Available online at www.sciencedirect.com

ScienceDirect

journal homepage: www.JournalofSurgicalResearch.com

Conditioned medium of adipose-derived stromal cell culture in three-dimensional bioreactors for enhanced wound healing

Sun Hyun Kwon, MS,[a,1] *Suk Ho Bhang, PhD,*[b,1] *Hyeon-Ki Jang, BS,*[c] *Taiyoun Rhim, PhD,*[d,**] *and Byung-Soo Kim, PhD*[a,e,*]

[a] *School of Chemical and Biological Engineering, Seoul National University, Seoul, Republic of Korea*
[b] *School of Chemical Engineering, Sungkyunkwan University, Suwon, Republic of Korea*
[c] *Interdisciplinary Program for Bioengineering, College of Engineering, Seoul National University, Seoul, Republic of Korea*
[d] *Department of Bioengineering, College of Engineering, Hanyang University, Seoul, Republic of Korea*
[e] *Institute of Bioengineering, Institute of Chemical Processess, Seoul National University, Seoul, Republic of Korea*

ARTICLE INFO

Article history:
Received 26 August 2014
Received in revised form
29 October 2014
Accepted 30 October 2014
Available online 6 November 2014

Keywords:
Conditioned medium
Human adipose-derived stromal cells
Neovascularization
Wound healing

ABSTRACT

Background: It was previously shown that human adipose-derived stromal cell (hADSC)-conditioned medium (CM) promotes wound healing. An essential part of the wound healing process is neovascularization in the wound bed.

Materials and methods: We hypothesized that CM prepared from hADSCs cultured as spheroids in three-dimensional suspension bioreactors (spheroid CM) would contain much higher concentrations of angiogenic growth factors secreted by hADSCs, induce a higher extent of neovascularization in the wound bed, and improve wound healing as compared with CM prepared by conventional monolayer culture (monolayer CM).

Results: The concentrations of angiogenic growth factors (i.e., vascular endothelial growth factor, basic fibroblast growth factor, and hepatocyte growth factor) in spheroid CM were 20- to 145-fold higher than those in monolayer CM. Either fresh medium, monolayer CM, or spheroid CM was administered to full-thickness wounds created on the dorsal aspects of athymic mice. The monolayer CM promoted wound healing as compared with fresh medium or no treatment. Importantly, wound closure was faster, and dermal and epidermal regeneration was improved in the spheroid CM-treated mice compared with that in the monolayer CM-treated mice.

Conclusions: The improved wound healing by spheroid CM may be attributed, at least in part, to enhanced neovascularization in the wound beds. The spheroid-based CM approach showed potential as a therapy for skin wound repair.

* *Corresponding author*. School of Chemical and Biological Engineering, Seoul National University, Seoul 151 744, Republic of Korea. Tel.: +82 2 880 1509; fax: +82 2 888 1604.
** *Corresponding author:* Department of Bioengineering, College of Engineering, Hanyang University, Seoul 133 791, Republic of Korea. Tel.:+82 2 2220 2347; fax: +82 2 2220 4347.
E-mail addresses: byungskim@snu.ac.kr (B.-S. Kim), rhim@hanyang.ac.kr (T. Rhim).
[1] These authors contributed equally to this work.
0022-4804/$ – see front matter © 2015 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.jss.2014.10.053

1. Introduction

Adipose-derived stromal cells (ADSCs) can be used for wound healing. Topical administration of ADSCs to wounds accelerated wound healing in animal models of traumatic wounds and diabetic wounds [1–3]. The administration of ADSCs significantly enhanced epithelialization and capillary formation in traumatic wounds [2]. Differentiation of ADSCs into keratinocytes and dermal fibroblasts has not been fully investigated. Instead, ADSCs contribute to wound healing at least partially through paracrine mechanisms. ADSCs are histologically located beneath the dermal fibroblasts in the skin. Thus, growth factors secreted by ADSCs may diffuse into the dermis and epidermis in cases of skin damage and accelerate wound healing. ADSCs are known to induce angiogenesis and promote healing in animal models of diabetic skin wound mainly through paracrine secretion of angiogenic and antiapoptotic factors, such as vascular endothelial growth factor (VEGF) and fibroblast growth factor (FGF2) [3]. *In vitro* studies have demonstrated that conditioned medium (CM) prepared by ADSC culture (ADSC-CM), which contains angiogenic growth factors such as VEGF, basic FGF2, hepatocyte growth factor (HGF), and stromal cell-derived factor 1 alpha (SDF-1α), enhanced type I collagen secretion and migration of cultured human dermal fibroblasts, and stimulated proliferation and migration of cultured human keratinocytes [1,4]. A previous study showed that inhibition of VEGF and FGF2 using neutralizing antibodies reversed the migration of human dermal fibroblasts in wounds and decreased wound healing in animals [5].

Despite the regenerative capacity of ADSCs, clinical application of ADSCs for wound healing may be limited because of poor survival of ADSCs administered in avascular wound beds, which may limit their regenerative capacity [6]. Furthermore, administration of ADSCs in humans can be hazardous because of the risk of inducing cancer [4]. Alternative therapeutic strategies are required to solve the previously mentioned problems while maintaining the advantages of ADSC transplantation. ADSC-CM may overcome these hurdles. Much of wound healing afforded by ADSCs can be achieved by treatment with cell-free ADSC-CM. Thus, it is expected that ADSC-CM sustains advantages by containing stem cell paracrine factors without the risk of poor survival of administered ADSCs or potential cancer development [7]. However, a few drawbacks, such as a low concentration of angiogenic factors, should be resolved before therapeutic utilization of ADSC-CM [8,9]. Otherwise, a large volume of CM should be repeatedly injected into the damaged tissues [10,11].

Previously, we showed that CM obtained from culturing ADSCs in three-dimensional bioreactors as spheroids contains larger amounts of angiogenic and antiapoptotic paracrine factors without any genetic modification [12]. Here, we hypothesized that this CM can enhance skin wound healing compared with that of CM obtained from conventional monolayer culture of ADSCs. The core part of ADSC spheroids is naturally exposed to mild hypoxic conditions because of limited oxygen and nutrient diffusion [13]. Therefore, these cells in spheroids would secrete more angiogenic paracrine factors to the culture medium. Moreover, a spheroid culture can maintain a higher cell density per unit volume of culture medium as compared with an ADSC monolayer culture. Thus, CM from ADSC spheroid culture has much higher concentrations of angiogenic and antiapoptotic paracrine factors as compared with CM from an ADSC monolayer culture [12]. We investigated whether human ADSC (hADSC) CM from spheroid culture can enhance skin repair efficacy as compared with that of CM obtained from monolayer culture or fresh medium (FM) in a mouse skin wound-closing model (Fig. 1A).

2. Materials and methods

2.1. *Isolation and culture of hADSCs*

hADSCs were isolated from lipoaspirates collected from consented patients and cultured as previously described [14]. hADSCs were cultured in 24 mL of α-minimum essential medium (α-MEM; Gibco BRL, Gaithersburg, MD), supplemented with 10% (v/v) fetal bovine serum (FBS; Gibco BRL), 100 U/mL of penicillin (Gibco BRL), and 100 μg/mL of streptomycin (Gibco BRL). To obtain CM from the hADSC monolayer culture, 6.0×10^6 hADSCs were cultured on a tissue culture plate (Corning Inc, Corning, NY) with a diameter of 15 cm containing 24 mL of α-MEM supplemented with 100 U/mL of penicillin and 100-μL/mL streptomycin for 2 d. Under these culture conditions, cells were approximately 80% confluent. hADSCs within five passages were used for all experiments.

2.2. *Culture of hADSC spheroids*

hADSCs (1×10^6 cells/mL) were placed in siliconized spinner flasks (Bellco, Vineland, NJ) and stirred at 70 rpm for 3 d for spheroid formation. hADSC spheroids were cultured with α-MEM supplemented with 10% (v/v) FBS (Gibco BRL), 100 U/mL of penicillin (Gibco BRL), and 100 μg/mL of streptomycin (Gibco BRL). To obtain CM from the hADSC spheroid culture, the medium was changed with α-MEM without FBS and the cells were cultured for 2 d.

2.3. In vitro *enzyme-linked immunosorbent assay*

The amounts of angiogenic growth factors in CM collected from hADSC cultures were determined using enzyme-linked immunosorbent assay kits (human VEGF, HGF, FGF2, and SDF-1α, R&D Systems, Minneapolis, MN) according to the manufacturer's instructions. Concentrations were expressed as the amount of growth factor per 10^6 cells at the time of harvest. The angiogenic factor secretion per cell was obtained by multiplying the growth factor concentration in the medium by the cell concentration.

2.4. *Wound treatment*

Athymic mice (BALB/c-nu, female 4-wk-old; SLC, Tokyo, Japan) were anesthetized with xylazine (20 mg/kg) and ketamine (100 mg/kg). A 4-cm^2 (2 cm × 2 cm) skin defect was made on the dorsal back of each mouse. Epidermis, dermis, subcutaneous tissue, and panniculus carnosus were removed, and