Journal of Developmental Biology and Tissue Engineering Vol. 3(7) pp. 80-84, July 2011
Available online http://www.academicjournals.org/jdbte
ISSN 2141-2251 ©2011 Academic Journals

Review

Challenges of bioengineering and endodontics

Anil Dhingra, Viresh Chopra and Shalya Raj*

Department of Conservative Dentistry and Endodontics, Subharti Dental College, Meerut (U.P.), Meerut, Uttar Pradesh India.

Accepted 15, June 2011

Stem cell research and scaffolding are now the buzz words in basic science pulp researches. The endodontic community needs to enhance its clinical understanding of the vital pulp and dentin, and embrace new treatment modalities. Several regenerative techniques have been described, while therapies involving stem cells, growth factors and scaffolds have been proposed. Each technique has its own advantages and disadvantages, although some techniques are still in a hypothetical stage or in an early stage of development. It has been accepted that the regenerative therapies could revolutionize the future endodontics with the synergitic confluence of advances in signaling pathways underlying morphogenesis and lineage stem/ progenitor cells by morphogens such as bone morphogenic proteins and synthetic scaffolds.

Key words: Stem cells, scaffolds, gene therapy, endodontics.

INTRODUCTION

Endodontology is the science that deals with endodontium. An endodontist is supposed to be an expert on diseases of the endodontium, and should be able to treat the diseases thereof. Presently, the most common therapy for the exposed and/or diseased pulp is total amputation. This crude therapy is unfortunate because today there are restorative materials that rarely require post retention for large restorations, that is, the root canal could still harbor a vital pulp if treated properly (Murray et al., 2007).

In endodontics, regenerative endodontic procedure refers to the regeneration of dentin pulp complex and even the whole tooth structure. Murray et al. (2007) defined it as a "biologically based procedure designed to replace damaged structures including dentin and root structures, as well as cells of the pulp dentin complex". Objectives of the regenerative endodontics can be outlined as regeneration of dentin or pulp-like tissue; regeneration of the damaged coronal dentin and regeneration of the reabsorbed root, cervical or apical dentin. To successfully achieve these, certain hurdles were outlined by Baum and Mooney in 2000. They threw light on some other important issues like, till now, all the researches have been done on animals like swine, mice, dogs and rabbits. Hence, the dangers of transplant rejection by the human cells are still hovering on success. Ethical concerns are another debatable topic among the researchers. Inspite of the obstacles faced, tissue engineering offers exciting opportunities for innovative collaborative research efforts, integrating the fields of medicine, developmental biology and physical sciences (Baum and O'Colonnell, 1995).

*Corresponding author. E-mail: shalyabhatnagar@gmail.com Tel: 09536635492.

STEM CELLS

Duailibi et al. (2006) define stem cells as "Quiescent cell populations present in low numbers in normal tissue, which exhibit the distinct characteristic of asymmetric cell division, resulting in the formation of two distinct daughter cells - a new progenitor/ stem cell and another daughter cell capable of forming a differentiated tissue".

The uniqueness of the tooth and its dentin pulp complex has a natural regenerative potential leading to the formation of tertiary dentin. Odontoblasts may survive mild injury, such as attrition or early caries, and secrete a reactionary dentin matrix. In cases of trauma with greater intensity, the pre existing odontoblasts may die leading to the recruitment and differentiation of new odontoblasts

and synthesis of an atubular dentin (Duailibi et al., 2006).

Dental stem niches and other stem cell sources for the development of teeth *in vitro* or *ex vivo* have been discovered. As tooth formation results from epithelial-mesenchymal interactions, two different populations of stem cells have to be considered: epithelial stem cells, which will give rise to ameloblast, and mesenchymal stem cells that will form the odontoblasts, cementoblasts, osteoblasts and fibroblasts of the periodontal ligament. Thus, tooth engineering using stem cells is based on their isolation, association and culture (Murray et al., 2007).

ISOLATION OF VARIOUS DENTAL STEM CELLS

Mesenchymal stem cells (MSC)

MSC can be isolated from different sources. It was first described in the bone marrow to have been extensively characterized *in vitro* by the expression of markers such as: STRO-1, CD146 or CD44.

Stem cells from human exfoliated deciduous teeth (SHED)

The isolation of post-natal stem cells from an easily accessible source is indispensable for tissue engineering and clinical applications. Recent findings demonstrated the isolation of mesenchymal progenitors from the pulp of human deciduous incisors. These cells were named SHED (Miura et al., 2003).

Adult dental pulp stem cells (DPSC)

After a dental injury, dental pulp is involved in a process called reparative dentinogenesis, where cells elaborate and deposit a new dentin matrix for the repair of the injured site. It has been shown that adult dental pulp contains precursors capable of forming odontoblasts under appropriate signals; among these signals are the calcium hydroxide or calcium phosphate materials, which constitute pulp-capping materials used by dentists for common dental treatments (Gronthoss et al., 2000).

Stem cells from the apical part of the papilla (SCAP)

Stem cells from the apical part of the human dental papilla (SCAP) have been isolated and their potential to differentiate odontoblasts was compared to that of the periodontal ligament stem cells (PDLSC). SCAP exhibit a higher proliferative rate and appears more effective than PDLSC for tooth formation. Importantly, SCAP are easily accessible since they can be isolated from the third molars of humans (Hargreaves et al., 2008).

Stem cells from the dental follicle (DFSC)

DFSC have been isolated from the third molars' follicle of humans and used to express the stem cell markers: Notch1, STRO-1 and nesting. These cells can be maintained in culture for at least 15 passages. STRO-1 positive DFSC that can be differentiated in cementoblasts *in vitro* have the ability to form cementum *in vivo.* However, immortalized dental follicle cells are able to re-create a new periodontal ligament (PDL) after *in vivo* implantation (Huang et al., 2008).

Periodontal ligament stem cells (PDLSC)

The PDL is a specialized tissue located between the cementum and the alveolar bone and plays the role of maintaining and supporting the teeth. Its continuous regeneration is thought to involve mesenchymal progenitors arising from the dental follicle. PDL contains STRO-1 positive cells that maintain certain plasticity since they can adopt adipogenic, osteogenic and chondrogenic phenotypes *in vitro* (Srisuwant et al., 2006).

Bone marrow derived mesenchymal stem cells (BMSC)

BMSC have been tested for their ability to recreate periodontal tissue. These cells are able to form *in vivo* cementum, PDL and alveolar bone after implantation into the defective periodontal tissues. Thus, bone marrow provides an alternative source of MSC for the treatment of periodontal diseases such as recession, vertical bone loss, etc (Abe, 2008).

In search of epithelium-originated dental stem cells

Although significant progress has been made with MSC, there is no information available for dental EpSC in humans. The major problem is that dental epithelial cells, such as ameloblast and ameloblast precursors are eliminated soon after tooth eruption. Therefore, epithelial cells that could be stimulated *in vivo* to form enamel are not present in human adult teeth (Gonclaves et al., 2007).

Epithelial stem cells from developing molars

Several studies describe the use of EpSC isolated from newborn or juvenile animals, usually from third molar teeth. In these studies, epithelia were removed and the cells were dissociated enzymatically. Precursors were then amplified and associated with MSC (originated from the same tooth) *in vitro* in contact with biomaterials such as collagen sponges or synthetic polymers (Gonclaves

et al., 2007).

Epithelial stem cells from the labial cervical loop of rodent incisor

The rodent incisor is a unique model for studying dental EpSC since, in contrast to human incisors or other vertebrates, this tooth grows throughout their life time. An EpSC niche, which is located in the apical part of the rodent incisor epithelium (cervical loop area), is responsible for a continuous enamel matrix production; thus, in this highly proliferative area, undifferentiated epithelial cells migrate toward the anterior part of the incisor and give rise to ameloblast (Huang et al., 2008).

GROWTH FACTORS

Growth factors have been described by Murray et al. (2007) as proteins that bind to receptors on the cell and induce cellular proliferation and/or differentiation. Many growth factors are quite versatile, simulating cellular division in numerous cell types, while others are more cell specific (Iohara, 2002; Murray, 2007). However, what regulates the abrupt transition of stem/progenitor cells from quiescent to active state in terms of proliferation, migration, differentiation and matrix secretion is still unclear.

Platelet derived growth factor (PDGF)

It consists of 2 disulphide bonded poly-peptide chains that are encoded by 2 different genes: P.D.G.F- A and P.D.G.F.-B (Iohara et al., 2006).

The primary effect of PDGF is that of a mitogen-initiating cell division. Thus, it has been characterized as a competence factor, that is, a growth factor that makes a cell competent for cell division. A progression factor, such as I.G.F.-1, is then necessary to induce mitosis. PDGF also causes replication of endothelial cells, causing budding of new capillaries (angiogenesis) (Iohara, 2006).

Insulin-like growth factors (IGF-I, II)

They are peptide growth factors with biochemical and functional similarities to insulin. Bone cells produce and respond to IGF's, and bone is a storage house for these factors in their inactive form. They are mitogens, but in fibroblastic systems, they appear as progression factors. In bone cell systems, they stimulate both proliferation of pre-osteoblasts, as well as the differentiation of osteoblasts, including Type I collagen synthesis. Thus, IGF increases both the number of cells synthesizing bone, as well as the amount of extra-cellular matrix deposited by each cell.

Combinations of PDGF and IGF have been tested in periodontal systems. The combination could potentiate the growth of multiple tissue types by combining a competence factor with a progression factor (Murray et al., 2007).

Transforming growth factor-β- (TGF- β)

It is a multifactorial growth factor, structurally related to bone morphogenic proteins, but functionally quite different. It has been shown to be chemotactic for bone cells, and may increase or decrease their proliferation depending on the differentiation state of the cells, culture conditions and concentration of TGF-β applied. If injected in a close proximity to the bone *in vivo*, it produces new cartilage and / or bone; however, it does not induce new bone formation when implanted away from a bony site. In spite of its effects on the augmentation of bone, no positive data have been reported on *in vivo* healing in a periodontal setting (Trantor et al., 2005).

Fibroblast growth factors (FGF)

They are family of at least 9 related gene products of which 2 major members are acidic FGF (a-FGF or FGF-1) and basic FGF (b-FGF or FGF-2). It can stimulate endothelial cells and periodontal ligament cell migration and proliferation, as well as bone cell replication (Nakashima et al., 2006).

Bone morphogenetic proteins (BMPs)

Bone morphogenic proteins are secreted by the oral epithelium layer (oral ectoderm) during early stages of odontogenesis (Dental lamina) (Nakashima et al., 2006).

SCAFFOLDS

The importance of scaffold material and design for tissue engineering has long been recognized. Several studies have proved the combinations of stem cells and scaffolds in the successful regeneration of periodontium, bone, pulp dentin complex and even the whole teeth in the field of dentistry and several organs and tissue outside our field, for the better health care of patients (Abe, 2008).

Co-polymers of polylactic acid and polyglycolic acid

The apparent disadvantage of these poly (α-hydroxy) acids is their degradation by hydrolysis resulting in decomposition products that are mostly metabolised to carbon-dioxide and water through the Kreb's cycle (Kim and Mooney, 1998).

Synthetic calcium phosphate ceramics

These were implemented as matrix materials for facilitating regeneration *in vivo*. The two most widely used forms of these bioceramics are:

1) Tricalcium Phosphate (TCP): This is a porous form of calcium phosphate, the most commonly used form being ß-TCP. The potential problem with the use of this material was that it regularly underwent physio-chemical dissolution too often after implantation.
2) Synthetic Hydroxyapatite: The problems associated with TCP led to the development of this second form of bioceramic. The rationale for developing this material relied, in part, on the fact that because the mineral naturally occurring in bone was hydroxyapatite, synthetic implants of the same material would be biocompatible with osseous tissue (Kim and Mooney, 1998).

Deorganified bone or anorganic bone

This is the hydroxyapatite skeleton that retains the microporous and macroporous structure of cortical and chancellors bone, remaining after chemical or low heat extraction of the organic component. Usually, bovine bone mineral is used for this purpose. Studies have demonstrated that natural bone mineral particles implanted in defects show a greater degree of incorporation into host osseous tissue and have a composite modulus of elasticity closer to that of natural bone (Murray et al 2007).

Collagen

Collagen is defined as a protein with 3 polypeptide chains, known as α-chains, each containing at least one stretch of the repeated amino acid sequence 'Gly-Xaa-Yaa', where 'Xaa' and 'Yaa' can be any amino acid, but often proline and hydroxyproline.

Collagen constitutes almost one-third of all protein in the body, and accounts for almost 60% of gingival connective tissue and 90% of the total protein in the bone (Murray et al 2007).

Chitosan (Poly-N-Acetyl Glycosaminoglycans)

Chitosan is a carbohydrate biopolymer obtained from chitin (extracted from anthropods). Chitin is second only to cellulose as the most abundant natural biopolymer. Chitosan is made by treating chitin with hot strong alkali which results in deacetylation of chitin (Kim and Mooney, 1998).

GENE THERAPY

The goal of gene-enhanced tissue engineering is to regenerate lost tissue by the local delivery of cells that have been genetically-enhanced to deliver physiologic levels of specific growth factors. The basis for this approach lies in the presence of a population of progenitor cells that can be induced, under the influence of these growth factors, to differentiate the specific cells required for tissue regeneration, with guidance from local clues in the wounded environment.

GENE DELIVERY

The application of gene therapy to treat exposed pulp by delivering DNA, RNA or antisense sequences alters gene expression within a target cell population in pulp tissue. The gene therapy manipulates cellular processes and responses. The transfected genes stimulate immune response, modify cellular information or developmental program, or produce a therapeutic protein with specific functions (Nakashima et al., 2006).

Vectors for gene transfer

Gene transfer should achieve a stable expression of transgene in a target cell in an appropriate form without side effects, such as interaction with host genome, toxicity, carcinogenic transformation and insertional mutagenesis (Nakashima et al., 2006).

Viral and non-viral gene therapy

Both viral vectors and non-viral vectors have been employed for gene transfer. Viral vectors are derived from viruses with either RNA or DNA genomes, such as Retrovirus, Lentivirus, Adenovirus, Adeno-associated virus and Herpes simplex virus.

Non-viral methods represent a simple and safer alternative to viral vectors. Simple quantitative production, low host immunogenicity and further recent advances in sustained gene expression and efficient and long-term gene expression are now making non-viral gene therapy more of a reality for human clinical medicine (Nakashima et al., 2006).

However, recent advances include:

1. Intravenous Infection at high hydrodynamic pressure: Plasmid DNA can be delivered to tissues *in vivo* by intravenous infection at high hydrodynamic pressure. It is possible in a practical sense to use a blood-pressure cuff in the limbs to achieve high pressures to deliver plasmid DNA. The delivery of DNA by coated metal microparticles by particle bombardment into cutaneous tissues has been useful. However, attendant issues include heat generation and transfer at the site of penetration of the microparticles (Nakashima et al., 2006).
2. Electroporation: Application of regulated electric pulses

in delivering genes to cells is electroporation. Electroporation is routinely used to deliver DNA to bacteria, yeast and mammalian cells in culture. Electroporation uses electric fields to create transient pores to facilitate entry of plasmid DNA. Electroporation *in vivo* was successfully used in muscle, skin, brain and liver. One of the limitations of electroporation is the tissue damage. Although electroporation is an efficient technique, it is an invasive method (Nakashima et al., 2006).

3. Ultrasound: The application of ultrasound leads to acoustic cavitation and produces cell membrane permeabilization, thereby promoting the delivery of plasmid DNA. Ultrasound contrast agents can improve cavitation. However, microbubbles and optison which are coated by albumin and contain octafluoro-propane gas were found to be superior for cavitation using ultrasound. The use of a combination of ultrasound and electro-poration was found to be better than either of these methods alone. The recent advances in the uses of ultrasound to drug and gene delivery has multiple therapeutic applications including regenerative medicine (Nakashima et al., 2006).

CONCLUSION

Overall, the future application of regenerative and tissue-engineering techniques to dentistry is one of the immense potentials capable of meeting a variety of patient needs. High-quality basic dental research is paramount to ensuring that the development of novel clinical treatments is supported by robust mechanistic data and that such approaches are effective. These efforts reveal how successful innovations in the field of dentistry can be guided by advances in basic research, highlighting the need for close partnerships between basic research and clinical scientists. This hypothesis might be a challenge to modern bioengineering and endodontics.

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