Harvesting Tissue from Teeth-related Structures as a Source of Stem Cells

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Submitted: 2020-11-10; **Accepted:** 2020-12-24; **Published Online**: 2021-01-12; **DOI**: *10.22037/rrr.v5i1.33483*

The tooth bud comprises of aggregation of cells demarcated as enamel organ, dental papilla, and dental follicle. They produce different parts of tooth and its supporting structures. More attention is paid to the teeth and its related structures as sources of mesenchymal stem cells (MSCs). Using dental sources has many advantages, such as easy access and less ethical consideration. MSCs from these tissues, besides *In vitro* achievements, showed effective results in clinical applications, especially in oral and maxillofacial regeneration. The case selection and how to harvest the tissue from teeth structures are rarely described. In this concise review, the criteria of subject and teeth selection as a source of MSC isolation were assessed and how to harvest the considered tissue was also discussed.

Keywords: Dental Pulp; Periodontal Ligament; Supporting Tooth Structure; Stem Cell

Introduction

Mesenchymal stem cells (MSCs) have two main properties of renewal and differentiation (1). They have been isolated from variety of adult tissues such as bone marrow, adipose tissue, and placenta. They are ideal sources of adult stem cells and their successful application in regenerative therapies was also shown (2, 3). Dental pulp (DP) was the first dental tissue which was considered for stem cell isolation. After successful isolation of MSCs from DP of permanent teeth, the researchers noted the other dental structures (3). The higher proliferation rate of stem cells from dental-related tissue compared to bone marrow was reported (4, 5). The MSCs from dental-related tissues can be differentiated into various cells including epithelial cells, vascular cells, adipocytes, odontoblasts, osteoblasts, neuronal cells, and muscular cells (2, 4, 6).

Using dental sources have major advantages including as easy access. Most of unerupted wisdom teeth or extracted teeth for orthodontic treatment are discarded (7), therefore, achieving these tissues is harmless without donor morbidity (8). As these teeth extracted for therapeutic reasons, the ethical consideration is less (3). Surprisingly, tooth is a unique tissue that can be used in immature and mature stages. With such benefits teeth and their related structures will be considered as effective sources for stem cell therapies (7, 8). There are several studies about the dental sources of MSCs (3, 9); however, case selection, teeth selection, and how to harvest the considered tissue is rarely described. The aim of this study was to review the teeth-related sources of MSCs and their isolation.

Participant selection as a donor

Almost all studies selected the healthy individuals without systemic disease as donors (7, 10). Pregnant women were excluded from the studies (1). The selected teeth have to be caries free and without periodontal , i.e., gingivitis and periodontitis, or periapical infections (10). In some papers it was suggested to rinse the mouth with 0.2% chlorhexidine mouthwash for one minute immediately before dental procedures of tissue harvesting or tooth extraction to decontaminate the oral cavity (5, 11-13). Professional dental plaque removal and oral hygiene instruction before tooth extraction also led to decreasing bacterial load. For ethical consideration, individuals usually undergo tooth extraction for

orthodontic reasons or unerupted wisdom teeth are selected. Therefore, mostly premolars and third molars were used for this purpose.

Dental pulp

DP is a soft tissue in the center of teeth crown and root comprising of vascular and neural network (Figure 1) (14). Dental pulp stem cells (DPSCs) were the first stem cells originating from dental tissues that showed high proliferative and colony formation capacity. DPSCs had more neurogenic and odontogenic capacity than bone marrow stem cells (4).

For pulp tissue harvesting, it was suggested to clean the teeth with 70% ethanol or iodine, after tooth extraction (13, 15). Another recommendation was washing the teeth using Dulbecco's phosphate buffered saline solution supplemented with antibiotics, i.e., penicillin and streptomycin), (16). To expose the pulp tissue, different methods were explained. High speed hand pieces with sterile diamond bar can be used to create routine access cavity. Another method was creating a 0.5–1.0 mm deep groove around the teeth with low-speed hand pieces and disc. Then, splitting the tooth with a chisel from the created groove. Whenever using hand pieces, having copious irrigation seems logical. Performing Crack on the tooth crown with a mini hammer or wire cutter was also described (13, 17). After pulp exposure, the tissue can be extracted using endodontic files, barbed broach, dental excavator, or pansies (11, 15, 16).

Periodontal ligament

The periodontal ligament (PDL) is a part of tooth supporting structure which anchor the tooth to the alveolar bone and play an important role in maintaining tooth in its position (Figure 1). The periodontal health in selected teeth for PDL stem cell isolation is crucial. Mechanical plaque removal and oral hygiene instruction before tooth extraction is suggested. The extraction must be done with forceps and the PDL tissue should not be injured with elevators (18). PDL tissue scraped with a sterile scalpel. Mostly, middle portion of PDL was used to avoid contamination with pulpal or gingival cells (12, 19, 20). To decrease tooth crown contamination, it was also suggested to cut tooth crown immediately after extraction using disk and high-speed handpiece under copious irrigation (18). After tooth extraction, some parts of PDL may remain attached to the alveolar bone. Some studies used this part as another source of PDL tissue for PDL stem cell isolation (20).

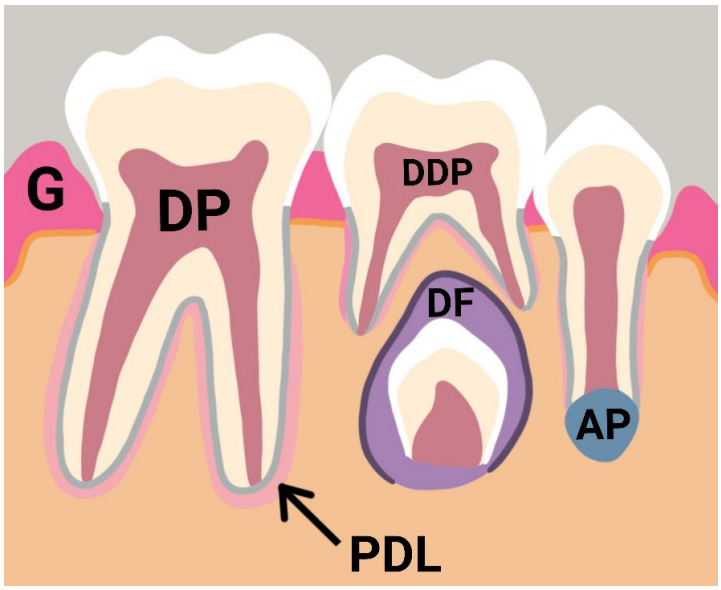

Figure 1. Schematic illustration of teeth-related structures as a source of stem cells. Apical papilla (AP), Dental follicle (DF), Dental pulp (DP), Deciduous teeth dental pulp (DDP), Gingiva (G), and Periodontal ligament (PDL).

Deciduous teeth

After successful stem cell isolation from tooth structure, deciduous teeth also noticed as sources of stem cells. Deciduous teeth exfoliate routinely after a certain time and without any harm. The exfoliated deciduous teeth contain some amount of DP which was used in the studies as a source of stem cells (Figure 1). The pulp stem cells from human exfoliated teeth are called SHED. It was shown that stem cells of deciduous teeth can be cryopreserved and it was suggested to use for creating a tooth bank (8). It was shown that the proliferation rate and differentiation properties of deciduous teeth stem cells were differed from permanent teeth (21).

Harvesting DP from deciduous teeth is similar to permanent teeth, but whenever the pulp is exposed from resorbed part of the tooth, there is no need for cutting the crown. Another source of stem cells which was retrieved from deciduous teeth was PDL. For PDL harvesting from deciduous teeth, the teeth with

complete root and without signs of root resorption or ankylosis were selected (12).

Dental follicle

Dental follicle (DF) is a connective tissue which is formed at cap stage of tooth development and surrounds enamel organ and dental papilla. It is the origin of tooth supporting structures (PDL, cementum, and alveolar bone proper). DF covers the crown of unerupted teeth and coordinates in tooth eruption (Figure 1) (22). Radiographically, DF appears as a radiolucent area around the crown of impacted teeth with normal width of approximately 2 to 2.5 mm (23). After tooth eruption, DF connect to the gingiva and undergoing structural changes (24). For achieving DF stem cells, only impacted teeth were selected. The teeth assessed radiographically and large follicular zone (>2.5-3mm) is considered as pathologic changes in follicular tissue. After teeth removal, DF attached to the crown teeth were separated using sterilized scalpel (25).

It must be considered that DF may undergo pathological changes like cystic or tumoral transformation (26, 27). Histopathologic studies on normal DF without any clinical and radiographic changes showed some pathological changes like inflammation, squamous metaplasia, or cystic transformation. These changes significantly increased in patients older than 20 years old (26, 28).

Apical papilla

In tooth development stages, the dental papilla is derived from ectomesenchyme, induced by overlying dental lamina. Dental papilla produces pulp and dentin and its apical portion is named apical papilla (AP) (29). The AP is a soft tissue loosely attached to the apices of immature teeth and its presence is essential for root completion and tooth development (Figure 1) (29-31). This tissue is separated from root canal pulp with a cell rich zone, histopathologically (30, 32). Its cellular and vascular components of apical papilla are less than the pulp tissue (30). For harvesting AP, only immature teeth were used, mostly, immature unerupted third molars. It was suggested to disinfect the teeth with 75% ethanol and wash with phosphate-buffered saline solution after extraction (31). The tissue at the apical portion of the root which is not framed by dentin (33) is gently separated using tweezers (30).

Gingiva

Gingiva is a part of supporting tooth structure which covers other parts of the periodontium (Figure 1) (34). It comprises of gingival epithelium and connective tissue, i.e., lamina properia. It seems that gingival connective tissue is the most accessible and abundant tooth-related structure for isolation of stem cells (34, 35). The gingival wound healed rapidly, therefore, the tissue was harvested during routine dental procedures, no more pain or discomfort was forced to the participants. Other advantages of gingival stem cells are rapid proliferation, stable morphology, uniformly homogenous property, and long-term capacity of maintaining the characteristics of MSCs (36). The donors with healthy periodontium and without history of periodontal disease were selected (1). The gingival connective tissue can be achieved conservatively after the tooth extraction from the inner site of the flap or during crown lengthening surgical procedures (29, 30).

Inflamed tissue

Although most of the studies selected non-inflamed tissue for MSC isolation, some studies isolated them from inflamed tissues. The inflamed pulp with irreversible pulpitis was assessed (36, 37). The diagnosis of irreversible pulpitis was made according to clinical signs such as spontaneous pain and pulp vitality was confirmed before access to the pulp tissue (36). The inflamed pulp with irreversible pulpitis were gained during root canal therapy and after pulpotomy. It was shown that MSCs from inflamed pulp preserved its proliferation and differentiation potential (36, 37). But more studies in this content is needed. The PDL of periodontally-affected teeth was also used as a source of MSCs. Sohelifar *et al.,* (18) used the PDL of hopeless teeth affected from periodontitis and found that viability and proliferation capacity of these stem cells was less than healthy PDL stem cells. Park *et al.,* used attached granulation tissue of apical portion of periodontal defects as a source of MSCs of inflamed PDL and showed the regenerative potential of these stem cells *ex vivo* (38). Using inflamed tissue

for MSC isolation is an interesting issue which bring the new source of stem cells without the need of tooth extraction but

more studies are needed to confirm the effectiveness of them.

Conclusion

In conclusion, tooth-related tissues are easily accessible sources for isolation of MSCs with less ethical consideration. Surprisingly, dental- related inflamed tissue was also a promising source of MSC isolation, but more studies needed to show their capacities in tissue regeneration. There is no obvious protocol for donor preparation or tissue harvesting, but summary of some suggestions was discussed here.

Acknowledgements

None

Conflict of Interest: 'None declared'.

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Please cite this paper as: Atarbashi-Moghadam F. Harvesting Tissue from Teeth-related Structures as a Source of Stem Cells. Regen Reconstr Restor. 2021;6 (1): e2. Doi: 10.22037/rrr.v5i1.33483.