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Brief Communication

Capturing the Regenerative Potential of Periodontal Ligament Fibroblasts

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The cell population within the periodontal ligament (PDL) tissue is remarkably heterogeneous[1]. Fibroblasts, a mixed population of cells, are the main cellular component of the PDL and the cell type most often studied for periodontal regeneration. Osteoblasts and osteoclasts are found on the bone side, while fibroblasts, macrophages, undifferentiated adult/mesenchymal stem cells, neural elements, and endothelial cells are found throughout the PDL. Epithelial rests of Malassez cells and cementoblasts are focused near the root surface. PDL tissue also includes loose connective tissue between dense fiber bundles that contain branches of the periodontal blood vessels and nerves[2]. The complexity of the PDL tissue, with its various cell types and cell progenitor components, explains the challenges involved in therapies to restore tissue following periodontal disease. Cementoblasts, osteoblasts, and endothelial cells must migrate, differentiate, and coordinately interact with a variety of soluble mediators to regenerate the periodontium[3]. Stem cells located in the PDL tissue are key contributors to this process[4]. Stem cells in the PDL are important not only for formation and maintenance of the tissue but also for repair, remodeling, and regeneration of adjacent alveolar bone and cementum[5]. Our laboratory has shown that progenitor cells isolated from PDL tissue by selection with cell surface markers STRO-1+ and CD146+ are capable of differentiating into chondrogenic, osteogenic, and adipogenic phenotypes under appropriate culture conditions[6]. Immortalized cell lines are genetically modified human cells that proliferate beyond the point at which their original in vivo primary culture counterparts become senescent[7]. Immortalized PDL cells have been genetically altered, with unknown effects on cellular processes. Also, many immortalized cell lines harbor intraspecies and interspecies cross-contamination. Authentication tests to confirm the identity of immortalized cell lines is now a requirement from funding agencies[8]. In order to study the regenerative potential of PDL fibroblasts, our laboratory uses primary cells for in vitro studies. Primary PDL cell cultures used at early passages have the advantage of maintaining the rich phenotypic and functional heterogeneity of fibroblasts in the original tissue. Here, we present a detailed method for culturing PDL fibroblasts, based on over 10 years of experience of culturing PDL cells from primary cultures, which has been effective in maintaining the progenitor phenotype of PDL stem cells.

Our laboratory uses a direct cell outgrowth technique to obtain primary cells, including stem cells, from the PDL. First, general data pertinent to the tissue source is documented, including the age, gender, and medical history of the patient, the type and clinical status of the tooth, and the reason for extraction. Next, the extracted tooth is placed into a conical tube containing transport medium consisting of any minimal essential medium supplemented with 1% penicillin, 1% streptomycin, 1% fungizone, and 10% fetal

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bovine serum. Tissue harvesting and transport to the laboratory in a biohazard container should be completed promptly to ensure maximum viability of cells. Once in the laboratory, the tube containing the tooth is opened inside a laminar flow hood to optimize sterile conditions (Figure 1A). The tooth is placed in a 100-mm tissue-culture dish and rinsed with sterile phosphate-buffered saline in an apico-coronal direction to minimize contamination from gingival fibroblasts and other contaminants in the portion of the tooth exposed to the oral cavity. Sterile forceps and a scalpel blade are used to carefully scrape and collect the PDL tissue from the middle third of the root (Figure 1B). Harvesting cells from this portion of the root is less likely to result in contamination by bacteria and fibroblasts from dentogingival fibers from the coronal portion or pulp tissue at the apical third of the root. The harvested PDL tissue is placed on a sterile tissue-culture dish and a sterile cover slip lightly coated on one edge with autoclaved laboratory-grade petroleum jelly is placed on top of the tissue (Figure 1C). This fixation step is important because tissues and cells detached from the culture plate will not adhere, grow, or migrate. After stabilization of the cover slip and PDL tissue, medium containing 10% fetal bovine serum, 1% penicillin, 1% streptomycin, and 1% fungizone is added to the culture dish.

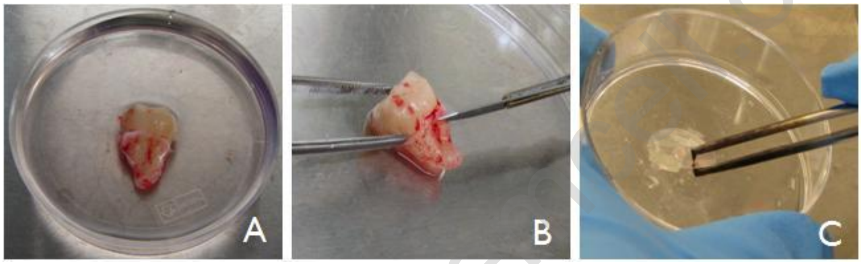

Figure 1 – A) Third molar placed in a tissue-culture dish inside the hood. (B) Close up of instruments used to hold the tooth and harvest the PDL tissue. C) Cover slip with autoclaved vacuum grease is placed over the freshly harvested PDL tissue.

PDL cell cultures are best incubated in a humidified atmosphere containing 95% air and 5% carbon dioxide at 37°C. Fibroblast outgrowths from the tissue are usually observed within 5–7 days but may take longer to develop (Figure 2A). This initial growth period is called passage zero (P0). When approximately 90% cell confluence is reached, cells are detached from the tissue-culture dish with EDTA and trypsin, collected, and split onto new tissue-culture dishes. During this next growth phase, called passage one (P1), PDL cells often grow in a parallel orientation to each other (Figure 2B). Cell morphology at subsequent passages (named P2, P3, P4, and so forth) is shown (Figure 2C).

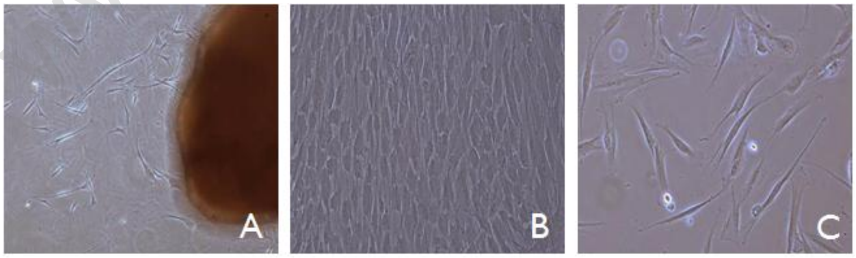

Figure 2 - A) PDL cell outgrowths derived from the PDL tissue (P0) using the explant technique (10x); B) Parallel orientation of PDL fibroblasts grown to confluence and observed in the P0 phase (20x); C) Non-parallel orientation of PDL fibroblasts observed in subsequent passages (20x).

The cell passage number at which PDL cells are used varies, and the optimal point for discontinuing cells of a given passage has not been firmly established. Since PDL fibroblast cultures undergo significant biochemical and phenotypical alterations as the passage number increases[9], we

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support the use of early-passage PDL fibroblasts to maintain a cellular response similar to that of the host tissue. Primary PDL cell cultures used at early passages have the advantage over cells used at later passages of maintaining the rich phenotypic and functional heterogeneity of fibroblasts in the original tissue.

The tissue explant technique described is advantageous because PDL cells isolated using this method exhibit mesenchymal stem cell-like properties, as exemplified by expression of the stem cell markers, STRO-1 and CD146 and their ability to differentiate into cementoblastic, osteogenic, chondrogenic, and adipogenic lineages given the appropriate differentiation media and conditions. Specifically, our laboratory has shown that primary PDL cells derived from this explant technique yield STRO-1+ cells that when treated with $Ca(OH)_2$ express cementum-specific proteins including protein tyrosine phosphatase-like, member A/cementum attachment protein (PTPLA/CAP) and cementum protein-1 (CEMP1)[6]. Furthermore, our laboratory has shown that 2.6% of primary PDL fibroblasts cultured with the explant technique express cell surface markers STRO-1+ and CD146+[10], which are used to isolate multipotent postnatal stem cells from the PDL[4]. Isolated STRO-1+/CD146+ cells accumulate cartilaginous macromolecules, mineralized calcium nodules, and lipid vacuoles under chondrogenic ,osteogenic, or adipogenic conditions, respectively. In addition, STRO-1+/CD146+ cells express cartilage-specific genes, early markers of osteoblastic differentiation, and adipogenic markers under appropriate culture conditions.

In conclusion, PDL tissues contain a rich diversity of cells, including stem cells. Therapies to regenerate the periodontium will depend upon obtaining cells from reproducible cell culture techniques that maintain the pluripotency of the PDL stem cells. Our laboratory has used the described cell culture technique to effectively study PDL stem cells in vitro, and future studies are necessary to determine the regenerative capacity of these cells *in vivo*.

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