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Tissue engineering - the promise of regenerative dentistry

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Abstract

The science of tissue engineering aims at the repair of damaged tissues as well as creates replacement of the lost ones. This is becoming a major component of the regenerative medicine by combining the principles of transplantation, materials science and bioengineering to restore a diseased or a damaged tissue to normal function. The earliest attempts at tissue replacement thousands of years back involved teeth and even in modern times, dentistry has continued to place considerable emphasis on the study and use of biocompatible materials. For most of the general dental practitioners restoration of lost tooth tissue, whether from disease or trauma, represents a significant proportion of their daily routine. Considering the current prevalence of the dental diseases, it can be said that the challenge and resource burden of restoring lost tooth tissue will be with us for many years to come. Tissue engineering will have a considerable effect on dental practice during the next coming years. The greatest effects will likely be related to the repair and replacement of mineralized tissues, the promotion of oral wound healing, correction of craniofacial abnormalities, integration of biocompatible prosthetic implant materials with the oral tissues, the regeneration of dental hard and soft tissues and the use of gene transfer adjunctively. The purpose of this brief review is to provide the general dental practitioner a background of tissue engineering, its accomplishments in dentistry and its future promises to the profession in the form of regenerative dentistry.

Keywords: Bioengineering; regeneration; gene transfer.

Introduction

Tissue engineering is a multidisciplinary field which involves the 'application of the principles and methods of engineering and life sciences towards the fundamental understanding of structure-function relationships in normal and pathological mammalian tissues and the development of biological substitutes that restore, maintain or improve tissue function' (Shalak and Fox, 1988). This field builds on the interface between materials science and biocompatibility, and integrates cells, natural or synthetic scaffolds, and specific signals to create new tissues. Tissue engineering is viewed as synonymous to ''regenerative dentistry'' because the goal of tissue engineering is to restore tissue function through the delivery of stem cells, bioactive molecules, or synthetic tissue constructs engineered in the laboratory. Tissue engineering in dentistry takes several forms from gene transfer to osteoinduction, osteoconduction, regeneration of hard and soft tissues and integration of prosthetic implants with human bone. Majority of the dental and maxillofacial procedures range from simple tooth restorations to major reconstruction of facial soft and mineralized tissues and so far, materials and treatment options available have provided the dentist with a limited ability to replace diseased, infected, traumatized, and lost tissues. Continuous research is going on in the field of regenerative dentistry at both pre-clinical and clinical levels; with some remarkable and promising results, most of these efforts involve different forms of tissue engineering. Following are the various forms of tissue engineering related to regenerative dentistry:

Tissue Conductive Approaches

An excellent example of a conductive (or passive) approach to tissue engineering is the dental implant. This is a relatively simple application because the devices used do not include either living cells or diffusible biological signals. Although the idea of replacing lost teeth dates back to antiquity, it was not until the middle-to-late 20th century that reproducible and predictable clinical success in using dental implants was achieved. Today, the use of implants in dentistry is widespread and is considered a standard treatment option in conjunction with prosthetic rehabilitation for replacing multiple and single teeth. Another relatively simple example of a conductive approach to tissue engineering that is widely used in dentistry is guided-tissue regeneration. This is used most often to regenerate the periodontal supporting structures and uses a

material barrier to create a protected compartment for selective wound healing.

Tissue Inductive Approaches

In contrast to passive tissue formation achieved with conductive approaches, a tissue-inductive approach activates cells near the tissue with specific signals. The impetus for this approach was the discovery of defined molecules — termed growth factors — that could lead to new bone (osteogenesis) and blood vessel (angiogenesis) formation.

Urist (1965) first demonstrated that new bone could be formed at a non-mineralizing site after implantation of powdered bone. This led to the isolation of the active ingredients (specific growth-factor proteins) from the bone powder, the eventual cloning of the genes encoding these proteins, and their now large-scale production by a number of companies (Cochran and Wozney 1999). These proteins—termed bone morphogenetic proteins, or BMPs—have been used in many clinical trials, including studies of non-healing long-bone fractures and periodontal tissue regeneration and are in the early phase of FDA review.

An alternative tissue-inductive approach to using diffusible growth factors involves placing specific extracellular matrix molecules on a scaffold support at a tissue site. These molecules have the ability to direct the function of cells already present at that site and, therefore, to promote the formation of a desired tissue type or structure. For example, a preparation of enamel proteins derived from pigs is used to promote new bone formation in periodontal defects (Heijl et al, 1997) while the protein laminin is being tested for its ability to improve gingival adhesion to dental implants. 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 for the necessary time. Typically, many of these proteins have short half-lives in the body, yet they must 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 protein at the sites of interest. Newer efforts involve the development of controlled release systems (Sheridan et al 2000). A somewhat similar approach involves delivering a gene that encodes the inductive factor instead of delivering the protein itself.

Bone Graft Products Used for Bone Tissue Engineering

Osteoinductive	Osteoconductive	Osteogenic
Demineralized freeze-dried bone allograft (DFDBA) Partially pure proteins (BMP) BMP-2 BMP-4 BMP-7 BMP-9	Freeze-dried bone Autograft Ceramics Bioglasses Coral-derived Deproteinized bovine bone Polylactic acid (PLA) polyglycolic acid (PGA)	Mesenchymal stem cells (MSC) Marrow Platelet-rich plasma (PRP) PRP + white blood cells (WBC) Emdogain Gene therapy Fibroblast growth factor (FGF) Peptide TP508 Peptide P15 Platelet-derived growth factor (PDGF)

Cellular Therapies

Over the last decade, the regenerative capacity of postnatal progenitor cells has increasingly emerged making these cells an attractive candidate for use in tissue-engineering applications. Whether these cells represent true pluripotent cells or more committed multipotent or oligopotent progenitors remains to be defined, but their capacity to differentiate into a multitude of cell types has been demonstrated abundantly (Pittenger et al 1999). Speculation, however, continues as to how these cells may function in tissue repair. Arguments for and against direct participation in the generation of new tissue or

creation of conducive environments for endogenous host cell differentiation have been raised (Wagers et al 2004). Substantial work has already progressed with these postnatal progenitors, with early studies concentrating on mesenchymal stem cells (MSCs) naturally residing within bone marrow. Several investigators have demonstrated this cell population to contribute to the regeneration of other mesenchymal tissues throughout the body, including bone, cartilage, muscle, ligament, tendon, adipose, and stroma (Pittenger et al 1999, Prockop 1997, Haynesworth et al 1992). Furthermore, using bone marrow aspirates from over 350 human donors, Pittenger and colleagues (1999) were able to show lineage specific differentiation of these 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 craniofacial tissue engineering. The promise of mesenchymal cells for the repair of craniofacial skeletal defects remains attractive with a readily available and cost-effective cell source in MSCs.

Distraction Osteogenesis

Distraction osteogenesis is a powerful form of endogenous tissue engineering, promoting bone formation through the gradual separation of osteogenic fronts. Despite its recent application to craniofacial surgery, the fundamental principles of distraction osteogenesis have existed since the early twentieth century (Codivilla 1905). In 1956, Ilizarov demonstrated this modality could be consistently applied to long bone reconstruction with acceptable morbidity. The first translation to intramembranous bone of the craniofacial skeleton was established in 1972 using a canine model and McCarthy (1992) performed the first human mandibular distraction. Since that landmark description, this technique has now become a standard tool for craniofacial surgeons to achieve clinically significant midface and mandibular advancement. As elaborated by Ilizarov, distraction osteogenesis incorporates rigid fixation with a several day latency period, followed by gradual distraction and stable fixation until radiographic and clinical assessment demonstrates the formation of a robust, mineralized regenerate (Ilizarov 1989, 1990). Despite ever-increasing experience, however, significant complications nonetheless continue to plague surgeons performing this procedure; overall morbidity rates as high as 35% have been described (Mofid et al 2001). Most commonly, soft-tissue infection, osteomyelitis, and pin-tract infection or loosening secondary to daily manipulation of exposed devices have been reported. Patient discomfort and incompliance also contribute to overall morbidity.

In the face of such concerns, however, overall results remain acceptable, with surgeons reporting good or excellent results in over 86% of patients and as a form of endogenous tissue engineering, distraction osteogenesis has spread rapidly throughout the field of craniofacial reconstruction and is currently the treatment of choice for several midface and mandibular deformities.

Gene Therapy

There are hundreds of clinical research protocols which have been approved worldwide for gene transfer in a range of conditions, including cystic fibrosis, muscular dystrophy and numerous malignancies. Many of these studies have shown promise and have yielded partial efficacy, but widespread clinical applications are yet to be achieved. The principal shortcoming in the field 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 present drawbacks. However, there is considerable research activity in this field. New vectors, both nonviral and viral, are being developed and are likely to offer advantages over current gene delivery systems. It is reasonable to expect that clinical gene transfer will be routine, for both primary and adjunctive therapies, within the next 10 to 20 years.

Craniofacial examples of using gene therapy are:

a. Either primary or adjunctive therapies for head and neck cancers.
b. A potentially novel approach to the treatment of severe chronic pain.
c. Engineering salivary gland function.

Gene-transfer techniques are being used as either primary or adjunctive therapies for head and neck cancers. Already several early-stage clinical studies have been conducted. Most of the focus has been on

squamous-cell carcinoma, and some incremental progress has been achieved.

Gene therapy also may offer a potentially novel approach to the treatment of severe chronic pain. Many studies have shown that genes can be readily transferred to cells in the central nervous system of animal models. Finegold et al (1999) showed that viral mediated transfer of the β-endorphin gene leads to effective analgesia in a rat pain model.

The loss of salivary gland parenchyma and, thus, the inability to make saliva may not look like a life threatening condition but it markedly affects the quality of life of the patient (e.g. patients receiving radiotherapy for head and neck cancer, patients with Sjogren's Syndrome). These patients experience dysphagia, rampant caries, mucosal infections (e.g. candidiasis), dysgeusia and considerable oral discomfort. Gene transfer has been used to treat these patients by making the surviving ductal cells secretory in nature and, thus, capable of fluid movement. This was achieved by the transfer of a gene coding for—the water channel aquaporin-1—into the radiation-surviving cells via a recombinant adenovirus. The virus, AdhAQP1, was tested in an irradiated rat model. Three days after being given AdhAQP1, these rats experienced an increase in fluid production to near normal levels (Delporte et al 1997).

Experimental models have been developed to create a blind end tube that would be suitable for engrafting in the buccal mucosa. The lumen of these tubes would be lined with compatible epithelial cells and be physiologically capable of unidirectional water movement. This system should be ready for clinical testing in near future and can be a valuable help in patients whose salivary parenchyma has been destroyed (O'Connell et al 1996, Wang et al 1999).

Salivary glands may also be seen well suited for gene therapeutics (using transferred genes as drugs). An obvious application for this concept is to augment saliva with gene products for upper-gastrointestinal tract disorders. Salivary secretions bathe the upper-gastrointestinal tract mucosa continuously, and thus both prophylactic and therapeutic applications can be achieved. Using rodent models, it was shown that after performing adenoviral-mediated gene transfer, human anticandidal peptide histatin 3 in rat salivary glands was expressed and this recombinant histatin 3 could kill fluconazole-resistant *Candida albicans* (O'Connell et al 1996). Experimental models have also showed that after adenoviral-mediated gene transfer, both human alpha-1-antitrypsin and human growth hormone could be secreted into the bloodstream from rat salivary glands (Kagami et al 1996, He et al 1998).

Conclusion

In the coming future, advances in bioengineering research will lead to the wide application of the regenerative dentistry into general dental practice to produce wonderful treatments and dramatically improve patients' quality of life. Tissue engineering has become the new frontier in dentistry. A past frontier was the introduction of amalgam restorative materials in the 1830s. As an interdisciplinary endeavor, tissue engineering brings the power of modern biological, chemical and physical science to real clinical problems. The impact of tissue engineering likely will be most significant with mineralized tissues, already the focus of substantial research efforts. These efforts will yield numerous clinical dental benefits, including improved treatments for intraosseous periodontal defects, enhanced maxillary and mandibular grafting procedures, perhaps more biological methods to repair teeth after carious damage and possibly even regrowing lost teeth. Present controversy surrounding tissue engineering related regenerative dentistry is not a bad thing, because it increases scrutiny of its safety, and helps educate the public and profession on its effectiveness and potential disadvantages.

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