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Local administration of stromal cell-derived factor-1 promotes stem cell recruitment and bone regeneration in a rat periodontal bone defect model

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ABSTRACT

Stromal cell-derived factor-1 (SDF-1) recruits adult stem/progenitor cells via its specific receptor, C–X–C motif receptor 4 (CXCR4), to promote heart, kidney and tendon regeneration, but little is known about the effects of SDF-1 on bone regeneration in periodontal diseases. The objective of this study was to investigate whether local administration of SDF-1 in a collagen membrane scaffold enhanced the recruitment of host stem cells and improved periodontal bone defect repair. To this end, bone defects were established on the buccal side of bilateral mandibles in Wistar rats. After application of collagen membranes loaded with SDF-1 or phosphate-buffered saline (PBS) to the defects, the effects of SDF-1 on stem cell recruitment, inflammatory cell responses, angiogenesis, osteoclastogenesis, scaffold degradation, and bone regeneration were evaluated. It showed that SDF-1 recruited host-derived mesenchymal stem cells and hematopoietic stem cells to the wound area and significantly reduced the CD11b+ inflammatory cell response. Moreover, SDF-1 increased vascular formation, induced early bone osteoclastogenesis, accelerated scaffold degradation, and promoted the quality and quantity of regenerated bone. Our results suggest that this cell-free approach by local administration of SDF-1 may be an effective strategy for development as a simple and safe technique for periodontal bone regeneration.

1. Introduction

Periodontitis can lead to attachment loss, alveolar bone resorption, and finally tooth loss. Regeneration of bone is the main goal for periodontal therapy. The development of tissue engineering techniques has led to new prospects for periodontal bone regeneration. Although researchers have studied the application of bone marrow mesenchymal stem cells (MSCs) [1], adipose-derived stem cells [2], periodontal ligament stem cells (PDLSCs) [3], and dental follicle cells [4] for periodontal bone regeneration, many barriers limit their application in clinical practice. The main limitations include donor site morbidity due to cell harvesting [5], high costs and time-consuming procedures for ex vivo cell handling [6], and even malignant transformation of the cells during expansion [7]. Therefore, stem cell therapy may be clinically applicable for incurable and fatal diseases but not periodontal therapy. The development of in situ tissue engineering techniques has overcome the aforementioned shortcomings of stem cell therapy. This approach focuses on stimulating and supporting the body's own regenerative capacities by recruiting circulating or residing MSCs to the defect site to undergo tissue-specific differentiation and participate in tissue repair [8]. Substantial recruitment of autologous stem cells can be achieved by increasing the local concentrations of cytokines and chemokines at the target site [9,10]. Cytokines and chemokines are important factors that regulate mobilization, trafficking and homing of stem/progenitor cells [11]. Among the various cytokines and chemokines, stromal cell-derived factor-1 (SDF-1) is a promising candidate for in situ tissue engineering.

SDF-1, also known as C–X–C motif ligand 12 (CXCL12), has been characterized as a chemokine that is vital for stem/progenitor cell recruitment to repair injured tissues for [12]. SDF-1 and its complementary receptor, C–X–C motif receptor 4 (CXCR4), play an important role in the development of embryonic organs [13] and maintaining the steady state of tissues after birth including blood homeostasis [14] and bone remodeling [15]. At the cellular level, binding of SDF-1 to CXCR4 results in cytoskeleton rearrangement and integrin activation that mediate directional migration of CXCR4+ cells towards high gradients of SDF-1 [16–18]. Numerous studies have indicated that the SDF-1/CXCR4 axis is necessary for MSCs to mediate tissue repair and regeneration. During the healing process of impaired brain [19], heart [20–22], muscle [23], liver [24], kidney [25,26], skin [27], tendon [28] and bone [29] tissues, elevation of SDF-1 levels at injury sites induces recruitment of CXCR4+ stem/precursor cells from blood circulation and local tissues, resulting in tissue-specific differentiation for trauma repair. Moreover, local delivery of SDF-1 to injured tissues mediates engraftment of

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circulating stem cells and precursor cells in damaged sites to promote organ repair and regeneration [21–23,27].

Studies of chemokine-based in situ tissue engineering have mainly focused on cardiovascular and bone diseases, and less on periodontal disease. It has been found that systemically delivered MSCs can migrate to injury sites and participate in periodontal bone repair and regeneration [30], which is the main rationale for the application of SDF-1 in periodontal therapy. Our previous work demonstrated that PDLSCs express CXCR4, and SDF-1 promotes the proliferation, migration and differentiation of PDLSCs in vitro [31]. However, the effects of local application of SDF-1 in vivo on the homing of stem/progenitor cells and periodontal bone regeneration have not been elucidated.

The present study aimed to test the hypothesis that SDF-1 plays a pivotal role in periodontal bone repair. Using rat mandibular bone defect models, it demonstrated that local delivery of SDF-1 recruited stem cell to injury sites, reduced inflammatory responses during the early phase of wound repair, and promoted the quantity and quality of newly formed bone. Our results suggest a new strategy for the therapeutic use of SDF-1 to promote periodontal bone regeneration.

2. Materials and methods

2.1. SDF-1 loading into absorbable collagen membranes

Absorbable collagen membranes (ZhengHai Biotechnology, Yantai, Shandong, China) were cut into 4 × 3 × 1 mm pieces and soaked in 100 μL of 50 μg/mL SDF-1 (sc-4654, Santa Cruz Biotechnology, Santa Cruz, CA, USA) according to a previous study [32] or PBS (control). The soaked scaffolds were then transferred to 6-well plates and incubated at 4 °C overnight.

2.2. Ethics statement

This study complied with the recommendations in the Guide for the Care and Use of Laboratory Animals of the Chinese Science and Technology Ministry. All experimental procedures were approved by the Committee on the Ethics of Animal Experiments of Shandong University (Permit Number: 201302070). All surgery was performed under chloral hydrate anesthesia, and all efforts were made to minimize suffering.

2.3. Animal mandibular bone defect model and SDF-1 delivery to the wound area

Forty adult Wistar rats (200–250 g, male) were used in this study. All animals were maintained in a temperature-controlled room with a 12-h light/dark diurnal cycle and had free access to food and water throughout the experiment. The rats were anesthetized with 10% chloral hydrate (0.4 mL/100 g body weight). After an extra-oral incision parallel to the inferior border of bilateral mandibles, subcutaneous tissues and masseter muscle were dissected. The mandibular buccal surface was exposed and a standardized buccal defect was made around the mandibular first molar with a round bur and a fissure bur at low speed with copious saline irrigation. The mandibular bone wound window was approximately 5 × 4 × 1.5 mm with the anterior margin at 1 mm distal to the front of the mandible and the coronal margin was approximately 1 mm apical of the crest of the alveolar bone. Left-side defects were implanted with SDF-1-loaded collagen membranes, while the right-side defects were filled with PBS-loaded collagen membranes as the control group.

2.4. Specimen collection and preparation for microcomputed tomography (μCT) and histological analyses

Rats were sacrificed at 3 days, 7 days, 4 weeks, and 8 weeks post-surgery (n = 10 for each time point). Animals were anesthetized with 10% chloral hydrate (0.4 mL/100 g body weight) and perfused through the heart with 100 mL saline and then 250 mL of 4% paraformaldehyde in phosphate buffer, pH 7.4. After μCT analysis, the bilateral mandibles were incubated in the same fixative overnight at 4 °C and then

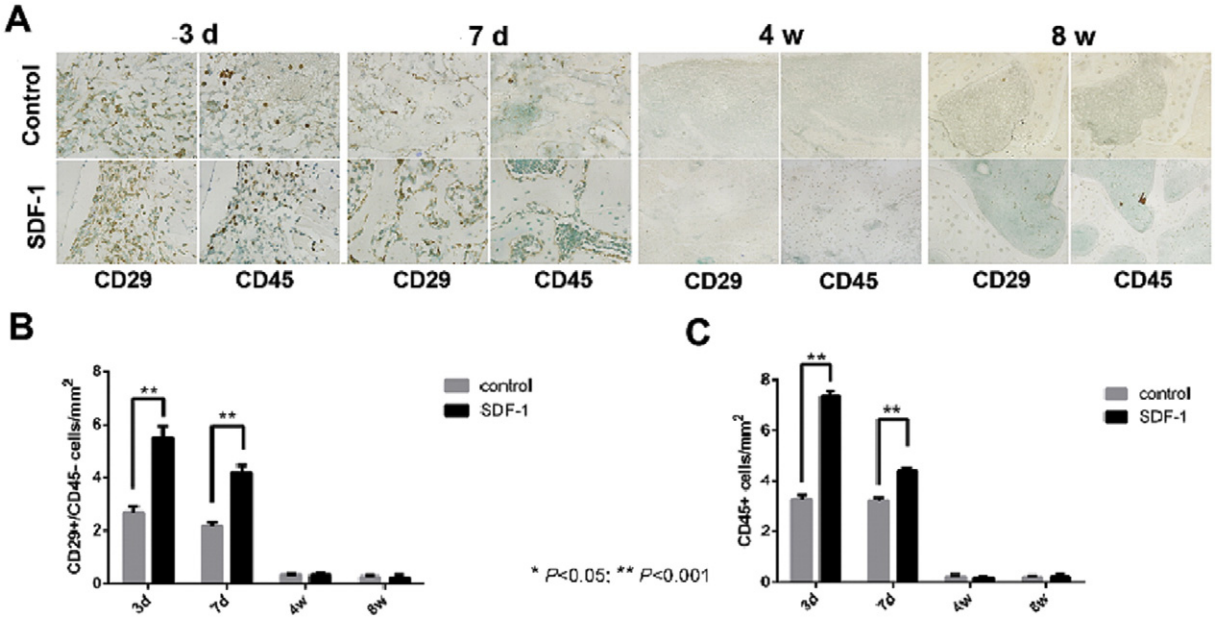

Fig. 1. SDF-1 increases engraftment of CD29 + /CD45 − MSCs and CD45 + HSCs in bone defects. (A) Immunohistochemical staining of CD29 (brown) and CD45 (brown) in serial sections at 3 days, 7 days, 4 weeks, and 8 weeks post-surgery. (B) Quantitative comparison of MSC numbers between control and SDF-1 groups as evaluated by CD29 + /CD45 − cells/mm^2. (C) Quantitative comparison of HSC numbers between control and SDF-1 groups as evaluated by CD45 + cells/mm^2. At day 3, the control group showed relatively low engraftment of MSCs and HSCs than SDF-1-treated group (A). At day 7, the density of MSCs and HSCs decreased in both groups, but the SDF-1 group still had a larger number of stem cells than the control group (A). At 4 and 8 weeks, the stem cells had almost disappeared in both groups (A). There was a significant ~2-fold increase in the number of engrafted MSCs and HSCs in the SDF-1 group than in the control group at both 3 and 7 days (B, C). The number of stem cells decreased and there was no difference between the two groups at weeks 4, and 8 (B, C). *P < 0.05; **P < 0.001.