AN ALTERNATIVE APPROACH TO CONVENTIONAL REGENERATIVE THERAPIES: CULTIVATING STEM CELLS FROM DECIDUOUS TEETH

An Undergraduate Research Scholars Thesis

by

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Submitted to the LAUNCH: Undergraduate Research office at
Texas A&M University
in partial fulfillment of requirements for the designation as an

UNDERGRADUATE RESEARCH SCHOLAR

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May 2021

Majors: Dental Hygiene, B.S.

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This project did not require approval from the Texas A&M University Research Compliance & Biosafety office.

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ABSTRACT

An Alternative Approach to Conventional Regenerative Therapies: Cultivating Stem Cells from Deciduous Teeth

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Healthcare professionals recommend many methods of restorative and therapeutic treatments, such as medicine, surgery, radiation, and rehabilitation to treat chronic diseases and conditions. However, evidence suggests that cultivating stem cells from permanent and deciduous teeth are an effective method to treat many oral and systemic conditions. There is a greater advantage to using stem cells from human exfoliated teeth (SHED) because of their differentiating capabilities, which allows for them to have diverse clinical applications. Deciduous teeth are often discarded after they are shed from the oral cavity, which reduces the likelihood of ethical considerations. In addition, stem cells obtained from teeth replicate at a

quicker rate and for a longer period of time than other types of stem cells from the body. The process of isolating SHED is relatively uncomplicated and unlikely to generate pain. Additionally, since the transplant is obtained from the same individual, the individual does not demonstrate adverse immune reactions or rejections. The host and recipient are the same; therefore, the person is a 100% match to the stem cell. Hence, when compared to other types of adult stem cell harvesting, acquiring stem cells from deciduous teeth is noninvasive. SHED demonstrates great potential in treating patients with periodontal disease, namely reducing gingival bleeding, increasing new attachment of the periodontal ligament, and decreasing pocket depths. Oral healthcare providers should consider recommending SHED in their clinical practice due to their multi-differentiation potential, self-renewal capacity, and reported ease of the procedure. Further research is needed on the potential impact of regenerative therapies on patients with special healthcare needs.

DEDICATION

To our friends, families, instructors, and peers who supported us throughout the research process.

ACKNOWLEDGEMENTS

Contributors

We would like to thank our faculty advisors, Dr. Kabani, Ms. Mallonee, and Ms. Brown for their guidance and support throughout the course of this research.

Thanks also go to our friends and colleagues and the department faculty and staff for making our time at Texas A&M University a great experience.

Finally, thanks to our families for their encouragement, patience, and love.

Funding Sources

Our undergraduate research did not receive any funding.

INTRODUCTION

Healthcare professionals recommend many methods of restorative and therapeutic treatments, such as medicine, surgery, radiation, and rehabilitation to treat chronic diseases and conditions. However, in 1868 the term "stem cell" was first introduced by a German biologist named Haeckel. In 1908 a Russian histologist, Alexander Maksimov who discovered the use of stem cells.[1] It was then that stem cells were declared for scientific use.[1] Growing evidence has shown that stem cells have confounding therapeutic abilities in orofacial, neurologic, corneal, cardiovascular, hepatic, diabetic, renal, muscular auto-immune conditions.[2]

A recent development in stem cell research has been the discovery of dental stem cell harvesting as a noninvasive method to treat various health conditions. Not only can stem cells from human exfoliated deciduous teeth (SHED) help treat many dental diseases, such as periodontal disease, they can also be used to establish normal function in many other organs. SHED has the potential to regenerate human cells and tissues, important in regenerative medicine. These therapies have the potential for clinical applications in numerous diseases.[2] The approach of stem cell harvesting has shown less legal implications due to the natural exfoliation of one's teeth. SHED has been used in bone regeneration for periodontal disease, diabetes, acute renal failure, Alzheimer's disease, and many others.[2] Therefore, SHED shows promising therapeutic and regenerative capabilities for advancing future medical innovations.

1. CURRENT SCIENCE REGARDING STEM CELL RESEARCH AND DECIDUOUS TEETH

Human deciduous teeth are altogether not the same as permanent teeth with respect to developing cycles, tissue structure, and function.[3] Dental pulp from permanent teeth stem cells are adult dental pulp stem cells (DPSC) and deciduous teeth stem cells are stem cells from human exfoliated deciduous teeth (SHED). Cultivating stem cells from permanent and deciduous teeth differ in numerous points of view. Multiple studies have shown that SHED has greater proliferation and survival rates than DPSC, but there are not any functional differences between DPSC and SHED because they both have the capability to proliferate into soft and hard tooth tissues such as, endothelial cells and dentin secreting odontoblasts which are both important cells to regenerate damaged teeth.[4,5]

In 2019, Naz et al. found that SHEDs growth rate was the first cell to be able to culture in approximately 4 to 16 days while the DPSCs cells took 9 to 16 days.[4] Naz et al. also used a trypan blue dye to test the integrity of SHED's and DPSC's cell membrane and it showed that for SHED it was a 94.19 +/- 1.65% viability and for DPSC it was a 99.4 +/- 0.9% viability.[4] This study shows that DPSC and SHED have the capability to be able to regenerate into bone and cartilage by differentiating into odontoblast like cells and produce dentin from the enzymatic method *in vivo*.[4] By using the enzymatic method, it shows there is a greater advantage because it releases multiple different types of cells that are able to regenerate in the dental pulp tissue.[4]

In spite the fact that stem cells share comparative properties, the stem cells vivo experiment, developmental, and restorative potential are desired for their undeveloped start and their possible anatomic location.[3] Teeth develop from oral ectoderm and neural-crest mesenchyme cells which are the epithelial components of the stem cells for continuously growing teeth that form from tissue layers.[3] The oral ectoderm and neural-crest mesenchyme cells compared to the embryonic stem cells show that they have similar pluripotent development because they are able to differentiate into many different cells types such as glia, neurons, bones, tendons, melanocytes, chondrocytes, endocrine cells, adipose cells, and many others.[3] The neural crest cells are the most wanted stem cells because they do not have a restriction capacity or a limiting number as the embryonic stem cells do. The deciduous teeth stem cells have the clonogenicity, self-renewal and proliferation capacity, expression pattern of specific markers, and multipotency that is needed for the use of cultivating and regenerating for the use of other types of medical treatment.[3]

The stem cells niche (SCN) is a remarkable microenvironment inside each tissue that directs stem/progenitor cell multiplication, endurance, relocation, destiny, and maturing, through explicit connection between the cells and through different bioactive atoms.[3] The development of deciduous teeth stem cells starts before birth and is continued up until eruption of the permanent teeth.[3] During the period of developing deciduous teeth stem cells they are not affected by genetic or environmental factors; therefore, deciduous teeth stem cells provide a great source of healthy stem cells that can be used for cultivating and regenerating for the use of medical treatments when compared to the permanent teeth stem cells.[3]

Stem cell banking of bone marrow and placental cord blood has been available for decades with one of the world’s first dental stem cell banks starting in 2004 at Hiroshima University in Japan.[5] Recently the United States, United Kingdom, and India have also started specializing in stem cell banking of teeth. Within the United States there are several places that have stem cell banking companies such as New York (StemSave), Indiana (Tooth Bank), and

Massachusetts (NDPL - National Dental Pulp Lab). These companies can still provide services anywhere in the United States through a collection kit that is mailed to the donor then shipped back to the stem cell bank overnight. The stem cell banking company will hold the viable cells and pulp tissue for as long as the donor is paying the annual or monthly storage fees. The annual cost to store the viable cells and pulp tissue can range from $115 to $600 or $1,500 to $2,500 if the donor would like to pre-purchase a 20-year storage plan. The banking companies also have an initial enrollment and processing fee that cost about $600.

With dental stem cell banking, there are many types of different protocols to follow for the sampling process. Most of the dental stem cell banking services recommend that dentists extract the deciduous tooth as soon as it becomes loose.[5] After the deciduous tooth is extracted or exfoliated, the tooth needs to be placed in a transport medium containing pasteurized bovine milk. The storage process has the biggest impact on keeping the pulp living for cultivating stem cells.[5] Once the tooth is out of the oral cavity and placed into the milk, it only has up to 48 hours before the tooth becomes non-vital for cultivating.[5] Bovine milk is one of the most recommended types of transport mediums for a tooth because of its biocompatibility, neutral pH, and naturally buffering properties.[5] Other types of transport medium such as soy milk or almond milk are not as ideal.[5] In order for the viable cells and pulp tissue to be successfully isolated, they must go through a process called cryopreservation which consists of extracting the viable cells and pulp tissue from the tooth and storing them in a frozen compartment with liquid nitrogen within the 48-hour window.[5]

2. LEGAL AND ETHICAL IMPLICATIONS

There has been a large amount of controversy regarding the stem cell harvesting of embryonic stem cells due to personal and moral beliefs; however, cultivating stem cells from exfoliated teeth can alleviate some of these concerns. The derived embryonic stem cells are collected at a developmental stage prior to the time that implantation would occur in the uterus.[5] Fertilization typically occurs in the oviduct, and after a few days a series of mitotic divisions occur as the embryo travels along the fallopian tube into the uterus.[5] During this stage of mitotic division the blastomeres are not defined to any certain type of cell.[5] In fact, each of these cells can take on any form of cell.[9] Induced pluripotent stem cells (iPSCs) can be obtained from the inner cell mass of within about a week by blastomere cells.[6] This is where the ethical and political controversy regarding human embryonic stem cells (hESC) is a concern due to the possible destruction of human embryos and the different opinions on when human life begins.[7]

The alternative to stem cell research is the harvesting of stem cells through exfoliated deciduous teeth. The deciduous teeth are discarded after they are shed from the oral cavity; therefore, this reduces the likelihood of ethical considerations.[3,8] Isolation of the dental pulp, retrieving stem cells, manipulating, and expanding cells *in vitro* is a straightforward process.[3] The majority of humans lose their teeth; therefore, creating an ideal opportunity to retrieve and store this source of stem cells.[8] In addition, stem cells obtained from teeth replicate at a quicker rate and for a longer period of time than other types of stem cells from the body.[8] Another benefit of dental stem cells from deciduous is that there are multiple opportunities to obtain the stem cells.[8] The process of isolating SHED stem cells is not difficult and unlikely to generate pain, and since the transplant is obtained from the same individual they do not demonstrate immune reactions or rejections.[1] The host and recipient are the same; therefore, the person is a 100% match to the stem cell. Hence, when compared to other types of stem cell harvesting methods, acquiring stem cells from teeth is noninvasive, not as dependent on timing, and much less expensive unlike embryonic stem cell harvesting.[8]

An associated concern of stem cell harvesting is the use of these stem cells for therapeutic cloning. Therapeutic cloning can be defined as the transfer of nuclear material from a somatic cell into an enucleated oocyte with the objective of obtaining embryonic stem cells that have the same genome as the donor.[9] The purpose for therapeutic cloning is to allow an individual's own cell, isolated from an embryo that is not planned to transfer into the uterus, to be used for regenerative and reproductive medicine.[9] This is not the same thing as reproductive cloning, where one is trying to create a person.[9] However, laws regarding therapeutic cloning and reproductive cloning are vague, making it difficult to differentiate between the two types. The ethical considerations of therapeutic cloning come from the source of embryonic stem cells, from aborted fetuses, unused zygotes, and the embryos that are unable to implant themselves in utero.[9] However, there is no supporting research tying the use of SHED or hDPSCs to therapeutic cloning. Therefore, this can reduce the associated apprehension of harvesting dental stem cells for other therapeutic reasons.

The emergence of SHED and hDPSCs have grown in popularity as a source for bone regeneration.[10] However, there is still no clear evidence on the types of teeth and the condition of the teeth most acceptable for stem cell isolation.[10] Nakajima conducted an *in vitro* study at Hiroshima University to test the success rate of SHED and hDPSCs. The successful isolation of SHED and hDPSCs appears to be associated with the age of the patient, the length of the remaining root, and the mechanical forces.[10] In the study, they used extracted permanent teeth from two canines, twelve premolars, and six wisdom teeth to obtain the periodontal tissue.[10] They also used exfoliated deciduous periodontal tissue from six incisors, ten canines, and six molars.[10] The results from isolation of SHED was successful in 18 out of 22 exfoliated deciduous teeth (82%), and hDPSCs was successfully isolated from 14 out of 20 permanent teeth extracted (70%).[10]

In 2017, Tsai et al. conducted an *in vitro* study to identify predictors of success rate of mesenchymal stem cell (MSC) isolation from deciduous teeth pulp.[9] The study was conducted using 161 deciduous teeth that were extracted at the pediatric dental clinic, the dental pulps were harvested using standard protocol.[9] In the study, the researchers collected four types of teeth: incisors, canines, molars, and supernumerary teeth.[9] They examined whether the tooth was present with caries or not and the severity of the caries.[9] Lastly, the pulp status was collected to determine if the tooth was present with pulpitis or non-present with pulpitis (yes/no).[9] In this study, 128 colonies of MSCs were acquired out of the 161 deciduous teeth, and the results showed a success rate of 79.5%.[9] The study found that the deciduous teeth that successfully yielded MSCs had less dental caries ($P < 0.001$) and they had less prevalence of pulpitis ($P < 0.001$).[9] In conclusion, the deciduous teeth that were not present with dental caries and pulpitis were 80% more suitable for MSC isolation and processing.[9]

3. APPLICATION TO PATIENT CARE AND/OR TREATMENT MODALITIES

Periodontitis is a common inflammatory disease affecting 15-50% of adults in developed countries.[6,11] Periodontitis is caused by infection of the periodontium, tissue inflammation, and excessive immune response to the oral bacteria, eventually leading to bone loss.[11] Consequently, periodontitis can lead to tooth loss if left untreated and therefore affects quality of life in these patients.[11] In 2018, Gao et al explained that multidose SHEDs resulted in periodontal tissue regeneration. In an induced periodontitis model, multidose of SHED was delivered in 7-day intervals. Results showed that SHED reduced gingival bleeding, increased new attachment of the periodontal ligament, decreased the differentiation of osteoclasts in the periodontium, and decreased pocket depths.[11] The results demonstrated a p-value of $P<0.05$ and is considered to be statistically significant.[11]

Cleft lip and palate are other conditions that have been treated with bone regeneration. Iliac bone and alveolar bone grafting are some examples of the conventional approaches in bone regeneration. These types of procedures have been shown to be quite invasive and have been associated with many complications, such as, scarring, fracture of the iliac bone, infection, and hematoma.[12] Harvesting SHED for bone regeneration is less invasive compared to conventional approaches and is therefore an accessible and promising tool in regenerating bone in cleft lip and palate patients.[12]

SHED also has the capability to form into pulp-like structures which may be useful in treating pulpitis or promoting restoration of pulp vitality.[13] In 2017, Li et al demonstrated that SHED can proliferate into dentin cells and endothelial cells. In this study, SHED was placed in a full-length human root canal and placed directly under the skin of nude mice.[13] The results were that SHED proliferated into functional dentin cells similar to that of normal dental pulp cells.[13] However, this study has only been done ectopically on mice models and further studies need to be conducted within the oral cavity in order to fully explore the ability of SHED.[13]

Dental pulp stem cells, specifically SHED, have shown great potential in not only dental medicine but also in many other fields of medicine.[2] Regenerative therapies from stem cells have been studied by scientists over the past three decades in hopes of finding minimally invasive medical treatments for patients with systemic diseases such as diabetes, acute renal failure, Alzheimer's disease, and many others.[2] SHEDS have shown high multi-differentiation potential, compared to DPSCs and other types of stem cells, and therefore can be used to regenerate different types of tissue.[2] SHEDS are also easily accessible and there is no need for immunosuppressive drugs because there is typically no immune rejection during transplantation.[2,13,14,15]

Repairing the nervous system is limited and there are very few procedures that are able to repair it structurally and functionally. In turn, there are few treatment options for patients with neurological diseases such as Parkinson's and Alzheimer's disease. In 2017, Li et al conducted a study in which SHED was injected in a rat model with Parkinson's disease.[13] In this study, 36 female rats with Parkinson's disease were placed into three random groups. One group was the negative control group, one group was injected with in-tact SHED, and one group was injected with SHED-derived spheres created in vitro, similar to neural crest cells. Within two weeks, the rat models showed behavior improvement and recovery that was sustained for up to six weeks. This study successfully demonstrates that SHED can differentiate into neuron-like cells and can be used therapeutically to improve neural function.[16] Similarly, in rats that experienced spinal cord injury, SHED injections helped improve motor functions due to the stem cells and the common embryonic developmental origin with cells in the nervous system.[13] Animal behavior was analyzed using a one-way analysis of variance (ANOVA, SPSS software) and then compared using Bonferroni (Dunn) comparison groups.[16] The results demonstrated a p-value of $P<0.01$ and is considered to be statistically significant.[16]

In 2012, Kerkis and Caplan conducted a study in which systemic and local delivery of SHED was given to dog models with Duchenne muscular dystrophy.[14] Six golden retriever muscular dystrophy (GRMD) dogs were given both local and systemic delivery of the stem cells.[6] Giving the dogs SHED intravenously showed less of an immune response than when given directly into the muscle by injection.[2] The effect of the paracrine system in these models triggered overall regenerative processes to begin to take place by progenitor stem cells which improved function in these dogs. This treatment effect may also be useful in patients with other autoimmune diseases that have few treatment options.[2] However, studies conducted with a larger sample size and potential human models need to be done in order to show validity of the results in this study.[14]

SHEDs show excellent immunomodulatory effects compared to other types of stem cells and therefore have been studied in therapies for autoimmune diseases.[13,17] Infusion of SHED showed a significant increase of Treg cells and decrease of T helper 17 cells. Treg cells prevent autoimmunity and T helper 17 cells promote autoimmunity and inflammation.[17] Therefore, SHED may be useful in treating autoimmune diseases and improving immunomodulatory properties.[17] These properties were studied in mice models with systemic lupus erythematous (SLE). SLE is an immune disease that destroys targeted organs such as the kidneys, joints, cardiovascular system, and nervous system.[17] The disease induces both deficiency in immune reactions as well as hyperactivity of the immune system.[3] Transplantation of SHED in these mice reversed the effects of SLE by reducing inflammation caused by T helper 17 cells in the blood. It also helped in reconstruction of trabecular bone and inhibited the action of osteoclasts which can improve the effects of SLE.[3] This study showed that the use of SHEDS showed promising potential in the therapeutic effects of stem cells compared to bone marrow mesenchymal stem cells.[3] This suggests that SHEDS can provide another treatment option in those with immune diseases.[3,13] However, more studies need to be conducted in order to further investigate the regenerative aptitude and safety of stem cell therapy in regenerative therapies.[2]

CONCLUSION

In conclusion, studies have shown there are many uses for deciduous teeth stem cells in not only dentistry but the medical field as well.[1,2,3,11,13,14,15,17,18] Deciduous teeth stem cells have the clonogenicity, self-renewal and proliferation capacity, expression pattern of specific markers, and multipotency that is needed for the use of cultivating and regenerating for the use of other types of medical treatment.[3] Compared to other types of stem cell harvesting, such as bone marrow stem cells, SHED has shown no ethical controversy or considerations due to the noninvasive way of harvesting them.[8] Dental clinicians must treat patients as a whole and be aware of their overall health and wellbeing. Patients with systemic diseases such as diabetes, Alzheimer's disease, or lupus experience manifestations that affect their oral health. Understanding and further researching treatment options provided by deciduous teeth stem cells can greatly benefit our patients. Most studies conducted have been in-vitro studies completed on animal models. Therefore, further research needs to be conducted on human models and with larger sample sizes in order to validate the effectiveness of SHED. SHED show great potential for new therapies in the clinical setting due to their multi-differentiation potential, self-renewal capacity, and the reported ease of accessibility in acquiring stem cells from human deciduous teeth.[2] Therefore, clinicians should consider further researching these types of regenerative therapies for their patients and future implementation into their own practices.

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