Tissue Engineering - A New Era in Endodontics : A Review

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Abstract

Pulpal injury after tooth injury is not easy to accomplish, because of the infected pulp requires tooth extraction or root canal therapy. Recently, there has been increasing interest in applying the concept of tissue regeneration to endodontics. Tissue Engineering is the science of designing and manufacturing of new tissue to replace damaged ones.

In the recent years, stem cell research has grown exponentially owning to the recognition that stem cell- based therapies have the potential to repair and restore the normal functional tissue. It is known that dental tissues are rich source of mesenchymal stem cells that are suitable for tissue engineering. The aim of the study was to review the potential use of different types of stem cells, most common growth factors and scaffolds used to control their differentiation.

Key words: Dental Stem cells, tissue engineering, regenerative endodontics

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Introduction

Although current root canal treatment modalities offer high levels of success for many conditions, an ideal form of therapy might consist of regenerative approach in which diseased or necrotic pulp tissue are removed and replaced with health pulp tissue to revitalize the teeth.

The American Association of Endodontists (AAE) states the following: "Regenerative endodontics is one of the most exciting developments in dentistry today and endodontists are at the forefront of this cutting-edge research. Regenerative endodontics uses the concept of tissue engineering to restore the root canals to a healthy state, allowing for continued development of the root and surrounding tissue. Endodontist' knowledge in the fields of pulp biology, dental trauma and tissue engineering can be applied to deliver biologically based regenerative endodontic treatment of necrotic immature permanent teeth resulting in continued root development, increased thickness in the dentinal walls and apical closure. These developments in regeneration of a functional pulp-dentin complex have a promising impact on efforts to retain the natural dentition, the ultimate goal of endodontic treatment."

Key Elements of Tissue Engineering

Stem cells
Growth Factors
Scaffolds

Stem Cells

Considered to be most valuable cells that can continuously produce unaltered daughters and furthermore, has the ability to generate cells with different and more restricted properties. During recent years, stem cells have been extensively used in many medical disciplines for the repair or regeneration of defective tissue and organs.

Embryonic and adult stem cells have been under intense investigation that focuses on the in vitro development of new organs such as hair, skin, and bone. Adult stem cells (ASC), which possess a potential of differentiation, can easily be isolated from the patient and after in vitro amplification or differentiation could be re-injected to same patient thus avoiding immune rejection. However, the knowledge in stem cell technology is increasing quickly in all the medical disciplines, and needs the new strategic approach in all fields, including reparative dentistry.[1]

Types of Stem Cells

- **Early embryonic stem cells**
- **Blastocyst embryonic stem cells**
- **Fetal Stem Cells**
- **Umbilical cord stem cells**
- **Adult stem cells**

Early Embryonic Stem Cells

The first step in human development occurs when the newly fertilized egg or zygote begins to divide, producing a group of stem cells called an embryo. These early stem cells are totipotent, i.e. possess the ability to become any kind of cell in the body.

Blastocyst Embryonic Stem Cells

Five days after fertilization, the embryo forms a hollow ball-like structure known as a blastocyst. Embryos at the blastocyst stage contain two types of cells: an outer layer of trophoblasts that eventually form the placenta, and an inner cluster of cells known as the inner cell mass that becomes the embryo and then develops into a mature organism. The embryonic stem cells in the blastocyst are pluripotent, i.e. having the ability to become almost any kind of cell in the body. However, the sourcing of embryonic stem cells is controversial and associated with ethical and legal issues, thus reducing their appeal for the development of new therapies.[2,3]

Fetal Stem Cells

After 8 weeks of development, the embryo is referred to as a fetus. Stem cells in the fetus are responsible for the initial development of all tissues before birth. Like embryonic stem cells, fetal stem cells are pluripotent.

Umbilical Cord Stem Cells

The lifeline of the fetus is the umbilical cord that transports nutrients and oxygen-rich blood from the placenta to the fetus. Blood from the umbilical cord contains stem cells that are genetically identical to the newborn baby. Umbilical cord stem cells are multipotent, i.e. they can differentiate into a limited range of cell types. Umbilical cord stem cells can be stored cryogenically after birth for use in future medical therapy.

Adult Stem Cells

This name is rather misleading, because infants and children also have stem cells. Thus, the term Postnatal Stem Cells is preferable. These stem cells reside in tissues that have already developed, directing their growth and maintenance throughout life. These cells are also multipotent.[3,4]

Postnatal stem cells have been found in almost all body tissues, including dental tissues. To date, following types of human dental stem cells have been isolated and characterized.

- **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 clinicalapplications.Miura et al., 2003 demonstrated the isolation of mesenchymal progenitors from the pulp of human deciduous incisors. These cells were named SHED (Stem cells from Human Exfoliated Deciduous teeth) and exhibited a high plasticity since they could differentiate into neurons, adipocytes, osteoblasts and odontoblasts. In vivo SHED cells can induce bone or dentin formation but, in contrast to dental pulp, DPSC failed to produce a dentin-pulp complex.[5]
- **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 (Mitsiadis and Rahiotis, 2004).[6] It has been shown that adult dental pulp contains precursors capable of forming odontoblasts under appropriate signals (About et al., 2000).[7] Dental pulp progenitors have not been clearly identified but some data suggest that pericytes, which are able to differentiate into osteoblasts, could also differentiate into odontoblasts. Tooth repair is a lifetime process thus suggesting that MSC might exist in adult dental pulp. The in vivo therapeutic targeting of these adult stem cells remains to be explored[7].
- **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 into

odontoblasts was compared to that of the periodontal ligament stem cells (PDLSC) (Sonoyama et al., 2006). 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 human third molars.[8,9]

- **Stem Cells From The Dental Follicle (DFSC).** DFSC have been isolated from follicle of human third molars and express the stem cell markers Notch1, STRO-1 and nestin (Morsczeck et al., 2005). These cells can be maintained in culture for at least 15 passages. STRO-1 positive DFSC can differentiate into cementoblasts in vitro (Kemounet al., 2007) and are able to form cementum in vivo. Immortalized dental follicle cells are able to re-create a new periodontal ligament (PDL) after in vivo implantation.[10,11]
- **Periodontal Ligament Stem Cells (PDLSC).** The PDL is a specialized tissue located between the cementum and the alveolar bone and has as a role the maintenance and support of 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 (Gay et al., 2007). It is thus obvious that PDL itself contains progenitors, which can be activated to self-renew and regenerate other tissues such as cementum and alveolar bone (Seo et al., 2004).[12]
- **Bone Marrow Derived Mesenchymal Stem Cells (BMSC).** BMSC have been tested for their ability to recreate periodontal tissue. These cells can form in vivo cementum, PDL and alveolar bone after implantation into defective periodontal tissues. Thus, bone marrow provides an alternative source of MSC for the treatment of periodontal diseases (Kawaguchi et al., 2004).[13] BMSC share numerous characteristics with DPSC and are both able to form bone-like or tooth-like structures. However, BMSC display a lower odontogenic potential than DPSC (Yu et al., 2007). Commitment could arise from conditioning of stem cells by their specific microenvironment or stem cell niche. As these cell populations display distinctive biological properties depending upon their tissue of origin, it remains to be explored which source might be used for an optimal tooth development for clinical application.
- **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 cells 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 (Honda et al., 2005, 2007).These approaches are promising for tooth formation and/or regeneration. However, the clinical application is difficult, if not unrealistic, since it would require the donation of a tooth germ from children. The use of autologous stem cells is desirable but raises the question of a good and reliable source.[15,16]

Culturing of Stem Cells Cell

Culture is a term that refers to the growth and maintenance of cells in a controlled environment outside an organism. A successful stem cell culture is one that keeps the cells healthy, dividing, and unspecialized.[17]

Dental pulp stem cells can be cultured by two methods:

1. The enzyme-digestion method in which the pulp tissue is collected under sterile conditions, digested with appropriate enzymes, and then the resulting cell suspensions are seeded in culture dishes containing a special medium supplemented with necessary additives and incubated. Finally, the resulting colonies are sub cultured before confluence and the cells are stimulated to differentiate.
2. The explant outgrowth method in which the extruded pulp tissues are cut into 2-mm3 cubes, anchored via microcarriers onto a suitable substrate, and directly incubated in culture dishes containing the essential medium with supplements. Two weeks time is needed to allow a sufficient number of cells to migrate out of the tissues.

Haung et al. compared both methods and found that cells isolated by enzyme-digestion had a higher proliferation rate than those isolated by outgrowth.[18]

Growth Factors

Growth factors are extracellularly secreted signals governing morphogenesis/organo-genesis during epithelialmesenchymal interactions. They regulate the division or specialization of stem cells to the desirable cell type, and mediate key cellular events in tissue regeneration including cell proliferation, chemotaxis, differentiation, and matrix synthesis. Many growth factors are quite versatile, stimulating cellular division in numerous cell types, while others are more cell-specific.

Some Growth Factors are Used To Increase Stem Cell Numbers Are:

- Platelet-Derived Growth Factor (PDGF),
- Fibroblast Growth Factor (FGF),
- Insulin-like Growth Factor (IGF),
- Colony-Stimulating Factor (CSF)
- Epidermal Growth Factor (EGF).
- Bone Morphogenetic Proteins (BMPs)

Bone morphogenetic proteins are multi-functional growth factors belonging to the transforming growth factor β superfamily. To date, about 20 BMP family members have been identified and characterized. They have different profiles of expression, different affinities for receptors and therefore unique biological activities in vivo. During the formation of teeth, BMPs dictate when initiation, morphogenesis, cytodifferentiation, and matrix secretion will occur. Without the BMP family of growth factors, the enamel knot would not be formed, and teeth would be unlikely to develop. BMPs, as well as other growth factors, have been successfully used for direct pulp capping. This has encouraged the addition of growth factors to stem cells to accomplish tissue engineering replacement of diseased tooth tissues.[19]

There are Two Strategies For the Use of Bmps For Dentin Regeneration.

- The first is in vivo therapy, where BMPs or BMP genes are directly applied to the exposed or amputated pulp.
- The second is ex vivo therapy, which consists of isolation of DPSCs, their differentiation into odontoblasts with recombinant BMPs or BMP genes, and finally their autogenous transplantation to regenerate dentin.
- However, a novel role has been suggested for BMP-4, which is secreted by mesenchymal cells, in the regulation of Hertwig's epithelial root sheath (HERS) during root development by preventing elongation and maintaining cellular proliferation. Therefore it has been utilized as an agent for regulating root formation in a variety of tissue engineering applications[20].

Scaffolds

A scaffold can be implanted alone or in combination with stem cells and growth factors to provide a physicochemical and biological three-dimensional microenvironment or tissue construct for cell growth and differentiation.[21,22]

Ideal Requirements of a Scaffold are

- Should be porous to allow placement of cells and growth factors.
- Should allow effective transport of nutrients, oxygen, and waste.
- Should be biodegradable, leaving no toxic byproducts.
- Should be replaced by regenerative tissue while retaining the shape and form of thefinal tissue structure.
- Should be biocompatible.
- Should have adequate physical and mechanical strength.

Types of Scaffold

a) Biological/Natural Scaffolds: These cons -ist of natural polymers such as collagen and glycosaminoglycan, which offer good biocompatibility & bioactivity. Collagen is the major component of the extracellular matrix and provides great tensile strength to tissues. As a scaffold, collagen allows easy placement of cells and growth factors and allows replacement with natural tissues after undergoing degradation. However, it has been reported that pulp cells in collagen matrices undergo marked contraction, which might affect pulp tissue regeneration.[23]

b) Artificial Scaffolds: These are synthetic polymers with controlled physicochemical features such as degradation rate, micro-structure, and mechanical strength[24], for example:

- Polylactic acid (PLA), polyglycolic acid (PGA), and their copolymers, poly lactic-co-glycolic acid (PLGA).

- Synthetic hydrogels include polyethylene glycol (PEG)- based polymers. Scaffolds modified with cell surface adhesion peptides, such as arginine, glycine, and aspartic acid (RGD) to improve cell adhesion and matrix synthesis within the three-dimensional network.
- Scaffolds containing inorganic compounds such as hydroxyapatite (HA), tricalcium phosphate (TCP) & calcium polyphosphate (CPP), which are used to enhance bone conductivity, and have proved to be very effective for tissue engineering of DPSCs.
- Micro-cavity-filled scaffolds to enhance cell adhesion.[24]

Clinical Applications of Tissue Engineering Concepts

Following are areas of research that might have application in the development of regenerative endodontic techniques:

1. Root canal revascularization via blood clotting
2. Postnatal stem cell therapy
3. Pulp implantation
4. Scaffold implantation
5. Injectable scaffold delivery
6. 3-dimensional cell printing
7. Gene therapy

The Key Procedures of the New Protocol Suggested For Treating Non-vital Immature Permanent Teeth are-

- Minimal or no instrumentation of the canal while relying on gentle but thorough irrigation of the canal system with sodium hypochlorite and chlorohexidine,
- Augmented disinfection by intra-canal medication with a triple-antibiotic paste (containing equal proportions of ciprofloxacin, metronidazol,&minocycline in a paste form at a concentration of 20 mg/ml) between appointments,[28] and
- Sealing of the treated tooth with mineral trioxide aggregate (MTA) and glass ionomer/resin cement upon completion of the treatment. Finally, periodical follow-ups are made to observe any continued maturation of the root.

Some investigators,[24,25] have induced hemorrhage in the root canal system by over-instrumentation, allowing a blood clot to form in the canal. Then MTA was placed over the blood clot. They considered that the initiation of a blood clot would provide a fibrin scaffold containing platelet-derived growth factors that would promote the regeneration of tissue within the root canal system. The induction of bleeding to facilitate healing is a common surgical procedure. It had been proposed earlier by Ostby[27] and Myers and Fountain [28] to guide tissue repair in the canal. However, there is a lack of histological evidence that a blood clot is required for the formation of repaired tissues in the canal space. Moreover, there have been no systematic clinical studies to indicate that application of this approach gives significantly better results than procedures that lack it. There is no current evidence-based guideline to help clinicians determine the types of cases that can be treated with this conservative approach. As mentioned above, the presence of radiolucency in the peri-radicular region can no longer be used as a determining factor, nor can the vitality test be used. In both situations, vital pulp tissue or an apical papilla may still be present in the canal and at the apex.[28,29]

Conservative approach should be chosen first by the clinicians, while apexification be performed in cases of failure.

Conclusion

Tissue regeneration in postnatal life recapitulates events that have occurred in the normal course of embryonic development and morphogenesis.

Both embryonic development and tissue regeneration are equally regulated through the interaction of selected and highly conserved families of proteins and gene products.

It is now accepted that the dental pulp harbors several niches of multipotential stem cells capable of self-renewal and differentiation.

Techniques to isolate and characterize human pulp stem cells and manipulate their growth under defined in vitro conditions must be established and optimized before cell therapy.

Current research is exploring the perfect formula for a reliable autogenous stem cell source, appropriate signaling molecule(s) and a scaffold that will promote controlled cell growth and differentiation

Tissue engineering using the triad of dental pulp progenitor/stem cells, morphogens, and scaffolds may provide an innovative and novel biologically-based approach for generation of clinical materials and/or treatments for dental disease.

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