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Human umbilical cord blood–derived mesenchymal stromal cells and small intestinal submucosa hydrogel composite promotes combined radiation-wound healing of mice

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Abstract

Background aims. Mesenchymal stromal cells (MSCs) are a promising agent for treating impaired wound healing, and their therapeutic potential may be enhanced by employing extracellular matrix scaffolds as cell culture scaffolds or transplant cell carriers. Here, we evaluated the effect of human umbilical cord blood–derived (hUCB)-MSCs and a porcine small intestinal submucosa (SIS)-derived extracellular matrix scaffold in a combined radiation-wound mouse model of impaired wound healing. *Methods.* hUCB-MSCs and SIS hydrogel composite was applied to the excisional wound of whole-body irradiated mice. Assessment of wound closing and histological evaluation were performed *in vivo*. We also cultured hUCB-MSCs on SIS gel and examined the angiogenic effect of conditioned medium on irradiated human umbilical vein endothelial cells (HUVECs) *in vitro*. *Results.* hUCB-MSCs and SIS hydrogel composite treatment enhanced wound healing and angiogenesis in the wound site of mice. Conditioned medium from hUCB-MSCs cultured on SIS hydrogel promoted the chemotaxis of irradiated HUVECs more than their proliferation. The secretion of angiogenic growth factors hepatocyte growth factor, vascular endothelial growth factor-A and angiopoietin-1 from hUCB-MSCs was significantly increased by SIS hydrogel, with HGF being the predominant angiogenic factor of irradiated HUVECs. *Conclusions.* Our results suggest that the wound healing effect of hUCB-MSCs is enhanced by SIS hydrogel via a paracrine factor-mediated recruitment of vascular endothelial cells in a combined radiation-wound mouse model.

Key Words: *angiogenesis, combined radiation wound, hUCB-MSC, HUVEC, HGF, paracrine effect, SIS gel*

Introduction

Effective cutaneous wound healing requires the highly coordinated integration of molecular biological events that lead to dermal repair, angiogenesis and epithelialization. However, irradiation-induced skin injury heals more slowly than normal wounds due to the effects of radiation on subcutaneous tissues, including capillary tissue and bone marrow. Radiation causes microvascular dysfunction and reduces the recruitment of regenerative cells to injured sites, thereby reducing levels of regulatory growth factors and the synthesis of extracellular matrix in the wound microenvironment. Furthermore, radiation reduces angiogenesis and prevents the optimal completion of wound repair [1,2].

Mesenchymal stromal cells (MSCs) have emerged as an effective agent for promoting wound healing, and their therapeutic effect is thought to be due to secreted paracrine factors [3,4]. MSCs transplanted in wound sites release proangiogenic, prosurvival and proimmunomodulatory factors and improve wound healing [5,6]. In particular, bone marrow–derived (BM)-MSCs secrete angiogenic factors including vascular endothelial growth factor (VEGF) and hepatocyte growth factor (HGF), which are key factors in vascular network remodeling during wound healing [7,8]. For this reason, the therapeutic effect of BM-MSCs has been studied in conditions associated with impaired wound healing, such as diabetes [9,10]. However, BM-MSC therapy requires special techniques

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and long periods of time to obtain sufficient quantities of cells. By contrast, it is easier to obtain sufficient quantities of umbilical cord blood-derived (UCB)-MSCs; they are also more immature and have fewer immunogenic properties than other types of adult MSCs, which could lead to more successful transplantation without rejection [11]. Furthermore, UCB-MSCs have been commercialized for cartilage repair as a first cell therapy product in Korea [12].

Most clinical trials have used injections of suspensions of therapeutic cells, including MSCs, in liquid carrier solutions, many of which have resulted in poor cell retention and reduced therapeutic efficacy [13]. To overcome this problem, efforts have been made to find natural materials that enhance stromal cell survival and have therapeutic potential [14,15]. In our laboratory, we have used porcine small intestinal submucosa (SIS), an extracellular matrix-based material, in conjunction with UCB-MSCs as an implantable scaffold for tissue repair. Porcine SIS is a commercially available, collagen-based biomaterial developed for vessel engineering [16]. SIS has been widely used in the clinic for tissue remodeling, particularly for providing structural support [17–19]. SIS has many advantages over other types of extracellular matrix. The porous, three-dimensional structure of SIS allows for cell adherence, proliferation and differentiation [20,21]. Furthermore, SIS is available in various forms, including sheets, sponges and gels, and has no immunogenic characteristics [22–24].

In the present study, we determined whether transplantation of UCB-MSCs and SIS hydrogel composite promotes wound healing and angiogenesis in an *in vivo* combined radiation-wound mouse model and enhances the angiogenic effects of UCB-MSCs *in vitro*.

Methods

Preparation of SIS hydrogel

Our method of preparing SIS hydrogel is based on previously described methods [20,24] with modifications. Commercially available porcine SIS sheet (Biodesign, Cook Medical) was cut into small pieces and pulverized using a Precellys 24 homogenizer (Bertin Technologies). The pulverized SIS was suspended in a solution of 1 mg/mL porcine pepsin (Sigma-Aldrich) in 0.01 mol/L HCl and stirred (60 rpm) constantly for 48 h at room temperature. The digested SIS solution was neutralized by 0.1 mol/L NaOH (pH 7.4) and freeze-dried to obtain soluble SIS matrix. The obtained SIS matrix was sterilized using ethylene oxide gas and pulverized again to yield the final SIS powder. The SIS powder was dispersed in phosphate-buffered saline (PBS) at a 20% w/v concentration. To prepare SIS gel-coated plates for hUCB-MSCs *in vitro* culture, 250 μL (5 mg/well) SIS suspension was poured into a 24-well plate and placed in an incubator at 37°C overnight to form a gel.

Culture of human UCB-MSCs

Human UCB-MSCs (hUCB-MSCs) were purchased from Medipost, and cells from passages 3 to 5 were used for *in vitro* experiments. The culture medium consisted of minimal essential medium-alpha (MEM-a) with 10% fetal bovine serum and 1% penicillin-streptomycin (Gibco-BRL). Green fluorescent protein (GFP) tagged hUCB-MSCs were generated by transduction of retrovirus-GFP for visualization of cells on SIS gel as previously reported [25]. A total of 4×10^4 cells/well were seeded onto SIS gel-coated or conventional 24-well plates. For *in vivo* experiments, only cells at passage 2 were used. Cells were expanded in culture medium at a density of 3×10^3 cells/cm^2 and cultured at 37°C in 5% CO_2. When cells reached the expected amount, they were trypsinized (0.05% trypsin/ethylenediamine-tetra-acetic acid solution, Gibco-BRL) and washed twice with PBS.

RNA extraction, reverse transcription, and real-time quantitative reverse transcription polymerase chain reaction

Cultured hUCB-MSCs on SIS gel were harvested, and total RNA was isolated using the RNeasy Mini Kit (Qiagen). RNA was resuspended in UltraPure DNase/RNase-Free distilled water (Invitrogen). RNA concentration was quantified using a Synergy HTX Multi-Mode Reader (Bio-Tek). Total RNA samples (1 μg) were reverse-transcribed into cDNA with oligo (dT) 18 primers using an Accu-Power RT PreMix (Bioneer). To quantify octamer-binding transcription factor 4 (*Oct4*), sex determining region Y-box 2 (*Sox2*) and *Nanog m*RNA level, real-time quantitative reverse transcription polymerase chain reaction (qRT-PCR) was performed using LightCycler (Roche Diagnostics). qRT-PCR mastermix consisted of 6 μL water, 1 μL forward (F) primer, 1 μL reverse (R) primer, 10 μL LightCycler-Fast Start DNA Master SYBR Green I, and 2 μL cDNA. The primer sequences were as follows: *Oct4*-F, 5′-GATGTGGTCCGAGTGTGGTT-3′; *Oct4*-R, 5′-AGCCTGGGGTACCAAAATGG-3′; *Sox2*-F, 5′-AAGGCCTCAGCACCTACCTA-3′; *Sox2*-R, 5′-TGCACCAGGTCTGAGTGTTC-3′; *Nanog*-F, 5′-GCCCTGCAGTACAACTCCAT-3′; *Nanog*-R, 5′-GACTTGACCACCGAACCCAT-3′; glyceraldehyde 3-phosphate dehydrogenase (GAPDH)-F, 5′-GGACTCATGACCACAGTCCATGCC-3′; GAPDH-R, 5′-TCAGGGATGACCTTGCCCACAG-3′. qRT-PCR was performed in triplicate at 95°C for 3 min, followed by 35 cycles of amplification (94°C for 30 s, 60°C for 30 s, 72°C for 30 s). The relative amounts of mRNA were determined by subtracting