

2017

Stem cells in bone regeneration in dentistry

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BOSTON UNIVERSITY
SCHOOL OF MEDICINE

THESIS

**STEM CELLS IN BONE REGENERATION
IN DENTISTRY**

by

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B.S., University of Rochester, 2016

Submitted in partial fulfillment of the
requirements for the degree of
Master of Science

2017

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ACKNOWLEDGEMENTS

I would like to thank my advisor, Dr. Theresa A. Davies, for her help and support.

STEM CELLS IN BONE REGENERATION

IN DENTISTRY

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ABSTRACT

In the recent years, stem cell research has leaped to the field of dentistry in the hopes of finding a method to ethically and efficiently develop better ways for bone regeneration. Stem cell research is the most valuable study in the dental field, and various types of adult stem cells have been found in the oral cavity, including stem cells from apical papilla, stem cells from human deciduous teeth, dental follicle stem cells, and dental pulp stem cells. These dental stem cells have the potential and ability to form specialized neuronal cells, which can be used for therapies. In addition to the benefit in the oral cavity, stem cells can serve as the front for therapeutic applications in the spinal cord, the brain, and other nerve regeneration treatments. This thesis will summarize existing studies involving stem cells and bone regeneration treatments and upcoming initial clinical trials using oral cavity stem cells for craniofacial bone regeneration.

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LIST OF ABBREVIATIONS

ALS.....	amyotrophic lateral sclerosis
BDNF.....	brain derived neurotrophic factor
BM.....	bone marrow
BMP.....	bone morphogenetic protein
CTP.....	connective tissue progenitors
DFSCs.....	dental follicle stem cells
DISCs.....	dental implant stem cells
DPSCs.....	dental pulp stem cells
FGF.....	fibroblast growth factor
GABA.....	gamma-aminobutyric acid
GBR.....	guided bone regeneration
GFSCs.....	gingival fibroblastic stem cells
GMSCs.....	gingival mesenchymal stem cells
hEM.....	human embryonic stem
iPS.....	induced pluripotent
stem MSC.....	mesenchymal stem cells
NSCs.....	neural stem cells
OI.....	osteogenesis imperfecta
PDGF.....	platelet derived growth factor
PDLSCs.....	periodontal ligament stem cells

SCAP.....stem cells from apical papilla
SHED.....stem cells from human exfoliated deciduous teeth
TGF-beta.....transforming growth factor beta
VEGFvascular endothelial growth factor

INTRODUCTION

Stem cells have been researched heavily in recent past. However, studies as early as 1924 by Alexander Maksimov identified a precursor mesenchymal cell within the bone marrow which would develop into more than one cell type. Stem cell biology is seen as one solution to the regeneration of neurons and as a potential tool for the replacement of damaged organs; unfortunately research still continues as there are many challenges associated with efficiently obtaining stem cells and the direction for which to go with modern science. The oral cavity has been shown to be a site where stem cells are found and thus suggest a very promising therapeutic approach to several structural defects, including bone generation and growth (DiPietro, 2014). A stem cell possesses both potency and self-renewal. Several review articles have been published, along with primary articles describing studies in order to identify a platform to regenerate tissue and stem cells, specifically utilization for bone regeneration (Avinash et al, 2017; Strong et al, 2017; Heng et al, 2017).

The capacity of bone regeneration has several limitations on reconstructive techniques and tissue engineering; however, its importance has a varying range as the skeleton itself acts as a regulator for homeostasis and the oral and craniofacial region have roles in several critical functions such as speech, mastication, and the effects of these on self-esteem and general overall health. There are many cases in which skeletal bone regeneration does not

occur spontaneously, such as a skeletal injury beyond a certain size (Strong et al, 2017). Additionally, there are many deformities, traumas, and levels of degenerative diseases in which large amounts of bone are necessary for reconstruction. Even though the “gold standard” for reconstruction is through bone grafts, alloplastic materials still remain part of the clinical process (Walmsley et al, 2016). The risk of infection through alloplastic materials combined with an insufficient amount of bone grafts has fueled the search for other approaches to repair large bone defects.

Bone Development and Wound Repair

Bone is composed of a mineral component and an organic matrix in a mineralized dense connective tissue. The organic matrix consists of mainly Type I collagen with Type III collagen. Between individual collagen molecules, hydroxyapatite is found and provides rigidity to bone. Mesenchymal stem cells, which can self-renew and differentiate into different cell lines, can play a role in bone development and fracture healing/bone remodeling (Walmsley et al, 2016). The development of the skeleton is aided by mesenchymal groups whose stem cells mature into cartilage and later into hypertrophic cartilage, which is then replaced by bone and marrow (Walmsley et al, 2016). During osteogenesis, the majority of osteoprogenitor cells produce extracellular matrix although there is a high cellular content present. There are a limited number of cells that can be made in already synthesized bone tissue. The bone remodeling process consists

of degradation of the extracellular matrix by the multinucleated osteoclast cells (Figure 1). Many different types of cells contribute to the generation of the complex 3 dimensional (D) bone tissue at the early stage of its formation, however once it reaches its mature state, only a few cells are abundant in the extracellular matrix (Gomez-Barrena et al, 2011).

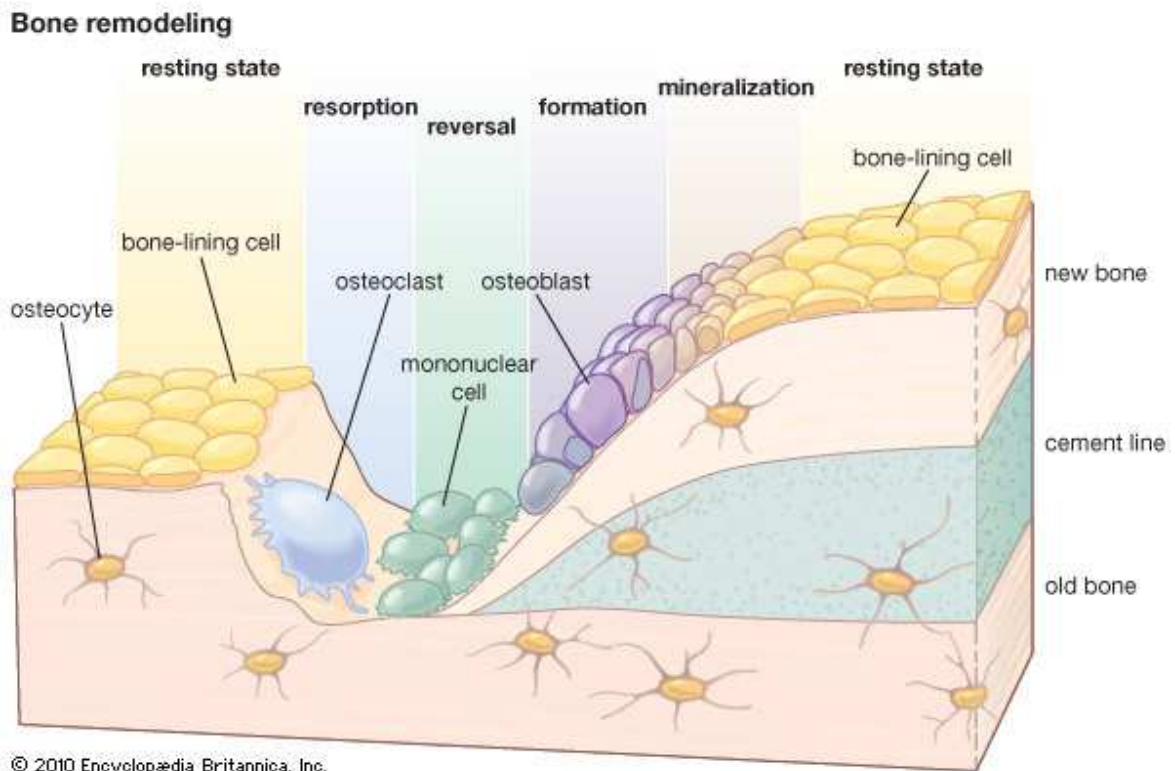


Figure 1: Bone Remodeling. Figure represents the growth process where mature bone tissue is taken out of the skeleton while new bone tissue is formed through osteocyte to an osteoclast then to an osteoblast. Figure taken from Encyclopedia Britannica, n.d.

After injury, the wound healing process begins and can be divided into several phases (Figure 2) (Shield Health, n.d.). The first phase, hemostasis, starts immediately after injury and hematoma formation occurs. During the second phase, inflammatory cells enter the clot and prevent infection and also initiate further cellular signaling cascades. During the proliferative phase or the third phase of wound healing, keratinocytes function to close the wound while the wound size decreases by myofibroblast contraction. The extracellular matrix is allowed to remodel itself and form a scar with a strength of 80% comparable to that of its original, unharmed skin. The clot, or hematoma, that is formed during the first phase, provides a temporary matrix in which cells can through migrate through during this wound healing process. The hematoma releases cytokines, and other growth factors such as TGF-beta, insulin-like growth factor, and epidermal growth factor.

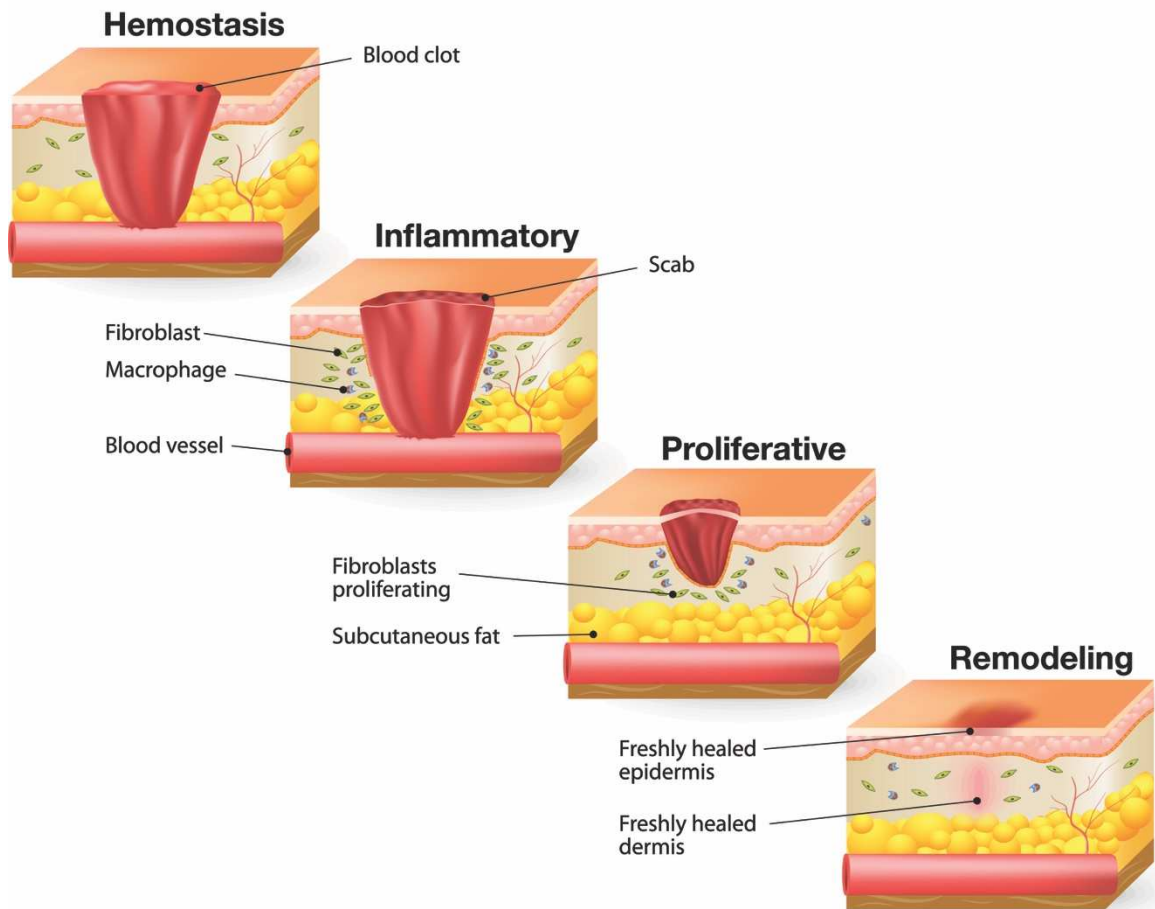


Figure 2: The Four Stages of Wound Healing. Wound healing entails four stages, hemostasis, inflammatory, proliferative and ultimately remodeling. Figure taken from Shield Health, n.d.

During the fracture healing process, hematoma formation occurs. After the hematoma forms, inflammatory cells enter the hematoma and prevent infection, releasing cytokines, growth factors, and other platelets which release TGF-beta (transforming growth factor beta) and PDGF (platelet derived growth factor). PDGF plays a significant role in blood vessel formation. Through these steps, it is that mesenchymal stem cell differentiation is able to proceed (Shakoori et al, 2017).

Bone tissue, as described, is capable of self-repair but in some instances, the defect is too large. Bone grafting is the gold standard for bone repair today, although the costs of this are considerable due to the additional surgical procedures required to harvest the bone. Furthermore, this process is hindered by the limited amount of donor material available. To resolve these issues, both allograft and xenograft based strategies have been proposed but it has been demonstrated that these run with high risks of rejection. Bone tissue engineering is an alternative strategy that has been explored. Much of this still has yet to be studied because of financial limitations and because of low efficiency of differentiation, inpatient variability, and the risk of ectopic bone growth (Fisher et al, 2016). Some studies that have clinical applications with bone tissue engineering are mentioned in the published studies section of this thesis. Connective tissue progenitors (CTP's) are used to describe the heterogeneous system of stem cells and progenitor cells that are present in native tissues, which

can proliferate and generate one or more connective tissues like bone, cartilage, fat, fibrous tissue, muscle, and blood. Colnot et al, 2012, has demonstrated the periosteum and endosteum and rich sources of osteochondral progenitor cells during fracture healing. Grafting experiments revealed that the transplanted periosteum generates both osteoblasts and chondrocytes during fracture repair, which transplanted endosteum generates primary osteoblasts. Circulating CTP's that are mobilized into circulation following an injury may also contribute to fracture healing. However, this same study showed that circulating cells only contribute a small number of cells in the fracture callus and thus therapeutic therapies are needed to make a difference in the healing process (Colnot et al, 2012). The most common source of CTPs is bone marrow from the iliac crest. Hermigou et al (2016) demonstrated successful treatment of diaphyseal nonunions with marrow-derived cells can be achieved as long as at least 50,000 CTPs are implanted at the site of the nonunion. Because native tissues have such a small number of CTPs, mesenchymal stem cells (MSCs) are very useful because of their different roles in bone repair. MSCs can differentiate into osteoblasts, trigger the division and differentiation of native CTPs, modulate cells of the immune system, and secrete trophic molecules that inhibit apoptosis and fibrosis and/or promote angiogenesis (Marcucio et al, 2015). While MSCs are the most frequently studied and characterized, several preclinical studies have also not demonstrated success, although clinical data is fairly limited. Other stem cell populations, like endothelial progenitor cells, can be more useful for other certain

therapeutic applications. Endothelial progenitor cells have been shown to contribute to bone and vasculature *in vitro*. One recent study suggested that therapy with endothelial progenitor cells was superior to MSC therapy in a bone defect model in the rat (Nauth et al, 2010). There are, however, still a number of barriers before stem cells can be fully clinically used in fracture healing and bone remodeling. Protocols for the expansion and differentiation of MSCs are established and repeatable; however, they differ depending on the *in vivo* niche from which the founding CTPs were isolated. Additionally, the concentration and prevalence of CTPs decreases in frequency and function with age. This should be able to be overcome with *in vitro* expansion of CTPs but very few clinical studies have been done in order to fully confirm this solution. The contribution of MSCs to new tissue formation is also unclear, because long-term engraftment of transplanted MSCs has not been readily observed. The effect of transplanted MSCs has largely focused on factors that MSCs may secrete although clinical trials have shown that MSCs reduce graft versus host disease (Prockop, 2013). Another improvement to make is the injection technique used in studies. Therapeutically, stem cells are often injected systemically or locally, but engraftment of cells delivered by this mechanism is generally low. Reducing strain by injecting cells at a slower rate, using a larger needle, or employing a viscous solution when injected may help improve cell viability (Marcucio et al, 2015).

Stem Cells

There are three categories of stem cells: adult stem cells, embryonic stem cells, and induced pluripotent stem cells (Figures 3 & 4) (Slide Share, n.d and Roberts, 2015).

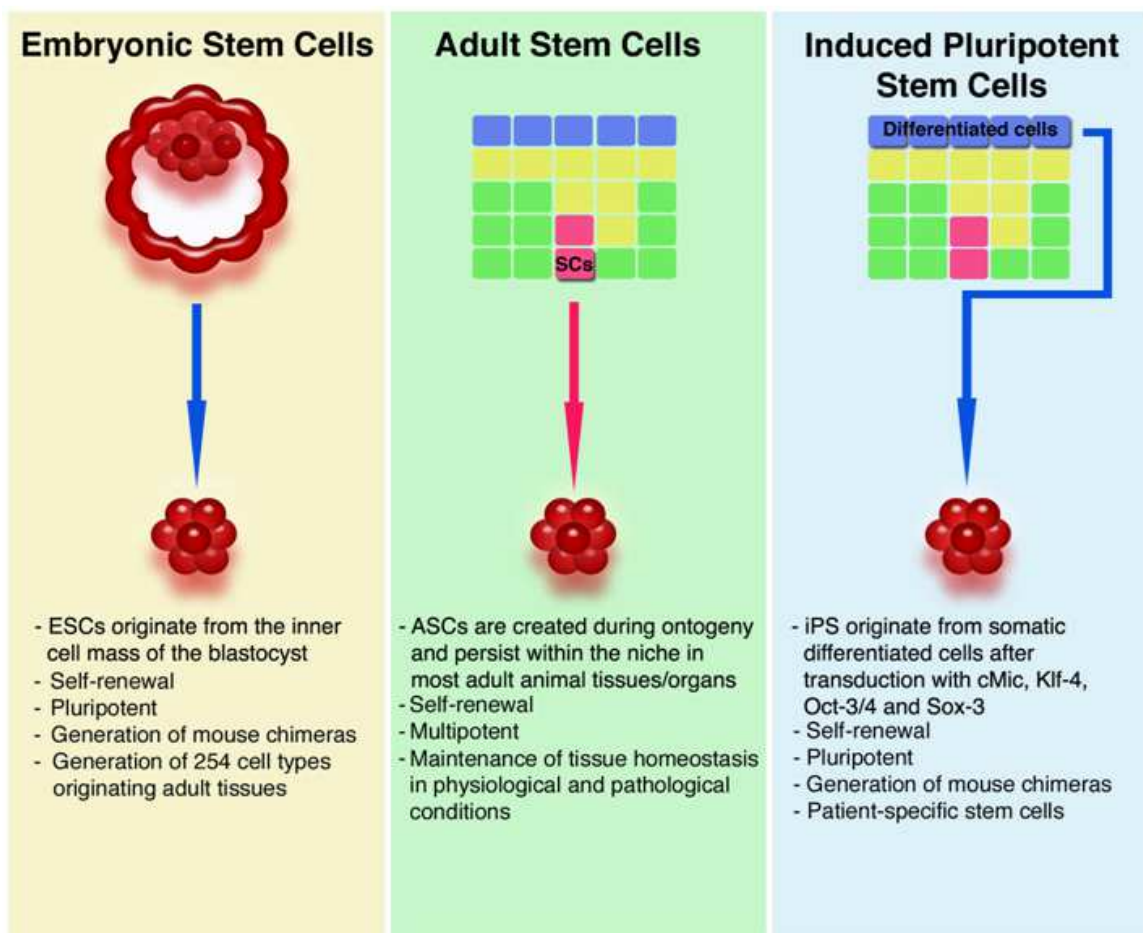


Figure 3: Types of Stem Cells. Summary of embryonic, adult, and induced pluripotent stem cells and their major characteristics (Figure taken from SlideShare, n.d.)

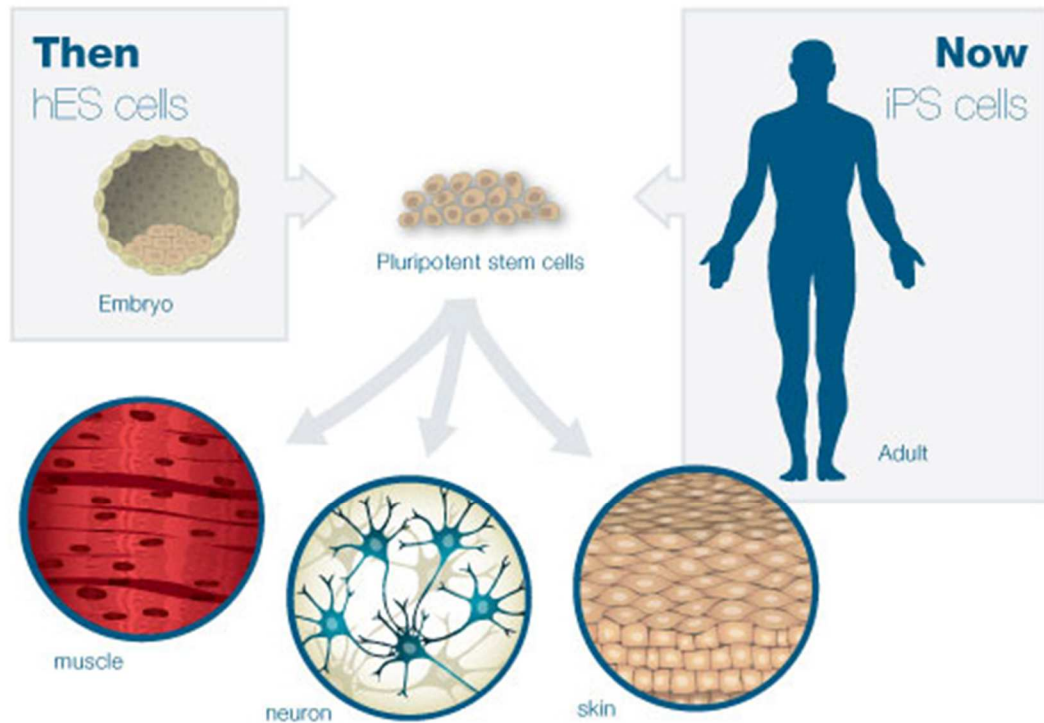


Figure 4: Three different categories of stem cells. Both human embryonic stem (hES) cells and induced pluripotent stem (iPS) cells are pluripotent: they can become any type of cell in the body. While hES cells are isolated from an embryo, iPS cells can be made from adult cells. Figure taken from Roberts (2015).

Adult stem cells are found in the bone marrow and periosteum, including the orofacial tissues like teeth, dental pulp, and supporting structures. Those from orofacial tissues can then be classified into two categories: dental stem cells and nondental oral stem cells. Types of dental stem cells include dental pulp stem (DPSC's), stem cells from human exfoliated deciduous teeth (SHED), and stem cells from apical papilla (SCAP) cells (Figure 5).

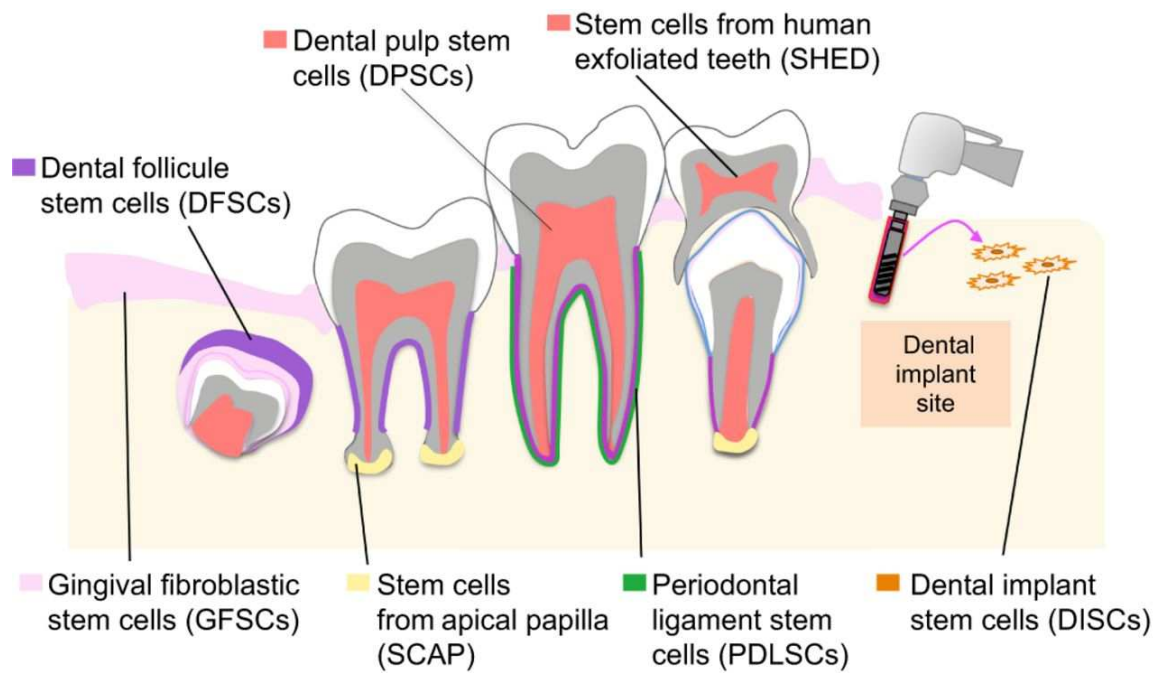


Figure 5: Overview of Dental Stem Cells. Different types of dental mesenchymal stem cells can be categorized by their location of origin and specific tissue. Figure taken from Sharp (2016).

Types of nondental oral stem cells include dental follicle stem cells (DFSC's), periodontal ligament stem cells (PDLSC's) and gingival mesenchymal stem cells (GMSC's) (Heng et al, 2017) (Figure 6). All of the adult stem cells are mesenchymal stem cells in character. Chondrocytes and osteoblasts are derived from a common mesenchymal stem cell (MSC), even though their process of differentiation is very different (Caplan, 1991). Adult MSC's, because of their presence in gingival connective tissue, have osteogenic potential and thus are capable of bone regeneration in mandibular defects. They also have been found to promote bone regeneration by inhibiting lymphocyte proliferation and cytokines and thus restricting the inflammatory response (Shakoori et al, 2017).

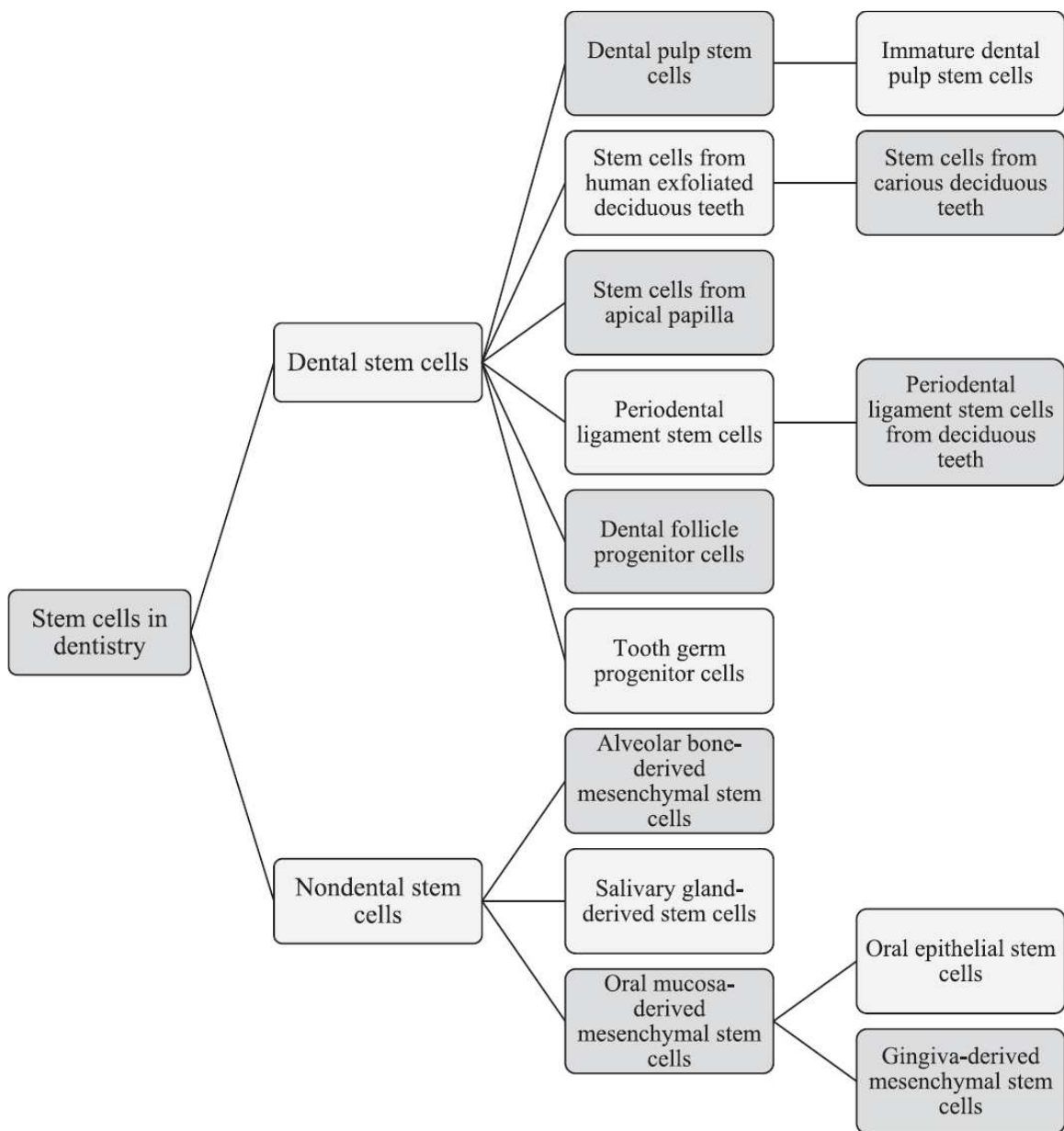


Figure 6: Classification of Stem Cells in Dentistry. Five various types of dental stem cells have been isolated including dental pulp stem cells, periodontal ligament stem cells, stem cells from exfoliated deciduous teeth, dental follicle progenitor cells, and stem cells from the apical papilla. Figure taken from Khazaei et al. 2016.

Regulatory T-cells and anti-inflammatory cytokines are also being recruited by these stem cells (Bansal & Jain, 2015). Embryonic stem cells are only found in the blastocyst stage of development while induced pluripotent stem cells are considered a new source of stem cells. It has been discovered that induced pluripotent stem cells are derived from adult cells by introducing four pluripotency genes, Oct4, Sox2, cMyc, Klf4 (Shakoori et al, 2017). Table 1 summarizes many of these results.

Table 1: Overview of studies describing stem cells and their roles in improving disease models (Shakoori et al, 2017)

MSCs	Markers	Animal Models	Studies	Disease Models
PDLSC	<i>STRO-1, CD146/MUC18</i>	Mouse Swine Swine	Seo et al, ²² 2004 Liu et al, ²³ 2008 Ding et al, ²⁴ 2010	Periodontitis Periodontitis
DPSC	<i>CD105⁺</i>	Dog Rat Rabbit Rabbit	Kerkis et al, ⁴² 2008 Gandia et al, ⁴³ 2008 Monteiro et al, ⁴⁴ 2009 Gomes et al, ⁴⁵ 2010	Muscular dystrophy Myocardial infarct Cerebral ischemia Chemical-induced corneal injury
SHED	<i>Oct-4, Nanog, SSEA-3, SSEA-4, TRA-1-60, TRA-1-81</i>	Rat	Wang et al, ⁴⁶ 2010	Parkinson disease
GMSC	<i>Oct-4, SSEA-4, STRO-1</i>	Mouse Mouse Mouse Rat Rat	Zhang et al, ³³ 2009 Zhang et al, ³² 2010 Su et al, ³⁵ 2011 Wang, 2011 ⁴⁷ Zhang et al, ⁴⁸ 2013	Colitis Wound healing Contact hypersensitivity Mandibular and calvarial defects Arthritis
SCAP	<i>STRO-1</i>	Swine	Sonoyama et al, ²¹ 2006	Tooth regeneration

There are non-dental stem cells that are also used for dental application. In a recent study by Cai et al (2016), a possible method was reported in which pluripotent stem cells derived from human urine were used for growing teeth in mice. The generated teeth in mice had physical properties similar to that of normal human teeth with few exceptions. The advantages to this approach included a noninvasive technique, and a low cost (Bansal & Jain, 2015). Furthermore, dental stem cells can also be used in a vast array of fields in medicine. A major advantage of dental stem cells is their increased availability through routine biological waste produced during dental treatments that would otherwise not be used (Heng et al, 2016). Some examples include applications like regenerating brain tissue, and heart therapies. A study in 2008 by de Mendonca et al, showed human dental pulp stem cells were used to reconstruct large-sized cranial defects in rats. These studies and applications are only a small example of the multiple applications and potential that stem cells of dental origin have in the ability of reconstructing not only skeletal bone but craniofacial structures as well. Many of the studies are confined to animal subjects and even though expanding rapidly, it is important to consider that stem cells are less potent than embryonic stem cells.

Signaling in Bone Regeneration

Bone regeneration is possible through highly organized and coordinated pathways. Several signaling molecules have been found to take place in the different phases described above and all lead to enhance MSCs in bone fracture (Shakoori et al, 2017). The first growth factors found to induce osteoblast differentiation were BMP2 and BMP7. There are also certain therapeutic agents that target the Wnt signaling pathway which also service as direct osteoblast inducers. Factors such as platelet derived growth factor (PDGF) or fibroblast growth factor (FGF) have roles of mitogens and thus serve to increase the number of bone producing cells and therefore increase vascularization. Vascular endothelial growth factor (VEGF) has a special role, where it not only induces new blood vessel formation but it has a direct effect on BMP production. Table 2 indicates several signaling processes in each of the bone regeneration phases (Hankenson et al, 2015).

Table 2: Studies that demonstrate extracellular signaling molecules to promote bone regeneration (Hankenson et al, 2015)

<i>Phase of Healing</i>	<i>Treatment</i>	<i>Subject</i>	<i>Outcome</i>
<i>Inflammation</i>	Pro-macrophage cytokine factor-1	Mouse	Enhanced soft callus formation
	Anti-TNF alpha antibody	Mouse	Fracture healed
	Erythropoietin	Mouse	Increased bone density
	Erythropoietin	Mouse	Enhanced consolidation of bony callus
<i>Cellular proliferation</i>	rhPDF-BB (PUT ABBV)	Human	Significant periodontal defect fill
	AMD3100	Mouse	Larger fracture callus and increased bone material density
	bFGF	Rat (diabetic)	Increased number of osteocytes and improved bone repair
<i>Angiogenesis</i>	DFO (HIF Stabilization)	Mouse	Increased bone formation
	VEGF	Mouse	Increased vascularity in soft tissue around fracture area
<i>MSC Differentiation</i>	Beta-catenin (stabilization of Wnt signaling)	Mouse	Enhanced fracture consolidation
	Anti-Dkk1	Mouse	Enhanced fracture callus formation
	Wnt3a	Mouse	Faster bone regeneration
	R-spondins	Mouse	Promotes bone formation in mouse models of osteoporosis
	P-15	Humans	Early incorporation of bone graft of lumbar fusion
<i>Bone remodeling</i>	RANKL inhibitor	Mouse	Delayed bone remodeling but enhanced bone strength

One should also consider the effects and consequences of producing MSCs for clinical applications. Precursors of MSCs have been found in blood vessels and most of human tissues and thus it is possible to obtain them from an unlimited number of organs. However, it can be challenging and could compromise the safety of the donor. In 2010, Lin et al concluded that for bone marrow (BM), the age of the donor is inversely correlated to the yield of MSCs obtained. The opposite has been found for adipose tissue derived MSCs and the age of the donor (Shi et al, 2005).

SPECIFIC AIMS OR OBJECTIVES

Current literature on stem cells in bone is aimed at developing therapeutic approaches for bone repair and bone regeneration. There are several downsides to bone grafting which makes this a promising alternative approach. Several initial studies have demonstrated that therapy with mesenchymal stem cells (MSC) have been helpful in regenerating bone in fracture healing. However, it is also important to recognize that these studies are done in animal models and the clinical translation to human subjects is quite challenging. This thesis will focus on introducing these studies, and explaining the current advances in those experiments, especially on oral bone regeneration/craniofacial regeneration.

- Discuss how stem cells work
- Discuss how bone regeneration works
- Identify studies of stem cell in skeletal bone regeneration
- Identify studies of stem cell in craniofacial regeneration
- Discuss the types of benefits that stem cell use can have in improving human disease conditions

From the objectives above, we hope to learn how stem cells are able to play a role in the betterment of bone regeneration.

PUBLISHED STUDIES

In vitro differentiation of neural-like cells from human teeth-derived stem cells has been studied by Nourbakhsh et al in 2011 (Figure 7) (Nakamura et al, 2009). Obtaining SHED is a better alternative in obtaining stem cells as it causes no harm or injury in children who lose primary teeth. Furthermore, DPSC isolation is possible even five days after tooth extraction, which demonstrates the usefulness of obtaining stem cells from children who are losing primary teeth (Nourbakhsh et al, 2011). The proliferation rate of SHED cells, however, is higher than DPSCs and BM-MSCs (Figure 8) (Nourbakhsh et al, 2011).

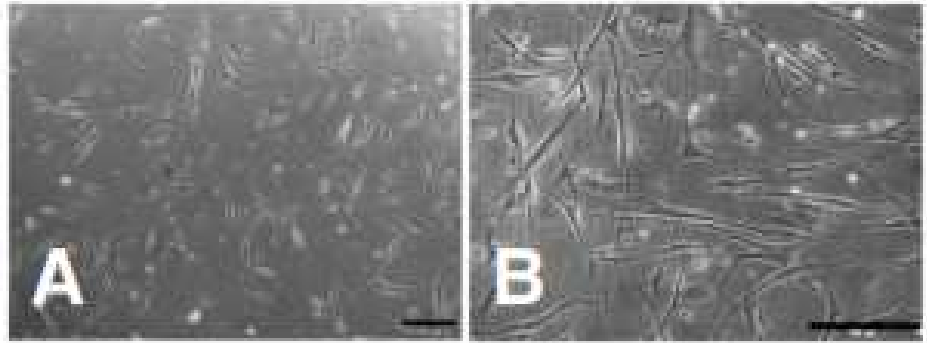


Figure 7: SHED Cell Differentiation: Image by phase contrast shows SHED cell differentiation five (A) and ten (B) days after neural induction. Figure taken from Nourbakhsh et al, 2011.

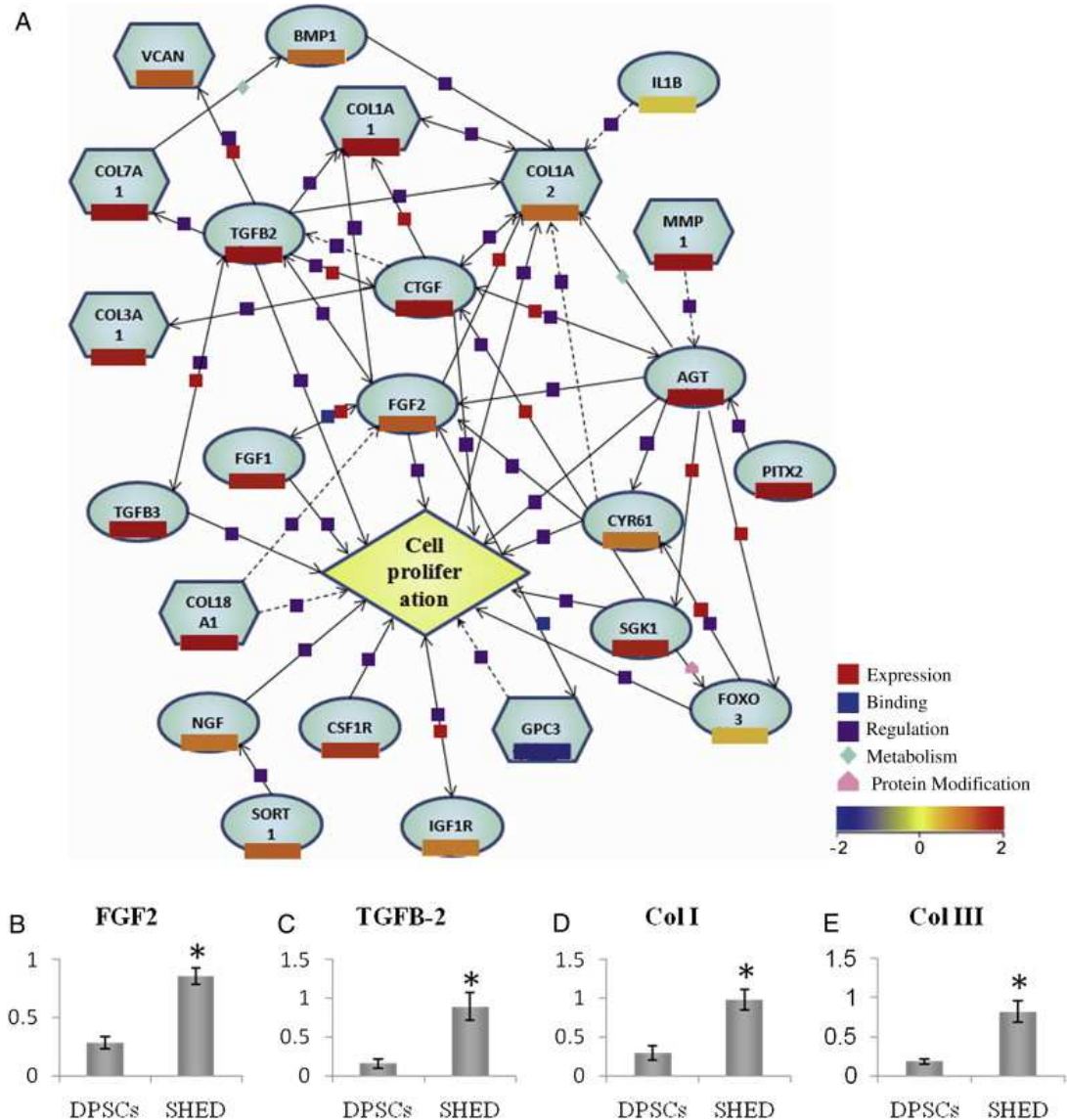


Figure 8: Network of cell proliferation. A) Pathway analysis of gene categories that were prominent in SHED compared with DPSCs. B-E) PCR analysis performed to validate the expression level of the genes in the cell proliferation pathway. FGF2, TGF-beta 2, Col I, and Col II had a higher expression in SHED compared with DPSCs. Figure taken from Nakamura et al, 2009.

Kerkis et al (2008) concluded that SHED cells cause no harm by implanting human SHED cells to golden retriever muscular dystrophy dogs; no immune rejection was present and the dogs presented with betterment in their bone engraftment and clinical conditions. After culturing SHED cells, viable cells with elongated shapes were detected after one day. Additionally, these cultured SHED cells expressed the marker ALP while the *ex vivo*-expanded SHED cells expressed the cell surface molecules STRO-1 and CD146, which are two early MSC markers found in bone marrow mesenchymal stem cells. In order to evaluate whether SHED cells could later differentiate into mineralized cells, the cells were supplemented with L-ascorbate-2-phosphate, dexamethasone, and inorganic phosphate. The results showed calcium accumulation *in vitro* (Kerkis et al, 2008).

Experimental evidence for bone tissue engineering was given support by a study performed by Quarto in 2001. This study published results obtained in three patients with several severe bone defects. Bone marrow stem cells were isolated and expanded *ex vivo* under the stimulation of several growth factors before the implantation of hydroxyapatite scaffolds for each bone defect. Through this implantation, all patients recovered limb function and after several months, good integration of the cells with the recipient was observed. After the implantation, the use of autologous bone marrow encased within a titanium cage with bone mineral blocks for reconstructive mandibular reconstruction was reported. This was implanted in the latissimus dorsi muscle for seven weeks to

allow for vascularization and growth before transplantation of the bone muscle flap. This treatment has been deemed successful in patients after 6-7 years of follow up (Quarto et al. 2001).

Stem cells have also been used in the treatment of several blood diseases and other types of diseases. Parkinson's disease is a movement disorder that is characterized by the damage of neurons in the midbrain, which results in stiffness, rest tremor, and bradykinesia. It has been shown that embryonic stem cells have the ability to differentiate into neural stem cells which in turn develop into dopaminergic neurons. A study published by Sonntag et al. (2007) showed that the combination of these cells with a bone morphogenic protein antagonist (Noggin) results in a higher production of stem cells that can differentiate into dopaminergic neurons. Venkataramana et al (2010) used mesenchymal cells and injected them into a ventricular area of seven patients with Parkinson's disease and observed improvement in several symptoms, including: gait, freezing episodes, and facial gestures. Induced pluripotent stem cells and fetal neural stem cells have also been used in a study for the treatment of Parkinson disease patients (Figure 9) (Venkataramana et al, 2010).

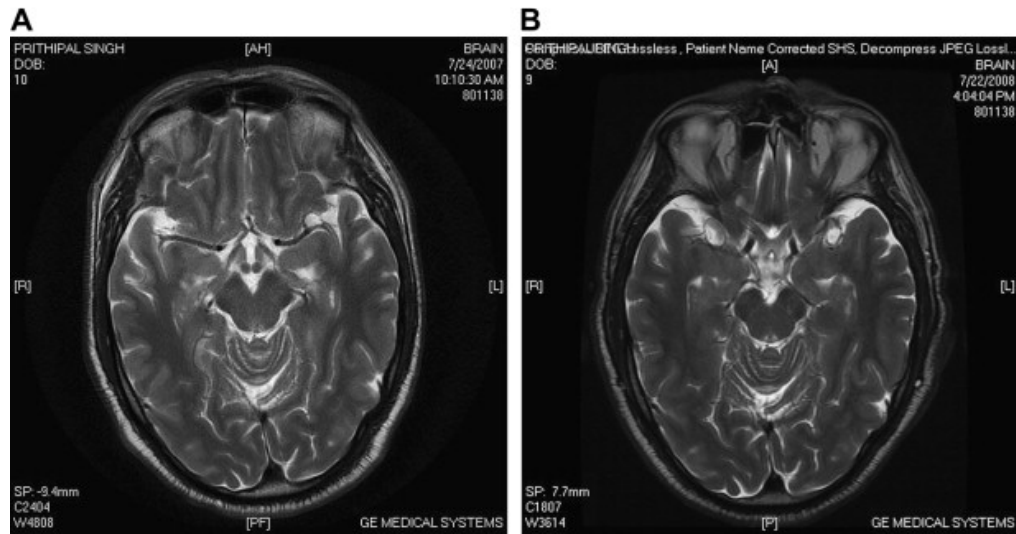


Figure 9: Imaging before and after stem cell transplantation. There was no abnormal evidence after stem cell transplantation nor were there any other significant changes in these images. Figure taken from Venkataramana et al, 2010.

However, the use of iPSCs have led to tumor in those patients (Wernig et al, 2008). Stem cells have also been used in the improvement of patients with amyotrophic lateral sclerosis (ALS), amyotrophic lateral sclerosis. It is a fatal neurodegenerative disease that is characterized with the destruction of neurons of the spinal cords and neurons of the brain. Xu et al in 2009 showed the replacement of human neural stem cells in mice resulted in their differentiation to neurons with GABA (gamma-aminobutyric acid) ergic phenotype which led to

beneficial effects for motor neurons and thus improved the symptoms. It has also been shown that astrocyte replacement through injected astrocytes called Glial-Restricted precursors increased survival in mice, slowed down respiratory disorders, and reduced motor neuron damage (Lepore et al, 2008).

Mesenchymal stem cells were used in muscles of mice with familial ALS; these cells caused glial cell factor secretion which served to increase the number of motor neuron cell bodies in the spinal cord and prolonged survival for 28 days (Suzuki et al, 2008). Alzheimer's disease is also a neurodegenerative disease which has shown improvement with the use of stem cells. Neural stem cells have been used because they have the ability to differentiate into neurons, astrocytes, and oligodendrocytes. Neural stem cells were injected into the basal part of the forebrain of rats and it was observed that the group which received the injection had a much greater number of cholinergic neurons versus the group that had not received any treatment (Table 3)(Xuan et al, 2009).

Table 3 : BDNF and NSCs increase the number of cholinergic neurons.
 Comparison of medial septum and vertical diagonal band of brain in rats. Table taken from Xuan et al, 2009.

	MS		VDB	
	Lesioned side	(%)	Lesioned side	(%)
Control group	42.47 ± 1.88		97.66 ± 2.77	
Lesioned group	15.28 ± 2.41*	35.9	50.08 ± 1.86*	50.7
NSCs-transplanted group	28.57 ± 1.53*,#	65.7	68.91 ± 2.45*,#	72.4
Combination-treated group	39.88 ± 2.33#,**	85.0	80.93 ± 2.52#,**	81.4

Embryonic and neural stem cells have also been shown to play a huge role in the treatment of spinal cord injury. The injection of embryonic stem cells, as demonstrated in a study by Kerr et al in 2010, increased neurological responses in treated mice compared with control mice. However, it was also found these cells can cause tumors and thus making it challenging to develop a study similar to that for humans. Olfactory ensheathing cells are special glial cells that only exist in the olfactory system and aid with the production of olfactory neurons. Lopez-Vales et al, in 2006, examined the use of these cells in rats and found that the injection of olfactory ensheathing cells improved performance and behavior with an increased regeneration of axons. In stroke, many studies have shown to facilitate the improvement of symptoms and have been shown to help in the protection of neurons, regulation of immune system, the increase of internal healing processes, and vascular regeneration. A study led by Gupta et al in 2007 demonstrated that autologous stem cells injected in children resulted in an improvement in their deteriorating condition. From an initial 12 children, 5 patients died due to cirrhosis, 4 patients recovered from cholangitis, and in 9 patients either liver stiffness or liver function was improved. Table 4 summarizes the different types of stem cells that have been used in the treatment for stroke (Larijani et al, 2012, Gupta et al, 2007).

Table 4: Different types of stem cells used in the treatment of stroke. Different types of cells used for the protection of neurons, regulation of immune system, increase of internal *healing processes*, and *vascular regeneration* are shown. (Larijani et al, 2012)

Type of cell	Effect	Type of Study (animal / human)
Neural progenitor cells derived from human Embryonic stem cells	Neural stem cells derived from human embryonic stem cells, differentiated to neurons, oligodendrocytes and astrocytes.	animal
Neural progenitor cells derived from embryos	Human Neurosphere cells cultured in vitro improved neurological activity in mice. Also some synapses between neurons derived from human embryonic stem cells and host neurons were produced.	animal
Immortalized cell lines	Transplantation of cells, that were immortalized through connection of transgene c-mycERTAM and then cultured in the presence of 4-hydroxy tamoxifen, to animal model resulted in reduction of functional impairments	animal
Stromal cells of human adipose tissue	Injection of stromal cells of human adipose tissue into the left ventricle of mouse brain showed that these cells have ability of survival, migration, and functional improvement after stroke.	animal
Peripheral blood cells	The injections of peripheral blood stem cells as well as cord blood stem cells in mice resulted in decrease of hyperactivity due to stroke and progression of motor asymmetry.	animal
Blood cells of human umbilical cord	Blood cells of Human umbilical cord after intravenous injection into rat brain, had the ability of survival, migration, and improvement of performance after stroke	animal
Mesenchymal cells	Intravenous administration of human mesenchymal cells to mice improved performance and reduced infarction rate and neuroprotective.	animal
Bone marrow stromal cells	Injection of bone marrow stromal cells increased axonal plasticity that can cause neurological functional improvement.	animal

Stem cells have also been shown to help with bone diseases, where mesenchymal cells will differentiate to chondrocytes and osteoblasts and lead to a fracture heal. Non union is an orthopedic issue which causes very prolonged hospitalization. In a study by Marcacci et al, 2007, bone marrow stromal cells were grafted in defective areas in four patients with large bone disorders. After some time, radiography and CT scans showed that bone healing had occurred. The same effect can be observed in osteogenesis imperfecta, a hereditary disorder that is characterized by bone fragility, bone density reduction, and connective tissue disorders. Mesenchymal cells were examined in humans in a study by Horwitz et al in 2002. Mesenchymal cells derived from bone marrow of donors were injected twice into six children with severe osteogenesis imperfecta (OI) who had been treated with normal bone marrow transplantation previously. These patients, in comparies to those patients that were the same age and sex but had not received treatment, improved an average of 70 percent with no side effect (Figure 10) (Horwitz et al, 2002).

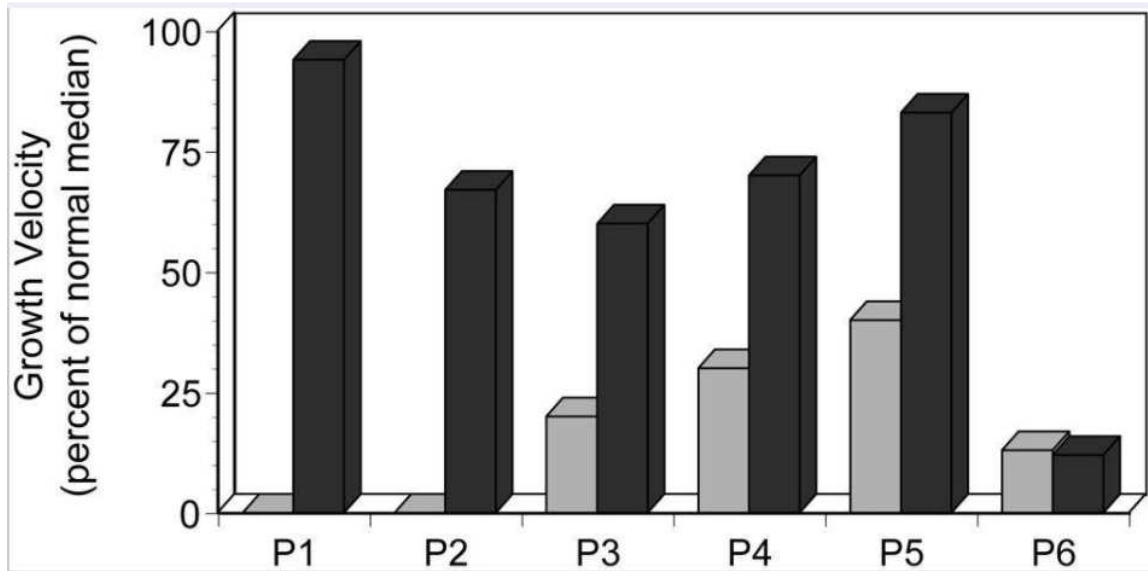


Figure 10: Growth Stimulation in Osteoimperfecta Patients. Growth velocity of the patients during the 6 months immediate before (light shadow) and after (dark shadow) the first MSC infusion. Figure taken from Horwitz et al, 2002.

Hypophosphatasia is a rare disorder which results in metabolic bone disorder because of reduction of tissue-nonspecific alkaline phosphatase activity. This disease in children comes in the form of rickets which leads to death in the first years of life because of weakness in respiratory muscles. No treatment by any drug has been found, however, Cahill et al in 2007, injected heterogeneous stem cells into three different locations intraperitoneally, subcutaneously, and intravenously. Four months after the injection, evidence showed increased mineralization and after seven years, the child was active and had demonstrated a reduced severity of the disease (Figure 10) (Cahill et al in 2007).

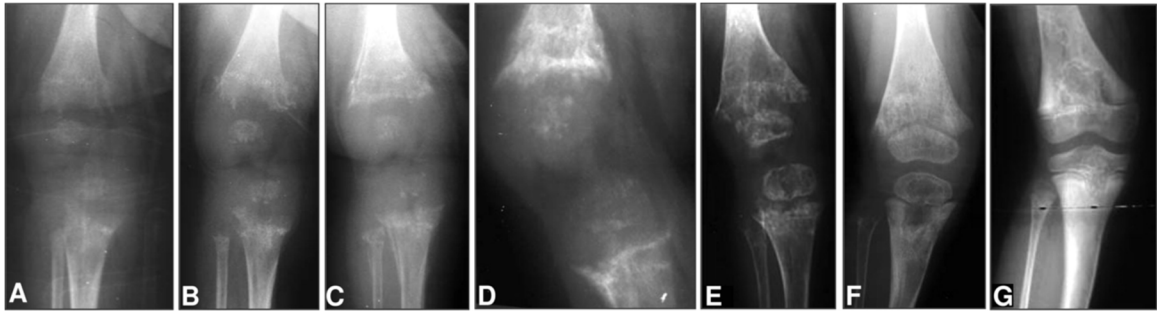


Figure 11: Knee radiographs before and after bone transplantation. Radiographs of the right knee comparison of before (Figures A-C) and after (D-G) bone transplantation. Significant improvement is shown in the reappearance of the epiphyses after treatment. Figure taken from Cahill et al in 2007.

The regeneration of ischemic cardiac muscle and vascular endothelium was studied by Jackson et al in 2001. To identify a source of stem cells capable of restoring damaged cardiac tissue, the group transplanted hematopoietic stem cells into irradiated mice by coronary artery occlusion for 60 minutes. The engrafted cells migrated into ischemic cardiac muscle and blood vessels, differentiated to cardiomyocytes and endothelial cells and finally contributed to the formation of functional tissue. When transplanted into the bone marrow of irradiated mice, these cells marked with the lacZ gene regenerated the hematopoietic system. It was also found that lacZ positive cells could also participate in neovascularization in regenerating heart tissue. The results of this study demonstrate the potential of hematopoietic stem cells in a strategy that could eventually be used in human patients (Jackson et al in 2001).

Several studies have also focused on stem cell therapy in craniofacial bone regeneration. A randomized controlled trial conducted by Kaigler et al in 2013 investigated tissue repair cell therapy through stem cells to reconstruct defects in craniofacial bone. The study involved twenty four patients who were randomized to receive either guided bone regeneration (GBR) or tissue repair cell transplantation. Bone biopsies were obtained and the patients underwent quantitative micro-computer tomographic and bone histomorphometric analysis. Oral implants were placed in the patients, restored, and loaded with tooth restorations; the patients were followed up for the treatment for 1 year following therapy. Following a 6 week period, it was shown there was greater radiographic

bone height in patients that received the tissue repair cell transplantation versus the group that received guided bone regeneration. At 12 weeks, the tissue repair cell group showed 80.1 bone fill while the guided bone regeneration group showed 74.6 bone fill. Figure 2 shows images of tissue repair cell and guided bone regeneration treatment sites at time 0, 6 weeks following treatment, and fully restored 1 year after the initial surgery. The study determined that in the guided bone regeneration group, the regenerated tissues at 6 weeks appeared fibrous and vascular, with many specimens being notably soft during biopsy. However, the tissues in the tissue repair cell group exhibited a bone like appearance clinically, achieved higher vascularity, and were more dense. Additionally, the study found there was a greater need for the guided bone regeneration group to receive secondary bone grafting procedures, compared to the tissue replacement cell transplantation group. In the GBR groups at 6 and 12 weeks there was sixfold greater implant exposure which needed more extensive secondary grafting, relative to the need in the tissue repair cell treated group. The sizes of the implants used in both groups were very similar and all implants achieved integration into the bone following 6 months after treatment. Bone core biopsies were also analyzed at 6 and 12 weeks; bone volume fraction and bone mineral density were measured. At 6 weeks, the bone volume fraction for the guided bone regeneration group was 13, compared to 28 for the tissue repair cell transplantation group. Bone repair in the tissue repair cell group had a greater than twofold bone mineral density, as well. However, upon the analysis at 6 and

12 weeks, no statistically significant differences were found in measures of percent bone area/tissue area (Kaigler et al, 2013).

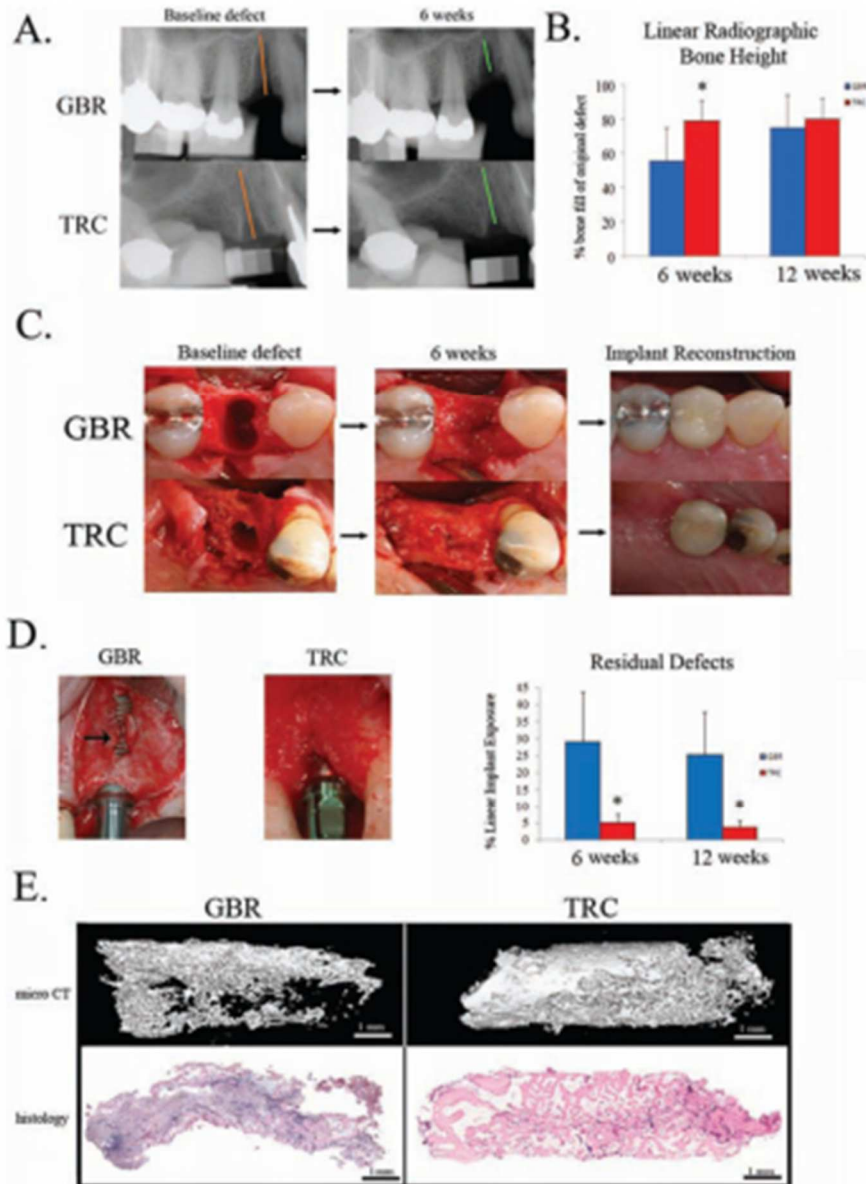


Figure 12: Comparing results for guided bone regeneration and tissue repair cell groups in implants A) Radiographic images of bone height density for guided bone regeneration groups and tissue repair cell groups B) Standardized digital radiography used to analyze linear changes in bone height C) Clinical photographs of the treatment site following removal of the tooth, at reentry after 6 weeks, and 12 months after treatment with full restoration with a crown D) Residual bone defects were noticed in some patients E) Tomographic and histomorphometric analyses used for bone volume fraction and bone mineral density. Figure taken from Kaigler et al in 2013.

DISCUSSION

Many studies are evaluating the use tissue engineering as regenerative medicine. In the studies described above and many other studies, the mechanism of intrinsic bone repair has been very effective with very little external intervention required. Bone tissue engineering has demonstrated to bring clinical relief and is especially important because it allows application to a greater number of patients, especially to those patients for whom traditional bone grafting procedures are unfeasible. The changing trend to acquire a developmental engineering approach in the reformation of bone follow closely the natural process of bone development as outlined in the introduction of this thesis, through the remodeling of hypertrophic cartilage templates via endochondral ossification (Fisher et al, 2016).

Even though the mentioned studies provide layers of evidence and help catalyze future studies in stem cells and bone regeneration, it is important to acknowledge that much is still left to be accomplished in this field. Both deciduous teeth and permanent teeth are able to differentiate into osteoblasts, adipocytes, and chondrocytes, although quantification results indicated that deciduous teeth exhibited better differentiation quality than permanent teeth. It was found that deciduous teeth are capable of differentiating both *in vitro* and *in vivo* into osteoblasts, adipocytes, odontoblasts, and even hepatocytes. Permanent teeth, however, were more appropriate for dental tissue regeneration and

neurodegenerative diseases (Govindasamy et al, 2010). This is important to acknowledge when choosing the most desirable cells for a specific outcome in bone regeneration. Not only knowing which cells differentiate most appropriately but also knowing which stem cells are most appropriate to use are key factors in bone tissue engineering.

Govindasamy et al, in 2010, also demonstrated dental pulp stem cells were likely to have the greatest potential for neural differentiation than any other stem cells. One possible test to evaluate the differentiation quality of varying cells is to test the presence of neuroal mRNA and determine whether that affects end point differentiation. This was done with deciduous and permanent teeth through the induction of neurospheres in which cells aggregate to floating spheres (Reynolds et al, 1992). An interesting finding was that neurospheres were found in a higher amount in permanent teeth compared to deciduous teeth. One of the most probable reasons for that finding is the fold expression of nestin that was found in permanent teeth versus deciduous teeth (Dahlstrand et al, 1995). Nestin is an essential part in the formation of neurospheres, and the abundant expression of nestin in permanent teeth could enable permanent teeth cells to differentiate more efficiently than deciduous teeth. There is not enough proof of that and it is something that would be a step forward in evaluating the use of dental stem cells in bone regeneration and reformation. Figure 12 gives a quick summary of how dentistry could be transformed in the next 10 years with these new possible methods as described above.

THE FUTURE OF DENTISTRY

Dentistry could be transformed in the next 10 years as researchers develop procedures to regrow dental tissue.

HARVESTING STEM CELLS

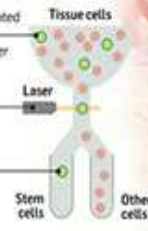
Adult stem cells can be extracted from tissue at several harvest sites for use in dental treatment. These cells can produce every type of tissue in the teeth and gum.

ISOLATING CELLS

The cells are treated with a green fluorescent marker that binds only to stem cells.

A laser beam illuminates cells.

Cells that glow green are stem cells and are sorted into a test tube.



RESEARCH

Treatments being studied at Nova Southeastern University:

REGENERATING A ROOT CANAL

A liquid base material, with the patient's stem cells, is put into the pulp area.

A constant blood supply supports the growth of new tissue and nerve vessels.



GROWING A NEW TOOTH

An incision is made in the gum to expose the "tooth socket" in

KNOCKED-OUT TEETH

The root of the tooth is treated with stem cells

Figure 13: Future of Dentistry. Figure showing the future of dental research in the next 10 years and how stem cells could be used to correct many oral issues, including bone reformation (Figure taken from Elevate Strategy Consulting , n.d. <http://elevatestrategyconsulting.com/innovation/stemcellsindentistry/>)

Several recent advances in biomaterials have led to a transition from non-porous, inert materials to more osteoconductive, more porous biomaterials. These can be used as delivery machines, such as porous ceramics of hydroxyapatite and beta-tricalcium phosphate loaded with MSC's (Mao et al, 2006). Mesenchymal stem cells can be obtained from the same individual and induced to differentiate into both osteogenic and chondrogenic cells (Nakayama et al, 2003).

There are many challenges ahead that limit the development and availability of these therapeutic treatment approaches. Additionally, between countries, the unequal resources, differences in access to opportunities by the local population, and variable standards of public and community health comprise another major hurdle to overcome to achieve success in this field.

REFERENCES

- Avinash, K., Malaippan, S., & Dooraiswamy, J. N. (2017). Methods of Isolation and Characterization of Stem Cells from Different Regions of Oral Cavity Using Markers: A Systematic Review. *International Journal of Stem Cells*, 10(1), 12–20. <https://doi.org/10.15283/ijsc17010>
- Cai, J., Zhang, Y., Liu, P., Chen, S., Wu, X., Sun, Y., ... Pei, D. (2013). Generation of tooth-like structures from integration-free human urine induced pluripotent stem cells. *Cell Regeneration*, 2(1), 6. <https://doi.org/10.1186/2045-9769-2-6>
- Caplan, A. I. (1991). Mesenchymal stem cells. *Journal of Orthopaedic Research*, 9(5), 641–650. <https://doi.org/10.1002/jor.1100090504>
- Colnot, C., Zhang, X., & Tate, M. L. K. (2012). Current insights on the regenerative potential of the periosteum: Molecular, cellular, and endogenous engineering approaches. *Journal of Orthopaedic Research*, 30(12), 1869–1878. <https://doi.org/10.1002/jor.22181>
- Dahlstrand, J., Lardelli, M., & Lendahl, U. (1995). Nestin mRNA expression correlates with the central nervous system progenitor cell state in many, but not all, regions of developing central nervous system. *Brain Research. Developmental Brain Research*, 84(1), 109–129.
- de Mendonça Costa, A., Bueno, D. F., Martins, M. T., Kerkis, I., Kerkis, A., Fanganiello, R. D., ... Passos-Bueno, M. R. (2008). Reconstruction of Large Cranial Defects in Nonimmunosuppressed Experimental Design With Human Dental Pulp Stem Cells: *The Journal of Craniofacial Surgery*, 19(1), 204–210. <https://doi.org/10.1097/scs.0b013e31815c8a54>
- DiPietro, L. A. (2014). Oral Stem Cells: The Fountain of Youth for Epithelialization and Wound Therapy? *Advances in Wound Care*, 3(7), 465–467. <https://doi.org/10.1089/wound.2012.0421>
- Elevate Strategy Consulting (n.d.) The Future of Dentistry. Retrieved on 8/1/17 from <http://elevatestrategyconsulting.com/innovation/stemcellsindentistry/>
- Encyclopedia Britannica, n.d. Bone Remodeling. Retrieved on 8/1/17 from <https://www.britannica.com/science/bone-anatomy>

Fisher SA, Doree C, Mathur A, Taggart DP, Martin-Rendon E. (2016) [Stem cell therapy for chronic ischaemic heart disease and congestive heart failure](#). *Cochrane Database Syst Rev*. 2016 Dec 24:12:

Govindasamy, V., Abdullah, A. N., Sainik Ronald, V., Musa, S., Che Ab. Aziz, Z. A., Zain, R. B., ... Abu Kasim, N. H. (2010). Inherent Differential Propensity of Dental Pulp Stem Cells Derived from Human Deciduous and Permanent Teeth. *Journal of Endodontics*, 36(9), 1504–1515. <https://doi.org/10.1016/j.joen.2010.05.006>

Heng, B. C., Lim, L. W., Wu, W., & Zhang, C. (2016). An Overview of Protocols for the Neural Induction of Dental and Oral Stem Cells In Vitro. *Tissue Engineering Part B: Reviews*, 22(3), 220–250. <https://doi.org/10.1089/ten.teb.2015.0488>

Hernigou P, Trousselier M, Roubineau F, Bouthors C, Chevallier N, Rouard H, Flouzat-Lachaniette CH. (2016) Stem Cell Therapy for the Treatment of Hip Osteonecrosis: A 30-Year Review of Progress.. *Clin Orthop Surg*. 2016 Mar;8(1):1-8. doi: 10.4055/cios.2016.8.1.1..

Jackson, K. A., Majka, S. M., Wang, H., Pocius, J., Hartley, C. J., Majesky, M. W., ... Goodell, M. A. (2001). Regeneration of ischemic cardiac muscle and vascular endothelium by adult stem cells. *The Journal of Clinical Investigation*, 107(11), 1395–1402. <https://doi.org/10.1172/JCI12150>

Jain, A., & Bansal, R. (2015). Current overview on dental stem cells applications in regenerative dentistry. *Journal of Natural Science, Biology and Medicine*, 6(1), 29. <https://doi.org/10.4103/0976-9668.149074>

Kaigler, D., Pagni, G., Park, C. H., Braun, T. M., Holman, L. A., Yi, E., ... Giannobile, W. V. (2013). Stem Cell Therapy for Craniofacial Bone Regeneration: A Randomized, Controlled Feasibility Trial. *Cell Transplantation*, 22(5), 767–777. <https://doi.org/10.3727/096368912X652968>

Kerkis, I., Ambrosio, C. E., Kerkis, A., Martins, D. S., Zucconi, E., Fonseca, S. A., ... Zatz, M. (2008). Early transplantation of human immature dental pulp stem cells from baby teeth to golden retriever muscular dystrophy (GRMD) dogs: Local or systemic? *Journal of Translational Medicine*, 6(1), 35. <https://doi.org/10.1186/1479-5876-6-35>

Khazaei M, Bozorgi A, Khazaei S, Khademi A. Stem cells in dentistry, sources, and applications. *Dental Hypotheses* [serial online] 2016 [cited 2017 Jul 28];7:42-52. Available from: <http://www.dentalhypotheses.com/text.asp?2016/7/2/42/183764>

- Larijani, B., Esfahani, E. N., Amini, P., Nikbin, B., Alimoghaddam, K., Amiri, S., ... Ghavamzadeh, A. (2012). Stem cell therapy in treatment of different diseases. *Acta Medica Iranica*, 50(2), 79–96.
- Lepore, A. C., Rauck, B., Dejea, C., Pardo, A. C., Rao, M. S., Rothstein, J. D., & Maragakis, N. J. (2008). Focal transplantation–based astrocyte replacement is neuroprotective in a model of motor neuron disease. *Nature Neuroscience*, 11(11), 1294–1301. <https://doi.org/10.1038/nn.2210>
- Lin CS1, Xin ZC, Deng CH, Ning H, Lin G, Lue TF. Defining adipose tissue-derived stem cells in tissue and in culture. (2010). *Histology and Histopathology*, (25), 807–815. <https://doi.org/10.14670/HH-25.807>
- Marcucio RS, Nauth A, Giannoudis PV, Bahney C, Piuizzi NS, Muschler G, Miclau T 3rd. (2016) [Stem Cell Therapies in Orthopaedic Trauma](#). *J Orthop Trauma*. 2015 Dec;29 Suppl 12:S24-7.
- Nakamura, S., Yamada, Y., Katagiri, W., Sugito, T., Ito, K., & Ueda, M. (2009). Stem Cell Proliferation Pathways Comparison between Human Exfoliated Deciduous Teeth and Dental Pulp Stem Cells by Gene Expression Profile from Promising Dental Pulp. *Journal of Endodontics*, 35(11), 1536–1542.
- Nourbakhsh, N., Soleimani, M., Taghipour, Z., Karbalaie, K., Mousavi, S.-B., Talebi, A., ... Baharvand, H. (2011). Induced in vitro differentiation of neural-like cells from human exfoliated deciduous teeth-derived stem cells. *The International Journal of Developmental Biology*, 55(2), 189–195. <https://doi.org/10.1387/ijdb.103090nn>
- Quarto R, Mastrogiacomo M, Cancedda R, Kutepov SM, Mukhachev V, et al. (2001) Repair of large bone defects with the use of autologous bone marrow stromal cells. *N Engl J Med* 344: 385–386.R.
- Reynolds, B. A., & Weiss, S. (1992). Generation of neurons and astrocytes from isolated cells of the adult mammalian central nervous system. *Science (New York, N.Y.)*, 255(5052), 1707–1710.
- Roberts, J (2015) Embryonic Stem Cell Controversy Ethics. Retrieved from <http://mirrorsmagazine.com/embryonic-stem-cell-controversy-ethics>
- Schemitsch, E., & Nauth, A. (2012). Stem cells for the repair and regeneration of bone. *Indian Journal of Orthopaedics*, 46(1), 19. <https://doi.org/10.4103/0019-5413.91630>

- Shakoori, P., Zhang, Q., & Le, A. D. (2017). Applications of Mesenchymal Stem Cells in Oral and Craniofacial Regeneration. *Oral and Maxillofacial Surgery Clinics of North America*, 29(1), 19–25. <https://doi.org/10.1016/j.coms.2016.08.009>
- Sharpe, P. T. (2016). Dental mesenchymal stem cells. *Development*, 143(13), 2273–2280. <https://doi.org/10.1242/dev.134189>
- Shield Health (n.d.). Four Stages of Wound Healing. Retrieved on 8/1/17 from <http://www.newhealthadvisor.com/wound-healing-process.html>
- SlideShare (n.d.) Stem Cell and Cloning. Retrieved 7/27/17 from <https://www.slideshare.net/PALWINDERGILL/stem-cell-and-cloning-presentation>
- Sonntag, K.-C., Pruszak, J., Yoshizaki, T., van Arensbergen, J., Sanchez-Pernaute, R., & Isacson, O. (2007). Enhanced Yield of Neuroepithelial Precursors and Midbrain-Like Dopaminergic Neurons from Human Embryonic Stem Cells Using the Bone Morphogenic Protein Antagonist Noggin. *Stem Cells*, 25(2), 411–418. <https://doi.org/10.1634/stemcells.2006-0380>
- Strong, A. L., Neumeister, M. W., & Levi, B. (2017). Stem Cells and Tissue Engineering: Regeneration of the Skin and Its Contents. *Clinics in Plastic Surgery*, 44(3), 635–650. <https://doi.org/10.1016/j.cps.2017.02.020>
- Suzuki, M., McHugh, J., Tork, C., Shelley, B., Hayes, A., Bellantuono, I., ... Svendsen, C. N. (2008). Direct Muscle Delivery of GDNF With Human Mesenchymal Stem Cells Improves Motor Neuron Survival and Function in a Rat Model of Familial ALS. *Molecular Therapy*, 16(12), 2002–2010. <https://doi.org/10.1038/mt.2008.197>
- Walmsley, G. G., Ransom, R. C., Zielins, E. R., Leavitt, T., Flacco, J. S., Hu, M. S., ... Wan, D. C. (2016). Stem Cells in Bone Regeneration. *Stem Cell Reviews and Reports*, 12(5), 524–529. <https://doi.org/10.1007/s12015-016-9665-5>
- Wernig, M., Zhao, J.-P., Pruszak, J., Hedlund, E., Fu, D., Soldner, F., ... Jaenisch, R. (2008). Neurons derived from reprogrammed fibroblasts functionally integrate into the fetal brain and improve symptoms of rats with Parkinson's disease. *Proceedings of the National Academy of Sciences*, 105(15), 5856–5861. <https://doi.org/10.1073/pnas.0801677105>
- Xu, N., Papagiannakopoulos, T., Pan, G., Thomson, J. A., & Kosik, K. S. (2009). MicroRNA-145 Regulates OCT4, SOX2, and KLF4 and Represses Pluripotency in Human Embryonic Stem Cells. *Cell*, 137(4), 647–658. <https://doi.org/10.1016/j.cell.2009.02.038>

Xuan, A. G., Luo, M., Ji, W. D., & Long, D. H. (2009). Effects of engrafted neural stem cells in Alzheimer's disease rats. *Neuroscience Letters*, 450(2), 167–171.
<https://doi.org/10.1016/j.neulet.2008.12.001>

VITA

