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The influence of cellular source on periodontal regeneration using calcium phosphate coated polycaprolactone scaffold supported cell sheets

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ABSTRACT

Cell-based therapy is considered a promising approach to achieving predictable periodontal regeneration. In this study, the regenerative potential of cell sheets derived from different parts of the periodontium (gingival connective tissue, alveolar bone and periodontal ligament) were investigated in an athymic rat periodontal defect model. Periodontal ligament (PDLC), alveolar bone (ABC) and gingival margin-derived cells (GMC) were obtained from human donors. The osteogenic potential of the primary cultures was demonstrated *in vitro*. Cell sheets supported by a calcium phosphate coated melt electrospun polycaprolactone (CaP-PCL) scaffold were transplanted to denuded root surfaces in surgically created periodontal defects, and allowed to heal for 1 and 4 weeks. The CaP-PCL scaffold alone was able to promote alveolar bone formation within the defect after 4 weeks. The addition of ABC and PDLC sheets resulted in significant periodontal attachment formation. The GMC sheets did not promote periodontal regeneration on the root surface and inhibited bone formation within the CaP-PCL scaffold. In conclusion, the combination of either PDLC or ABC sheets with a CaP-PCL scaffold could promote periodontal regeneration, but ABC sheets were not as effective as PDLC sheets in promoting new attachment formation.

1. Introduction

Periodontitis is a common inflammatory disease that results in irreversible destruction of the tooth supporting structures and eventually leads to tooth loss [1]. The ultimate goal of periodontal treatment is to restore the structure and function of the damaged periodontium. This is challenging as it requires the restoration of both hard and soft tissues, with the formation of functionally oriented periodontal ligament fibers which insert into newly formed cementum and alveolar bone. In the past few decades, various procedures, including root conditioning, bone grafting, guided tissue regeneration (GTR) and the application of biological factors have been utilized in order to promote periodontal regeneration. Unfortunately, currently regenerative procedures have had limited success in achieving this goal [2,3].

Recent advances in progenitor cell biology and tissue engineering have enabled the development of cell-based therapeutics that are aimed at achieving periodontal regeneration with greater efficacy and predictability [2,4]. A variety of cell types, including bone marrow mesenchymal stem cells, adipose-derived stem cells and dental follicle cells, have been shown to promote periodontal regeneration to various degrees in animal models [5–7].

The progenitor capacity of periodontal tissue itself has also been extensively studied during the past few decades [8]. Although studies utilizing the progenitor capacity of cells derived from

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periodontal tissues have mainly focused on periodontal ligament-derived cells (PDLC), recent studies have demonstrated that alveolar bone and gingival tissue may also contain progenitor cells that can be used for periodontal regeneration [9–12]. These cell sources are important given the highly specialized nature of the various periodontal components, and their ready availability from redundant tissues obtained from periodontal and oral surgery. However, the periodontal regenerative capacity of cells derived from different periodontal tissues has not as yet been compared in an *in vivo* model.

The delivery of intact cell sheets onto a diseased tooth root is an attractive periodontal regeneration approach as it simulates the anatomical features of the periodontal ligament, whose presence is necessary for reforming the periodontal attachment between alveolar bone and root surface cementum. Intact cell sheets for transplantation can be prepared using thermo-responsive culture flasks [13]. Using this technique, cells are detached from the culture flask by lowering the temperature, without the need for enzymatic treatment. This results in cell sheets with an intact extracellular matrix. The cell sheet method has been used to promote periodontal regeneration in various periodontal defect models, and the safety and efficacy of PDLC sheets has been evaluated both *in vitro* and *in vivo* in pre-clinical studies [14–18]. Although the results of these studies suggest that therapy based on cell sheet engineering can be effective and practical for clinical periodontal regeneration, an inherent challenge of the cell sheet transplantation approach is the difficulty in stabilizing and securing the cell sheet within the periodontal defect. Therefore, there is a need to identify effective strategies for the delivery of cell sheets into periodontal defects. To this end, the ideal characteristics of a cell sheet 'carrier' scaffold to be used for periodontal regeneration would include the ability to stabilize the cell sheets on the root surface, as well as facilitate bone formation within the periodontal defect.

Our hypothesis was that the combination of an osteoconductive scaffold and cell sheets prepared from primary periodontal tissue derived cell cultures would result in a construct that facilitates periodontal regeneration by not only recreating the complex hierarchical structure of periodontal tissues, but also ensuring that the cells are secured to the denuded root surface within the periodontal defect. The first aim of this study was to compare the regenerative potential of cell sheets produced by human primary cell cultures derived from different periodontal tissues in an athymic rat periodontal defect model. The second aim was to assess the suitability of a previously characterized electrospun calcium phosphate coated polycaprolactone (CaP-PCL) scaffold [19] to support and stabilize a cell sheet at the periodontal defect site.

2. Materials and methods

The experimental design of the study is summarized in Fig. 1A. Briefly, cell sheets obtained from well characterized primary cell cultures were harvested intact using thermoresponsive culture plates and delivered into rat periodontal defects

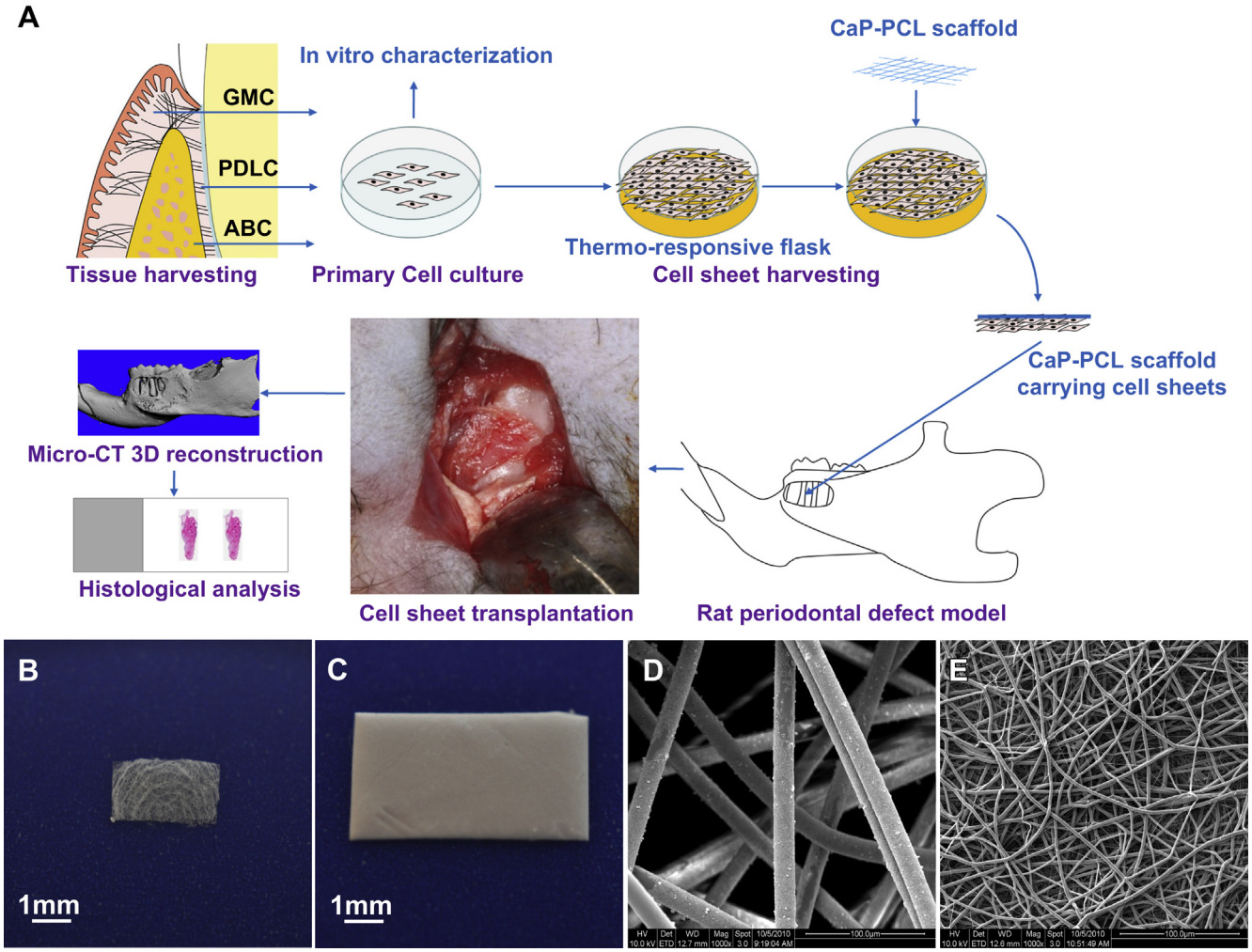

Fig. 1. A: Flow chart of cell culture, cell sheet harvesting and transplantation. B and D: morphology of the CaP-PCL scaffold; C and E: morphology of the covering membrane; D and E display the morphology of the structures under Scanning Electron Microscopy (SEM).