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ORIGINAL ARTICLE

Injectable adipose tissue combined with stem cells for soft-tissue augmentation: A pilot study for dental applications

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Abstract *Background/purpose:* Bone resorption and soft-tissue defects are the typical physiologic responses after tooth extraction. Various dental ridge augmentation techniques have been applied and lack of the soft tissue is the major factor causing the failure. We propose that the adipose-derived stem cell can be useful in soft-tissue augmentation in dental applications. The objective of this study was to optimize the operation procedures for the isolation of adipose stem cells and tissues. Accelerated clinical protocols for effective transplantation of adipose tissue with high amount of adipose stem cells shall be developed.
Materials and methods: Operation parameters were designed and optimized for the extraction of adipose tissue-derived stromal vascular cells. The optimized accelerated procedure was washing the lipoaspirate samples one time. Collagenase was then added and samples were incubated in a water bath for 30 minutes at 37°C and centrifuged at 1200*g* for 3 minutes. A mouse animal model was applied to evaluate the soft-tissue-filling effects using the optimized procedure.
Results: The animal model tests demonstrated the filling and regeneration of the soft tissues with significant angiogenesis.

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Conclusion: This pilot study demonstrated the feasibility of soft-tissue augmentation applications.

Introduction

Alveolar bone resorption is the typical physiologic response following tooth extraction.[1] Soft-tissue defects in conjunction with severe bone defects are commonly observed clinically. In addition, ridge defects present important challenges for aesthetic dentistry. Several ridge augmentation techniques have been developed to replace tissue loss, including soft tissue or/and alveolar bone.[2–5] The critical factor for successful ridge augmentation is the coverage of soft tissues. It is indicated that mucosal dehiscence and premature exposure of the bone graft are the most common failure mechanisms.[6] Therefore, prior to performing grafting procedures, plastic periodontal procedures to augment soft tissue may be necessary. The traditional procedures include the use of free gingival grafts, subepithelial connective grafts, and various types of roll and pedicle flaps.[7–10] However, these advanced surgical procedures are highly technique sensitive and usually result in postoperative complications. The development of a rapid protocol for soft-tissue augmentation is therefore needed.

Injectable adipose tissue obtained from patients by liposuction can be used as autologous filling materials to improve multiple defect healing.[11] This also presents a nonsurgical alternative for augmenting soft tissues. So far, injection of adipose tissue has safely been applied in cosmetic medicine. Adipose tissue is not only suitable as a soft-tissue filling material, but also serves as a plentiful source of adipose-derived stem cells (ADSCs). Clinical studies of ADSCs for the regeneration of periodontal tissues have shown that the ADSCs with platelet-rich plasma can regenerate alveolar bone, cementum, and periodontal ligament.[12,13] ADSCs in combination with functional biomaterials could be used to accelerate bone healing in peri-implant defects caused during dental implant placement.[14]

ADSCs are contained in adipose tissue-derived stromal vascular fraction (SVF) cells, which are freshly isolated from adipose tissue by enzymatic digestion. ADSCs can further be selected and expanded by culturing extracted SVF cells in plastic,[15] and if the SVF cells are harvested, a sufficient number of these cells can be used clinically without the need for expansion of ADSCs. SVF cells supplemented fat tissues can improve calcification, fibrosis, and reduce the transplant absorption rate as these cells secrete vascular endothelium growth factor, fibroblast growth factor, and tissue growth factor-beta.[16–20] Therefore, injectable adipose tissue supplemented with SVF cells has the potential for augmenting soft tissues in clinical applications. It is suggested that SVF cells contain ADSC-assisted autologous adipose tissue that may assist in augmentation of ridge defects healing.

SVF cell-assisted autologous adipose tissue augmentation is a process that relies on the isolation of SVF cells. However, most methods of isolation of SVF cells are tedious and time consuming. Similar methods reported in the literature for isolating the adipose cells and their principles are shown in Figure 1. At present, a typical isolation process takes ~3 hours. During this period, patients, doctors, and healthcare personnel are forced to wait until the completion of stem cell extraction through a surgical procedure. As a result, such isolation process may increase the cost and affect the quality of the care. In addition, each step of the isolation process may affect the cell viability. With the increasing number of experimental procedures, the viability of the extracted cells starts to decrease.

The objective of this study was to develop an accelerated procedure for adipose tissue augmentation. A series of experiments were designed to optimize the parameters for extraction of SVF cells. Animal tests were carried out to evaluate soft-tissue augmentation with adipose tissue and SVF cells extracted using the optimized procedure presented herein.

Materials and methods

Harvest of human adipose tissue

Human adipose tissue was harvested from individual female donors during liposuction surgery using a 16-gauge suction cannula under a pressure of 760 mmHg. All procedures were approved by the Institutional Review Board of Tri-Service General Hospital, Taipei, Taiwan with the informed consent of the donors (TSH-IRB-100-05-143). Adipose tissue specimens were preserved at 4°C within 3 days after the surgery for subsequent use.

Isolation of human adipose tissue-derived SVF cells

The collected fat tissue was divided into 15 mL samples for experiments. The adipose tissue was washed using Dulbecco's phosphate-buffered saline (DPBS; Sigma-Aldrich, St. Louis, MO, USA) at 1200*g* for 3 minutes. The tissue was then digested with an equal amount of 0.2% collagenase I (Sigma-Aldrich) in DPBS at 37°C for various amounts of time (30 minutes, 45 minutes, or 60 minutes). The collagenase was inactivated using Dulbecco's modified Eagle's medium (DMEM; HyClone, Logan, UT, USA) with 10% (v/v) fetal bovine serum (FBS; HyClone); the digested tissue was then centrifuged at different forces (600*g*, 1200*g*, or 2800*g*) for various periods (1 minute, 3 minutes, or 5 minutes; UNIVERSAL 320 R-1406-01; Andreas Hettich GmbH & Co.KG, Tuttlingen, Germany). The supernatant was removed and

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