



## Review Article

## Stem cells: Isolation, preservation and application in dental surgery

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## ARTICLE INFO

## Article history:

Received 28-05-2020

Accepted 05-06-2020

Available online 21-07-2020

## Keywords:

Stem cells

Dental pulp

## ABSTRACT

In the past few years, third molars have garnered great attention in regenerative medicine as a potential source of pluripotent stem cells. They offer easy accessibility, plasticity coupled with less invasive harvesting procedure in comparison to the traditional sources of stem cell but do not have the ethical issues associated with other sources of stem cells. Various sources of dental stem cells include stem cells from adult human dental pulp like that of third molars, stem cells from human primary exfoliated deciduous teeth, periodontal ligament stem cells, and dental follicle Stem Cells from human third molars.

Dental pulp stem cells have mesenchymal stem cell like qualities namely differentiation into multiple lineages and an inherent capacity to renew its own population. When exposed to specific stimuli, these are capable of differentiating into neurons, adipocytes, osteocytes and chondrocytes, etc. hence allowing applications in various systemic illnesses. Dental stem cells appear to retain their stem cell properties following cryopreservation making their storage and mass application easy.

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## 1. Introduction

Personalized stem cell-based therapy is the most promising means of treating an illness which may be otherwise difficult to cure. The main postnatal objective of stem cells is to repair and regenerate tissues. Stem cells can be hematopoietic or mesenchymal origin. Stem cells can be isolated from several oral tissues such as craniofacial bone, dental pulp, PDL, dental follicle, tooth germ, apical papilla, oral mucosa, gingival, and periosteum.<sup>1</sup> Dental stem cells are most commonly found in the third molars, which are generally regarded as medical waste. Because the erupting third molar is in an early stage of development it is capable of yielding an optimum quantity of dental pulp tissue for the isolation of DPSCs. Although the percentage of DPSCs decreases with age, a population of these cells was always present, even in older patients.<sup>2</sup> Zhang et al. reported no loss in differentiation ability of frozen stored pulp derived stem

cells from third molars when compared with non-frozen control.<sup>3</sup>

## 1.1. Dental pulp stem cells

DPSCs were the first type of DSC derived from dental pulp and were isolated by enzymatic digestion of the pulp tissue of the human-impacted third molars. These multipotential cells exhibited a typical fibroblast-like morphology.<sup>4</sup> Isolation of DPSC was performed and with various differentiation media, their dentinogenic, osteogenic, adipogenic, neurogenic, chondrogenic, and myogenic differentiation potential were demonstrated.<sup>5</sup> DPSCs and endothelial cells have a synergistic effect.<sup>6</sup> DPSCs have shown the greatest potential to produce a high volume of mineralized matrix and show promise for use in regenerative dental therapies.<sup>7</sup>

## 1.2. Dental follicle stem cells

Dental Follicle Progenitor Cells isolated from follicle of human third molars displayed fibroblast-like morphology.<sup>8</sup>

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The *in vitro* studies demonstrated the multilineage potential of DFPCs to undergo osteogenic, adipogenic, and neurogenic differentiation.<sup>9</sup> STRO-1-positive DFSCs can differentiate into cementoblasts *in vitro* and are able to form cementum *in vivo*. DFPC showed their potential to form enamel matrix derivatives (EMDs).<sup>10</sup> DFSCs are able to re-create a new PDL after *in vivo* implantation.<sup>11</sup>

### 1.3. Tooth collection

Following criteria must be fulfilled:<sup>12</sup>

1. Only vital teeth should be harvested.
2. Teeth with infection and any pathology is not preferred.
3. Periodontally compromised teeth cannot be harvested.
4. Teeth should have a sufficient amount of pulp.
5. Extracted tooth is preferred over exfoliated tooth.
6. Informed consent from the donor.

### 1.4. Guidelines during harvesting

Procedure should be done under sterile environment. Prophylactic antibiotic usage should be 1h before and antiseptic mouthwash just before the extraction. Extraction should be carried out carefully avoiding breaking the crown. Care should be taken to avoid contamination of extracted teeth and immediately transfer into the container.<sup>12</sup>

### 1.5. Post extraction protocol

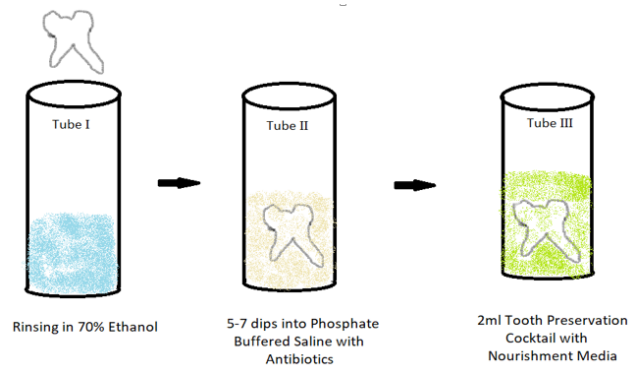
Gently wash the tooth in 70% ethanol by dipping inside tube I. Using antibiotics (100 µg/ml streptomycin and 100 IU/ml penicillin) in phosphate buffered saline, rinse the tooth in tube II by dipping it five to seven times according to the size of teeth so as to remove ethanol.

Add 2 ml of Tooth Preservation Cocktail (TPC) over the tooth in tube III. Sample must then be embedded in TPC and nourishment media. Leave it for 5 min. Over the TPC in tube III, add 1 ml of nourishment medium. Seal the tube with paraffin film after proper labelling.<sup>12</sup>

A thermette is used to store the vial. It is basically a carrier to avoid changes in the sample due to change in temperature. The carrier, in turn, is placed into an insulated metal transport vessel. With the help of the insulated transport vessel, the thermette maintains a hypothermic state for the safe transportation of the sample throughout the journey. This procedure is described as Sustentation. Both time and temperature hold critical importance for viability of the stem cells. The time from harvesting to arrival at the processing storage facility should not exceed 40 hours.<sup>13</sup>

### 1.6. Isolation of stem cells

Tooth surface is cleaned by washing it three times with Dulbecco's Phosphate Buffered Saline without Mg<sup>2+</sup> and



**Fig. 1:**

Ca<sup>2+</sup> ions (DPBS), followed by Povidone Iodine. This is because magnesium and calcium facilitate clumping and cell binding. The pulp is retrieved either by using sterile barbed broach or by cutting the tooth using diamond disc along with coolant to avoid damage to the pulp.<sup>12</sup> There are two widely used methods for the isolation of DPSCs: the explant method and the enzymatic digestion method of the pulp tissue.<sup>14,15</sup> Stem cell rich pulp can also be flushed out with salt water from the centre of the tooth.<sup>16</sup>

Upon isolation, Dispase and Collagenase Type I are used at 37°C for 1 hour to digest the tissue. Trypsin- EDTA can also be used. Isolated cells are passed through a 70 µm filter to obtain single cell suspensions. Subsequently, the cells are cultured in a Mesenchymal Stem Cell (MSC) medium that comprises of alpha modified minimal essential medium with 2mM glutamine, supplemented by 15% Foetal Bovine Serum (FBS), 0.1Mm L-Ascorbic Acid Phosphate, 100U/ml Penicillin and 100ug/ml Streptomycin at 37°C and 5% CO<sub>2</sub> in air. Isolated colonies may usually take 24 hours to become visible. Different cell lines can be obtained such as odontogenic, adipogenic and neural by making changes in the MSC medium.<sup>16</sup> The cells are then sorted using fluorescence activated cell sorting system and passed through appropriate stem cell markers.<sup>12</sup>

## 2. Preservation Techniques

After successful isolation, the stem cells so obtained can be preserved by either cryopreservation or by magnetic freezing.

### 2.1. Cryopreservation

It is the process of preserving cells or whole tissues by cooling them to sub-zero temperatures.<sup>17</sup> The cells are preserved in liquid nitrogen vapor at a temperature of less than -150°C. For a vial, 1.5 ml of freezing medium containing 1-2x 10<sup>6</sup> cells is considered an optimal quantity. Too low or high cell number may decrease recovery rate. Care must be taken to avoid ice injury which is a frequent

cause for failure of a sample during tissue cryopreservation. Slow and rate-controlling freezing reduced the ice injury of cryopreserved living cells. To prevent cell damage vitrification can be utilised, which freezes cells quickly before ice crystals can form, is an efficient approach used to cryopreserve oocytes and embryos.<sup>18</sup>

## 2.2. Magnetic freezing

Cell Alive System (CAS) used by Hiroshima University. Applying a weak magnetic field to water or cell tissue will lower the freezing point of that body by up to 6-7 degrees Celsius. The idea of CAS is to completely cool an object below freezing point without the occurrence of freezing, and achieving a state of uniformly distributed low temperature. Once the object is uniformly cooled, the magnetic field is turned off to snap freeze the object by preventing water from crystallising into ice. Maintaining a CAS system is a lot cheaper than cryogenics and more reliable as well.<sup>19</sup>

## 2.3. Therapeutic uses

Can be identified as systemic and dental uses:

## 2.4. Systemic applications

### 2.4.1. Neurological disorders

DPSCs originate from the cranial neural crest and have neural characteristics such as the expression of neurotrophins.<sup>20</sup> DPSC exerted a neuroprotective effect in in-vitro models of Alzheimer's and Parkinson's diseases.<sup>21</sup> Transplanting differentiated neural stem cells isolated from dental pulp improved motor disability and reduced infarct volume in stroke.<sup>22</sup> For surgically induced crush injury to the optic nerve, rat DPSCs promoted neurotrophin-mediated survival of rat ganglion cells and axon regeneration.<sup>23</sup> DPSC helped in nerve injury treatment by reconnection of damaged axons.<sup>24</sup>

### 2.4.2. Angiogenesis and vasculogenesis

Iohara et al. have shown that SP of dental pulp cells has a property of vasculogenesis which is being viewed as a potential treatment for ischemic heart disease.<sup>25</sup>

### 2.4.3. Liver disease

Stem cells from third molars were differentiated into hepatocytes in cell culture, and in an animal model of liver disease, they prevented liver fibrosis and increased levels of albumin and bilirubin.<sup>26</sup>

### 2.4.4. Diabetes mellitus

The use of differentiated stem cells or islet transplantation for replenishing the lost insulin-producing cells could be an alternative approach to the conventional insulin-based therapy for diabetes.<sup>27</sup> The potential of DPSC

to differentiate into pancreatic cell lineage resembling islet-like cell aggregates was reported.<sup>28</sup> Reversal of hyperglycaemia to the normal level in experimental diabetic mice was reported.<sup>29</sup>

### 2.4.5. Regenerative ocular therapy

Since both cornea and DPSC share similar embryonic origin, DPSC differentiated effectively into keratocytes in vitro to generate a tissue-engineered corneal stromal-like tissue construct and to function as keratocytes in vivo without eliciting overt rejection.<sup>30</sup>

### 2.4.6. Bone tissue engineering

Enhanced mineralization, protein secretion, and an upregulated osteo-related gene profile resulted from immobilization and, interestingly, immobilization triggered osteogenic differentiation of DPSC without the use of induction factors in the medium.<sup>31</sup>

A tendency to increase the bone mineral density was observed when DPSCs were implanted in the granular deproteinized bovine bone (GDPB) scaffold.<sup>32</sup> Lucaciu et al. used DFSCs from impacted teeth to improve bone regeneration on titanium implants surfaces.<sup>33</sup>

## 2.5. Dental applications

### 2.5.1. Regenerative endodontic therapy

Complete pulp regeneration with neurogenesis and vasculogenesis occurred in an adult canine model with pulpectomy and with autogenous transplantation of pulp CD105 + SP cells with stromal cell-derived factor-1 (SDF-1).<sup>34</sup> Another preclinical trial using autologous "mobilized" DPSCs to the pulpectomized teeth of dogs showed regeneration of pulp tissue with no adverse effects and the treated teeth showed recovery of pulp.<sup>35</sup>

### 2.5.2. Dentin regeneration

The DPSCs migrate, proliferate, and differentiate into odontoblasts, which then synthesize matrix on scaffolds to form the tertiary dentin at the damaged sites.<sup>36</sup>

### 2.5.3. Regenerative periodontal therapy

Research findings established that the cells derived from the PDL can differentiate into osteoblasts or cementoblasts to contribute to periodontal regeneration.<sup>37</sup> The multipotent PDLSCs were first isolated from the PDL of extracted teeth. These cells were shown to be clonogenic and able to differentiate into adipocytes, osteoblasts, and cementoblast-like cells both in vitro and in vivo.<sup>38</sup>

### 2.5.4. Bioengineered tooth

The whole tooth regeneration by tissue engineering currently uses two methods: scaffold method and cell aggregates method. So far as regeneration of tooth by

scaffold method is concerned, a biodegradable polymer membrane or collagen sponge scaffold is used to arrange the stem or precursor cells in proper spatial orientation; in order to generate an artificial tooth germ.<sup>39</sup> The cell aggregates method, dental epithelial tissue, and mesenchymal cell pellets are dispersed in a well-controlled culture condition to create an artificial tooth germ. The tooth germ formed in this method mimics a tooth germ of the early inductive stage of tooth development where cell-to-cell and epithelial–mesenchymal interactions are predominant.<sup>40</sup>

Hung et al. were able to utilize DPSC to form tooth-like structures in rabbit alveolar sockets but there was no visible tooth eruption in any of the graft sites.<sup>41</sup>

### 3. Tooth Bank

Tooth Banking may be defined as the process of storing the dental stem cells with a view to harness their ability to regenerate into various cell types.

The first commercial tooth bank was established as a venture company at National Hiroshima University of Japan in 2004 called Three Brackets.

Other tooth and stem cell banking companies known internationally include Stem Save Inc. from New York, USA; BioEden from Austin, Texas, USA and Store-A-Tooth (Provia Laboratories) from Littleton, Massachusetts, USA. In India, the credit of introducing the concept of Dental Stem Cell banking goes to Stemade Biotech.<sup>42</sup>

At the very basic level, human teeth banks can be founded in dental education institutions and colleges as it allows ethical donation and handling of teeth by dentists and students whether for preclinical or research-based utilization.

### 4. Recent Advances

1. Implants have a 5% chance of being rejected by the body. In a study it was found that adding stem cells derived from apical root papillae improve the acceptance rate with better osseointegration.<sup>43</sup>
2. 3-D bioprinting of osseous tissue has been successfully carried out using osseoinductive cell lines such as those from dental origin and used it along with appropriate scaffolds and growth inducing factors. This gives rise to custom made grafts which are similar in molecular and cellular makeup made with patient's own tissues.<sup>44</sup>
3. Precision controlled symmetry, surface texture and rigidity of scaffolds developed under micro and Nano technology have also proven to be great success when used with dental stem cells, specially in the micro-topographical features of tooth such as pits, ridges and grooves.<sup>45</sup>
4. Dental pulp stem cells have been recently found to have difference in mitochondrial dynamics and activity which ranges from most favourable in children's

exfoliated teeth to least favourable in patients with Rhett syndrome due to reduced mitochondrial potential and distribution. Thus proving that mitochondrial profile should be evaluated before making differentiating lines of cells from dental stem cells.<sup>46</sup>

### 5. Possible Limitations

The biggest challenge is the unawareness amongst general public regarding the potential benefits of stem cell and tooth banking.

The specific quantity and quality of stem cells available deteriorates with age of the patient, further complicating the process. Pathogenic contamination via bacterial, fungal and viral agents must be avoided.

Further application, research and understanding are required for the dental stem cells to be used confluent with current and future trends in healthcare.

### 6. Conclusion

The need of the hour is to understand each step of the process well and apply it in every field of regenerative medicine possible. The potential challenges posed must be overcome to achieve optimal results. Previously, stem cell harvesting was invasive and traumatic but now a simple extraction of an unwanted tooth could potentially be a cure-all, undertaken by a dentist.

It is an accessible, less invasive, affordable and convenient option to save which must be utilized as we expect better advances in its applications in the near future.

### 7. Source of Funding

None.

### 8. Conflict of Interest

None.

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**Cite this article:** Agarwal A, Sharma S, Krishnan Y, Aeran H. Stem cells: Isolation, preservation and application in dental surgery. *Int J Oral Health Dent* 2020;6(2):103-108.