Cartilage Repair by Human Umbilical Cord Blood-Derived Mesenchymal Stem Cells With Different Hydrogels in a Rat Model

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ABSTRACT: This study was carried out to assess the feasibility of human umbilical cord blood-derived mesenchymal stem cells (hUCB-MSCs) in articular cartilage repair and to further determine a suitable delivering hydrogel in a rat model. Critical sized full thickness cartilage defects were created. The hUCB-MSCs and three different hydrogel composites (hydrogel A; 4% hyaluronic acid/30% pluronic (1:1, v/v), hydrogel B; 4% hyaluronic acid, and hydrogel C; 4% hyaluronic acid/30% pluronic/chitosan (1:1:2, v/v)) were implanted into the experimental knee (right knee) and hydrogels without hUCB-MSCs were implanted into the control knee (left knee). Defects were evaluated after 8 weeks. The hUCB-MSCs with hydrogels composites resulted in a better repair as seen by gross and histological evaluation compared with hydrogels without hUCB-MSCs. Among the three different hydrogels, the 4% hyaluronic acid hydrogel composite (hydorgel B) showed the best result in cartilage repair as seen by the histological evaluation compared with the other hydrogel composites (hydrogel A and C). The results of this study suggest that hUCB-MSCs may be a promising cell source in combination with 4% hyaluronic acid hydrogels in the in vivo repair of cartilage defects. © 2015 Orthopaedic Research Society. Published by Wiley Periodicals, Inc. J Orthop Res 33:1580–1586, 2015.

Keywords: cartilage repair; human umbilical cord blood mesenchymal stem cells; hyaluronic acid; pluronic; chitosan

Recent progress in stem cell biology has enabled diverse repair applications to the articular cartilage defect. Since the articular cartilage is an avascular tissue, it has a limited self-healing potential, leading to a high incidence of unresolved cartilage-related injuries.[1] Therefore, various therapeutic methods have been attempted for articular cartilage repair including marrow stimulation, autologous chondrocyte implantation (ACI)[2] or autologous chondrocytes with scaffolds.[3] However, these methods have the disadvantage of size limitation, limited donor cell availability, and donor site morbidity.

Mesenchymal stem cells (MSCs) have been used for cartilage repair, bone marrow being the most common sources, achieving certain level of hyaline-like cartilage regeneration.[4,5] However, the collecting procedure of bone marrow from the donor is invasive and unfeasible as a routine method.[6] Therefore, we focused on human umbilical cord blood-derived MSCs (hUCB-MSCs) as a novel cell source. These cells have various advantages in that they are relatively easy to collect and have a high expanding capacity compared with bone marrow or adipose-derived MSCs.[7–9]

Scaffold intended for cartilage regeneration should fulfill many requirements, including adequate nutrient transport, adhesion to the defect site, degradability, and proper mechanical function.[10] Among various materials, hydrogels are most commonly explored. Hydrogels are attractive because they possess several advantages, such as high cell seeding efficacies and the abilities to transport nutrients, fill defects of any size and suspend cells homogenously, and injectability as a liquid that gels at body temperature and rebuild the three-dimensional structure.[11,12]

Hyaluronic acid (HA) is a natural non-sulfated glycosaminoglycan (GAG) that is widely distributed throughout the extracellular membrane of all connective tissues in humans and other animals, and is also found in articular cartilage as well. Due to its excellent biocompatibility, biodegradability, and gel-forming properties, HA and its derivatives have been widely explored as hydrogels in tissue engineering.[10,13] Pluronics are synthetic polymers composed of tri-block copolymers of poly (ethylene oxide)-poly(propylene oxide)-poly (ethylene oxide).[14] They are typical thermosensitive and biodegradable polymers exhibiting sol-gel transitions in water with an increase in temperature.[15] Hydrogels prepared from pluronic are thermosensitive synthetic polymers, which form gels above its lower critical solution temperature. Chitosan is a linear polysaccharide, which is a partially deacetylated derivative of chitin, a natural polysaccharide, and shows structural and functional similarity to the natural GAG.[16] Chitosan has been investigated in various tissue engineering applications in recent years due to its biocompatibility, biodegradability, low immunogenicity, and cationic nature.[16,17]

In cartilage repair, chondrogenic differentiation of hUCB-MSCs has been studied in other laboratories, however, their role in animal cartilage repair has rarely been evaluated.[18,19] Furthermore, proper hydrogels for delivering hUCB-MSCs have not been systemically investigated either. Therefore, we have applied the composites of hUCB-MSCs and different hydrogels (hyaluronic acid, hyaluronic acid: pluronic [1:1] and

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hyaluronic acid: pluronic:chitosan [1:1:2]) in critical-sized full-thickness cartilage defect and evaluated hUCB-MSCs as a novel cell sources in cartilage repair and determined the most suitable delivering hydrogel using a rat model.

MATERIALS AND METHODS

Animals

Fifteen Spraque-Dawley rats (Orient Bio Inc., Seungnam, Korea) were included in this study. All procedures and experimental protocols were reviewed and approved by the Institutional Animal Care and Use Committee at our institution (Samsung Medical Center, Seoul, Korea). This study also followed the institutional and National Institutes of Health guidelines for laboratory animal care.

Isolation and Culture of hUCB-MSCs

Human umbilical cord blood was collected from umbilical veins after neonatal delivery by an independent cord blood bank with informed consent from pregnant mothers. MSCs were isolated and cultured as previously published.[20] Specifically, mononuclear cells were isolated using density gradient centrifugation in Ficoll ($d = 1.077$ g/ml, Sigma, St. Louis, MO). Separated mononuclear cells were cultured in α-minimum essential medium (α-MEM, Gibco, Carlsbad, CA) supplemented with 15% fetal bovine serum (FBS, HyClone, Logan, UT) and maintained at 37°C in a humidified 5% CO_2 atmosphere with media changes twice weekly. Approximately 3 weeks after plating, fibroblast-like adherent cells were observed and when the cells reached 80% confluence, they were trypsinized (0.25% trypsin, HyClone) and resuspended in culture medium (α-MEM supplemented with 10% FBS).

Experimental Design

The experiment was performed in three groups (hydrogel A, B, C) to evaluate hUCB-MSCs as a novel cell sources as well as three different hydrogels. In each group ($n = 5$), hUCB-MSCs + hydrogel was transplanted into the 'experimental knee' (right knee) and hydrogel only was transplanted into the "control knee" (left knee). Different hydrogels in each groups were hydrogel A-4% HA (LG life Science, Daejon, Korea): 30% pluronic (Sigma, St.Louis, MO) (1:1, v/v), hydrogel B-4% HA, hydrogel C-4% HA: 30% pluronic:chitosan (Sigma, St. Louis, MO) (1:1:2, v/v).

Surgical Procedures

Before surgery, the animals were anaesthetized by inhalation of 5% ether and xylene (1 ml/kg) and ketamine (3ml/kg). The patella was everted through a medial parapatellar approach. The full-thickness defect of 2 mm in diameter and 3 mm in depth at the trochlear groove of the distal femur was created using a motorized drill. The knees were thoroughly irrigated with normal saline. The mixtures (20 μl) of the hUCB-MSCs (1×10^7 cells/ml) and the three different hydrogels were transplanted to the experimental knee. Hydrogels without hUCB-MSCs were implanted to the control knee. The arthrotomy was closed with interrupted nylon sutures, and the skin closed with continuous nylon sutures. Intramuscular antibiotics were injected for a week. Rats were allowed to move freely in their cages after surgery. In two knees a postoperative partial rupture of the skin suture occurred with the joint capsule still closed, which were re-sutured within the first postoperative days and no further complications were seen.

Gross Appearance and Scoring

Rats were sacrificed 8 weeks after transplantation and the harvested samples were first examined grossly. Gross appearance was graded according to the International Cartilage Repair Society (ICRS) macroscopic evaluation including parameters of "degree of defect repair, integration to border zone and macroscopic appearance for cartilage repair" with maximum scores of 12.[21]

Histology and Histological Evaluation

Harvested tissues were fixed in 10% formaldehyde solution (Sigma) for 24 h, and decalcified using a 5% nitric acid solution for an additional 3 days. Tissues were embedded in paraffin wax and 4 μm thick slides were prepared, parallel to and as close as possible to, the edge facing the center of the defect.

For histological evaluation, the serial sections were stained with Masson's trichrome for collagen contents and safranin O for GAG distribution. For Masson's trichrome staining, tissue sections were stained using a kit (BBC Biochemical, Seattle, WA). Specifically, sections were incubated in Masson trichrome for 5 min and differentiated in 5% phosphotungstic acid for 10 min. Tissue sections were subsequently stained in Aniline blue solution for 5 min, and excess stain removed by washing with 0.2% acetic acid. For safranin O staining, sections were stained with fast-green (Sigma, 0.05%) for 3 min, rinsed in tap water, and incubated in 0.1% safranin-O solution (Sigma) for 10 min. The histological results were also graded semi-quantitatively according to a modified O'Driscoll score.[22] The nature of the predominant tissue (cellular morphology, Masson's trichrome staining of the matrix), structural characteristics (surface regularity, structural integrity, thickness, and bonding to the adjacent cartilage) and freedom from the cellular changes of degeneration (hypocellularity and chondrocyte clustering) were analyzed from Masson's trichrome and safranin O stained images with maximum scores of 22.[22] Two observers blinded to sample codes scored the sections independently. Statistical analysis was performed using the Mann–Whitney U test (SPSS, version 14). A p-value of less than 0.05 was considered statistically significant.

Collagen II Immunostaining

For type II collagen immunostaining, sections were deparaffinized, washed with PBS and treated with 0.3% (v/v) hydrogen peroxide in methanol for 15 min to inactivate the endogenous peroxidases. Sections were incubated with an anti type-II collagen monoclonal antibody (1:200; Millipore Corporate, Billerica, MA) at 4°C overnight. After washing in PBS, the sections were incubated for 1 h with an HRP-conjugated anti-mouse secondary antibody. The reactivity was detected by using the EnVisionTM FLEX System-HRP (DAKO, Carpinteria, CA) and developed using a 3, 3'-diaminobenzidine chromogen (DAB, Vector Laboratories, Burlingame, CA) substrate kit according to the manufacturer's instructions.

RESULTS

Gross Appearance

In the gross appearance, no signs of overgrowth, degenerative change or inflammation were observed in any of the knees. Eight weeks postoperatively, the defects of both knees had produced repaired tissues that were pearly-white and firm (Fig. 1A). The

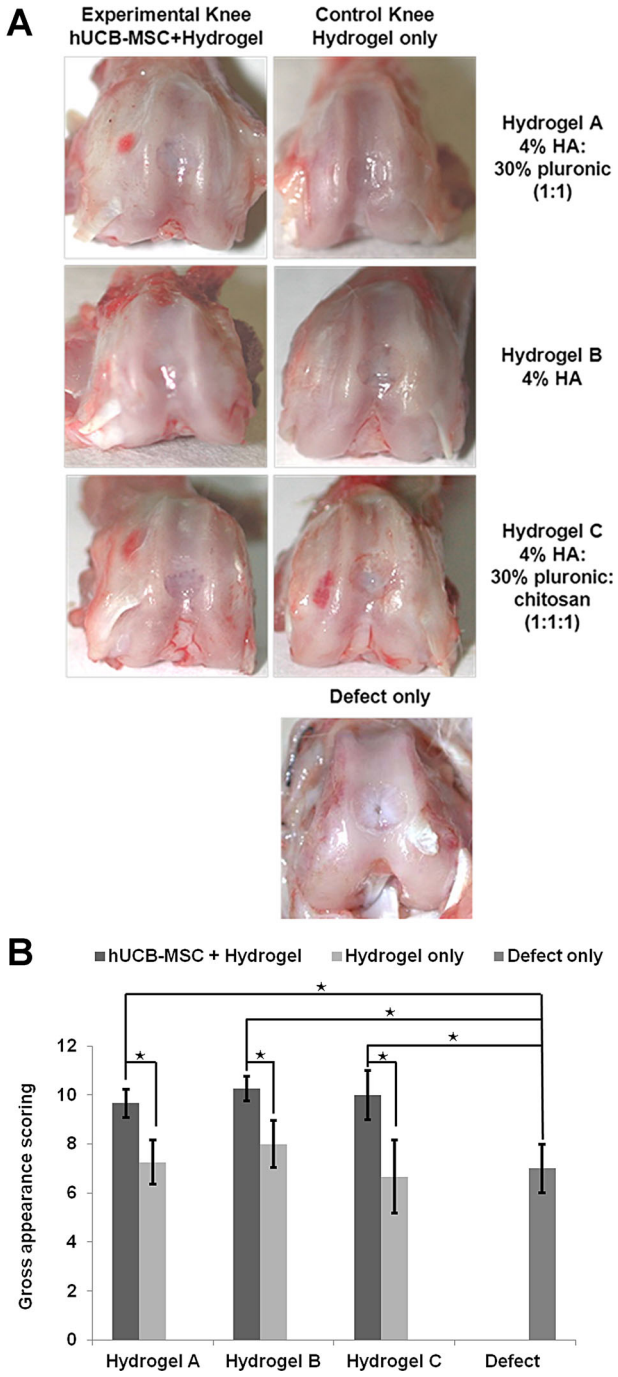

Figure 1. Gross appearance of articular cartilage defects in a rat model at 8 weeks post-transplantation. (A) The experimental knees with hUCB-MSCs and hydrogels and control knees with hydrogel only are shown. (B) ICRS gross appearance scores in hUCB-MSCs + hydrogel versus hydrogel in hydrogel A, B, and C and defect only. ($n = 5$/group, $^{*}p < 0.05$).

repaired tissues of the control knee were slightly irregular, looked fibrillated, and the margins of the defects were clearly recognizable. In contrast, repaired tissues in the experimental group resembled articular cartilage, well-integrated to the adjacent cartilage, and they had restored the contour of the femoral condyles (smooth articular surface without fissures or cracks). When the gross appearance was scored according to the ICRS evaluation system, the average scores in all experimental knees (hUCB-MSCs + hydrogel; hydrogel A 9.7, hydrogel B 10.3, hydrogel C 10.0) were significantly higher than those in the control knees (hydrogel only; hydrogel A 7.3, hydrogel B 8.0, hydrogel C 6.7) ($p = 0.006$, 0.004, 0.006) and those in defect knees without hydrogels (defect only; 7.0) (Fig. 1B). Among the three different hydrogels, the gross appearance did not show differences in the overall repair assessment.

Histological Examination and Grading

Representative images of Masson's trichrome and safranin O staining are shown in Figure 2 and Figure 3. In experimental knees with hUCB-MSCs, the cartilage repair was overall superior compared with the control knees. The defect had cartilaginous tissue that was well stained with Masson's trichrome and safranin O. The articular surface was smooth and intact, and bonded to the adjacent cartilage. In particular, the repaired sites with hUCB-MSCs and 4% HA (Figs. 2 and 3) showed superior structural characteristics (smooth and intact surface, and bonded to the adjacent cartilage with similar thickness) and normal cellularity without chondrocyte clusters. However, the cartilages in control knees without cells showed incomplete repair, mainly expressed as the slight Masson's trichrome and safranin O staining and irregular cartilage surfaces. Decreased repaired cartilage thickness was also observed (Figs. 2 and 3). The same sized (2 mm diameter and 3 mm deep) defects were created, and the cartilage repair with no transplanted cells or hydrogel was observed as a control. At 8 weeks, the defects also showed incomplete repair and a lack of integration, indicating poor cartilage repair (Figs. 2 and 3). The results show that this size of articular cartilage defect in a rat joint is a critical sized defect which cannot heal by itself.

Masson's trichrome and safranin O stained images were semi-quantatively analyzed using modified O'Driscoll scores (Fig. 4). As shown in Fig. 4A, there was a significantly higher repair score in the defects treated with the composite of hUCB-MSCs and hydrogels (hydrogel A 13.0, hydrogel B 18.3, hydrogel C 15.0) compared with that of hydrogel only (hydrogel A 9.7, hydrogel B 11.0, hydrogel C 11.0) in all groups ($p = 0.018$, 0.006, 0.006, respectively), and with that with containing no transplanted cells or no hydrogel (10.6). These findings indicate that hUCB-MSCs play a role in cartilage repair. Among the experimental knees, hUCB-MSCs with hydrogel B (4% HA) show an overall superior cartilage repair (Fig. 4B).

To investigate type II collagen following transplantation of hUCB-MSCs and hydrogels, regenerated tissues were analyzed using immunostaining. Immunohistochemical staining of tissue sections with a type II collagen antibody show that the proteins were

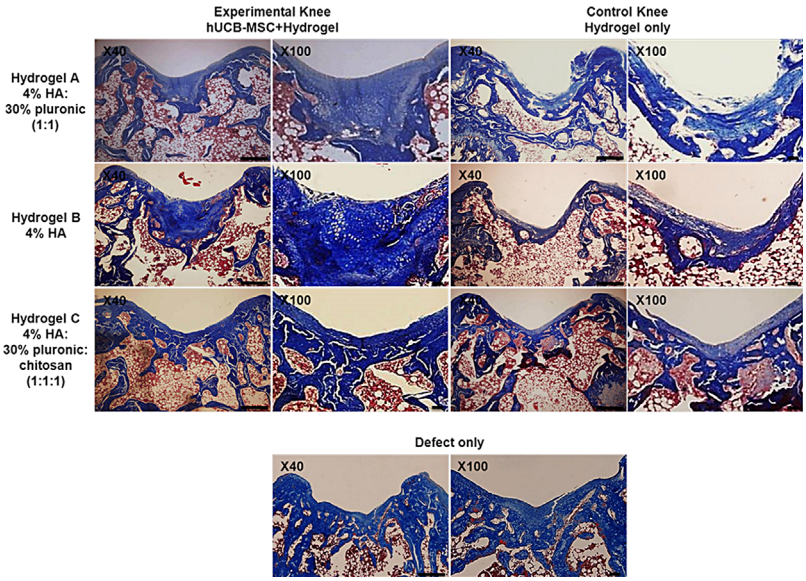

Figure 2. Masson's trichrome staining at 8 weeks post-transplantation in the experimental and control knees. Light red or pink indicates cytoplasm and dark brown to black shows cell nuclei. (Scale bar = 500 μm).

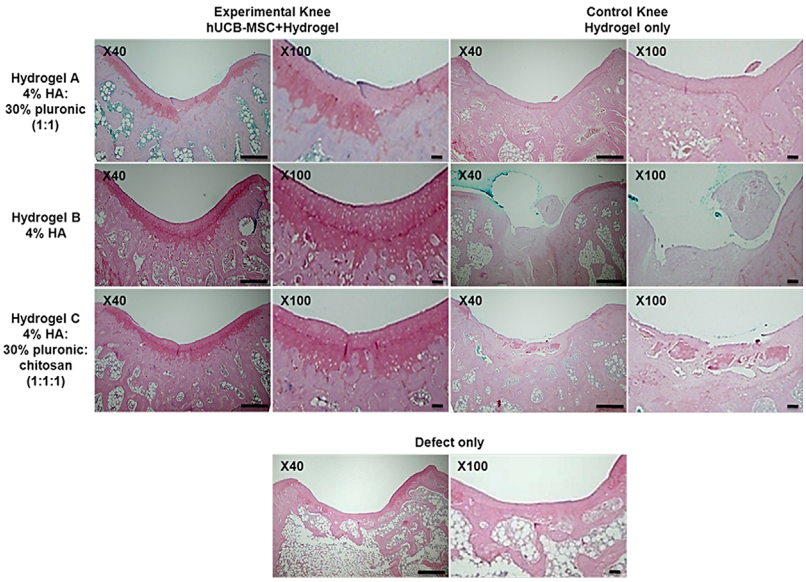

Figure 3. Safranin O staining at 8 weeks post-transplantation. Cartilage repair in the experimental knees was superior compared with the control knees. (Scale bar = 500 μm).

expressed at detectable levels in the repaired cartilage from the experimental groups and were not expressed in the control groups (Fig. 5). In particular, in the experimental groups, the regenerated cartilage has a similar staining pattern to the surrounding articular cartilage, with positive staining of the extracellular matrix around the chondrocyte-like cells at the site of the repair tissue (Fig. 5). These results indicate that hUCB-MSCs function 8 weeks post-injection and that the newly formed tissue was in the process of forming cartilage. Among the experimental groups, type II collagen is strongly expressed in hUCB-MSCs with hydrogel B (4% HA) transplanted knee (Fig. 5). In the control knees without transplanted cells, the repair tissue is positive for type-II collagen only around the junction with the normal cartilage, and the matrix is stained very weakly.

DISCUSSION

The present study demonstrate that the transplantation of hUCB-MSCs with hydrogel composite in cartilage defects led to improvements in cartilage repair compared with both hydrogel only and defect only. Among the three different hydrogels, the gross appearance did not show the differences. The hUCB-MSCs with 4% HA hydrogel composite showed superior cartilage repair in histological evaluation compared with hUCB-MSCs with other hydrogels composite.

The results of this study show the possibility of hUCB-MSCs as a novel cell source in cartilage repair. Recently, MSCs have received much attention in the field of cartilage repair because of their self-renewal capacity and multi-lineage differentiation potentials, including chondrocytes.[5] There are several sources of multipotential MSCs such as adipose tissue,

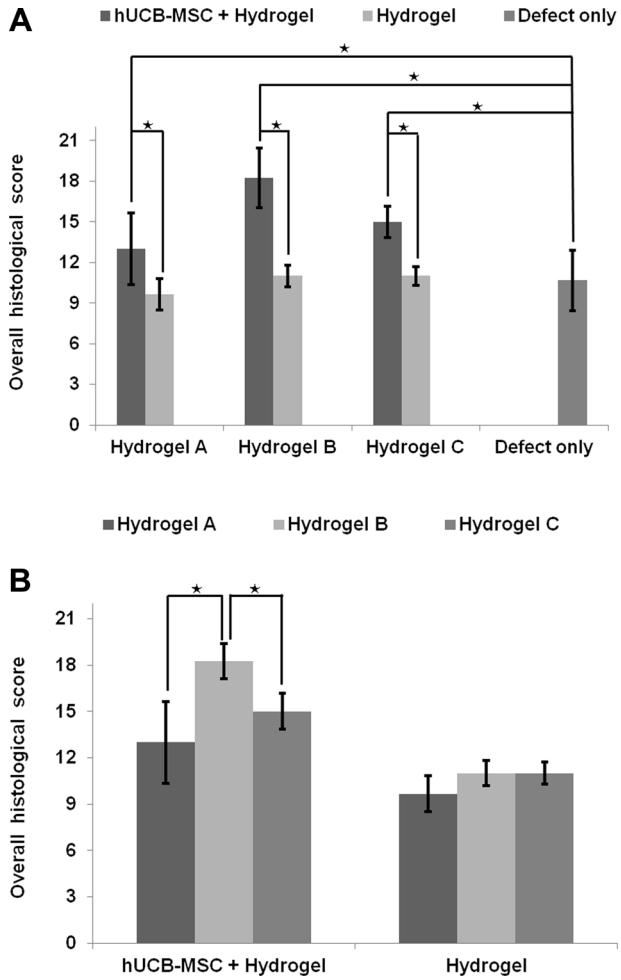

Figure 4. Semi-quantitative analysis of repair tissue in cartilage defects. (A) Comparison between experimental knee (hUCB-MSCs and hydrogel) and control knee (hydrogel only), and (B) among different hydrogels and defect only. Stained images were analyzed using a modified O'Driscoll scoring system and statistical difference was denoted by $p < 0.05$ ($n = 5$).

periosteum, synovial membrane, muscle, dermis, pericytes, blood, bone marrow, and trabecular bone.[23] Of these, bone marrow and adipose tissues were considered to be the source of a common pool of multipotent cells that gain access to various tissues via the circulation, subsequently adopting characteristics that meet the requirements of maintenance and repair of a specific tissue type. However, bone marrow-derived MSCs often showed bone formation[24,25] and adipose-derived MSCs showed decreased cartilage repairing potency.[6,26] The present study shows the superior cartilage repair with hUCB-MSCs up to 8 weeks without bone formation or degenerated cartilage repair, showing the possibility of hUCB-MSCs as a cell novel source in cartilage repair. In addition, hUCB-MSCs have many more advantages as a cell source in cellular therapy. Firstly, they showed more than 1,000-fold expanding capacity compared with bone marrow-derived MSCS.[20] Secondly, they are more beneficial in aspects of donor site morbidity compared with bone marrow-derived MSCs, which are commonly harvested from the posterior iliac bone. Finally, hUCB-MSCs are less immunogenic and can escape the host immune surveillance,[27,28] and the fact that no obvious sign of immunological response were seen in this study, in spite of xeno-transplantation supports this.

In explaining cartilage repair with hUCB-MSCs, the mechanism is not well-known. It has not been clear whether hUCB-MSCs migrate onto the injury site and repair the cartilaginous tissue directly, or whether they secrete bioactive molecules that trigger or support the tissue repair system in the microenvironment, "paracrine effects."[29,30] The hUCB-MSCs have shown the chondrogenic differentiation potential.[31] Recently, the paracrine actions of hUCB-MSCs were shown to promote differentiation to chondroprogenitor cells in vivo.[32] In that study, in order to investigate whether the newly formed cartilage is

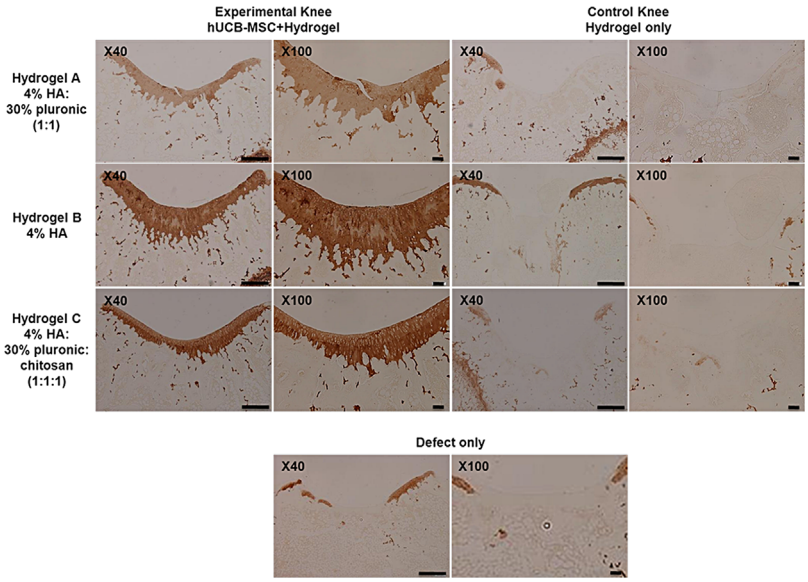

Figure 5. Type II collagen immunostaining. Eight weeks post-transplantation, the repaired tissue of the experimental knee had a strong affinity for the anti-type II collagen antibody. The cartilage of the control knee did not stain. (Scale bar = 500 μm).

generated via the paracrine actions of MSCs, transplanted hUCB-MSCs was observed with reverse transcriptase polymerase chain reaction of human gene and PKH 26 labeling in the regenerated site. The transplanted hUCB-MSCs disappeared within 4 to 8 weeks post-transplantation. Certain studies have also reported that labeled transplanted MSCs gradually disappeared in regenerated tissue over time following transplantation.[33,34] Although we cannot exclude the possibility of chondrogenic differentiation of transplanted MSCs, an interaction between hUCB-MSCs and subchondral progenitor cells initiated by paracrine action may play an important role in cartilage repair.

The addition of pluronic and chitosan to HA did not increase chondrogenesis in comparison with HA alone. Articular cartilage is a load bearing tissue. We think that the material used for successful cartilage repair should mimic viscoelastic properties such as compressive, friction, and tensile strength to bear large deformations and motions.[35] For example, sufficient surface and tensile properties are up to 0.1–2 MPa in order to function in the high shear joint environment. The mechanical property of pluronic is known to be less than 1 KPa, whereas hyaluronic acid is approximately 29–149 KPa.[10,36] Therefore, HA has better mechanical property to provide a proper environment for chondrogenesis compared with pluronic gel. Chitosan is one of the promising materials with various advantageous such as good biocompatibility and mechanical property, antibacterial activity, and ability to bind to growth factors.[37] However, based on the inferior repair with chitosan addition, we speculate that chitosan would not match properly to the viscoelastic property changes during repair process compared with HA.

Certain limitations our study has should be considered. First, the mechanism of the hUCB-MSCs for cartilage repair is currently partially known.[32] These issues should be investigated in future studies. Second, the study period of 8 weeks is relatively short. The result of longer-term study is unknown. This is an initial study in therapeutic approach to investigate the feasibility of hUCB-MSCs in articular cartilage repair. Some studies to evaluate the cartilage repair using a rat model have analyzed at 8 weeks.[38,39] Thus, we chose 8 weeks as the end point. Finally, magnetic resonance imaging to monitor the quality of repair tissue was not performed. MRI for T2 mapping was expensive and technically demanding.

In the present study, we have determined that 4% HA as a suitable delivering hydrogel in cartilage repair using a rat model. To date, various types of hydrogels have been proposed for articular cartilage repair.[13] Several studies suggest that polymers are required to accommodate enough cells and to keep cells in the defect lesion. However, few studies have compared the quantitative cartilage repair process in animal models with composite of MSCs and various hydrogels. In this study, hydrogels with pluronic, HA and chitosan were evaluated. They were biocompatible, biodegradable, and had hydrogel-forming properties.[10,13] Four percent HA was turned out to be the most suitable hydrogels. The superiority of HA hydrogel correlates with the previous findings that HA possesses biological cues to promote chondrogenesis.[40]

In conclusion, the current study suggest that hUCB-MSCs are a promising cell source with many advantage and the composite of hUCB-MSCs and 4% HA are a feasible and effective treatment for cartilage repair.

AUTHORS' CONTRIBUTIONS

YBP contributed research design, drafting the paper, and interpretation of data. MS contributed research design, drafting the paper, and interpretation of data. CHL contributed acquisition, analysis, and interpretation of data. JAK contributed revising the paper, and acquisition, analysis of data. CWH contributed research design, drafting the paper, and approval of the submitted and final versions. All authors read and approved the final submitted manuscript.

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REFERENCES

1. Guettler JH, Demetropoulos CK, Yang KH, et al. 2004. Osteochondral defects in the human knee: Influence of defect size on cartilage rim stress and load redistribution to surrounding cartilage. Am J Sports Med 32:1451–1458.
2. Brittberg M, Lindahl A, Nilsson A, et al. 1994. Treatment of deep cartilage defects in the knee with autologous chondrocyte transplantation. N Engl J Med 331:889–895.
3. Willers C, Chen J, Wood D, et al. 2005. Autologous chondrocyte implantation with collagen bioscaffold for the treatment of osteochondral defects in rabbits. Tissue Eng 11:1065–1076.
4. Wakitani S, Goto T, Pineda SJ, et al. 1994. Mesenchymal cell-based repair of large, full-thickness defects of articular cartilage. J Bone Joint Surg Am 76:579–592.
5. Caplan AI 2005. Review: mesenchymal stem cells: cell-based reconstructive therapy in orthopedics. Tissue Eng 11:1198–1211.
6. Liu TM, Martina M, Hutmacher DW, et al. 2007. Identification of common pathways mediating differentiation of bone marrow- and adipose tissue-derived human mesenchymal stem cells into three mesenchymal lineages. Stem Cells 25:750–760.
7. Erices A, Conget P, Minguell JJ. 2000. Mesenchymal progenitor cells in human umbilical cord blood. Br J Haematol 109:235–242.
8. Wagner W, Wein F, Seckinger A, et al. 2005. Comparative characteristics of mesenchymal stem cells from human bone marrow, adipose tissue, and umbilical cord blood. Exp Hematol 33:1402–1416.
9. Kern S, Eichler H, Stoeve J, et al. 2006. Comparative analysis of mesenchymal stem cells from bone marrow, umbilical cord blood, or adipose tissue. Stem Cells 24:1294–1301.

10. Kim IL, Mauck RL, Burdick JA. 2011. Hydrogel design for cartilage tissue engineering: A case study with hyaluronic acid. Biomaterials 32:8771–8782.
11. Hoffman AS 2002. Hydrogels for biomedical applications. Adv Drug Deliv Rev 54:3–12.
12. Park KM, Lee SY, Joung YK, et al. 2009. Thermosensitive chitosan-Pluronic hydrogel as an injectable cell delivery carrier for cartilage regeneration. Acta Biomater 5: 1956–1965.
13. Spiller KL, Maher SA, Lowman AM. 2011. Hydrogels for the repair of articular cartilage defects. Tissue Eng Part B Rev 17:281–299.
14. Khattak SF, Bhatia SR, Roberts SC. 2005. Pluronic F127 as a cell encapsulation material: Utilization of membrane-stabilizing agents. Tissue Eng 11:974–983.
15. Chen WJ, Huang JW, Niu CC, et al. 2009. Use of fluorescence labeled mesenchymal stem cells in pluronic F127 and porous hydroxyapatite as a bone substitute for posterolateral spinal fusion. J Orthop Res 27:1631–1636.
16. Ragetly GR, Griffon DJ, Lee HB, et al. 2010. Effect of chitosan scaffold microstructure on mesenchymal stem cell chondrogenesis. Acta Biomater 6:1430–1436.
17. Suh JK, Matthew HW. 2000. Application of chitosan-based polysaccharide biomaterials in cartilage tissue engineering: A review. Biomaterials 21:2589–2598.
18. Arufe MC, De la Fuente A, Fuentes I, et al. 2011. Umbilical cord as a mesenchymal stem cell source for treating joint pathologies. World J Orthop 2:43–50.
19. Wang L, Tran I, Seshareddy K, et al. 2009. A comparison of human bone marrow-derived mesenchymal stem cells and human umbilical cord-derived mesenchymal stromal cells for cartilage tissue engineering. Tissue Eng Part A 15:2259–2266.
20. Yang SE, Ha CW, Jung M, et al. 2004. Mesenchymal stem/progenitor cells developed in cultures from UC blood. Cytotherapy 6:476–486.
21. van den Borne MP, Raijmakers NJ, Vanlauwe J, et al. 2007. International Cartilage Repair Society (ICRS) and Oswestry macroscopic cartilage evaluation scores validated for use in Autologous Chondrocyte Implantation (ACI) and microfracture. Osteoarthritis Cartilage 15:1397–1402.
22. Song SU, Cha YD, Han JU, et al. 2005. Hyaline cartilage regeneration using mixed human chondrocytes and transforming growth factor-beta1- producing chondrocytes. Tissue Eng 11:1516–1526.
23. Tuan RS. 2006. Stemming cartilage degeneration: Adult mesenchymal stem cells as a cell source for articular cartilage tissue engineering. Arthritis Rheum 54:3075–3078.
24. Gao J, Dennis JE, Solchaga LA, et al. 2001. Tissue-engineered fabrication of an osteochondral composite graft using rat bone marrow-derived mesenchymal stem cells. Tissue Eng 7:363–371.
25. Shao X, Goh JC, Hutmacher DW, et al. 2006. Repair of large articular osteochondral defects using hybrid scaffolds and bone marrow-derived mesenchymal stem cells in a rabbit model. Tissue Eng 12:1539–1551.
26. Sakaguchi Y, Sekiya I, Yagishita K, et al. 2005. Comparison of human stem cells derived from various mesenchymal tissues: Superiority of synovium as a cell source. Arthritis Rheum 52:2521–2529.
27. Ren G, Zhang L, Zhao X, et al. 2008. Mesenchymal stem cell-mediated immunosuppression occurs via concerted action of chemokines and nitric oxide. Cell Stem Cell 2: 141–150.
28. Ding Y, Bushell A, Wood KJ. 2010. Mesenchymal stem-cell immunosuppressive capabilities: Therapeutic implications in islet transplantation. Transplantation 89:270–273.
29. Granero-Molto F, Myers TJ, Weis JA, et al. 2011. Mesenchymal stem cells expressing insulin-like growth factor-I (MSCIGF) promote fracture healing and restore new bone formation in Irs1 knockout mice: Analyses of MSCIGF autocrine and paracrine regenerative effects. Stem Cells 29:1537–1548.
30. Chen L, Tredget EE, Wu PY, et al. 2008. Paracrine factors of mesenchymal stem cells recruit macrophages and endothelial lineage cells and enhance wound healing. PLoS One 3: e1886.
31. Yang S-E, Ha C-W, Jung M, et al. 2004. Mesenchymal stem/progenitor cells developed in cultures from UC blood. Cytotherapy 6:476–486.
32. Jeong SY, Kim DH, Ha J, et al. 2013. Thrombospondin-2 secreted by human umbilical cord blood-derived mesenchymal stem cells promotes chondrogenic differentiation. Stem Cells 31:2136–2148.
33. Koga H, Muneta T, Ju YJ, et al. 2007. Synovial stem cells are regionally specified according to local microenvironments after implantation for cartilage regeneration. Stem Cells 25:689–696.
34. Sato M, Uchida K, Nakajima H, et al. 2012. Direct transplantation of mesenchymal stem cells into the knee joints of Hartley strain guinea pigs with spontaneous osteoarthritis. Arthritis Res Ther 14:R31.
35. Huey DJ, Hu JC, Athanasiou KA. 2012. Unlike bone, cartilage regeneration remains elusive. Science 338:917–921.
36. Levett PA, Hutmacher DW, Malda J, et al. 2014. Hyaluronic acid enhances the mechanical properties of tissue-engineered cartilage constructs. PLoS ONE 9:e113216.
37. Di Martino A, Sittinger M, Risbud MV. 2005. Chitosan: A versatile biopolymer for orthopaedic tissue-engineering. Biomaterials 26:5983–5990.
38. Lee JM, Im GI. 2012. SOX trio-co-transduced adipose stem cells in fibrin gel to enhance cartilage repair and delay the progression of osteoarthritis in the rat. Biomaterials 33:2016–2024.
39. Song JQ, Dong F, Li X, et al. 2014. Effect of treadmill exercise timing on repair of full-thickness defects of articular cartilage by bone-derived mesenchymal stem cells: An experimental investigation in rats. PLoS One 9:e90858.
40. Aulin C, Bergman K, Jensen-Waern M, et al. 2011. In situ cross-linkable hyaluronan hydrogel enhances chondrogenesis. J Tissue Eng Regen Med 5:e188–e196.