THE USE OF STEM CELLS IN DENTISTRY AND DEVELOPMENTS FOR THE FUTURE

BY
HEIDI SWINHOE

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ABSTRACT

Stem cells have the ability to divide and self-renew to produce different cell types. They therefore have a key role in providing new methods of regenerative medicine such as replacing missing tissues and treating diseases. Focussing on the field of dentistry, the dental pulp of a tooth and other oral and
maxillofacial tissues are a rich source of adult mesenchymal stem cells. They not only provide a source but also an ideal therapeutic target for stem cells. Stem cells throughout dentistry are becoming of great clinical interest due to their ability in restoring and regenerating teeth themselves without the risk of rejection by the immune system, as well as providing less ethical controversy than the use of embryonic stem cells. Oral tissue derived stem cells provide a prospective foundation for many future developments in stem cell related medicine. The article outlines the sources of dental stem cells and examines research into bone regeneration, creation of a biotooth, the use of laser to stimulate stem cells, and the use of dental pulp cells in the creation of a corneal graft.

INTRODUCTION

Stem cells are undifferentiated cells with the extraordinary potential to divide and develop into any type of cell in the body. One key characteristic of stem cells is their ability to serve as an internal repair system by self-renewing whilst maintaining the potential to develop into other mature cells with distinctive features and specific functions such as cells of the blood, heart, skin, muscles and bones. In some organs such as bone marrow and the gut, stem cells will regularly divide to repair worn out or damaged tissues, whereas in other organs such as the pancreas or the heart, stem cells only divide under special conditions. Stem cells can be divided into two groups: Embryonic stem cells (found in embryos) and adult stem cells which can be found in tissues such as bone marrow, skin, adipose tissue and dental pulp. [7]

Due to their unique properties, stem cells have the aptitude to become an important tool in tissue engineering and regenerative medicine. They offer a new potential for treating diseases such as heart disease or diabetes and for the restoration, conservation and improvements of tissue function. However, much work remains to be done in the laboratory and clinic to understand how to use these cells in cell based reparative medicine.

Embryonic and adult stem cells each have their advantages and disadvantages regarding their potential use in regenerative medicine. One key difference is the number and type of differentiated cells that they can become. Embryonic stem cells are able to become all cell types, they are known as pluripotent, whereas adult stem cells are thought to only be able to differentiate into limited cell types of their tissue origin - multipotent. Embryonic stem cells can also be grown moderately easily in culture unlike adult stem cells which are more challenging to isolate as they are rare in mature tissues. Adult stem cells however are believed to be less likely to initiate rejection after transplanting them. This is because these cells can be derived from the
patient’s own cells, expanded in culture, adapted into a specific cell type and reintroduced into the patient. Adult stem cells are therefore a key part of achieving successful tissue regeneration in the years to come.

In dentistry, tissue engineering research has become more popular due to the familiarity among dentists with tissue regeneration techniques, for example the use of tertiary dentin in dental pulp. The development of techniques which could eventually create whole new teeth is ongoing and a key factor in finalising this technology is the use of adult stem cells. Recently, mesenchymal stem cells were demonstrated in the dental pulp, periodontal ligament and dental follicle of teeth (Figure 1 and 2). These stem cells could be used in tissue engineering applications including periodontal and bone regeneration. Not only can such cells be used in the regeneration and restoration of teeth, they can be used in the tissue engineering of other vital organs within the mouth such as the tongue and salivary glands[3] also further field such as corneal stromal regeneration[6]. Discussing all the possible uses of these cells from the dental pulp would be beyond the scope of the report, however I will go into further detail in the areas which I believe could have great potential for the future.

Figure 1 - Shows the anatomy of the mouth and a tooth. It includes the different types of teeth positioned in the jaw (Incisors, Canines, Premolars and Molars), the Lips, the Tongue, the teeth roots and Jaw bone. The tooth consists of Enamel, Dentin, Pulp, the periodontal ligament and Cementum. (http://www.daviddarling.info/encyclopedia/T/teeth.html)
This article proposes to outline the sources of stem cells from dental and oral tissues. The discussion includes an example of successful stem cell use in the field of prosthodontics. I will then discuss some dental stem cell work which is currently at very early stages, and suggest how this may lead onto future treatments in humans.

**DISCUSSION**

**Types of stem cells used in dentistry**

**Adult Stem cells**

An adult stem cell is an undifferentiated cell found among differentiated cells in a tissue or organ. It can renew itself, and can differentiate yielding some or all of the specialised organ cell types. Their primary role is the maintenance and repair of that tissue within the living organism. Research on adult stem cells began in the 1950s with the discovery of two types of stem cells, namely haematopoietic-forming types of blood cells, and a second population called bone marrow stromal stem cells, or mesenchymal cells. These can generate bone, cartilage and fat that supports the formation of blood and connective tissue. Further types of adult stem cells have subsequently been discovered: neural stem cells, epithelial cells and skin stem cells.

**Induced pluripotent stem cells (iPSCs)**

These are adult stem cells, altered to become pluripotent. They are designed to express genes important for maintaining the defining properties of embryonic stem cells. Tissue derived from iPSCs are nearly identical to the cell donor, therefore helping to avoid rejection.

**Sources of Stem Cells from Dental / Maxillofacial Tissues**

Adult mesenchymal stem cells have been isolated from many oral and maxillofacial structures, being a rich source of stem cells oral tissues are likely to be not only a source but also a therapeutic target area for stem cell treatments, as research translates into clinical therapies.
Mesenchymal stem cells (MSCs)

Mesenchymal Stem cells originally isolated from bone marrow are amongst the most promising stem cells for clinical applications. When cultured they are identified by their adherence to tissue culture treated plastic. They have now also been found in skin, adipose tissue, and various dental tissues. [3]

Bone marrow derived MSCs robustly form bone in the patient (in vivo) making them ideal for bone regeneration therapy. They can be harvested by bone marrow aspiration from the iliac crest, however this is a painful and invasive procedure. Several studies have shown an age related decline in osteogenic (ability to grow bone) efficacy, suggesting donor age is important. BMSCs can be isolated from craniofacial tissues during dental surgical procedures such as implants (Figure 5 and 6), wisdom tooth extraction, and orthodontic osteotomy. Clinical and animal studies show that autologous membraneous bone harvested from a craniofacial site and grafted to a craniofacial site results in higher bone volume than when harvested from the iliac crest or rib (which is endochondral bone) . It also appears that the age of the patient has less effect. These apparent advantages may however be offset by the fact the collectable volume is very small, 0.03 mls compared with 0.5 mls from the iliac crest.

**Stem cells derived from Dental Tissue**

Figure 2- Shows the close up anatomy of a tooth. Including the Enamel, Dentin, Dental pulp, Cementum, Periodontal ligament, Nerve and blood supply the Root and the Crown. (www.nlm.nih.gov)
Dental Pulp Stem cells

Adult human stem cells have been identified in the dental pulp (Figure 2) (DPSCs in 2000, and MSC like cells have subsequently been isolated from the dental pulp of human exfoliated deciduous teeth (SHED) [3]. These cells importantly have the specific ability to regenerate the dentin -pulp complex. SHEDs can induce bone -like matrix formation by recruiting host cells. This may be explained by the fact that in deciduous teeth root resorption is accompanied by new bone formation around the root.

Periodontal Ligament Stem Cells

The periodontal ligament (Figure 2) is another source of adult stem cells, and these can be isolated from extracted teeth. They possess the ability to regenerate periodontal tissue such as the alveolar bone, periodontal ligament and cementum.

Dental Follicle Stem Cells DFSCs

Developing dental tissues such as the dental follicle, mesenchyme, and apical papilla SCAP, have also been identified as a source of MSC like cells. Some of these have high proliferation activity and can differentiate in vitro (in the laboratory) into lineages of different germ layers, osteoblasts, neural cells and hepatocytes. It is possible that developing dental tissues provide a better source of immature stem cells than developed tissues. Practically these are a readily accessible supply of stem cells as these tissues are often discarded as medical waste.

Oral Mucosa and Periosteum

The oral mucosa and the gingiva have both been shown to contain stem cells with multipotency and rapid expansion ex vivo. They are a highly attractive tissue source due to the ease of isolation and clinical abundance.

The periosteum is a specialised connective tissue covering the bone surface. Cells derived from the periostium have strong osteogenic potential and have been used of oro-facial bone regeneration.
Induced pluripotent stem cells have been generated from various oral mesenchymal cells such as SCAP, DPSCs, SHED, and buccal mucosal and gingival fibroblasts. Due to their high proliferation rate and high expression of endogenous reprogramming factors, and easy accessibility cells from an oral source are ideal for dentists and researchers. They will be very important in the future in the development of innovative treatments such as regeneration of full structure missing jaw bones, salivary glands and missing teeth.

**Practical Applications and projects for the future**

In dentistry tissue engineering is a new frontier in regeneration of new tissue, structures and organs. Good detention and strong bone is important for mastication, and speech as well as aesthetic reasons. Tooth loss may occur from periodontal disease, caries or traumatic injury. Alveolar bone resorption (Figure 3) occurs after tooth loss and when severe makes is difficult to restore missing teeth with denture or dental implant treatment.

![Figure 3 – Shows gradual alveolar bone resorption after tooth loss.](http://doctorspiller.com/Bone_Grafting/bone_grafting.htm)

**Alveolar Ridge Augmentation**

Prosthodontics is the branch of dentistry concerned with the design and replacement and fitting of artificial replacements for teeth. Dental implants, usually titanium (Figure 5 and 6) are used to replace missing teeth but their fixation requires good anchorage into the alveolar bone. Different approaches to alveolar ridge augmentation are being evaluated. Long established stem cell based technologies have used the in vitro preparation of tissue engineered bone
Several authors report successful growth of bone graft in humans, providing a reliable basis for the insertion of dental implants [8].

A more recent technique called cell sheet based bioengineering has been used where the cell to cell contact in the newly engineered tissue construct remains intact which should be beneficial. This also is an in vitro process with the construct being re-implanted. From studies it is understood that as well as the stem cell source, a scaffold for the cells to adhere to, specific growth factors are necessary to support the proliferation of cells to form the graft. (Figure 4)

A completely different approach could be in the dental clinic,” chair-side cellular grafting”. This approach uses freshly processed patient derived bone marrow, containing MSCs, haemopoietic stem cells and angiogenic cells mixed with a scaffold and growth factors as a grafting material. This is convenient as it is prepared and introduced in the clinic. However one difficulty is the accessibility of the bone marrow in the dental clinic in particular if the iliac crest is to be used.

Figure 4 – Shows a labelled flow diagram of a tissue engineering approach. (Hiroshi E. ( 2012) Stem Cells in dentistry – Part II: Clinical applications in Journal of Prosthodontic Research 56 (2012) 229-248)
Despite early success of differing approaches events following the transplantation are still poorly understood. It is not known whether the new bone is formed from the surviving implanted cells, or the host osteogenic cells. Furthermore culture expansion of the BMSCs may alter the cells’ biological functions, which can affect the immune response by the recipient. Much further understanding of the immune response to transplanted tissue need to be established, before this early human work can be translated into routine dental practice.

Creation of a Biotooth

The ideal goal of regenerative medicine is the generation of a fully functional tissue or organ. There has been promising research recently in which a Chinese team have grown rudimentary tooth like structures in culture.[9]

Developmentally during tooth formation the odontogenic potential shifts at bud stage-embryonic day 12, from dental epithelium to dental mesenchyme. The epithelium differentiates into cells called ameloblasts and forms enamel, and the mesenchyme forms the dentin, cementum and dental pulp.

It has been shown by Arakaki et al that mouse iPSCs can be differentiated into ameloblasts through interactions with dental epithelium. The team used human urine as a source of induced pluripotent stem cells[2]. These were differentiated into epithelial sheets which were then recombined with mouse dental mesenchymes in vitro. These recombinants were then...
transplanted and grown in the mouse kidney sub-renal capsule. After three weeks the researchers found fibrous cysts within which there were rudimentary tooth like structures. These structures contained dental pulp, dentin enamel space and enamel organ (Figure 2). However these structures were found in only 30% of the mice.

From this exciting study we can postulate that with further advances that human the bioengineering of human teeth may be possible in the future.

The culture system proposed would comprise of human urine induced pluripotent stem cells, and their combination with human tooth stem cells derived from the patient’s dental tissue such as dental pulp cells or the periodontal ligament which are the most powerful for tooth engineering. Using the patients’ own tissue would avert the likelihood of immunogenic reactions. However the formation of enamel is critical for hardness of the tooth and epithelial stem cells are required for enamel formation. One problem exhibited in the mice teeth structures was inadequate enamel development. The lack of available epithelial cells in adults is a major obstacle, yet to be overcome. Dental epithelial cells could be isolated from newborn or young animals, but their use in humans would be dangerous due to immunoreactions. Dental epithelial stem cells could also be isolated from the tooth germ of children’s third molar, having been saved for future use. However subjecting a child for extraction surgery is ethically unsound. It may be that the solution to the enamel generation problem could be the use of an artificial crown for the biotooth.

Implanting the biotooth during the growing phase may well influence the development and root implantation into the alveolar bone and strengthen the biotooth, as stimulation from use affects the growth of tissues as opposed to lack of such stimulus in culture.

Clearly humans have different types of teeth, namely incisors, canines, premolars and molars fulfilling very different functions. Further understanding of odontogenesis is required to ensure the finished biotooth grows into a predetermined specific tooth type.

**Tooth Repair using dental stem cells stimulated by laser**

Very recent research conducted in Harvard USA has discovered an exciting new development in the stimulation of dental stem cells by light [10]. Since the advent of medical laser therapy in 1960 medics have acquired anecdotal evidence to support the theory that low level light can stimulate many biological processes such as rejuvenating skin and stimulating hair growth.
Praveen Arany used low power laser to trigger human dental stem cells to form dentin [1]. Initially mice had holes drilled into the molar teeth. Then they were exposed to laser therapy, and had temporary caps applied to the teeth. After twelve weeks, microscopy and high resolution x-rays confirmed the lasers had induced enhanced dentin formation. The team have also identified the precise molecular mechanism responsible for simulation the regenerative effects of the laser. The laser acts in a dose dependent manner to induce reactive oxygen species which in turn activated latent transforming growth factor 1.

**Future developments**

Much stem cell work to date is conducted in vitro, with transplantation of the resultant organ into the recipient. It would be major progressive step if instead of the in vitro manipulation of stem cells, these specialised cells could be targeted within the target tissue itself, encouraging the tissue to regrow and repair itself. With the precise molecular pathway requiring activation now having been identified assumptions can be made that low level laser light may be of use in stimulation of other processes, such as tissue healing. This process of using non ionising low power laser could be applied to humans, with the possibility of stimulation human dental stem cells. It may be possible that instead of tooth loss through decay and damage laser may have role to play in the regeneration of teeth in vivo. This would be very elegant way of delivering tooth repair technique which is simple and non-invasive and easily conducted in the dental chair.

In bringing the two exciting new techniques together there is potential that such a laser may be used to stimulate the further growth and proliferation of cells within a transplanted, growing biologically engineered tooth to enhance the implantation process.

**Dental Stem cells for use in non-oral sites**

The cornea is the transparent outer tissue of the eye serving as a physical barrier and has the refractive power to focus light onto the retina. The corneal stroma is a dense avascular tissue made up of type 1 collagen bundles in a highly organised structure. Corneal blindness is a terrible affliction of millions of people worldwide. The current treatment is by corneal grafting which faces numerous difficulties. Corneas are harvested from cadavers, and donor tissues are in short supply, with many grafts failing long term due graft rejection.
Dental pulp contains a population of adult stem cells and similarly to corneal stroma, is developed embryonically from the cranial neural crest. DPCS isolated from the third molar have been shown to have the ability to differentiate into keratocytes, the cells of the corneal stroma. I propose that with further research and development human dental pulp cells may be used in the treatment of corneal stromal disease. A very recent publication by Funderburgh from the department of Ophthalmology in the University of Pittsburgh has demonstrated both in vitro and in vivo potential. [6]

Dental pulp cells were differentiated in vitro, and have expressed molecules characteristic of keratocytes both at gene and protein levels. These were cultured on nanofibre substrates, and have been grown into corneal stroma like constructs. The Funderburgh study used mice as the recipient where the constructs were injected into the mouse stroma. DPCs produced corneal stroma extracellular matrix containing human collagen type 1, which did not affect corneal transparency or induce an immune rejection response. I propose that this demonstrates that human DPCs have distinct potential in human tissue engineering therapies for corneal stromal blindness.

**CONCLUSION**

Growing evidence demonstrates clearly that the oral and maxillofacial region is a rich source of adult stem cells. Accessibility however, of stem cells from bone marrow in the iliac crest and liposuction from extra-oral tissue is not an easy operation for dentists. On the other hand, orofacial bone marrow, the salivary glands and dental tissues are more assessable sources but the isolation of stem cells from these areas may prove inconvenient as it requires surgery or the extraction of teeth. However, recently, exfoliated deciduous teeth have proven to be a rich source of mesenchymal stem cells and could be the answer to many issues (particularly accessibility) within regenerative medicine. This evidence has led to the recent development of tooth banking.

Stem cells from human exfoliated deciduous teeth (SHED) act as an easy collection site for efficient extraction of stem cells and have extensive differentiation ability. In 2003, Miura et al confirmed that they are able to differentiate into a greater variety of cell types than many other postnatal mesenchymal stem cells. Therefore the ethical limitations related to the use of embryonic stem cells along with the limitations of readily accessible sources of multi-potent postnatal stem cells make SHED a suitable alternative for tissue engineering. Storing SHED
has enormous potential in future treatment of diseases such as Parkinson’s and Alzheimer’s along with repairing connective tissues, dental tissues and bone. Harvesting stem cells at the right point of development is the key to successful stem cell therapy; exfoliated teeth usually receive blood flow until the last minute which is indicative of cell viability however SHED is preferred after extraction rather than an exfoliation to prevent a compromised blood supply. SHED are a convenient replacement for storing umbilical cord blood as they can be collected every time a milk tooth is lost rather than immediately after birth. This means that parents have more time to decide whether to store their child’s teeth or not. Tooth banking also costs less than one third of the cost of umbilical cord blood storage.[5] SHED could have a huge impact on the future of medical breakthroughs and the research is very encouraging.

The development in research involving stem cells is a very exciting field for medicine and dentistry. Dental sources in particular, are promising as they are readily accessible, and have no ethical obstacles unlike the use of embryonic stem cells. There has been a huge degree of progress over recent years, however much is still to be understood as of yet. Stem cells provide us with a positive outlook on future advances in regenerative medicine and many ‘incurable’ diseases.

Currently it is best to practice to maintaining preventative care, for example, keeping a healthy diet, good oral hygiene and mouth care with regular flossing, brushing and dental check-ups.

References


10. Researchers use light to coax stem cells to repair teeth report by Wyss Institute wyss.harvard.edu/viewpressrelease/155/researchers-use-light-to-coax-stem-cells-to-repair-teeth