Soluble IL-6R promotes chondrogenic differentiation of mesenchymal stem cells to enhance the repair of articular cartilage defects using a rat model for rheumatoid arthritis

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Abstract

Objective

Although articular cartilage contributes to smooth joint motion, once damaged this functionality cannot be recovered. Activation of the IL-6/STAT3 signalling pathway contributes to chondrogenic differentiation of mesenchymal stem cells (MSCs), indicating a role for soluble IL-6R (sIL-6R) during chondrogenesis in vitro. The aim of this study is to develop a novel therapeutic tool for regenerative medicine of articular cartilage.

Methods

Human bone marrow-derived MSCs were pre-treated with sIL-6R to direct their differentiation into chondrocytes, then seeded on a poly-lactic-co-glycolic acid (PLGA) sheet to enhance the localised residence of MSCs. The material was implanted into knee joint spaces of antigen-induced arthritis (AIA) rats, an animal model of rheumatoid arthritis (RA). After 8 weeks, the effects of the implantation on articular cartilage repair were assessed by x-ray image and staining with safranin O (S-O), aggrecan and human leukocyte antigen (HLA).

Results

Swelling of knees in AIA rats, but not sham-treated rats, was observed. AIA rats implanted with PLGA and sIL-6R-treated MSCs showed similar knee joint imaging to sham rats using x-ray; however, those with PLGA alone, or with PLGA with MSCs, did not. Rats implanted with PLGA and sIL-6R-treated MSCs, but not PLGA alone or PLGA with MSCs, showed positive imaging by S-O staining as well as human aggrecan. HLA was not detected in the knees of any of the rats.

Conclusion

PLGA and sIL-6R-treated MSCs help to repair articular cartilage with high efficacy. Thus, the application of this promising strategy to regenerative medicine for articular cartilage in patients with RA is anticipated.

Key words

articular cartilage repair, mesenchymal stem cells, antigen-induced arthritis, polylactic-co-glycolic acid, soluble IL-6 receptor

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Introduction

Articular cartilage covers the ends of bones and contributes to smooth joint motion. This tissue is composed of chondrocytes and cartilage extracellular matrix (ECM), including type II, IX, and XI collagen, aggrecan (ACAN), and proteoglycan, leading to the formation of hyaline cartilage. The articular cartilage is a structurally unique tissue that lacks blood vessels and nerves, and is in a low-nutrient, low-oxygen environment.

Rheumatoid arthritis (RA) is a systemic autoimmune disease characterised by chronic inflammatory synovitis and the destruction of articular cartilage and bone tissue (1). Without appropriate treatment, the patients experience reduced muscle strength associated with limited joint function (2, 3). In particular the destruction of the articular cartilage, but not bone tissue, correlates with the physical disorder of RA (4, 5). Therefore, the repair of articular cartilage is a prerequisite for improvement of activity of daily living (ADL) and quality of life (QOL) (4, 5). The goal of treatment for RA is to achieve clinical or structural remission through the use of methotrexate (MTX) as the first line and disease-modifying antirheumatic drugs (DMARDs), in addition to biological DMARDs including tumour necrosis factor (TNF) inhibitor, interleukin (IL)-6 inhibitor, cytotoxic T-lymphocyte-associated protein 4 immunoglobulin (CTLA-4 Ig), or small-molecular compounds including Janus kinase (JAK) inhibitor (6, 7). However, a proper therapeutic tool to repair articular cartilage damage in RA patients remains to be developed.

Previously, two types of osteochondral transplantations have been applied toward this difficult task. One is autologous osteochondral transplantation, which involves grafting articular cartilage taken from a patient into the affected area of the same patient (8). The pathological features of the articular cartilage improve over the short term, whereas the long-term effects are inconsistent (8, 9). The second technique is allogeneic osteochondral transplantation with the goal of repairing widespread defects in the articular cartilage, which has shown successful results in terms of articular cartilage improvement (10). However, several issues remain that need to be addressed, such as the need for the adaptation of the donor's graft size to that of the recipient, and assessment of the condition of the recipient's health with or without infection (11, 12).

Recently, an alternative strategy using mesenchymal stem cells (MSCs) has been focused on. Once articular cartilage is damaged, it is thought that MSCs differentiate into chondrocytes to repair the cartilage tissue. However, we have reported endogenous MSCs actually exhibit a poor capacity for self-repair. Furthermore, even if MSCs were injected intra-articularly (IA) into the body, the cells diffuse into peripheral blood and reside in the non-affected regions. As a result, it is difficult to provide functional recovery of articular cartilage once damaged (13). To enable MSCs to localise and reside in the affected area, a scaffold is needed for implantation. Poly-lactic-co-glycolic acid (PLGA) is a commonly used scaffold composed of both poly-glycolic acid (PGA) and poly-lactic acid (PLA). PLGA has several advantages, such as biodegradability, *i.e.* it disintegrates in the body, low immunogenicity, is an efficient carrier of drugs to the target tissue, forms a scaffold for regeneration of cartilage defects through the support of cell residence and cell differentiation. Notably, we found that MSCs differentiate into chondrocytes even when MSCs seeded on PLGA plugs are cultured without any cytokine stimulation (14). However, bone marrow-derived MSCs from *IL-1Ra* knockout mice, but not *wild-type mice*, which exhibit pathological conditions like RA, show a low capacity for self-renewal or osteoblastic differentiation (15). This suggests that endogenous MSCs from RA patients might have a low ability for cell differentiation, including chondrocytes. Therefore, it is necessary to use exogenous MSCs, which have a normal ability for chondrocyte differentiation, for implantation. However, it remains unclear about the efficacy of PLGA with MSCs on articular cartilage repair in the destroyed joint of RA.

In this study PLGA sheets were used to enhance the localised residence of MSCs at the joints. Previously we found that sIL-6R stimulation promotes differentiation of bone marrow-derived MSCs into chondrocytes via *in vitro* cell pellet culture (16). The results indicated a critical role for soluble IL-6 receptor (sIL-6R) during chondrogenesis *in vitro*. However, these results have not been proven *in vivo*. To define the role of sIL-6R in articular cartilage repair which may be related to mechanism of chondrogenesis *in vitro*, we performed the following study. MSCs were pre-treated with sIL-6R to efficiently induce differentiation of MSCs into chondrocytes. PLGA and sIL-6R-treated MSCs were then implanted into an AIA rat model with partial destruction of articular cartilage as a representative animal model for RA.

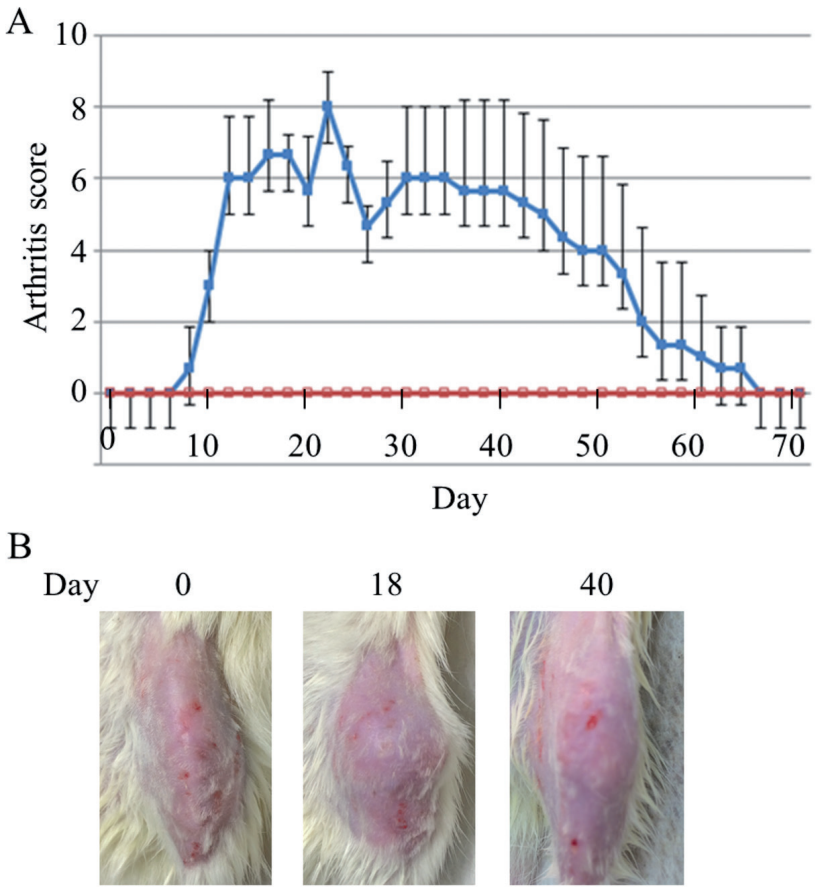

Fig. 1. Evaluation of arthritis in AIA or sham rats.
A: Arthritis score in ankles of AIA Lewis rats (n=5) or sham rats (n=5) with or without injection of mBSA into the joint space, respectively. Arthritis was scored by the extent of swelling in rats' ankle as described in *Materials and methods*. Blue and red plots show arthritis score of AIA rats or sham rats, respectively.
B: Representative macroscopic findings of knees from 5 AIA rats. Respective knees at day 0, day 18 and day 40 after mBSA injection. AIA: antigen-induced arthritis; mBSA: albumin methylated from serum.

Materials and methods

Reagents

Bone marrow-derived human MSCs purchased from Lonza (Walkersville, MD) were cultured in mesenchymal stem cell growth medium (MGM BulletKit, Lonza) in cell culture dishes at 37°C under 5% CO_2 atmosphere as described previously (13). The MSCs were seeded at a density of 100,000 cells/cm^2 in a 24-well plate, and then treated with or without either sIL-6R or IL-6 overnight. The cells were then seeded on a sterilised PLGA sheet (1.5×1.5 cm^2; 50:50, lactic acids: glycolic acids; Teijin) as a scaffold, and further cultured for 24 hours. They were then used for the experiments described below.

Preparation of rats with AIA

Fifty female Lewis rats were purchased from Charles River (Yokohama, Japan). They were provided and bred with standard rat chow and water. To induce AIA, albumin methylated from serum (mBSA, Sigma Aldrich, St. Louis, A1009) was emulsified in an equal amount of complete Freund's adjuvant (CFA), and injected intradermally (total 500μL) in the tail of 8-week-old rats, and mBSA was then injected intra-articularly (IA) (total 50μL) as described previously (17). The severity of arthritis in each limb was assessed every 2 days using an established clinical score method with a score of 0 to 4 (13).

Preparation of MSCs and scaffold for implantation

At about 60 days after injection of mBSA into joint spaces, PLGA nanofibre sheets with or without MSCs treated in the presence or absence of either sIL-6R or IL-6 were implanted into the joint spaces of the bilateral knees of thirty rats with AIA. The open joint caves were then ligated with 4–0 silk at three points. This experiment was approved by the ethics committee of the University of Occupational and Environmental Health, Japan.

X-ray and micro-CT

Knee joints of pre-implantation (IMP) rats were evaluated by x-ray (Sofron, Tokyo) or micro-CT (Hitachi Aloka Medical, Tokyo) as described previously (13). At 4 weeks after MSCs implantation, the rats were sacrificed. Knee joints of post-IMP rats were also evaluated via both methods. Quantification of tibial bone mass was carried out using Image J as described previously (18).

Histological examination

Rat knee joints were harvested, fixed in 10% formaldehyde, decalcified in ethylene diaminetetraacetic acid (EDTA) bone decalcifier for 25 days, and embedded in paraffin. They were then subjected to histological examinations, including haematoxylin and eosin (HE), safranin O (S-O), and immunohistochemistry (IHC) as described previously (13, 16, 19, 20). Synovium invasion is generally characterised by hyperplastic synovial lining cells and infiltration

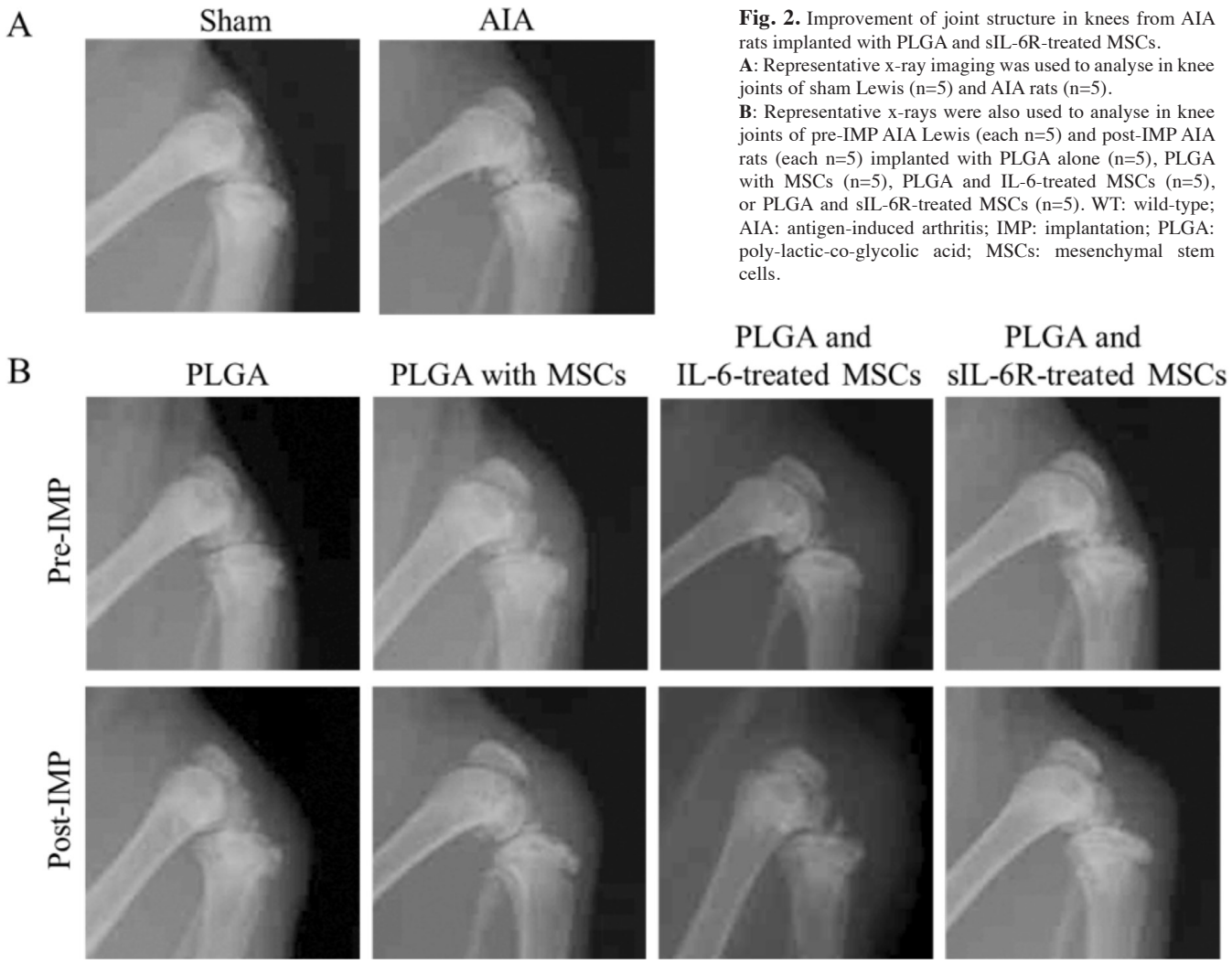

Fig. 2. Improvement of joint structure in knees from AIA rats implanted with PLGA and sIL-6R-treated MSCs.
A: Representative x-ray imaging was used to analyse in knee joints of sham Lewis (n=5) and AIA rats (n=5).
B: Representative x-rays were also used to analyse in knee joints of pre-IMP AIA Lewis (each n=5) and post-IMP AIA rats (each n=5) implanted with PLGA alone (n=5), PLGA with MSCs (n=5), PLGA and IL-6-treated MSCs (n=5), or PLGA and sIL-6R-treated MSCs (n=5). WT: wild-type; AIA: antigen-induced arthritis; IMP: implantation; PLGA: poly-lactic-co-glycolic acid; MSCs: mesenchymal stem cells.

of inflammatory cells in synovium, and was studied on the basis of indicators of hyperplastic synovial lining cells in synovium stained with HE. The invasion characterised by hypertrophic synovial lining cells decreases HE-positive area in joints because synovium shows negative staining for HE. Positive areas were measured by BZ-X analyzer on an All-in-one fluorescence microscope (KEYENCE, Osaka, Japan). For IHC analysis, antigen retrieval was performed by soaking biopsy specimens on slides in 5 mM sodium citrate solution in phosphate-buffered saline containing 0.05% (v/v) Tween 20 (PBST) (pH 6.0). Slides were blocked with serum-free protein block (Dako, 2016-08), and then incubated with mouse monoclonal antibodies that specifically recognise human aggrecan (ACAN, Santa Cruz biotechnology, Saint Luis, sc-33695), collagen II (col II, LifeSpan BioSciences, Seattle, LS-C18868), or HLA A/B/C (human leukocyte antigen, Abcam, Cambridge, UK, ab70328) for 2 h, which were diluted 1:200 in Can Get Signal immunostain Solution A (Toyobo). The slides were washed with PBST, incubated with anti-mouse IgG secondary antibodies conjugated with horse radish peroxidase (HRP)-labeled polymer (DakoCytomation, Glostrup, Denmark) for 1 h, and then visualised by treatment with DAB Chromogen (3,3' diaminobenzidine, DakoCytomation, #K3465). Nuclei were visualised with Mayer's haematoxylin (MERCK, 1:1000 dilution in PBST). For mounting, the sections were rinsed in water, dehydrated in graded ethanol (100% ethanol for 30 s × 3), cleared in xylene (for 30 s × 2), and sealed using Multi mount 480 (Matsunami, FM48001). HE, S-O, and immunohistochemical quantifications were carried out using BZ-X analyser on an All-in-one fluorescence microscope.

MSCs treatment and real-time PCR

Human MSCs at subconfluent conditions were trypsinised, and aliquots of 10^5 cells/well were added to a 24-well plate (Corning). For differentiation into chondrocytes, cells were cultured in a commercial chondrogenic induction medium (hMSC Differentiation BulletKitchondrogenic; Lonza) in the absence or presence of 25 ng/ml of human soluble IL-6 receptor (sIL-6R; R&D Systems) or TNF-α (PeproTech) for 1 day or 5 days. Total RNA extraction followed by real-time PCR was performed as described previously (19). The TaqMan® Gene Expression Assay (Applied BioSystems) primer/probe pairs used in this study were as follows: *type II collagen* (*COL2A1*, Hs00264051_m1) and *glyceraldehyde-*

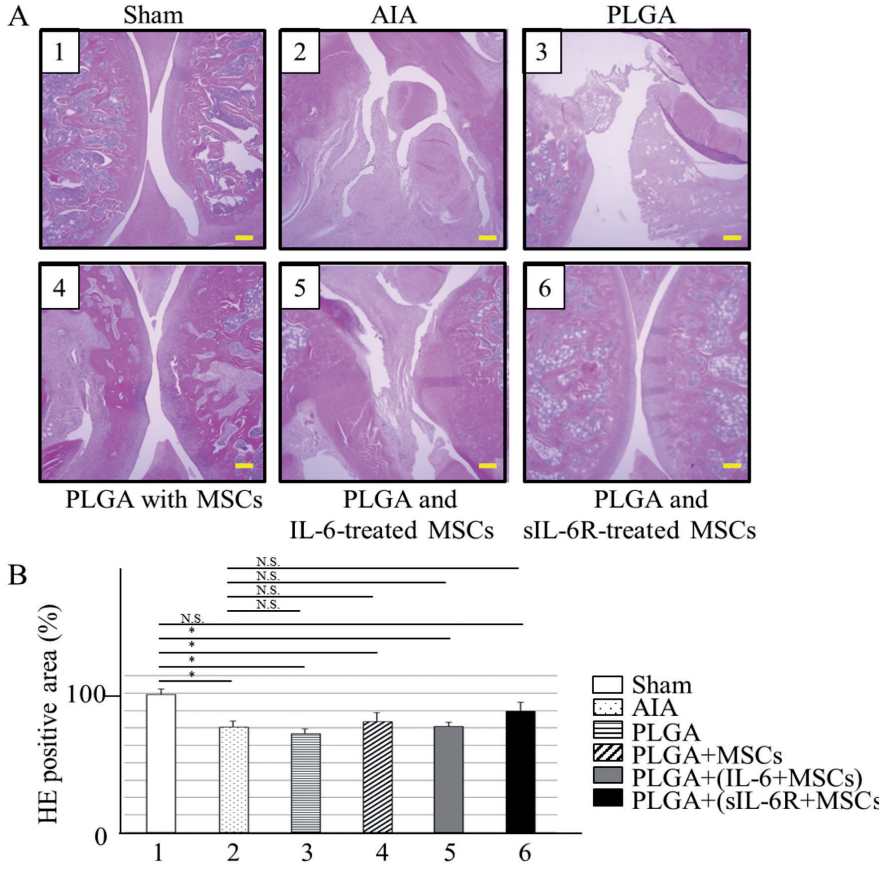

Fig. 3. Suppression of synovitis in knee tissues from AIA rats implanted with PLGA and sIL-6R-treated MSCs.
A: HE staining was performed in biopsy specimens of joints of sham Lewis rats (n=5), AIA rats (n=5), AIA rats implanted with PLGA alone (n=5), PLGA with MSCs (n=5), PLGA and IL-6-treated MSCs (n=5), or PLGA and sIL-6R-treated MSCs (n=5). Representative images are shown. Scale bar, 100 μm. **B**: The histological scores were measured. Quantification is shown. All quantitative data are expressed as mean ± standard deviation (SD) (each n=5). *p<0.01 *vs*. sham rat. HE: haematoxylin-eosin; AIA: antigen-induced arthritis; PLGA: poly-lactic-co-glycolic acid; MSCs: mesenchymal stem cells; NS: no statistically significant difference.

3-phosphate dehydrogenase (*GAPDH*, Hs99999905_m1). *COL2A1* mRNA levels were expressed relative to that of *GAPDH* mRNA.

Statistical analysis

All quantitative data are expressed as mean ± standard deviation (SD). Multiple comparisons were tested for statistical significance by ANOVA. A p-value <0.05 was considered significant. Statistical analyses were performed using SPSS statistical software (v. 21.0; SPSS, Inc., Chicago, IL).

Results

Evaluation of arthritis in AIA rats or sham rats

A rat model of AIA that mimics the pathological features of RA was used as described previously (17). Joint swelling was detected in the limbs of all rats at 10 days after immunisation with mBSA via intra-articular injection (IA). The score based on the extent of its swelling showed a peak value after 1 month due to inflammation caused by antibodies to mBSA antigen, and then returned to baseline at about 2 months (Fig. 1A, see blue plot). In contrast, no swelling was detected in the limbs of sham rats (see red plot). Swelling was detected in the knee and ankle joints of AIA rats (Fig. 1B).

Implantation of materials into AIA rats

PLGA sheet was rinsed with PBS before implantation to ensure free sIL-6R was not transferred (Supplementary Fig. S1A). A series of implantations were performed according to the indicated scheme (Suppl. Fig. S1B). Possible effects of the implantation on joints regeneration, but not inflammatory regulation, were analysed in the following experiments using AIA rats as a model for RA.

Ameliorated joint structure in knee tissues of AIA rats implanted with PLGA and sIL-6R-treated MSCs

X-ray imaging was used to study the phenotypes of the knee joints of the rats (Fig. 2). At day 66 the knee joints of AIA rats, but not the sham rats, showed irregular structures (Fig. 2A), suggesting partial destruction of surficial bone tissue. After implantation of PLGA alone, PLGA with MSCs, or PLGA and IL-6-treated MSCs compared with pre-implantation, joint structure was unaffected (Fig. 2B). Importantly, implantation of PLGA and sIL-6R-treated MSCs into AIA rats improved the phenotype of joint structures compared with pre-IMP AIA rats, and further resulted in joint structures similar to those of sham rats (Fig. 2B), suggesting a role of the implantation in the repair of bone tissue. Micro-CT findings showed that irregular bone structure was detectable in knee joints of AIA rats, but not sham (Suppl. Fig. S2A). Bone destruction was sustained in AIA rats even if PLGA alone, PLGA with MSCs, or PLGA and IL-6-treated MSCs were implanted (Suppl. Fig. S2B). Notably, tibial bone structures in AIA rats implanted with PLGA and sIL-6R-treated MSCs were comparable with those in sham although no significant difference was observed between groups (Suppl. Fig. S2C). These results indicate that this technique may benefit the improvement of bone structure, in this case partial repair of surficial bone tissue.

Synovitis suppression in knee joints of AIA rats implanted with PLGA and sIL-6R-treated MSCs

Biopsy specimens of knee joints from rats were created to study the pathological features in depth. HE staining was performed to examine synovium invasion (Fig. 3A). Invasion was detected in knee joints of AIA rats, but not sham. Furthermore, after AIA rats were implanted with PLGA and sIL-6R-treated

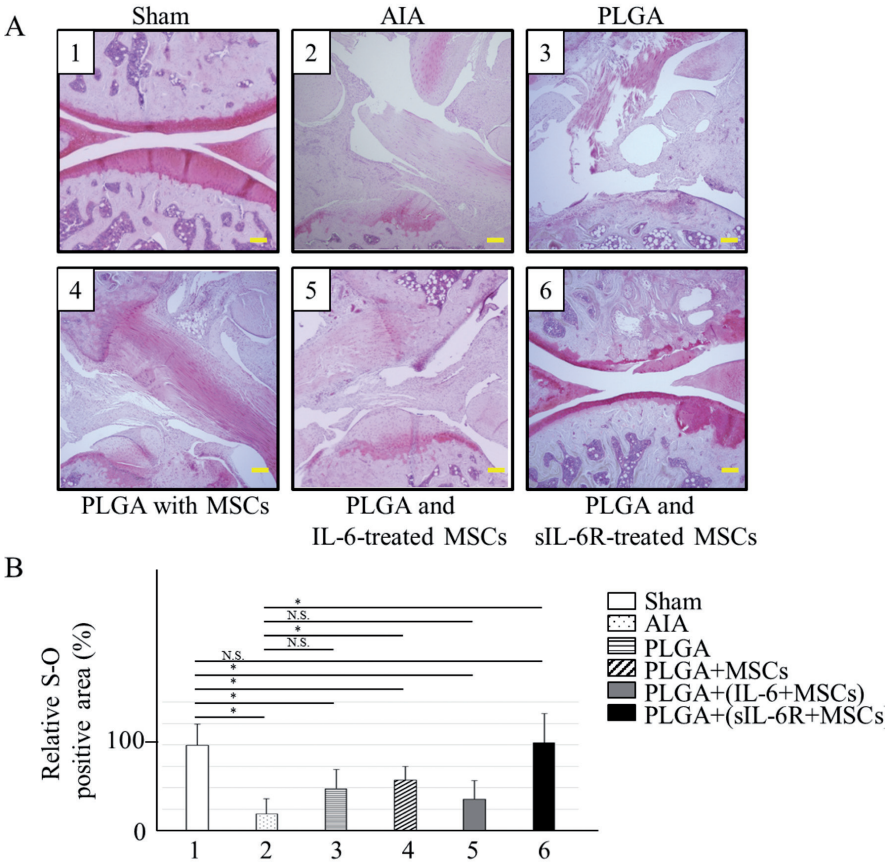

Fig. 4. Repair of articular cartilage tissue of AIA rats implanted with PLGA and sIL-6R-treated MSCs. **A**: S-O staining to examine the deposition of proteoglycan was evaluated in biopsy specimens of joints of sham Lewis rats (n=5), AIA rats (n=5), AIA rats implanted with PLGA alone (n=5), PLGA with MSCs (n=5), PLGA and IL-6-treated MSCs (n=5), or PLGA and sIL-6R-treated MSCs (n=5). Representative images are shown. Scale bar, 100 μm.
B: Quantification is shown. All quantitative data are expressed as mean ± standard deviation (SD) (each n=5). *p<0.01 *vs*. sham rat. S-O: safranin O; AIA: antigen-induced arthritis; PLGA: poly-lactic-co-glycolic acid; MSCs: mesenchymal stem cells; NS: no statistically significant difference.

MSCs, the abnormal phenotype including hyperplastic synovial lining cells were morphologically recovered to levels equal to the control phenotype of sham rats; but those with PLGA alone, or PLGA with MSCs, or with PLGA and IL-6-treated MSCs, were not. Quantification for the respective HE staining of biopsy is shown in Figure 3B.

Increased amounts of proteoglycan in articular cartilage defects of AIA rats implanted with PLGA and sIL-6R-treated MSCs

Little is known about the effect of MSCs implantation on the functionality of articular cartilage. To address this question, S-O staining, which detects proteoglycans of the cartilage ECM, was carried out (Fig. 4A). Knee joints of AIA rats, but not sham, showed irregular and reduced staining for S-O. Little S-O staining was detected in knee joints in AIA rats implanted with PLGA alone, PLGA with MSCs, or with PLGA and IL-6-treated MSCs. After AIA rats were implanted with PLGA and sIL-6R-treated MSCs, abnormal phenotype of articular cartilage in knee joints of AIA rats appeared recovered with similar levels of regular and dense staining for S-O in sham rats. Quantification for the respective S-O staining of biopsy is shown in Figure 4B.

In addition, the staining for type II collagen (col II), another cartilage ECM component was analysed (Suppl. Fig. S3). Knee joints of AIA rats, but not sham, showed irregular and faint staining for col II. Col II staining was detected in knee joints in AIA rats implanted with PLGA alone, PLGA with MSCs, or with PLGA and IL-6-treated MSCs. After AIA rats were implanted with PLGA and sIL-6R-treated MSCs, abnormal phenotype of articular cartilage in knee joints of AIA rats appeared recovered, with equal levels of positive staining for col II compared to sham rats. Quantification for the respective col II staining of biopsy is shown in Suppl. Fig S3B. After MSCs were cultured with chondrogenic induction medium with or without sIL-6R or TNF-α for 5 days *in vitro*, sIL-6R, but not TNF-α, showed up-regulation of *col II* (Suppl. Fig. S4). These results indicated that this strategy contributes to proper repair of articular cartilage.

Differentiation of sIL-6R-treated MSCs with PLGA into chondrocytes in articular cartilage defects

It remains unknown whether either implanted human MSCs or endogenous rat MSCs contribute to improving the articular cartilage phenotype. IHC was performed using specific antibodies that recognise human ACAN, but not rat ACAN, to address this issue (Fig. 5). Articular cartilage from osteoarthritis (OA) patients, but not *WT* or AIA rats, showed positive staining for human ACAN (Fig. 5A). Furthermore, positive staining of human ACAN, but not rat ACAN, was only detected in knee joints from AIA rats implanted with PLGA and sIL-6R-treated human MSCs as shown in 400X image (see arrows in lower panel of Fig. 5B) corresponding to black and open square of the 40x image (upper panel); those with PLGA alone, with PLGA with human MSCs, or with PLGA and IL-6-treated human MSCs exhibited none. This supports the idea that the implantation of PLGA and sIL-6R-treated human MSCs leads to differentiation into human chondrocyte around the affected area.

Low immunogenicity of chondrocytes from implanted MSCs

In this system the immunogenicity, which is generally related to expression of HLA and co-stimulators, of chondrocytes derived from MSCs is unclear. To clarify this point, we assessed HLA expression by IHC. Positive imaging for HLA A/B/C, but not isotype control IgG, was detected in human skin tissue

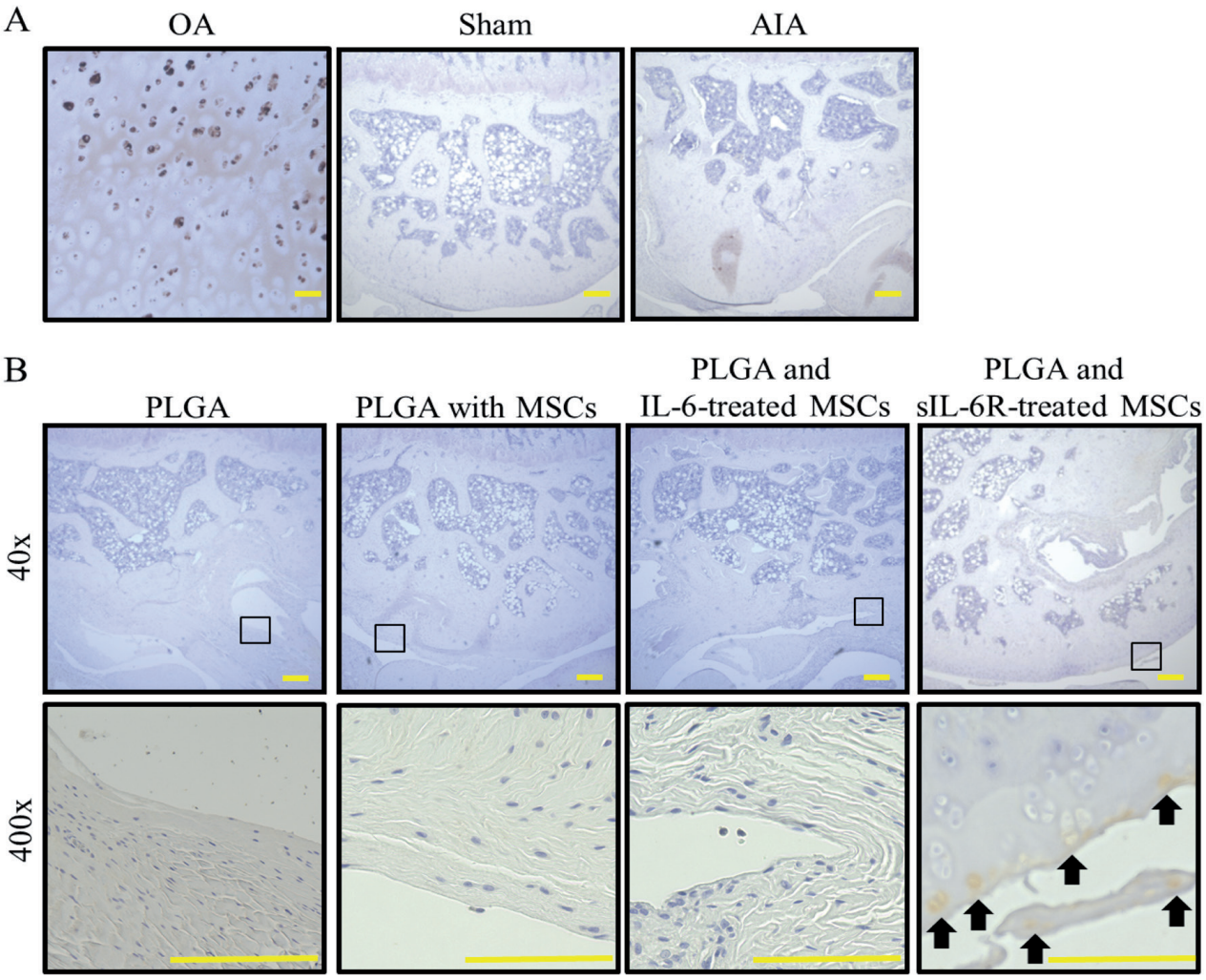

Fig. 5. Human MSCs differentiation into human chondrocytes in articular cartilage of AIA-rats implanted with PLGA and sIL-6R-treated MSCs.
A and **B**: IHC using specific antibodies against human ACAN was performed in biopsy specimens of joints of human OA patients (n=2), sham Lewis rats (n=5), AIA rats (n=5), AIA rats implanted with PLGA with human MSCs (n=5), PLGA and IL-6-treated human MSCs (n=5), or PLGA and sIL-6R-treated human MSCs (n=5).
B: 40x images (upper panels) and 400x images (lower panel) in the joints implanted with the indicated materials are shown. Representative IHC data on expression of ACAN are shown. 400x images correspond to black and open square of the 40x image. Arrow shows positive staining for ACAN. IHC: Immunohistochemistry; OA: osteoarthritis; AIA: antigen-induced arthritis; PLGA: poly-lactic-co-glycolic acid; MSCs: mesenchymal stem cells; ACAN: aggrecan. Bar, 100 μm.

controls (Fig. 6A). Negative staining for HLA was also observed in the articular cartilage of sham rats, AIA rats implanted with, PLGA alone, PLGA with MSCs, PLGA and IL-6-treated MSCs, or PLGA and sIL-6R-treated MSCs (Fig. 6B-C). These results suggest the possibility that chondrocytes derived from implanted MSCs express little to no HLA. To study potential excessive immune reaction following MSCs implantation, IHC was done using anti-CD4 antibodies to examine T-lymphocyte invasiveness. No T-lymphocytes were detected in the articular cartilage of sham rats, or AIA rats implanted with PLGA with MSCs, PLGA and sIL-6R-treated MSCs (data not shown). These results indicate that this strategy shows low immunogenicity in the body.

Discussion

We previously reported that after PLGA and MSCs were implanted into the ankles of a collagen-induced arthritis (CIA) rat model, subsequent inflammation was suppressed (13). However, it remains unknown whether PLGA and sIL-6R-stimulated MSCs could form articular cartilage *in vivo*, even though we found that sIL-6R-treated MSCs differentiate into chondrocyte *in vitro* (16). It was also unclear whether MSCs implantation contributes to articular cartilage repair of RA model animal, such as CIA or AIA. Thus, the aim of this study was to clarify the effect of sIL-6R-treated MSCs implantation on joint regeneration in an animal model for RA. In this study, we found that implantation of PLGA and sIL-6R-treated MSCs into AIA rats resulted in the proper reversal of the phenotype. In particular, the implantation provided direct repair of articular cartilage.

To avoid ectopic calcification, a special vehicle is needed in advance to direct MSCs into chondrocyte differentiation. To this end, many mechanisms underlying differentiation of MSCs into chondrocytes have been studied. *In vit-*

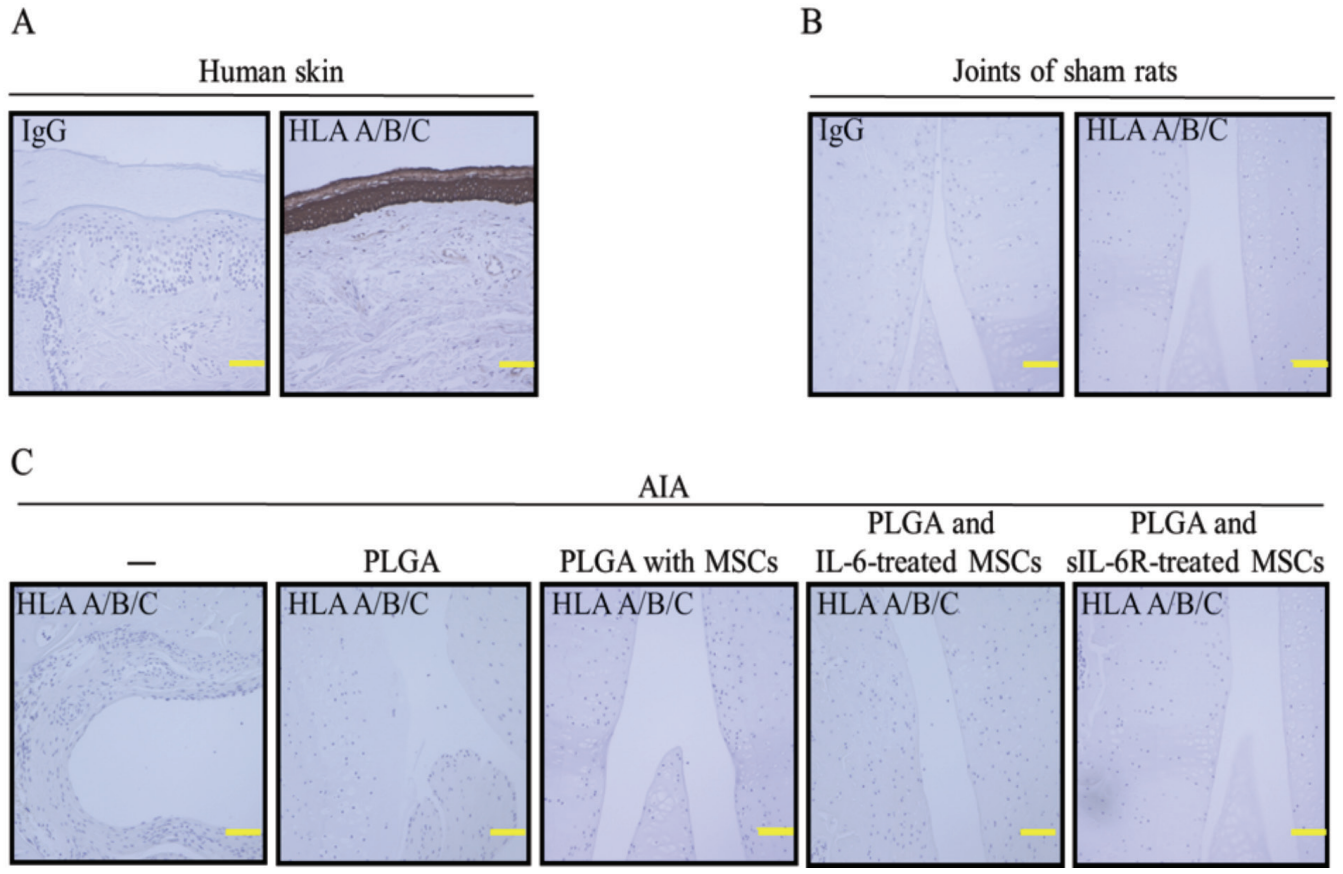

Fig. 6. Low immunogenicity of chondrocytes derived from implanted MSCs.
IHC using specific antibodies against HLA A/B/C was performed in biopsy specimens of **A**: human skin, **B**: joints from sham Lewis rats (n=5), or **C**: AIA rats (n=5) or the AIA rats implanted with PLGA alone (n=5), PLGA with MSCs (n=5), PLGA and IL-6-treated MSCs (n=5), or PLGA and sIL-6R-treated MSCs (n=5). Representative IHC data are shown. IgG: immunoglobulin G; HLA: human leukocyte antigen; AIA: antigen-induced arthritis; PLGA: poly-lactic-co-glycolic acid; MSCs: mesenchymal stem cells. Bar, 100μm.

ro studies showed that TNF-α, IL-1β, or IL-17 inhibit MSCs differentiation into chondrocytes (19, 21-24). TNF-α or IL-1β inhibit the smad pathway, and concomitantly down-regulate the *Sox9* gene, encoding the master regulator of chondrocyte differentiation (23, 24). In contrast, IL-17 suppresses protein kinase A (PKA) activity, and subsequently inactivates Sox9 via low levels of its phosphorylation (19). Taken together, the pro-inflammatory cytokines not only destroy joints, but also suppress the ability of differentiation of MSCs into chondrocytes. Unexpectedly, after MSCs that highly produce IL-6 are stimulated with sIL-6R, subsequent activation of IL-6 signalling elicits chondrocyte-related gene expression, leading to enhancement of chondrocyte differentiation (17). This evidence shows critical aspects of regenerative medicine for articular cartilage. However, it remained unclear whether sIL-6R-stimulated MSCs indeed achieve the repair of articular cartilage.

In this study, efficacy, functionality, and safety of a special tool composed from PLGA and sIL-6R-treated MSCs are assessed in the repair of articular cartilage of RA-model rats. In particular, implantation of MSCs treated with sIL-6R induced repair. Until now, implantation of PLGA alone into the affected joints of a rabbit model of osteochondral defects provided repair of bone and cartilage tissues, leading to proper coverage of the cartilage defect (25). This finding indicates that endogenous MSCs can adhere to PLGA, and then help in the repair of articular damage.

This study could not completely clarify the functionality of chondrocytes that reside in articular cartilage repaired by this strategy. We did identify production of proteoglycan, col II, and ACAN in the ECM of articular cartilage. It will be important to examine the expression status of type X collagen as one of the deep layer markers in cartilage tissues, lubricin as articular lubrication, and hyaluronic acid as a chondrotrophic factor (16, 26, 27).

It is important to evaluate whether this strategy contributes to efficient differentiation of MSCs into chondrocytes. We note the following two points. 1) Activation of IL-6 signalling induces MSCs differentiation into chondrocytes (16) as mentioned above. In fact, this strategy elicits production of proteoglycan, col II, or ACAN in articular cartilage, indicating formation of hyaline cartilage-like tissue. However, this signalling also contributes to differentiation of MSCs into osteoblasts (28). Therefore, we cannot deny a possibility that implanted MSCs form ectopic calcification via differentiation into osteoblasts. To confirm proper repair of articular cartilage, examination is needed as to whether there is ectopic calcifica-

tion in the affected area. As osteoblasts exhibit Ca^{2+} deposition, it is possible to clarify the existence of ectopic calcification by von Kossa staining. 2) Joints of patients with RA are exposed to various types of inflammatory cytokines (1). As mentioned above, TNF-α, IL-1β or IL-17 inhibit MSCs differentiation into chondrocytes whereas sIL-6 enhances its differentiation (19, 21-24). Therefore, even if this strategy is effective to promote MSCs differentiation into chondrocytes, it may be difficult to apply this system to patients with RA that suffer from cytokine storm. To tackle such an intractable problem, inflammation will need to be suppressed in advance using biological DMARDs. Such a strategy might result in the efficient repair of articular cartilage in limited non-inflamed joints.

In general, chondrocytes express low levels of HLA class I A/B/C and class II DR/PR/QR (29), indicating that chondrocyte immunogenicity is low. However, the immunogenicity of chondrocytes differentiated from MSCs treated by sIL-6R remains unclear. As shown in Figure 6, chondrocytes induced by this strategy expressed relatively little HLA A/B/C, emphasising the low immunogenicity of articular cartilage repaired in this case. Thus, this technique provides safe repair of articular cartilage.

We reported previously that MSCs exert anti-inflammatory property by production of TGF-β at an early stage (13). It is also reported that a series of effects might be induced possibly by pro-generative properties of MSCs at a late stage, such as chondrocyte differentiation that we observed in this study. One month after implantation of MSCs seeded on PLGA, but not MSCs alone, we found small fibre of PLGA within the implantation space. These findings suggest that MSCs persist at its implantation space. In fact, IHC analysis showed positive staining for human aggrecan in the implantation area, indicating that chondrocytes from MSCs remained in the area.

Little is known about how much articular cartilage repair is induced by this strategy. AIA rats as a RA model exhibit mild destruction of articular cartilage. In the present study, this strategy contributed to the proper repair of articular cartilage. Articular cartilage repair in mono-iodoacetate-induced arthritis (MIA) rats, as an OA model, shows widespread destruction of articular cartilage (30). Therefore, it is of interest to evaluate the utility of this system in the repair of articular cartilage after implantation of PLGA and sIL-6R-treated MSCs into MIA rats.

Collectively, this promising technique may be available for use in regenerative medicine of articular cartilage in the future. However, further study is necessary to conclude whether or not the implantation strategy using PLGA and sIL-6R-treated MSCs is a potent therapeutic tool for the repair of destroyed articular cartilage frequently detected in patients with RA.

Conclusions

To develop a novel therapeutic tool for regenerative medicine of articular cartilage, we applied MSCs treated with sIL-6R that can differentiate into chondrocytes using a RA model rat. Implantation of sIL-6R-treated MSCs seeded on PLGA around knee joints markedly suppressed synovial inflammation in a RA model rat. The implanted sIL-6R-treated MSCs seeded in PLGA differentiate into chondrocyte and then produce cartilage ECM within articular cartilage. Immunogenicity of chondrocytes derived from the sIL-6R-treated MSCs appears low. The implantation strategy using PLGA and sIL-6R-treated MSCs may be promising for the repair of destroyed articular cartilage detected in patients with RA.

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