

Bone marrow cells immunomagnetically selected for CD271+ antigen promote *in vitro* the repair of articular cartilage defects

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Abstract

Objective: The purposes of this project were to quantify the cells expressing the mesenchymal stem cell (MSC) marker CD271 in synovial membranes from human osteoarthritic (OA) and healthy joints, and to determine if those CD271 cells were involved in spontaneous human cartilage repair and were beneficial for the repair of human articular cartilage defects.

Methods: The coexpression of CD44/CD271, CD90/CD271, and CD105/CD271 antigens was determined by immunofluorescence in OA and healthy synovial membranes and during spontaneous cartilage repair. Isolated MSCs from the bone marrow of four OA patients (mean age: 64 years) were magnetically separated into MSC CD271+ and MSC CD271- subsets. The separated cell subsets were then implanted into 2 mm focal defects of articular cartilage. These implants were cultured in chondrogenic differentiation medium supplemented with recombinant human transforming growth factor-beta3 for 8 weeks. The repair tissues were analyzed by histochemistry (hematoxylin-eosin and safranin O) and immunohistochemistry for collagen types I and II.

Results: Cells expressing the CD271 antigen were diffusely distributed in OA synovial membranes and localized in the subintimal zone in healthy synovial membranes. The number of cells expressing MSC markers was higher in OA synovial membranes than in synovia from healthy joints, corresponding to the highest level of coexpression of CD90/CD271 antigens (9.8% vs. 2.6%). Spontaneous repair tissue showed more cells expressing the CD271 antigen (9.9% ± 4.0%). The highest levels of expression were found to be associated with CD44; 64% of positive CD271 cells coexpressed the CD44 antigen. In both implant cell types, the repair tissue morphology resembled articular cartilage, having an extracellular matrix with a hyaline aspect and numerous lacunae containing cells, and was immunopositive for collagen types I and II. Statistical analyses of the repair tissue demonstrated that the implantation of MSC CD271+ provided such benefits as a greater filling of the chondral defect and better integration between the repair tissue and native cartilage. Safranin O staining of repair tissue was negative in implants of MSC CD271- but more positive in implants with MSC CD271+. The overall histologic score for CD271- implants was 9.5 ± 0.89 and 12.19 ± 1.01 for CD271+ implants.

Conclusions: Synovial membranes from OA patients contain more cells expressing CD271 antigen than those from healthy joints, and spontaneous cartilage repair tissue contains cells positive for CD271 antigen. These data suggest the involvement of CD271 antigen in spontaneous cartilage repair and indicate that the cell subset MSC CD271+ provides higher quality chondral repair than the CD271- subset.

Introduction

Mesenchymal stem cells (MSCs) are multipotent cells that have the capacity to differentiate into various mesodermal tissues, including bone, cartilage, and adipose tissue.¹ Because there is no specific marker for MSCs, these cells are characterized according to their plastic adherence, fibroblastic morphology,² and the presence of surface markers (e.g., CD44, CD54, CD71, CD90, and CD166) or their absence (e.g., CD14, CD34, and CD45).³

MSCs have been recently identified in human articular cartilage from patients with osteoarthritis (OA).⁴ These progenitor cells have been implicated in the process of cartilage repair, although their role in the pathogenesis of OA is incompletely understood.^{5,6} Further, MSCs in synovial fluid were more numerous in patients with OA than in healthy individuals,⁷ but a detailed quantification of the MSCs in synovial membranes has not been performed.

In vitro^{1,8,9} and *in vivo*¹⁰ studies of clonally derived MSCs demonstrated that the MSC population consists of subsets that have different expression of markers and different capacities for cellular differentiation. These results suggest that a specific cell population may be advantageous for tissue repair. An immunoisolation method has been used to obtain MSC populations positive for specific markers, such as STRO-1,^{10,11} CD105,^{12,13} and CD271, also known as low affinity nerve growth factor receptor.^{14,15} Recent studies have indicated that the population of MSCs resulting from selection for cells expressing CD271 showed a greater clonogenic potential and a higher capacity for adipogenic and osteogenic differentiation.^{14,15}

In the present study, we analyzed the differences in the incidence of CD271 cells from synovial membranes from OA patients and healthy joints. We also determined whether CD271+ cells are involved in spontaneous cartilage repair. We observed that CD271+ cells are more abundant in OA patients and are present in the spontaneous repair tissue of OA cartilage. To evaluate whether CD271+ cells are implicated in articular cartilage repair and whether they are able to reach damaged cartilage through the vascular system, we determined the capacity of bone marrow (BM)-derived CD271+ cells to repair articular cartilage defects.

Materials and Methods

Harvest of synovial tissues and articular cartilage

Synovia from 20 OA patients (mean age: 65 years) and 6 patients with healthy joints (mean age: 47 years) were obtained aseptically during hip joint prosthetic replacement surgery. The synovial membranes were kept in sterile Eagle's minimum essential medium (Cambrex) supplemented with 10,000 IU/mL penicillin and 10,000 µg/mL streptomycin. Synovial membrane samples from each patient were also immersed in isopentane/liquid N₂ and held at -80°C until processed for immunostaining.

Normal articular cartilage from donors with no history of joint disease was provided by the Tissue Bank and the Autopsy Service at A Coruña Hospital. All tissues were obtained from femoral heads, sliced full thickness excluding mineralized cartilage and subchondral bone, and placed in Dulbecco's modified Eagle's medium (DMEM) (Lonza) with 20% fetal bovine serum (FBS) (Gibco) and 10,000 IU/mL penicillin and 10,000 µg/mL streptomycin.

This study was approved by the local ethics committee and informed consent was obtained from all donors.

Immunofluorescence of synovial membranes and articular cartilage

Frozen sections (four healthy and four OA synovial membrane tissue samples) of 4 µm were cut, mounted on Superfrost Plus microscope slides, and fixed in acetone at 4°C for 10 min. Monoclonal antibodies against CD44, CD90, CD105, and CD271 were used for immunofluorescent assays (Supplementary Table S1; Supplementary Data are available online at www.liebertonline.com/tea).

For double immunostaining for CD44/CD271 and CD90/CD271, sections were first exposed to mouse anti-human CD44 antibody (1:100) or mouse anti-human CD90 antibody (1:400), then incubated with rabbit anti-mouse fluorescein isothiocyanate (FITC)-labeled antibodies (1:10). For CD105/CD271 double immunostaining, the sections were first exposed to monoclonal anti-human CD105-FITC antibody (1:10). The sections were then exposed to mouse anti-human CD271 antibody (1:1,000) and incubated with goat anti-mouse R-phycoerythrin (PE)-labeled antibodies (1:20). Nuclei were counterstained with DAPI (100 ng/mL, 20 min). Immunofluorescence was green (FITC) for CD44, CD90, and CD105, and red (PE) for CD271.

Immunofluorescence incubations were for 30 min at room temperature in the dark. Between incubations, the sections were washed in phosphate-buffered saline. Negative staining controls were obtained by omitting the primary antibody and incubating with the secondary antibodies conjugated with FITC or R-PE. The sections were mounted in Glycergel (Dako Corporation).

Isolation and culture of BM-derived mesenchymal cells

BM samples (N=4) were obtained from hip OA patients (mean age: 64.3 years; age range: 35–82 years). Informed consent was obtained from each donor according to the guidelines of the local ethics committee.

BM-derived mesenchymal cells were extracted by washing the BM with 50 mL of DMEM, 10% FBS with a 1.1 mm x 25 mm gauge needle (BD Microbalance™ 3) and a 20 mL syringe (Kendall Monoject Syringe). The resulting cell suspension was filtered through a sterile 40 µm filter and centrifuged at 300 g for 8 min. Then, 1×10^5 cells were plated in a culture flask. The nucleated cells were cultured in DMEM supplemented with 20% FBS and penicillin/streptomycin (10,000 IU/mL) (both from Gibco BRL, Life Technologies, Barcelona, Spain) in a humidified 5% CO₂ atmosphere at 37°C. After 48 h, nonadherent cells were removed.

When the BM-derived cells became confluent, they were sub-cultured using trypsin–ethylenediamine tetraacetic acid treatment (Sigma-Aldrich). To reduce contamination by fibroblasts, the resultant cell suspension was subjected to a preplating technique.¹⁶ The basis for this technique is that MSCs require more time to attach to the culture dish than fibroblasts. Therefore, cells that had not adhered within 20 min were resuspended in fresh medium and reseeded. All experiments in this study were performed with cells from the second passage. Cells were counted and assessed for viability using Trypan blue 0.4% (Sigma) dye exclusion.

Immunomagnetic isolation of BM-derived mesenchymal cells CD271– and CD271+

The cell suspension (N=4) was washed with magnetic cell sorting (MACS) buffer containing phosphate-buffered saline at pH 7.2, 0.5% bovine serum albumin (BSA), and 2 mM ethylenediamine tetraacetic acid and stored at 4°C. The cells were then resuspended in MACS buffer at a concentration of 107 cells per 80 mL and incubated first with CD271-APC for 10 min at 4°C and then with Anti-(low affinity nerve growth factor receptor)-APC Microbeads for 15 min at 4°C. The resultant cell suspension was then loaded onto a MiniMACS Separator column (Miltenyi Biotec) and placed in the magnetic field of a MACS Separator, and the column was washed with MACS buffer. The magnetically labeled CD271+ cells were retained on the column, whereas the unlabeled cells run through; this cell fraction was depleted of CD271+ cells. After removal of the column from the magnetic field, the magnetically retained CD271+ cells were eluted as the positively selected cell fraction. The resulting CD271+ and CD271– cells were centrifuged at 300 g for 8 min. Then, 1×10^5 cells were plated in a culture flask. These nucleated cells were cultured in DMEM with 20% FBS and penicillin/streptomycin in a humidified 5% CO₂ atmosphere at 37°C.

BM-derived mesenchymal cells characterization by flow cytometry

The phenotypic characterization of the BM-derived cells (N=4) was achieved using the formaldehyde-saponin method. Cell suspensions from freshly isolated synovial membranes and from the second passage were incubated for 1 h at 4°C with mouse anti-human monoclonal antibodies conjugated with fluorochromes (Supplementary Table S1). A goat anti-mouse IgG FITC-conjugate (Southern Biotechnology Associates, Inc.) was used as a secondary antibody when necessary. A minimum of 10,000 cell-events per assay were analyzed on a FACsCalibur cytometer (Becton Dickinson) and the resulting data were processed using CellQuest software (Becton Dickinson).

Statistical analysis was performed using the Mann–Whitney U-test. The results are expressed as percentage of marker cells (mean ± standard deviation). Differences are considered statistically significant if $p < 0.05$.

Chondral defects and implants of BM-derived mesenchymal cells CD271– and CD271+

In full-thickness cartilage slices, on the surface, were drilled chondral defects (2 mm diameter and 2–3 mm deeper). Then were made cylinder (6 mm diameter) (Biopsy Punch Stiefel) with the chondral defects inside. Chondrogenic medium (Lonza) alone was used for the control group, whereas 2×10^5 CD271+ or CD271– cell subsets were seeded over the chondral defect produced. Samples were then transferred to 96-well plates with 150 µL of chondrogenic medium and incubated for 4 and 8 weeks at 37°C in a humidified 5% CO₂ atmosphere, after which the samples were fixed in 4% paraformaldehyde

and embedded in paraffin. The repair tissues were then analyzed by histochemical staining with hematoxylin-eosin and safranin O or by immunohistochemical methods for collagen types I and II.

Immunoperoxidase staining of paraffin tissue sections

The implants were fixed in 4% paraformaldehyde and paraffin-embedded, and then 4- μ m-thick sections were placed on Superfrost Plus slides (Menzel-Glaser) and incubated with monoclonal antibodies (Supplementary Table S1). Some sections were enzymatically pretreated to facilitate exposure of various epitopes (Supplementary Table S1). The peroxidase/DAB ChemMate™ DAKO EnVision™ detection kit (Dako Cytomation) was employed to detect the antigen-antibody reactions. Negative staining controls were created by omission of the primary or secondary antibodies.

Morphometric analysis

For each synovium sample, five consecutive sections were examined using an Olympus BX61 microscope connected to a digital camera (Olympus DP70). Images of four fields were acquired for each section. At least 2×10^3 cells were counted for each staining experiment. Cells with DAPI nuclear staining and cells with single (CD271) or double (CD44/ CD271; CD90/CD271 or CD105/CD271) immunostaining were independently quantified and the respective percentages calculated.

The results represent different simultaneous analyses of two antigens. In each sample, the percentage of cells positive for each marker was calculated by dividing the number of cells expressing the marker by the number of cells stained with DAPI; the percentage of cells simultaneously expressing two markers was calculated by dividing the number of cells positive for two markers by the number of cells stained with DAPI. The results are expressed as mean % \pm standard deviation.

The International Cartilage Repair Society scoring system¹⁷ was used for the macroscopic evaluation of cartilage repair (Table 1). The results are expressed as mean \pm standard deviation. Three different experiments with six samples at each time point were carried out, so there were 18 samples at each time point. The groups were compared using the two-tailed Mann-Whitney U-test or the Student's t-test. Differences were considered significant when $p < 0.05$.

Table 1. International Cartilage Repair Society Macroscopic Evaluation of Cartilage Repair After 8 Weeks of Culture

International Cartilage Repair Society parameters	CD271+	CD271-
Degree of defect repair (in level with surrounding cartilage, 4; 75% repair of defect depth, 3; 50% repair of defect depth, 2; 25% repair of defect depth, 1; 0% repair of defect depth, 0)	2.33 \pm 0.84 (n=6)	1.00 \pm 0 (n=9)
Integration to border zone (complete integration with surrounding cartilage, 4; demarcating border <1 mm, 3; 3/4 th of graft integrated, 1/4 th with a notable border >1mm width, 2; 1/2 of graft integrated with surrounding cartilage, 1/2 with a notable border >1mm, 1; from no contact to 1/4 th of graft integrated with surrounding cartilage, 0)	3.50 \pm 0.55 (n=6)	2.33 \pm 0.50 (n=9)
Macroscopic appearance (intact smooth surface, 4; fibrillated surface, 3; small, scattered fissures or cracks, 2; several, small or few but large fissures, 1; total degeneration of grafted area, 0)	3.30 \pm 0.46 (n=30)	2.29 \pm 0.37 (n=26)
Overall repair assessment (Grade I: normal, 12; Grade II: nearly normal, 11-8; Grade III: abnormal, 7-4; Grade IV: severely abnormal, 3-1)	10.02 \pm 0.81 (n=41)	7.48 \pm 0.63 (n=29)
Total	19,16 \pm 2,65	13,10 \pm 1,50

The results are expressed as the mean_standard deviation.

Statistical analysis

All statistical analyses were performed using SPSS 16.0 software for Windows; p-values < 0.05 were considered statistically significant.

Results

Topographic location and quantification of cells expressing CD271 antigen in healthy and OA synovial membranes

There was an important difference in the site of cells expressing the MSC marker CD271 in OA and healthy synovial membranes. In healthy specimens, cells expressing CD271 were located in the intimal lining (Fig. 1). In OA specimens, the distribution of CD271+ cells was more diffuse and they were located deeper than the subintimal layer, around veins in the perivascular matrix.

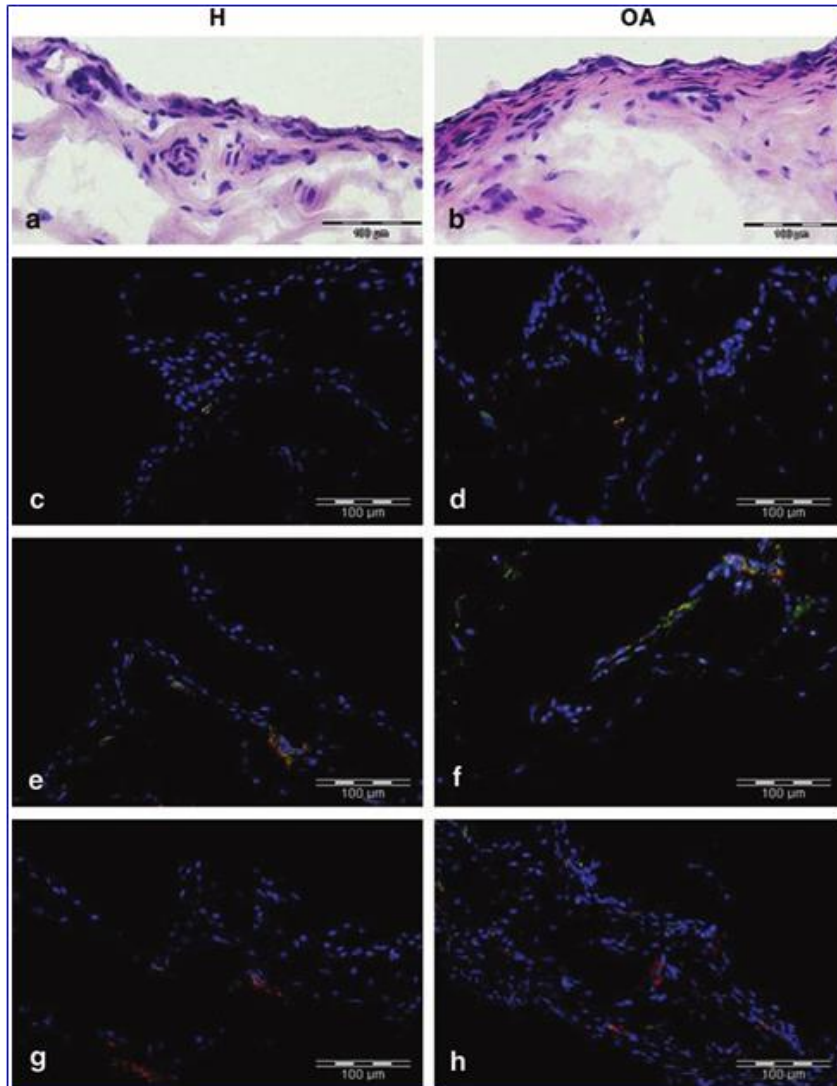


FIG. 1. Cells expressing the CD271 antigen with CD44, CD90, and CD105 antigens in synovial membranes shown by immunofluorescence. (a, b) Hematoxylin–eosin stain. (c–h) Monoclonal antibodies were used for the immunofluorescent detection of cells expressing CD271 alone or simultaneously with CD44, CD90, or CD105. CD44, CD90, or CD105 immunoreactivity appears green, whereas CD271 immunoreactivity appears red. (c, d) CD44 and CD271 double immunofluorescence of (a) healthy and (b) osteoarthritic (OA) synovial membranes. (e, f) CD90 and CD271 double immunofluorescence of (a) healthy and (b) OA synovial membranes. (g, h) CD105 and CD271 double immunofluorescence of (a) healthy and (b) OA synovial membranes. H, healthy synovial membranes. Magnification: (a, b) x 100; (c–h) x 400. Color images available online at www.liebertonline.com/tea.

Cells expressing the mesenchymal markers CD44, CD90, CD105, and CD271 were quantified using double immunofluorescent staining of frozen tissues, simultaneously analyzing two antigens. The percentages of cells expressing MSC markers were higher in OA than in healthy synovial membranes (Fig. 2). However, only the simultaneous expression of CD44/CD271 and CD90/CD271 antigens was significantly higher in OA than in healthy synovial membranes (1.09% vs. 2.2% for CD44/CD271, and 2.1% vs. 8.1% for CD90/CD271) ($p < 0.05$ for both sets of data).

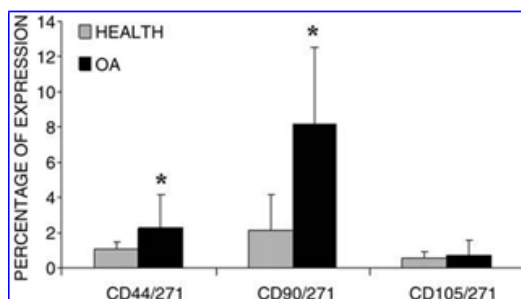


FIG. 2. Quantification of cells expressing mesenchymal stem cell (MSC) markers in synovial membranes using immunofluorescence. Graph showing percentages of synovial membrane cells simultaneously expressing CD44 and CD271, CD90 and CD271, and CD105 and CD271. Results are expressed as the mean \pm standard deviation. * $p < 0.05$.

The association of expression of the CD271 antigen with the expression of CD44, CD90, and CD105 antigens showed differences among these markers and between healthy and OA synovial membranes. The level of association of the expression of the CD271 antigen with the CD44 antigen (Fig. 3a) was moderate in healthy synovial membranes (56.8% \pm 18.2% of CD271+ cells were also positive for the CD44 antigen), but this level was higher in OA synovium, where 88.0% \pm 12.2% of CD271+ cells were positive for CD44. On the other hand, the expression of CD271 antigen was associated with the expression of the CD90 antigen in both healthy and OA synovial membranes (Fig. 3a). More than 80% of CD271+ cells in healthy and OA synovial membranes coexpressed the CD90 antigen. The expression of the CD271 antigen showed a low level of association with the expression of the CD105 antigen in both healthy and OA synovial membranes (Fig. 3a). Only 31.2% \pm 23.6% of CD271+ cells in healthy and 26.9% \pm 29.7% in OA synovial membranes were also positive for the CD105 antigen.

The expression of the CD44 antigen was strongly associated with that of the CD271 antigen (Fig. 3b); 81.8% \pm 11.1% of CD44+ cells in healthy and 68.7% \pm 2.7% in OA synovial membranes were CD44/CD271. The association of expression of the CD90 antigen with the CD271 antigen was significantly different ($p < 0.05$) between healthy and OA synovial membranes (Fig. 3c). In healthy synovial membranes, 37.8% \pm 12.1% of CD90+ cells also expressed CD271, whereas in OA synovium, 82.6% \pm 13.2% of CD90+ cells were also CD271+. The expression of the CD105 and CD271 antigens showed a lower level of association (Fig. 3d); 38.5% \pm 22.7% of CD105+ cells in healthy and 58.3% \pm 41.7% in OA synovial membranes were also positive for the CD271 antigen.

To summarize, the expression of the CD271 antigen was strongly associated with the expression of CD90 in both healthy and OA synovial membranes and with the expression of the CD44 antigen in OA synovial membranes. The expression of the CD271 antigen, however, was less strongly associated with the expression of the CD105 antigen in both healthy and OA synovial membranes.

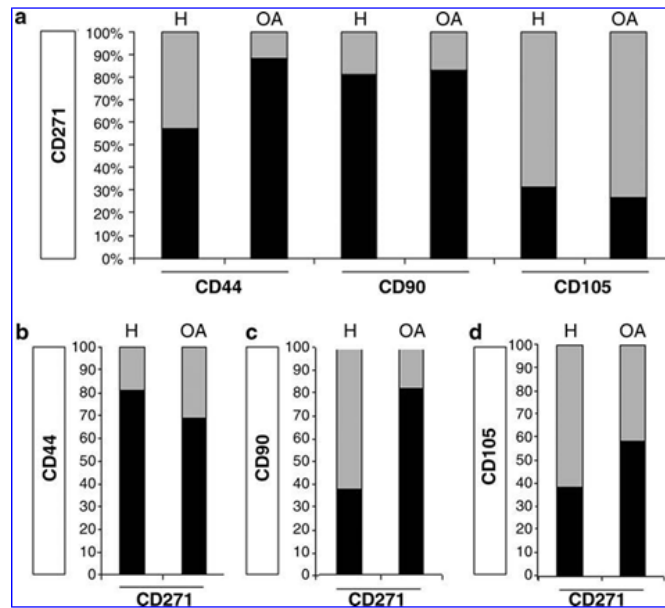


FIG. 3. Association of CD271 antigen expression with the expression of CD44, CD90, and CD105 antigens in synovial membranes. Each column represents the percentage of cells positive for one antigen also positive for another antigen (black solid box) in healthy (H) and OA synovial membranes. (a) The percentage of CD271+ cells expressing CD44, CD90, and CD105 antigens. (b) The percentage of CD44+ cells expressing the CD271 antigen. (c) The percentage of CD90+ cells expressing the CD271 antigen. (d) The percentage of CD105+ cells expressing the CD271 antigen.

Study of MSC markers in spontaneous repair tissue

The analysis of tissues localized in OA-damaged cartilage (spontaneous repair tissue) (Fig. 4a–d) showed a high percentage of cells expressing CD271 ($9.9\% \pm 4.0\%$) (Fig. 4e). Most CD271+ cells were found to be associated with CD44; 64% of CD271+ cells coexpressed the CD44 antigen (Fig. 4e). The coexpression of CD271 with CD90 was low; only 8% of CD271+ cells were also CD90+. The CD105 antigen was not present in spontaneous repair tissue.

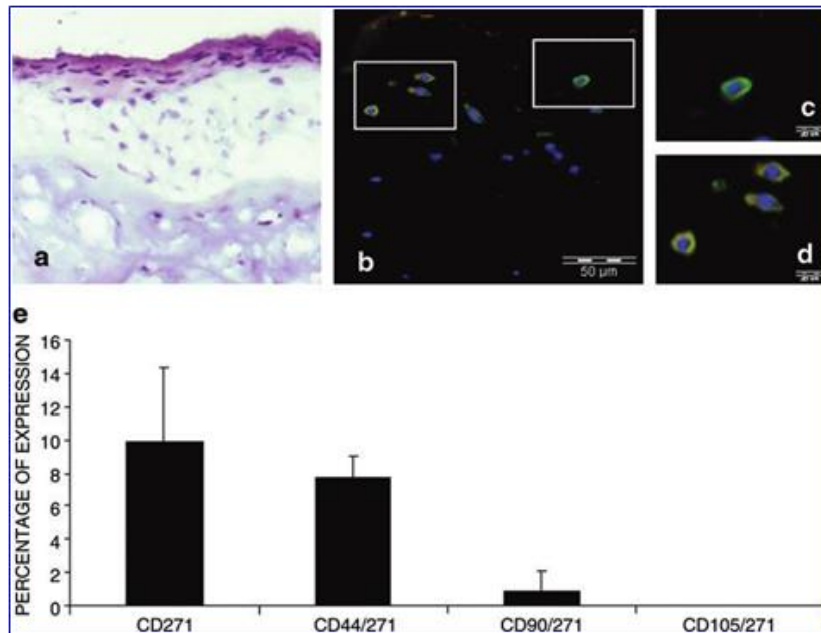


FIG. 4. Cells expressing CD44 and CD271 antigens in spontaneous repair tissue of OA articular cartilage. Monoclonal antibodies were used in frozen sections for the immunofluorescence detection of CD44 and CD271. (a) OA articular cartilage repair tissue stained with hematoxylin–eosin. (b) Detection of CD44 (green) and CD271 (red) antigens (panels c and d are higher magnifications of insets in panel b). (c) Cells expressing CD44 antigen. (d) Cells coexpressing CD44 and CD271 antigens. Scale bars: (a, b) 50 μ m; (c, d) 20 μ m. (e) Graph showing the percentage of cells in spontaneous repair tissue of OA articular cartilage that express CD271, as well as cells simultaneously expressing CD44 and CD271, CD90 and CD271, or CD105 and CD271. The results are expressed as the mean \pm standard deviation. Color images available online at www.liebertonline.com/tea.

In vitro repair of chondral defects with BM-derived CD271+ and CD271– cells

The phenotypic characterization of CD271– and CD271+ BM-derived mesenchymal cells (Fig. 5) demonstrated that both cellular populations are negative for the hematopoietic antigens CD34 and CD45 and had similar positive percentages for the antigens CD44, CD73, and CD90. These populations showed substantial differences only in the positive percentages for CD29 (CD271–: 74.26% \pm 22.87%; CD271+: 56.58% \pm 32.31%), CD105 (CD271–: 41.22% \pm 32.91%; CD271+ 20.81% \pm 24.82%), and CD166 (CD271–: 52% \pm 31.68%; CD271+: 44.05% \pm 36.65%). Both cell populations have adipogenic, osteogenic, and chondrogenic capacity (Fig. 6).

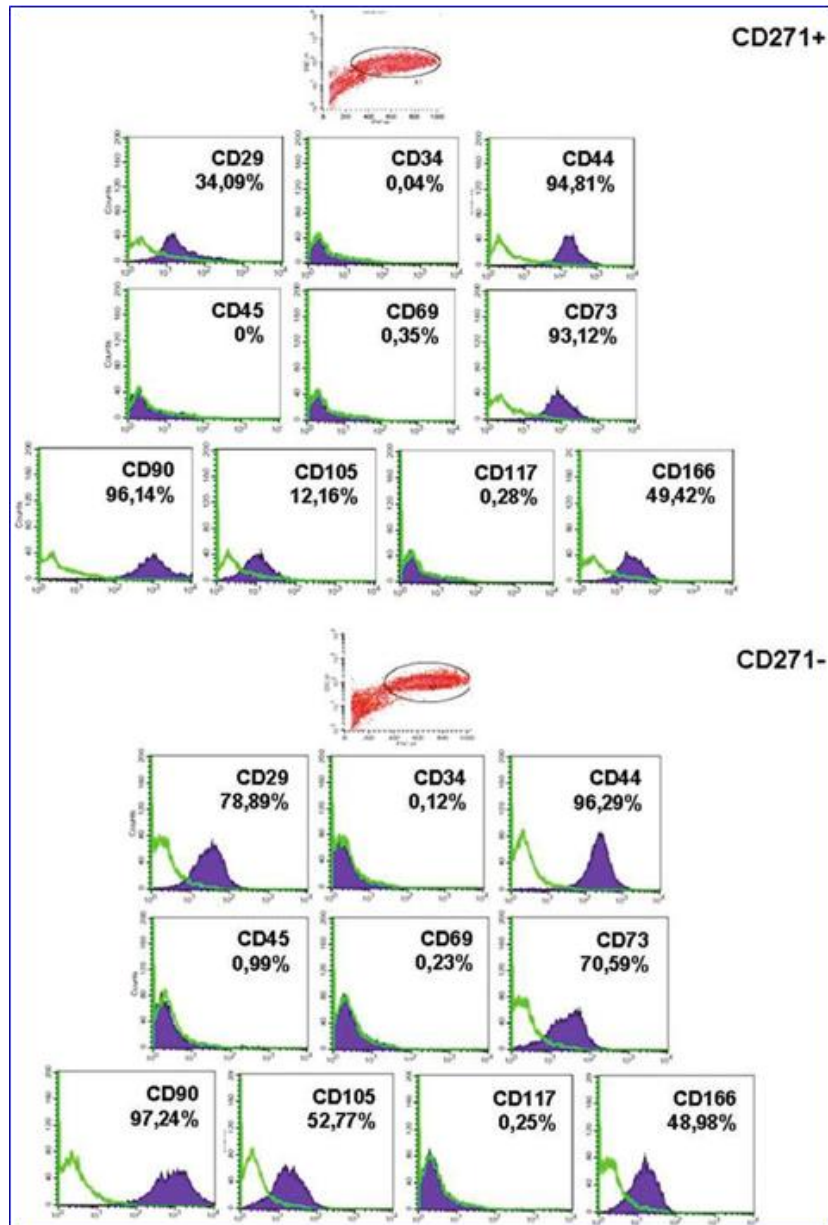


FIG. 5. The phenotypic characterization of CD271⁻ and CD271⁺ bone marrow-derived mesenchymal cells. Cells were harvested, centrifuged, washed, and counted before cell phenotype characterization using the formaldehyde-saponine method. The antibodies listed in Supplementary Table S1 were used for this procedure. Predetermined optimal amounts of anti-mouse or anti-human monoclonal antibodies were added directly to 2×10^5 cells for 1 h at 4°C. A minimum of 10,000 cell events per assay were analyzed with a FACsCalibur flow cytometer. This figure shows representative histograms of multipotential MSCs by FACs. The purple line signifies the specific antibody, whereas the green line represents the isotype control. Color images available online at www.liebertonline.com/tea.

After creation of the chondral defect described above, native cartilage discs showed depletion of proteoglycans in the cartilage zone located at the edge of the injury. This depletion occurred in cartilages of both the control and CD271⁺ or CD271⁻ cell implant groups; the deterioration was more evident during longer culture periods. The chondral defect affected the superficial and transitional zones of the cartilage. The surface of the defects was irregular, with numerous small cavities distributed along the lesion edge.

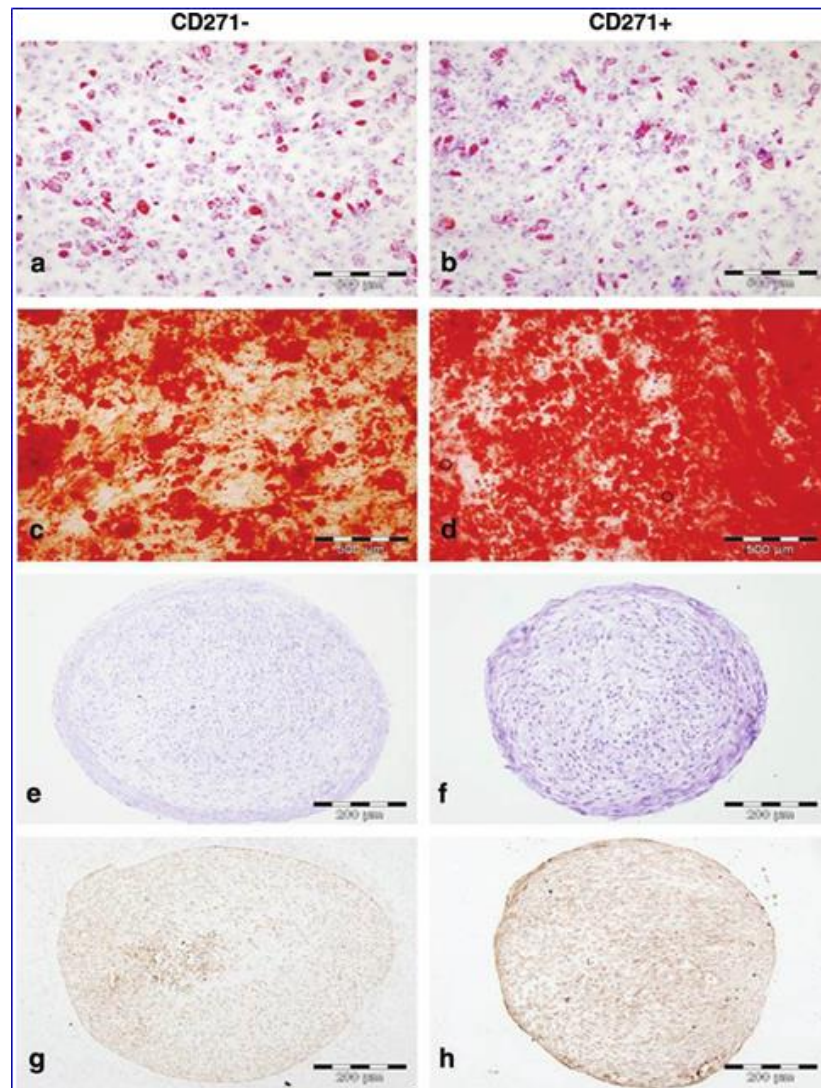


FIG. 6. Chondrogenic, adipogenic, and osteogenic capacities of CD 271+ and CD 271-. Adipogenesis (a, b). The presence of adipocytes was assessed by detection of lipidic drops with oil red stain. Osteogenesis (c, d). Scale bar value is 500 μ m. The presence of calcium deposits was demonstrated by alizarin red stain. Chondrogenesis (e-h). Scale bar value is 200 μ m. It was assessed by incubation of cells in micropellet culture with chondrogenic differentiation medium and transforming growth factor-b3; micromasses were stained for proteoglycans with toluidine blue and immunodetection of type II collagen. Color images available online at www.liebertonline.com/tea.

No repair was observable in chondral defects receiving only chondrogenic medium (control group). In contrast, chondral defects receiving MSC CD271+ or CD271- (implant groups) showed abundant repair tissue after 8 weeks of culture (Fig. 7). In both implant groups, the repair tissue morphology showed an extracellular matrix with a hyaline aspect and numerous lacunae containing round cells, resembling articular cartilage. The cell density was higher in repair tissue than in the native cartilage and in both types of implants the expression of collagen types I and II was observed.

Statistical analyses of the repair tissue at 8 weeks of culture showed significant differences in tissue morphology, filling of the defect, cellularity, integration of newly formed tissue, cell morphology, and matrix staining between implant groups (Table 1). The degree of defect repair was less in the implants with MSC CD271- ($1.00\% \pm 0\%$) than in those with MSC CD271+ (2.33 ± 0.84). Safranin O stain of the repair tissue was negative in the implants with MSC CD271- and moderately positive in implants with MSC CD271+. The integration between repair tissue and the adjacent normal cartilage was of better quality in the MSC CD271+ implants. The overall histologic score for CD271- implants was 13.10 ± 1.50 and 19.16 ± 2.65 for CD271+ implants.

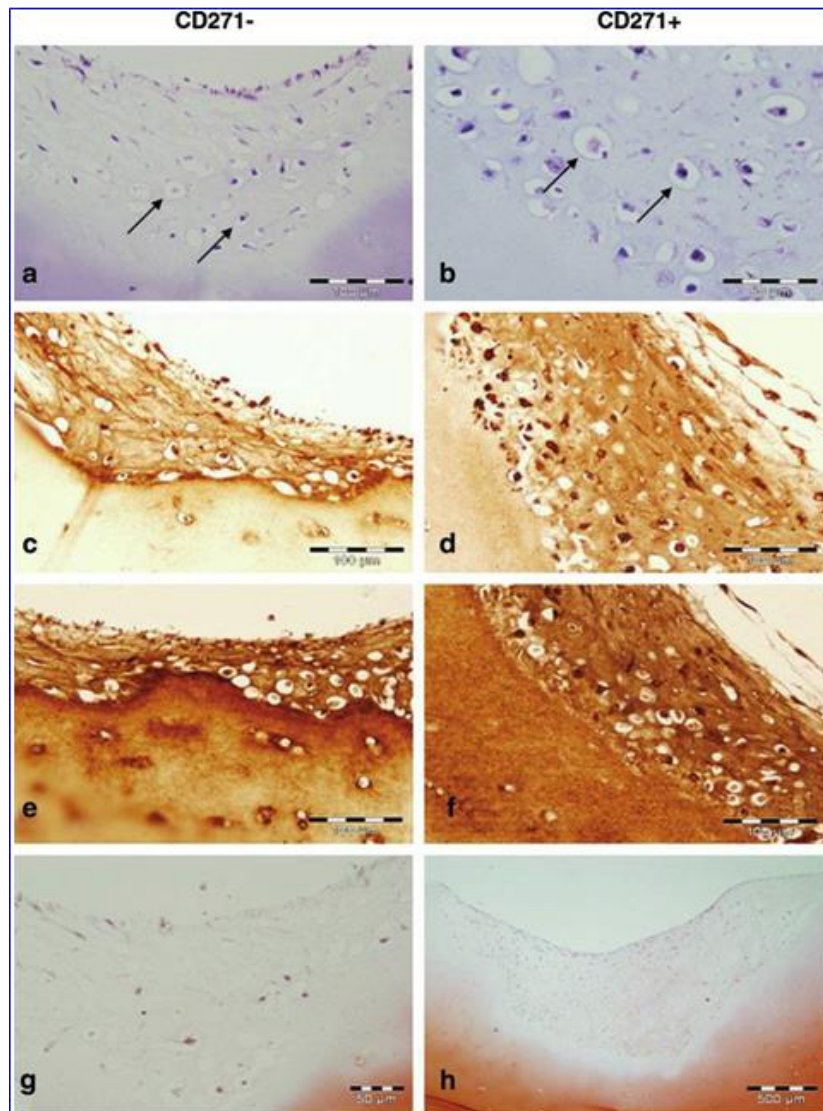


FIG. 7. *In vitro* chondral defect repair. Repair of chondral defects with implants of MSC CD271- (a, c, e, g) and MSC CD271+ (b, d, f, h). In both groups of implants, the repair tissue showed extracellular matrix with a hyaline aspect and numerous lacunae containing rounded cells (a, b, arrows) and the expression of collagen type I (c, d) and type II (e, f). Safranin O stain of the repair tissue was negative in the MSC CD271- implants and moderately positive in MSC CD271+ implants (g, h). Color images available online at www.liebertonline.com/tea.

Discussion

This study demonstrates for the first time that human synovial tissues and the spontaneous repair tissue of OA articular cartilage contain cells expressing the CD271 antigen. The expression of CD271 antigen in MSCs has been previously described in some human tissues, including BM,^{14,15,18,19} the joint fat pad,²⁰ and fetal membranes.¹⁵ CD271 mRNA has been reported in BMMSCs.²¹ Other research has shown that CD271 is a key marker of MSCs in BM and other tissues. The isolation of MSCs positive for CD271 using antibodies directed against the CD271 antigen should provide a phenotypically and functionally homogeneous cell population^{14,22} with high potentials for clonogenicity and differentiation into the multiple lineages of mesenchymal cells.^{14,15}

We observed that the expression of CD271, CD44, and CD90 antigens, but not of CD105 antigen, is significantly higher in OA than in healthy synovial tissues. Because these antigens have been identified as MSC markers from BM, adipose tissue, and synovial membranes,^{23,24} these results suggest a greater presence of MSCs in OA synovia than in healthy synovia.

The expression of the CD271 antigen was highly associated with the expression of CD44 and CD90 antigens in OA synovial membranes, whereas its association with the expression of the CD105 antigen was lower. Further, the phenotypic analysis of the immuno-selected cell population for the CD271 antigen showed that only 2.3% of the cells expressed the CD105 antigen. These results suggest that the MSC CD271+ subset has unique phenotypic characteristics different from other MSC subsets, such as MSC CD105+.

We found that 64% of the cells in OA articular cartilage spontaneous repair tissue that express the CD271 antigen are also positive for CD44, another marker for MSCs.¹ A recent study found that chondrocyte populations sorted for the CD44 marker antigen generate cartilaginous tissue of superior quality compared to that of unsorted cells.²⁵ These data suggest that CD271 is a marker for MSCs implicated in the repair mechanism of damaged cartilage.

The presence of cells expressing MSC markers in OA cartilage spontaneous repair tissue raises the question of the source of these cells. In chondral defects the cells that promote cartilage repair are MSCs from the same cartilage⁵ or MSCs from neighboring tissues, such as synovial membranes²⁶ or the fat pad.²⁰ It seems possible that MSCs from neighboring tissues migrate through the synovial cavity to the chondral defect and participate in the process of cartilage repair. Another possibility is that MSCs were transported to neighboring tissues through the vascular system, and from there migrate to the damaged cartilage. It has been shown that in osteochondral defects BMMSCs are included in a fibrin clot that migrates to the lesion and promotes repair tissue.²⁷

We observed an increase in the expression of CD271 in OA synovial membranes and a higher expression of CD271 antigen in cells located in OA articular cartilage repair tissue. Curiously, in human arthritic synovial fluid, the CD271 antigen was found on the surface of MSCs,⁷ but MSCs in normal bovine synovial fluid were negative for CD271.²⁸ Taken together, these findings suggest that, in the cartilage degenerative process, CD271+ MSCs from BM are activated and mobilized to travel through the vascular system. These cells first arrive at the synovial membrane then reach the damaged cartilage.

To summarize, synovial membranes from OA patients contain more cells expressing CD271 antigen than do those from joints with no cartilage damage, and spontaneous cartilage repair tissue contains cells positive for CD271 antigen. These findings imply the involvement of CD271+ MSCs in the repair mechanism for damaged articular cartilage. To evaluate the possible mobilization of CD271+ cells through the vascular system to arrive at damaged cartilage, we determined the capacity of CD271+ cells isolated from BM to repair articular cartilage defects in a chondrogenic medium. The repair of chondral defects in animal models using MSCs injected into defects closed with periosteum²⁹ or embedded in collagen gel³⁰ indicates that the MSCs can differentiate according to the biological medium in which they are grown.

This is the first *in vitro* study showing that implants of MSCs expanded in monolayer culture can repair focal defects in human articular cartilage. Using a relatively short 8-week culture time, both MSC CD271- and MSC CD271+ implants formed repair tissue that showed both cartilaginous (extracellular matrix immunopositive for collagen type II with a hyaline aspect and numerous lacunae containing rounded cells) and fibrous (immunopositive for collagen type I) characteristics.

Studies of autologous chondrocyte implants show that, 1 year after implantation, fibrocartilaginous morphology regions and hyaline morphology regions are coexistent in the same biopsy regions.^{31,32,33} Further, the expression of an aggrecanase activity higher than metalloprotease activity³³ and the expression of collagen mRNA indicate the prechondrocytic state (type IIA) and differentiated chondrocytes (type IIB) occur in repair tissue.^{34,35} These data suggest that the regeneration of articular cartilage probably occurs through turnover and remodeling of an initial fibrocartilaginous matrix using enzymatic degradation and synthesis of collagen type II,³³ and that this process is prolonged over time.³⁶

The quality of repair tissue is limited by the *in vitro* model. In an animal model, several cell types could contribute to the repair of cartilage, such as chondrocytes from native cartilage, implanted chondrocytes or stem cells, and stem cells from periosteum, perichondrium, and/or BM.^{29,37} In our *in vitro* model, the cell types are restricted to native cartilage chondrocytes and implanted MSCs. Other important limitations are the absence of biochemical signals from surrounding tissues stimulating chondrogenesis and differentiation, biomechanical stimuli, and the use of short culture times. The lack of data over the long process of regeneration of cartilage and the limitations of our *in vitro* model may explain the coexistence of fibrous and cartilaginous characteristics in the repair tissue.

Although both implant groups participated in the tissue repair process, the MSC CD271+ implants were found to provide improved repair. CD271+ cells achieved more filling of the chondral defect, better integration of the repair tissue with the native cartilage, improved regularity of the repair tissue surface, and greater chondrocytic morphology of the repair tissue cells. Further, repair tissue after MSC CD271+ implants showed moderate staining by safranin O, indicating the presence of proteoglycans.

A factor that can affect the quality of repair tissue is the homogeneity of the MSC CD271+ population. In this study, we used an immunomagnetic method of cell isolation to obtain an MSC population positive for CD271. This cell population was practically homogenous for CD44 and CD90 antigens, but only low percentages of cells expressed the CD29 and CD166 antigens. A comparative analysis between BM-MSC CD271 (bright) and CD271 (dim) populations as screened by flow cytometry showed that only the CD271 (bright) population was positive for established MSC markers and contained fibroblast colony forming units.²²

For the effective clinical use of MSCs in the treatment of focal cartilage defects, one critical investigation will be to determine the optimal population of MSCs for implantation to repair articular cartilage damage. Our results suggest that the MSC CD271+ subset promotes the repair of articular cartilage defects.

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Disclosure Statement

No competing financial interests exist.

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