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Effects of human umbilical cord blood–derived mesenchymal stromal cells and dermal fibroblasts on diabetic wound healing

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Abstract

Background aims. A previous study demonstrated that human umbilical cord blood–derived mesenchymal stromal cells (hUCB-MSCs) have superior wound-healing activity compared with fibroblasts *in vitro*. However, wound healing *in vivo* is a complex process that involves multiple factors. The purpose of this study was to compare the effects of hUCB-MSCs and fibroblasts on diabetic wound healing *in vivo*. This study especially focused on collagen synthesis and angiogenesis, which are considered to be the important factors affecting diabetic wound healing. *Methods.* Porous polyethylene discs were loaded with either fibroblasts or hUCB-MSCs, and a third group, which served as a control, was not loaded with cells. The discs were then implanted in the back of diabetic mice. During the first and the second week after implantation, the discs were harvested, and collagen level and microvascular density were compared. *Results.* In terms of collagen synthesis, the hUCB-MSC group showed the highest collagen level (117.7 ± 8.9 ng/mL), followed by the fibroblast group (83.2 ± 5.2 ng/mL) and the no-cell group (60.0 ± 4.7 ng/mL) in the second week after implantation. In terms of angiogenesis, the microvascular density in the hUCB-MSC group was 56.8 ± 16.4, which was much higher than that in the fibroblast group (14.3 ± 4.0) and the no-cell group (5.7 ± 2.1) in the second week after implantation. *Conclusions.* These results demonstrate that hUCB-MSCs are superior to fibroblasts in terms of their effect on diabetic wound healing *in vivo*.

Key Words: *angiogenesis, collagen, diabetic wound healing, fibroblast, human umbilical cord blood–derived mesenchymal stromal cell*

Introduction

Various cell transplantation therapies have been developed to promote wound healing in diabetic patients. Cell transplantation can improve the overall wound condition by adjusting to the wound environment and secreting growth factors essential for wound healing. In addition, transplanted cells can provide suitable conditions for cell migration and mitosis by producing extracellular matrices [1–5].

Various types of skin substitutes composed of either allogeneic or autologous fibroblasts and/or keratinocytes have been commercialized [6,7]. Although allogenic fibroblasts, derived from healthy donors, are readily available, they may carry the risk of immunologic reactions or cross-infections. Autologous fibroblasts are free from these drawbacks. However, they require a long culture time, and, in diabetic patients, autologous fibroblasts may not have sufficient capacity to stimulate wound healing [8].

Mesenchymal stromal cells (MSCs) may hold great promise in treating diabetic wounds because they have the advantages of both allogeneic and autologous cells. MSCs demonstrate low levels of immunity-assisted rejection and have the ability to divide without apoptosis [9,10]. It has been demonstrated that even after 20 or 30 cycles of cell doubling in culture, they retain their initial stem cell properties. Accordingly, MSCs have attracted much attention in the bioengineering field [11]. Bone marrow stroma is one of the main sources of MSCs. Previous studies performed by our group demonstrated that bone marrow–derived MSCs (BM-MSCs) synthesize higher amounts of collagen, fibroblast growth factor (FGF) and vascular endothelial growth factor (VEGF) *in vitro*, compared with dermal fibroblasts. Furthermore, they showed greater activity in terms of granulation tissue formation, epithelialization and angiogenesis *in vivo*, indicating their potential use in accelerated wound healing [9,12,13]. However, the number of MSCs in the BM decreases with aging,

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and procurement is relatively invasive. In addition, because of issues related to approval by the U.S. Food and Drug Administration (FDA), it has become difficult for a clinician to use cultured BM-MSCs for the purpose of wound healing these days [14].

Meanwhile, a new commercial drug that contains human umbilical cord blood–derived MSCs (hUCB-MSCs) has been developed and approved by the Korean regulatory agency to help regenerate the knee cartilage. These cells can be obtained in massive quantities without significant ethical issues. In addition, cord blood stem cells are more immature than adult MSCs and can proliferate easily *in vitro* [14]. Because the immune system is undifferentiated, successful transplantation without rejection has been reported [15–17]. A previous *in vitro* pilot study performed by our group demonstrated that hUCB-MSCs have superior wound-healing activity compared with both healthy and diabetic fibroblasts [18].

However, wound healing of a diabetic ulcer *in vivo* is a complicated process influenced by various factors. Therefore, the purpose of this study was to compare the effect of hUCB-MSCs and healthy fibroblasts on diabetic wound healing *in vivo*. In particular, the authors focused on collagen synthesis and angiogenesis, which are the essential factors for diabetic wound healing.

Methods

This study was approved by the Institutional Animal Care and Use Committee at Korea University (#KUACUC-2016-54) and the Institutional Review Board of Korea University Guro Hospital (#KUGH15366-001). All animals were treated humanely, and all investigations were conducted according to the principles expressed in the Declaration of Helsinki. The cell donors provided informed consent for use of their cells for research purposes.

Culture of dermal fibroblasts and hUCB-MSCs

Healthy fibroblasts used for the experiments were obtained from cryopreserved cells from the dermis of healthy adults (n = 3).

Healthy fibroblasts were cultured in Dulbecco's Modified Eagle's Medium/Ham's F-12 nutrient (DMEM/F-12; Gibco) containing 10% fetal bovine serum (FBS; Gibco). The fibroblasts were dissociated by trypsinization, diluted 2.7-fold in Dulbecco's phosphate-buffered saline without Mg^{2+} and Ca^{2+} (DPBS; Gibco) and collected by centrifugation at 450*g* for 17 min. The cells were washed twice in 40 mL of DPBS and resuspended in 5 mL of DPBS. Cell density was measured with a hemocytometer, and cell viability was assessed using the trypan blue dye exclusion assay. Third-passage cells were used for all the experiments in this study.

Human UCB-MSCs were purchased from Medipost [19]. According to the manufacturer's protocol, hUCB samples were obtained from the umbilical vein of infants after deliveries with informed maternal consent. A 16-gauge needle from a hUCB collection bag containing 23 mL of CPDA-1 anticoagulant (Greencross) was inserted into the umbilical vein, and the hUCB was allowed to flow under gravity. UCB harvests were performed in all cases within 24 h of collection, with a viability of more than 90%. Mononuclear cells were isolated from the hUCB by means of centrifugation through a Ficoll-Hypaque gradient (density, 1.077 g/cm^3, Sigma-Aldrich). After washing the separated mononuclear cells, they were suspended in α-minimum essential medium (α-MEM; Gibco), supplemented with 10% FBS (Hyclone) and 25 mg/mL gentamycin and seeded at a concentration of 5×10^6 cells/cm^2. Cultures were maintained at 37°C in a humidified atmosphere containing 5% CO_2, with a biweekly change of culture medium. When the monolayer of fibroblast-like adherent cell colonies reached 80% confluence within 1–3 weeks, the cells were trypsinized (0.25% trypsin, HyClone), washed, resuspended in culture medium (α-MEM supplemented with 10% FBS) and sub-cultured. Three different cell lines were used in the study (n = 3, Figure 1).

Preparation of polyethylene discs

Porous polyethylene (Medpor; Stryker) was used for the experiments. The blocks of polyethylene were cut using a 5-mm punch into 5-mm-diameter and 3-mm-thick discs. Four discs were soaked in a 50-mL centrifuge tube containing 4×10^6 cells and 1 mL of thrombin (Baxter) for 10 min, allowing the cells to infiltrate into the pores of the polyethylene structure. After the porous polyethylene discs had been loaded with the cell-thrombin composite, they were moved to a 96-well culture plate. Two hundred microliters of fibrinogen (Baxter) was added to each well to form a fibrin cuff that kept the infiltrated cells in the polyethylene pores. After 10 min, the polyethylene discs were taken out from the plate, and the surplus fibrin was removed from the exterior of the disc. Seventy-two polyethylene discs were divided equally into three groups according to the cells with which they had been mixed. In groups I, II and III, the discs were not loaded with cells (fibrin only, control group), loaded with fibroblasts or loaded with hUCB-MSCs, respectively.

Implanting and harvesting of the discs

Twelve genetically diabetic black mice (C57BLKS, J-m^{+}/$^{+}$Lepr^{db}; Institute for Animal Reproduction),