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ORIGINAL ARTICLE

Chondrogenic differentiation of human bone marrow and adipose tissue-derived mesenchymal stem cells

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Summary
Congenital abnormalities, various diseases and injuries may result in the degeneration of articular cartilage. Recently, stem cell therapy has offered new treatment possibilities for this condition. The aim of our study was to verify the chondrogenic differentiation potential of human bone marrow mesenchymal stem cells (BMSCs) and adipose tissue-derived mesenchymal stem cells (AMSCs) *in vitro* in the presence or absence of transforming growth factor beta (TGF-β1). Human BMSCs and AMSCs from healthy donors were collected during orthopaedic surgeries and expanded *in vitro* to obtain a sufficient quantity of cells; their chondrogenic differentiation was studied in the pellet culture system. Spontaneous chondrogenesis occurred in both BMSC and AMSC pellet cultures and was similar in both TGF-β1 treated and untreated pellet cultures. BMSC pellets contained more cells with a chondrogenic phenotype. The presence of TGF-β1 led to a decrease in the levels of collagen type I mRNA and to increased levels of collagen type II mRNA only in the BMSC pellet culture. Our results demonstrate that although both mesenchymal cell types can be used in cartilage tissue engineering, the chondrogenic potential of human BMSCs is higher than that of AMSCs.

Keywords: cartilage – collagen mRNA – mesenchymal stem cell – pellet cell culture – TGF-β1

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INTRODUCTION

Congenital abnormalities, diseases and injuries may result in the degeneration of articular cartilage, which seriously decreases the life quality of affected individuals in all age groups. Treatment of these patients is difficult because mature cartilage has a very limited capacity for self-healing due to its intrinsic properties (Buckwalter 1998). In many

cases, when osteoarthritis develops a surgical intervention (e.g. total joint replacement) is the only option (Tuli et al. 2003). Recent techniques of articular cartilage defect repair involve an autologous osteochondral cylinder, periosteum or perichondrium, transplantation, autologous chondrocyte implantation or fresh osteochondral allograft implantation (Brittberg et al. 1994). Many of these strategies appear to be promising, but they involve invasive tissue collection and suffer from size restrictions, low mitotic potential and the senescence of chondrocytes expanded *in vitro* (Tew et al. 2000). Moreover, cultured chondrocytes undergo a dedifferentiation process, gradually changing their morphology to a fibroblast-like shape, and the production of type II collagen is replaced by the production of collagen type I (Schnabel et al. 2002) typical for fibrocartilage.

Several studies have focused on the utilization of adult mesenchymal stem cells (MSCs) isolated from bone marrow as an alternative to the use of autologous chondrocytes (Bosnakovski et al. 2005, Mackay et al. 1998). MSCs isolated from bone marrow are multipotent progenitor cells and can differentiate in culture into osteoblasts, chondrocytes, adipocytes and myoblasts (Pittenger et al. 1999). They may be used in autologous transplantation protocols, and bone marrow as a source of cells has been already approved for the treatment of haematopoietic diseases. Even though the plasticity of adult stem cells is frequently debated (Wagers and Weismann 2004), spontaneous *in vitro* chondrogenic differentiation (Bosnakovski et al. 2004) or differentiation in the presence of growth factors such as transforming growth factor-β (TGF-β), bone morphogenetic proteins (BMPs) or insulin-like growth factor-1 (IGF-1) (Indrawattana et al. 2004, Schmitt et al. 2003) in a pellet culture system has been reported. More recently, it has been shown that adipose tissue-derived MSCs obtained from liposuction can differentiate into various types of cells, including chondrocytes (Gimble and Guilak 2003, Huang et al. 2004, Zuk et al. 2001). The potential of utilizing these cells is very attractive for cartilage tissue engineering because subcutaneous fat is abundant in the human body and the liposuction procedure is minimally invasive for the patient.

In this study, we compared the chondrogenic potential of three different pellet culture systems isolated from three different sources of cells. Chondrogenesis was induced under *in vitro* conditions either spontaneously or by the addition of TGF-β1 to the pellet culture system. We isolated human bone marrow- and adipose tissue-derived MSCs and compared their surface markers using FACS analysis; the chondrogenic potential of MSCs from both sources was compared to chondrocytes isolated from the hyaline cartilage.

MATERIALS AND METHODS

Procurement of samples

Bone marrow, adipose tissue and articular cartilage were collected from healthy donors during orthopaedic surgeries, always following patient's informed consent. All sampling procedures were performed in accordance with The Helsinki Declaration and were approved by the ethical committee of Motol Hospital in Prague.

Isolation and cell culture

Bone marrow-derived MSCs (BMSCs) were isolated from fresh samples, and supplemented with 20 U/ml heparin in a sterile physiological solution as described previously (Bosnakovski et al. 2004; Pittenger et al. 1999). To separate nucleated cells, gradient centrifugation using Ficoll-Paque (Amersham Biosciences, Sweden) at 1800 rpm for 30 min was performed. The obtained cells were washed twice in phosphate-buffered saline (PBS) then centrifuged at 1200 rpm for 5 min. Pellets were resuspended in a complete culture medium consisting of α-MEM (Gibco, USA), 10% fetal bovine serum (FBS, PAA, Austria), 100 U/ml Penicillin and 100 μg/ml Streptomycin (PAA, Austria). The cells (2×10^5 cells/ml) were plated in 100 mm Petri dishes and were cultured at 37 °C in a humidified atmosphere containing 5% CO_2. After 24 hours, non-adherent cells were removed by changing the culture medium. During subsequent cultivation, the medium was refreshed every three days. After 7 – 10 days, cells were detached by 0.25% trypsin (Gibco, USA) and sub-cultured up to the third passage.

Adipose tissue-derived MSCs (AMSCs) were obtained from freshly isolated subcutaneous fat as described previously (Gimble and Guilak 2003). Briefly, lipoaspirates were carefully rinsed with PBS and mechanically disaggregated with a scalpel. Subsequently, adipose specimens were digested by 0.1% collagenase (Serva, Germany) in PBS supplemented with 1% human albumin (Grifols, Spain) for 60 min at 37 °C with agitation at 75 rpm. The released cells and residual adipose tissue were centrifuged at 1200 rpm for 5 min. The supernatant layer of oil, fat and primary adipocytes and the underlying layer of collagenase were aspirated. The pellet was resuspended in PBS with 1% human albumin following centrifugation at 1200 rpm for 5 min. The final pellet was resuspended in a complete culture medium consisting of D-MEM/F12 1:1 (Gibco, USA) with 10% FBS, 100 U/ml Penicillin and 100 μg/ml Streptomycin. The cells were seeded at a density of 2×10^5 cells/ml in 100 mm Petri dishes and were incubated in a humidified atmosphere of 5% CO_2 at 37 °C. Non-adherent cells were aspirated with the medium after 24 h of cultivation and fresh medium was added. The culture medium was changed every

three days. To obtain a sufficient number of AMSCs, the cells were sub-cultured three times.

Chondrocytes were obtained from meniscal biopsies of the knee joint according to protocols published previously (Brittberg et al. 1994). Briefly, cartilages were washed with PBS and mechanically disaggregated using a scalpel. The obtained specimens were digested with 0.25% trypsin for one hour, followed by digestion with 0.1% collagenase for 12 h at 37 °C. D-MEM/F12 1:1 with 10% FBS was added to the released cells, which were then centrifuged at 1200 rpm for 10 min. The pellet was resuspended in D-MEM/F12 1:1 with 10% FBS, 100 U/ml Penicillin and 100 µg/ml Streptomycin. The cells were seeded at a density of 2×10^5 cells/ml in 100 mm Petri dishes and were incubated in a humidified atmosphere of 5% CO_2 at 37 °C. The chondrocytes were sub-cultured twice and the culture medium was refreshed at three day intervals.

Phenotypic characterization

BMSCs and AMSCs from the third passage were analyzed by direct and indirect immunofluorescence, according to protocols specific for each antibody. In each case, 10 000 events were acquired and analyzed by a BD FACSAria flow cytometer using BD FACSDiva software (Becton Dickinson, USA). The following antibodies were used for cell staining: anti-CD29-PE, anti-CD44-FITC, anti-CD90-FITC (BD Pharmigen, USA); anti-CD45-PE-Cy5, anti-CD235a-PE (DakoCytomation, Denmark); anti-CD34-FITC, anti-HLA Class I-FITC, anti-HLA DR+DP (Exbio, Czech Republic); anti-human fibroblast surface protein (Sigma, USA) with a secondary FITC-conjugated donkey anti-mouse IgG antibody (Chemicon, USA). Non-immune mouse isotypes served as respective controls (BD Pharmigen, USA).

Pellet culture

For chondrogenic differentiation, a three dimensional pellet culture system was used. Pellets were formed by the centrifugation of 2×10^6 BMSCs or AMSCs at 1500 rpm for 10 minutes in 15 ml polypropylene tubes (TPP, Switzerland). The chondrogenic medium consisted of DMEM/F12 1:1, 10% FBS, 100 U/ml Penicillin, 100 µg/ml Streptomycin and 10 ng/ml TGF-β1 (R&D systems, USA). Spontaneous chondrogenic differentiation was performed under the same culture conditions but without the addition of TGF-β1. Chondrocytes from the second passage cultured as pellets were used as a control. All tubes were maintained in an incubator at 37 °C with a humidified atmosphere of 5% CO_2. The culture medium was carefully refreshed every third day for 21 days.

Histological staining and immunohistochemistry

After 21 days, the pellets were fixed in 4% paraformaldehyde for 4 hours, then dehydrated in ethanol and embedded in paraffin. Sections with a thickness of 5 µm were cut from paraffin blocks, deparaffinized with xylene and rehydrated with distilled water. The obtained sections were stained with hematoxylin and eosin (Sigma, USA) to visualize the morphology of the cells and with Alcian blue to visualize the acid mucopolysaccharides. Masson's trichrome (Sigma, USA) was utilized to visualize the collagenous matrix. The presence of collagen type II was detected by immunohistochemistry. A monoclonal antibody directed against collagen type II (Chemicon, USA) was used in accordance with the manufacturer's recommendations. As a negative control, the primary antibody was omitted and staining with only the secondary antibody was performed. As a positive control, cartilage tissue from meniscal biopsies of the knee point was used.

Transmission electron microscopy

Samples for transmission electron microscopy (TEM) were fixed in 2.5% glutaraldehyde (Serva, Germany), pH 7.2, at 4 °C for 4 h. After fixation, samples were rinsed in PBS and postfixed in 2% osmium tetraoxide (Serva, Germany) for 2 h, then rinsed in distilled water and dehydrated in a graduated series of ethanol. Subsequently, the samples were embedded in EPON and cut into semi-thin sections. Ultra-thin sections were mounted on 200 mesh copper grids, then double stained using uranyl acetate and lead citrate (Serva, Germany) and examined using a Philips Morgagni 268 transmission electron microscope.

RNA extraction and real-time reverse transcription polymerase chain reaction (RT-PCR)

Immediately after the termination of chondrogenic differentiation, template mRNA was isolated from all samples using an Oligotex Direct mRNA micro kit (Qiagen, USA) in accordance with the manufacture's protocol. The expression of the genes of interest (collagen I, collagen II, aggrecan) was analyzed using a QuantiTect SYBR Green RT-PCR kit (Qiagen, USA) on a BioRad IQ5 optical system (Bio-Rad laboratories, USA). β-actin was chosen for an internal control as a housekeeping gene. The collected raw data were evaluated using the BioRad program. The mRNA levels of the target genes were normalized by dividing their value by the value of the ß-actin mRNA level.

Statistical analysis

Data from three independent experiments were evaluated and expressed as the mean ± standard error. The Student's t-test was used to compare quantitative parameters, $p < 0.05$ was considered significant.

RESULTS

Cell cultures

Primary isolated chondrocytes had a typical polygonal appearance (Fig. 1a). During subsequent passages (up to the third passage) they gradually lost their original shape and showed a fibroblast-like morphology (Fig. 1b), which corresponds to the well-known fact that chondrocytes undergo a de-differentiation process when expanded *in vitro* (Schnabel et al. 2002).

When primarily plated, BMSCs and AMSCs grew as isolated colonies. After 7 – 10 days they were sub-cultured and grew in a confluent monolayer. Both types of cultured cells had a bipolar to polygonal fibroblast-like shape (Fig. 1c, d). During further sub-cultivation in monolayers (up to the third passage), they maintained this morphology; no changes in their morphology or their proliferative activity were observed.

Phenotypic characterization

The results of phenotypic characterization from third passage cells showed that almost all of the BMSCs and AMSCs were CD29, CD44 and CD90 positive. All cells were HLA Class I positive and HLA Class II negative. In contrast, no instances of the expression of the haematopoietic lineage markers CD34 and CD45 were found. Moreover, BMSCs and AMSCs did not express anti-human fibroblast surface protein. The phenotypic characterization is summarized in Table 1.

Table 1**. Immunophenotypic comparison of human bone marrow stromal cells (BMSCs) and mesenchymal cells isolated from adipoid tissue** (AMSCs).

Analyzed marker	*MSCs*	*AMSCs*
CD29	+	+
CD34	–	–
CD44	+	+
CD45	–	–
CD90	++	++
CD235a	–	–
HLA Class-I	++	++
HLA Class II (DR+DP)	–	–
Human fibroblast surface protein	–	–

Symbols: – negative, + positive (≤ 50%), ++ positive (≥85%).

Pellet morphology

In all our cell cultures, independently of the presence of TGF-β1, the condensation of the pellets into single aggregates was observed after 24 hours. The morphology of the pellets was identical in BMSC, AMSC and chondrocyte cultures. During cultivation the size of the pellets continually increased, and the pellets became opaque. On termination of the experiment, the average size of the pellets cultivated in the presence of TGF-β1 was 5.2 ± 0,8 mm^2, whereas the pellets cultivated without TGF-β1 were significantly smaller, 3.4 ± 0,6 mm^2.

Histology and immunohistochemistry

Photomicrographs of haematoxylin and eosin stained sections (Fig. 2a-c) showed a homogenous cell distribution in all pellets. The periphery of each pellet consisted of one or two layers of elongated cells (Fig. 2g-i). The centre region contained both elongated cells with lightly stained nuclei, corresponding to loose mesenchymal tissue, and rounded cells with darkly stained nuclei and surrounded by extracellular matrix, which were similar to chondrocytes (Fig. 2j-l). Pellets of BMSCs and chondrocytes contained more rounded cells than did pellets of AMSCs; the predominant shape of cells in the middle of the AMSC pellets was elongated. Moreover, the density of the cells in the AMSC pellets was lower when compared with the BMSC and chondrocyte pellets. Alcian blue staining showed deposits of acid mucopolysaccharides in the proximity of the cells in all of the pellets, regardless of TGF-β1 treatment (Fig. 2m-o). The intensity of Alcian blue staining slightly decreased from chondrocytes through BMSCs to AMSCs.

Masson's trichrome staining showed that the cells in each group were separated by extensive regions of diffuse extracellular matrix with a high collagen content. (Fig. 3a-c). Interestingly, no influence of TGF-β1, at the concentration employed, on cell morphology or extracellular matrix production was observed with haematoxylin and eosin (Fig. 2d-f) or Masson's trichrome staining (Fig. 3d-f). Immunostaining for collagen

type II revealed its presence in all of the pellet cultures, more intensely in the centre of the pellets; the immunostaining intensity was highest in the chondrocyte pellets and lowest in the AMSC pellets (Fig. 3m-o). In TGF-β1 treated pellets, the production of collagen type II was not significantly changed in comparison with cultures without the growth factor (Fig. 3j-l).

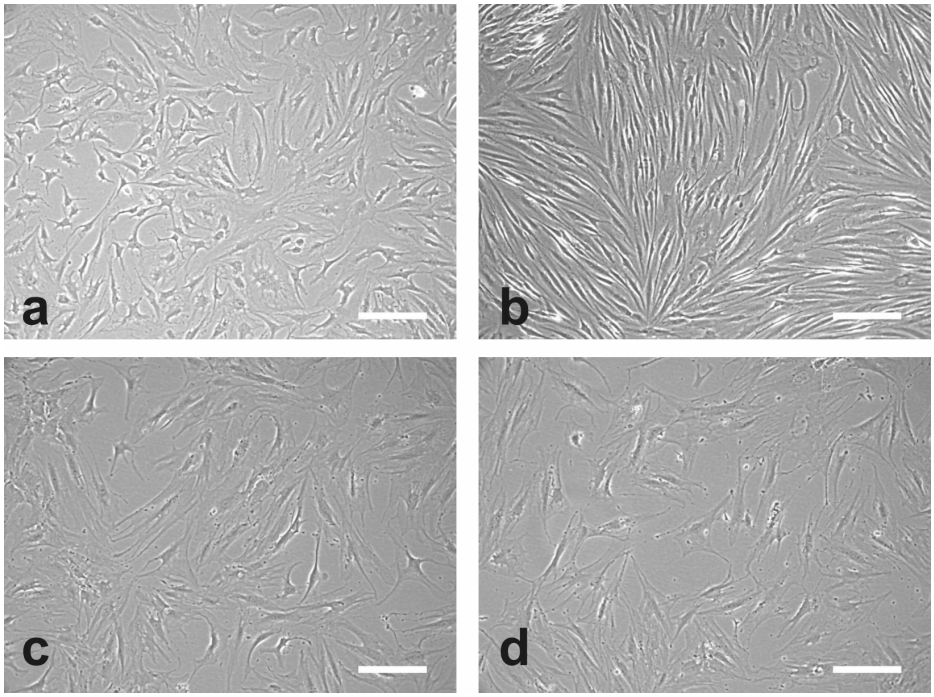

Fig. 1. **Cell cultures growing in a monolayer under *in vitro* conditions.** a. Chondrocytes, first passage. b. Chondrocytes, third passage. Note the elongation of the cells, associated with their dedifferentiation. c. BMSCs, third passage. d. AMSCs, third passage. Both types of cultured mesenchymal stem cells had a bipolar to polygonal fibroblast-like shape. Scalebar = 100 μm.

Transmission electron microscopy

TEM images (Fig. 4) showed normal ultra-structure of BMSCs, AMSCs and chondrocytes. Chondrocytes were in all instances surrounded with newly synthesized ECM. The cells contained nuclei with nucleoli and excessive endoplasmatic reticulum and transport vesicles, which are typical for neocartilage formation. Pathologic changes (apoptosis, necrosis) were not observed in any sample.

Collagen and aggrecan real-time RT-PCR

A quantitative analysis of collagen mRNA showed that the levels of collagen type I and II mRNA in both BMSCs and AMSCs in the pellet culture system, without the addition of a growth factor, are significantly lower than the levels found in chondrocytes (Fig. 5). In pellet cultures with the addition of TGF-β1, the amount of collagen type I mRNA was lower in both BMSCs and AMSCs than the amount found in pellet cultures without TGF-β1 (Fig. 5a). Cultivation in the presence of TGF-β1 led to a massive increase in the level of collagen type II mRNA in the BMSCs, surpassing the level seen in chondrocytes cultured in the same differentiation media. The amount of collagen type II mRNA in AMSCs did not change in the presence of TGF-β1 (Fig. 5b).

We were able to detect aggrecan mRNA in chondrocyte pellet cultures cultivated with or without TGF-β1, but no aggrecan mRNA was detected in any of the mesenchymal stem cell pellet cultures after three weeks of cultivation.

DISCUSSION

The utilization of autologous chondrocytes in order to repair articular cartilage defects dates from 1994, when the first 23 patients treated for isolated defects of the knee cartilage due to trauma or osteochondritis dissecans were reported by Brittberg et al. (1994). Although this technique is currently widely accepted as the treatment for focal chondral lesions, its use is hindered by two important factors. The chondrocytes must be

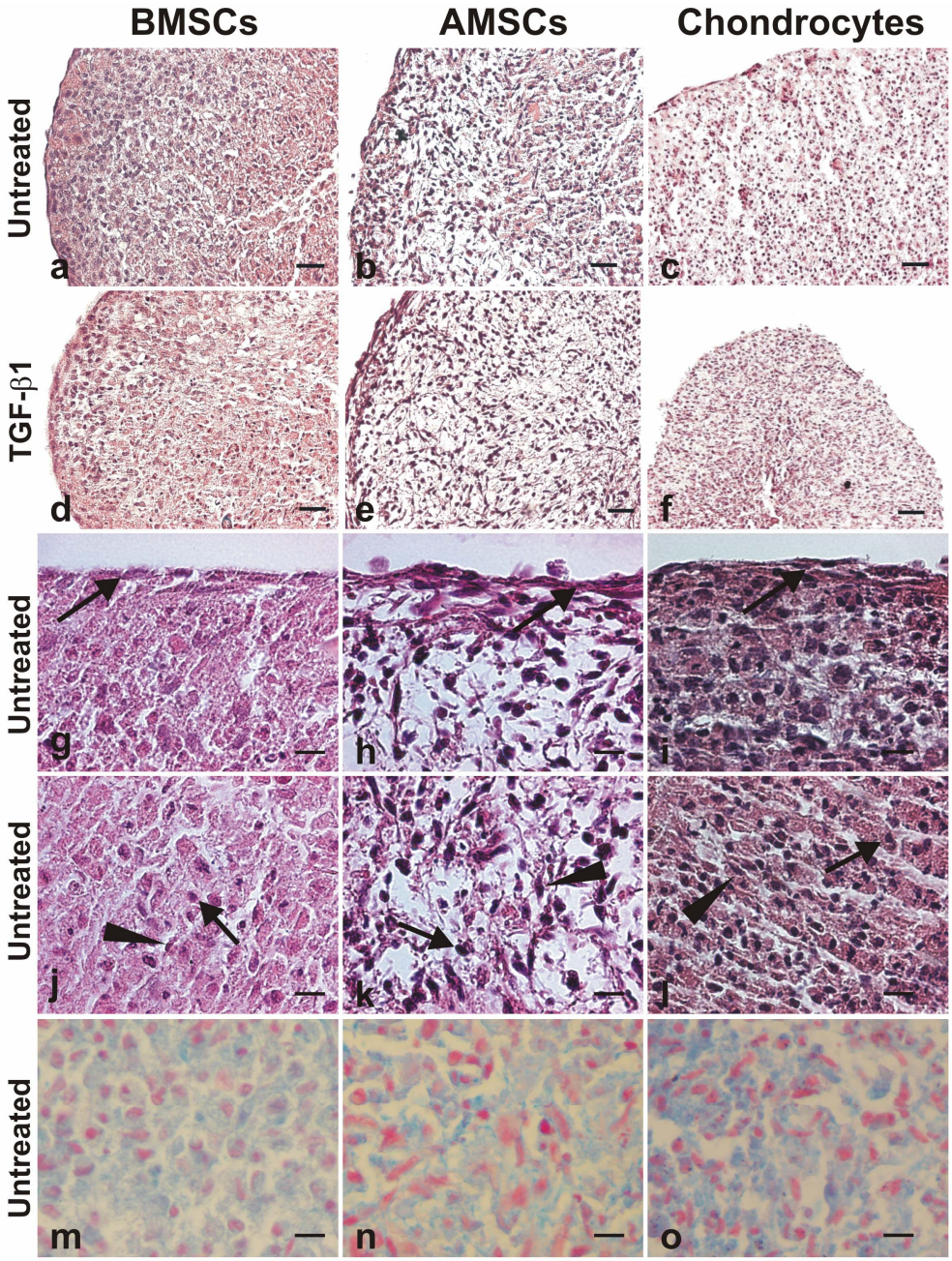

Fig. 2. **Hematoxylin and eosin staining of BMSCs, AMSCs and chondrocytes cultured in a pellet culture system on day 21.** a-f. Overview image. Scalebar = 100 μm. g-i. Periphery of the pellets; arrow marks a layer of elongated cells. j-l. Central region of the pellets; arrows mark rounded (chondrocyte-type) cells, arrowheads mark elongated (mesenchymal-type) cells. m-o. Alcian blue staining of the pellets, the cell nuclei are stained with Nuclear fast red.
Scalebar = 10 μm.

arthroscopically obtained from healthy articular cartilage; the necessity of two consecutive surgeries increases the risk for the remaining cartilage. It is also well known that chondrocytes lose in time their morphological and biochemical characteristics and became unsuitable for cartilage tissue engineering when expanded *in vitro* (Schnabel et al. 2002).

For these reasons, attempts were made to find another source of cells that are easily accessible

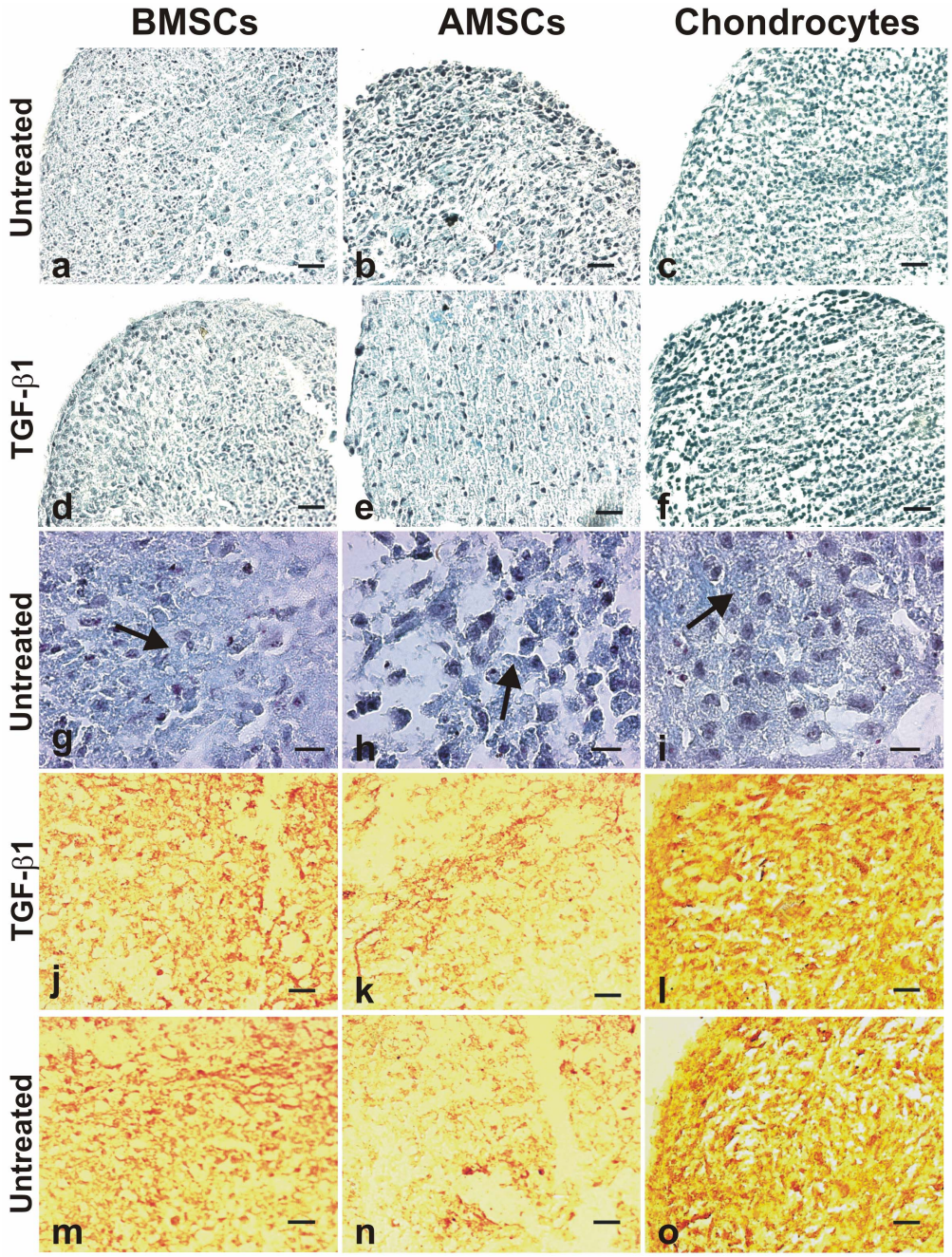

Fig. 3. **The histology of BMSCs, AMSCs and chondrocytes cultured in a pellet culture system on day 21.** a-f. Masson's trichrome staining. Scalebar = 100 μm. g-i. Masson's trichrome staining of the central region of the pellets; arrows mark the diffuse extracellular matrix surrounding the cells. Scalebar = 10 μm. j-o. Immunohistochemical staining using a monoclonal antibody against collagen type II. Scale bar = 100 μm.

and have a chondrogenic differentiation capacity. Several studies with MSCs of both animal and human origin isolated from the bone marrow have been performed in this respect (Bosnakovski et al. 2004, Mackay et al. 1998). Unfortunately, the utilization of BMSCs has some limits associated with patient discomfort and the pain of bone marrow harvesting. As a promising alternative to BMSCs, some studies have focused on the use of AMSCs, which can be easily isolated in large

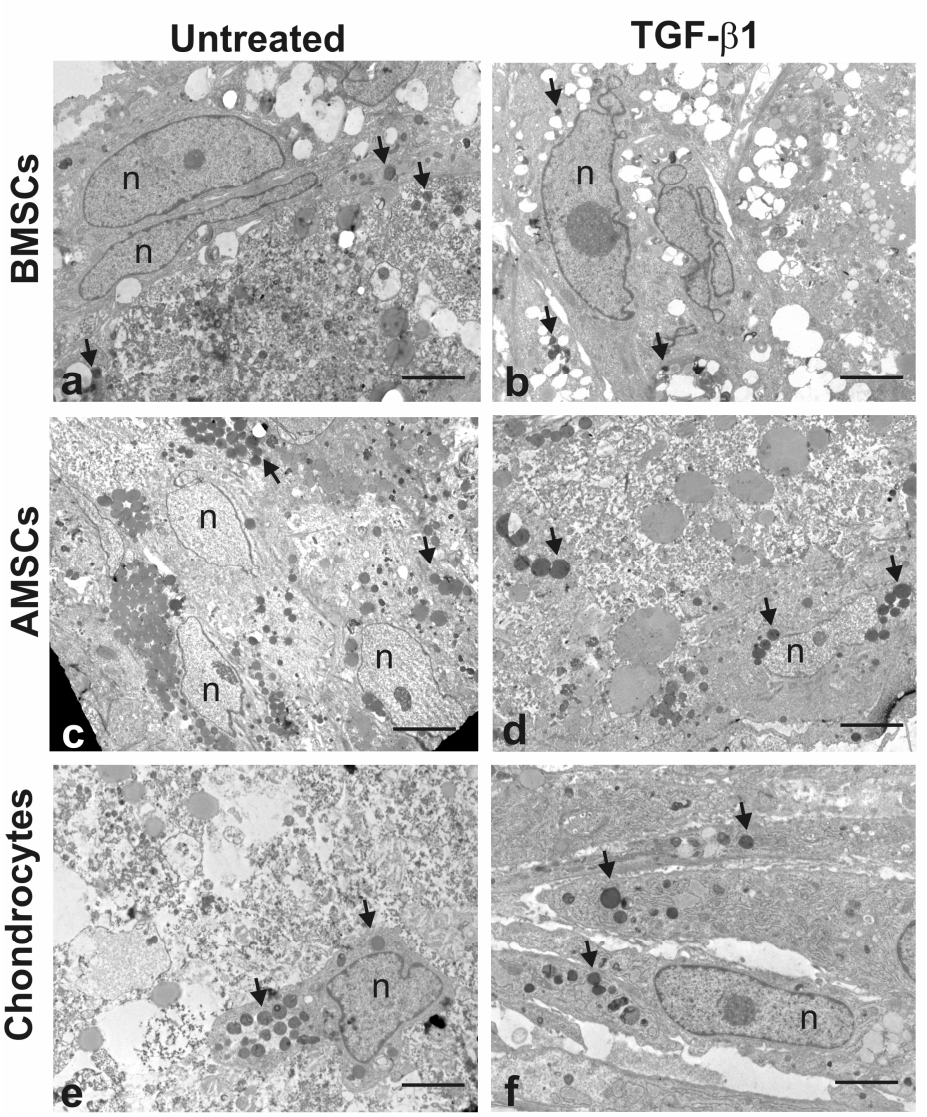

Fig. 4. **Transmission electron microphotographs of BMSCs, AMSCs and chondrocytes cultured in a pellet culture system on day 21.** Arrows show transport vesicles, n = nucleus. Scalebar = 1 μm.

numbers with minimal discomfort to the patient (Zuk et al. 2001). Moreover, bone marrow harvesting yields only a small number of stem cells for clinical use, usually requiring *in vitro* expansion, whereas adipose tissue is plentiful and easy to obtain. Furthermore, large numbers of AMSCs can be harvested from a relatively small amount of adipose tissue (Zuk et al. 2001), eliminating the need for lengthy culture expansion.

We found a similar phenotype of AMSCs and BMSCs when examining cell surface markers characteristic of mesenchymal stem cells. We then studied the possibility of differentiating AMSCs and BMSCs towards a chondrocyte phenotype.

In order to study differentiation into chondrocytes, many authors utilize a pellet culture system in which chondrogenesis is induced by growth and differentiation factors such as TGF-β, BMP or IGF (Indrawattana et al. 2004, Schmitt et al. 2003). The most commonly used factor is TGF-β1, which acts through a multimeric complex involving two membrane serine/threonine kinase receptors and an intracellular signaling pathway involving a cascade of Smad proteins (Miyazono 2000).

The chondrogenic nature of the differentiated cells in our pellet cultures was supported by the following findings: 1) the expression of a cartilaginous matrix within the processed cell pellets, containing collagens and proteoglycans (not shown) as demonstrated by histological staining and immunohistochemical analysis; 2) the presence

of transport vesicles on TEM microimages, and 3) the expression of collagen type II mRNA, as confirmed by real time RT-PCR analysis.

We found spontaneous chondrogenesis in both AMSCs and BMSCs without the addition of TGF-β1, which was previously believed to be necessary (Johnstone et al. 1998, Mastrogiacomo et al. 2001). These results are in accordance with the findings of Bosnakovski and co-workers, who reported the spontaneous chondrogenic differentiation of bovine BMSCs in pellet culture (Bosnakovski et al. 2004); the pellet culture conditions obviously mimic the cellular condensation process and hypoxic environment occurring during *in vivo* chondrogenesis (Stott et al. 1999).

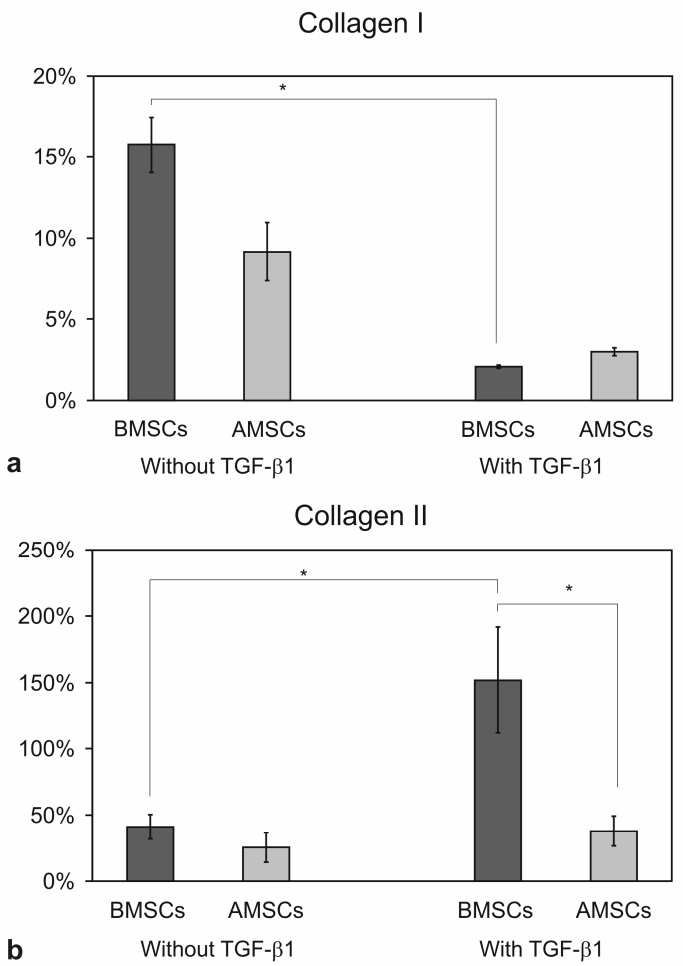

Fig. 5. **RT-PCR analysis of collagen mRNA, normalized by dividing the collagen values by the value of the ß-actin mRNA level.** The values are expressed as the percent of collagen production in chondrocytes under the same cultivation conditions. * statistically significant

Although our results showed that pellets treated with TGF-β1 were larger when compared to pellets cultured without TGF-β1, we have not found any visible differences in the histology of the pellets with and without TGF-β1 treatment. Histological analysis demonstrated that TGF-β1 treatment did not have any effect on cell morphology, which was also proved by TEM analysis. The effect of TGF-β1 is evidently more complex, and this factor alone may not lead to the proper genesis of mature chondrocytes in all cell types. Many authors stress the importance of TGF-β1 on cartilage maturation (Bosnakovski et al. 2006); however, some authors report that TGF-β1 is only one of the factors that maintain chondrocytes in a dedifferentiated state and prevent

their abnormal terminal differentiation (Galera et al. 1992, Yang et al. 2001).

Real-time RT-PCR analysis revealed significantly lower levels of collagen type I mRNA in TGF-β1-treated BMSCs and AMSCs. This is in agreement with other findings showing that collagen type I is down-regulated during chondrogenic differentiation (Kosher et al. 1986a). The amount of collagen type II mRNA in pellet cultures without growth factor was not significantly different between pellets of BMSCs and AMSCs, and both cell types showed approximately the same extent of spontaneous chondrogenic differentiation. In TGF-β1-treated pellets, the transcription of collagen type II mRNA was up-regulated in BMSCs, while in AMSCs it remained unchanged, suggesting a lower chondrogenic potential of AMSCs in comparison with BMSCs. Similarly, immunohistochemical labelling indicated a higher chondrogenic potential of BMSCs in comparison with AMSCs.

We did not detect any aggrecan mRNA in any of the mesenchymal stem cell pellet cultures. This negative result might be due to the timing of our aggrecan analysis (after three weeks in pellet culture). It has been demonstrated that aggrecan accumulates at the onset of chondrogenesis, coincident with cellular condensation (Kosher et al. 1986b). In other work (Huang et al. 2004), lipoaspirate cells in pellet cultures expressed aggrecan in 2 day processed pellet cultures, and the aggrecan expression was lost after 2 weeks of chondrogenic induction, since it overlapped with collagen type II expression.

The question of which cells (AMSCs or BMSCs) are better replacements for chondrocytes in the cartilage tissue engineering is still under debate (Lee et al. 2004, Sakaguchi et al. 2005). Strem and Hedrick (2005) have analyzed AMSCs and BMSCs from the same patients under identical conditions and found that AMSCs had greater chondrogenic ability than did BMSCs. However, Winter et al (2003) demonstrated that while AMSCs and BMSCs are not significantly different in their ability to undergo chondrogenesis in two-dimensional cultures, BMSCs exhibited enhanced chondrogenesis in three dimensional cultures. We found a high chondrogenic potential of BMSCs in our experiments. Our results also show that TGF-β1 may not be the ideal bioactive molecule for chondrogeneic differentiation; other growth factors and their combinations, such as TGF-β2, TGF–β3, BMP-6 and IGF-1, have recently been investigated (Im et al. 2006, Indrawattana et al. 2004).

Although pellet culture is far from clinical application in cartilage replacement, the ability of MSCs to differentiate into chondrocyte-like cells appears promising. *In vitro* experiments are still far from the use of stem cells in clinical trials. There are several problems that have to be solved before transplantation into living organisms. In many cases the mechanisms by which transplanted cells contribute to tissue regeneration are poorly defined, and questions about stem cell plasticity, transdifferentiation and cell fusion remain under debate. However, the treatment of tendons by autologous BMSC in horses has yielded promising results (Smith et al. 2003). In addition, problems with cell fusion are mainly a concern when undifferentiated stem or progenitor cells are injected (Collins et al. 2007, Lyon and Harding 2007). In cartilage tissue engineering, the cells will always have to be predifferentiated *in vitro*, and this may decrease the occurrence of cell fusion after *in vivo* transplantation. AMSCs seem to have a lower chondrogenic potential than do BMSCs; however, their differentiation in biomaterials, or in the presence of growth factors other than TGF-β1 or their combination, needs to be examined. Both types of the evaluated MSCs may therefore play an important role in cartilage tissue engineering.

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