REVIEW

Advancements in dental pulp stem cells for potential therapeutic applications

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Dental pulp stem cell research has provided new understanding regarding the subtle differences from the more commonly studied mesenchymal stem cells. The ability to differentiate into various types of cells, the ease of extraction, and ability to maintain clonogenic properties in cell cultures have led dental pulp stem cells to be an attractive alternative focus in stem cell therapeutic research. The recent research advancements that have led an endeavor for application in medicine and therapeutics are reviewed.

Research has led to acknowledgment that stem cells possess the ability to self-renew and differentiate into many different types of cells within the body. Stem cells can be isolated from various parts of the body that include neural tissue, skin, retina, bone marrow and dental epithelium. Furthermore, stems cell can be distinguished into two distinct categories, embryonic and adult stem cells. Embryonic stem cells are pluripotent, meaning they have the potential to differentiate into all possible types of somatic cells and can theoretically divide an infinite amount of times. However, further research in human embryonic stem cells is stunted by their controversial harvesting method.

Harvesting human embryonic stem cells requires embryos to be destroyed in early blastocyst stages through immune-surgery. The majority of embryonic stem cell research performed is on animal cells, making it difficult to apply it toward therapeutic potential for humans.

Fortunately, adult stem cell research does not hold such ethical restrictions because stem cells theoretically can be found in all types of tissue samples. The shortcoming however is that adult stem cells harvested from different tissue samples can potentially have different characteristics distinguished by their development potential, making them less versatile than the embryonic stem cells. Adult stem cells are limited by their proliferation potential and can either be bi-potent or unipotent progenitor cells. They may differentiate only into mature cells of the type of tissue from which they were harvested. However some adult stem cells do show multpotency potential. This observed phenomena in adult stem cells is further investigated by focusing on dental pulp stem cells.

Dental pulp stem cells (DSPCs) are extracted from dental pulp tissue. The sites of extraction are usually from teeth. The molar teeth are the most used in extraction and research. The ease of extraction compared to other adult stems cells that may require bone marrow surgery or other invasive methods has led dental pulp stem cells to become a considerable candidate in stem cell research. Recent research demonstrates dental pulp stem cells exhibit various traits sought after in developing therapeutic applications. Researchers see dental pulp stem cells as a potential tool for one day regenerating teeth in patients due to the dentin regeneration capabilities observed. Research also shows the potential that dental pulp stem cells may exhibit in other fields of therapy in addition to dentistry. Therapies involving neurology, oncology, and transplantation are some of the applications for which dental pulp stem cells may be useful.

DPSC Characteristics

Dental pulp stem cells are pluripotent adult mesenchymal stem cells that are isolated from dental pulp using enzymatic digestion and various differentiation media. Though they can be extracted from various types of human teeth, the third molar teeth are usually the preferred site of extraction due to the supporting evidence of enhanced proliferation the cells exhibit compared to other teeth (*1*). A unique characteristic of DPSCs is their ability to create a dentin-pulp-like complex structure similar to the complex seen in adult normal teeth. The dentin-pulp-like complex forms through the mineralization of tubules lined with odontoblasts which contain blood vessels made of fibrous tissue (*2*).

In vivo, DPSCs possess the important stem cell property of self-renewal immunoreactivity to human DSP antibody. They are able to proliferate into different kinds of cell types that include chondrocytes, osteoblasts, adipocytes, muscle and neural cells (*3*). Important features of DPSCs are their abilities to maintain differentiation function and proliferation function after cryopreservation, considered important for handling stem cells in a laboratory setting (*4*). Ease of storage by cryopreservation has led to the rising trend of using dental banks to preserve teeth extracted from patients (*5*).

With the introduction of different culturing methods, scaffolds, proteins or cells, direct control of differentiation and proliferation of DPSCs has been observed. Short-term treatment using tumor necrosis factor-alpha directly enhances the ability of DPSCS to form cell colonies, migrate, and differentiate into other cell lineages. DPSCs can also be downregulated by various proteins. Full-length amelogenin, for example, has been observed to directly slow down the proliferation of DPSCs (*6*). Therefore, DPSCs are an attractive field of research.

Dentin Pulp Regeneration

Due to the unique ability to create a dentin-pulp-like complex, DPSCs have

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been seen as a plausible tool to restore or repair tertiary dentin of damaged teeth and restore pulp tissue lost when infected root pulps are removed. Typically, DPSCs migrate to the damage site of dentin where they proliferate and differentiate into odontoblasts. They begin to form the dentin matrix (*7*). Researchers are currently trying to regenerate adult teeth by applying materials that allow DPSCs to differentiate into dentin. A variety of culturing materials can be used to increase the amount of dentin DPSCs produce.

DPSCs have exhibited favorable cell-surface interactions with *Emdogain*, a protein-based gel designed to promote regeneration of soft tissues, resulting in better mineralization and osteogenic differentiation compared to materials such as mineral trioxide aggregate or platelet derived growth factor– BB (*7*).

Furthermore, introduction of different proteins can also influence the amount of dentin DPSCs can produce. The bone morphogenetic protein 2 has been demonstrated to directly influence stem cell differentiation into odontoblasts, resulting in faster dentin formation (*8*). It is important to make DPSCs more efficient in producing dentin because this enhances their potential to be used as a therapy tool.

A clinical trial performed in 2009 showed how much potential DPSCs have in regenerating dental pulp tissue. In a comparative study, patients had both third molars extracted, with one of the molars serving as a control and the other serving as the test molar. The test molar side received a collagen sponge with DPSCs inside, while the control molar side had a collagen sponge without DPSCs. After a three month period, the test molar was completely regenerated, showing no signs of infection or inflammation, and had higher expression levels of bone morphogenetic protein 2 and vascular endothelial growth factor compared to the control molar (*9*). The results of the clinical trial suggest how the recovery of patients' teeth who have received teeth extraction or other teeth operations will one day be altered by using DPSCs in a dentistry setting.

Bone Regeneration

Bone reconstruction following a major surgery is accomplished by harvesting the bones from the patient's own body. For example, the scapula, the iliac bone, and the fibula are typically used to reconstruct the craniofacial area of a patient. The procedure is often costly, time-consuming, and harmful to the patient. Therefore, there is a growing demand for alternative bone grafts that can be used instead of the more traditional grafts. The demand has led advancements in regenerative medicine that may revolutionize the way bone fractures and defects are treated using stem cells, due to their potential for differentiation into osteogenic cells. Several studies have shown the vast potential of the bone regeneration properties DPSCs possess when paired with scaffolds. Furthermore, the variation on the type of material the scaffold is composed of has been observed to have a direct impact on the proliferation and differentiation for the DPSCs. For example, a study found that the transplantation of DPSCs within scaffolds that are composed of collagen, fibroin, and hydroxyapatite phosphate ceramic particles showed no significant difference in the amount of healing observed in the critical-sized bone defects of rats and mice, when compared to scaffolds that did not have any stem cells at all (*10*).

However, current research using advanced 3D-printed polycaprolactone scaffolds containing freeze-dried platelet-rich plasma has demonstrated significantly increased DPSCs bone formation when compared to the traditional scaffolds, allowing for greater potential for practical use in clinical trials (*11*). In another study, a collagen scaffold containing human amniotic fluid has also shown promise in reconstructing critical-sized cranial bone defects in animal models (*12*). Therefore, research on the promotion of bone formation using different scaffolds is a major focus to further enhance the bone healing process of DPSCs.

Further advancements have moved away from traditional standard scaffolds entirely by using the novel treatment of gold nanoparticles within a calcium phosphate cement which has been observed to significantly improve the osteogenic differentiation, proliferation and cell adhesion of DPSCs (*13*). The researchers concluded that the gold nanoparticles allowed the DPSCs to bind more easily to the calcium phosphate medium, allowing the greatly improved differentiation and proliferation in comparison with the calcium phosphate medium.

Cartilage Regeneration

A preliminary study determined that, in addition to being able to differentiate into bones, DPSCs also have the capability to regenerate articular cartilage. The DPSCs were cultured along with isolated rabbit chondrocytes on a chondrogenic culture medium and were found to express collagen II and aggrecan *(14)*. The cultured medium was then implanted in a rabbit model containing cartilage damage. Significant regeneration of the cartilage was observed. The study suggests that DPSCs are able to further enhance the regeneration of damage cartilage (*14*). The finding is important because current pharmacological treatment results in poor regeneration. The research conducted thus far has shown that DPSCs are a powerful candidate to help treat bone and cartilage defects. However further research is needed to before the technology can be utilized.

Peripheral nervous system regeneration

The regenerative capacity of the central nervous system and the peripheral nervous system is fairly limited by the healing capacity of the body. Therefore, therapies that try to repair injuries to the brain, spinal cord or nerves are usually costly and provide suboptimal results that typically involve implicating different strategies such as allografts, autografts, and nerve conduits that are often derived from the patient's own nerve tissue. For example, a current problem doctors face when treating patients with peripheral nerve injuries (PNI) is that culturing and acquiring the Schwann cells needed for the repair of the damaged nerves is often

a difficult and slow process that requires resection of other peripheral nerves from the patient (*15*).

Schwann cells are important for treating PNI because they play a major role in initiating repair of peripheral nerve damage as well as providing physical guidance for trophic support of axonal regeneration. Fortunately, researchers have been able to use transmission electron microscopes to confirm that DPSCs are able to differentiate into Schwann cells that possess the capability to express neurotrophic and neuroprotective properties that allow them to bind with collagen type 1 hydrogels and myelinated neurites that can promote neurite outgrowth *in vitro* (*16*).

The ability of DPSCs to differentiate into Schwann cells and maintain neurogenic properties is an important discovery that may one day help treat PNI more efficiently by offering an alternative faster method of culturing Schwann cells with the additional benefit of not being as invasive to the patients.

Furthermore, in order to further investigate if the DPSCs are able to regenerate nerve tissue once differentiated into Schwann cells *in vivo*, the same group of researchers transplanted *NeuroWrap* conduits filled with engineered neural tissue aligned with DPSCs onto rats that had damaged sciatic nerve gaps. A similar allograft treatment was performed for comparison, without any DPSCs. Following an 8-week recovery period, immunohisto-chemistry and ultrastructural analysis of the DPSCs conduit revealed significant neural repair with ingrowing neurites, blood vessels, and myelinated nerve fibers (*17*).

These results offer a potential solution to the problem doctors face when treating a long-gap nerve repair scenario because current treatments using allografts does not provide the same nerve regeneration and vascularization. Future research is needed to optimize the usage of the DPSCs engineered *NeuroWra*p conduit and improve the extent of recovery of the damaged peripheral nerves before the technology can be applied in a therapeutic setting.

Central Nervous System Regeneration

Similar to the peripheral nervous system, the central nervous system also has difficulties in terms of its regeneration capacity. Damages afflicting the brain or spinal cord resulting from neurodegenerative disease, strokes, or traumatic injuries are usually detrimental, leaving devastating effects for those afflicted. However, the ability of DPSCs to promote neurogenesis in the central nervous system is a promising characteristic that may be utilized to treat such injuries.

In one study, the immunostaining of hippocampal slices extracted from adult mice revealed that the transplanted DPSCs were able to differentiate into neuronal cell lineages and express brain-derived neurotrophic factor, a critical protein involved in neurogenesis (*18*). The observed slices demonstrated that the DPSCs stimulated the growth of neurons within the hippocampus outer edges and produced action potentials. Researchers hope, that with further experiments, DPSCs may prove to be a potential treatment of neurodegenerative diseases such as Alzheimer's disease by restoring function to damaged neurons in the brain.

Another DPSC study suggests potential utility as a treatment for diabetic neuropathy, a progressive nerve damage disease affecting the legs and feet, commonly afflicting diabetics with high blood pressure *(19)*. Rats were partly frozen in an 80°C freezer for 6 months to stimulate the nerve damage cause by diabetic neuropathy. The DPSCs used in the study were previously cryopreserved and thawed before being cultured. For comparison, the researchers also simultaneously carried out a similar experiment using freshly extracted DPSCs. The ability to ameliorate diabetic polyneuropathy was demonstrated in both experiments by the increasing sciatic nerve blood flow and sciatic nerve conduction velocity observed following the DPSCs transplantation in the unilateral hind limb skeletal muscles. An injection of streptozotine was given 8 weeks before the transplantation to promote diabetes. The evaluation took place 4 weeks later. In addition to being able to alleviate the damage caused diabetic neuropathy, the DPSCs demonstrated functionality, despite being cryopreserved (*19*). Further study is required before clinical trials are implemented, but the current results suggest that DPSCs are a promising treatment for diabetic polyneuropathy.

Muscle Regeneration

The muscular system has effective muscle repair and regeneration mechanisms that can regenerate most acute injuries, though such healing processes may be hampered by slow recovery time, and chronic diseases such as muscular dystrophy that can repress the muscular system. Fortunately, according to recent research, DPSCs may be a potential candidate to one day speed up the regeneration process of typical muscle injuries and slow down and reverse the damage caused by muscular dystrophy (*20*).

According to a study performed on isolated DPSCs, the healing potential in wound healing assays of mice demonstrated for the first time the differentiation of perivascular smooth muscle cells and the differentiation and regeneration of skeletal muscle cells and epithelial tissue. The researchers also used wound healing assays treated with phosphate buffer saline (PBS) to compare with the results of the DPSCs assays. In contrast to the 40% epithelial coverage in the PBS assay, the assays treated with DPSCs displayed complete epithelial coverage of the afflicted area. Significant amounts of collagen synthesis and fibroblast proliferation were observed in the DPSCs assays that resembled the characteristics of normal skin. Although the direct molecular mechanism is not understood, it is evident that the DPSCs acted mainly through paracrine signaling and vascularization. The study showed that the DPSCs achieved the more efficient healing process by secreting important growth factors, such as HGF, TIMP-2 ANG, VEGF, and PDGF-BB, which are involved in the extracellular matrix and angiogenesis, by improving the oxygen delivery and migration of keratinocytes (*20*).

In a separate study performed by the same group of researchers, the effects of

DPSCs injection on dystrophic mice was analyzed. The dystrophic mice were injected in the left tibialis anterior limb. The right untreated limbs were used as controls *(21)*. Twenty days after the injection, the mice were sacrificed and the DPSCs engraftment of the muscle tissue, as well as the control limbs, were subjected to immunofluorescence analysis. The analysis revealed that the DPSCs located themselves mostly in the interstitial space among fibers. Integration of DPSCs occurred within the smooth muscular fiber blood vessels. The expression of α-smooth muscle actin and vWF proteins was also significantly higher within the DPSC-injected muscle, factors researchers believe are important to the revascularization process of muscles to slow down dystrophic symptoms. Further analysis revealed that type II fast-glycolytic fibers, a fiber known to be affected by muscle dystrophy (*21*), persisted in high frequency in the DPSC-injected muscle (*20*). The therapeutic potential to cure muscular dystrophic disorders and further the healing process has made DPSCs a focal point in regenerative research.

Conclusions

Stem cell research has led to tremendous progress in understanding the capabilities that DPSCs possess in terms of regeneration, proliferation, and differentiation potential, as well as the materials and molecules that can enhance or induce different results. Though a fairly new research area, DPSCs show promising results that are likely to revolutionize the medical industry by changing the traditional methods used to treat injuries and diseases. In addition to being more cost effective, patients may no longer have to make the harmful decisions to use their own bodies for grafts to treat their injuries. However, further research is needed until the technology can be fully implemented in a human medical or therapeutic setting. Researchers will need to make sure that the body will not reject the stem cell transplants. Any adverse effects that stem cell transplantation may cause have yet to be studied. All concerns that may arise must addressed before stem cells are seen as a novel treatment.

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