

## ORIGINAL ARTICLE

# Comparison of human umbilical cord blood-derived mesenchymal stem cells with healthy fibroblasts on wound-healing activity of diabetic fibroblasts

Jae-A Jung<sup>1</sup>, Young-Don Yoon<sup>1</sup>, Hyup-Woo Lee<sup>2</sup>, So-Ra Kang<sup>3</sup> & Seung-Kyu Han<sup>1</sup>

1 Department of Plastic Surgery, Korea University College of Medicine, Seoul, South Korea

2 Department of Laboratory Medicine, College of Medicine, Kangwon National University, Kangwon, South Korea

3 Department of Plastic and Reconstructive Surgery, Ewha Womans University School of Medicine, Seoul, South Korea

**Key words**

diabetic wound healing; fibroblast; human umbilical cord blood stromal cells

**Correspondence to**

Seung-Kyu Han, MD, PhD  
Department of Plastic Surgery  
Korea University Guro Hospital  
148 Guro-Dong  
Guro-Ku  
Seoul  
South Korea  
E-mail: pshan@kumc.or.kr

doi: 10.1111/iwj.12849

Jung J-A, Yoon Y-D, Lee H-W, Kang S-R, Han S-K. Comparison of human umbilical cord blood-derived mesenchymal stem cells with healthy fibroblasts on wound-healing activity of diabetic fibroblasts. *Int Wound J* 2018; 15:133–139

**Abstract**

Various types of skin substitutes composed of fibroblasts and/or keratinocytes have been used for the treatment of diabetic ulcers. However, the effects have generally not been very dramatic. Recently, human umbilical cord blood-derived mesenchymal stromal cells (hUCB-MSCs) have been commercialised for cartilage repair as a first cell therapy product using allogeneic stem cells. In a previous pilot study, we reported that hUCB-MSCs have a superior wound-healing capability compared with fibroblasts. The present study was designed to compare the treatment effect of hUCB-MSCs with that of fibroblasts on the diabetic wound healing in vitro. Diabetic fibroblasts were cocultured with healthy fibroblasts or hUCB-MSCs. Five groups were evaluated: group I, diabetic fibroblasts without coculture; groups II and III, diabetic fibroblasts cocultured with healthy fibroblasts or hUCB-MSCs; and groups IV and V, no cell cocultured with healthy fibroblasts or hUCB-MSCs. After a 3-day incubation, cell proliferation, collagen synthesis levels and glycosaminoglycan levels, which are the major contributing factors in wound healing, were measured. As a result, a hUCB-MSC-treated group showed higher cell proliferation, collagen synthesis and glycosaminoglycan level than a fibroblast-treated group. In particular, there were significant statistical differences in collagen synthesis and glycosaminoglycan levels ( $P=0.029$  and  $P=0.019$ , respectively). In conclusion, these results demonstrate that hUCB-MSCs may have a superior effect to fibroblasts in stimulating diabetic wound healing.

**Introduction**

Diabetic foot ulcers frequently present a difficult handling problem for wound specialists. There is a complex pathophysiological connection between diabetes and wound-healing impairment. Vascular, neurological, immunological and biochemical problems lead to the hindrance of tissue restoration. Severe impairment of key cell activities related to wound healing is another important factor in diabetic ulcers. Fibroblasts cultured from diabetic patients demonstrate weakened production of growth factors and lower proliferative potential. Additionally, there is an overload of metalloproteinases and a diminution in

**Key Message**

- previous studies demonstrated that stem cells have superior wound-healing capabilities when compared with both healthy and diabetic fibroblasts; in the meantime, a novel commercial drug that uses human umbilical cord blood-derived MSCs (hUCB-MSCs) has been developed and has achieved recognition by the Korean Food and Drug Administration (FDA) for helping the regeneration of knee cartilage
- as this cell line is already approved for clinical use, it would be easy to commercialise for diabetic wound healing if it has a superior effect on wound healing

- therefore, the present study was designed to compare the treatment effect of hUCB-MSCs with that of fibroblasts on diabetic wound healing *in vitro*
- a hUCB-MSC-treated group showed higher cell proliferation, collagen synthesis and glycosaminoglycan level than a fibroblast-treated group; in conclusion, hUCB-MSCs may have a superior effect to fibroblasts in stimulating diabetic wound healing

their natural inhibitors. All of these factors may cause a collapse of growth factors and extracellular matrix components such as fibronectin (1,2).

Recently, there has been increasing interest in the use of cell-based therapies for diabetic wounds. These therapies are designed to modulate the levels of biological molecules, including growth factors and extracellular matrices that may promote wound healing (3,4). Various types of skin substitutes composed of either allogeneic or autologous fibroblasts and/or keratinocytes have been commercialised (5,6). Although allogeneic cells, derived from healthy donors, are already available, they may carry the risk of immunological reactions or cross-infections. Autologous cells are free from these drawbacks. However, they require a long culture time, and in diabetic patients, autologous cells may not have sufficient capacity to stimulate wound healing (7).

Mesenchymal stem cells (MSCs) may be a good alternative for treating diabetic wounds because they have the advantage of containing both allogeneic and autologous cells. MSCs demonstrate low levels of immunity-assisted rejection and can divide without apoptosis (8,9). It was demonstrated that even after 20 or 30 cycles of cell doubling in culture, they still retain initial stem cell properties. Accordingly, MSCs have attracted much interest in the bioengineering field (10). Bone marrow (BM) stroma is one of the main sources of MSCs. Previous studies performed by our group demonstrated that BM-derived MSCs (BM-MSCs) synthesise higher amounts of collagen, fibroblast growth factor (FGF) and vascular endothelial growth factor (VEGF) *in vitro*, as compared with dermal fibroblasts (8,11,12). However, the number of MSCs in the BM decreases with age, and procurement is relatively invasive. In addition, because of issues regarding approval by the Food and Drug Administration (FDA), it is difficult for a clinician to use cultured BM-MSCs for wound healing these days (13).

In the meantime, a novel commercial drug that uses human umbilical cord blood-derived MSCs (hUCB-MSCs) has been developed and has achieved recognition by the Korean FDA for helping the regeneration of knee cartilage. Human UCB-MSCs can be obtained in massive quantities without significant ethical issues. Besides, cord blood stem cells are more immature than adult MSCs and can proliferate easily *in vitro* (13). Because the immune system of a newborn is relatively immature than that of an adult, cord blood-derived MSCs have been successfully transplanted without causing rejection (14,15). A previous study performed by our group demonstrated that hUCB-MSCs have superior wound-healing capabilities when compared with both healthy and diabetic fibroblasts (16). However, the result was not enough to confirm

the direct interaction of the hUCB-MSC and diabetic wound healing.

Therefore, the present study was designed to evaluate the effect of cell therapy using the hUCB-MSC on the diabetic wound healing. The goal of this *in vitro* study was to compare the effect of hUCB-MSCs with that of healthy fibroblasts on cell proliferation, collagen synthesis and glycosaminoglycan (GAG) synthesis, which are the crucial contributing elements in wound healing, of diabetic fibroblasts.

## Materials and methods

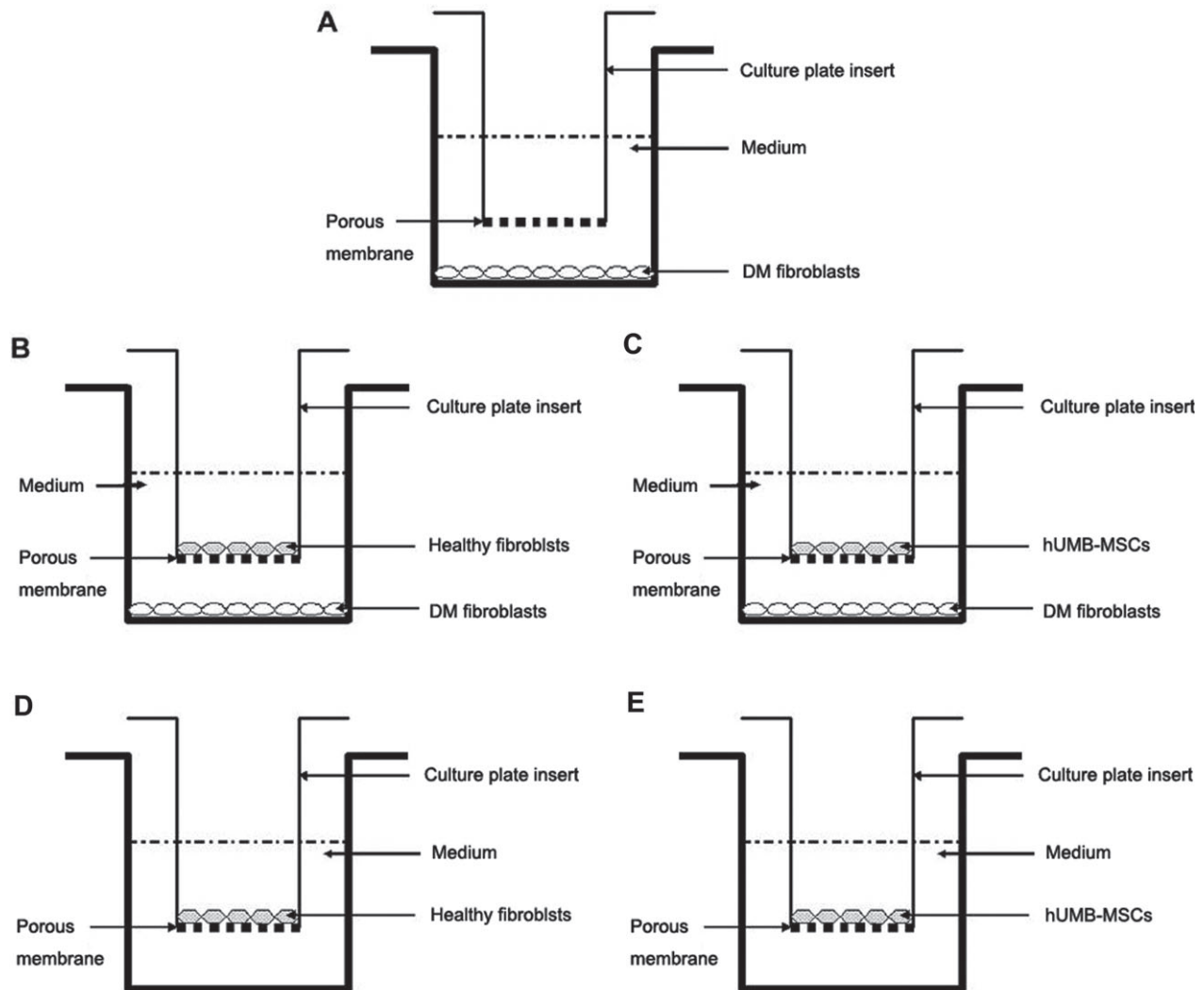
This study was approved by the authors' institutional review board. The cell donors provided informed consent for their cells to be used for research purposes.

### Isolation and culture of healthy fibroblasts, diabetic fibroblasts and hUCB-MSCs

Healthy and diabetic fibroblasts used for the experiments were obtained from cryopreserved cells from the dermis of healthy ( $n = 5$ ) and diabetic ( $n = 2$ ) adults.

Both types of fibroblasts were cultured in Dulbecco's modified Eagle's medium/Ham's F-12 nutrient (DMEM/F-12; Gibco, Grand Island, NY) containing 10% foetal bovine serum (FBS; Gibco). The fibroblasts were dissociated by trypsinisation, diluted 2.7-fold in Dulbecco's phosphate-buffered saline without  $Mg^{2+}$  and  $Ca^{2+}$  (DPBS; Gibco) and collected by centrifugation at 450g for 17 minutes. The cells were washed twice in 40 ml of DPBS and re-suspended in 5 ml of DPBS. Cell density was counted with a haemocytometer, and viability was assessed using a trypan blue dye exclusion assay. Third-passage cells were used for all the experiments in this study.

Human UCB-MSCs were purchased from Medipost Co Ltd. (Seoul, Korea) (17). According to the manufacturer's protocols, hUCB samples were obtained from the umbilical vein of deliveries with informed maternal consent. A 16-gauge needle from a hUCB collection bag containing 23 ml of CPDA-1 anticoagulant (Greencross Co., Yongin, Gyeonggi-do, Korea) was inserted into the umbilical vein, and the hUCB was allowed to flow with gravity. UCB harvests were performed in all cases within 24 hours 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<sup>3</sup>, Sigma, St. Louis, MO). After washing the separated mononuclear cells, they were suspended in  $\alpha$ -minimum essential medium ( $\alpha$ -MEM; Gibco), supplemented with 10% FBS (HyClone, Logan, UT) and 25 mg/ml gentamycin and seeded at a concentration of  $5 \times 10^6$  cells/cm<sup>2</sup>. Cultures were maintained at 37°C in a humidified atmosphere containing 5% CO<sub>2</sub>, with a bi-weekly change of culture medium. When the monolayer of fibroblast-like adherent cell colonies reached 80% confluence within 1–3 weeks, the cells were trypsinised (0.25% trypsin, HyClone), washed, re-suspended in the culture medium ( $\alpha$ -MEM supplemented with 10% FBS) and sub-cultured. Five different cell lines were used in the study ( $n = 5$ ).



**Figure 1** Illustrations of the coculture of the cells. A, group I; B, group II; C, group III; D, group IV; and E, group V.

### Coculture of the cells

Diabetic fibroblasts were seeded on 24-well culture plates with DMEM/F-12 containing 5% FBS, 350 mg/dl of glucose and 25 mg/ml gentamycin at 37°C in a 5% CO<sub>2</sub>/95% air atmosphere at 100% humidity and left for 24 hours to attach. Next, the medium was replaced with serum-free medium, and 3- $\mu$ m pore-sized polycarbonate culture plate inserts (Millipore, Billerica, MA) were placed in the culture well. Human UCB-MSCs or healthy fibroblasts were seeded in the culture plate insert. To prevent direct attachment between the hUCB-MSCs/healthy fibroblasts and diabetic fibroblasts, the porous membrane of the insert was kept 1 mm above the well bottom. The initial number of healthy fibroblasts, hUCB-MSCs and diabetic fibroblasts seeded was  $1.5 \times 10^4$  cells per well.

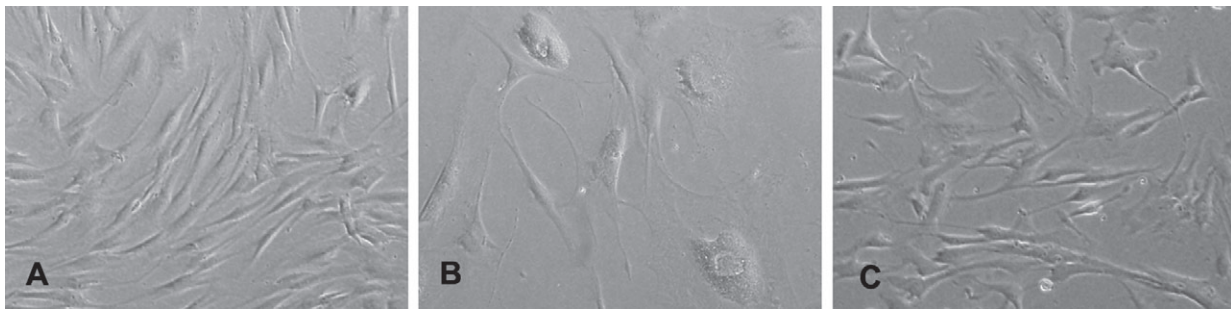
Five different treatment groups were used. In group I, diabetic fibroblasts were cultured with an insert containing no cells and served as a control. In groups II and III, healthy fibroblasts or hUCB-MSCs were added to each culture plate insert. Diabetic fibroblasts from two donors were treated with healthy fibroblasts isolated from five donors and five different

hUCB-MSC cell lines for coculture ( $n = 10$ ,  $2 \times 5$  each). In groups IV and V, diabetic fibroblasts were not seeded in the well, and only healthy fibroblasts or hUCB-MSCs within the inserts were incubated (Figure 1).

### Evaluation of cell proliferation, collagen synthesis and GAG synthesis

#### Cell proliferation assay

After incubation for 3 days, the inserts and the cells inside were removed, and the cell proliferation of the diabetic fibroblasts was evaluated. Cell proliferation was determined with a 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide assay (MTT; Sigma). Briefly, 10  $\mu$ l of 5 mg/ml MTT was mixed with 100- $\mu$ l DMEM. Then, 100  $\mu$ l of the mixture was added to each well containing a cell monolayer. After incubating at 37°C for 3 hours, 100  $\mu$ l of 0.04 M HCL in propan-2-ol was added to each well and mixed completely to dissolve the blue formazan crystal. Then, 100  $\mu$ l of the solution was transferred to a 96-well plate, and the absorbance was measured at a test



**Figure 2** Human healthy fibroblasts (A), diabetic fibroblasts (B) and hUCB-MSCs (C) in a monolayer culture (original magnification  $\times 100$ ).

wavelength of 570 nm with a reference wavelength of 630 nm using an enzyme-linked immunosorbent assay (ELISA) reader. Each sample was analysed in triplicates and averaged.

### Collagen synthesis assay

To measure the amount of collagen produced, the collagen type I carboxy-terminal propeptide enzyme immunoassay was performed using a Metra C1CP kit (Quidel; San Diego, CA) according to the manufacturer's instructions. Simply, 100  $\mu$ l of diluted culture supernatant was added to each well of several monoclonal anti-C1CP antibody-coated plates, and the plates were incubated at room temperature for 2 hours. Next, 100  $\mu$ l of rabbit anti-C1CP antiserum was added for 50 minutes, followed by the addition of 100  $\mu$ l of goat anti-rabbit alkaline phosphatase conjugate for the next 50 minutes. The reaction was stopped, and the collagen synthesis was measured at 405 nm.

The amount of collagen synthesised by the diabetic fibroblasts treated with healthy fibroblasts was calculated by subtracting the collagen level of group IV from that of group II. The amount of collagen synthesised by the diabetic fibroblasts treated with hUCB-MSCs was calculated by subtracting the collagen level of group V from that of group III. Each sample was analysed in triplicate and averaged.

### Glycosaminoglycan synthesis assay

Levels of sulphated GAG were measured in culture media using human GAG ELISA kits (YEHUA biological technology Co.; Shanghai, China) according to the manufacturer's instructions. Briefly, 50  $\mu$ l of a predetermined dilution sample was added to each well and incubated for 30 minutes at 37°C. After washing buffer was added to every well, 50  $\mu$ l of HRP-Conjugate reagent enzyme was added, and the mixture was incubated for 30 minutes at 37°C. This washing step was repeated. After 50  $\mu$ l of Chromogen Solution A and 50  $\mu$ l of Chromogen Solution B were added to each well, GAG levels were determined by measuring the absorbance at 450 nm using an ELISA reader.

The amount of GAG synthesised by the diabetic fibroblasts treated with healthy fibroblasts was calculated by subtracting the GAG level of group IV from that of group II. The amount of GAG synthesised by the diabetic fibroblasts treated with hUCB-MSCs was calculated by subtracting GAG level of group V from that of group III. Each sample was analysed in triplicate and averaged.

### Statistical analysis

Each sample was analysed in triplicate and the results averaged. The results were presented as mean  $\pm$  standard deviation (SD) of untransformed data. Statistical comparisons were performed using the Mann–Whitney *U*-test, with *P*-values  $< 0.05$  being considered statistically significant. The statistical analysis was performed using SPSS ver. 20.0 for Windows (SPSS, Chicago, IL).

### Results

Monolayer cultures of human dermal fibroblasts consisted of homogeneously spindle-shaped cells. Most hUCB-MSCs were larger and had a more polygonal shape (Figure 2).

### Cell proliferation

After incubating for 3 days, the average number of diabetic fibroblasts in group III (hUCB-MSC treated group) was  $3.6 \pm 0.2 \times 10^4$  per well, which was higher than in group II (healthy fibroblast treated group:  $3.4 \pm 0.2 \times 10^4$  per well) and group I (control:  $3.2 \pm 0.2 \times 10^4$  per well). However, there were no significant statistical differences between the groups (Figure 3). No cells were observed at the bottom of the culture wells in groups IV and V after removing the cell-containing inserts.

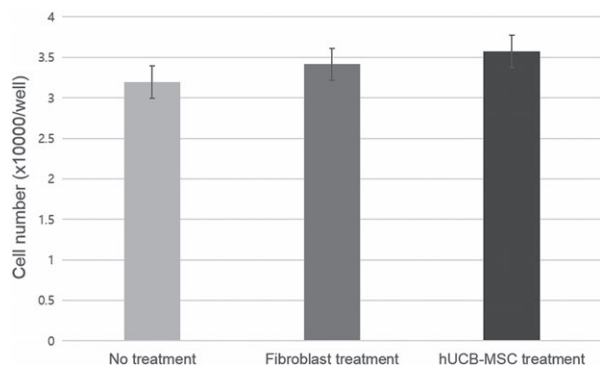
### Collagen synthesis

Collagen levels in groups I, II and III were  $101.0 \pm 39.6$ ,  $298.5 \pm 20.7$  and  $283.4 \pm 29.7$  ng/ml, respectively. The collagen levels in groups IV and V were  $194.6 \pm 19.7$  and  $160.2 \pm 15.0$  ng/ml, respectively.

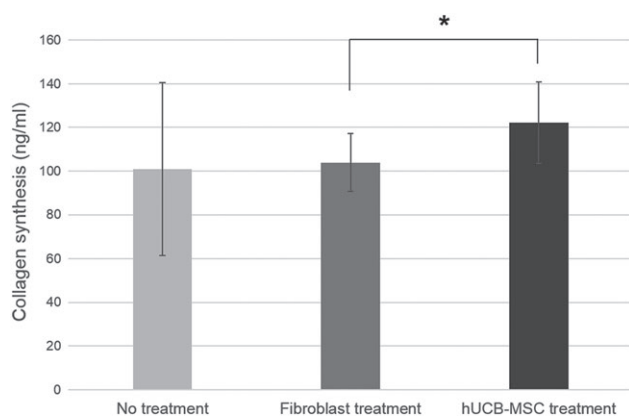
The amount of collagen synthesised by the diabetic fibroblasts treated with hUCB-MSCs was larger ( $122.2 \pm 18.7$  ng/ml) than that treated with healthy fibroblasts ( $103.9 \pm 13.2$  ng/ml, *P* = 0.029; Figure 4).

### Glycosaminoglycan synthesis

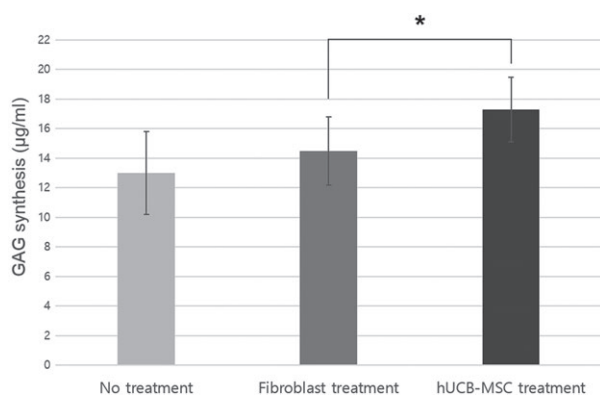
GAG levels in groups I, II and III were  $13.0 \pm 2.8$ ,  $35.5 \pm 2.3$   $\mu$ g/ml and  $40.3 \pm 3.4$   $\mu$ g/ml, respectively. The GAG levels in groups IV and V (healthy fibroblast only and hUCB-MSCs only groups) were  $21.0 \pm 2.5$  and  $23.0 \pm 3.7$   $\mu$ g/ml, respectively.



**Figure 3** Cell proliferation of the three groups.



**Figure 4** The amounts of collagen synthesised by diabetic fibroblasts (\* $P < 0.05$ ).



**Figure 5** The amounts of glycosaminoglycan (GAG) synthesised by diabetic fibroblasts (\* $P < 0.05$ ).

The amount of GAG synthesised by the diabetic fibroblasts treated with hUCB-MSCs was larger ( $17.3 \pm 2.2 \mu\text{g/ml}$ ) than that treated with healthy fibroblasts ( $14.5 \pm 2.3 \mu\text{g/ml}$ ,  $P = 0.019$ ; Figure 5).

## Discussion

Over the past 10 years, many different bioengineered skin substitutes have been introduced for clinical use (18,19).

Bioengineered skin products have concentrated on both allogeneic and autologous cells. A cryopreserved human fibroblast-derived dermal substitute (Dermagraft; Shire Regenerative Medicine Inc., San Diego, CA) (20) and a bi-layered living human skin equivalent (Apligraf; Organogenesis Inc., Canton, MA) (6) are representative allogeneic skin substitutes that are reportedly effective for treating diabetic foot ulcers. Using non-cryopreserved fresh human fibroblast allografts was also found to be a safe and worthy treatment for diabetic foot ulcers (1,21). Recently, a new bioengineered dermal substitute composed of cultured autologous fibroblasts seeded on a hyaluronic acid sheet (Hyalograft 3D; ChaBio & Diostech, Seoul, Korea) was developed. However, in several cases, the autologous primary cells are not accessible or are not available in sufficient numbers and do not have proliferative capacity to be viable for skin bioengineering.

Multi-potent stem cells and progenitor cells hold great promise for addressing the need for viable cell sources. Most studies have shown them to have great promise in promoting wound healing (8,10,11).

MSCs from human umbilical cord blood can be gained and refined without difficulty in comparison with stem cells from other sources. As hUCB-MSCs are plentiful, simple to gain and proliferate to up to four times the number of cells in 3–5 days in adequate culture conditions *in vitro*, they can be cultured to a larger scale. The most significant benefit of hUCB-MSCs is their decidedly low immunogenicity (13). In contrast with BM-MSCs, the amount of hUCB-MSCs are not influenced by the general health condition of the recipient (22). On the basis of these advantages, a mixture of hUCB-MSCs and sodium hyaluronate (CARTISTEM<sup>®</sup>; Medipost Co Ltd) is produced as a single-dose cellular therapeutic agent for cartilage regeneration in patients with cartilage defects of the knee due to aging, trauma or degenerative diseases. After receiving the approval of the Korean FDA, this product has become the world's first allogeneic, off-the-shelf stem cell drug and is now accessible in Korea.

The therapeutic potential of hUCB-MSCs has been widely explored, and its use has been evaluated in a broad range of diseases; however, its effect on the healing of chronic wounds has not been studied to the same degree (15). There have been a few studies that applied hUCB-MSCs to advance wound healing. Zhao *et al.* (23) reported that hUCB-MSCs specifically localise at focused ulcerated wounds and may support the epithelialisation of diabetic foot ulcers in rats. Luo *et al.* (24) showed that applying hUCB-MSCs locally may not only increase wound-healing speed but may also advance the quality of wound healing in mice. Tark *et al.* (25) conducted a delayed wound-healing study using the diabetic mouse model and demonstrated that hUCB-MSCs had a positive effect on wound healing, especially at TGF- $\beta$  levels. However, most of the studies on hUCB-MSCs are limited animal studies, and the basic mechanisms have not yet been clearly explained. You *et al.* (16) attempted to assess the cell characteristics and phenotypes *in vitro* in comparison to healthy and diabetic fibroblasts. That was the first quantitative comparison of MSCs from human UCB and somatic cells. However, the study could not demonstrate the role of the hUCB-MSCs allograft on the activity of diabetic wound healing. Therefore, the present study was

performed to show the treatment effect of the hUCB-MSCs allograft on the activity of diabetic fibroblasts.

Prior to this study, the authors designed a preliminary study to decide the appropriate pore size for the microporous polymeric membrane, which serves as a barrier against direct contact between the cells within the insert and the diabetic fibroblasts in the monolayer culture outside the insert but allows extracellular matrices and cytokines produced by the cells within the insert to be successfully transported to the diabetic fibroblasts for stimulation. It was confirmed that the optimal pore size for the membrane is between 3 and 8  $\mu\text{m}$  (data not shown). In the present study, a polycarbonate culture plate insert with 3- $\mu\text{m}$  micro-pores was used to separate the hUCB-MSCs or healthy fibroblasts from the diabetic fibroblasts attached to the bottom of the culture plate. Therefore, hUCB-MSCs and fibroblasts could not pass through the insert but secreted molecules such as growth factors and collagen could. In clinical applications, the division of the transplanted cells with a microporous polymeric membrane may possibly surmount the major disadvantages of traditional cell transplantation methods.

The results of the study showed that the graft of both healthy fibroblasts and hUCB-MSCs stimulated the activity of diabetic fibroblasts. Additionally, the activity of diabetic fibroblasts cultured with hUCB-MSCs was greater than that with healthy fibroblasts. The previous *in vitro* study by the authors demonstrated that hUCB-MSCs are superior to fibroblasts in the production of major growth factors associated with wound healing but are not in collagen synthesis (16). Consequently, the results of the present study show that although hUCB-MSCs do not secrete much more extracellular matrix components by themselves than fibroblasts, treatment with hUCB-MSCs stimulates extracellular matrix synthesis, including collagen, of diabetic fibroblasts much greater than that with fibroblasts.

Clinical application of allogeneic cells might be limited by the possibility of graft rejection caused by the immunological obstacle. However, previous studies have shown that hUCB-MSCs are 'immunologically safe' for application in allogeneic cell therapies. Oh *et al.* (13) showed that hUCB-MSCs can inhibit the allogeneic responsiveness of human lymphocytes because they inhibit the proliferation of lymphocytes and decrease the formation of immunostimulatory cytokines. Lee *et al.* (26) used a humanised mouse model to prove that the low immunogenicity of allogeneic hUCB-MSCs can be retained *in vivo*. Possible disadvantages of autologous cells can be overestimated in patients with chronic wounds because of the weakened cell activity. For instance, even BM-MSCs show diminished growth when cultured from patients with chronic wounds (27,28). The therapeutic advantages of using allogeneic MSCs from healthy donors might overcome the drawbacks of autologous MSCs derived from patients with chronic wounds. Therefore, hUCB-MSCs, rather than BM-MSCs or other somatic cells, may be the better option in stem cell-based bioengineered products for wound healing.

However, wound healing of a diabetic ulcer *in vivo* is a complicated process influenced by various factors, and it is difficult to understand the specific role of hUCB-MSCs transplantation on the all-round process of wound healing because they can develop into fibroblasts or other cell types and become quiescent. Further well-established animal model studies of diabetes

will be needed to definitely determine the role of hUCB-MSCs transplantation on diabetic wound healing.

## Conclusion

In the present *in vitro* research, the authors performed a comparison between the influence of allograft of hUCB-MSCs and that of healthy fibroblasts on cell proliferation, collagen synthesis and GAG synthesis, which are the major contributing elements for wound healing, using diabetic fibroblasts. The diabetic fibroblasts treated with hUCB-MSCs showed greater increases in collagen and GAG synthesis than those treated with healthy fibroblasts. The authors conclude that the treatment of diabetic wounds with hUCB-MSCs may be a far better option than with fibroblasts.

## Acknowledgements

This study was supported by the Ewha Womans University scholarship of 2015. The authors have declared no conflicting interests. None of the authors has a financial interest in any of the products, devices or drugs mentioned in this manuscript.

## References

- Han SK, Choi KJ, Kim WK. Clinical application of fresh fibroblast allografts for the treatment of diabetic foot ulcers: a pilot study. *Plast Reconstr Surg* 2004;**114**(7):1783–9.
- Embil JM, Papp K, Sibbald G, Tousignant J, Smiell JM, Wong B, Lau CY, The Canadian Becaplermin Study Group. Recombinant human platelet-derived growth factor-BB (becaplermin) for healing chronic lower extremity diabetic ulcers: an open-label clinical evaluation of efficacy. *Wound Repair Regen* 2000;**8**(3):162–8.
- Satoh H, Kishi K, Tanaka T, Kubota Y, Nakajima T, Akasaka Y, Ishii T. Transplanted mesenchymal stem cells are effective for skin regeneration in acute cutaneous wounds. *Cell Transplant* 2004;**13**(4):405–12.
- Papanas N, Eleftheriadou I, Tentolouris N, Maltezos E. Advances in the topical treatment of diabetic foot ulcers. *Curr Diabetes Rev* 2012;**8**(3):209–18.
- Warriner RA 3rd, Cardinal M, Investigators T. Human fibroblast-derived dermal substitute: results from a treatment investigational device exemption (TIDE) study in diabetic foot ulcers. *Adv Skin Wound Care* 2011;**24**(7):306–11.
- Steinberg JS, Edmonds M, Hurley DP Jr, King WN. Confirmatory data from EU study supports Apligraf for the treatment of neuropathic diabetic foot ulcers. *J Am Podiatr Med Assoc* 2010;**100**(1):73–7.
- Hehenberger K, Heilborn JD, Brismar K, Hansson A. Inhibited proliferation of fibroblasts derived from chronic diabetic wounds and normal dermal fibroblasts treated with high glucose is associated with increased formation of l-lactate. *Wound Repair Regen* 1998;**6**(2):135–41.
- Han SK, Yoon TH, Lee DG, Lee MA, Kim WK. Potential of human bone marrow stromal cells to accelerate wound healing *in vitro*. *Ann Plast Surg* 2005;**55**(4):414–9.
- Conget JA, Minguell JJ. Phenotypical and functional properties of human bone marrow mesenchymal progenitor cells. *J Cell Physiol* 1999;**181**(1):67–73.
- Kim JB, Chun KW, Han SK, Kim WK. Effect of human bone marrow stromal cell allograft on proliferation and collagen synthesis of diabetic fibroblasts *in vitro*. *J Plast Reconstr Aesthet Surg* 2010;**63**(6):1030–5.
- Lee CH, Han SK, Choi WI, Kim WK. Effect of human bone marrow stromal cells and dermal fibroblasts on collagen synthesis and epithelialization. *Ann Plast Surg* 2007;**59**(6):713–9.

12. Han SK, Chun KW, Gye MS, Kim WK. The effect of human bone marrow stromal cells and dermal fibroblasts on angiogenesis. *Plast Reconstr Surg* 2006;**117**(3):829–35.
13. Oh W, Kim DS, Yang YS, Lee JK. Immunological properties of umbilical cord blood-derived mesenchymal stromal cells. *Cell Immunol* 2008;**251**(2):116–23.
14. Malgieri A, Kantzari E, Patrizi MP, Gambardella S. Bone marrow and umbilical cord blood human mesenchymal stem cells: state of the art. *Int J Clin Exp Med* 2010;**3**(4):248–69.
15. Kim JY, Jeon HB, Yang YS, Oh W, Chang JW. Application of human umbilical cord blood-derived mesenchymal stem cells in disease models. *World J Stem Cell* 2010;**2**(2):34–8.
16. You HJ, Namgoong S, Han SK, Jeong SH, Dhong ES, Kim WK. Wound-healing potential of human umbilical cord blood-derived mesenchymal stromal cells in vitro—a pilot study. *Cytotherapy* 2015;**17**(11):1506–13.
17. Yang SE, Ha CW, Jung MH, Jin HJ, Lee MK, Song HS, Choi SJ, Oh W, Yang YS. Mesenchymal stem/progenitor cells developed in cultures from UC blood. *Cytotherapy* 2004;**6**(5):476–86.
18. Pomahac B, Svensjo T, Yao F, Brown H, Eriksson E. Tissue engineering of skin. *Crit Rev Oral Biol Med* 1998;**9**(3):333–44.
19. Falanga V, Sabolinski M. A bilayered living skin construct (APLIGRAF) accelerates complete closure of hard-to-heal venous ulcers. *Wound Repair Regen* 1999;**7**(4):201–7.
20. Marston WA, Hanft J, Norwood P, Pollak R, Dermagraft diabetic foot ulcer study Group. The efficacy and safety of dermagraft in improving the healing of chronic diabetic foot ulcers: results of a prospective randomized trial. *Diabetes Care* 2003;**26**(6):1701–5.
21. Han SK, Kim HS, Kim WK. Efficacy and safety of fresh fibroblast allografts in the treatment of diabetic foot ulcers. *Dermatol Surg* 2009;**35**(9):1342–8.
22. Jin HJ, Bae YK, Kim M, Kwon SJ, Jeon HB, Choi SJ, Kim SW, Yang YS, Oh W, Chang JW. Comparative analysis of human mesenchymal stem cells from bone marrow, adipose tissue, and umbilical cord blood as sources of cell therapy. *Int J Mol Sci* 2013;**14**(9):17986–8001.
23. Zhao QS, Xia N, Zhao N, Li M, Bi CL, Zhu Q, Qiao GF, Cheng ZF. Localization of human mesenchymal stem cells from umbilical cord blood and their role in repair of diabetic foot ulcers in rats. *Int J Biol Sci* 2013;**10**:80–9.
24. Luo G, Cheng W, He W, Wang X, Tan J, Fitzgerald M, Li X, Wu J. Promotion of cutaneous wound healing by local application of mesenchymal stem cells derived from human umbilical cord blood. *Wound Repair Regen* 2010;**18**(5):506–13.
25. Tark KC, Hong JW, Kim YS, Hahn SB, Lee WJ, Lew DH. Effects of human cord blood mesenchymal stem cells on cutaneous wound healing in leprdb mice. *Ann Plast Surg* 2010;**65**(6):565–72.
26. Lee M, Jeong SY, Ha J, Kim M, Jin HJ, Kwon SJ, Chang JW, Choi SJ, Oh W, Yang YS, Kim JS, Jeon HB. Low immunogenicity of allogeneic human umbilical cord blood-derived mesenchymal stem cells in vitro and in vivo. *Biochem Biophys Res Commun* 2014;**446**(4):983–9.
27. Rodriguez-Menocal L, Shareef S, Salgado M, Shabbir A, Van Badiavas E. Role of whole bone marrow, whole bone marrow cultured cells, and mesenchymal stem cells in chronic wound healing. *Stem Cell Res Ther* 2015;**6**:24.
28. Nuschke A. Activity of mesenchymal stem cells in therapies for chronic skin wound healing. *Organogenesis* 2014;**10**(1):29–37.