Mesenchymal Stem Cells Transplantation to the Knee Joint of Hartley Strain Guinea Pig with Spontaneous Osteoarthritis

INTRODUCTION
Osteoarthritis (OA) of the knee joint is a major public health problem and its prevalence is expected to increase dramatically and rapidly over the next 20 years with an increasingly aged population. It is therefore desirable to develop less technically demanding but effective therapies for knee OA such as cell transplantation with or without scaffold enhancement.

The objective of this study was to determine whether intra-articular injection of MSCs suspended in hyaluronic acid (HA) solution into the knee joint enhances the repair of degenerated cartilage in an animal model of spontaneous OA. We used the Hartley strain guinea pigs because these animals spontaneously develop degenerative cartilage changes in the knee joint that mimic those of human OA.

METHODS
Commercially-available human MSCs (Lot. 0PT-2501; Lonza, Walkersville, MD) were cultured, labeled with carboxyfluorescein diacetate succinimidyl ester (CFDA-SE), suspended in either phosphate-buffered-saline (PBS) or hyaluronic acid (HA), and injected into the knee joints of 7-month-old male Hartley strain guinea pig. The control animals were injected with either PBS or HA alone. The animals were sacrificed at week 1, 3, and 5 after injection and the knee joints were harvested. The experimental protocol strictly followed the Animal Care Ethical Committee Guideline for Experimental Study of our University Medical Faculty and institutional review board (IRB) guidelines for Stem Cell Research.

Macroscopic Examination
The surface of the tibial plateau were exposed. India ink (2 ml) was injected onto the joint surfaces with a syringe, and 1 minute later, the surfaces were washed with physiological saline. The staining pattern of the cartilage surface was observed macroscopically. The gross findings were classified and scored.

Histological and Immunohistochemical Examinations
Serial 10-µm thick, 20 frontal frozen sections in each knee were prepared carefully in order to include the severely degenerated area not covered by meniscus. The sections were stained with HE, toluidine blue and safranin O using standard methods. The severity of knee OA was assessed using the modified Mankin criteria.

The tissue sections were also stained with a primary polyclonal antibody, raised in rabbits against type II collagen (1:200 dilution; COSMO BIO, Tokyo). These sections were counterstained with nuclear marker DAPI.

Cells labeled with CFDA-SE can be visualized by fluorescence microscopy using standard fluorescein filter sets. The labeled cells were quantified in 500×500 µm² areas of tibial frontal section (original magnification, x100).

Immunoblot Analysis for Collagen Type II Protein
Immunoblot analysis was also carried out. Total protein (80µg/lane) extracted from the cartilage was transferred down to polyvinylidene difluoride membrane. The membrane was then incubated with the collagen type II primary antibody (1:20 dilution; COSMO BIO, Tokyo), followed sequentially by anti-mouse IgG antibody. The antibody binding was visualized using a FluorChem™ 8000 System (Alpha Innotech Corporation, San Leandro, CA). The size of each band for collagen type II was normalized to β-actin (Lot. no. IMG-5142A, 1:1000; Imgenex, San Diego, CA).

RESULTS
Macroscopic Findings
In the HA+MSC group, the surface of the medial tibial plateau was relatively smooth and the staining intensity was weaker at the same time interval. The macroscopic OA score was significantly lower in the HA+MSC group than the PBS group.

Histological Findings
In the PBS and PBS+MSC groups, significant fibrillation was observed in the articular surface, together with loss of chondrocytes from the tangential to the radial layer. Matrix staining was reduced for all treatment groups apart from HA+MSC. In the HA group, only minor fibrillation of the articular surface was observed, however, weak matrix staining and cell depletion were also observed. The HA+MSC group showed large numbers of chondrocytes with cluster formations in the radial layer of the articular cartilage. Furthermore, the matrix around the cell clusters was strongly stained at week 5 after injection.

At week 5 after injection, significant differences were noted between the OA scores of the PBS and HA+MSC groups, and between the PBS+MSC and HA+MSC group.

Immunohistochemical and Western Blot Analyses
The immunostaining for type II collagen was stronger and more extensive around the chondrocyte-like cells at week 5 in the HA+MSC treated group compared to the other 3 treatment groups. Similarly, Western blot analysis showed higher levels for type II collagen in the HA+MSC group, relative to the PBS group. Quantitative analysis confirmed these findings.

Fluorescent Microscopic Findings
A few cells labeled with CFDA-SE were found within the cartilage at week 1 after injection of PBS+MSC. The labeled cells gradually disappeared from the cartilage at weeks 3 and 5 after injection. In contrast, for the HA+MSC group, the labeled cells appeared both adhered to the surface and scattered within the superficial and transitional layers of the cartilage at week 1 after injection of HA+MSC, but they were more frequent in the transitional layer and showed a columnar arrangement at weeks 3 and 5.

DISCUSSION
In the present study, we evaluated the feasibility of intra-articular injection of MSCs suspended in HA for the treatment of knee OA. Our results at week 5 after injection showed histologically-confirmed partial repair of the articular cartilage, compared to the other three treatment groups. In vivo tracing techniques using CFDA-SE labeled cells and fluorescence microscopy demonstrated that the intra-articularly injected MSCs in HA migrated throughout the osteoarthritic cartilage.

Immunostaining for type II collagen was observed in the matrix around residual chondrocytes in the radial layer at week 5, in addition to pericellularly and in the matrix between labeled MSCs in the transitional layer. These findings indicated chondrogenic differentiation of the injected MSCs. These results imply intra-articular differentiation of the injected MSCs into chondrocytes and their subsequent proliferation; in addition, while HA is known to influence chondrocyte metabolism, the MSCs may also produce trophic factors which could stimulate the residual chondrocytes.

Our study has certain limitations that include the use of non-physiologic conditions for xenogeneic MSCs transplantation, possible deterioration of the repaired cartilage after injection, identification of the optimum timing of injection during OA progression or lack of optimizing the molecular weight of HA for the treatment. Further studies are needed to clarify these points.

SIGNIFICANCE
The results of the present study suggest that intra-articular injection of MSCs with HA is a potential beneficial therapy for OA. This scaffold-free, minimally invasive treatment seems to retard the progression of spontaneous OA and stimulates the regeneration of articular cartilage.

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