Cytotherapy, 2017; 19: 543–554

International Society for Cellular Therapy
ISCT®

VASCULAR CELLS

Stromal vascular fraction shows robust wound healing through high chemotactic and epithelialization property

DONG-SIC CHAE[1,*], SEONGHO HAN[2,*], MINA SON[3] & SUNG-WHAN KIM[3]

[1]*Department of Orthopedic Surgery, International St. Mary's Hospital, Catholic Kwandong University College of Medicine, Incheon, Republic of Korea,* [2]*Department of Family Medicine, College of Medicine, Dong-A University, Busan, Republic of Korea, and* [3]*Department of Medicine, College of Medicine, Catholic Kwandong University, Gangneung, Republic of Korea*

Abstract

Background. Although human stromal vascular fraction (SVF) has been regarded as an attractive stem cell source, its therapeutic mechanism in wound healing has not been fully elucidated. *Aims.* In this study, we investigated the molecular characteristics and therapeutic property of SVF for wound healing. *Methods.* Microarray data showed that SVF cells are enriched with a higher level of wound healing or epithelium development–related genes and micro RNA. *Results.* Quantitative polymerase chain reaction (PCR) and reverse transcriptase PCR results revealed that the epithelialization growth factor, epidermal growth factor (EGF), chemokines, stromal cell–derived factor (SDF-1 or CXCL12), neutrophil-activating protein-2 (NAP-2 or CXCL7), chemokine receptors (CXCR1, CCR2 and CCR3) and wound healing genes were up-regulated in SVF compared with those in adipose-derived mesenchymal stem cells (ASCs). An *in vitro* scratch wound closure experiment demonstrated that co-culture with SVF substantially accelerated the wound closure of fibroblasts. Wounds in nude mice were created by skin excisions followed by injections of SVF with Pluronic hydrogel. SVF implantation highly accelerated wound closure and increased cellularity and re-epithelialization. In addition, the transplanted SVF exhibited high engraftment rates in the wound area, suggesting direct benefits for cutaneous closure. *Conclusions.* Taken together, these data suggest that SVF possesses high therapeutic capability for wound healing via the secretion of epithelialization and chemotactic growth factors and enhanced engraftment properties.

Key Words: *chemokine, epidermal growth factor, Pluronic hydrogel, stromal vascular fraction, wound healing*

Introduction

Proper healing of a cutaneous wound requires the well-arranged elements of biological and molecular events of inflammation, epithelialization, granulation, neovascularization and reorganization [1]. Various cytokines and growth factors regulate the growth and differentiation of keratinocytes [2].

Epidermal growth factor (EGF) is one of the most important growth factors in skin wound healing and epithelialization. EGF induces keratinocytes and fibroblasts to migrate, grow and accelerate wound healing [3]. EGF also promotes the formation of granulation tissue [4]. Eleven EGF family members are known: EGF, heparin binding EGF-like growth factor, transforming growth factor-α, amphiregulin, epigen, epiregulin, β-cellulin and the neuregulins (NRGs) NRG-1, NRG-2, NRG-3 and NRG-4 [5,6]. The members of this protein family have highly similar structures and functions.

Stem cells derived from adipose, bone marrow, peripheral blood, cord blood, amnion and other tissues enhance the repair of damaged tissue [7–13]. In fact, recent studies indicate that adipose-derived stem cells from *in vivo* animal studies promoted human dermal fibroblast proliferation by direct cell-to-cell contact and

*These authors contributed equally to this work.

Correspondence: **Sung-Whan Kim**, PhD, International St. Mary's Hospital, 25, Simgok-ro 100beon-gil, Seo-gu, Incheon 404-190, Republic of Korea. E-mail: swkim@cku.ac.kr

(Received 22 December 2016; accepted 17 January 2017)

ISSN 1465-3249
http://dx.doi.org/10.1016/j.jcyt.2017.01.006

by secretory-induced paracrine activation, which significantly reduced the wound size and accelerated the re-epithelialization from the edge [11].

One of the most abundant and easily isolated stem cell sources is adipose tissue. It has been reported that the total yield of isolated stem cells from adipose tissue is 40-fold greater than from bone marrow [14–16]. SVF cells are a variable cell population that includes fibroblasts, circulating hematopoetic cells, pericytes, endothelial cells, mesenchymal stromal cells (MSCs) and pre-adipocytes [17]. Numerous articles already reported that SVF cells contain large numbers of MSC-like cells that can be induced to differentiate into chondrogenic, myogenic, adipogenic and osteogenic lineages [18]. In fact, freshly isolated SVF cells are widely used in cosmetic or plastic surgery due to the easy accessibility and isolation method. Cultured SVF cells could be made to MSCs and they might have the similar activity of fresh SVF cells. However, cultured MSCs have additional challenges, such as high cost, long time, contamination or other xenogenic infections like mycoplasma, viruses and other uncovered pathogens.

Recently, rat stromal vascular fraction (SVF) improved deep partial thickness burn wound healing [19]. However, the therapeutic mechanisms of freshly isolated SVF derived from human adipose tissues in wound healing have not yet been fully elucidated.

In this study, we investigated the therapeutic mechanisms and capabilities of human SVF compared with adipose-derived mesenchymal stromal cells (ASCs) in an excisional wound animal model. In addition, we used the degradable hydrogel, Pluronic F-127, to test its biocompatibility with SVF and its behavior during the wound healing process. The three-dimensional environment of a hydrogel can enhance the engraftment and differentiation of injected stem cells [20].

Materials and methods

SVF isolation

Human SVF cells were obtained from the resection of subcutaneous fat portions after receiving informed consent from healthy donors using the guidelines approved by the institutional review board of Catholic Kwandong University. The SVF was separated using a modification of a previously described method [18]. Briefly, the fat tissue was digested with 0.075% collagenase in phosphate-buffered saline (PBS) solution for 1 h at 37°C. The digested fat tissues were then separated by centrifugation at 800*g* for 10 min at 4°C. The cell pellet was resuspended in red blood cell lysis buffer and incubated for 10 min at 4°C. Finally, the suspended cells were filtered with a 100-μm mesh filter (BD). Nucleated cell counts were performed using an automated cell counter.

Cell culture

Human dermal fibroblasts (HDFs) and human umbilical vein endothelial cells (HUVECs) were obtained from the American Type Culture Collection. Human ASCs were cultured using SVF cells derived from healthy donors. Briefly, HDFs and ASCs were cultured in low-glucose Dulbecco's modified Eagle's medium (DMEM; Gibco) supplemented with 10% fetal bovine serum (FBS), 100 U/mL penicillin and 100 mg/mL streptomycin (Gibco) [21,22]. Human umbilical vein endothelial cells (HUVs) were cultured in endothelial growth medium (EGM-2; Lonza).

Flow cytometry

Cells were suspended in PBS containing 1% bovine serum albumin. Cells were incubated with fluorescein isothiocyanate- or phycoerythrin-conjugated monoclonal antibodies specific for CD14, CD29, CD31, CD44, CD45, CD73, CD 90, CD105, CD166 and rat anti-mouse immunoglobulin (Ig)G. All antibodies were from BD. Isotypically identical IgG was used as a control. Cells were stained and fixed in 2% paraformaldehyde and analyzed using a flow cytometer (BD).

Microarray and gene set enrichment analysis

Microarray analysis was performed using Human Gene 2.0 ST array (Affymetrix, Inc.) (Supplementary Figure S1A). Gene set enrichment analysis (GSEA) was performed using a GSEA program (http://www.broad.mit.edu/gsea/msigdb/index.jsp). Wound-healing gene sets were collected based on the gene ontology (GO) data base (http://www.geneontology.org/). $P < 0.05$ and $Q < 0.15$ were considered statistically significant. Micro RNA (miRNA) microarray was performed using human miRNA 4.0 (Affymetrix) (Supplementary Figure S1B).

Real-time polymerase chain reaction and reverse transcriptase polymerase chain reaction analysis

Real-time polymerase chain reaction (PCR) and reverse transcriptase (RT)-PCR assays were performed according to previously reported methods with modifications [23]. Briefly, the total RNA was isolated from cells using RNA-Stat reagent (Iso-Tex Diagnostics) according to the manufacturer's instructions. The concentrations of RNA were determined by a NanoDrop ND-1000 spectrophotometer (NanoDrop Technologies). Then, the extracted RNA was reverse transcribed using TaqMan Reverse Transcription Reagents (Applied Biosystems) according to the manufacturer's instructions. The synthesized complementary DNA (cDNA) was subjected to quantitative real-time PCR (qRT-PCR) or RT-PCR using