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RESEARCH ARTICLE

TISSUE ENGINEERING IN PERIODONTICS: RECENT UPDATES AND SYSTEMATIC REVIEW

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ABSTRACT

Tissue engineering is an interdisciplinary field that applies principles and methods of engineering and the life sciences towards the development of biological substitutes that restore, maintain, and improve the function of damaged tissues and organs. 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 aim of this article is to bring awareness among the dentists about the huge potential associated with the use of stem cells in a clinical setting, as well as the growth factors promote true regeneration of the periodontal attachment apparatus and the use of combination protein therapeutics which is commercially available can provide more predictable, faster, less invasive, less traumatic, and efficient outcome for the patient.

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INTRODUCTION

Tissue engineering is a contemporary area of applied biomedical research aimed at developing procedures and biomaterials for the fabrication of new tissues to replace damaged tissues and is based on the principles of cell biology, developmental biology and aims to replace dead or dying ones. The main requirements for producing an engineered tissue are the appropriate levels and sequencing of regulatory signals, presence and numbers of responsive progenitor cells, an appropriate cellular matrix or carrier and an adequate blood supply. (Carini *et al*., 2007) Issue engineering was proposed as a possible technique for regenerating lost periodontal tissues by Langer and colleagues in 1993. (Nakahara, 2006) The purpose behind writing this brief review has been to integrate the evidence of research related to tissue engineering so as to implement them in our daily practice and results of clinical pilot studies using dental stem cells for bone repair-tissue engineering using dental stem cells-future challenges, future trends, and importance of dental surgeons role. Tissue engineering or regenerative medicine has been defined as 'an interdisciplinary field that applies the principles of engineering and life sciences toward the development of biological substitutes for the repair or regeneration of tissue or organ function". Regenerative medicine emphasizes a transition from organ replacement with bonier or biocompatible, durable materials to a focus on cell-based organ replacement. (Huang *et al*., 2009) Tissue engineering can be divided into two types: (Dimitrios Tziafas *et al*., 2010)

Ex-vivo tissue engineering

Extracorporeal or ex-vivo tissue engineering involves the expansion, differentiation, or modification of progenitor cells in culture, which then organize into functional tissues through cell-cell signaling,biomolecules production, and formation of extracellular matrix. This engineering requires the harvest of progenitor cells at various stages of differentiation for expansion and maturation on appropriate scaffolds in culture and subsequent implantation.

In-vivo or in-situ tissue engineering

In-vivo or in-situ tissue engineering depends on the in-vivo proliferation and differentiation of progenitor cells on scaffolds, or on the administration of growth factors that recruit progenitor cells, and enhance their expansion and differentiation. In-vivo tissue engineering using progenitor cells in which the patient acts as his or her own bioreactor obviates cellular patterning and microcirculation, as most progenitor cell-containing constructs self-assemble into histologically recognizable tissues. As with extracorporeal tissue engineering, techniques using autologous progenitor cell transplantation are limited by the need for progenitor cell harvest, and at times, ex-vivo expansion prior to implantation.

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This type of in-situ tissue engineering is designed to compensate for a deficiency in the numbers or function of progenitor cells, as may occur with previous irradiation, scarring, or compromised vascularity. When the in-vivo availability of progenitor cells is not a cause for concern, the use of growth factors or other external stimuli to recruit and stimulate proliferation and differentiation of progenitor cells *in vivo* is an attractive, but complicated, endeavour.

Preclinical and clinical accomplishments of tissue engineering

It can be categorized into four categories

Another, widely used and relatively simple example of a conductive approach to tissue engineering is guided tissue regeneration. It is used to regenerate the periodontal supporting structures and uses a material barrier to create a protected compartment for selective wound healing (Mahmoud Torabinejad and Hadi Faras, 2012). (Figure 1 and 2)

Inductive approaches

The inductive approach activates cells situated close to the damaged or deficient tissue with specific signals. Urist first demonstrated that new bone could be formed at a no mineralizing site after implantation of powdered bone.

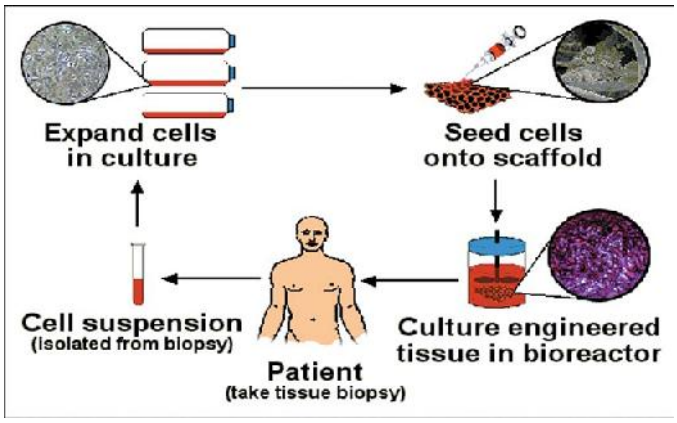

Figure 1. Illustration depicting the three approaches to the engineering of a tissue conductive, inductive and cell transplantation

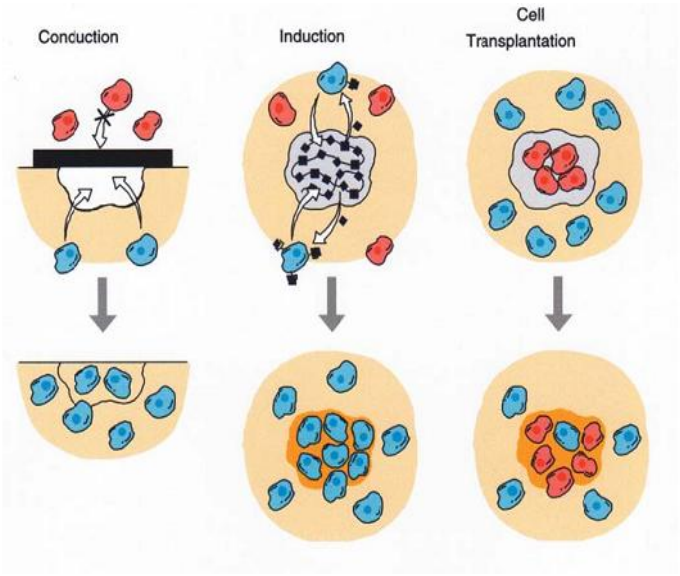

Figure 2. Illustration depicting the three approaches to the engineering of a tissue. (a) In conductive approaches, a scaffold can serve as a barrier controlling which cells can infiltrate the defect site and initiate repair. The barrier may be resorbed over time or removed surgically. (b) Inductive approaches can involve the release of bioactive molecules that bind only to specific host cells with receptors for the molecules. The desired cells migrate into the defect and begin to deposit new extracellular matrix. (c) Cells from a donor source are seeded directly onto a polymer scaffold in vitro, and the cell/scaffold construct is subsequently implanted into the defect site in the cell transplantation approach. The transplanted cells, along with host cells that migrate into the defect, repair the site with new tissue that is structurally and functionally integrated with the host tissue

Conductive (passive) approaches

Conductive approaches utilize biomaterials in a passive manner to facilitate the growth or regenerative capacity of existing tissue. A dental implant is an example of a conductive (or passive) approach to tissue engineering. Today, implants are considered a standard treatment option, in conjunction with prosthetic rehabilitation, for replacing multiple and single teeth.

This discovery led to the isolation of the active ingredients from the bone powder, the cloning of the genes encoding these proteins, and their now large scale production by a number of companies. These proteins, termed bone morphogenetic proteins or BMPS, have been used in many clinical trials, including in studies on no healing long bone fractures and periodontal tissue regeneration, and are presently in the earlyphase of FDA review. The identification of proteins that

promote new blood vessel formation, and their clinical application, followed closely the identification and use of the BMPS. Judah Folkman was the first to recognize that specific molecules regulate new blood vessel formation. Several such molecules are now known that either promote or inhibit this process. These have found several applications, including in the induction of new vessel formation to bypass blocked arteries. An alternative tissue-inductive approach involves placing specific extracellular matrix molecules on a scaffold support at the tissue site. These molecules will have the ability to direct the function of cells already present at that site and, therefore, to promote the formation of a desired tissue or structure. For example, a preparation of enamel proteins derived from pigs is used to promote new bone formation in periodontal defects. The Forsyth researchers induced the growth of small, recognizable tooth crowns within a period of 30 weeks from cells obtained from immature teeth of 6-month-old pigs seeded onto biodegradable polymer scaffolds and placed in a rat host. (Figure 1 and 2) For tissue induction to be successful clinically, it is critical to deliver the appropriate biologically active factors to the desired site at the appropriate dose and for the necessary time. Typically, many of these proteins have short half-lives in the body, yet they need to be present for an extended period to be effective. Up until now, clinicians and researchers have addressed these concerns by delivering extremely large doses of the protein at the sites of interest. More recently, the efforts have been to develop controlled-release systems. A somewhat similar approach involves the delivery of a gene that encodes for the inductive factor, instead of delivering the protein itself. An unresolved issue in tissue engineering is whether multiple protein signals, perhaps presented in a specific sequence, may be necessary to develop fully functional tissues. (Kavitarani B Rudag, 2012)

Cell transplantation

Cell transplantation is an extremely attractive option when the inductive for a specific tissue factors are not known, when a large tissue mass or organ is needed, or when tissue replacement must be immediate. The greatest success in this area has been the development of a tissue-engineered skin equivalent. Skin is the first FDA approved tissue engineered products for clinical use. A similar approach has also been developed for replacement of oral mucosa. (K.M. *et al*., 2005) The designing of polymer scaffolds with the appropriate mechanical and derivative properties has allowed investigators to engineer new cartilaginous tissues in animal models with precisely defined sizes and shapes (e.g., nasal septum and ear), which makes this method potentially useful for craniofacial reconstruction. Two approaches are currently being studied for the development of vasculature to support the metabolic needs of the organs and for the integration of the engineered organ with the host. The first involves transplantation of endothelial cells on the scaffold with the tissue cells of interest. Transplanted endothelial cells can increase the vasculature in polymer scaffolds and integrate with growing host capillaries. The second approach uses localized delivery of inductive angiogenic factors at the site of the engineered tissue. Experiments on mice show that tooth rudiments can be formed in vitro cultures of nondental stem cells, and complete teeth and associated bone can be obtained when these rudiments are transferred to adult mice[9]. (Figure 1 and 2) Gene therapy: However, gene transfers to well-differentiated cells arguably can be viewed as one way of engineering a tissue. In the clinical setting, gene transfer has been used in the treatment of two children suffering from a severe combined immunodeficiency resulting from an inherited reduced production of the enzyme adenosine deaminase (ADA). These patients were treated with a procedure termed ex vivo gene therapy. In this method, the ADA gene was transferred to their lymphocytes in the laboratory and these modified cells were then reinfused into the patients. Both patients are alive today.

However, it is not possible to conclude that their survival was the result of gene transfer because conventional therapy was also administered along with the genetically modified cells. Hundreds of clinical research protocols have been approved worldwide for gene transfer in a wide range of conditions, including cystic fibrosis, muscular dystrophy, and numerous malignancies. The principal problem is the lack of adequate gene transfer vectors to deliver foreign genes to host cells. Most often modified viruses are used, but all common viruses have their drawbacks. There is considerable research activity taking place in this field. New vectors, both nonviral and viral, are being developed and are likely to offer many advantages over current gene delivery systems. It is reasonable to expect that clinical gene transfer therapy will be routine, as both primary and adjunctive therapy, within the next 10-20 years. (Darnell Kaigler *et al*., 2006) A stem cell is essentially the building block of the human body. The stem cells inside an embryo will eventually give rise to every cell, organ and tissue in the foetus's body. Unlike a regular cell, which can only replicate to create more of its own kind of cell, a stem cell is pluripotent. When it divides, it can make any one of the 220 different cells in the human body. Use of the term *"Stem cell"* dates back at least to William Sedgwick, who used it to describe the regenerative properties of plants in 1886.All tissues originate from stem cells. A stem cell is commonly defined as a cell that has the ability to continuously divide and produce progeny cells that differentiate (develop) into various other types of cells or tissues. (Bramnemark *et al*., 1969) Stem cells are commonly defined as either embryonic/fetal or adult/postnatal. The reason why it is important to distinguish between embryonic and postnatal stem cells is because these cells have a different potential for developing into various specialized cells (i.e. plasticity). Researchers have traditionally found the plasticity of embryonic stem cells to be much greater than that of postnatal stem cells. The plasticity of the stem cell defines its ability to produce cells of different tissues. Stem cells are also commonly subdivided into totipotent or all competent, pluripotent, and multipotent categories according to their plasticity. (Bramnemark *et al*., 1969)

Classification of stem cells

Stem cells can typically be divided into four types:

- **Embryonic stem cells** - Stem cells taken from human embryos
- **Fetal stem cells**- Stem cells taken from aborted fetal tissue
- **Umbilical stem cells** - Stem cells take from umbilical cords
- **Adult stem cells** - Stem cells taken from adult tissue

Morphogens are extracellular secreted signals governing morphogenesis during epithelial-mesenchymal interactions. Growth factors are proteins that bind to receptors on the cell and induce cellular proliferation and/or differentiation. Many growth factors are quite versatile, stimulating cellular division

in numerous cell types, while others are more cell-specific. Currently, a variety of growth factors have been identified, with specific functions that can be used as part of stem cell and tissue engineering therapies. Many growth factors can be used to control stem cell activity, such as by increasing the rate of proliferation, inducing differentiation of the cells into another tissue type, or stimulating stem cells to synthesize and secrete mineralized matrix The regeneration of most tissues usually requires the expression of various growth factors, whose effects may be mitogenic, chemotactic, morphogenic, or apoptotic depending on the cell type to which the growth factor is exposed, the growth factor concentration, and the presence of other growth factors. The morphogenetic signaling networks include the five major classes (embryonic tooth development) Tumor necrotic factor (TNF) families: These families exhibit redundant and reiterative signaling, each with distinct temporal and spatial expression during initiation, patterning formation and morphogenesis, and cytodifferentiation. Although five distinct families of morphogens are involved in embryonic tooth development, BMPs appear to be sufficient for tooth regeneration in adults. BMP family members are sequentially and repeatedly involved in embryonic tooth development. (Francisco Banchs and Martin Trope, 2004) A wide variety of growth factors have been considered for dental and orthopedic applications. Polypeptides such as platelet-derived growth factor have been shown to play an important role in wound healing. Much attention has been directed toward investigations of biomaterials for the delivery of bone morphogenetic protein to treat defects in bone: tricalcium phosphate, collagen, and hydroxyapatite ceramic. It is important, however, to consider that the biomaterial used to localize the soluble regulator at the implant site, also needs the properties of a matrix material compatible with the functional needs of a scaffold into which the tissue will regenerate. The biochemical stimulation of tissue regeneration requires the development of delivery systems that are able to deliver growth factors at an appropriate rate, in a proper dose, for an adequate length of time, and that will also provide appropriate scaffolding for cellular ingrowth and proliferation. The interactions of biomolecules in the control of tissue growth and development have yet to be fully elucidated. (Cell cycle. wikipedia, free encyclopedia.www.google.com)

Scaffold materials

The primary function of the scaffold in tissue engineering is to provide a template to introduce the progenitor stem cells to the specific site of interest and to provide interim mechanical stability for tissue growth and integration. Additional requirements of the scaffold include the following:

- Provide a space to host cells – a three-dimensional (3D) structure is required
- Has the ability to transfer nutrients to the cells and remove their waste without adverse effects on the cells
- Should be biodegradable – at a rate comparable to extracellular matrix production
- Should be biocompatible
- Should be non-toxic
- Should be easy to manufacture

The role of the scaffold in tissue engineering is to provide a matrix of special configuration on which seeded cells may grow to produce the desired tissue or organ. Biomaterials used as scaffolds are broadly classified into two categories – naturally derived and synthetic. Advantages of naturally derived scaffolds include the ability to support cellular invasion and proliferation. Synthetic materials offers ease of processing and mechanical strength. Biomaterials can also be divided into ceramics and polymers. These biomaterials may be produced into solid blocks, porous sponges or foams or hydrogels. Over the last decade there has been significant interest in biocompatible biodegradable scaffold materials including synthetic biodegradable polymers. (Huang, 2009)

Clinical applications of tissue engineering concepts

Guided tissue regeneration

Nyman and Karring in the 1982 were the first ones to have proposed the use of guided tissue regeneration for periodontal regeneration, which marked the evolution of periodontal regeneration technologies using tissue engineering.

Protein based approaches (Murakami *et al*., 2003; Nevins *et al*., 2003)

The use of growth and differentiation factors evolved tissue engineering to its next level and has been the most popular tissue engineering approach for regeneration of periodontal tissues. Several growth factors have been used including Transforming growth factor β, Bone morphogenetic proteins (super family members); Basic fibroblast growth factor and Platelet derived growth factor.

Enamel matrix derivative

The rationale for the clinical use of enamel matrix derivative is the observation that enamel matrix proteins are deposited onto the surfaces of developing tooth roots before cementum formation. (Gestrelius *et al*., 1997) Enamel Matrix Protein (EMPs) are commercially available as Emdogain which have been known to effect periodontal regeneration. Recent data from a systematic review indicates that biologically EMPs cause an increase in cell attachment of epithelial cells, gingival fibroblasts, and PDL fibroblasts.

Platelet rich plasma

Since physiologic concentrations of growth factors may not be sufficient to stimulate local bone formation, the use of exogenous growth factors to supplement endogenous biological mediators has been explored. (Okuda *et al*., 2003)

Role of rhbmp-2 in periodontal regeneration

The identification and development of recombinant human bone morphogenetic protein-2 (rhBMP-2) has lead to the commercial availability for the first time of an osteoinductive autograft replacement (INFUSE® Bone Graft). rhBMP-2 is a homodimeric protein consisting of two BMP-2 protein subunits. Studies provide an important insight that space provision appears critical to draw clinically significant benefits from a BMP construct.

- rhBMP 2 has been combined with ACS atellocollagen sponge (ACS) (McKay *et al*., 2007)

- rhBMP2 has also been used in a DFDBA/fibrin clot carrier (Sigurdsson *et al*., 2001)
- rhBMP2 and calcium phosphate cement matrix (Seeherman *et al*., 2006)

Hanisch O Tatakis reported that rhBMP-2/ACS at 1.5 mg/cc, INFUSE® Bone Graft, induced significant bone formation suitable for implant placement. Additional clinical studies are needed to evaluate rhBMP2 in combination with other materials for further potential applications.

Cell based approaches

Cell transplantation using autologous cells is expected to play a central clinical role in the future. Dental cell seeding attempts have attempted to regenerate the periodontal tissues since 1990s. Attempts have been made to create the target tissue in the laboratory by culturing and proliferating mesenchymal cells together with scaffolds, before transplanting them into the body**.** Huang and Zhang have set forward a hypothesis of transplanting PDL cell obtained from the periodontium of autogenous extracted teeth, such as the third molar and premolar for orthodontic purposes sheets when cultured using the cell sheet engineering approach into the implant beds before inserting the implants. (Huang and Zhang, 2010)

Gene delivery based approaches

Numerous tissue regeneration studies have investigated various gene delivery techniques. These techniques involve a gene encoding a therapeutic protein being introduced into the cells which can then express the target protein. This technique avoids the problems associated with the protein delivery method by maintaining constant protein levels at the site of the defect. (Huang and Zhang, 2010)

Challenges ahead

1. Structural and functional complexity of the periodontium The fact that more than one tissue must be reconstructed, namely alveolar bone, periodontal ligament, root cementum, and gingiva, makes it much more difficult to find both the right combination and the doses of growth factors.
2. To overcome the rapid clearance of growth factors, a carrier system must be found that stores and releases the growth factors over a longer period of time so that their resident time is prolonged. Although many carrier systems have been tested, none of them appears to be ideal.
3. While high developmental and therapeutic costs appear justified for severe skeletal conditions such as non-unions, open fractures, spinal fusion, and large bone defects, for example in the mandible, the same cannot necessarily be said for relatively small and non-life-threatening periodontal defects where preventive and maintenance measures are still mandatory.

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