

## Investigation of dental pulp stem cells isolated from discarded human teeth extracted due to aggressive periodontitis



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### ABSTRACT

Recently, human dental pulp stem cells (DPSCs) isolated from inflamed dental pulp tissue have been demonstrated to retain some of their pluripotency and regenerative potential. However, the effects of periodontal inflammation due to periodontitis and its progression on the properties of DPSCs within periodontally compromised teeth remain unknown. In this study, DPSCs were isolated from discarded human teeth that were extracted due to aggressive periodontitis (AgP) and divided into three experimental groups (Groups A, B and C) based on the degree of inflammation-induced bone resorption approaching the apex of the tooth root before tooth extraction. DPSCs derived from impacted or non-functional third molars of matched patients were used as a control. Mesenchymal stem cell (MSC)-like characteristics, including colony-forming ability, proliferation, cell cycle, cell surface antigens, multi-lineage differentiation capability and *in vivo* tissue regeneration potential, were all evaluated in a patient-matched comparison. It was found that STRO-1- and CD146-positive DPSCs can be isolated from human teeth, even in very severe cases of AgP. Periodontal inflammation and its progression had an obvious impact on the characteristics of DPSCs isolated from periodontally affected teeth. Although all the isolated DPSCs in Groups A, B and C showed decreased colony-forming ability and proliferation rate ( $P < 0.05$ ), the decreases were not consistent with the degree of periodontitis. Furthermore, the cells did not necessarily show significantly diminished *in vitro* multi-differentiation potential. Only DPSCs from Group A and the Control group formed dentin-like matrix *in vivo* when cell-seeded biomaterials were transplanted directly into an ectopic transplantation model. However, when cell-seeded scaffolds were placed in the root fragments of human teeth, all the cells formed significant dentin- and pulp-like tissues. The ability of DPSCs to generate dental tissues decreased when the cells were isolated from periodontally compromised teeth ( $P < 0.05$ ). Again, increased periodontal destruction was not necessarily followed by a decrease in the amount of dentin- and pulp-like tissue formed. These findings provide preliminary evidence that periodontally compromised teeth might contain putative stem cells with certain MSC properties, as long as the vitality of the pulp has not been totally damaged. Whether these cells can serve as a source of autologous multipotent MSCs for clinical regenerative therapies warrants further investigation with larger sample sizes and various types of periodontitis.

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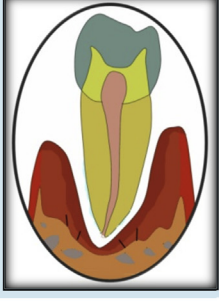

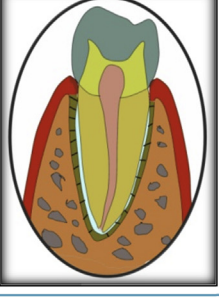

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## 1. Introduction

Stem cells constitute the source of differentiated cells for generating tissues during development and have attracted interest for regenerating tissues that are diseased or damaged postnatally. In recent years, we have witnessed major advances in both our understanding of stem cell biology and the practice of stem cell-based therapies in many clinical settings [1]. Over this same period, stem cell research has grown exponentially due to the

recognition that the use of stem cells as therapeutics has the potential to improve the quality of life of patients with a wide variety of medical conditions, ranging from Alzheimer's disease to cardiac ischemia to bone or tooth loss [2]. Mesenchymal stem cells (MSCs) represent a population of nonhematopoietic fibroblast-like cells that display the capacity to self-renew and differentiate into multiple lineages, including osteoblasts, adipocytes and chondrocytes, and these cells divide to replenish dying cells and regenerate damaged tissues. These unspecialized cells were originally

**Table 1**  
Group design and inclusion criteria for teeth before extraction.

Groups	No. of teeth	Schematic representations	Dental radiographs	Inclusion criteria
Group A	3			<ul style="list-style-type: none"> <li>➤ need to be removed due to irreversible periodontitis</li> <li>➤ bone resorption approaches the apex of the tooth root</li> <li>➤ apical foramen is not included in the inflamed area</li> </ul>
Group B	4			<ul style="list-style-type: none"> <li>➤ need to be removed due to irreversible periodontitis</li> <li>➤ bone resorption partially approaches the apex of the tooth root</li> <li>➤ apical foramen is partially included in the inflamed area</li> </ul>
Group C	5			<ul style="list-style-type: none"> <li>➤ need to be removed due to irreversible periodontitis</li> <li>➤ bone resorption reaches the apex of the tooth root</li> <li>➤ apical foramen is completely included in the inflamed area</li> </ul>
Control	3			<ul style="list-style-type: none"> <li>➤ may be removed due to impaction or because the tooth is non-functional</li> <li>➤ without periodontitis and the bone around the root is basically healthy</li> <li>➤ no inflammation around the apical foramen</li> </ul>

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