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Periodontal tissue regeneration by combined implantation of adipose tissue-derived stem cells and platelet-rich plasma in a canine model

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Abstract

Background aims. One goal of periodontal therapy is to regenerate periodontal tissues. Stem cells, growth factors and scaffolds and biomaterials are vital for the restoration of the architecture and function of complex tissues. Adipose tissue-derived stem cells (ASCs) are an ideal population of stem cells for practical regenerative medicine. In addition, platelet-rich plasma (PRP) can be useful for its ability to stimulate tissue regeneration. PRP contains various growth factors and may be useful as a cell carrier in stem cell therapies. The purpose of this study was to determine whether a mixture of ASCs and PRP promoted periodontal tissue regeneration in a canine model. *Methods.* Autologous ASCs and PRP were implanted into areas with periodontal tissue defects. Periodontal tissue defects that received PRP alone or non-implantation were also examined. Histologic, immunohistologic and x-ray studies were performed 1 or 2 months after implantation. The amount of newly formed bone and the scale of newly formed cementum in the region of the periodontal tissue defect were analyzed on tissue sections. *Results.* The areas of newly formed bone and cementum were greater 2 months after implantation of ASCs and PRP than at 1 month after implantation, and the radiopacity in the region of the periodontal tissue defect increased markedly by 2 months after implantation. The ASCs and PRP group exhibited periodontal tissue with the correct architecture, including alveolar bone, cementum-like structures and periodontal ligament-like structures, by 2 months after implantation. *Conclusions.* These findings suggest that a combination of autologous ASCs and PRP promotes periodontal tissue regeneration that develops the appropriate architecture for this complex tissue.

Key Words: *adipose-derived stem cells, adult stem cells, canine, periodontal disease, periodontal tissue regeneration, platelet-rich plasma*

Introduction

Periodontitis results in the loss of connective tissue and bone support and is a major cause of tooth loss in adults (1). The ultimate goal of periodontal therapy is to regenerate the periodontal tissues that are lost as a result of periodontitis (2). Several treatments for periodontal tissue regeneration, including bone grafts (3,4), guided tissue regeneration (5,6) and application of an enamel matrix derivative (7), have been developed through basic research and used in the clinical dental arena. However, achieving a complete reconstruction of periodontal tissues in a predictable manner is difficult (8) because the periodontium, including alveolar bone, periodontal ligament and cementum tissue, has a limited capacity for regeneration once damaged (9).

These limitations have led to the emergence of a new field—tissue engineering. Tissue engineering, which has the goal of regenerating a functional living tissue, is an extremely complex process that requires the temporally and spatially appropriate occurrence of multiple events. Current paradigms in tissue engineering often involve a combination of mesenchymal stromal cells (MSCs) and progenitor cells, growth factors and cytokines and scaffolds/biomaterials. The implantation of MSCs that can differentiate into osteoblasts (10), cementoblasts or periodontal ligament-forming cells (11) has been viewed as a future approach for periodontal tissue regeneration.

More recently, several procedures using bone marrow-derived MSCs have been examined for their ability to correct periodontal osseous defects in a canine model (11–13). Although the results of these studies showed greater periodontal tissue regeneration than current clinical therapies and suggest that

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clinical applications may be possible, cell harvesting from bone marrow for use in the clinical dental arena is a concern. Although bone marrow is the most abundant source of MSCs, other tissues, such as periosteum (14), muscle (15), synovial membrane (16) and adipose tissue (17–19), also possess MSCs. Adipose tissue, in particular, is attractive because of its accessibility and abundance. Adipose tissue-derived stem cells (ASCs) are isolated from fat tissue (20,21). Similar to MSCs, ASCs can differentiate into cells and tissues of mesodermal origin, such as adipocytes (19,20), cartilage (22,23), bone (24,25) and skeletal muscle (26,27). More recent *in vitro* data have shown that ASCs not only regenerate mesodermal tissues but may contribute to both ectodermal and endodermal tissues (28,29).

Identifying a suitable cell carrier is also important to establish effective methods for stem cell therapies. Platelet-rich plasma (PRP) has been used clinically in humans since the 1970s for its wound healing properties, which are attributed to the high levels of growth factors and secretory proteins in PRP (30). The growth factors in PRP enhance the recruitment, proliferation and differentiation of cells involved in tissue regeneration (31). The use of a combination of ASCs and PRP has been reported in periodontal tissue engineering (32), wound healing (33), tendon repair (34) and bone regeneration (35). These reports suggest that PRP may be a useful component of stem cell therapies.

A previous report revealed that the combination of ASCs and PRP produced some regenerated periodontal tissue, including alveolar bone, cementum-like structures and periodontal ligament-like structures (32). However, the efficacy of implanted ASCs in periodontal tissue regeneration has yet to be demonstrated in a large animal model. In this study, we examined the utility of combined implantation of autologous ASCs and autologous PRP in periodontal tissue engineering in a canine periodontal tissue defect model.

Methods

Experimental animals

After receiving approval from the Committee of Research Facilities for Laboratory Animal Science of Nippon Medical School (approval number 17-058), beagle dogs 9 or 10 months old weighing 8–10 kg (Japan Laboratory Animals Inc, Tokyo, Japan) were used in this study (n = 8).

Cell harvesting and primary cell culture

Animals were anesthetized with thiamylal sodium (3 mg/kg) and isoflurane. The inguinal fat pads were harvested from eight dogs, extensively washed with phosphate-buffered saline (Gibco-BRL, Grand Island, NY, USA), finely minced, and enzymatically digested at 37°C for 40 min with 0.1% collagenase (Wako, Osaka, Japan). An equal volume of control medium (Dulbecco's modified Eagle's medium containing 10% fetal bovine serum and 1% antibiotic/antimycotic; all from Gibco-BRL) was added to neutralize the collagenase. The cell suspension was centrifuged at 1300 rpm (260*g*) for 5 min to obtain a high-density ASC pellet, which was re-suspended in control medium. The cells were plated at a concentration of 5×10^5 cells per 100 mm^2 tissue culture dish (Becton-Dickinson, Franklin Lakes, NJ, USA) and maintained in control medium at 37°C in 5% CO_2.

Generation of class III periodontal tissue defects

The canine periodontal tissue defect model was generated as previously described (11,13). Briefly, class III periodontal tissue defects, in which the tissue defect penetrates to the root bifurcation of the tooth, were generated in eight dogs. All surgical procedures were performed under general anesthesia with thiamylal sodium (3 mg/kg) and isoflurane. To minimize bleeding in the surgical field, 2% xylocaine and epinephrine (1:80,000 dilution) (Astellas Pharma, Inc, Tokyo, Japan) was administered locally. The bilateral second, third and fourth premolars (P2, P3 and P4) in the mandibular body of each dog were selected for experimentation. Following sulcular incisions, the mucoperiosteal flaps were raised, and class III bifurcation defects were generated surgically at P2, P3 and P4. The height from the top of the dental root bifurcation to the reduced alveolar crest in the class III defect was 5 mm. Denuded root surfaces were prepared to remove all periodontal ligament and cementum (Figure 1). The generated periodontal tissue defects were marked with a notch (5 mm from the coronal) on the surface of the dental root.

Preparation and analysis of PRP

Before implantation of ASCs, 20 mL of whole blood was drawn from each of the eight dogs with a 23-gauge needle (Terumo, Tokyo, Japan) into tubes containing 3.8% sodium citrate. The blood was centrifuged in a standard laboratory centrifuge (Kubota 3740; Kubota, Tokyo, Japan) for 10 min at 450*g*, after which a long cannula was used to harvest the plasma supernatant and the buffy coat (which consists of platelets and leukocytes) into a neutral tube. To concentrate the platelets, the tubes were centrifuged at 800*g* for 15 min. The infra-natant