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Dental Pulp Stem Cell: A review of factors that influence the therapeutic potential of stem cell isolates

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Abstract. Undifferentiated stem cells are being studied to obtain information on the therapeutic potential of isolates that are produced. Dental Pulp Stem Ccell (DPSC) may provide an abundant supply of highly proliferative, multipotent Mesenchymal Stem Cells (MSC), which are now known to be capable of regenerating a variety of human tissues including bone and other dental structures. Many factors influence DPSC quality and quantity, including the specific methods used to isolate, collect, concentrate, and store these isolates once they are removed. Ancillary factors, such as the choice of media, the selection of early versus late passage cells, and cryopreservation techniques may also influence the differentiation potential and proliferative capacity of DPSC isolates. This literature review concludes that due to the delicate nature of DPSC, more research is needed for dental researchers and clinicians to more fully explore the feasibility and potential for isolating and culturing DPSCs extracted from adult human teeth in order to provide more accurate and informed advice for this newly developing field of regenerative medicine.

Keywords: Dental Pulp Stem Cell (DPSC); isolation; culture; cryopreservation; media; differentiation; biomarkers

1. Introduction

Many organs and tissues require the ability to replace cells as a normal part of the aging process, as well as in response to damage, injury or infection Ennis *et al*. (2013). Research has now revealed that many of these organ and tissue systems have resident populations of somatic stem cells, which are capable of asymmetrical replication Raveh-Amit *et al*. (2013). The process of asymmetric replication results in two daughter cells - one that retains the undifferentiated stem cell properties and the other that is capable of replacing dead, dying or injured cells Jones and Klein (2013).

Stem cells are defined as immature, undifferentiated cells that can self-replicate and are able to differentiate into at least two different cell types. In order to be called a stem cell, the cell must

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meet both of these criteria Rai *et al*. (2013). Stem cells remain in their undifferentiated state until they receive a signal to develop into a specialized cell. Stem cells may exhibit three different key features including self-renewal, clonogenicity, and stemness (Chopra *et al*. 2013).

1. Self-renewal: The ability of stem cells to proliferate extensively into vast numbers of cells.
2. Clonogenicity: The ability of stem cells to proliferate into a colony of cells.
3. Stemness: The undifferentiated nature of the stem cell.

Each of the primary germ layers of the developing embryo, ectoderm (or outside layer), endoderm (or inside layer) and mesoderm (or middle layer) give rise to tissues that will ultimately host their own populations of resident stem cells Rinaldi and Benitah (2014). These tissue-specific stem cell populations maybe further classified, based upon their potential for differentiation into the various cell types found within the tissue or organ system Shilpa *et al*. (2013). Although stem cells from embryonic tissues are capable of differentiating into every possible cell type found within the body, these are typically harvested from developmental or fetal tissues - which may limit their availability and potential for therapeutic use King and Perrin (2014). Most adult tissues host other types of stem cells, including multipotent, pluripotent and oligopotent stem cells capable of differentiation into more than one cell type, as well as unipotent stem cells that produce specialized cells of only one type Kolios and Moodley (2012), Shilpa *et al*. (2013).

Although adult stem cells may be harvested and retrieved from a variety of tissues and organs, this may involve costly and invasive procedures, such as bone marrow or liposuction aspiration Liao and Chen (2014). However, recent clinical studies have shown that dental pulp from extracted teeth may provide an abundant supply of highly proliferative, multipotent mesenchymal stem cells (MSC), which are now known to be capable of regenerating a variety of human tissues including bone and other dental structures Verma *et al*. (2014). In addition, dental pulp-derived stem cells (DPSC) have also been demonstrated to be capable of differentiating into many other lineages, including osteoblasts, chondroblasts, adipocytes, as well as vascular and neural tissues Tatullo *et al*. (2014).

Stem cells from dental tissues originate from the neural crest cells and the mesenchymal cells during fetal development. Dental tissues are formed from both epithelial and mesenchymal stem

Stem cell classification	
Totipotent	Stem cells that have the potential to differentiate into every possible cell types found in the human body. Examples of totipotent stem cells include the initial cell of embrological development, the zygote
Pluriopotent	Stem cells that can differentiate into nearly all cell types in the body. Examples are embryonic cells that are derived from the inner cells of the blastocyst that form the ectoderm, mesoderm, and endoderm in the growing embryo
Multipotent	Stem cells that can differentiate into more than one cell type, but is limited in its ability to differentiate. Examples are hemopoietic stem cells that differentiate into many different types of blood cell types, or stem cells in the brain that differentiate into neural cells and glia
Oligopotent	Stem cells that can only differentiate into few cell types. Examples are lymphoid or myeloid cells
Unipotent	Stem cells that only produce specialized cells of their own type in an indefinite supply. Unipotent stem cells are important in transplant procedures such as epithelial skin cells which are used in skin grafts

Classification of Stem Cells from Dental Tissues	
SHED	Stem cells from human exfoliated deciduous teeth (SHED) were first discovered by Dr. Songtao Shi, a pediatric dentist. SHED were found to be highly proliferative clonogenic cells with the potential of differentiating into a variety of cell types including neural cells, odontblasts, and adipocytes. In vivo, SHED have induced bone and dentin formation. Deciduous teeth are naturally shed which makes them an easily accessible source of post mesenchymal stem cell Miura *et al*. (2003)
SCAP	Stem cells from the apical part of the papilla (SCAP) are clonogenic fibroblastic type cells that were isolated by Sonoyama *et al*. (2006). The dental papilla contributes to tooth formation and then becomes part of the dental pulp tissue in mature teeth. SCAP have higher expression of survivin and telomerase than Dental Pulp Stem Cells (DPSC), and also has a unique cell surface marker, CD 24 that is not found in other stem cells derived from dental tissues. SCAP have been used with a combination of other dental tissue derived stem cells to reproduce dentin and cementum in swine. This tissue can be derived from forming third molars, and may be a unique source of cells that may be used in bioengineering of tissues. Sonoyama *et al*. (2006)
DFSC	Stem cells from the dental follicle (DFSC) were isolated from the periodontium surrounding third molars prior to tooth eruption in 2005 Morsczeck *et al*. (2005). They found DFSC to be unique in that they expressed higher amounts of insulin-like growth factor-2 transcripts than in human mesenchymal stem cells. Stem cell markers Notch-1 and Nestin were identified on these cells prior to culture with Dexamethasone. DFSC showed signs of differentiation and expressed osteocalcin and bone sialoprotein post culture
PDLSC	Periodontal ligament stem cells (PDLSC) are clonogenic, highly proliferative cells that have been isolated from the periodontal ligament (PDL) of extracted third molars Seo *et al*. (2004). PDLSC express stem cell markers STRO-1 and CD146/MUC18. On culture, PDLSC differentiated into cemtoblast-like cells, adipocytes, and collagen-forming cells. These cells have a potential for reconstruction of tissues destroyed by periodontal disease as they formed a cementum/PDL-like structure when they were transplanted in vivo into rodents
TGPCs	Tooth Germ Progenitor cells (TGPCs) were isolated by Ikeda *et al*. (2007) from discarded third molars. They found these cells to be highly proliferative, and found them capable to differentiate in vitro into osteoblasts, neural cells, and hepatocytes. The cells were transplanted into rats and it was found that they prevented the progression of liver fibrosis. TGPCs have promise for cell based therapy to treat liver diseases
SPCs	Side Population Cells (SPCs) have been isolated from dental pulp in different species including bovine, canine, and porcine Iohara *et al*. (2006). Flow cytometry was used to exclude these cells from dental pulp by DNA biding dye Hoechst 33342. Bmi 1 was expressed in SPC which suggests longer self-renewal capacity and proliferative lifespan. SPCs were cultured and then identified with appropriate protein cell markers showing differentiation of cell types conducive of dentiongenesis, chondrogenesis adipogenesis, and neurogenesis
DPSCs	Dental pulp stem cells (DPSCs) are derived from the pulp tissue of permanent teeth and have been found to be highly proliferative, and clonogenic. DPSCs were first extracted from third molars and were recognized as stem cells due to the presence of mesenchymal stem cell surface markers in 2000 Lin *et al*. (2009, 2000). *In vivo* studies have shown DPSCs are capable of osteogenic, neurogenic, chondrogenic, adipogenic, dentinogenic, and myogenic differentiation Hyang *et al*. (2009). The majority of these studies have focused on odontogenic differentiation, with limited evidence for the potential of osteogenic potential

cells. Epithelial stem cells differentiate into ameloblasts and form the enamel of the teeth, while mesenchymal stem cells differentiate into various lines of cells that form the dentin of the teeth, and the bone and soft tissue of the periodontium. Dental derived stem cells are the most accessible of all adult stem cells and can be isolated from the dental pulp of both deciduous and permanent teeth, the periodontal ligament, the apical region of developing teeth, and other tooth derived tissues. Based on the location from witch the stem cells are retrieved from a more definitive classification is made of these cells Bluteau *et al*. (2008), Shilpa *et al*. (2013).

2. Sources

Many factors are known to influence DPSC quantity and quality, although one of the most basic may be the source of the dental pulp itself Sedgley and Botero (2012). For example, some evidence has recently emerged demonstrating that stem cells from human exfoliated deciduous (or primary) teeth (SHED) may exhibit faster growth and proliferation, as well as a more expansive array of potential cellular phenotypes and differentiation potentials Daltoé *et al*. (2014). Additionally, SHED may also exhibit slightly greater rates of survival after short-term freezing and storage than DPSCs derived from extracted, permanent (adult) teeth Dziubińska *et al*. (2013). However, due to the recent nature of these discoveries, the vast majority of the population is not able to make use of these reservoirs as they have already developed their permanent dentition, and those currently in need of stem cell therapy are unlikely to have had an opportunity to save any viable dental pulp from exfoliated primary teeth Tirino *et al*. (2011). Moreover, some studies have provided evidence that the quantity of healthy pulp derived from primary or deciduous teeth may, in fact, be insufficient to productively harvest SHED Tandon *et al*. (2010).

Based upon this knowledge, many researchers are now focused on exploring the role of permanent or adult teeth as a reservoir for the acquisition of DPSCs Vishwanath *et al*. (2013). One possible source of DPSCs from healthy, permanent, adult tooth extraction are orthodontic clinics, where extraction of premolars and molars remains common practice among the four million patients in the United States- often approaching or exceeding 30% of all patients Lee *et al*. (2008). Although no comprehensive review of DPSC by tooth type has been performed, recent work by this group has revealed that tooth type did not affect either quality or quantity of DPSC isolates - however, the age of donor was found to be a significant factor Hung *et al*. (2013). In fact, the most recent study provides further evidence that permanent teeth extracted from younger donors may yield DPSCs with higher growth, proliferation and differentiation without regard to tooth type Kellner *et al*. (2014).

3. Isolation

Other factors may also influence DPSC quality and quantity, including the specific methods used to isolate, collect, concentrate, and store these isolates once they are removed Hung *et al*. (2013). For example, the two most common methods used for DPSC isolation are enzymatic dissociation (DPSC-ED), where enzymes are used to digest the matrix and other biological materials comprising the dental pulp, and direct outgrowth (DPSC-OG) which allows for DPSCs to naturally dissociate from the pulp over the course of several weeks in laboratory cultures Karamazadeh *et al*. (2012). Some studies have demonstrated that DPSC-ED are more likely to

give rise to heterogeneous populations of faster growing cells due to the enzymatic activity that may facilitate release of DPSC embedded within this matrix Huang *et al*. (2006). However, other studies have suggested that cellular damage or destruction may result from using this method, suggesting that DPSC-OG may be a less a destructive alternative - although this tends to give rise to fewer and largely homogeneous DPSC populations with more limited differentiation potential Jeon *et al*. (2014). Research from this group confirmed these findings and has used the direct outgrowth method for all subsequent isolation procedures Alleman *et al*. (2013).

Within these heterogeneous populations of DPSC derived from the pulp of permanent dentition, there are more specific sub-populations that include stem cells from the apical papilla (SCAP), dental follicle (DFSC), periodontal ligament (PDLSC), as well as non-specific DPSCs Sonoyama *et al*. (2008), Ponnaiyan (2014). The dental papilla contributes to tooth formation and becomes part of the dental pulp tissue in the mature dentition. Stem cells isolated from the dental papilla (SCAP) have been demonstrated to produce dentin and cementum in animal models and have been shown to express comparatively higher levels of the survivin protein, as well as a unique cell surface marker (CD24) not found in other dental derived stem cells DPSCs Sonoyama *et al*. (2006).

However, stem cells have also been isolated from the dental follicle, more specifically from the peridontium surrounding third molars prior to eruption, which are capable of differentiation into bone lineages with expression of osteocalcin and bone sialoprotein - and can be separated based upon their comparatively higher expression of insulin-like growth factor (IGF-2) Viale-Bouroncle *et al*. (2014). Finally, stem cells derived from the periodontal ligament (PDLSC) express the biomarkers STRO-1 and CD146 and are capable of forming cementum-like cells, adipocytes and collagen forming cells Seo *et al*. (2004). Other research from this group has confirmed these findings, suggesting that multiple sub-populations and lineages may be derived from dental pulp and the associated tissues that has distinct phenotype and differentiation capabilities and potential. Loveland *et al*. (2014).

4. Culture and cryopreservation

Finally, other research has suggested that ancillary factors, such as the choice of media, the selection of early versus late passage cells, and cryopreservation techniques may also influence the differentiation potential and proliferative capacity of DPSC isolates Perry *et al*. (2008). For example, there is some evidence to suggest that no serum (serum-free) media may facilitate the preferential selection and expansion of DPSC bearing specific stem cell biomarkers, while other studies found similar results using low or limited serum media Hirata *et al*. (2010), Ferro *et al*. (2014). In addition, the selection of early- versus late-passage populations may also preserve a more diverse array of potential DPSC sub-populations, which has been repeatedly confirmed in more recent studies Suchanek *et al*. (2009), Coppe *et al*. (2009), Govindasamy *et al*. (2010), Ferro *et al*. (2012). Finally, some evidence suggests that cryopreservation methods and materials may also directly influence the survival rate and therapeutic potential of DPSC, suggesting more research into this area may be needed Woods *et al*. (2009), Lindemann *et al*. (2014), Davies *et al*. (2014).

5. Potential applications of DPSC

Tissue engineering has been described as an application of combining life sciences with the principles of engineering to develop biological substitutes that restore, maintain, or improve tissue function. Langer *et al*. (2005). There are numerous potential applications of DPSC that span from regeneration of dental tissues, differentiation of DPSC's into various tissues, treatment by infusion of MSC's to treat many systemic diseases due to their immunomodulatory properties, and in tissue regeneration.

DPSC's were first identified in human dental pulp by Gronthos in 2002, and were found to be able to regenerate a dentin/pulp-like complex and fibrous tissue containing blood vessels in an arrangement similar to normal human teeth. Peng *et al*. (2009). Since this time many studies have shown the potential of DPSC's to be used to replace or differentiate into many different tooth/oral born tissues. Sonoyama *et al*. (2006), Huang *et al*. (2008) concluded that DPSC engineering can be used for tooth root regeneration. PDL-like tissue has been shown to develop from periodontal progenitor cells. Kramer *et al*. (2004).

Outside of the potential of forming tooth and other oral structures, it has been shown that they can be used as an infusion of MSCs to treat a myriad diseases due to their immunomodulatory properties. Rasmusson (2006), Uccelli *et al*. (2006). Diseases that are currently treated or are in clinical trial include inflammatory diseases such as colitis, ischemic injury, renal failure, COPD, lung injury, lung fibrosis, acute pancreatitis, sepsis, and periodontitis. Autoimmune diseases include systemic lupus erythematosus, systemic sclerosis, Crohn's disease, diabetes, encephalomyelitis model of multiple sclerosis, and rheumatoid arthritis. Graft-vs-host disease, asthma, allergic contact dermatitis, Parkinson's disease, myocardial infarction, liver fibrosis, lung fibrosis, and stroke are other examples of diseases that can potentially be treated with MSC infusion. DPSCs have reservoirs of MSCs and as previously discussed are very accessible. Li *et al*. (2013), Sonoyama *et al*. (2008) showed that DPSCs inhibit the proliferation of stimulated T cells which would make them appropriate for the suppression of T cell mediated reaction in allogeneic bone marrow transplantation.

In contrast to being used to repair damaged tissues, DPSC's may also be used in plastic surgery for facial soft tissue reconstruction and in augmentation surgeries. Rai *et al*. (2013). Several studies have been sited showing the potential of DPSCs to regenerate tissues such as bone, cornea, skin, and neuronal. De Souza *et al*. (2013).

6. Conclusions

Although there are several for-profit organizations that have begun to offer services specific for the extraction, processing and long-term storage of DPSC, evidence-based recommendations are limited regarding the viability and potential applications of DPSCs in order to provide patients (and parents) interested in banking these tissues for future possible usage. Due to the delicate nature of the isolation, culture and storage process, extraction and processing fees, combined with the additional monthly long-term storage, may result in costs that exceed many thousands of dollars before (or if) these cells are needed. More research, however, will be needed for dental researchers and clinicians to more fully explore the feasibility and potential for isolating and culturing DPSCs extracted from adult human teeth in order to provide more accurate and informed advice for this newly developing field of regenerative medicine.

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