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RESEARCH ARTICLE

Enhanced treatment of articular cartilage defect of the knee by intra-articular injection of Bcl-xL-engineered mesenchymal stem cells in rabbit model

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Abstract

Direct intra-articular injection of mesenchymal stem cells (MSCs) has been proposed as a potential cell therapy for cartilage defects. This cell therapy relies on the survival of the implanted MSCs. However, the arduous local environment may limit cell viability after implantation, which would restrict the cells' regenerative capacity. Thus, it is necessary to reinforce the implanted cells against the unfavourable microenvironment in order to improve the efficacy of cell therapy. We examined whether the transduction of an anti-apoptotic protein, Bcl-xL, into MSCs could prevent cell death and improve the implantation efficiency of MSCs in a rabbit model. Our current findings demonstrate that the group treated with Bcl-xL-engineered MSCs could improve cartilage healing both morphologically and histologically when compared with the controls. These results suggest that intra-articular injection of Bcl-xL-engineered MSCs is a potential non-invasive therapeutic method for effectively treating cartilage defects of the knee.

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Keywords cartilage tissue engineering; mesenchymal stem cells; cartilage defects; Bcl-xL; cell therapy; intra-articular

1. Introduction

Articular cartilage is an avascular tissue, which enables smooth movement and shock absorption at joints. Due to the limited capacity of cartilage to repair itself, injuries to the cartilage usually do not heal spontaneously (Hunziker, 2002). Effective treatment of cartilage defects is still a difficult and challenging problem for surgeons.

Studies have proved that mesenchymal stem cells (MSCs) are appropriate for clinical applications because they can be easily isolated and have the potential to form a variety of tissues (Pittenger *et al*., 1999). In addition, MSCs are considered good vehicles of cell-mediated gene therapy because of their accessibility and ease of manipulation *in vitro* (Gafni *et al*., 2004). Recent studies have also shown that if MSCs are co-cultured with chondrocytes, the MSCs could progress to the chondrogenic differentiation without being induced (Murphy *et al*., 2003; Wakitani *et al*., 2002).

Most of the current techniques used to resurface cartilage defects are invasive, and conservative treatment has been shown to be superior to these invasive methods (Ochi *et al*., 2001; O'Driscoll, 1998). The direct intra-articular injection of MSCs has been proposed as a potential cell therapy approach for repairing cartilage defects. When cell-based therapy is employed, there is the additional challenge of retaining the viability of the implanted cells in the defect (Buckwalter *et al*., 1998).

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Several studies have shown that many cells implanted into the defects are lost due to cell death caused by a lack of oxygen and nutrition after implantation (Shintani *et al*., 2001; Zhang *et al*., 2001; Li *et al*., 2004). Limited cell viability after implantation into the defects restricts the implanted cells' survival and regenerative capacity, which then severely affects the efficiency of the cell therapy. Thus, in order to improve the efficacy of cell therapy, it is necessary to reinforce implanted cells against the harmful microenvironment (Melo *et al*., 2004).

Bcl-xL, one of the most important anti-apoptotic members of the Bcl-2 family, interacts with Bax, a pro-apoptotic member, and prevents the release of cytochrome *c* and subsequent apoptosis (Eskes *et al*., 2000). It can also block apoptosis by acting downstream of Bax activation via inhibition of Apaf-1 and caspase-9 association (Rossé *et al*., 1998). The latest studies have suggested that Bcl-xL can also block apoptosis induced by the Fas–FasL pathway (Biswas *et al*., 2001). Given these findings, it is evident that Bcl-xL can play an important anti-apoptotic role by blocking multiple apoptotic pathways stimulated by hypoxia, hyponutrition, inflammatory response and other proapoptotic factors.

Based on these studies, we proposed the use of intra-articular injection of Bcl-xL-engineered MSCs suspended in rat tail collagen gel for the treatment of cartilage defects, thus combining the regenerative potential of Bcl-xL-engineered MSCs with a non-invasive and scaffoldless technique.

2. Materials and methods

2.1. Animal groups

Forty-three New Zealand rabbits (5 months old, mean weight 3.0 kg) were used for the experiments. Forty rabbits were randomly assigned to four groups according to different therapeutic methods. In each group, four rabbits were sacrificed at 6 weeks from the time of the injection, and six rabbits were sacrificed at 12 weeks. Three rabbits were treated with an injection of Bcl-xL–GFP MSCs. All animals received humane care in compliance with the *Guide for the Care and Use of Laboratory Animals* (NIH Publication No. 85-23, revised 1996). The operative procedure and the care of the rabbits were performed under the regulation of the Experimental Animal Centre, the Fourth Military Medical University.

2.2. Construction of the recombinant plasmids

The *Bcl-xL* full-length gene from *Homo sapiens* (NM_138578.1) was amplified by PCR using two primer sets that consisted of: a sense primer, 5′-TTTGAATTCATGTCTCAGAGCAACCGGGAG-3′, and an antisense primer, 5′-TTTCTCGAGTCATTTCCGACTGAAGAG-3′, for pcDNA3; a sense primer, 5′-TTTCTCGAGATGTCTCAGAGCAACCGGGAG-3′, and an antisense primer, 5′-TTTGAATTCTTTCCGACTGAAGAGTGAGCC-3′, for pEGFP-N2. The *Xho*I/*Eco*RI-digested PCR products were ligated into *Xho*I/*Eco*RI-digested pcDNA3 vector and *Eco*RI/*Xho*I-digested pEGFP-N2 vector (BD Biosciences Clontech), using T4 DNA ligase. The recombinant plasmids named pcDNA3–Bcl-xL and pEGFP–N2–Bcl-xL were confirmed by restriction endonuclease digestion and DNA sequence analysis, and the pEGFP–N2–Bcl-xL vector could express a Bcl-xL–GFP fusion protein.

2.3. Isolation and culturing of MSCs

The MSCs were isolated from the iliac crest marrow of rabbits in a separate procedure 3 weeks prior to surgery; 2 ml marrow was aspirated with a transfixion pin into a syringe containing 0.5 ml heparin. The aspirated marrow was centrifuged and rinsed with low-Dulbecco's modified Eagle's medium (L-DMEM; Gibco). The pelleted components were resuspended with adequate amounts of L-DMEM supplemented with 15% fetal bovine serum (FBS; Gibco), L-glutamine (Sigma) and 1% antibiotics (100 U/ml penicillin, 100 mg/ml streptomycin; Sigma) before being distributed into one 25 cm^2 cell culture flask per rabbit. The cells were grown at 37 °C and 5% CO_2 for 3 days before the first medium replacement. After this, medium was replaced every 2–3 days. The adherent cells were grown to 90% confluence to obtain samples, defined here as passage zero (P_0) cells.

2.4. Identification of MSCs

When 80–90% of cells reached confluency, MSCs were seeded at a density of 3×10^5 cells/cm^2 and cultured for up to 3 weeks in osteogenic medium (H-DMEM containing 10% FBS, 50 μM ascorbic acid, 10 mM glycerophosphate and 1 μM dexamethasone; Sigma), and alizarin red stain was used for examining calcification of the extracellular matrix. Chondrogenic differentiation was induced using the high-density culture technique. Briefly, 3×10^5 cells were cultured in chondrogenic medium (H-DMEM containing 1% FBS, 50 μg/ml ascorbic acid, 6.25 μg/ml insulin and 10 ng/ml transforming growth factor-β1; Sigma) for 3 weeks. The pellet was gently overlaid so as not to detach the cells. Chondrogenesis was confirmed by toluidine blue and safranin-O stains. The stainings were observed under a microscope and photographed.

2.5. Transfecting MSCs with the *Bcl-xL* gene by a liposome-mediated method

Before transfection, 1×10^7 MSCs of the fifth generation were inoculated into the 75 cm^2 cell culture flask without antibiotic medium. Recombinant plasmid (pcDNA3–Bcl-xL and pEGFP–N2–Bcl-xL) and mock vector (pcDNA3) were transfected into subcultured MSCs in cell culture flask of 80–90% confluency with LipofectAMINE 2000

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reagent (Invitrogen), according to the manufacturer's instructions.

2.6. Western blot analysis

Forty-eight hours after genetic modification, Bcl-xL protein was separated by SDS–PAGE and transferred to nitrocellulose membranes (Amersham Biosciences). Membranes were incubated with primary antibodies that recognize Bcl-xL (1 : 500; Cell Signaling), GFP (1 : 1000; Santa Cruz Biotechnology) overnight at 4 °C in phosphate-buffered saline with Tween-20 (PBST) with 5% w/v non-fat dry milk. The membranes were next incubated with horseradish peroxidase-conjugated secondary antibody (1 : 2000; ZhongShan) for 2 h at room temperature. Western blots were visualized using an enhanced chemiluminescence kit (Pierce).

2.7. Indirect immunofluorescence staining

Indirect immunofluorescence was performed as follows. The cells were fixed with freshly prepared 3% paraformaldehyde for 10 min and permeabilized with 0.5% Triton X-100 for 10 min. After a 1 h incubation with 3% bovine serum albumin (BSA)/phosphate-buffered saline (PBS), the cells were incubated with anti-Bcl-xL (1 : 200; Cell Signaling) in PBS with 3% BSA for 2 h at room temperature, washed and then incubated with a Cy3-conjugated avidin–biotin complex (1 : 200; Sigma) for 1 h at room temperature. In order to identify the nuclei, the samples were counterstained with DAPI 25 μg/ml for 2 min. Finally, the cells were washed and mounted on slides. Cells were imaged by fluorescence microscope (Olympus BX51; Olympus).

2.8. Multiple differentiation of Bcl-xL-engineered MSCs

The genetically modified MSCs underwent osteogenic and chondrogenic differentiation, using the methods previously described, at 48 h post-transfection.

2.9. Analysing apoptosis and dead rate of MSCs

Forty-eight hours after transfection, the Bcl-xL MSCs, MSCs and mock vector-MSCs were treated with hyponutritional preconditioning of L-DMEM without FBS for 24 h. The evaluation of apoptosis was carried out using flow cytometry. Study data were displayed as two-colour dot-plots with FITC-Annexin V vs. PI (Sigma).

2.10. Preparation of rat tail collagen gel

Collagen was extracted from tendons as previously described (Mio *et al.*, 1996). Tendons were excised from rat tails and sheared. Some connective tissues were removed carefully, washed twice with PBS and then soaked in 4 mM acetic acid. After having been stirred for 2 days at 4 °C, collagen was extracted. The extracts were centrifuged for 30 min at 10 000 × *g* and supernatant was collected into another distilled vessel for use. Protein concentration in the collagen was 1.0–1.5 mg/ml, measured with an ultraviolet spectrophotometer.

2.11. Creation of the chondral defect

All the rabbits were anaesthetized with intramuscular (i.m.) ketamine (40 mg/kg). The knees were shaved and disinfected with Iodophors solution. At the initial operation, a medial parapatellar incision was made on the skin, and a medial arthrotomy was performed with a No. 15 blade. The patella was dislocated laterally and the knee placed in full flexion. A cartilage defect was created in the weight-bearing area of the medial femoral condyle in either knee joint, using a corneal trephine (No. 1 604 646, diameter 4.5 mm; Huaya, Shaanxi, China). The base of the defect was smoothened by a surgical scalpel. Care was taken to ensure that the subchondral bone was not breached. The defect size was 4.5 and 1.0 mm deep and, on average, this was 80% of the medial femoral condyle width. Careful haemostasis, irrigation with sterile saline and a layered closure was performed to ensure a good cure of the wound, and the rabbits were not immobilized. All of the operations were performed by the same two primary authors.

2.12. *In vivo* tracing of MSCs

The pEGFP–Bcl-xL-engineered MSCs suspended in rat tail collagen gel were injected into the knee synovial capsule 3 days after the surgery that created the cartilage defect. After 2 weeks, the frozen sections of the specimens were examined using a fluorescence microscope (Olympus).

2.13. Healing of the chondral defects

Three days after the surgery of creating the chondral defect, a small incision was made on the knee synovial capsule again. When the gel almost coagulated, about 6×10^6 Bcl-xL MSCs suspended in 0.5 ml rat tail collagen gel were injected onto the chondral defect. Then a layered closure was performed. The three control groups had separate injections of 6×10^6 MSCs suspended in 0.5 ml rat tail collagen gel, 6×10^6 mock vector-MSCs suspended in 0.5 ml rat tail collagen gel and 0.5 ml rat tail collagen gel.

2.14. Histological staining

In each group, four rabbits at 6 weeks and six rabbits at 12 weeks from the time of the injection were sacrificed by injection of a lethal dose of thiopentone. The entire

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knee was surgically removed and the distal part of the femur was harvested, fixed in 10% paraformaldehyde for 24 h and then decalcified in 0.5 M EDTA, pH 7.4, for about 3 weeks, dehydrated and embedded in paraffin. Serial sections cut longitudinally at a length of 4 μm were stained with haematoxylin and eosin (H&E), Masson's trichrome, safranin-O or toluidine blue.

2.15. Semi-quantitative histological scoring

We selected the Wakitani score for the histological scoring system (Wakitani *et al*., 1994). Three researchers analysing the slides were blinded to reduce observational bias.

2.16. Terminal deoxynucleotidyl transferase-mediated deoxyuridine triphosphate nick end labelling (TUNEL) assay

Sections were stained with the TUNEL method using a FragEL™ DNA Fragmentation Detection Kit (Calbiochem®) for special staining of DNA fragmentation and apoptotic bodies. Paraffin-embedded tissue sections were deparaffinized and hydrated following standard procedures. Specimens were covered with 100 μl 20 μg/ml proteinase K at room temperature for 20 min for permeabilization. After washing with TBS, specimens were rinsed with 100 μl 1× TdT equilibration buffer and incubated at room temperature for 20 min. Next, the specimens were treated with 60 μl TdT labelling reaction mixture. Finally, the slides were incubated in a humidified chamber at 37 °C for 1 h before detection using a fluorescence microscope (Olympus).

2.17. Statistical analysis

Numerical data are presented as mean ± standard deviation (SD) of the mean. The differences between the means of two groups were compared with the use of the Student–Newman–Keuls test. The Kruskal–Wallis test was used to test for significant differences among the four groups, and pairwise comparison was performed using the Wilcoxon test. $p < 0.05$ was considered statistically significant.

3. Results

3.1. Overexpression of Bcl-xL in genetically modified MSCs

To evaluate the expression of Bcl-xL protein in the genetically modified MSCs *in vitro*, western blot analysis and indirect immunofluorescence (Figure 1B, C) were performed on cell samples at 48 h post-transfection. Expression of Bcl-xL protein and Bcl-xL–GFP fusion protein was confirmed by Western blot analysis of cell culture supernatants (Figure 1A). Expression of the internal housekeeping β-actin gene was at the same level for all samples. Our observation showed that the MSCs were successfully modified to express Bcl-xL.

3.2. Bcl-xL-engineered MSCs maintain their multi-differentiation capacity

The isolated cells were identified to be MSCs by testing their multi-differentiation capacity (data not shown). To determine whether the Bcl-xL modification affected the differentiation capacity, the genetically modified MSCs underwent osteogenic and chondrogenic differentiation. Bcl-xL-modified MSCs underwent the osteogenic differentiation condition for 3 weeks and the osteoblast-like cells showed positive staining for alizarin red. Chondrogenesis was assessed by staining for aggrecan and after 3 weeks of culture under chondrogenic conditions (see Supporting information, Figure S1). These results indicate that Bcl-xL MSCs retain their multi-differentiation potential capacity into osteogenic and chondrogenic lineages.

3.3. Bcl-xL-modified MSCs protect against apoptosis *in vitro*

To test the capability of Bcl-xL MSCs to protect against apoptosis *in vitro*, the Bcl-xL MSCs, MSCs and mock vector-MSCs were treated with L-DMEM without FBS for 24 h. Then an evaluation of apoptosis was carried out using flow cytometry. Quantitative assay results showed that the number of Annexin V-FITC-positive and PI-negative cells was decreased from 12.2% of MSCs and 14.7% of mock vector-MSCs to 6.2% of Bcl-xL MSCs (Figure 2). The results of flow cytometry showed that the apoptotic cell number was significantly reduced by the *Bcl-xL* genetic modification.

3.4. Improving healing effect in Bcl-xL-modified MSCs treated group by macroscopic observations

To determine whether the transplanted MSCs were present in the knee, histological assessment of specimens treated with pEGFP–N2–Bcl-xL-MSCs was performed using fluorescence microscopy. The results confirmed that the injected cells were found in the neocartilage (Figure 3).

At 6 weeks after injection, partial filling of the defects was seen in typical Bcl-xL–MSC-treated, MSC-treated and mock vector-MSC-treated specimens, and there was not much difference in the appearance of specimens from these three groups. There was almost no healing seen in the rat tail collagen gel-treated group.

At 12 weeks post-injection, almost complete healing was seen in typical Bcl-xL–MSC-treated specimens.

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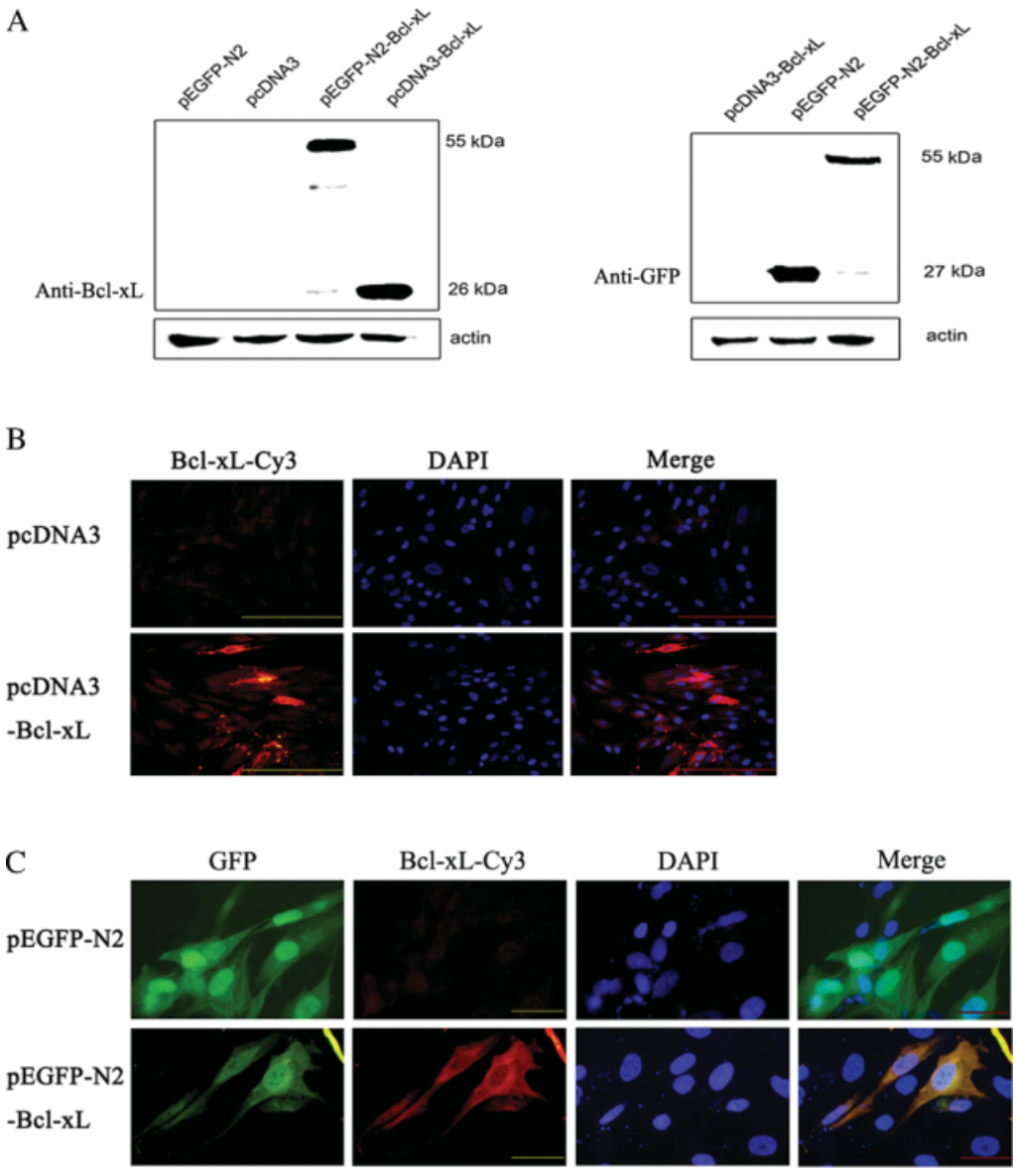

Figure 1. (A) Western blot analysis of concentrated cell culture medium from MSCs transfected with *Bcl-xL* gene after 48 h of culture. Blots were probed with anti-Bcl-xL and anti-EGFP antibody, as indicated. (B) Indirect immunofluorescence analysis to demonstrate the expression of protein Bcl-xL in MSCs transiently transfected with pcDNA3-Bcl-xL for 48 h. Scale bar = 200 μm. (C) Indirect immunofluorescence analysis to demonstrate the fusion protein GFP–Bcl-xL in MSCs transiently transfected with pEGFP–N2–Bcl-xL for 48 h. Scale bar = 50 μm

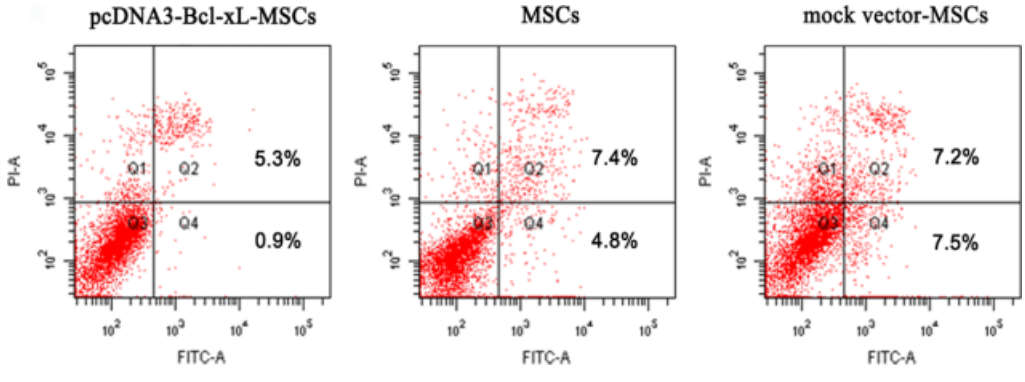

Figure 2. Flow cytometry analysis of the effect of Bcl-xL transfection on the MSCs apoptotic rate in experimental group and two control groups. The numbers of apoptotic cells are the summation of apoptotic cells in the Q2 and Q4 quadrants. The numbers of apoptotic cells are 12.2% MSCs, 14.7% mock vector-MSCs and 6.2% Bcl-xL MSCs

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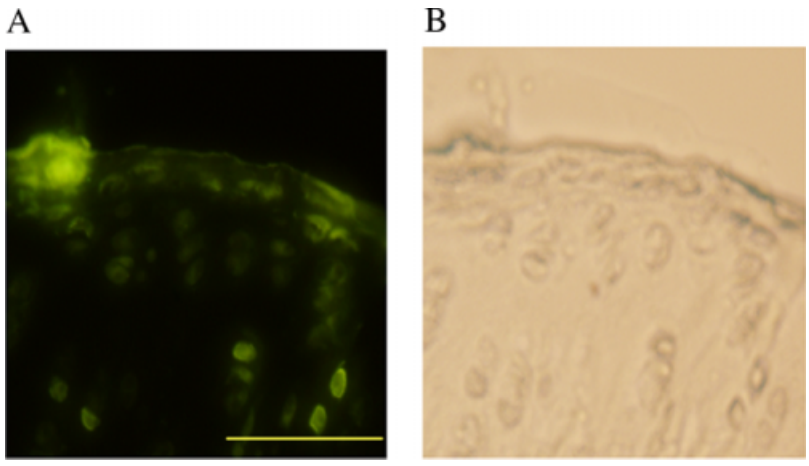

Figure 3. pEGFP–Bcl-xL-engineered MSCs allowed *in vivo* tracing and the injected MSCs were confirmed to be found in the neocartilage, using a fluorescence microscope. (A) The image that was captured in the scotopic field. (B) The image that was captured in the bright field. Scale bar = 200 μm

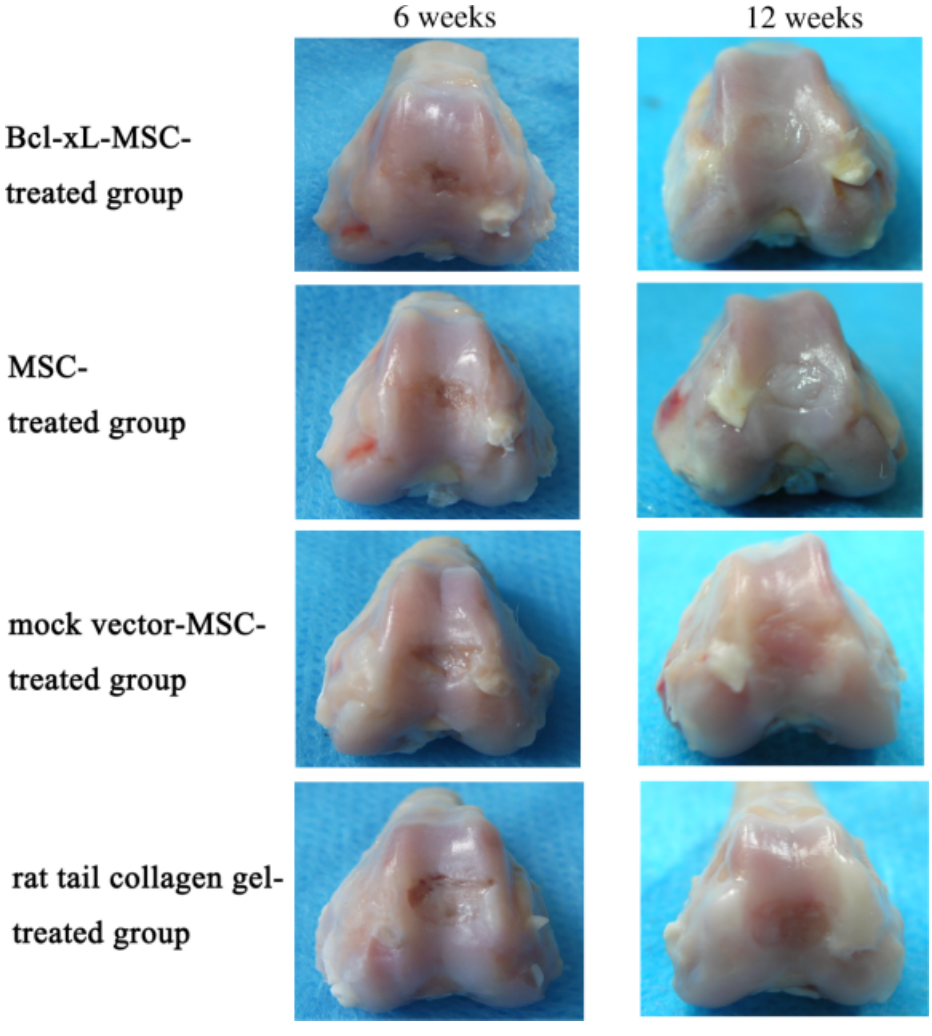

Figure 4. Gross observation of the repaired chondral defects in the Bcl-xL MSC-treated group, MSC-treated group, mock vector–MSC-treated group and rat tail collagen gel-treated group at 6 and 12 weeks post-injection

Healing tissues in most specimens contained smooth surfaces and were of good thickness. In mock vector-MSC-treated and MSC-treated specimens, partial filling of the defects was seen, which demonstrated discernible edges, irregular surfaces and insufficient thickness. There were small parts of reparative tissue filling in the rat tail collagen gel-treated group, and the margins of the defects were clearly distinguishable (Figure 4).

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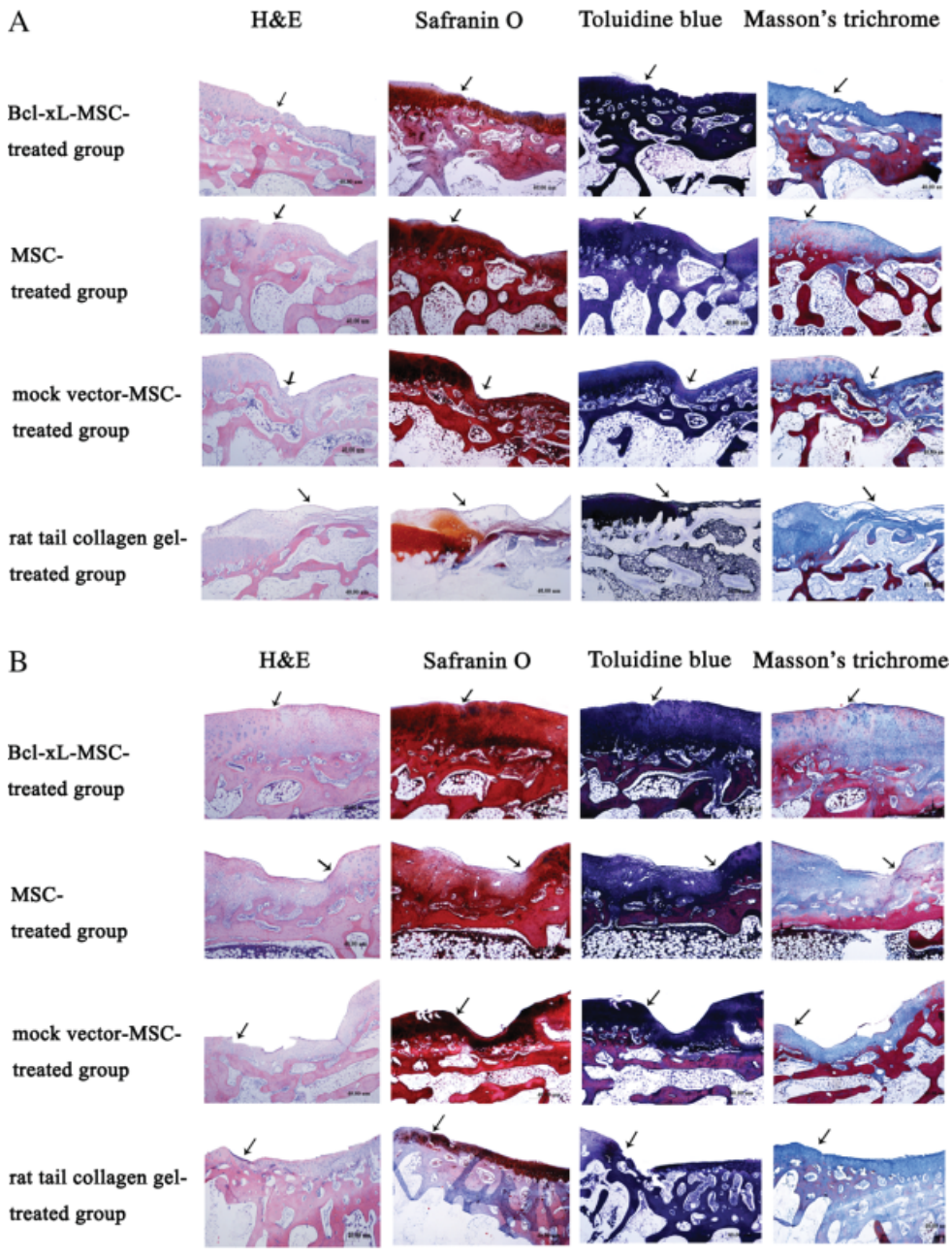

Figure 5. Histological stains of repaired tissue. (A) Microimages of repaired chondral defects at 6 weeks after injection. (B) Microimages of repaired chondral defects at 12 weeks after injection. The positions of the defects are indicated by arrows. Scale bar = 40 μm

3.5. Enhanced treatment effect in Bcl-xL-modified MSCs-treated group by histological observations and grading

At 6 weeks post-injection, the appearance of the repair tissue in the Bcl-xL–MSC-treated group was fibrocartilage-like cartilage. This repair tissue was thin and there was relatively poor integration with the normal cartilage. The defects in the MSC-treated group and mock vector-MSC-treated group were partially filled with patchy areas of metachromatic staining. The rat tail collagen gel-treated group demonstrated poor healing and had no reparative tissue (Figure 5A).

At 12 weeks post-injection there was marked improvement in the quality of the repair tissue found in the Bcl-xL–MSC-treated group compared with the three controls. The tissue was hyaline-like, with apparent integration, thickness and surface regularity. However, metachromasia demonstrated in most specimens that most of the reparative cells had an appearance distinguishable from that of hyaline cartilage. At higher magnification, the cells resembled highly differentiated chondrocytes at the bottom layer and differentiated fibrocartilage in most parts of the neocartilage. The defects in the MSC-treated group and mock vector-MSC-treated group were partially filled with fibrocartilage, with patchy areas of metachromatic staining. This repair tissue was thin and there was

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relatively poor integration with the normal cartilage. The group treated with rat tail collagen demonstrated poor healing and the reparative tissue, if any, was very thin and had an irregular surface (Figure 5B).

At 6 weeks post-injection, the semi-quantitative Wakitani scores were 8.5, 7.5, 7.0 and 8.0 for the Bcl-xL–MSC-treated group; 8.5, 9.0, 10.0 and 9.5 for the MSC-treated group; 10.0, 10.0, 9.5 and 10.5 for the mock vector-MSC-treated group; and 13.0, 13.0, 14.0 and 13.0 for the rat tail collagen gel-treated group. Statistical analysis showed that, overall, there was a significant difference among the four groups ($p < 0.05$). Pairwise comparison using the Mann–Whitney test showed no significant difference between the Bcl-xL–MSC-treated and MSC-treated groups, the MSC-treated and the mock vector-MSC-treated groups, or the mock vector-MSC-treated and the rat tail collagen gel-treated groups.

At 12 weeks after injection, the semi-quantitative Wakitani scores were 3.0, 4.5, 3.5, 2.0, 3.5 and 5.5 for the Bcl-xL–MSC-treated group; 6.5, 7.0, 8.5, 6.5, 9.0 and 7.0 for the MSC-treated group; 7.5, 8.0, 10.5, 6.5, 7.5 and 8.0 for the mock vector-MSC-treated group; and 12.0, 13.0, 13.0, 13.0, 12.0 and 13.0 for the rat tail collagen gel-treated group (Table 1). Statistical analysis showed that there was a significant difference among the Bcl-xL–MSC-treated, MSC-treated and rat tail collagen-treated groups, and among the Bcl-xL–MSC-treated, mock vector-MSC-treated and rat tail collagen-treated groups ($p < 0.05$). Pairwise comparison using the Wilcoxon test showed no significant difference between the MSC-treated group and the mock vector-MSC-treated group, and it did show significant differences between the Bcl-xL–MSC-treated group and the three control groups, respectively. And there were also differences between the MSC-treated/mock vector-MSC-treated group and the rat tail collagen gel-treated group ($p < 0.05$).

Table 1. Summary of Wakitani scores at 6 and 12 weeks after injection

Groups	*n*	Scores
6 weeks		
Bcl-xL-MSC-treated group	4	8.5
		7.5
		7.0
		8.0
MSC-treated group	4	8.5
		9.0
		10.0
		9.5
Mock vector-MSC-treated group	4	10.0
		10.0
		9.5
		10.5
Rat tail collagen gel-treated group	4	13.0
		13.0
		14.0
		13.0
12 weeks		
Bcl-xL-MSC-treated group	6	3.0
		4.5
		3.5
		2.0
		3.5
		5.5
MSC-treated group	6	6.5
		7.0
		8.5
		6.5
		9.0
		7.0
Mock vector-MSC-treated group	6	7.5
		8.0
		10.5
		6.5
		7.5
		8.0
Rat tail collagen gel-treated group	6	12.0
		13.0
		13.0
		13.0
		12.0
		13.0

3.6. Enhanced survival of the genetically modified cells *in vivo* by TUNEL assay

TUNEL-positive apoptotic cells were observed clearly within the islets as light green-stained, small, round cells, which could always be found in the cell aggregates. At 6 weeks after injection, only a few TUNEL-labelled cells were found in sections of the Bcl-xL–MSC treated group, which was similar to the situation of normal cartilage. However, more positive signals were observed in the sections of the mock vector-MSC treated group and the MSC-treated group (Figure 6). This suggested that the overexpression of Bcl-xL could markedly enhance the survival of the genetically modified cells *in vivo*.

4. Discussion

Mesenchymal stem cell-based therapies for the regeneration of cartilage have gained popularity over the last few years. However, the cell therapy for treatment of cartilage defects requires sufficient cells for regeneration. Using an anti-apoptosis strategy by transducing the anti-apoptotic protein Bcl-xL may be an alternative to ensure sufficient cell numbers for effective therapy. In this study, we wanted to test our hypothesis that intra-articular injection of Bcl-xL-engineered MSCs that were suspended in rat tail collagen gel could adhere to the site of injury and regenerate cartilage *in vivo*. Our results at 12 weeks post-injection in the Bcl-xL–MSC-treated group showed definitive evidence of improved healing of cartilage, histologically and morphologically, when compared with the three control groups (statistically significant at $p < 0.05$). The TUNEL method has been generally used to detect cell death *in situ*. In our study, TUNEL staining demonstrated that the number of apoptotic cells in the repair tissue in the Bcl-xL-engineered MSC group were lower than those in the MSC group and the mock vector-MSC group at 6 weeks post-injection. *In vivo* tracing techniques using GFP-labelled cells and fluorescence microscopy confirmed that the injected MSCs were present in the neocartilage.

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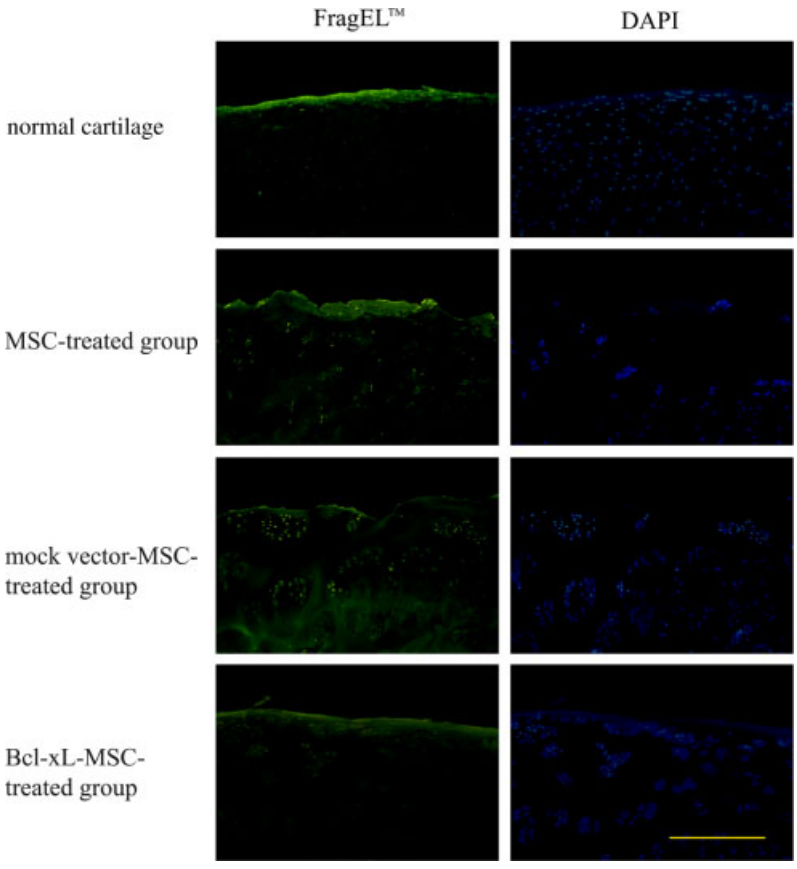

Figure 6. Comparison of TUNEL results in the Bcl-xL MSC-treated group, MSC-treated group, mock vector–MSC-treated group and normal cartilage at 6 weeks post-injection. Scale bar = 200 μm

There are two different ways to apply MSCs to therapy. One is to use a suitable matrix or scaffold seeded with stem cells *in vitro* (Jackson *et al*., 1999). The alternative is to differentiate stem cells *in vivo* by co-culturing with the targeting cell type. Many studies have demonstrated that MSCs can survive and expand without a scaffold. In addition, injected stem cells have been recovered in viable form in a goat knee with simulated arthritis (Barry *et al*., 2003; Lee *et al*., 2007). Other scientists reported that MSCs could survive and differentiate into cartilage on osteochondral defects *in vivo* without being induced (Tatebe *et al*., 2005). Murphy *et al*. (2003) even demonstrated stimulated regeneration of articular cartilage via intra-articular injection of MSCs into an osteoarthritic knee in the goat model. Our results at 6 weeks after injection indicate that MSCs might need more time to achieve chondrogenic differentiation *in vivo* than *in vitro*.

In our study, we chose rat tail collagen gel as the carrier for MSCs, which is permitted for use as a carrier for intra-articular injection by the state food and drug administration (SFDA) of the People's Republic of China. Initially, we postulated that rat tail collagen alone might facilitate the migration and adherence of MSCs to the defect. However, specimens at 12 weeks after injection in the rat tail collagen gel-treated group showed that a single application of rat tail collagen had inferior ability to generate repair tissue.

In summary, we have confirmed that genetic modification of MSCs with the anti-apoptotic *Bcl-xL* gene resulted in a high survival rate *in vitro* and enhanced the survival of engrafted MSCs at the site of cartilage defect in the taxing *in vivo* environment. Genetically engineered Bcl-xL expression in cells using a non-viral vector could be an effective strategy for increasing cell survival after cell implantation while minimizing the potential risks. Intra-articular injection of anti-apoptotic Bcl-xL-engineered MSCs may provide a novel and effective approach in the treatment of cartilage defects. However, we acknowledge that the number and body type of animals in this study were small and that the effect seen in this animal study may be different from that seen in humans.

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Supporting information on the internet

The following supporting information may be found in the online version of this article:

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