Intra-Articular Injection of Human Meniscus Stem/Progenitor Cells Promotes Meniscus Regeneration and Ameliorates Osteoarthritis Through Stromal Cell-Derived Factor-1/CXCR4-Mediated Homing

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INTRODUCTION

Tearing of the meniscus frequently occurs during athletic injuries to the knee [1]. As meniscus possesses poor self-regenerative capacity upon injury [2], meniscus degeneration is currently the most common treatment modality [3]. However, meniscus regeneration is associated with complications that may ultimately lead to osteoarthritis (OA) [4–8]. Hence, it is imperative to repair meniscal tears rather than perform surgical excision [9–12].

Over the last two decades, tissue-engineering strategies have emerged to repair injured meniscus [13, 14]. To date, various cell types have been examined in meniscus tissue engineering such as bone marrow mesenchymal stem cells (B-MSCs), synovium MSCs (S-MSCs), meniscal fibrochondrocytes, and chondrocytes [15–19]. However, these cell sources are associated with donor site morbidity. Moreover, the outcomes are unsatisfactory with these cell types because of poor proliferative capacity and ossification [20]. Therefore, a new source of seed cells is needed to promote the regeneration of injured meniscus. A recent study showed that multipotent stem cells could be present in the meniscus [21, 22]. This finding raises the possibility of using meniscus-derived stem/progenitor cells for meniscal regeneration in vivo. However, the effect of human meniscus-derived stem/progenitor cells (hMeSPCs) on meniscus regeneration and OA prevention in vivo and the mechanisms involved in the migration of injected hMeSPCs have not yet been reported.

Systemically transplanted mesenchymal stem cells (MSCs) have been previously shown to migrate to injured tissues and contribute to tissue regeneration [23]. The migration of MSCs is mediated by stromal cell-derived factor-1 (SDF-1) that is upregulated at injury sites and its cognate receptor CXCR4 expressed on the migratory cells [24–29]. However, it is still unknown whether the SDF-1/CXCR4 chemokine axis will facilitate the migration of intra-articular injected MeSPCs to promote meniscus repair in vivo. In this study, we demonstrated that intra-articular injection of hMeSPCs enhances meniscus regeneration.
through SDF-1/CXCR4-mediated homing, underscoring its potential use for meniscus repair in the clinic.

**Materials and Methods**

**Monoclonal Selection of hMeSPCs**

Human meniscus tissue was digested with collagenase (3 mg/ml) for 6 hours. The cells from the digested tissue were cultured in low-glucose Dulbecco’s modified Eagle’s medium (DMEM) supplemented with penicillin-streptomycin and 20% (vol/vol) fetal bovine serum. After passage 0, they were seeded at a very low density to form colonies on 6-cm dishes (300 cells in one dish). The putative cells isolated from human meniscus were designated as hMeSPCs [30]. All hMeSPCs in this study were of polyclonal origin. hMeSPCs between passages 1 and 3 were used for all experiments.

**Flow Cytometry**

Cells (5 x 10^5) were incubated with 1 μg phycoerythrin (PE)- or fluorescein isothiocyanate (FITC)-conjugated mouse antibodies specific to human cell surface markers, including CD34 (FITC), CD90 (FITC), CD44 (PE), CD45 (FITC), and CD166 (FITC) for 1 hour at 4°C. The nonconjugated mouse-specific antibody to human CD105 was incubated with 1 x 10^6 cells for 1 hour at 4°C. After washing, the cells were incubated with FITC-conjugated rabbit anti-mouse immunoglobulin G for 45 minutes on ice and analyzed using a Coulter Epics XL flow cytometer.

**Evaluation of Multipotent Differentiation Potential of hMeSPCs**

The multipotent differentiation potential of hMeSPCs toward the adipogenic, osteogenic, and chondrogenic lineages was evaluated in vitro according to established protocols [30]. Positive induction of adipogenesis, osteogenesis, and chondrogenesis was confirmed by Oil Red O staining, alkaline phosphatase staining, and safranin O staining, respectively.

**Colony Formation Assay and Expression Analysis of Meniscus-Related Genes by MeSPCs, S-MSCs, and B-MSCs**

Human MeSPCs, S-MSCs, and B-MSCs were harvested from total knee arthroplasty of donors with osteoarthritis, as described previously [30]. Twelve days after initial seeding, some of the dishes were stained with 1% (wt/vol) crystal violet solution (Sigma-Aldrich, St. Louis, MO, http://www.sigmaaldrich.com) in methanol, and the number of colonies (with diameter >2 mm) was counted. Total cellular RNA was isolated by lysis in TRIzol (Invitrogen, Carlsbad, CA, http://www.invitrogen.com), followed by a one-step phenol chloroform-isomyl alcohol extraction, as described by the manufacturer’s protocol. Polymerase chain reaction (PCR) analysis of collagen I and collagen II expression was performed, as described previously [30], and the results are presented as target gene expression normalized to glyceraldehyde-3-phosphate dehydrogenase. The primer sequences used for PCR analysis in this study are listed in Table 1.

**In Vitro Chemotaxis Assay**

To determine whether recombinant human SDF-1α (rhSDF-1α) can regulate the migration of hMeSPCs, an in vitro chemotaxis assay was performed using a Transwell system (Costar 3422; Corning Inc., Corning, NY, http://www.corning.com), as described previously [27, 30]. Briefly, 1 x 10^5 cells suspended in 100 μL serum-free DMEM plus 0.5% (wt/vol) bovine serum albumin were placed within the upper chamber. To induce chemotaxis, 60 μL rhSDF-1α (200 ng/ml) in serum-free DMEM was added to the lower chamber (−/+). Additionally, to observe chemokinesis, rhSDF-1α was also added to the upper chambers (+/+). To evaluate whether rhSDF-1α promoted the migration of hMeSPCs through the SDF-1/CXCR4 axis, hMeSPCs were pretreated with AMD3100 (10 μg/ml; CXCR4-specific antagonist) for 2 hours at 37°C in some experimental groups (AMD3100 –/+). Twenty-four hours later, the upper surface of the filters was scraped free of hMeSPCs and debris. hMeSPCs that had migrated through the filter were fixed in 4% (v/v) paraformaldehyde and subjected to 4',6-diamidino-2-phenylindole (DAPI) staining (Beyotime Institute of Biotechnology, Jiangsu, China). The total number of cells on the lower surface of the membrane was counted under light microscopy in five randomly selected fields (x400). The data were expressed as mean number of cells per high-power field (cells per HPF) ± SD, and were subsequently analyzed for statistically significant differences between experimental groups.

**Expression of SDF-1 After Meniscectomy**

The injured meniscus tissues were harvested from the meniscectomy groups, as well as from the sham groups (n = 3 in each time point) at 1, 2, and 3 weeks postsurgery. The mRNA expression levels of SDF-1 within injured meniscus were then analyzed, as previously described [30]. The primer sequences used in this study are listed in Table 1.

**In Vivo Chemotaxis and Loss-of-Function Assay**

One week after meniscectomy, 1,1'-dioctadecyl-3,3',3'-tetramethy-lindocarbocyanine perchlorate (Dil)-stained hMeSPCs (6 x 10^5 cells in 50 μL phosphate-buffered saline [PBS], pretreated with 10 μg/ml AMD3100 for 2 hours at 37°C) were injected into the right knee. As control, the left knee was injected with normal hMeSPCs in PBS alone. Four weeks after operation, the Kodak in vivo FX small animal imaging system was used to evaluate the migration of injected hMeSPCs within the meniscus defect. This experiment was repeated three times.

**Meniscectomy and hMeSPC Injection**

Six female rats weighing 200–220 g were used in this study. The rats were treated with cyclophosphamide (150 mg/kg) 24 hours

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**Table 1. List of primer sequences used for real-time polymerase chain reaction**

| Genes  | 5’-3’   | Primers                        | Production size (bp) |
|--------|---------|--------------------------------|----------------------|
| SDF-1  | Forward | AGGCCATATGTCGCCAGAGCCA          | 309                  |
|        | Reverse | GGTACAAAGGCGCAAGAGGA            |                      |
| Collagen I | Forward | CGATGATTCGCCAGTGGTGA            | 246                  |
|        | Reverse | CATCGAAGCTGACGCTGTA             |                      |
| Collagen II | Forward | GGCAGCGCTGGTCTAGGGTT            | 570                  |
|        | Reverse | GCCCTGCTACACCATTTTCC            |                      |
| GAPDH  | Forward | GCAAGTTCAACGCGCAG              | 141                  |
|        | Reverse | GGCGATGACTCCAGCAG               |                      |

Abbreviations: GAPDH, glyceraldehyde-3-phosphate dehydrogenase; SDF-1, stromal cell-derived factor-1.
before the meniscectomy. The anterior half of medial meniscus was removed at the level of the medial collateral to create a defect [18]. hMeSPCs (6 x 10⁶ in 50 µl PBS) were injected into the right knee at 1 week and 2 weeks after meniscectomy, whereas the same volume of PBS was injected into the left knee as control. After euthanasia, three meniscuses of rats from each experimental group were subjected to histological evaluation at the 4-week and 12-week time points. Treatment of animals was in accordance with standard guidelines approved by the Zhejiang University Ethics Committee.

Cell Labeling and Detection
The hMeSPCs used for in situ repair of meniscus were pre-stained with Dil/6-carboxyfluorescein diacetate (CFDA). To evaluate the survival of implanted hMeSPCs in the meniscus defect, a noninvasive Kodak in vivo FX small animal imaging system was used to analyze the samples at 4 and 12 weeks postmeniscectomy [32]. Reverse-transcriptase polymerase chain reaction showed that hMeSPCs expressed higher levels of collagen II as compared with B-MSCs and S-MSCs. Scale bars = 50 µm (Ba, Bc), 100 µm (Aa, Bb), 500 µm (Ab, Ac). *, p < .05 versus control group. Abbreviations: ALP, alkaline phosphatase; B-MSCs, bone marrow-derived mesenchymal stem cells; COL I, collagen I; COL II, collagen II; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; MeSPCs, meniscus-derived stem/progenitor cells; P, passage; S-MSCs, synovium-derived mesenchymal stem cells.

Histology
Hematoxylin and eosin and safranin O staining were performed, as described previously [33]. Macroscopically, regeneration of the injured meniscus was evaluated by area assay, and the degeneration of femoral and tibial articular cartilage was evaluated directly after ink staining [34]. Histological scoring was performed, as described previously [33]. Briefly, four sections from each sample were graded blindly by three observers. Histology evaluation was performed using the International Cartilage Repair Society visual histological assessment scale, including surface, matrix, cell distribution, and depth.

Transmission Electron Microscopy
At 4 and 12 weeks postsurgery, tissue specimens from the hMeSPC-treated and control groups were fixed according to standard procedures for transmission electron microscopy (TEM) to assess the cell morphology of the regenerated meniscus [30].

Immunostaining
A series of 8-µm-thick sections were used for immunohistochemical staining. Rabbit anti-Col1 (Anbo Biotechnology Co., San Francisco, CA, http://www.anbobio.com), mouse anti-Col2 (Calbiochem, San Diego, CA, http://www.emdbiosciences.com), rabbit anti-Col10 (Abcam, Cambridge, U.K., http://www.abcam.com), and rabbit anti-Hif-2α (Abcam), together with goat anti-mouse (Beyotime) or goat anti-rabbit (Beyotime) secondary antibodies, were used to detect the expression of these proteins within the degenerated articular cartilage [35].

Statistical Analysis
All quantitative data sets are expressed as mean ± SD. Student’s t test was performed to assess whether there were statistically significant differences in the results of different data sets, with a value of p < .05 being considered significantly different.
RESULTS

Characterization of hMeSPCs

A subpopulation of meniscus cells attached and formed colonies 10–12 days after initial seeding (Fig. 1A). The colonies were heterogeneous in morphology at P0, possibly reflecting differences in cell origin from the meniscus tissue. A multipotent homogeneous population of MSC-like cells became apparent after further culture (Fig. 1B). These results confirmed the clonogenicity and multipotency of meniscus-derived cells in vitro, as previously reported [21]. Flow cytometry results showed that the cells expressed high levels of MSC markers, including CD44 (89.39%), CD90 (99.36%), CD105 (95.58%), and CD166 (96.23%), but not the hematopoietic markers CD34 (1.47%) and CD45 (0.06%) (Fig. 1C). Together, these results suggest that hMeSPCs possess stem cell properties similar to MSCs. However, MeSPCs exhibited higher clonogenicity and displayed a higher level of collagen II expression than B-MSCs ($p < .05$) and S-MSCs (Fig. 1D, 1E), which is indicative of their unique features.

Migration of hMeSPCs Was Mediated by SDF-1/CXCR4 In Vitro and In Vivo

To investigate the direct influence of SDF-1 on the migration of hMeSPCs, the following in vitro and in vivo studies were performed. In the in vitro study, it was observed that SDF-1 significantly ($62.79 \pm 27.53$ vs. $14.39 \pm 3.56$/HPF; $p < .05$) increased the numbers of migratory hMeSPCs on the lower surface of the membrane, as compared with the untreated control (Fig. 2Aa, 2Ab). The number of hMeSPCs that migrated in response to SDF-1 was decreased when SDF-1 was introduced into both the upper and lower chambers at the same time ($p < .05$; n = 3 wells per group). Black arrows show the anterior half of medial meniscus. Scale bars = 50 mm (A, C), 5 mm (A, inset); 50 mm (B); Abbreviations: CFDA, 6-carboxyfluorescein diacetate; DiI, 1,1'-dioctadecyl-3,3,3',3'-tetramethylindocarbocyanine perchlorate; MeSPCs, meniscus-derived stem/progenitor cells; N, number; SDF-1, stromal cell-derived factor-1α; W, weeks.

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concentrations (Fig. 2Aa–2Ac). To further investigate the effects of the SDF-1/CXCR4 chemokine axis on hMeSPC migration, AMD3100-pretreated (2 hours) hMeSPCs were placed into the upper chambers. The results showed that AMD3100 abolished the chemotactic effects of SDF-1 on hMeSPCs significantly (12.43 ± 4.08 vs. 62.79 ± 27.53/HPF; p < .01; n = 3 wells per group), suggesting that the signaling events leading to migration of hMeSPCs are transmitted through the SDF-1/CXCR4 chemokine axis (Fig. 2Aa–2Ad).

Before performing the in vivo study, we first analyzed the expression of SDF-1 in the injured meniscus at 1, 2, and 3 weeks after meniscectomy. Real-time PCR results showed that the expression of SDF-1 started to increase at 1 week after meniscectomy (Fig. 2B). Subsequently, the expression of SDF-1 increased significantly after 2 weeks (13.42 ± 4.57 vs. 2.24 ± 0.87; p < .01) and remained elevated at 3 weeks after meniscectomy.

Based on the observation that SDF-1 promoted MeSPC migration in vitro and that expression of SDF-1 was upregulated within the injured meniscus in vivo, we further investigated the effect of meniscus injury on the migration of intra-articular injected hMeSPCs in vivo through the SDF-1/CXCR4 chemokine axis. One week after meniscectomy, Dil-stained hMeSPCs were injected into the left knee. As control, the AMD3100-pretreated hMeSPCs were injected into the right knee. As shown by gross morphology and image tracking (Kodak in vivo FX small animal imaging system), injected hMeSPCs adhered to the injured meniscus at 3 weeks after injection (Fig. 2C). By contrast, in the AMD3100-pretreated group, the adhesion of hMeSPCs was almost inhibited. These results suggest that the SDF-1/CXCR4 chemokine axis mediated the migration and adhesion of injected hMeSPCs.

Intra-Articular Injection of hMeSPCs Promoted Meniscus Repair

We further examined the effects of intra-articular implanted hMeSPCs on meniscus repair in vivo (Fig. 3A). Compared with the PBS control, intra-articular injection of hMeSPCs induced significantly more neo-tissue formation and extracellular matrix (ECM) deposition in the meniscal defect at 4 weeks postmeniscectomy (Fig. 3B, black arrows; Fig. 3C, 1.09 ± 0.48 vs. 0.28 ± 0.18; p < .05). At 12 weeks postmeniscectomy, there were no significant differences between the hMeSPC injection group and the control group in terms of gross morphology (Fig. 3B, black arrows; Fig. 3C, 1.78 ± 1.25 vs. 0.81 ± 0.34; p > .05). It is a major limitation of our in vivo study that rat meniscus could heal spontaneously in the control group. For future studies, larger animal models, such as sheep, dog, and pig [36], are required to demonstrate the clinical efficacy of intra-articular injection of MeSPCs for meniscus repair. TEM imaging also showed that the cells of the hMeSPC injection group were morphologically more similar to that of normal meniscus with rounded shape (Fig. 4A, black arrows), and with their collagen fibrils being more ordered and larger than that of the control group (Fig. 4B), at both the 4- and 12-week time points.

To evaluate the contribution of injected hMeSPCs to meniscus regeneration, hMeSPCs were labeled with Dil and CFDA before injection and traced in vivo (top right panels of Fig. 2C). Dil and CFDA signals were detected at the injury site at 4 and...
Meniscus Stem/Progenitor Cells for Meniscus Repair

W, weeks. inducible factor 2 of COL I, COL X, and HIF-2 that the osteoarthritis process was suppressed within the hMeSPC-postinjection, immunohistochemistry of the cartilage of tibia showed articular injection of human MeSPCs (hMeSPCs). At 4 and 12 weeks CXCR4 chemokine axis. The results positively confirmed that:jured meniscus through induced cell homing via the SDF-1/

Progression of OA

We further examined the therapeutic effect of hMeSPCs in an animal model of OA. Craters, present on the surface of the medial femoral condyle and medial tibial cartilage of the control, were undetectable in the hMeSPC-treated cartilage surface at 12 weeks postmeniscectomy (Fig. 5A; supplemental online Fig. 2A). Compared with the control, intra-articular injection of hMeSPCs reduced surface irregularities (Fig. 5B–5D, p < .05; supplemental online Fig. 2B, 2C). Immunohistochemistry analysis demonstrated that, in contrast to the control, hMeSPC treatment decreased the expression of the OA markers collagen I and X but maintained high collagen II expression, thus indicating that hMeSPCs ameliorated the degeneration of cartilage (Fig. 6; supplemental online Fig. 3).

Figure 6. The suppression of experimental osteoarthritis after intra-articular injection of human MeSPCs (hMeSPCs). At 4 and 12 weeks postinjection, immunohistochemistry of the cartilage of tibia showed that the osteoarthritis process was suppressed within the hMeSPC-treated group with high COL II expression and low expression levels of COL I, COL X, and HIF-2α. Scale bars = 50 μm (COL II bottom row, COL I, COL X, HIF-2α), 100 μm (COL II top row). Abbreviations: COL I, collagen I; COL II, collagen II; COL X, collagen X; HIF-2α, hypoxia-inducible factor 2α; MeSPCs, meniscus-derived stem/progenitor cells; W, weeks.

12 weeks postmeniscectomy, as shown by both the small animal fluorescent imaging system and fluorescence microscopy (supplemental online Fig. 1A, 1B), thus suggesting that injected hMeSPCs may contribute to meniscus regeneration. However, we didn’t detect the percentage of the cells administered remain at the subsequent time points. It is another limitation of our in vivo study.

Intra-Articular Injection of hMeSPCs Suppressed the Progression of OA

This study was performed to evaluate our hypothesis that intra-articular injected hMeSPCs can enhance the regeneration of injured meniscus through induced cell homing via the SDF-1/CXCR4 chemokine axis. The results positively confirmed that:

(hMeSPCs) with the characteristics of both mesenchymal stem cells and cartilage phenotype was successfully isolated from human meniscus and characterized; (b) the SDF-1/CXCR4 chemokine axis can mediate the migration of hMeSPCs in vitro, as well as promote the trafficking of hMeSPCs to the meniscus injury site in vivo; (c) intra-articular injection of hMeSPCs enhanced the regeneration of meniscus injury in vivo within a rat model, resulting in more physiological structure of the ECM; (d) intra-articular injection of hMeSPCs also suppressed the process of osteoarthritis in vivo through the inhibition of hypoxia-inducible factor 2α (HIF-2α). Collectively, these results demonstrated that intra-articular injection of hMeSPCs has great potential for clinical applications in meniscus regeneration and OA prevention in the future.

hMeSPCs Can be an Ideal Seed Cell for Meniscus Regeneration In Vivo

Tissue engineering is a multidisciplinary field comprising an intersection of biology, engineering, and clinical applications, the ultimate objective of which is to grow and maintain living tissues both in situ and ex vivo. Most researchers agree that no tissue-engineered construct can be derived without cells. Recently, some studies suggest that multipotent stem cells are present in the meniscus [21, 22]. Our previous study found that stem cells can be isolated from rabbit meniscus and that these cells possess immunosuppressive properties. Intra-articular injection of meniscus derived stem cells promoted meniscus regeneration and maintained joint space width. These data thus suggest that meniscus-derived MSCs have great potential for the regeneration of meniscus injuries. Nevertheless, to date, no report has yet mentioned the injection of human meniscus-derived MSCs for regeneration of injured meniscus. In our study, we found that 35% of cells derived from human meniscus formed circular colonies, which is higher than the proportion of putative adult stem cells derived from other human tissues, such as bone marrow and synovium. We also found that hMeSPCs displayed higher expression of collagen II compared with the putative stem cells derived from synovium and bone marrow. Hence, we selected hMeSPCs as seed cells for our in vivo study. The in vivo study demonstrated that the hMeSPC-transplanted group yielded better histological staining results. We observed that the hMeSPC-transplanted groups exhibited higher density of ECM deposition with round meniscal cells within it, thus indicating the key role of hMeSPCs in the process of meniscus healing. Our findings suggested a new strategy of articular cartilage protection through meniscus regeneration induced by intra-articular injection of hMeSPCs for patients undergoing meniscectomy. Meniscus regeneration is feasible using this tissue-engineering technique.

Injected hMeSPCs Enhance the Regeneration of Injured Meniscus Through the SDF-1/CXCR4 Chemokine Axis

A number of studies have previously demonstrated that intra-articular injection of cells is an effective treatment strategy for regenerating injured meniscus. For example, Murphy et al. [37, 38] found that local delivery of adult MSCs to injured knee joints could stimulate the regeneration of meniscal tissue. Sekiya and colleagues [18] reported that the injected stem cells differentiated directly into meniscal cells within the meniscal defect, but the mechanisms by which these cells adhered to the injury sites are not well understood. SDF-1, a major chemokine of the bone

Discussion

This study was performed to evaluate our hypothesis that intra-articular injected hMeSPCs can enhance the regeneration of injured meniscus through induced cell homing via the SDF-1/CXCR4 chemokine axis. The results positively confirmed that:
Intra-Articular Injected hMeSPCs Suppress the Process of OA In Vivo

In our study, we found that intra-articular injection of hMeSPCs can delay or reduce the progression of OA induced by meniscotomy, including the degree of cartilage degeneration, osteophyte formation, and subchondral sclerosis. Some researchers have hypothesized that the neo-meniscal tissue in the treated groups is associated with protection against degenerative changes induced by OA after meniscal injury [4, 37]. It is thought that MSCs may have a direct role in cartilage protection through direct remodeling of the articular cartilage or by acting to preserve subchondral or trabecular bone based on the relationship between early bone remodeling and OA development [40, 41]. However, Sekiya et al. [42] found that only a small proportion of the cells adhered to the cartilage defect after injection, which is similar to our previous results [22]. These results suggest that other mechanisms may be inferred. Many studies have shown that the destruction of articular cartilage is involved in the upregulation of catabolic factors, such as matrix metalloproteinases (MMP1, MMP3, MMP9, MMP12, and MMP13) and aggrecanase-1 [43–45]. HIF-2α expression was inhibited in our hMeSPC-treated group. Chun and colleagues also demonstrated that gene knockout of HIF-2α in mice could suppress cartilage destruction, thus suggesting that HIF-2α plays a leading role in causing osteoarthritis [46]. In our study, we observed that HIF-2α expression was inhibited in our hMeSPC-treated group. Therefore, this suggests that hMeSPCs may impede OA progression by inhibiting HIF-2α expression.

CONCLUSION

Our study demonstrated for the first time that intra-articular injection of hMeSPCs promoted meniscus regeneration through SDF-1/CXCR4-mediated migration and ameliorated the progression of OA. Our study highlights the potential of using intra-articular injection of hMeSPCs for the treatment of meniscus injuries in the clinic.

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AUTHOR CONTRIBUTIONS

W.S.: conception and design, collection of data, data analysis and interpretation, manuscript writing; J.C.: collection and assembly of data, data analysis and interpretation, manuscript writing; T.Z., L.C., W.Z., and Z.F.: collection and assembly of data; B.C.H.: collection of data, data analysis and interpretation; H.-W.O.: conception and design, provision of study material, data analysis and interpretation, final approval of manuscript; Z.Y. and X.C.: conception and design, provision of study material, data analysis and interpretation, final approval of manuscript. The authors indicate no potential conflicts of interest.
An allogenic cell-based implant for meniscal using a novel biomaterial. Am J Sports Med 2012;14:R31.

Spontaneous osteoarthritis. Arthritis Res Ther 2008;10:223.

Baker BM, Nathan AS, Huffman GR et al. Tissue engineering with meniscus cells derived from surgical debris. Osteoarthritis Cartilage 2009;17:336–345.

Horie M, Sekiya I, Muneta T et al. Intra-articular injected synovial stem cells differentiate into meniscal cells directly and promote meniscal regeneration without mobilization to distant organs in rat massive meniscal defect. Stem Cells 2009;27:878–887.

Sato M, Uchida K, Nakajima H et al. Direct transplantation of mesenchymal stem cells into the knee joints of Hartley strain guinea pigs with spontaneous osteoarthritis. Arthritis Res Ther 2012;14:R31.

Harris MT, Butler DL, Boivin GP et al. Mesenchymal stem cells used for rabbit tendon repair can form ectopic bone and express alkaline phosphatase activity in constructs. J Orthop Res 2004;22:998–1003.

Segawa Y, Muneta T, Makino H et al. Mesenchymal stem cells derived from synovium, meniscus, anterior cruciate ligament, and articular chondrocytes share similar gene expression profiles. J Orthop Res 2009;27:435–441.

Shen W, Chen X, Chen J et al. The effect of incorporation of exogenous stromal cell-derived factor-1 alpha within a knitted silk-collagen sponge scaffold on tendon regeneration. Cell Transplant 2009;18:433–441.

Chen X, Song XH, Yin Z et al. A novel strategy incorporated the power of mesenchymal stem cells to allografts for segmental bone tissue engineering. J Orthop Res 2009;27:1276–1287.

Ji JF, He BP, Dheen ST et al. Interactions of chemokines and chemokine receptors mediate the migration of mesenchymal stem cells to the impaired site in the brain after hypoglossal nerve injury. Stem Cells 2004;22:415–427.

Tögel F, Isaac1, Hu Z et al. Renal SDF-1 signals mobilization and homing of CXCR4-positive cells to the kidney after ischemic injury. Kidney Int 2005;67:1772–1784.

Ratajczak MZ, Zuba-Surma E, Kucia M et al. The pleiotropic effects of the SDF-1-CXCR4 axis in organogenesis, regeneration and tumorigenesis. Leukemia 2006;20:1915–1924.

Shen W, Chen X, Chen J et al. The effect of cell-derived factor 1 on stem-cell homing and tissue regeneration in ischaemic cardiomyopathy. Lancet 2003;362:697–703.

Li B, Aspden RM. Composition and mechanical properties of cancellous bone from the femoral head of patients with osteoporosis or osteoarthritis. J Bone Miner Res 1997;12:641–651.

Koga H, Shimaya M, Muneta T et al. Local adherent technique for transplanting mesenchymal stem cells as a potential treatment of cartilage defect. Arthritis Res Ther 2008;10:R84.

Yatabe T, Mochizuki S, Takizawa M et al. Hya1uronan inhibits expression of ADAMTS4 (aggrecanase-1) in human osteoarthritic chondrocytes. Ann Rheum Dis 2009;68:1051–1058.

Sato T, Konomi K, Fuji K et al. Prostaglandin EP2 receptor signalling inhibits the expression of matrix metalloproteinase 13 in human osteoarthritic chondrocytes. Ann Rheum Dis 2011;70:221–226.

Hui W, Litherland GJ, Elias MS et al. Leptin produced by joint white adipose tissue induces cartilage degradation via upregulation and activation of matrix metalloproteinases. Ann Rheum Dis 2012;71:455–462.

Yang S, Kim J, Ryu JH et al. Hypoxia-inducible factor-2alpha is a catabolic regulator of osteoarthritic cartilage destruction. Nat Med 2010;16:687–693.

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