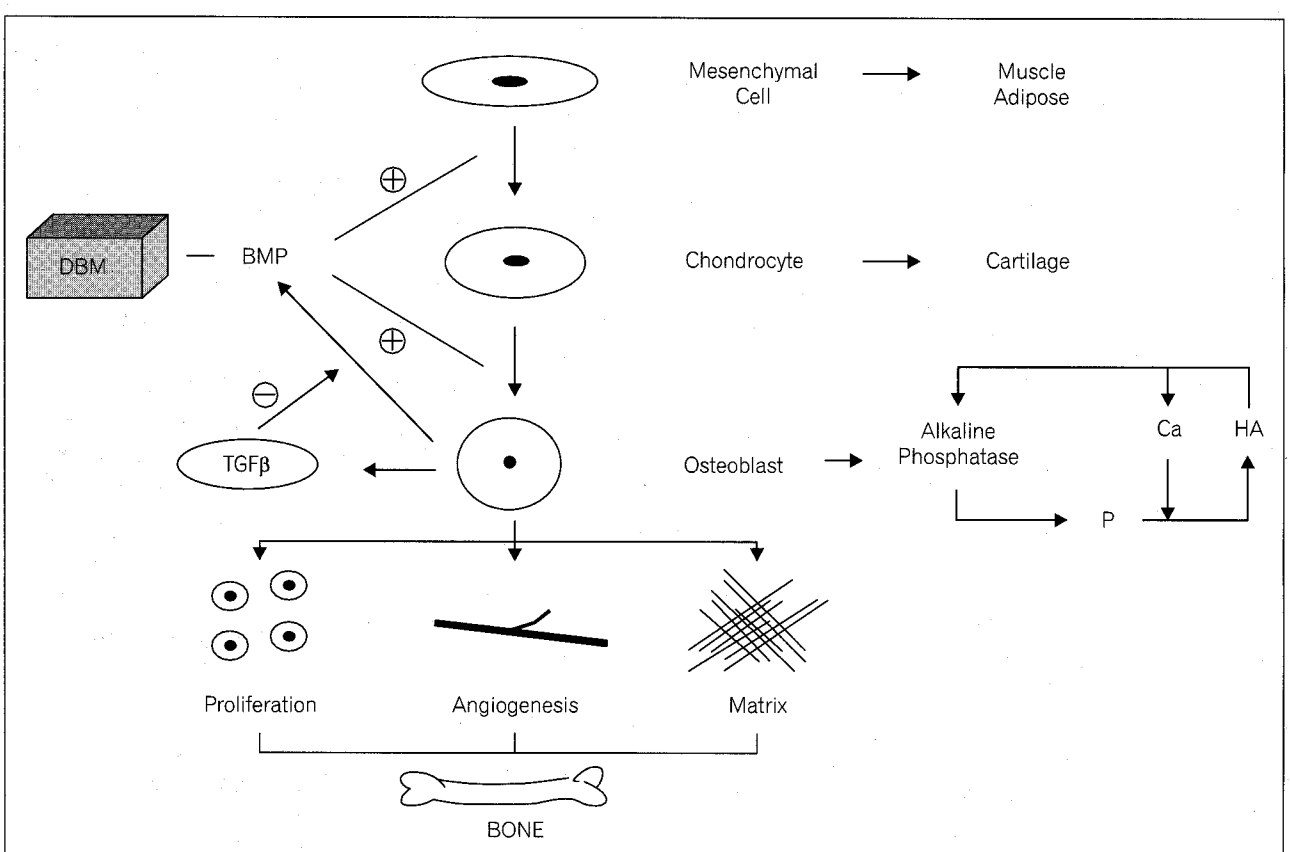

Fig. 2. Role of BMP in osteoduction.

concentration of BMP-2 was increased, bone formation was observed earlier and cartilage and bone were formed concurrently. The osteoinductive response induced by BMP-4 and BMP-5 seems to be weaker than that of BMP-2.[24] Cartilage and bone formation was induced in athymic mice by Chinese hamster ovary cells transfected with either the murine BMP-6 gene[33] or the BMP-4 gene.[34]

Implanted rhBMP-2 enhanced the healing of femur defects in rats[31] and sheep,[35] and mandible defects in rats[36] and dogs.[37] Recombinant human BMP-7 (OP-1) enhances the healing of segmental ulnar defects in rabbits[27] and dogs,[26] and stimulates the differentiation of cartilage from periochondrium tissue.[38] Recently, rhBMP-2 has been shown to stimulate the repair of bone and hyaline-like cartilage in experimental osteochondral defects in rabbits,[39] and has been shown to accelerate the healing process when a tendon graft is transplanted into a bone tunnel.[40]

BMP enhances the differentiation of mesenchymal or muscle cells into chondrocytes and osteoblasts. Osteoblasts release BMP and TGF, and the latter inhibits further BMP release. Osteoblast-derived alkaline phosphatase catalyzes the formation of hydroxyapatite (HA), and these growth factors promote the formation of bone[41] (Fig. 2).

BMP delivery systems

Bone morphogenetic proteins have been shown to stimulate the production of bone *in vivo* when combined with an appropriate carrier material such as collagen, tricalcium phosphate, or polylactic acid.[42] An ideal functional carrier that is also compatible with human tissues should possess the following characteristics: -high affinity for BMP and host bone promote the delivery and/or function of BMP; lack of interference with bone repair; lack of toxicity and immunogenicity; weight bearing capacity and mechanical strength; ease of manipulation; compatibility of sterilization; biodegradability. Synthetic polymers have most of the characteristics required of the ideal BMP carrier.[41]

Poly-D,L-lactide-co-glycolide (PLGA) copolymers are good carriers for BMP and promote the induction of new bone formation. Further, PLGA copolymers, with recombinant human bone morphogenetic proteins (rhBMP-2), had a greater effect in inducing new bone formation and resorbing implanted material than active demineralized freeze-dried bone allografts alone.[43] PLGA capsules containing rhBMP-2 regenerated bone in rat femur defects[44] and in segmental defects of the rabbit's radius.[45] Bioerodible PLGA particles loaded with rhBMP-2 were suspended in either carboxymethylcellulose (CMC) or methylcellulose (MC) implants. The CMC implants appeared to encourage bone growth even in the absence of BMP, and when BMP was added, new bone formed earlier. CMC may influence new bone formation because it is hydrophilic. MC is less hydrophilic and may cause undue inflammation.[46] Rat mandibular defects were implanted with rhBMP-2 with or without osteopromotive membrane. rhBMP-2 was delivered using bio-absorbable PLGA beads plus allogenic blood as carriers. After 24 days, defects treated with membrane and rhBMP-2 in the PLGA carrier were totally bridged with regenerated bone.[47]

Freeze-dried poly-L-lactic acid discs mixed with BMP may be effective at healing rat skull defects.[48] Composites of semipurified BMP and polylactic acid-polyethylene glycol block copolymer (PLA-PEG), and composites of BMP, PLA-PEG and PLGA were implanted under the fasciae of the dorsal muscles of mice. After three weeks, both the BMP/PLA-PEG and BMP/PLA-PEG/PLGA composites were absorbed and replaced by newly induced bone with hematopoietic marrow. The BMP/PLA-PEG/PLGA composites were also implanted in large segmental bone defects in the tibiae in rabbits. Twelve weeks after implantation, the bone defect was completely restored by a newly formed bone mass of the original thickness and structure.[49]

BMP, associated with N, N-dicarboxymethyl chitosan, was used to induce or facilitate the repair of articular cartilage.[50] rhBMP-2, reconstituted with insoluble collagenous bone matrix, was sufficient to repair craniotomy defects in the rat.[51] BMP-beta tricalcium phosphate (TCP) composite also regenerated bone in skull trephine defects in dogs.[52] And a TCP-monocalcium phosphate monohydrate cement was also determined to be an effective carrier of rhBMP-2 in rat femoral defects.[53] Poloxamer 407 proved to be efficient at delivering BMP.[54]

EPIDERMAL GROWTH FACTOR

Epidermal growth factor (EGF), a 53-amino acid mitogenic polypeptide present in many mammalian

species, is one of a number of growth factors being investigated for their potential to expedite the healing process.[55] EGF and TGF-α are distinct mitogenic peptides (of 53 and 50 amino acids, respectively) that interact with the same cell-surface receptor despite their limited sequence homology. Though they are antigenically distinct, they seem to do so with similar binding affinities, and have both been implicated in processes ranging from carcinogenesis and central nervous system development to craniofacial morphogenesis and wound healing.[56] EGF has been shown to stimulate keratinocyte division *in vitro* and epidermal regeneration *in vivo*.[57] It has also been shown to have an effect on mesenchymal cells by producing marked proliferation of the dermis in partial-thickness wounds and by increasing the tensile strength of surgical incisions.[58] EGF is a naturally occurring mitogen, which in its recombinant form is under intensive investigation for therapeutic use. Receptor activation by EGF induces up-regulation of the syntheses of specific proteins and the proliferation and differentiation of the corneal epithelium, keratocytes, and endothelium both *in vivo* and *in vitro*.[59]

Wound healing is a localized process, which involves inflammation, wound cell migration and mitosis, neovascularization and the regeneration of the extracellular matrix. Recent data suggests the actions of wound cells may be regulated by the local production of peptide growth factors, which influence wound cells through autocrine and paracrine mechanisms. EGF may play an important role in normal tissue wound healing, such as, in skin, cornea, and the gastrointestinal tract. EGF treatment accelerated healing of gastroduodenal ulcers, and also increased the tensile strength of skin incisions in rats and corneal incisions in rabbits, cats, and primates.[60]

EGF delivery systems

Experimental studies in animals have demonstrated that the topical application of epidermal growth factor accelerates the rate of epidermal regeneration of partial-thickness wounds and second-degree burns. Donor sites treated with silver sulfadiazine containing epidermal growth factor demonstrated an accelerated rate of epidermal regeneration in all 12 patients compared with paired donor sites treated with silver sulfadiazine alone.[57]

EGF and extracellular matrix (ECM) molecules (collagen type IV, and chondroitin sulfate) were also investigated, as surface-grafted biomolecules. They stimulate cell attachment, proliferation, and function by signaling only from the basal side of cultured cells.[61]

Treatment with EGF in a Carbopol gel carrier for a period of 8 hours resulted in significant wound healing enhancement ($p < 0.05$). The optimum EGF loading in the gel was determined to be 0.4%. A slowly releasing gel was suggested to be an effective way of delivering EGF to the corneal surface.[62]

In vitro and *in vivo* studies have shown that EGF has the potential to improve ligament healing. Gene therapy approaches may represent a new alternative in delivering these specific growth factors to the anterior cruciate ligament (ACL). The aim of this study was to investigate the feasibility of three different gene therapy approaches (direct-, fibroblast-, and myoblast-mediated gene transfer) to the ACL. This new technology based on gene therapy and tissue engineering may allow a persistent expression of selected growth factors to enhance ACL healing following injury.[63]

A single application of irradiated EGF gene transfected fibroblasts to wounds can thus continuously deliver the transgene *in vivo* and could be used to administer drugs to the wound bed during the crucial initial seven days of wound-healing.[64]

FIBROBLAST GROWTH FACTOR

The fibroblast growth factor (FGF) family has nine members. Among them, the best characterized are acidic fibroblast growth factor (FGF-1) of 16 KDa and basic fibroblast growth factor (FGF-2) of 17 KDa.[65] The fibroblast growth factor (FGF) family modulates function in various cell types including fibroblasts, chondrocytes, endothelial cells, smooth muscle cells, and astrocytes.[66]

When injected or ingested, bFGF is rapidly degraded and loses its mitogenic activity primarily by sustained release.[67] Prolonged storage and encapsulation were accomplished by binding bFGF to heparin-sepharose beads. Various combinations of FGF and heparin complexed to fibrin were investigated *in vitro*.[68] DNA replication of fibroblasts grown either on or within fibrin matrices was enhanced in the presence of both FGF and high doses of heparin incorporated in the fibrin. To develop a reliable carrier

system, various carriers were investigated, including, the release of FGF from plaster of Paris (PLP),[69] fibrin scaffold for the delivery of FGF-1,[70] *in vivo* release of bFGF from biodegradable gelatin hydrogel carrier.[17] bFGF, which is a potent mitogen, induces neovascularization[71-78] and osteogenesis[79-87] and enhances nerve regeneration.[6,88,89]

FGF delivery systems for neovascularization

bFGF is known as heparin-binding growth factor because of its high affinity for heparin and sulfate, which are abundantly present in the ECM of endothelial cells.[71] When the vascular wall is damaged, bFGF can be released through several mechanisms,[72] and proliferation of endothelial cells will be induced. bFGF was sorbed into microspheres of acidic and basic gelatin with different isoelectric points. CMC incorporation slowed down the biodegradation and vascularization effect of bFGF-incorporating gelatin microspheres.[73] Neovascularization was induced around the implanted site of the bFGF-incorporating acidic gelatin hydrogel, but a prolonged vascularization effect was not achieved by the bFGF-incorporating basic gelatin hydrogel.[74] Due to an initial large burst in bFGF release, probably because of the down regulation of bFGF receptor, only transient vascularization occurred.

The treatment of myocardial ischemia based on the use of pro-angiogenic growth factors induced the growth of new blood vessels to supply the myocardium at risk.[75] A single intrapericardial injection of bFGF in a porcine model improved myocardial perfusion and function in the ischemic territory, but these benefits were not seen in saline- or heparin-treated ischemic animals. Also the administration of growth factors is emerging as a new therapeutic approach for the enhancement of collateral vessel formation.[76] FGF-2 administered with heparin proved the most effective method of enhancing angiogenesis, when compared to FGF-2 alone, FGF-2 plus heparan sulfate or FGF-2 coated heparin agarose beads.

bFGF, endothelial cell growth factor (ECGF) and a penetrance enhancer (dimethyl sulfoxide) were applied to composite grafts.[77] A group of bFGF and ECGF showed a 40% increase in vascular ingrowth but did not increase growth survival. Recently, *de novo* adipogenesis using a mixture of basement membrane extract (Matrigel) and the bFGF-incorporated gelatin microspheres provided a new idea for the tissue engineering of adipose tissue.[78]

FGF delivery systems for bone regeneration

FGF-2 was found to improve endosteal bone formation in rat long bones after intravenous administration, but to a limited extent,[79-81] and to moderately promote fracture healing.[82] FGF-2 delivered on a collagenous carrier was shown to stimulate bone healing of segmental bone defects in rabbit femurs,[83] enhance ectopic bone formation in rats[84] and promote bone ingrowth in titanium chambers placed in rat tibia.[65] However, FGF-2 effects on the ectopic bone formation rate were dose-dependently biphasic.[85] Minipellets incorporating atelocollagen were prepared and analyzed.[83] At doses of 1.4 microgram approximately 90% of the defects were filled with new bone and cartilage within 6 weeks of minipellet implantation. But an injection of 2 microgram of FGF solution into bony defects had no effect on the repair of segmental bony defects. In other cases, an increased number of osteocytes was found in the newly formed bone at sites treated with the lower rhFGF-2 doses, whereas the high-dose rhFGF-2 caused a return to control levels.[86] Morphometrical analysis revealed that the new bone area in the 1-ng group was significantly larger than that in the 0-ng group,[87] but in the 100-ng FGF-2 group, new bone formation seemed suppressed. Continuous slow administration of a small amount of FGF-2 may thus accelerate bone-derived osteogenic cytokine-induced new bone formation.

Applications of FGF in nerve regeneration

bFGF was shown to enhance the *in vitro* survival and neuritic extension of various types of neurons, including dorsal root ganglion cells.[6,88] This cell culture experiment was performed in the context of peripheral nerve regeneration.

FGF-9 improved the survival of acetylcholinesterase-positive neurons, increased their mean soma size and up-regulating their choline acetyltransferase (ChAT) activity.[89] The ChAT-promoting effect of FGF-9 was approximately as potent as that of nerve growth factor (NGF) and was greater than those of bFGF, ciliary neurotrophic factor and glia-derived neurotrophic factor. Although the effective delivery of exogenous FGF-9 into the central nervous system remains a problem, FGF-9 may be a promising candidate for therapeutic trials in Alzheimer disease.

NERVE GROWTH FACTOR

The role of neurotrophic factors in the maintenance and survival of neuronal cells has been the subject of numerous studies.[90] Neurotrophic factors are polypeptides known to regulate the survival and differentiation of nerve cells during the development of the peripheral and central nervous systems. Specific neurotrophins such as nerve growth factor (NGF), neurotrophin-3 (NT-3) and brain derived neurotrophic factor (BDNF) have shown to protect nerve cells in a number of experimental models of neurodegenerative diseases, such as, Parkinson disease, Alzheimer disease, and amyotrophic lateral sclerosis, in much the same way as specific neurotrophic factors have been shown to stimulate the regenerative growth of both peripheral and central nerve fibers.[91]

NGF exists as a 7 S complex containing 2 α, 2 β, and 2 γ subunits held together by two gram-atoms of Zn^{2+}. These subunits are readily separated and each has been sequenced and cloned. Only the β-NGF dimer possesses biological activity. It is associated with two α-NGF and two γ-NGF subunits, which belong to the glandular kallikrein family of serine proteinases. The γ-NGF subunit is an active serine proteinase capable of processing the precursor form of β-NGF, whereas, α-NGF is an inactive serine proteinase. The structure of 7 S NGF could be used as a starting point to design inhibitors that prevent NGF binding to its receptors, as a potential treatment of neurodegenerative disease. The role of the α-subunit remains unclear, although it is required for stable interaction of the β- and γ-subunits in vitro.[92]

NGF delivery systems

NdGF and other neurotrophic factors have been shown to promote neuritic extension after injury. The difficulty in effectively delivering these substances over a protracted time course that promotes maximal, directed growth has been an obstacle to achieving the maximal benefits from these substances. Nowadays, problems with continuous and localized delivery of specific neurotrophins, single or in combination, into the nervous system appears to be the most important obstacle preventing more widespread clinical application.[93]

The use of transferrin (Tf) as a brain drug delivery vector proven as effective at transporting biotinylated therapeutics as OX26, and avoided the disadvantages of its antigenicity. Transferrin receptors are concentrated on the plasma membrane of brain endothelial cells and mediate the transcytosis of transferrin (Tf) through the blood-brain barrier. This property allows transferrin to act as the brain drug transporter vector. NGF was conjugated to transferrin using the avidin/biotin technology, and its brain-uptake efficiency was increased.[94]

The effectiveness of a specific growth factor/extracellular media incorporated in a biodegradable non-neural nerve conduit material was also investigated for enhancement of axonal regeneration. Numerous experiments were carried out on the incorporation of hyaluronic acid (HA) inside a newly manufactured nerve conduit material from fresh human amnionic membrane. Results of these investigations showed that NGF/HA treatment improved axonal regeneration across the amnionic tube nerve conduit 45% more so than the non-treated amnionic tube group.[95]

Agarose hydrogel scaffolds were engineered to stimulate and guide neuronal process extension in three dimensions *in vitro*. Using the bifunctional cross-linking reagent 1,19-carbonyl diimidazole the extracellular ECM protein laminin (LN) was covalently coupled to agarose hydrogel. Compared to the unmodified agarose gels controls, LN-modified agarose gels significantly enhanced neurite extension from three dimensionally (3D) cultured embryonic day 9 (E9) chick dorsal root ganglia, and PC12 cells.[96]

A NGF-releasing biodegradable microsphere is currently under investigation to protect striatum against excitotoxic damage. NGF-loaded poly (d,l-lactide-co-glycolide) microspheres resulted in a sustained release of NGF for at least one month *in vitro*. Microspheres implanted in the intact striatum still contained NGF after 2.5 months and they were totally degraded after 3 months. After quinolinic acid infusion, the lesion size in the group treated with the NGF-releasing microspheres was found to be reduced by 40% compared with the control group.[97]

As these devices, aimed at delivering neurotrophic factors to brain cells, are further developed and perfected, it is very likely that their use will greatly contribute to the amelioration of a variety of neurogenic disorders.[6]

PLATELET-DERIVED GROWTH FACTOR

Platelet-derived growth factor (PDGF) was initially observed as a fibroblast growth-promoting activity present in serum but lacking in plasma.[98] PDGF (−30 kDa) consists of two distinct disulphide-linked peptide chains, termed A and B, that share a 60 percent sequence identity, and can be expressed as homodimers (PDGF-AA and PDGF-BB) or as a heterodimer (PDGF-AB).[99,100] Binding of PDGF to several plasma and extracellular matrix proteins, including α_2-macroglobulin may modulate its biological activity.[6,102] Since PDGF is a strongly cationic protein, it should bind to negatively charged extracellular matrix proteins.[6] Also, binding of PDGF to the extracellular part of either receptor type leads to dimerization of the receptor molecules, followed by activation of the receptor protein-tyrosine kinase, and the generation of phosphorylation-mediated signals that initiate the biological response.[101,103] PDGF is not detectable in the circulation and its biological half-life is less than 2 minutes when injected intravenously.[104] PDGF is both a locally produced and locally acting growth factor.[6] Smooth muscle cells and fibroblasts synthesize only the PDGF-A chain, whereas endothelial cells and macrophages synthesize both the PDGF-A and PDGF-B chains.[105-108]

PDGF as a mitogen is able to induce density-inhibited cells to reach the first arrest point in the cell cycle, called the competence point, hence it is classified as a 'competence factor'.[109] Peptides such as insulin-like growth factor-I (IGF-I) and EGF act later in the cell cycle, and are termed 'progression factors'. They exhibit potent synergy with PDGF *in vitro* and *in vivo*.[6] PDGF is mitogenic for fibroblasts, partly through its ability to induce the synthesis of autocrine factors.[6] It also enhances fibroblast production of fibronectin, hyaluronic acid and collagenase.[110,111]

PDGF is a potent mitogen and chemotactic factor for cells of mesenchymal origin, including periodontal ligament cells and osteoblasts.[112-118] Enhancement of periodontal tissue regeneration using PDGF has been demonstrated in beagle dogs and monkeys.[119-123] Though PDGF has superior activity in tissue regeneration, rapid clearance of PDGF due to its short half-life result in difficulties at maintaining therapeutic concentrations after injection. This has lead to the administration of extremely high doses above 10 μg for bone regeneration.[113] PDGF and IGF-I are important anabolic growth factors for bone regeneration, because both adsorb to the bone mineral matrix in a concentration-dependent fashion.[124,125] PDGF in concentrations equal to or greater than 50 ng/ml demonstrated a significant stimulation of periodontal ligament cells adherence to periodontal diseased root surfaces.[126] Resting zone chondrocyte (RC) cells were pretreated with recombinant human PDGF prior to implantation. Pretreatment of the RC cells with PDGF promoted the retention of a hyaline-like chondrogenic phenotype. There was also a marked increase in cartilage formation in PDGF treated cells.[127]

Topically applied recombinant human PDGF is a new pharmacologically active therapy for chronic, neuropathic, lower extremity diabetic ulcers.[128] In a rabbit ear dermal ulcer model, PDGF was unique among several factors tested, including TGF-β_1, FGF and EGF, in significantly enhancing both granulation tissue volume and the degree of re-epithelization.[17] It also stimulated granulation tissue formation in normal and diabetic rats.[19]

Platelets release granule products and products of the coagulation process which deposit locally. The sequential migration of neutrophils, monocytes and fibroblasts into wounds begins immediately and continues over the first several days. Activated wound macrophages and fibroblasts result in the *de novo* synthesis of growth factors, other cytokines and extracellular matrix proteins including collagen.[129] PDGF and IGF-I have been shown to regulate DNA and protein synthesis in bone cells *in vitro* and to interact synergistically to enhance soft tissue wound healing *in vivo* despite their short half-lifes.[115] A combination of PDGF and EGF promoted human colonic fibroblast-dependent wound repair activities.[130] PDGF also stimulates neointimal formation and vascular regeneration.

PDGF and TGF β1 stimulated neointima cells *in vitro* and neointimal formation *in vivo*.[131] HGF and PDGF also act in coordination to promote the proliferation and migration of smooth muscle cells in the earlier phases of neointimal formation.[132] Experiments with an *in vitro* growth chamber model in the rat, consisting of a silicone shell containing a dissected femoral vascular bundle, revealed that recombinant PDGF-BB, when incorporated into a rapidly dissolving collagen type film, induced the generation of *de novo* tissue around the femoral vascular bundle.[133]

PDGF-BB delivery systems

New bone formation induced by DBM was significantly enhanced by PDGF or transforming growth factor beta (TGF-β) (60 ng of each growth factor) after adsorption on microcrystals of hydroxyapatite, which indicated that a suitable carrier was required for the stimulation of osteoinduction.[117] It is essential that a carrier system is developed to maintain PDGF-BB at therapeutic concentrations at wound sites for healing period of up to 4 weeks to obtain enhanced bone regeneration.[123] Khouri et al. developed a collagen disk which delivers rPDGF-BB either as a rapid pulse or by slow release. Sustained delivery of rPDGF-BB caused continuous growth of the tissue and was more effective than pulsed delivery.[134] The local delivery of PDGF and TGF-1 significantly increased neointimal thickness at the neck of porcine aneurysms using collagen sponge. The chitosan/TCP sponge carrier system was fabricated as a sustained delivery system of PDGF-BB for bone regeneration.[135] Extrudable ethylene-vinyl acetate (EVA) copolymer delivery systems capable of sustained release of PDGF-BB were developed for human osteoblast proliferation and differentiation.[136] In previous study, EVA, bovine serum albumin and PDGF-BB were combined and coated onto a stainless-steel Kirshner wire (K-wire). PDGF-BB released from the K-wire delivery system stimulated thymidine uptake in human bone cell cultures. Differences in porosity and tortuosity of the EVA rod accounted for the different release kinetics observed.[137] For enhanced regeneration of both soft and hard tissue components of the periodontium, a combination of 3 μg of recombinant PDGF-BB and IGF-I in a methylcellulose gel was prepared. Compared to controls receiving placebo gel, PDGF-BB/IGF-I treated sites showed increased height and total area of new bone after 2 to 5 weeks.[138] Poly α-hydroxyacids are known to be degraded principally by non-specific hydrolysis *in vivo*. Porous poly-L-lactide (PLLA) membranes have been developed and PDGF-BB was incorporated into such a membrane for periodontal regeneration. The membrane maintained a sustained release of PDGF-BB and degraded gradually during the regeneration period.[127]

TRANSFORMING GROWTH FACTOR-β

TGF-β is a secreted multifunctional protein that regulates cell proliferation, differentiation and extracellular matrix metabolism.[139-141] TGF-β is member of the TGF-β superfamily, which consists of three groups, TGF-β, the activins and the BMPs. Five subtypes have been demonstrated, and three of these are found in all mammalian species.[140, 142] The active TGF-β is 25 kDa homodimer of disulfide-linked subunits. TGF-β performs various function on different tissues, stimulating mesenchymal cells and inhibiting ectodermal cells.[143] A variety of potential clinical applications for this growth factor has been suggested, including the enhancement of soft and hard tissue healing, control of chronic inflammatory diseases associated with fibrosis and the suppression of autoimmune diseases.[144] TGF-β usually circulates in the blood stream in latent form with a half life of 90 min, while the active form of TGF-β, is cleared from the circulation in a few minutes.[145]

TGF-β presents its signals to the cell by binding to specific large transmembrane receptors on the surface of the target cell. Binding to the extracellular domain of the receptor triggers the intracellular domain, which generally activates a protein kinase. The kinase cascade activates transcription of affected gene into mRNA, which is then translated into protein to be secreted.[146] TGF-β is secreted by cells as a biologically inactive latent precursor. Latent TGF-β is generally found as a complex of active TGF-β, a latency-associated peptide (LAP) and the latent TGF-β binding protein.[141] The release of mature TGF-β from LAP is thought to be necessary for the interaction of TGF-β with cell-surface receptors. Latent TGF-β is activated by glycosidase, resulting in a change of carbohydrate structure in LAP domain.[147] Recently, an extracellular matrix protein, thrombospondin (TSP), was found to activate latent TGF-β via a novel mechanism which does not require proteolytic activity.[148] The possible mechanism of such activation may be via a conformational change in the LAP induced by the binding of TSP.

Some binding proteins play important roles in targeting TGF-β to proper locations after synthesis and secretion.[149,150] Active TGF-β binds to multiple extracellular matrix components, such as, IV collagen, fibronectin, thrombospondin, decorin and heparin. Binding of TGF-β to these components can modulate its activity, serve as a reservoir for the growth factor,[151,152] and also play a role in delivering it to the cell-surface receptors.[153]

TGF-β delivery systems to enhance wound healing

TGF-β mediates tissue embryogenesis, normal cellular physiology, inflammation and tissue repair.[154] The wound healing response of full-thickness skin defects in rabbit to TGF-β, incorporated in a collagen scaffold, was evaluated.[155] Though greater inflammatory response was found in the collagen scaffold-treated group, the fastest epithelialization and contraction rates were associated with TGF-β and collagen. This study demonstrated that TGF-β delivered through a collagen scaffold enhanced the healing process and showed promise for future clinical applications. In general, TGF-β shows great promise for use in the therapy of poorly healing wounds.[153,156] However, the fibrogenic potential of TGF-β becomes apparent after repeated injections of higher doses.[157] The deleterious effects of TGF-β when administered in excess requires the development of a controlled delivery system.

TGF-β delivery system for bone repair

TGF-β is produced by osteoblasts and stored in the bone matrix, making bone the largest reservoir of TGF-β in the body.[158] It stimulates osteoblast-like cells to proliferate and synthesize collagen in culture,[159] and increase bone thickness when applied adjacent to periosteum *in vivo*.[160] A single application of human recombinant TGF-β1 in a 3% methylcellulose gel to skull defects created in rabbits induced a dose-dependent increase in intramembranous bone formation.[161]

Although bone has a remarkable capacity for regenerative growth, there are many clinical situations in which the bony repair process is impaired. There still exists a need for an effective method of delivering TGF-β1 to the osseous defect site to promote bone healing. A biodegradable controlled release system for TGF-β1 comprised of poly (DL-lactic-co-glycolic acid) and DBM has been described.[162]

TGF-β1 is found in the periosteum at an early stage in fractures, and enhances the proliferation of mesenchymal cells and osteoblasts in experimental bone defects.[163-165] Whereas, BMPs induce bone in heterotopic sites, TGF-β1 depends on orthotopic application such as subperiosteal injection.[165] TGF-β1 enhances the healing of experimentally created defects of the skull in rabbits,[120,166] bone ingrowth in porous titanium rods[167] and tricalcium phosphate coated implants in dogs.[168]

Rh TGF-β1 was incorporated into biodegradable microparticles of blends of poly (DL-lactic-co-glycolic acid) and poly (ethylene glycol) to create a delivery vehicle for growth factor.[169] The TGF-β1 released from the microparticles enhanced the proliferation and osteoblastic differentiation of marrow stromal cells cultured on poly (propylene fumarate) substrate. The cells showed significantly increased total cell number, ALP activity, and osteocalcin production compared to cells cultured without TGF-β1. These results suggest that controlled release of TGF-β1 from the PLGA/PEG blend microparticles may modulate cellular response bone healing at a skeletal site.

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