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# Secretory factors of human chorion-derived stem cells enhance activation of human fibroblasts

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**Abstract**

*Background aims.* Wound healing remains a principal challenge in modern medical science. Chorion-dervied stem cells (CDSCs), isolated from human placenta, have largely been overlooked, and few studies on their potential in wound healing have been conducted. In this study, we investigated the functional characteristics of CDSCs compared with adipose-derived stem cells (ASCs) on human fibroblasts (HFs). *Methods.* We analyzed CDSCs by means of flow cytometry to confirm their mesenchymal stromal cell characteristics. We then evaluated the paracrine effects of CDSCs on HFs in a co-culture system and focused on fibroblast proliferation, migration and collagen synthesis. To explore the potential of CDSCs in wound healing, CDSC- and ASC-secreted factors were compared by use of a cytokine antibody array. *Results.* CDSCs had morphology similar to MSCs and expressed a mesenchymal stromal cell phenotype. HF proliferation and migration increased more than 5-fold when co-cultured with CDSCs. Furthermore, Western blot and reverse transcription–polymerase chain reaction analysis showed that expression of collagen (types I and III) in fibroblasts was upregulated 2-fold when co-cultured with CDSCs. Cytokine array results of CDSC-conditioned medium and ASC–conditioned medium revealed the presence of growth factors known to influence wound healing, including interleukin -6, interleukin -8, monocyte chemotactic protein 1 and regulated on activation, normal T cells expressed and secreted. *Conclusions.* Our data demonstrated that CDSCs are functionally similar to ASCs, promote HF activation, and secrete growth factors that influence wound healing. Therefore, we suggest that CDSCs are potentially applicable in wound healing.

**Key Words:** *adipose-derived stem cells, chorion, fibroblasts, mesenchymal stromal cells, wound healing*

## Introduction

Wound healing, an extremely complex and dynamic process that involves interactions among various cells, growth factors and tissue, remains a principal challenge in modern medical science. Conventional wound treatment includes antibiotics, debridement with or without grafting, compression bandages and advanced therapy with the use of bioengineered skin substitutes and growth factors [1]. Such methods are usually effective; however, in large, chronic wounds they may be time-consuming and turn out to be refractive. Stem cell therapy has been suggested as another solution for wound healing [1,2].

Mesenchymal stromal cells (MSCs) may be isolated from bone marrow, adipose tissue, the umbilical cord, placenta and other adult tissue. MSCs are undifferentiated cells that have high proliferative ability, self-renewal potential and the potential to differentiate and develop into the mesodermal lineage [3–5]. Adipose-derived stem cells (ASCs) are a more readily available source of these MSCs than is bone marrow and contain a more abundant cell concentration [6–8]. In addition, ASCs secrete growth factors such as basic fibroblast growth factor, keratinocyte growth factor, transforming growth factor-$\beta$ (TGF-$\beta$), hepatocyte growth factor and vascular endothelial growth factor. These factors control damaged cells at the wound site [9]. ASC-conditioned medium (CM) promotes proliferation and collagen synthesis in human fibroblasts (HFs) and accelerates wound healing in animal models [10]. Reports also show that ASCs combined with acellular dermal matrix improve wound repair in healing-impaired diabetic rats [11]. The significant effect of ASCs on wound healing has been investigated extensively [12–15].

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Chorion-derived stem cells (CDSCs), isolated from human placenta, may be an alternative source of MSCs [16,17]. The human placenta, previously considered disposable postpartum tissue, is an abundant source of human stem cells that is capable of proliferation and differentiation into the mesodermal lineage *in vitro* [18]. Recent reports demonstrated that placental tissue and placenta-derived stem cells improve wound healing in the healing-impaired diabetic model. For instance, dehydrated human amnion/chorion tissue allograft enhances wound repair in diabetic rats [19]. Placental mesenchymal stromal cells induce wound healing by enhancing angiogenesis in the diabetic Go-To Kakizaki model and can differentiate into endothelial-like cells [20]. Other reports demonstrated that amniotic MSCs, which originate from the amnion membrane, enhance wound healing in diabetic NOD/SCID mouse models and increase re-epithelialization and cellularity [21]. However, there is a paucity of studies on the functional characterization and the wound-healing potential of CDSCs [22].

To confirm the functional characteristics of CDSCs more clearly, we compared them with ASCs through the use of several wound-healing experiments. In this study, we analyzed the functional characteristics of CDSCs compared with ASCs as a positive control on HF activation. We analyzed CDSCs with the use of flow cytometry to confirm their MSC characteristics. We then evaluated the paracrine effects of CDSCs on HFs through the use of a co-culture system and focused on fibroblast proliferation, migration and collagen synthesis. We also used comparative analysis to investigate the factors secreted by CDSCs and ASCs to explore the potential application of CDSCs in wound healing.

## Methods

### *Cell isolation and culture*

Informed consent was obtained from subjects in accordance with the regulations of the Institutional Review Board of Seoul St Mary's Hospital, Seoul, Korea.

Human adipose tissue was obtained from tissue that was disposed after liposuction or fat tissue resection. The obtained samples were washed with phosphate-buffered saline (PBS; Wisent Inc, Quebec, Canada) and digested with 0.1% collagenase type I (Sigma-Aldrich, St Louis, MO, USA) in Celltibator (Medikan, Seoul, Korea) for 30 min at 37°C. The digested samples were washed twice with Dulbecco's modified Eagle medium (DMEM; Gibco, Carlsbad, CA, USA) containing 10% fetal bovine serum (FBS; Wisent Inc), 1% antibiotic-antimycotic (Gibco) and centrifuged at 1300 rpm for 3 min. The stromal vascular fraction was filtered through a 100-μm strainer (BD Biosciences, San Jose, CA, USA). After centrifugation, the supernatant was discarded. The pellet was resuspended in culture medium, seeded in culture dishes and incubated overnight at 37°C in 5% $CO_2$ atmosphere. To remove debris, cells were washed with PBS and fresh culture medium was added. The cells were cultured until they reached 90% confluence and were passaged after trypsinization. The cells used for analysis were from passages 3 to 5. ASC morphology was photographed with the use of an inverted microscope (Olympus CKX41, NY Microscope Co, Hicksville, NY, USA).

HFs were acquired from healthy patients who underwent split-thickness skin grafts for skin defects caused by trauma. The obtained skin samples were washed with PBS and separated into dermis and epidermis with the use of Dispase II (Roche, Basel, Switzerland) for 2 h at 37°C in a 5% $CO_2$ atmosphere. The collected dermis was digested with 0.1% collagenase type II (Sigma-Aldrich) in the Celltibator for 30 min at 37°C. The procedures are in accordance with a previous protocol.

CDSCs at passage 1 were obtained from the Department of Obstetrics and Gynecology, the Catholic University of Korea Seoul, Korea. CDSCs were cultured in DMEM containing 10% FBS and 1% antibiotic-antimycotic. When the cells reached 90% confluence, CDSCs were passaged after trypsinization and used for analysis at passage 3–5. CDSC morphology was photographed with the use of an inverted microscope (Olympus).

### *Flow cytometry*

For flow cytometry analysis, cultured ASCs and CDSCs were trypsinized and resuspended in Hank's balanced salt solution (Wisent Inc) containing 2% FBS. The cells were incubated with monoclonal fluorescein isothiocyanate–conjugated antibodies for cluster of differentiation (CD)34, CD90, highly polymorphic human leukocyte antigen (HLA)-DR (BD, San Jose, CA, USA) and CD105 (AbD Serotec, Oxford, United Kingdom) or with phycoerythrin-conjugated antibodies for CD13, CD73, CD45 and CD146 (BD) for 30 min. As a negative control, cells were incubated with isotype control immunoglobulin G1 (BD). Incubated cells were fixed with 0.5% paraformaldehyde and were analyzed on a fluorescence-activated cell sorting (FACS) Vantage SE (Becton Dickinson, Franklin Lakes, NJ, USA) with the use of Summit 5.2 software (Beckman Coulter, Pasadena, CA, USA).