Review Paper

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TISSUE ENGINEERING AND DENTISTRY

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ABSTRACT

Tissue engineering is a novel and highly exciting field of research that aims to repair damaged tissues as well as create replacement (bioartificial) organs. Several examples of preclinical and clinical progress are presented. These include passive approaches, such as dental implants, and inductive approaches that activate cells with specific molecular signals. Tissue engineering will have a considerable effect on dental practice during the next 25 years. The greatest effects will likely be related to the repair and replacement of mineralized tissues, the promotion of oral wound healing and the use of gene transfer adjunctively.

KEYWORDS: Tissue engineering; dentistry; molecular

INTRODUCTION

Dental and medical treatment for loss of tissue or end-stage organ failure is required for millions of Americans each year. The field of tissue engineering has developed over the past decade to re-create functional, healthy tissues and organs in order to replace diseased, dying, or dead tissues. As they relate to the oral-maxillofacial apparatus, hard and soft tissue defects secondary to trauma (e.g, car accidents), congenital defects (e.g., cleft palate), and acquired diseases (e.g., cancer, periodontal disease) are a significant health problem.[1] The principal objectives of the current clinical approaches to tissue replacement and reconstruction were to alleviate pain and to restore mechanical stability and function. Current strategies used for treatment of lost tissues include the utilization of autogenous grafts, allografts, and synthetic materials (alloplasts). Although all of these treatment approaches have had successes and have been major advances in medicine, each of them has limitations. One of the major shortcomings with autografts, as well as allografts, is the fact that humans do not have significant stores of excess tissue for transplantation. Other restrictions, particularly related to replacing lost bone, include donor site morbidity, anatomic and structural problems, and elevated levels of resorption during healing.[2] Compounded with this, in the case of allografts, there always exists the possibility of eliciting an immunologic response due to genetic differences, as well as inducing transmissible diseases.[3] On the other end of the spectrum lies synthetic material replacements (e.g., dental implants). Common with all foreign implanted materials, as part of a natural defense mechanism, the body has a tendency to encapsulate foreign materials in a thin, fibrous membrane. As it relates to the dental implant, the fibrous capsule created by the immune response can potentially wall off the implant from its new environment and can prevent the implant from achieving true osseointegration,[4] ultimately leading to failure. Furthermore, if implants do achieve initial osseointegration, the changing needs of the body often will lead to failure over time. The advent of viable tissue engineering will have an effect on therapeutic options available to oral health specialists. This, in turn, will have implications for curriculum content at the predoctoral and postgraduate levels, as well as for continuing professional education programs for practicing dentists.

PRECLINICAL AND CLINICAL ACCOMPLISHMENTS

Conductive approaches

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. 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.[5]

Tissue Induction

The inductive approach uses activation of cells situated close to the damaged or deficient tissue with specific signals. New bone could be formed at a nonmineralizing site after implantation of powdered bone. 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.[6,7] These proteins, termed bone morphogenetic proteins or BMPS, have been used in many clinical trials, including in studies on nonhealing long bone fractures and periodontal tissue regeneration, and are presently in the early phase 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.[8] 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.[9] 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.[10] 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 halflives 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 controlledrelease systems.[11] 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.

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. For example, 250,000 ft^2 of skin tissue can be manufactured from a 1 in ^{2} sample of starting tissue.[12] A similar approach has also been developed for replacement of oral mucosa.[13,14] The designing of polymer scaffolds with the appropriate mechanical and degradative 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.[15,16] 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 typed of interest. Transplanted endothelial cells can increase the vasculature in polymer scaffolds and integrate with growing host capillaries.[17] The second approach uses localized delivery of inductive angiogenic factors at the site of the engineered tissue.[11] Experiments on mice show that tooth rudiments can be formed in in vitro cultures of nondental stem cells, and complete teeth and associated bone can be obtained when these rudiments are transferred to adult mice.[18]

Gene therapy

Generally, gene therapy is not considered to be an example of tissue engineering. However, gene transfers to welldifferentiated 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 resultin

from an inherited reduced production of the enzyme adenosine deaminase (ADA).[19] 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.[20,21] 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.

Safety Measures in Gene Therapy

There is unanimity among experts that gene therapy trials should only be carried out under certain safety rules. The nature and scope of these rules and their legislative basis are, however, matters of controversy. As far as the legal framework is concerned, one side argues that safety is adequately ensured through the network of existing regulations. The other side criticizes the current situation as a tangle of legal regulations and expresses grave doubts that this takes adequate account of the specific hazards of gene therapy techniques. An overview of the international regulatory mechanisms shows clearly that, despite widely varying legislative approaches, the emphasis in (legislative) efforts everywhere is on patient safety and biological safety.[22] For example:

- There are strict test criteria for pharmaceuticals (which also apply to gene therapy), and this is one way of limiting the risks associated with gene therapy. Thus, licensing of gene therapy projects is subject to demanding requirements.
- There are ethics commissions present in all the countries; these commissions serve to ensure the maximum possible safety for the patient. In all the important countries (except Italy), the opinion (at least 'consultative') of the ethics commissions has to be obtained before approval is granted for conduct of gene therapy trials in humans.
- Another important safeguard is the professional ethical regulations covering the clinical applications of gene therapy. In the overwhelming majority of regulatory systems these are concerned (inter alia) with:
- Adequate clinical pre-trials
- Risk-benefit reviews in the use of gene therapy techniques on humans
- Prior patient education and consent
- Consultation with an ethical commission
- In addition to the specific statutory regulations there are also the general statutes on civil and criminal liability, which apply on a subsidiary basis. Biological safety is ensured through various forms of legislation. All countries have a national (official) licensing authority. These are also the basis for establishing a common European licensing authority responsible for biological safety in member states. Besides the common features indicated above, there are also differences in the ethics commissions of various countries, for example, in terms of the statutory basis of the ethics commissions, the commission's responsibilities, and the binding nature of their votes.
- Under French law, there is a separate act (the 'Loi Huriet') covering the duties and responsibilities of the ethics commissions. In German law, the ethics commission's powers are covered by section 40 I of the Drugs Act and, in Austria, by sections 30 et seq. of the Genetic Engineering Act. In Italy, on the other hand, there is no special regulation covering the responsibilities of the ethics commissions.
- In the USA, the responsibilities of the local ethics commissions are limited to projects promoted by the National Institutes of Health. The licensing procedure in the UK operates at two levels: Besides local ethics commissions,

the central ethics commissions must also give its approval for every gene therapy project. With respect to the binding nature of their votes, some national ethics commissions have a purely advisory status, as for example in France. In other countries (e.g., USA, Austria, UK, and Denmark) the commission's vote is more important and can result in refusal of approval.

A look into the Future of Dentistry

Engineered tissues will find many applications in dentistry within the next few years. However, reconstruction of complex tissue defects, which would require multiple cell types, has not yet been attempted in the craniofacial complex, even in preclinical trials. Such a goal will likely take about 10-15 years to realize

Mineralized tissue defects

Considerable research activity is focused on applying the principles of tissue engineering to dental and craniofacial structures, probably because of the ease of access to these sites and the extent and nature of the clinical problems. As a departure from the reliance of current clinical practice on durable materials such as amalgam, composite, and metallic alloys, biological therapies utilize mesenchymal stem cells, delivered or internally recruited, to generate craniofacial structures in temporary scaffolding biomaterials. Craniofacial tissue engineering is likely to be realized in the foreseeable future and represents an opportunity that dentistry cannot afford to miss.[23] BMPS and other growth factor–rich preparations are being applied with a variety of natural and synthetic scaffolds. There may be an advantage to be gained from using polymers that are allowed to flow into a defined site, rather than those that are fixed or implanted.[24,25]

Gene Therapy

There are several examples of the use of gene therapy in the craniofacial area, e.g., in head and neck cancers.[26-28] In the next decade, clinicians will likely be able to use gene transfer technologies as part of the standard treatment of neoplasms.

Engineering Salivary Gland Function

There are many circumstances involving tissue loss that are non-life threatening yet that markedly affect quality of life, e.g., the loss of salivary gland parenchyma and the consequent inability to make saliva; without saliva, these patients experience dysphasia, rampant caries, mucosal infections, etc. For such patients, a program has been developed to create a 'blind end' tube that would be suitable for engrafting in the buccal mucosa.[29] 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 within 10 years. The major salivary glands are inviting targets for gene transfer, mainly because of the ease of access to the parenchymal cells. Gene transfer has been used to treat patients undergoing ionizing radiation and those with Sjogren syndrome who had some remaining nonsecretory, ductal epithelial cells. The initial aim was rather simplistic: To make the surviving ductal cells secretory in nature and, thus, capable of fluid movement. The major impediment to fluid flow from nonsecreting ductal cells was the absence of a pathway for water in their luminal membranes. So, the strategy was to transfer the 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.[30]

ETHICAL CONCERNS

There is a significant amount of debate among researchers in the biochemical community about at least two major ethical concerns related to tissue-engineered products. The first, tissue procurement, is also a manufacturing concern. For many tissue-engineered products (such as skin equivalents and bioartifical organs), visible cells are an essential component. If the patient's own cells cannot be amplified in an adequate and timely manner, enabling them to be used in the tissue-engineered device (i.e., a cell autograft), then cells must be derived from another tissue. This situation raises a number of significant ethical issues; for example, should the tissue source be another person or can animal tissue (i.e., a xenograft) be used? If the source is to be another person (i.e., a cell allograft), should the donor be paid for the tissue sample (such as skin or liver)? Such a policy may induce people in financial distress to 'donate' their tissues. Since fetal tissues often have more growth potential

than adult tissues, should fetal tissues be used as a cell source? If, as with organs for transplantation, there are not enough cellular sources to meet the demand for any particular tissue-engineered device, how does one decide who will get the products-on the basis of need or ability to pay? For several cell-based tissue engineering products, the use of animal cells has been explored. Perhaps the most significant effort has been in the development of an artificial pancreas through the use of porcine cells. Recently, researchers have called for a moratorium on research using cellular xengrafts, in large part, because of a hypothetical risk[31-33] that an animal (in this case, porcine) virus might cross the species barrier and perhaps mutate and result in serious human disease. Although this possibility, with respect to a porcine virus, is hypothetical and there is no evidence that such an event could occur, there is recognition in the research community that the AIDS virus apparently had its origin in primates and 'jumped species' though the human consumption of infected animals. The proposed moratorium on xenograft research recognizes such potential societal implications and would allow public and legislative discussion of xenograft use. Not surprisingly, there is no agreement on this issue, although the dialogue has generally focused attention on the ethical consideration in tissue engineering.[34]

CONCLUSION

Dental practice has always been affected by new technologies, be it the development of high-speed handpieces, the modern restorative materials, or tissue engineering. Tissue engineering brings the power of modern biological, chemical, and physical science to solve real clinical problems. This should yield numerous clinical benefits in dentistry, e.g., improved treatment for intraosseus periodontal defects; enhanced maxillary and mandibular grafting procedures, possibly even allowing lost teeth to be regrown; use of devices such as an artificial salivary gland and muscle (tongue) or mucosal grafts to replace tissues lost through surgery or trauma.

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