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INTERNATIONAL JOURNAL OF CURRENT RESEARCH

International Journal of Current Research
Vol.2, Issue, 1, pp. 106-113, January, 2011

ISSN: 0975-833X

REVIEW ARTICLE

RECOMBINANT PROTEINS IN THE FUTURE OF PERIODONTAL THERAPEUTICS: A REVIEW

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ARTICLE INFO

Article History:

Received 5th October, 2010
Received in revised form
18th December, 2010
Accepted 28th December, 2010
Published online 6th January, 2011

Key words:

Periodontal regeneration,
Recombinant proteins,
Growth factors,
Carrier matrices

ABSTRACT

Regeneration of periodontal tissues has been the ultimate goal of periodontal therapy. Conventional therapies may result only in repair of the periodontal tissues. Numerous regenerative techniques utilising autogenous bone grafts, allografts had been devised but they have significant limitations. Improved understanding of cellular and molecular biology of periodontal wound healing has lead to the application of exogenous growth factors in periodontal defects as they play a critical role in cellular events. Experimental studies utilizing recombinant human growth factors like platelet derived growth factor, bone morphogenetic protein supports periodontal regeneration using minimally invasive techniques. These biological molecules can be conjugated to tissue-engineered carrier constructs, which are artificial analogues of extracellular matrix. These carrier matrices protect, localize and release the recombinant proteins at the appropriate time . This review article highlights the role of various recombinant growth factors and the carrier constructs in periodontal wound healing and its limitations in clinical use.

INTRODUCTION

A major goal of periodontal therapy continues to be regeneration of attachment structures of teeth including new bone, periodontal ligament and cementum which have been destroyed by periodontal diseases. Although a number of treatment modalities are available, clinicians seek more predictable regenerative therapies that are less technique sensitive, leading to faster tissue regeneration that is applicable to the broad array of periodontal and peri-implant defects encountered by them daily. In recognition of the need for a potent regenerative agent, tissue engineering investigations have proceeded towards a biomimetic approach utilising recombinant proteins. Tissue engineering combines three critical components - conductive scaffolds, signaling molecules and cells for periodontal regeneration.

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In a normal wound healing process, multiple cytokines act in concert to regulate the cellular functions of various cell types within and adjacent to a wound in nearly all tissues, including the periodontium (Murakami *et al.,* 2003). Signaling molecules like growth factors and morphogens, are capable of stimulating cellular events and have received a great amount of attention with regard to periodontal regeneration. Growth factors have pleiotropic effects, that can regulate migration, attachment, proliferation and synthesis of specialised proteins (Saygin *et al.,* 2000). Morphogens acts mostly by osteoinduction, which is, the differentiation of stem cells into bone forming cells. The binding of these signaling molecules to its receptors triggers a series of events by which extracellular signals are transduced into the cell, leading to the stimulation or repression of gene expression. Growth factors are very frequently not in adequate quantities in grafts of autogenous or allogenic sources. For instance, the yield of extracted and partially

purified bone morphogenetic proteins only amounts to 1 µg/kg of fresh bone. Allogenic bone that is freeze dried and decalcified, frequently does not have any bone morphogenetic protein despite it being specifically prepared so that these proteins are expressed.(Schwart et al,1998).Hence these techniques do not provide appropriate quantity of growth factors to cause periodontal regeneration. Recombinant technologies could therefore be a more predictable manner by which host tissues may be modulated to regenerate lost tissues.

Recombinant proteins are derived from recombinant DNA which is a form of artificial DNA created by combining two or more DNA sequences that would not normally occur together. They are produced by removing the specific DNA sequence from a human cell and transfecting it into a bacterial plasmid. The plasmid is then transfected into host cells like yeast or Chinese hamster ovarian cells capable of large scale growth (Lynch 2008). With the advances in recombinant technology, proteins may be now synthesised ,concentrated ,purified and packaged in large, sterile quantities. Regenerative periodontal procedures are more predictable by combining these proteins with suitable matrices which would aid in specific cell adhesion, proliferation, differentiation.

To date only 3 recombinant growth factor products have been approved by the US Food and Drug Administration (FDA) for therapeutics. They are Recombinant human platelet derived growth factor –BB (rhPDGF-BB) gel, rhPDGF-BB gel with β-tricalcium phosphate, Recombinant human bone morphogenetic proteins-2 (rhBMP-2) with type 1 collagen sponge. The clinical indications for these proteins is de novo regeneration of bone and/or periodontal complex as well as an adjunct to local socket grafting and smaller ridge augmentation procedure.

Recombinant human platelet derived growth factor (rhPDGF)

PDGF family includes four genes located on different chromosomes which encodes PDGF-A, PDGF-B, PDGF-C, PDGF-D isoforms (Fredriksson *et al.,* 2004). Platelets, macrophages, endothelial cells are all sources of PDGF. It is released locally during clotting by blood platelets at the site of soft or hard tissue injury, stimulating a cascade of events that leads to wound healing response (Lynch 1999). PDGF is chemotactic and mitogenic for cells that will differentiate into osteoblasts, cementoblasts and periodontal ligament cells. They can stimulate formation of granulation tissue, which is a prerequisite for wound healing and bone regeneration.

Recombinant PDGF-BB is a 98% pure recombinant protein developed using conventional recombinant expression techniques under highly controlled conditions. It contains approximately 1000 times higher concentration of PDGF than the level commonly obtained through platelet concentration (Huang JS, 1983). The host cells for rhPDGF-BB are yeast which synthesise and secrete them. These proteins are separated by sophisticated analytical protein chemistry techniques, sterile filtered and formulated into a dose specified for clinical use. (Lynch 2008). The rhPDGF solution comes in a physiological buffer to be mixed with tricalcium phosphate,bone allograft or another conducive matrix.
In 2005, rhPDGF with tricalcium phosphate (GEM 21S,Biomimetic Therapeutics) was approved for periodontal indications such as treatment of intrabony and furcation defects and gingival recession associated with periodontal defects. Tricalcium phosphate which fulfils PDGF pharmacokinetics is calibrated to wound healing biology and provides localization of PDGF at the wound site for appropriate period of time and at an optimal and biologically active dose. The half life of PGDF is 4 hours approximately. The therapeutic dose of PDGF may decrease by 50% after the first 4 hours of its release.

Recombinant human PDGF applied to root surfaces increased proliferation of periodontal ligament cells, cementoblasts, osteoblasts, perivascular cells and endothelial cells (Wang 2004) and showed increase in bone and cementum formation (Lynch 1991) . In a clinical study with 38 human subjects rhPGDF-BB with insulin growth factor-1 (IGF-1) has shown significant vertical alveolar bone height and periodontal osseous defect fill (Howells 1997). In a histological analysis, rhPGDF-BB treated intrabony defect and furcation defect sites showed regeneration of cellular cementum, functional, vascular, mature periodontal ligament and alveolar bone.(Nevins 2003).The authors subsequently conducted a prospective, blinded and randomized controlled clinical trial in 180 human subjects to test the safety and efficacy of rhPGDF-BB delivered with β-tricalcium phosphate for advanced periodontal osseous defects. The rhPGDF-BB treated sites promoted a larger gain of clinical attachment level and had greater linear bone gain and percentage of defect fill than did the sites that received β-tricalcium phosphate alone. Root resorption, ankylosis, inflammation and adverse tissue responses were absent. (Nevins 2005). The rhPDGF improves bone healing at tooth extraction sites (Cardaropoli, 2003), peri-implant bone areas (Berglundh, 2003) as well as in patients with diabetes or osteoporosis. PDGF-BB with bone graft material covered with a collagen membrane in recession sites provides comparable results to connective tissue grafts,

without the need for a second surgical site. Thus this approach reduces the procedure time and eliminate the morbidity and risk associated with the harvest site (MCGuire 2006).

Recombinant human bone morphogenetic protein (rhBMP)

BMPs belong to transforming growth factor β super family. Dr.Urist discovered that BMP induced cartilage bone and marrow formation when implanted intramuscularly in rodent models. BMP may act as growth factor, differentiation factor and as chemotactic agents. They stimulate angiogenesis and migration, proliferation and differentiation of stem cells from the surrounding mesenchymal cells into cartilage and bone forming cells in an injury. DNA probes are used to obtain the human complementary DNA sequence of BMP which is cloned and spliced into a viral expression vector. Chinese hamster ovarian cells or *Escherchia coli* transfected to become carriers have been used to produce rhBMP in large quantities for therapeutic evaluation (Yi-Hao Huang, 2008).

The rhBMP-2/Absorbable Collagen Sponge (ACS) is cleared by the FDA in 2007 for maxillary sinus augmentation and localized alveolar ridge augmentations for defects associated with extraction sockets. The ACS is a bovine type I collagen matrix that is soak loaded with a BMP solution before surgical implantation. A 4 month phase I clinical study assessed the safety and technical feasibility of using rhBMP-2/ACS for maxillary floor sinus augmentation. The overall mean height resulted for maxillary sinus floor augmentation was 8.5 mm. No serious or unexpected immunological side effects observed. The most frequent adverse effects were facial edema, oral erythema, pain and rhinitis (Boyne, 1997). The results from the subsequent phase II studies suggests that rhBMP/ACS appears to be safe and effective alternative to bone grafts in patients requiring maxillary sinus floor augmentation procedures. The induced bone assumed the qualities of the contiguous maxillary resident bone allowing the placement and long term functional loading of endosseous implants (Boyne, 2005).

The rhBMP-2 in a calcium phosphate cement matrix (α-BSM) proves to be a formidable technology for the augmentation of maxillary sinus together with placement of titanium implants. Recombinant human osteogenic protein-1 (rhOP-1) /demineralized bone matrix also has the potential to induce bone formation following sinus augmentation (Groenveld, 1999). rhBMP/ACS combined with bovine bone mineral adapted to extraction socket and alveolar ridge showed limited clinically irrelevant increase in bone height. Histological evaluation showed normal bone indistinguishable from resident bone. The rhBMP-2 coated with bovine bone mineral (Biooss) combined with GBR (Guided Bone Regeneration) using a resorbable collagen membrane were used to augment alveolar ridge in conjunction with the placement of endosseous implants. (Cochran, 2000). rhBMP-2 in a demineralised freeze-dried bone allograft/fibrin carrier or in a calcium phosphate cement matrix (α-BSM) (Wikesjo, 2002) might have substantial clinical benefits in augmenting alveolar ridge defects, allowing placement and osseointegration of endosseous implants.

Non or slowly resorbable biomaterials, such as a bovine bone mineral, may displace rhBMP -2 induced bone formation, when used for only indications resulting in poor bone quality (Barboza, 2000, Sigurdsson, 1996).But the use of space providing rapidly resorbing biomaterials for rhBMP-2 allows clinically relevant bone formation and osseointegration that is indistinguishable than from that in the adjoining resident bone. Alveolar augmentation with BMP appears to be less promising than that of maxillary sinus augmentation. rhBMP/ACS appears to be unpredictable for only indications as it is vulnerable to tissue compression. Lack of structural integrity makes the newly formed bone sparsely trabecular, barring placement of endosseous implants. To overcome the limitations, rhBMP-2/ACS has been sandwiched with various bone biomaterials to enhance its structural integrity in support of bone formation. However the long term reliability of such induced bone formation has been questioned (Tatakis, 2002). Inlay indications such as space providing intrabony defects may be treated successfully using rhBMP-2 constructs with lesser structural integrity. Implants treated with rhBMP-2 /ACS revealed significant bone formation compared to controls. The newly formed bone exhibited osseointegration to the titanium implants. Peri-implant defects in dogs that received rhBMP-2/ACS with expanded polytetrafluoroethylene (ePTFE) resulted in bone formation filling the large dome shaped space provided by the ePTFE device. (Wikesjo, 2003). rhBMP-2/ACS supports re-osseointegration of endosseous implants exposed to peri-implantitis (Hanisch, 1997). Implants exposed to functional loading for 12 months exhibited no significant difference between implants placed into rhBMP-2/ACS induced and resident bone for any parameter evaluated. rhOP-1 with autologous bone marrow can be considered for reconstruction of a mandibular resection defect. The newly formed bone assumes the characteristics of the adjacent resident bone allowing placement, osseointegration re-osseointegration and functional loading of endosseous implants (Warnke 2004).

Recombinant human transforming growth factor-β (rhTGF-β)

TGF-β is a homodimeric peptide with multifunctional actions controlling growth, differentiation and function of a broad range of target cells. Thus far five distinct TGF-βs have been identified. TGF- β s,which are relatively abundant in bone matrix (Hauschka *et al.,* 1986, Seyedin *et al.,* 1986), have been shown to affect bone metabolism, through modulation of both osteoclastic and osteoblastic cell differentiation and activity (Strong *et al.,* 1991). Administration of TGF- β has been shown to result in intramembranous and endochondral bone formation (Joyce *et al.,* 1990, Beck *et al.,* 1991) thus broadening the range of its application for regenerative procedures. TGF-β1 supports wound healing by augmenting angiogenesis, fibroblast collagen formation and the production of extracellular matrix (Hauschka, 1986). It also has a role in recruiting and stimulating osteoprogenitor cells to proliferate providing a pool of early osteoblasts. Human recombinant transforming growth factor beta 1 (rhTGF-b1) stimulates migration and proliferation of a broad range of mesenchymal cells as well as extracellular matrix synthesis by connective tissue cells (Massague, 1990). TGF β1 with barrier membrane greatly enhances bone regeneration in osseous oral defects (Ruskin, 2000).

The TGF-β3 isoform is considered to be far more potent as regulator of functions associated with osteogenesis and angiogenesis than TGF β1 or β2. The rhTGF- β3 in matrigel significantly enhanced periodontal tissue regeneration in class III furcation defects created surgically in adult baboons. There was striking vascularisation seen in periodontal ligament and vascularisation is a prerequisite for osteogenesis. (Teare *et al.,* 2008).

Recombinant human basic fibroblast growth factor (rhbFGF)

Basic fibroblast growth factor (bFGF) is a member of the heparin binding growth factor family that participate in the early stage of the wound healing process (Bikfalvi, 1997). It was initially purified from bovine pituitary and belongs to the large FGFfamily of structurally related protein. Periodontal ligament cells express a great number of FGF receptors and that bFGF induces mitogenesis in these cells. This may cause an increase of the clonal frequency of multi potent undifferentiated cells in periodontal ligament cells and suggests that application of bFGF may enhance wound healing in the periodontal tissues (Takayama 1998). It is a mitogenic protein for fibroblast cells and is involved in tissue remodelling and regeneration. bFGF stimulates quiescent endothelial cells and tissues inducing both morphogenesis and proliferation. Local application of rhBGF stimulates bone formation at the applied site as well as it is effective in healing impaired ulcers (Okumura, 1996). bFGF accelerates healing of periodontal tissue especially cementum and periodontal ligament in an experimental model of root surface defects in beagle dogs. (Yasako Sato, 2004).Topical application can enhance considerable periodontal regeneration in artificially created class II furcation bone defects of beagle dogs (Murakami *et al.,* 2003).

Recombinant human growth differentiation factor (rhGDF)

Recombinant human growth/differentiation factor-5 (rhGDF-5) are members of the transforming growth factor-b (TGF-b) superfamily . It is also known as cartilage derived morphogenetic protein-1 (Hotten *et al.,* 1994, 1996). Expression of GDF-5, -6, and -7 genes in bovine and rat tooth germs at the root-forming stage in the dental follicle suggests that they have an important regulatory roles in the development of the periodontal attachment (Morotome *et al.,* 1998, Sena *et al.,* 2003). GDF-5 plays critical roles in mesenchymal cell recruitment inducing cartilage and bone formation as well as tendon/ligament cell differentiation in morphogenesis. (Hotten *et al.*, 1996).

It promotes PDL cell proliferation by affecting extracellular matrix metabolism (Nakamura *et al.,* 2003). GDF-5 appears to be a promising therapeutic agent for periodontal wound healing/regeneration as it supports and accelerates periodontal tissue formation. (Moore *et al.,* 2009). The rhGDF-5, rhGDF-7, induces bone less aggressively compared with rhBMP-2 and rhBMP-7 and it allows regeneration of all periodontal tissues without ankylosis and root resorption (Herberg 2008). The injectability of the novel rhGDF-5/ Poly lactic-co-glycolicacid (PLGA) construct is considered to support minimally invasive regenerative procedures (Cortellini and Tonetti, 2001, 2007) and ease-of-use application in contained and non-contained periodontal defects (Herberg 2008). The construct stimulated periodontal wound healing/regeneration and it appears to be a safe technology (Kwon, 2010).

Implantation of rhGDF-5/ β-tricalcium phosphate into one wall intrabony defects has a greater potential to support the regeneration of the periodontal attachment . Long-term studies are necessary to confirm the uneventful maturation of the regenerated tissues. (Lee, 2010).

Carrier matrices

Extracellular matrix (ECM) present in the tissues act as a substratum for cell adhesion and serves as a reservoir for growth factor. It also regulates the proliferation, movement, and differentiation of the cells living within it. The extracellular matrix protects the growth factor from the local microenvironment and thereby decreases its degradation rate (Flaumenhaft *et. al.,* 1990). In tissue engineering, analogs of ECM are made which can act as a scaffold for cell adhesion, proliferation and differentiation. These scaffolds or carrier matrices are also used as vehicles to deliver recombinant human growth factors in an attempt to achieve a constant therapeutic level at the injured site. The scaffolds localize, protect and release the growth factor at appropriate times They should be biocompatible and non-toxic so that they does not elicit any adverse immune reactions. Carrier construct maintains the shape of the defect and prevent the infiltration of unwanted cells that may hinder regeneration. Scaffolds have been synthesized from an array of synthetic and natural biomaterials. Collagen, matrigel , fibrin, chitosan and hyaluronate are biodegradable natural polymers.The mechanical properties of collagen are modified by means of cross-linking agents to increase stability and alter the rate of degradation (Rosso, 2005). Polymers like polylactic acid, polyglycolic acid and ceramic hydroxyapatite are synthetic ECM analogs. Their properties are altered such that it regulates cell behaviour and provide tissue ingrowth. The undesirable degradation profile of synthetic hydroxyapatite and polymers can be modified depending on the clinical need.

Fabrication of porous, absorbable matrices are significant advances in tissue engineering. The porous structure is required to accomodate cells, signalling molecules and thereby facilitates regeneration. The pore diameter and orientation has critical role in the formation of tissue. The matrix should provide support to the applied loads during tissue regeneration. Synthetic calcium phosphate ceramics as matrix are suitable substances for load bearing as they are stiff structures with high modulus of elasticity. However, these materials are nonresorbable and thereby affect remodelling of neighbouring bone. Scaffolds have to be resorbable such that it allows new tissue formation at the site of implantation. Recombinant human platelet derived growth factor has been combined with β-tricalcium phosphate, which is a well established resorbable bio-ceramic that is used as a bone graft. β-tricalcium phosphate is a purified multicrystalline porous form of calcium phosphate with a calcium to phosphate ratio similar to that of natural bone mineral.

The efficacy of various materials have been evaluated as carrier technology for recombinant human bone morphogenetic protein. Inorganic scaffolds like hydroxyapatite, β-tricalcium phosphate, calcium carbonate,calcium sulphates and organic biomaterials like allogeneic or xenogeneic collagen, poly-α-hydroxy acids have been used with BMP. The recombinant human bone morphogenetic protein-2 /absorbable collagen sponge construct appears to be effective for a number of periodontal indications, including significant augmentation of maxillary floor sinus, and the alveolar ridge.

Poly lactic-co-glycolic acid (PLGA), a bioresorbable synthetic polymer, dissolved in biocompatible organic solvent (Herberg *et al.,* 2008) has been used with recombinant human growth differentiation factor. The rhGDF-5/PLGA construct is designed as a two-component system which requires simple admixing of the lyophilized rhGDF-5 and the paste-like PLGA carrier before administration. When brought in contact with body fluids like blood, the rhGDF-5/PLGA construct solidifies and forms a highly porous scaffold in situ. The rhGDF-5/PLGA construct is used in minimally invasive periodontal procedures (Herberg, 2008). Recombinant human transforming growth factor-b3, delivered by Matrigel,as carrier, induces alveolar bone, periodontal ligament and cementum (Teare *et al.,* 2008). Delivery vehicles have to be specific for each recombinant human growth factor and for each clinical target. The need is to construct a programmable carrier matrix that will deliver growth factors at an optimal and biologically active dose during the appropriate phase of wound healing.

DISCUSSION

To overcome or to counterbalance the limitations of the currently available products clinicians continue to seek material that could provide spectacular periodontal regeneration. Recombinant proteins appear to be promising and it holds solution to a number of clinical problems. The rhPDGF with tricalcium phosphate fulfils the histologic criteria for periodontal regeneration when administered in intrabony and furcation defects. rhBMP-2/ACS construct showed significant augmentation of maxillary floor sinus, alveolar ridge and osseointegration of endosseous implants. It is also effective in reosseointegration of implants associated with peri-implant defects. The rhGDF-5/PLGA construct did not support the formation of an epithelial attachment and allowed periodontal wound healing/regeneration in surgically created periodontal pockets. The currently availabe recombinant growth factors provide limited clinical benefits. This may be attributed to inappropriate doses and delivery systems. The optimal clinical doses

for various recombinant proteins needs to be specified for each periodontal indications. Most of the commercial carriers that deliver recombinant proteins lack the ability to adhere cell adhesion molecules or resorb too quickly. It is essential for any carrier matrix to maintain its structural integrity at the target site while releasing the growth factors in the desired concentration over time. Also, it should resorb at appropriate time so that it does not hinder bone formation and compromise the physiology and biochemical properties. There is a clinical need to design and develop a programmable delivery system scaffold that will release the recombinant growth factors at a therapeutic dose at the right time for a regenerative outcome. Also, recombinant technology relies on the inherent ability of the transfected cells like yeast or Chinese hamster cells which could produce a recombinant human growth factor of diminished biologic activity. Ultimately wound healing requires various growth factors to act together to regulate the cellular events. Recombinant protein therapy which offers single growth factor may not be adequate to achieve the desirable effects. Improved regenerative products needs to be synthesized by combining highly concentrated, pure signaling proteins. Such combination products would represent an emerging trend in regenerative therapeutics.

Conclusion

Based on the improved understanding of cellular and molecular biology of periodontal wound healing the recombinant technology comprising of growth factors and carrier construct is employed in periodontal regeneration. The future of periodontal therapy is heading towards less invasive treatment regimens with more predictability. Recombinant proteins represents a major evolution in regenerative therapies and has the potential to become a new standard of care broadening the scope of clinical practice. Knowledge in this area needs to be broadened, by potentiating the delivery of recombinant growth factors in appropriate carrier constructs creating a new paradigm, with a profound beneficial effects with regard to periodontal tissue regeneration.

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