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## Utilization of Stem Cells in Dentistry: A Review

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For centuries, scientists have known that certain animals can regenerate missing parts of their bodies. Humans actually share this ability with animals such as starfish which has the ability to regenerate lost arms and can grow an entire new limb. Researchers define stem cells as the clonogenic cells with the capability of self renewal and multi-lineage differentiation and also have the capabilities of generating complex tissues and various organs.

**KEYWORDS:** Stem cells, Regenerative Medicine, Regeneration

### INTRODUCTION

Regenerative medicine is a newly evolving branch of modern medicine that deals with cell based therapies which use healthy cells cultured in the laboratory to replace damaged cells in adult organisms to treat disease. Regenerative medicine aims to repair or re-grow parts or tissues which are lost as a consequence of disease or injury. One of the building blocks of this therapy is —“stem cell”. Stem cells have the capability to multiply manifolds and convert or differentiate into any specialized cell types of the body. Hence, the potential of these invaluable assets could even be projected as far as, sometime in the near future, to replace organ transplantation.<sup>1</sup>

Stem cells in dentistry: Birth of stem cell research took place way back in 1953, when Leroy Stevens identified teratoma like cells in testicles of inbred mice and in 2003, Dr. Songtao Shi, who is a pediatric dentist, discovered baby tooth stem cells by using the deciduous teeth of his six year old daughter, he was luckily able to isolate, grow and preserve these stem cell’s regenerative ability, and named them as SHED (Stem cells from Human Exfoliated Deciduous teeth).<sup>2</sup>

Stem cell therapy in dentistry: The goal of modern restorative dentistry is to functionally and cosmetically restore the tooth structure. Till recently, a variety of synthetic materials were developed to restore the damaged tooth structure.

Although these materials have proved to be effective, they do not exhibit the same mechanical and physical properties as naturally formed dentine and enamel.

Regeneration of dental hard tissue: Natural dental hard tissues, i.e. dentin, enamel and cementum exhibit little or no regenerative capability. Hence, there is a need for the replacement of the tooth tissue. Tissue engineering is a novel and highly exciting field of research. With tissue engineering techniques, it may be possible to repair damaged tissues or even create replacement organs. Tissue engineering can help in the regeneration of enamel and dentin to restore the lost tooth structure in future.<sup>3</sup>

i) **Enamel regeneration:** The enamel spends the remainder of its lifetime vulnerable to wear, damage, and decay. Although researchers have experienced some success in producing enamel-like and tooth-like tissues, problems remain to be solved before the technology can be tested in humans. One of the issues has been how to produce, in culture, a sufficient number of enamel-forming cells. There are reports that a new technique is being developed for culturing cells that have the capacity to produce enamel.<sup>4</sup>

A gene has been identified by Maria Zoupa et al., responsible for the formation of enamel, which is

a key component of the teeth. The experiments were accomplished in mice carrying a deletion of the transcription factor *Tbx1*, a gene that plays a principal role in several human malformations. Studies demonstrate that a direct link exists between impaired *Tbx1* function and enamel defects.<sup>5</sup>

ii) **Dentine regeneration:** The dentine–pulp complex displays exquisite regenerative potential in response to injury. The postnatal dental pulp contains a variety of potential progenitor/stem cells, which may participate in dental regeneration. A population of multipotent mesenchymal progenitor cells known as dental pulp stem cells with high proliferative potential for self-renewal has been described and is important for the regeneration of the dentine.<sup>6</sup>

iii) **Cementum regeneration:** Although there are differences in the organization of bone and cementum, it is not clear if distinct cell types form them or they are formed by a bone-forming cell that has different environmental cues. Distinguishing between these two possibilities has been difficult because, to date, there is no specific marker for cementum or cementocytes.<sup>7</sup>

Cultures of murine or primary human cementum-derived cells (HCDCs) have been established using a collagenase pre-treatment as had been established previously for the culture of trabecular bone cells. Thus formed cells (human cementum-derived cells) form bone like tissues that have osteocyte or cementocyte like cells embedded within a mineralized matrix.

The mineralized tissue was lined with a layer of cells that were somewhat more elongated than osteoblasts, and the human cementum-derived cell matrix was somewhat less cellular than that produced by bone marrow stem cells. Unlike bone marrow stem cell transplants, which developed lamellar bone, the human cementum-derived cell matrix was found to contain unorganized collagen bundles, as is seen in cementum. These results show that cells from normal human cementum can be isolated and expanded in vitro. Furthermore, these cells are capable of differentiating and forming a cementum-like tissue when transplanted into immunocompromised mice.<sup>8</sup>

iv) **Regeneration of pulp:** Regenerative endodontic procedures can be defined as biologically based procedures which are designed to replace damaged structures including dentin and root structures, as well as the cells of the pulp-dentin complex.

#### **Regenerative approaches in Endodontics - These techniques are**

a. **Post natal stem cell therapy:** The simplest method to administer the cells of appropriate regenerative potential is to inject the post natal stem cells into the disinfected root canal systems after the apex is opened. The post natal stem cells can be derived from multiple tissues including skin, buccal mucosa, fat and bone. One recent approach could be to use the dental pulp stem cells that have been taken from the umbilical cord, which are mostly disease and pathogen free.<sup>9</sup>

There are several advantages to an approach using postnatal stem cells. First, autogenous stem cells are relatively easy to harvest and to deliver by syringe, and the cells have the potential to induce new pulp regeneration. Second, this approach is already used in regenerative medical applications, including bone marrow replacement, and a recent review has described several potential endodontic applications.

b. **Pulp implantation:** In pulp implantation, the cultured pulp tissue is transplanted into cleaned and shaped root canal systems. The pulp tissue is grown in sheets in vitro on biodegradable polymer nanofibers or on sheets of extracellular matrix proteins such as collagen I or fibronectin. The limitation of this technique is that specialized procedures may be required to ensure that the cells properly adhere to the root canal walls.<sup>10,11</sup>

c. **Scaffold implantation:** Pulp stem cells must be organized into a three-dimensional structure that can support cell organization and vascularization. This can be accomplished by using a porous polymer scaffold which is seeded with pulp stem cells. In pulp exposed teeth, dentin chips have been found to stimulate reparative dentin bridge formation. Dentin chips may provide a matrix for pulp stem cell attachment and they may also be a reservoir of growth factors. The natural reparative activity of the pulp stem cells in

response to the dentin chips provides some support for the use of scaffolds to regenerate the pulp dentin complex.

**d. Injectable scaffold delivery:** Tissue engineered pulp tissue is seeded into the soft three-dimensional scaffold matrix, such as a polymer hydrogel. Hydrogels are injectable scaffolds that can be delivered by syringe, they have the potential to be noninvasive and are easy to deliver into the root canal systems. In theory, the hydrogel may promote pulp regeneration by providing a substrate for cell proliferation and differentiation into an organized tissue structure. Despite these advances, hydrogels are at an early stage of research and this type of delivery system, although promising, has yet to be proven to be functional *in vivo*.<sup>9</sup>

**e. Three dimensional cell printing:** The three dimensional cell printing technique can be used to precisely position cells and this method has the potential to create tissue constructs that mimic the natural tooth pulp tissue structure. The ideal positioning of cells in a tissue engineering construct would include placing odontoblastic cells around the periphery to maintain and repair dentin, with fibroblasts in the pulp core supporting a network of vascular and nerve cells.

**f. Gene therapy:**

Gene therapy has been recently used as a means of delivering genes for growth factors, morphogens, transcription factors and extracellular matrix molecules locally to the somatic cells of individuals, with resulting therapeutic effect. The gene can stimulate or induce a natural biological process by expressing the molecules which are involved in the regenerative response for the tissue of interest. Both an *in-vivo* and *ex-vivo* approach can be used for gene therapy. One use of gene delivery in endodontics would be to deliver mineralizing genes into the pulp tissues to promote tissue mineralization.<sup>10</sup>

There has been little or no research in this field, except for the work of Rutherford. He transfected ferret pulps with cDNA-transfected mouse BMP-7 failed to produce a reparative response, suggesting that further research is needed to optimize the potential of pulp gene therapy.

## BONE REGENERATION

Adult mesenchymal stem cells (hMSC) isolated from bone marrow aspirates can effectively differentiate into osteoblasts and can serve for rebuilding of bone defects in the oromaxillofacial region by bone regeneration. Bone marrow mesenchymal cells can differentiate into bone, cartilage, muscle, blood vessels and nerves in culture or by transplantation into the tissues.<sup>12</sup>

They may be useful for treatment of periodontal diseases, osteoporosis, bone fractures, augmentation of the alveolar ridge of maxilla and mandible, sinus lift, filling of large bone defects after surgical treatment of cancer or after injury and repair of inborn bone.

## PERIODONTAL REGENERATION

Periodontal regeneration is the reproduction or reconstitution of a lost or injured part of the periodontium so that the form and function of lost structures is restored. The strategy of periodontal tissue regeneration therapies has been to control inflammation and stimulate stem progenitors to regenerate new periodontal tissues. Recent advances in stem cell biology and regenerative medicine have presented opportunities for tissue engineering as well as gene-based approaches in periodontal therapy.<sup>13</sup>

Periodontal ligament stem cells (PDLSC) are a unique population capable of forming an ectopic cementum/ PDL-like structure. However, although cultured PDL stem cells have shown promising results when implanted into surgically created periodontal defects in rats (Seo et al, 2004).<sup>14</sup>

Stem cells from human exfoliated deciduous teeth (SHED), another population of dental-derived stem cells, were observed in immunocompromised mice to induce cells to differentiate into osteoblasts and osteocytes, resulting in the synthesis of new bone. However, this cell strain was unable to form periodontal ligament and root cementum. Recently, Precursors cells (PCs) isolated from human dental follicle of wisdom teeth were characterized by Morscheck et al.<sup>15</sup> These cells were able to create *in vitro* a structure similar to a periodontal

membrane composed of fibroblast phenotype cells and calcified structures, full of alkaline phosphatase and bone sialoprotein.

### **STEM CELLS IN SINUS AUGMENTATION**

In a study conducted by Yadollah S.S et al (2008) it was shown that sinus augmentation can successfully be done using beta-tricalcium phosphate, hydroxyl apatite and human mesenchymal stem cells. Mesenchymal stem cells (MSCs) are a better source because they are able to proliferate under low oxygen tension and differentiate when the oxygen level rises. Depending on the micro-environment mesenchymal stem cells have the ability to differentiate into osteoblasts. In animal experiments stem cell application in combination with a bio material (BioOss) show lamellar bone formation and bone invasion into the micropores.<sup>16</sup>

### **REPAIR OF CLEFT LIP & PALATE DEFECTS**

Cleft lip and palate (CLP), one of the most frequent congenital malformations, affects the alveolar bone in the great majority of the cases, and the reconstruction of this defect still represents a challenge in the rehabilitation of these patients. One of the current most promising strategies to achieve this goal is the use of bone marrow stem cells (BMSC) however, isolation of bone marrow stem cell from iliac bone, which is still the mostly used graft in the surgical repair of these patients, confers site morbidity to the donor. Therefore, in order to identify a new alternative source of stem cells with osteogenic potential without conferring morbidity to the donor, orbicular oris muscle (OOM) fragments were used which are regularly discarded during cheiloplasty of CLP patients. Cells were obtained from OOM fragments of four unrelated CLP patients. These cells, through flow cytometry analysis, were mainly positively marked for five mesenchymal stem cell antigens (CD29, CD90, CD105, SH3, and SH4), while negative for hematopoietic cell markers, CD14, CD34, CD45, and CD117, and for endothelial cell marker, CD31. After induction under appropriate cell culture conditions, these cells were capable to undergo chondrogenic, adipogenic, osteogenic, and skeletal muscle cell differentiation, as evidenced by immunohistochemistry.<sup>17</sup>

### **REGENERATION OF IRRADIATED SALIVARY GLANDS**

Yearly, worldwide more than 500,000 new head and neck cancer patients are treated with radiotherapy. Co-irradiation of salivary glands may lead to xerostomia resulting in permanent loss of saliva production. This loss of gland function after radiation is thought to be due to a loss of stem cells that are no longer able to replenish saliva-producing acinar cells. Therefore, stem cell therapy could be utilized to prevent radiation-induced damage to the salivary gland. BMSCs, when mobilized to the blood circulation, are able to contribute to the regeneration of acinar cells and blood vessels of irradiated mice salivary glands, resulting in increasing saliva production. Since only low percentages of acinar cells were Bone Marrow-derived, the engrafted BMCs seem to secrete micro-environmental factors which stimulate radiation-surviving salivary gland stem/progenitor cells.<sup>18</sup>

Optimal recovery of irradiated salivary glands could possibly be obtained by increasing functional salivary gland stem cell numbers. This can be accomplished by in vivo administration of keratinocyte growth factor, which results in the formation of almost normal levels of acinar cell numbers and saliva production.<sup>19,20</sup>

### **MANAGEMENT OF ORAL CANCER**

There is increasing evidence that the growth and spread of cancers is driven by a small subpopulation of cancer stem cells (CSCs) – the only cells that are capable of long-term self-renewal and generation of the phenotypically diverse tumour cell population. It is reported that 132 Stem cells can be damaged in certain cancer treatments. The cancer stem cell hypothesis is a departure from traditional models of Oncogenesis, which proposed that genetic alterations transform mature, differentiated cells into cancer cells. Cancer stem cells, however, help explain two of the most challenging and demoralizing aspects of cancer: remission and recurrence. Often, radiation or chemotherapy halts the malignancy, sometimes to the point where it can no longer be detected, yet the disease returns.

Cancer stem cells may be especially resistant to eradication for two reasons. First, current

chemotherapies selectively target rapidly dividing cells, but stem cells tend to divide at a slow rate. Second, normal stem cells congregate in niches—specific physical areas within an organ that protect stem cells. Cancer stem cell niches have been found in the brain and proposed elsewhere, making radiation and chemotherapy treatment even more complicated. If even a small number of cancer stem cells survive an assault, they can once again give rise to full-fledged cancer as metastases result from the surviving cancer stem cells that travel through the body.

### WHOLE TOOTH REGENERATION

Tooth-like tissues have been generated by the seeding of different cell types on biodegradable scaffolds. A common methodology is to harvest cells, expand and differentiate cells in vitro, seed cells onto scaffolds, and implant them in vivo, in some cases, the scaffolds are re-implanted into an extracted tooth socket or the jaw. Ikeda et al. (2009)<sup>21</sup> reported a successful fully functioning tooth replacement in an adult mouse achieved through the transplantation of bioengineered tooth germ into the alveolar bone in the lost tooth region. This technology was proposed as a model for future organ replacement therapies.

### PRACTICAL ASPECTS OF DENTAL STEM CELLS

In addition to the above sources of dental stem cells in extracted third molars and exfoliating deciduous teeth, every year millions of healthy teeth that possess stem cells are routinely discarded as medical waste in the course of normal dental care, e.g., from extracted deciduous teeth and permanent teeth and the developing tooth germ of impacted third molars. Instead of discarding these sources, stem cells from these patients' teeth can be readily cryopreserved for future clinical application.

In our day to day practice of dentistry, stem cells have been identified in diseased or injured teeth such as primary incisors extracted because of disease, crown fractured adult teeth resulting from trauma, and inflamed dental pulp.<sup>22</sup>

### CONCLUSION

Stem cells have more roles to play in medicine and dentistry. The complete restoration of the

physiologic, structural & mechanical integrity of the native tissue structure is fascinating fact and it's a way far to reach the hands of mankind. Advances in adult stem cell biology have provided a great deal of impetus for the biomedical community to translate these findings into clinical application.

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