

Dentistry: Are Stem Cells the Future?

Tova Zemel

Tova Zemel will graduate in June 2019 with a Bachelor of Science degree in Biology.

Abstract

Stem cell research is currently advancing in every area of medicine. New information about regenerating stem cells is being uncovered on a daily basis. An area of stem cells that has not been focused on until recently is the use of dental stem cells. The objective of this paper is to elucidate the most current research about dental stem cells. Much of what is discussed in this paper has not been implemented yet, and is still in clinical trials. Dental stem cells are important because they could be an alternative way of treating caries, performing root canals, and other traumas to teeth instead of the non-biomaterial currently used. Present research involving dental stem cells includes analyzing the differences between the five dental stem cells, testing out optimal in vitro conditions of dental stem cell proliferation, and implementing lasers in regeneration.

Introduction

For many people, the word dentist brings up memories of fear and pain. Although dentist visits may be traumatic for some people, they are quite necessary. However, there may be a way to help patients with dental caries and trauma to avoid this unpleasantness. By expanding modern technology through ongoing research, regeneration of teeth and neighboring tissue without drills, tools, and cement may very well be the next advancement in dentistry.

The term 'stem cell' was first coined by Haeckel, a German biologist, in 1868. In 1908, Alexander Maksimov, a Russian histologist, suggested the existence of hematopoietic stem cells. Dental stem cells were not acknowledged until 1932, when Feldman, a stomatologist, was able to prove that dental pulp stem cells did grow under certain biological conditions. After this discovery, dentists were able to implement tooth therapy for dental pulp regeneration by using a dentine filling which stimulated pulp. In 2000, Gronthos et al. discovered and isolated the first dental pulp stem cells (DPSCs) (Bansal, Jain, 2015).

Stem cells are undifferentiated cells that can continuously regenerate and differentiate into mature cells. Further research may prove stem cells can be an alternative method of regeneration and repair in lieu of traditional medical procedures (Park, et al., 2016). There are two types of stem cells both based upon the stage of growth in which they are isolated. The first type of stem cells is embryonic stem cells (ESCs). These stem cells are totipotent, capable of giving rise to an embryo or any cell type. They are derived from the embryonic blastocyst. Embryonic stem cells would be the preferred stem cells for regeneration because they have the greatest differentiation ability, but there are ethical concerns about destroying the blastocyst embryo to isolate the stem cells. Therefore, ESC's are no longer being used to regenerate damaged tissues. The second type of stem cells are adult stem cells, such as bone marrow stem cells. These adult stem cells do not pose ethical questions because they are taken from postnatal, matured tissue. However, they do have limited differentiation potential compared with embryonic stem cells (Sunil, 2016).

A big step in stem cell research was the discovery of induced pluripotent stem cells (iPSCs). Induced pluripotent stem cells are made by "recreating" cells by putting new genes that resemble those genes of ESCs into iPSCs. Induced pluripotent stem cells are useful because scientists have reprogrammed them from their original somatic cell-like state into an embryonic-like

state. The use of iPSCs eliminates the limitations that adult stem cells have, such as lifespan and differentiation, without causing any ethical problems. Also, while cells from other parts of the body may cause the body to reject adult stem cells, iPSCs are not rejected because of their embryonic state. Induced pluripotent cells look the same, have the same differentiation potential, and have the same formation as the cells they are programmed to become. Induced pluripotent cells can react with and become neuron, pancreas, cardiac myocytes, and renal lineage cells (Yun-Jong, et al., 2016).

Methods

This document was written by researching peer reviewed scholarly articles and medical journals to assess the newest research and methods used in tooth regeneration. The research question was analyzed and reviewed from many different perspectives. Proquest, Ebsco, and Medline databases were accessed through Touro College Library online and Pubmed. Only research articles published within the last five years were referenced.

Discussion

Studies in the differentiation of dental epithelial cells conclude that iPSCs can differentiate into oral-related cells for generative purposes. Induced pluripotent stem cell technology needs suitable cells for the implementation of genes. Dental pulp cells have been shown to be able to help with the reprogramming of iPSCs. An advantage that dental pulp stem cells have over other cells used in reprogramming is their efficiency, multipotency, and accessibility (Yun-Jong, et al., 2016). During reprogramming, iPSCs are able to form colonies faster than mesenchymal stem cells and bone marrow stem cells (Sunil, 2016). However, iPSCs have recently been connected with tumor formation while adult stem cells have not been, so they may not be as beneficial in regeneration as originally proposed. Nonetheless, no conclusions can be drawn yet as these matters are still undergoing investigation in clinical trials (Takebe, et al., 2017).

Bone marrow is the most common tissue to retrieve adult stem cells from. However, the number of stem cells in bone marrow decreases with age, requiring an alternative place from which to recruit them (Newaskar, 2013). The use of dental stem cells may be the solution in response to the limitations bone marrow stem cells possess. Because dental stem cells are easily accessible and are often removed at some point in one's life,

they make a good alternative to bone marrow stem cells. These teeth contain dental pulp that have stem cells which have similar characteristics to bone marrow stem cells but the amount of cells do not decrease with age (Sunil, 2016).

Dental-tissue-derived stem cells are used because they can differentiate into other odontogenic cells. Since their discovery, dental stem cells have shown the ability to multi-differentiate into specifically osteogenic, odontogenic, adipogenic, and neurogenic cells. Although bone marrow cells do have the same differentiation ability, differences have been noted between the dental cells and bone marrow multipotent stem cells. It was found that dental stem cells appear to be more prone to odontogenic development, while stem cells derived from bone marrow are more prone to osteogenic development, making dental stem cells a more viable option for repair of dental trauma (Yun-Jong, et al., 2016).

Recently, researchers have been encouraging people to save dental stem cells from extracted teeth, and store them in a cell bank in case a need for regeneration does arise (Newaskar, 2013). When it comes to storing these cells in the US however, there are rules and regulations regarding isolation and storage. Dental pulp stem cells are separated into two categories. The first category produces products that are “minimally manipulated.” This means that the cells are used in a homologous manner in clinical trials. The second category of DPSC, produces products that are used in a non-homologous way, significantly altering the biological functions of the cell by using certain enzymes. The FDA does not approve the use of many enzymes to extract cells. Therefore, cells are isolated using the explant culture, which is a technique that cultures cells from tissues or organs which will eventually migrate to the top of the dish which will then be isolated and stored (Ducret et al., 2015).

Human stem cells are removed from either deciduous teeth or third molars. The tooth germ is put in sterile physiological saline and is placed in the lab within two hours of extraction. The success of the stem cells implantation is based upon time and temperature, so everything must be done accurately. The tooth is cleaned by washing with buffered saline (Dulbecco's Phosphate Buffered Saline without Ca^{++} and Mg^{++}). Povidine iodine and PBSA are used to disinfect the tooth. Once the tooth is disinfected, the pulp tissue is removed with forceps or a dental excavator. The tissue is digested and the cells that are distinct are isolated and filtered. The filtration allows for single cell suspensions (Bansal, 2015). The cells are placed onto growth plates, and tissue pieces are placed onto plates containing more growth medium. The stem cells are then incubated in a humidified atmosphere with carbon dioxide. These cells are left to incubate for ten to fifteen days. Cells are dissociated with trypsin, an enzyme that breaks down proteins so that the cells can stick to walls in which they are being held, and are incubated with antibodies such as CD34, CD35, CD73, CD90, CD105, CD133, CD166 for a total of 45 minutes. After 45 minutes, the

cells are washed and placed with more conjugated antibodies (Newaskar, 2013).

The stem cells from this medium can be preserved by cryopreservation which cools the cells or tissues at subzero temperatures. This causes all biological activity to stop, but pulp death does not occur because that process is stopped as well due to the subzero temperature conditions (Newaskar, 2013). The best cells for cryopreservation are cells harvested near the end of log phase growth (Bansal, Jain, 2015). Magnetic freezing is another method, which allows the cell to remain intact by forcing the temperature to drop below freezing point so that the cell does not have time to freeze and expand and therefore remains intact. Through this process, dental stem cells can be isolated and preserved until they are needed (Newaskar, 2013).

This cell banking process used is time consuming and costly. The cultures used also have the ability to get contaminated by microorganisms in the lab because they are left out for a while. It has been suggested to shorten cell processing before the cryopreservation step to avoid the time wasted and contamination that may occur. Taking dental pulp and cryopreserving it without culture would be the quickest way to go about this process. However, the percent recovery of cells would be very low and only a small amount of healthy cells would be left in the tissue. This would occur because without sitting in culture, the cryopreservation agent wouldn't infiltrate to the center of the tissue. Instead, letting the cells sit in culture (along with the cryopreservation agent) for 5 days allows the agent to infiltrate into the middle of the culture and around 90% of cells would be recovered. These cells would also be able to be easily isolated because they would migrate toward the edges even after five days (Takebe, et al., 2017).

Tooth-derived stem cells are named according to the part of the tooth from which they are isolated (Park, et al., 2016). There are five known types of tooth-derived stem cells: dental pulp stem cells (DPSC), periodontal ligament stem cells (PDLSC), stem cells from apical papilla (SCAPs), dental follicle progenitor cells (DFPC) and stem cells from human exfoliated deciduous teeth (SHEDS). These stem cells come from the tooth but are slightly different depending on which place in the tooth they originate (Benavides, et al., 2018).

Dental mesenchyme is also known as 'ectomesenchyme' because of its early interaction with neural crest. Dental tissues are specialized and are more restricted in their differentiation and do not undergo remodeling the same way that bone marrow tissue does.

Dental pulp cells can easily differentiate into odontoblasts. Odontoblasts are cells that have neural crest origin. Dental pulp cells can be transported in vitro to help regenerate other cells in the mouth. As reported for bone marrow mesenchymal stem cells (BMMSC) the DPSC also have different morphology and different rates of growth. Even within the same colony, different

cell growth and size can be observed. If DPSCs are placed on dentin, some of them will convert into odontoblasts. DPSCs, however, are not limited to oral-related issues. In vitro, they can multi-differentiate into adipose-like and neurogenic-like cells. They also have the potential to differentiate into osteogenic, myogenic, and chondrogenic cells. Their multipotency and rate at which they divide is greater than that of BMSCs. This makes them a better candidate for mineralized tissue regeneration (Yun-Jong, et al., 2016).

The second type of dental stem cells, PDLSCs, are found in the periodontal ligament. The periodontal ligament is located between the tooth and alveolar bone (Lymperi, 2013). These cells have the ability to differentiate into osteoblasts, cementoblasts, adipocytes, and chondrocytes. Because of their location, they can be used for periodontal ligament and cementum tissue inside the mouth (Park, et al., 2016).

After PDLSC's are isolated, they display spindle shapes and have microtubule cytoskeleton. PDLSCs were analyzed in depth and were found to have 3235 proteins. The majority of proteins were mainly part of the cellular metabolism network and showed high proliferation ability (Taraslia, 2018).

The third type of cells, SCAPs, are located in the upper dental papilla. They are usually isolated from third molars and the apex of the tooth (Lymperi, 2013). They are also able to be isolated while the tooth is developing, when the tooth still has immature roots. These cells could produce dentin in vivo. The SCAPs survive pulp necrosis (pulp death) because they are close to the periapical tissue vasculature. This means that after an endodontic disinfection, SCAPs can still give rise to primary odontoblasts which would help in the completion of root formation (Bansal, Jain, 2015).

The fourth type of dental stem cell, DFPCs, are located in the dental follicle surrounding tooth germ in early tooth formation. In vitro, these cells have a fibroblast and plastic-like appearance. In comparison to DPSC, DFPC had higher pluripotent reprogramming factors and developmental factors. This allows DFPC to have greater regeneration of pulp than DPSC. However, more research still needs to be conducted to help assess which dental stem cells are the best for oral regeneration (Karamzadeh, et al., 2017).

The fifth type of dental stem cell, SHEDs, come from exfoliated teeth. It has a faster and higher growth rate and can differentiate into BMSCs and DPSCs (Park, et al., 2016). They can also differentiate into a greater variety of cell types than DPSCs (Gabiec, et al., 2017). SHEDs have the same morphology as PDLSCs, but are larger in size. There are 2032 proteins that were found in SHEDs, with the majority functioning during cell adhesion, motility, regulation of localization and migration (Taraslia, 2018). SHEDs can be good candidates for regeneration purposes and specifically for the enhancement of orofacial bone regeneration in which these characteristics are greatly required (Bansal, et al., 2015).

The table below (Table 1) summarizes the different abilities of each dental cell in vivo and in vitro, along with bone marrow mesenchymal stem cells (BMMSC) for comparative measure.

The main differences in DPSC and BMMSC are in their proliferative ability and how they develop in vitro. Both are limited to dividing into other stem cells according to where they were in vivo. For example, the neurogenicity of dental stem cells is better than that of BMMSC because they come from the neural crest, while BMMSC does not (Yun-Jong, 2015).

	In Vivo -Multipotentiality	In Vitro
DPSC	Oseto/dentigonic Adipogenic Chondrogenic Myogenic Neurogenic (Kim B, et al., 2012)	Dentin pulp like complex Odontoblast-like cells Bone-like tissue (Lymperi, et al., 2013)
SHED	Dentinogenic Adipogenic Chondrogenic Neurogenic (Kim B, et al., 2012) Osteo-induction, and uniquely the ability to recruit bone forming cells and tissue formation (Gabiec, et al., 2017)	Dentin pulp like tissue Odontoblast like cells No dentin pulp like formation Bone formation (Lymperi, et al., 2013)
SCAP	Dentinogenic Adipogenic Chondrogenic Myogenic Neurogenic (Kim B, et al., 2012)	Dentin pulp like complex Odontoblast-like cells (Lymperi, et al., 2013)
PDLSC	Osteo/cementogenic Adipogenic Chondrogenic Neurogenic (Kim B, et al., 2012)	Differentiate into cells that have the properties of cementoblasts, osteocytes, and fibroblast (Gabiec, et al., 2017)
DFPC	Cementogenic Odontogenic Adipogenic Chondrogenic Myogenic Neurogenic (Yun-Jong, 2015)	Adipogenic Odontogenic (Lymperi, 2013) Cementum matrix formation and osteoblast formation. (Gabiec, et al., 2017)
BMMSC	Odontogenic Osteogenic Adipogenic Myogenic Neurogenic (Yun-Jong, 2015)	BMMSCs have both ectopic and orthotopic tissue formation. Bone and bone marrow like, cartilage, muscle, and neuronal cell tissue formation (Yun-Jong, 2015)

Table 1

The above chart is of vital importance because in many research centers, biological flipper teeth as well as implementing of a new tooth are trying to be successfully formed in vitro. So far the experiments have been able to be carried out in animals, but the formation of a tooth still needs much improvement (Gabiec, et al., 2017).

Clinical Trials:

The five dental stem cells have been discovered relatively recently, and now further researchers can study alternative ways

to use them. All of the following studies are currently in clinical trials and have not yet been implemented in dental clinics and private practices.

A study was done last year to determine if dentin repair could become biologically more enhanced if the formation of natural dentin could be stimulated by mobilizing the resident stem cells that are in the pulp of the tooth. The study was done *in vitro* and *in vivo* with the molars of mice.

When one loses a tooth due to dental caries or other trauma, the most commonly used treatment is placing cement in the empty space. The cement which is usually made up of calcium or silicon, never fully disintegrates. Due to this lack of regeneration, the normal tooth mineral level will never be like it once was. In general, when the soft dentin-like tissue gets exposed, a natural repair process starts to take place. Mesenchymal stem cells that are already there move and differentiate into new odontoblast-like cells that create new dentin, called tertiary dentin. When the dentin repairs itself, it creates a new, thin layer of dentin which is then able to protect the pulp from the surroundings. The problem is that this only works for small injuries. Large injuries cannot be fixed with reparative dentin (Neves, et al., 2017).

This restoration process takes place by the Wnt/B-catenin pathway. Wnt/B – cat (a signaling molecule) is an early response for stimulating cellular repair in all tissues. Axin-2, a repressor protein, inhibits Wnt/B pathway. Glycogen synthase kinase 3 (GSK – 3), a component of the Wnt/B transduction pathway, phosphorylates B- catenin and axin, in the absence of the Wnt ligand/receptor binding. This leads to degradation and death of the protein (Neves, et al., 2017). Therefore when the tooth is still developing, and B- catenin is not working, the development of the tooth stops (Han, et al., 2014). In the presence of the ligands, GSK does not work. Because it does not function, the B- catenin does not get phosphorylated, but enters the nucleus, where it interacts with Lef/ Tcf transcription factors. One of these transcription factors is Runx2, which helps reform the tissue into odontoblastic formation (Han, et al., 2014). These transcription factors are able to regulate expression of target genes, including Axin -2. Studies done showed that, following tooth damage, the number of receptors on Axin 2, and therefore Wnt/B signaling, is increased. It is theorized that if a Wnt signaling agonist were added to the stem cells, it may help the formation of reparative dentin. In this way, dentine can be restored following removal of a cavity (Neves, et al., 2017).

Recently, a study was done with the use of Tideglusib, a small molecule that inhibits GSK3 and upregulates Wnt activity, which is currently in clinical trials for the treatment of neurological disorders. This could help with Alzheimer's disease by upregulating Wnt activity (Boutajangout, Wisniewski, 2014). Other small molecules that inhibit GSK3 were also used in experimentation and upregulated WNT activity as well. In this study, three GSK3 inhibitors – BIO, CHIR9902, and Tideglusib were used to arouse

tertiary dentin formation after inducing with pulp exposure (Neves, et al., 2017).

Mouse dental cells were incubated with the three inhibitors. The results showed that BIO induction of Axin 2 was much greater than CHIR9902 and Tideglusib levels. To protect the tooth, a glass piece was placed on top of the sponge in the enamel part because enamel does not regrow from these drugs, only the dentin part does. As part of this experiment, a sponge was soaked with these three inhibitors and placed into holes in mice molars. Sponges were left in the teeth (they were able to dissolve) and the teeth were analyzed at 4 and 6 weeks. Treated teeth and the controls were compared.

It was discovered that mineralization did indeed, increase with all three agonists. When controls were analyzed, there was no obvious mineralization detected. Compared to controls containing collagen sponge and MTA, more reparative dentin was formed with GSK-3 inhibitors and the newly formed odontoblast-like cells expressed high levels of axin -2.

This method can be a very effective way of replacing fillings on teeth. A great way of implanting this procedure would be by using biomaterial, such as Kolspon (collagen sponge), which is soaked in the GSK-3 inhibitors. Wnt agonists elevate Wnt activity which is an immediate response to tooth damage. If Wnt is upregulated with a sponge, mineralization happens much faster and completely finishes the tissue formation (Neves, et al., 2017).

Another ongoing study is testing for the optimal conditions *in vitro* for stem cell formation. The past few years have brought about much new insight into hypoxia-based response. Hypoxia is a deficiency in the amount of oxygen in a biotic environment. Oxygen has been known for quite a few years to have a variety of effects on adult stem cells. Because of this knowledge, new regenerative ideas can be implemented to help enhance the use of stem cells *in vitro*, especially with the targeting of cellular oxygen sensors (Janji, et al., 2017).

When stem cells are used *in vitro*, their differentiation fate is determined by transcription factors and cell cycle regulators such as Oct-4, Sox2, c-Myc, and downstream signaling pathways (Zhou, et al., 2014). It has been shown, although not yet implemented, that low oxygen levels can activate Oct-4 to help maintain stem cell properties (Ratajczak, et al., 2016). Also, ongoing research has shown that hypoxia may induce the expression of hypoxia-inducible factor (HIF-1). HIF-1 regulates the expression of target genes that affect cell proliferation, differentiation, apoptosis, and embryonic development (Zhou, et al., 2014). Through HIF-1, angiogenin is able to work (Ratajczak, et al., 2016).

Based on the effects of hypoxia transcription factors in other stem cells, an experiment was done to try and ascertain if hypoxia stimulates production of angiogenin in the pulp (Janji, et al., 2017). Angiogenin (ANG), a growth factor contained in tissues, causes angiogenesis, the generation of new blood vessels. (Ratajczak, et al., 2016). Also known as ribonuclease 5, angiogenin

is a secreted protein that regulates cell proliferation and differentiation in other cell models during angiogenesis. Angiogenin binds to endothelial and smooth muscle cells. This causes cell migration, invasion, proliferation of the endothelial cells. They can also cause tube like structures to form. Because of these abilities, angiogenin can be used in regenerative techniques. In vivo, small amounts of angiogenin are found relatively early on during hard and soft tissue regeneration. There are several pro-angiogenic factors, including vascular endothelial growth factor, fibroblast growth factor, and endothelial growth factor, which stimulate the growth of angiogenin. The transcription factor HIF-1 helps induce angiogenin as well (Janji, et al., 2017).

Angiogenin is found in other mesenchymal stem cells, such as those derived from the umbilical cord. This has been shown to help with the function and growth of the tissue which could help with infertility due to its ability to create blood vessels (Zhang, et al., 2017).

The results obtained are relevant to preconditioning approaches for cell therapy and tissue engineering. The goal is to improve the pro-angiogenic capacity of transplanted cells (Janji, et al., 2017). In this experiment, human dental pulp cells (DPC) were extracted from third molars. Three different cultures were set up. First, a monolayer culture of DPC was collected and incubated overnight. Then, a spheroid culture of DPC was collected and lastly, a tooth slice organ culture was used. All these samples were rendered hypoxic. The degree of hypoxia used was based on previous studies done with stem cells and hypoxia (Janji, et al., 2017).

Angiogenin was increased in the monolayer culture of DPC when rendered with hypoxia. When angiogenin was first expressed in normoxic conditions, the increase of angiogenin did not reach significant levels at the mRNA level. However, at the protein level, angiogenin was increased after the hypoxia was added compared to the normoxic control. Inhibitor studies were also done with echinomycin, which inhibits HIF-1 function. Echinomycin is a peptide antibiotic that blocks the binding of HIF-1 α (subunit of HIF-1). This study was done to help figure out the role of HIF-1 mechanism. mRNA production of angiogenin was reduced in the presence of hypoxia and echinomycin. Protein production after hypoxia was implemented was also reduced in the presence of echinomycin. It was deduced from this data that under hypoxic conditions, HIF-1 activity is needed for the angiogenin to be increased on monolayer cultures of DPC.

In order to make the sample more like the dental pulp matrix, in addition to the monolayer used, a spherical culture model (3D model) was also used. At the mRNA level, no significant increase of angiogenin occurred. The same was found at the protein level. It was concluded that the effects of hypoxia and the hypoxia mimetic agents that were used did not have the same effect on 3D cultures as it did in the monolayer culture. Cells from the 3D model in different environments are possibly less

sensitive to hypoxia than those of the monolayer. This would be due to the possibility that the cells which are deep within the 3D sphere have already reached low levels of oxygen. The third culture of the tooth sliced organ culture model was then tested. Angiogenin was produced in the dental pulp before and even more so after hypoxia was added.

Overall, when comparing the response at the mRNA levels compared to that of the protein level especially in the tooth slice model, angiogenin did in fact increase in response to hypoxia. The kinetics of angiogenin has yet to be determined because the amount of time left for the effect of hypoxia in other stem cells to take place was not consistent with the time frame of the oral stem cells (Janji, et al., 2017).

The reason why this is a promising technique in the future of regenerative endodontics is targeting cellular oxygen sensors with hypoxia did in fact increase pro-angiogenic activity. In conclusion, this current research is the first of its kind to apply hypoxia and hypoxia mimetic agents in creating a greater scope of angiogenin in dental pulp cells. This specific experiment concluded that although successful, the results depend on the in vitro model and the HIF-1 α activity. Knowing how to modulate angiogenin in dental pulp stem cells is a stepping stone for future studies which will research the role of angiogenin in pulp regeneration. This study will also be very important for understanding cell therapy and tissue engineering in regenerative endodontics (Hong, et al., 2018).

Table 2 contains a list of just some of the factors contained in dental stem cells that help with angiogenesis stimulation (Ratajczak, et al., 2016). Although not yet tested with hypoxia, if any of these are somehow stimulated, angiogenesis may very well be increased.

The use of laser for stem cell regeneration is also being researched. Studies previously conducted have shown that lasers can help in the differentiation of stem cells. The lasers used in these studies were high powered lasers and the effects of low power laser (LPL) were never fully studied in dental stem cells. LPL had previously been used for therapy to help osteoarthritis, inflammation, and soft tissue injuries. When DPSCs were placed in vitro, the light from LPL (810 nm light- infrared light) did not enhance the growth of DPSC. However, when LPL was used to treat a culture for 21 days under optimal conditions, dentinogenesis did occur.

The success of LPL depends on both the biological conditions and the way in which it is applied. There are two ways to apply the laser. The first method uses the laser in a continuous motion and the second way is applying it in pulsed waves. Pulsed waves were shown to be more efficient due to the time in between pulses that allowed the light to penetrate.

Non-ionizing LPL does not affect the stem cells directly but affects the growth factors that regenerate the teeth. The light from the laser hits water, gets excited, absorbs the energy, and

Factor	Function	Population
Angiogenin (ANG)	Endothelial proliferation and migration activation of smooth muscle cells indirect degradation of basement membrane.	DPSCs, SCAPs, FSCs
Angiotensin -1 (ANGPT1)	Endothelial survival, migration, and matrix adhesion. endothelial sprouting and vessel stabilization	DPSCs, SCAPs, and FSCs.
Angiotensin-2 (ANGPT2)	Endothelial proliferation, migration, and sprouting in the presence of VEGF	PDLSC
Basic fibroblast growth factor (bFGF)	Endothelial proliferation, migration, and differentiation. induction of proteolytic enzyme release	DPSC, SCAP, SHED, and PDLSCs
Colony Stimulating factor (CSF)	Endothelial proliferation, migration, and differentiation. induction of proteolytic enzyme release	DPSCs
CXC chemokines, for example, interleukin-8 (IL-8)	Endothelial survival, proliferation, migration, and tube formation. Induction of MMP production	DPSCs
Hepatocyte growth factor (HGF)	Endothelial proliferation, migration, and tube formation, proliferation of vascular smooth muscle cells, stimulation of VEGF and PIGF	SCAP, SHED
Insulin-like growth factor-1 (IGF-1)	Endothelial proliferation, migration and tube formation. stimulation of VEGF and plasminogen activator production downregulation of endothelial apoptosis	PDLSC
Platelet-derived growth factor (PDGF)	Endothelial proliferation, migration, and differentiation. stimulation of VEGF expression proliferation of vascular smooth muscle cells and pericytes vessel stabilization	DPSC

Table 2

transfers energy to dissolved oxygen. This results in the formation of a mild amount of radical oxygen species (ROS). ROS then activate TGF β -1 which cause dentinogenic differentiation. The low levels of ROS are around when the cell is still a stem cell but when ROS is present in mild amounts it helps with differentiation, proliferation, and migration of the stem cells (Hong, et al., 2018).

All the methods mentioned in this paper have not yet been

used aside from trials in the laboratory. Last year, a study was done to assess the awareness of dental pulp cells to those whom it matters most, the dental professionals. Around one hundred eighty-nine dental professionals were involved in this study and over 90% of them had heard of dental stem cells. Only 81% were aware of the use of dental stem cells through the internet or journals. Some, however, were aware due to their undergraduate training. (This would probably be referring to the younger generation). Not everyone knew about banking dental stem cells, and the prospective uses that dentists may be able to apply to improve the restoration of teeth. Those who knew about dental stem cells thought that the main obstacles with implementing dental stem cells were the costs as well as lack of full knowledge about the procedures (most are still in clinical trials). Further awareness can be created by introducing seminars and lectures discussing this topic (Chitroda, et al., 2017).

Conclusions

Dental stem cells are definitely the future in dentistry. There are so many experiments being done that may very well change the future in how a yearly trip to the dentist may look like. Researchers are optimistic about tooth regeneration and regeneration of other traumas in the mouth using stem cells. However, before regeneration using dental stem cells becomes reality, trials need to be conducted on humans. As of 2018, many trials have only successfully been experimented on animals (Benavides, et al., 2018). Although there may still be a long way to go, dental stem cells are becoming more and more of a reality and may very well be our children's only association with cavities.

References

- Bansal R, Jain A. Current overview on dental stem cells applications in regenerative dentistry. *Journal Of Natural Science, Biology, And Medicine*[serial online]. January 2015;6(1):29-34. Available from: MEDLINE, Ipswich, MA. Accessed June 21, 2018.
- Bansal R, Jain A, Mittal S. Current overview on challenges in regenerative endodontics. *Journal of Conservative Dentistry*. 2015;18(1):1-6. <https://search.proquest.com/docview/1646775626?accountid=14375>. doi: <http://dx.doi.org/10.4103/0972-0707.148861>.
- Benavides A, Macri, Diana, RDH, B.S.D.H., M.Ed. Research: The promise of stem cells in dentistry: Although not all procedures done with stem cells have succeeded, the rapid advances have shown positive and beneficial outcomes. *Dent Assist*. 2018;87(2):9-11. <https://search.proquest.com/docview/2051212834?accountid=14375>.
- Boutajangout A, Wisniewski T. Tau-based therapeutic approaches for Alzheimer's disease - A mini-review. *Gerontology*. 2014;60(5):381-5. <https://search.proquest.com/docview/1564632688?accountid=14375>. doi: <http://dx.doi.org>.

org/10.1159/000358875.

Chitroda P, Katti G, Attar N, Shahbaz S, Sreenivasarao G, Patil A. Stem cells in dentistry: A study regarding awareness of stem cells among dental professionals. *Indian Journal of Dental Research*. 2017;28(6). <https://search.proquest.com/docview/1980711085?accountid=14375>. doi: http://dx.doi.org/10.4103/ijdr.IJDR_771_16.

Ducret M, Fabre H, Degoul O et al. Manufacturing of dental pulp cell-based products from human third molars: current strategies and future investigations. *Front Physiol*. 2015;6. doi:10.3389/fphys.2015.00213.

Gabiec K, Wyrzykowska K, Dąbrowska Z, Antoniak M, Dąbrowska E. Stem cells are the hope of modern stomatology. *Progress in Health Sciences*. 2017;7(1):175-181. <https://search.proquest.com/docview/1918837356?accountid=14375>.

Han N, Zheng Y, Li R, et al. β -Catenin enhances odontoblastic differentiation of dental pulp cells through activation of Runx2. *PLoS One*. 2014;9(2). <https://search.proquest.com/docview/1497948562?accountid=14375>. doi: <http://dx.doi.org/10.1371/journal.pone.0088890>.

Hong BK, Ku YB, Seonwoo H, et al. Effects of pulsing of light on the dentinogenesis of dental pulp stem cells in vitro. *Scientific Reports (Nature Publisher Group)*. 2018;8:1-11. <https://search.proquest.com/docview/1993385517?accountid=14375>. doi: <http://dx.doi.org/10.1038/s41598-018-19395-x>.

Janji K, Edelmayer M, Moritz A, Agis H. L-mimosine and hypoxia can increase angiogenin production in dental pulp-derived cells. *BMC Oral Health*. 2017;17. <https://search.proquest.com/docview/1905049552?accountid=14375>. doi: <http://dx.doi.org/10.1186/s12903-017-0373-6>.

Karamzadeh R, Eslaminejad MB, Sharifi-Zarchi A. Comparative in vitro evaluation of human dental pulp and follicle stem cell commitment. *Cell Journal*. 2017;18(4):609-618. <https://search.proquest.com/docview/1902175234?accountid=14375>. doi: <http://dx.doi.org/10.22074/cellj.2016.4727>.

Lymperi S, Ligoudistianou C, Taraslia V, Kontakiotis E, Anastasiadou E. Dental Stem Cells and their Applications in Dental Tissue Engineering. *The Open Dentistry Journal [serial online]*. July 26, 2013;7:76-81. Available from: MEDLINE, Ipswich, MA. Accessed June 21, 2018.

Newaskar V. Banking stem cells isolated from tooth germ of human third molar: A literature review. *Clinical Dentistry (0974-3979) [serial online]*. May 2013;7(5):28-33. Available from: Dentistry & Oral Sciences Source, Ipswich, MA. Accessed June 21, 2018.

Neves VCM, Babb R, Chandrasekaran D, Sharpe PT. Promotion of natural tooth repair by small molecule GSK3 antagonists. *Scientific Reports (Nature Publisher Group)*. 2017;7:39654. <https://search.proquest.com/docview/1899507119?accountid=14375>.

Park Y, Cha S, Park Y. Regenerative Applications Using Tooth Derived Stem Cells in Other Than Tooth Regeneration: A Literature Review. *Stem Cells International [serial online]*.

2016;2016:9305986. Available from: Medline, Ipswich, Ma. Accessed May 27, 2018.

Ratajczak J, Bronckaers A, Dillen Y, et al. The neurovascular properties of dental stem cells and their importance in dental tissue engineering. *Stem Cells International*. 2016. <https://search.proquest.com/docview/1820548606?accountid=14375>. doi: <http://dx.doi.org/10.1155/2016/9762871>.

Takebe Y, Tatehara S, Fukushima T, et al. Cryopreservation method for the effective collection of dental pulp stem cells. *Tissue Engineering Part C: Methods*. 2017;23(5):251-261. <https://search.proquest.com/docview/1898742526?accountid=14375>. doi: <http://dx.doi.org/10.1089/ten.tec.2016.0519>.

Taraslia V, Lymperi S, Anastasiadou E, et al. A High-Resolution Proteomic Landscaping of Primary Human Dental Stem Cells: Identification of SHED- and PDLSC-Specific Biomarkers. *International Journal Of Molecular Sciences [serial online]*. January 5, 2018;19(1) Available from: MEDLINE, Ipswich, MA. Accessed June 21, 2018.

Sunil, P. (2016). Induced pluripotent stem cells in dentistry. *Journal of Pharmacy and Bioallied Sciences*, 8 doi:<http://dx.doi.org/10.4103/0975-7406.191960>

Yun-Jong, P., Cha, S., & Young-Seok, P. (2016). Regenerative applications using tooth derived stem cells in other than tooth regeneration: A literature review. *Stem Cells International*, doi:<http://dx.doi.org/10.1155/2016/9305986>

Zhang Y, Xia X, Yan J, et al. Mesenchymal stem cell-derived angiogenin promotes primordial follicle survival and angiogenesis in transplanted human ovarian tissue. *Reproductive Biology and Endocrinology*. 2017;15. <https://search.proquest.com/docview/1884849854?accountid=14375>. doi:

<http://dx.doi.org/10.1155/2014/890675>

Zhou Y, Fan W, Yin X. The effect of hypoxia on the stemness and differentiation capacity of PDLC and DPC. *BioMed Research International*. 2014. <https://search.proquest.com/docview/1507747798?accountid=14375>. doi: <http://dx.doi.org/10.1155/2014/890675>.