ORIGINAL RESEARCH PAPER

Prosthodontics

TISSUE ENGINEERING - A REVIEW

KEY WORDS: Therapy, growth factors, regeneration; tissue engineering

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ABSTRACT

The development of effective therapies to treat the disease and regenerate tissue is an important goal of today's medicine. Regeneration of tissue is perhaps one of the most complex processes to occur in the body.

INTRODUCTION

Tissue engineering is a multidisciplinary field, which involves the application of the principles and methods of engineering and life sciences to help in the development of biological substitutes to restore, maintain or improve the function of damaged tissues and organs.

Langer and colleagues proposed tissue engineering as a possible technique for regenerating the lost tissues.

The goal of tissue engineering is to promote healing, and ideally, true regeneration of a tissue's structure and function, more predictably, more quickly, less invasively, and more qualitatively than allowed by previous passive techniques. The tissue engineering approach to bone and tissue regeneration combines three key elements to enhance regeneration.

A. Progenitor cells
B. Scaffold or supporting matrix
C. Signaling molecules

A. PROGENITOR CELLS:

The regenerative capacity of postnatal progenitor cells has increasingly emerged making these cells an attractive candidate for use in tissue-engineering applications.

Periosteal cells:

The cultured periosteum has the capacity to differentiate into an osteoblastic lineage and expresses tissue related genes. Yamamiya et al. showed cultured periosteum combined with platelet-rich plasma and hydroxyapatite induced clinical improvements in human infrabony defects.

Gingival epithelium and fibroblast:

Gingival epithelial sheets derived from human gingival tissues were developed and applied clinically as a treatment for chronic desquamative gingivitis. Transplantation of gingival epithelial sheets induced a reduction in inflammation and the gain of a healthy epithelial junction and connective tissue. Mohammadi et al., applied autologous gingival fibroblasts for patients with insufficient attached gingiva and showed the increase in width of keratinized tissue.

Bone marrow-derived mesenchymal stem cells: Using bone marrow aspirates from over 350 human donors, Pittenger and colleagues (1999) showed lineage specific differentiation of MSCs into fat, cartilage, and bone under appropriate in vitro culture conditions. Not only did the human bone marrow derived MSCs demonstrate ability to extensively proliferate, but these cells also were capable of guided differentiation into multiple cell types, establishing a provocative cell source for potential tissue engineering.

B. SCAFFOLD OR SUPPORTING MATRICES: The major roles for supporting matrices are listed below

1. It serves as a framework, which maintains the shape of the defect. It provides physical support for the healing area so that there is no collapse of the surrounding tissue into the wound site.
2. It serves as a 3D substratum for cellular adhesion, migration, proliferation and production of extracellular matrix.
3. It serves as a barrier to restrict cellular migration in a selective manner.
4. It serves potentially as a delivery vehicle for growth factors.

BIOMATERIALS USED AS SCAFFOLDS

Ceramics Natural and synthetic HA (hydroxyapatite) and beta tricalcium phosphate (TCP) are ceramics used in bone tissue engineering. They are biocompatible, osteoconductive and being protein free, they stimulate no immunological reaction. HA (hydroxyapatite) was one of the first biomaterial to be used as a scaffold. It may be derived from bovine bone or coralline or made of a pure synthetic material. TCP is a naturally occurring material comprising of calcium and phosphorous and is used as a ceramic bone substitute.

Polymers These include synthetic polyesters, such as polyglycolic acid, polylactic acid and polycaprolactone and natural polymers like collagen fibrin, albumin, hyaluronic acid, cellulose, chitosan, polyhydroxyalkanoates, polyhydroxyalkanoates, alginate, agarose and polyamino acids.

Synthetic polyesters PGA (polyglycolic acid) is a polymer of glycolic acid. It was the first polymeric scaffold used in tissue engineering. It is insoluble in water. It is also used as suture material, and as implants for bone fracture fixation. PLA (polylactic acid) is the polymer of lactic acid. PLA is more hydrophobic than PGA and more resistant to hydrolysis. Copolymers of PGA have been used for many types of biomaterials, including sutures (vicryl). PLGA (polylactic-co-glycolic acid) is a copolymer of PGA and PLA. Due to its biocompatibility, controlled structural and mechanical properties, tailored degradation rates, and its potential as growth factor delivery vehicles, it has been considered as the prime candidate for use in regenerative medicine and dentistry.

NATURAL POLYMERS

Collagen

1. Collagen foam: These are fabricated by freeze-drying a solution of collagen and placed in a mold of desired configuration. After physical or chemical crosslinking of sufficient intensity and duration, foam scaffolds become resistant to contraction by tissue cells and exhibit decreased or increased resistance to breakdown by col lagenase, depending on the cross-linking regimen.
2. Collagen fiber: Fibers with diameters of 300-nm and above have been made on a commercial scale. They can

be formed into wools by tangling in a scanning electron micrograph of the wool, into which cells are easily seeded. When cross-linked by methods that do not alter the native 67-nm cross-banding, the fibers are considerably more resistant to collagenase than are foam or gel scaffolds.

3. Collagen membrane scaffolds: Collagen membranes can be prepared by allowing collagen in solution to dry on a surface to which it will not bind, like Teflon or polyethylene. To promote formation of fibrils the solution is neutralized and warmed to 37°C, allowing the collagen to polymerize and form the fibrils. Before it begins to gel, the solution is spread on a suitable surface and allowed to dry. Membranes may be cross-linked by a variety of methods to improve their wet strength. For example, aldehydic cross-linking will prevent cell attachment, and UV cross-linking will reduce resistance to collagenase.

D. SIGNALING MOLECULES IN TISSUE ENGINEERING:
In order to enhance the in vivo efficacy, incorporation of various bioactive molecules into scaffolding materials have been brought into practice. This incorporation facilitates sustained release of bioactive molecules (growth factors) for longer periods of time. Several bioactive molecules have demonstrated strong effects in promoting wound repair in preclinical and clinical studies.

PLATELET DERIVED GROWTH FACTOR Kohler and Lipton (1974) and Ross et al. (1974) discovered that the material released from platelets is the principal source of mitogenic activity present in serum, and is responsible for the growth of many cells in culture that are serum dependent. This activity was later localized to the alpha granules within platelets by Witte et al. 1978, Kaplan et al. 1979 and called platelet derived growth factor (Ross and Vogel, 1978).

FIBROBLAST GROWTH FACTOR Fibroblast growth factor is the member of heparin binding growth factor family. There are 7 forms of fibroblast growth factor. It can be isolated from normal tissues in two forms: Acidic FGF (aFGF) and basic FGF (bFGF). Besides its name its activity exists beyond that of fibroblast and includes a wide variety of cell types such as smooth muscles, endothelial cells, chondrocytes and osteoblasts. Being a mitogen for fibroblasts, osteoblasts, chondrocytes, smooth muscle cells, skeletal myoblast, it has a profound effect on soft tissue and bone healing. FGF also stimulates angiogenesis, DNA synthesis and cell replication.

BONE MORPHOGENETIC PROTEINS These are 15 different types of proteins identified to date as a part of transforming growth factor-β superfamily. Bone morphogenetic protein-2 is a disulfide-linked homodimer. It helps undifferentiated pluripotent cells to differentiate into cartilage and bone forming cells. Along with β-FGF, it stimulates angiogenesis. It also stimulates alkaline phosphatase activity, thereby promoting bone formation. Thorarinn J. Sigurdsson (1995) did a study on beagle dog with artificially created 5 mm deep bone defects and concluded that rhBMP-2 treated sites showed higher alveolar bone level when compared with the control sites.

INSULIN LIKE GROWTH FACTOR This class of growth factors is also referred to as somatomedins. IGF-I is known as somatomedin C and IGF-II has been called multiplications timulating activity. Insulin like growth factor-I is found in substantial levels in platelets and is released during clotting along with the other growth factors. It is a potent chemotactic agent for vascular endothelial cells resulting in increased neovascularization. IGF-1 has strong effect on fibroblasts mitogenesis and protein synthesis in vitro. It promotes osteogenesis and cementogenesis. IGF-II is the most abundant growth factor in the bone and it also promotes parameters of bone formation but is not as potent as IGF1.

TRANSFORMING GROWTH FACTOR-β TGF-β was originally identified because it can induce non-transformed cells to grow in soft agar. It is found in highest concentration in bone and platelets. TGF-β is encoded by three different genes TGF-β1, TGF-β2, and TGF-β3. TGF-β is a strong promoter of extracellular matrix production. It selectively stimulates fibroblast proliferative activity. It stimulates type I collagen, fibronectin and osteocalcin biosynthesis, as well as bone matrix deposition and chemotaxis of osteoblast. On the other hand, TGF-β decreases synthesis of metalloproteinases and plasminogen activator, and also increases the synthesis of tissue inhibitor of metalloproteinases and plasminogen activator inhibitor (PAI), thus resulting in the decrease of connective tissue destruction.

RECENT ADVANCEMENTS IN TISSUE ENGINEERING
GENE THERAPY refers to the treatment of a disease by means of a genetic manipulation. Genetic information is transferred to the target cells, which enables them to synthesize a protein of interest to treat disease. Gene transfer is accomplished through the use of viral [retroviruses, adenoviruses (Ad) and adenoassociated viruses (AAV)] and non-viral vectors (plasmids and DNA polymer complexes. Gene vectors can be introduced directly to the target site (in vivo technique), or selected cell can be harvested, expanded, genetically transduced, and then reimplanted (in vitro technique).

Bone morphogenetic proteins gene delivery An experimental study in rodents by Lieberman and Colleagues demonstrated gene therapy for bone regeneration, with results revealing that the transduction of bone marrow stromal cells with rh BMP-2 lead to bone formation within an experimental defect comparable to skeletal bone. Another group was similarly able to regenerate skeletal bone by directly administering Ad5/BMP-2 providing further evidence for the ability of in vivo and in vitro bone engineering. Gene therapy presents certain advantages when compared with other therapies. Because both cell transplantation and laboratory cell culturing are not needed, gene therapy may be safer and more cost-effective than cell-based therapies.

Implantation of live cells Effective augmentation techniques to treat more challenging esthetic concerns, such as open interproximal spaces and other severe oral soft tissue deficiencies, though, are not currently available but cell-based therapies may change.

Use of tissue engineered human fibroblast derived dermal substitute to increase the amount of keratinized tissue HF-DDS is a tissue engineered human dermal replacement graft manufactured through a three dimensional cultivation of human diploid fibroblast cells on a polymer scaffold. Human fibroblast cell strains are obtained from newborn foreskins and are cultured by standard methods. The fibroblasts remain metabolically active after implantation and deliver growth factors key to neovascularization, cell migration and differentiation. Unlike keratinocytes which carry surface human leukocyte antigens that may cause allograft rejection phenomenon, implantation of allogenic human fibroblasts does not stimulate an immune response. The tissue engineered HF-DDS graft is safe and capable of generating keratinized tissue without the morbidity and the clinical difficulties associated with donor site surgery.

Bilayered cell therapy: A tissue engineered skin substitute as an alternative to tissue from palate Bilayered cell therapy is a living bilayered tissue engineered skin substitute constructed of type 1 bovine collagen and viable allogenic human fibroblasts and keratinocytes isolated from human foreskin. BCT is morphologically, biochemically and metabolically similar to human skin. Its cell proliferation rate is similar to that of human skin. Mitotic activity occurs in the

basal keratinocytes of the epidermis and in the fibroblasts within the matrix. The keratinocytes produce growth factors and cytokines that act as signals between cells and help to regulate normal wound healing. Bilayered cell therapy exhibits a synergistic interaction between epidermal and dermal layers. It enhances cell and tissue differentiation through cell: Matrix, cell: Cell and cell: Environment interactions. BCT is safe and capable of generating keratinized tissue without the morbidity and potential difficulties associated with donor site surgery (McGuire and Todd Scheyer).

CONCLUSION

A number of studies have reported that progenitor cells, in conjunction with different physical matrices and growth factors, have the capacity to regenerate soft tissues in vivo.

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