JOURNAL OF TISSUE ENGINEERING AND REGENERATIVE MEDICINE
J Tissue Eng Regen Med (2009).
Published online in Wiley InterScience (www.interscience.wiley.com) **DOI:** 10.1002/term.235

RESEARCH ARTICLE

Passaged human chondrocytes accumulate extracellular matrix when induced by bovine chondrocytes

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Abstract

A source of sufficient number of cells is a major limiting factor for cartilage tissue engineering. To circumvent this problem, we developed a co-culture method to induce redifferentiation in bovine articular chondrocytes, which had undergone dedifferentiation following serial passage in monolayer culture. In this study we determine whether human osteoarthritic (OA) and non-diseased passaged dedifferentiated chondrocytes will respond similarly. Human passaged chondrocytes were co-cultured for 4 weeks with primary bovine chondrocytes and their redifferentiation status was determined. Afterwards the cells were cultured either independently or in co-culture with cryopreserved passaged cells for functional analysis. The co-culture of passaged cells with primary chondrocytes resulted in reversion of their phenotype towards articular chondrocytes, as shown by increased gene expression of type II collagen and COMP, decreased type I collagen expression and extracellular matrix formation *in vitro*. Furthermore, this redifferentiation was stable, as those cells not only formed hyaline-like cartilage tissue when grown on their own but also they could induce redifferentiation of passaged chondrocytes in co-culture. These data suggest that it may be possible to use autologous chondrocytes obtained from osteoarthritic cartilage to form tissue suitable to use for cartilage repair. Copyright © 2009 John Wiley & Sons, Ltd.

Received 29 April 2009; Revised 15 September 2009; Accepted 15 October 2009

Keywords **tissue regeneration; co-culture; redifferentiation; osteoarthritis; chondrocytes**

1. Introduction

Avascularity, low cellularity and abundant extracellular matrix tissue are natural obstacles for the regeneration of articular cartilage. Treatment for diseases, characterized by irreparable cartilage degradation such as osteoarthritis, is mainly restricted to symptom management and/or replacement with a synthetic prosthesis. More recently there has been interest in developing ways to repair damaged cartilage using biological approaches. Treatments such as autologous chondrocyte implantation, mosaicoplasty or microfracture are promising but they have their limitations (Hunziker, 2002). Development of new approaches have been hampered by lack of access to sufficient numbers of cells that exhibit the phenotype of articular chondrocytes and do not show evidence of hypertrophic differentiation. One way to increase chondrocyte numbers is to culture the cells in monolayer *in vitro*. However, under these conditions the cells dedifferentiate, obtain a fibroblast-like morphology and lose their chondrocytic characteristics, as evidenced by down-regulation of collagen type II and aggrecan expression and increased expression of collagen type I (Holtzer *et al*., 1960). Concomitant with these changes is an inability of these cells to accumulate proteoglycan-rich extracellular matrix (ECM) and form cartilage tissue. Interestingly several studies have demonstrated that these passaged cells acquire some mesenchymal stem cell characteristics, suggesting that these cells have some plasticity (Tallheden *et al*., 2003).

Until a few years ago we considered cell fate and terminal differentiation to be unidirectional, but recent studies on cell reprogramming have changed this view

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dramatically (Park *et al*., 2008; Takahashi *et al*., 2007). Those experiments indicated that under the appropriate conditions the phenotype of a cell can be modulated, suggesting that it may be possible to generate cells suitable to use for cartilage repair. Several approaches have been used to modulate the phenotype of passaged chondrocytes. Among them, placing passaged cells in three-dimensional (3D) culture and growing the cells with or without growth factors and/or hormones has been the predominant approach utilized to stimulate redifferentiation of these cells (Chubinskaya *et al*., 2007; Francioli *et al*., 2007; Hauselmann *et al*., 1994; Schulze-Tanzil *et al*., 2002). However, the presence of exogenous growth factors such as members of the TGFβ superfamily may not always be desirable for tissue engineering (Chubinskaya *et al*., 2007; Yang *et al*., 2004). Under some conditions these treatments may trigger hypertrophy, as indicated by expression of collagen type X (Cancedda *et al*., 1995). An alternative way to induce redifferentaion is to use a co-culture approach. Cartilage is a recognized paracrine organ, as it secretes humoral factors which influence the proliferation and differentiation of neighbouring cells, as seen during cartilage degradation and wound healing (Bos *et al*., 2001; Dreier *et al*., 2001). *In vitro* culture studies have shown that cartilage tissue or isolated chondrocytes are able to influence other chondrocytes or mesenchymal progenitor cells (MPCs) via production of soluble paracrine factors (Ahmed *et al*., 2007; Hwang *et al*., 2007; Jikko *et al*., 1999; Locker *et al*., 2004). It has been reported that co-culture of cartilage tissue with MPCs delays progression towards hypertrophic differentiation by the latter cells (Ahmed *et al*., 2007).

We have shown previously that by providing proper environmental cues small numbers of bovine primary chondrocytes can induce stable redifferentiation in passaged bovine chondrocytes when placed in co-culture (Ahmed *et al*., 2009; Gan and Kandel, 2007). In this study we examine whether primary bovine chondrocytes can induce redifferentiation of human passaged chondrocytes, obtained from non-arthritic or OA cartilages, under the same co-culture conditions and if the induced change in phenotype is stable.

2. Materials and methods

2.1. Cell source, isolation and expansion

Articular cartilage was harvested under sterile conditions from bovine metacarpo-phalangeal joints (6–9 months old) within 24 h of death, as described previously (Gan and Kandel, 2007). Cartilage from two or three joints was combined to obtain sufficient cells for each experiment. Briefly, chondrocytes were isolated from the cartilage by enzymatic digestion with 0.1% collagenase A (Roche Diagnostics, Germany) for 18 h at 37 °C. The cells were resuspended in DMEM (Gibco, Rockville, MD, USA) containing 20% fetal bovine serum (FBS; HyClone, Logan, UT, USA), seeded in monolayer at a density of 2000 cells/cm^2 and grown at 37 °C, 95% relative humidity and 5% CO_2. When cells reached ~80% confluence, they were washed with PBS and harvested by incubating for 5 min with 1% trypsin–EDTA at 37 °C. Cells were resuspended in DMEM (20% FBS) and were cultured again at the same initial seeding density (bP2). Chondrocytes were obtained from human OA cartilage harvested from hip joints removed for end stage disease. Non-diseased chondrocytes were obtained from joints resected during the surgical removal of tumours. No adjuvant chemotherapy had been administered and the unaffected joint had been resected only to facilitate tumour removal. Use of these tissues was approved by the hospital research ethics board. The cells were isolated using the same enzymatic digestion procedure and were passaged as described above for the bovine cells. Aliquots of primary bovine (bP0) and twice passaged human (hP2) cells were also preserved in liquid nitrogen for later use.

2.2. Co-culture on filter inserts

P2 (1.6×10^6 cells/filter) and bP0 cells (0.4×10^6 cells/filter) were combined together and seeded onto type II collagen coated Millicell® culture plate inserts (60 mm^2; Millipore Co., Bedford, MA, USA). To grow 3D cultures, the inserts were placed in modified chondro-inductive medium (MCIM) which contained 1 : 1 F-12:DMEM (HG) (Cat. No. 11 965, Gibco), 20% FBS, 3 mM $MgCl_2$, 10 mM $NaHCO_3$ and 100 µg/ml ascorbic acid. The cells were grown for up to 4 weeks. As controls, P2 cells (1.6×10^6 cells/filter) and bP0 cells (0.4×10^6 cells/filter) were grown alone under the same conditions.

2.3. Cell labelling and subsequent culture conditions

bP0 cells were labelled with green fluorescent dye, carboxyfluorescein diacetate succinimidyl ester (25 µM CFDA SE, Vybrant™ in PBS; Molecular Probes, Eugene, OR, USA), as described previously (Ahmed *et al*., 2009). Briefly, unlabelled P2 cells (1.6×10^6 cells/filter) and CFDA-labelled bP0 cells (0.4×10^6 cells/filter) were combined together and were cultured as above. At the end of 4 weeks the newly formed tissue was digested with 0.1% collagenase A for 5 h. The cells were centrifuged, washed twice and resuspended in Ham's F-12 containing 5% FBS. The CFDA-labelled P0 cells and unlabelled P2 cells were sorted by flow cytometry, as described below. The sorted P2 cells (0.4×10^6 cells/filter) were then co-cultured with a new batch of cryopreserved P2 cells (1.6×10^6 cells/filter) for 4 additional weeks. As a control, cryopreserved P2 and sorted P2, now considered differentiated P2 cells (dP2) cells (1.6×10^6 cells/filter) were cultured separately (Figure 1).

DOI: 10.1002/term

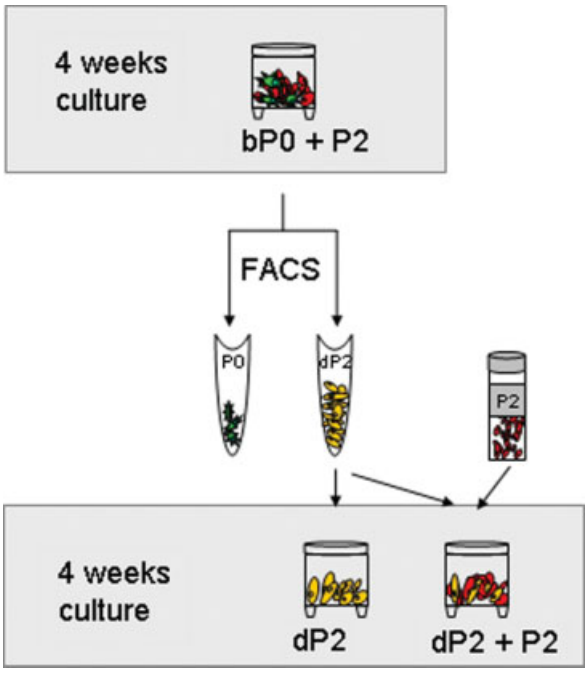

Figure 1. Schematic diagram of experimental set-up. CFDA-labelled primary bovine chondrocytes bP0 (green) were co-cultured with unlabelled human P2 cells (red) on filter inserts. After 4 weeks the labelled cells were separated from the unlabelled cells by FACS. The sorted unlabelled P2 cells (dP2) were cultured for 4 more weeks alone or in co-culture with cryopreserved P2 cells. Tissue analysis was carried out at the end of each culture cycle

2.4. Flow cytometry

The cells were harvested as described above and sorting was performed with a FACSAria cell sorter equipped with blue and red lasers (BD Bioscience, Franklin Lakes, NJ, USA). Doublets were excluded from cell populations by side scatter with SSC-H and FSC-W dot-plots. CFDA-positive cells were selected and sorted using the positive cell sorter's purity option, and sorted populations were reanalysed to confirm purity. The percentage ratio of P2 to P0 cells was determined.

2.5. Histological and immunohistochemical evaluation

The filter inserts were harvested, fixed in 10% neutral buffered formalin and embedded in paraffin. Sections (5 μm) were cut and stained with either toluidine blue to demonstrate the presence of sulphated proteoglycans or haematoxylin and eosin to visualize the tissue by light microscopy.

For immunohistochemical staining paraffin-embedded tissue sections were first digested with 0.4% w/v pepsin w/v (Sigma Aldrich, St. Louis, MO, USA), blocked with 5% v/v horse serum (Sigma Aldrich) and then incubated for 1 h at room temperature with antibody reactive with type II collagen (1/100, monoclonal, MS-306-P: Labvision, Fremont, CA, USA). This was followed by incubation with biotinylated horse anti-mouse secondary antibody (1/100 dilution; Vector Laboratories, Burlington, ON, USA). Vectasain Elite ABC kit (Vector Laboratories) and diaminobenzidine was used to visualize immunoreactivity. The tissue was counterstained with haematoxylin and examined under fluorescence microscope.

2.6. Tissue analysis

The harvested tissue was digested in papain (Sigma Aldrich; 40 μg/ml in 20 mM ammonium acetate, 1 mM EDTA and 2 mM DTT) for 48 h at 65 °C. Aliquots of this digest were assayed separately for DNA content and for accumulation of proteoglycan and collagen. DNA content was assayed to determine tissue cellularity, using the Hoechst dye 33 258 assay (Polysciences, Washington, PA, USA) and fluorometry (excitation, $\lambda = 365$ nm; emission, $\lambda = 458$ nm). Standard curves were generated using calf thymus DNA (Sigma Aldrich). Proteoglycan content was estimated by spectrophotometric quantification ($\lambda = 525$ nm) of sulphated glycosaminoglycans using the dimethylmethylene blue dye binding assay (Polysciences) (Goldberg and Kolibas, 1990). The standard curves were generated with bovine trachea chondroitin sulphate A (Sigma Aldrich). To determine the total collagen content, papain-digested tissue was hydrolysed with 6N HCl at 110 °C for 18 h and the hydroxyproline content was quantified spectrophotometrically ($\lambda = 560$ nm), using the chloramine-T/Ehrlich's reagent (Sigma Aldrich). Standard curves were generated using L-hydroxyproline (Sigma Aldrich). Collagen content was calculated by multiplying the hydroxyproline value by a factor of 10, as the hydroxyproline content represents approximately 10% of the total collagen weight (Bonaventure *et al*., 1994).

2.7. RNA isolation and reverse transcription

For isolation of total RNA frozen tissue was homogenized by mortar and pestle and RNA was extracted using Trizol® (Gibco). 0.5 μg RNA was reverse-transcribed with Superscript II reverse transcription kit (Invitrogen) in 20 μl of total volume of FirstStrand buffer and 0.1 M DTT for 50 min at 42 °C, followed by a 15 min extension period at 72 °C. Each reaction contained 40 U/μl recombinant ribonuclease inhibitor RNase OUT™, 50 μg/ml random hexamers, 10 mM dNTPs and 200 U SuperScript II enzyme.

2.8. Relative quantitative PCR

SYBR green dye I and Realplex2 Master Cycler (Eppendorf, Germany) were used for relative quantitative PCR (qPCR), according to the manufacturer's instructions, and the data were analysed with Mastercycler EP Realplex. Briefly, 1 μl cDNA was amplified in 20 μl final volume with 0.2 μM each primer suspended in 1× QuantiFast

DOI: 10.1002/term

SYBR Green Master Mix (Qiagen, Germany). Amplification parameters were identical for each primer pair; after the initial 10 min at 95 °C to activate the enzyme, 15 s denaturation at 95 °C was followed by 30 s annealing at 60 °C, and amplification data were collected for 40 cycles. Mean relative quantification (RQ) values from three independent experiments were calculated with the $\Delta\Delta$Ct method, using 18S rRNA as endogenous control and primary bovine chondrocytes as a calibrator. Primers were designed with Primer3 software (manufactured at Qiagen, Canada) and each primer pair's efficiency was tested with the formula $10^{(1/S)} - 1$, where S is the slope of the curve. Only primers with >90% efficiency were used.

2.9. Statistical analysis

Three to four independent sets of experiments from different samples were used for statistical analysis. Each sample was performed in triplicate and the data were pooled and presented as mean ± standard error of the mean (SEM). Results were analysed using a one-way analysis of variance (ANOVA) and all pairwise comparisons between groups were conducted using the Tukey *post hoc* test. $p \leq 0.05$ was considered to be statistically significant.

3. Results

3.1. Passaged human chondrocytes do not make cartilage tissue

To determine whether chondrocytes isolated from non-arthritic or OA human tissue retain the ability to form tissue after cell expansion, the freshly isolated cells were plated in monolayer culture at a low seeding density, passaged twice, and these (P2) cells were then placed in chondro-inductive 3D culture. After 4 weeks, histological assessment demonstrated that both the non-arthritic (Figure 2A, B) and OA (Figure 2E, F) passaged

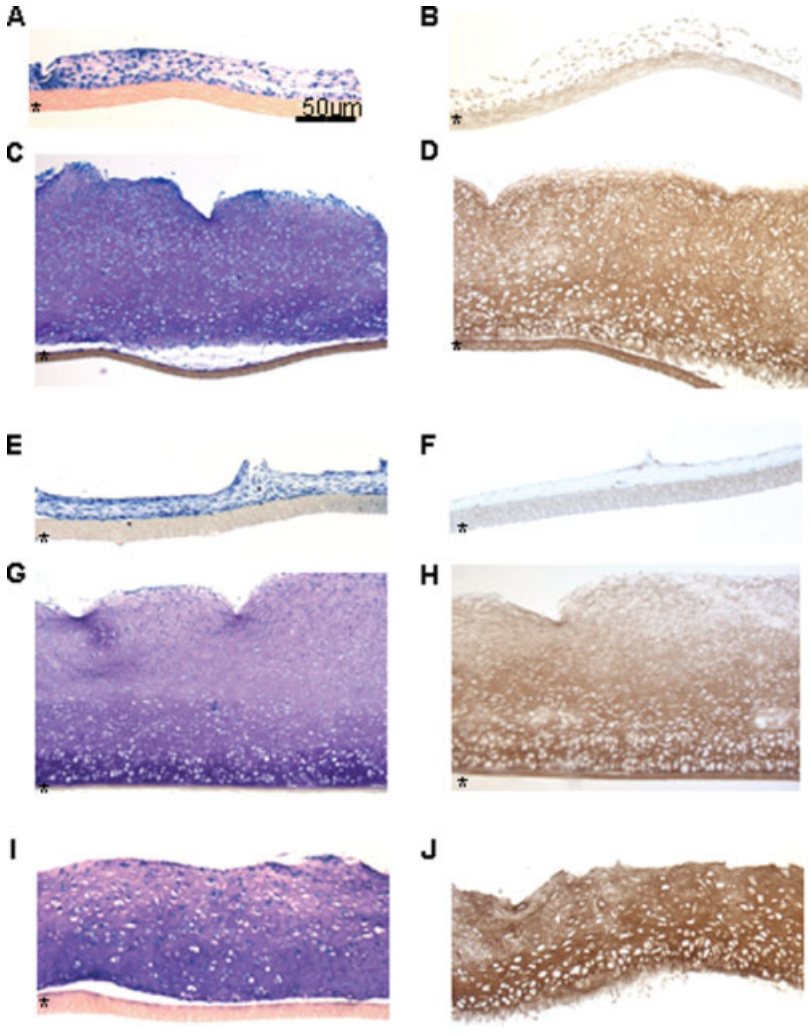

Figure 2. Microscopic examination of the tissue formed by the co-cultured passaged cells. Photomicrographs of the histological appearance of tissue formed by P2 chondrocytes from non-arthritic (A, B) and osteoarthritic (E, F) cartilage, cultured alone or with bovine P0 cells (C, D, non-arthritic; G, H, osteoarthritic) for 4 weeks. P0 cells were grown alone as a control (I, J). All images are the same magnification; A, C, E, G and I, toluidine blue; B, D, F, H and J, collagen type II immunostaining. *Filter inserts; $n = 4$

DOI: 10.1002/term

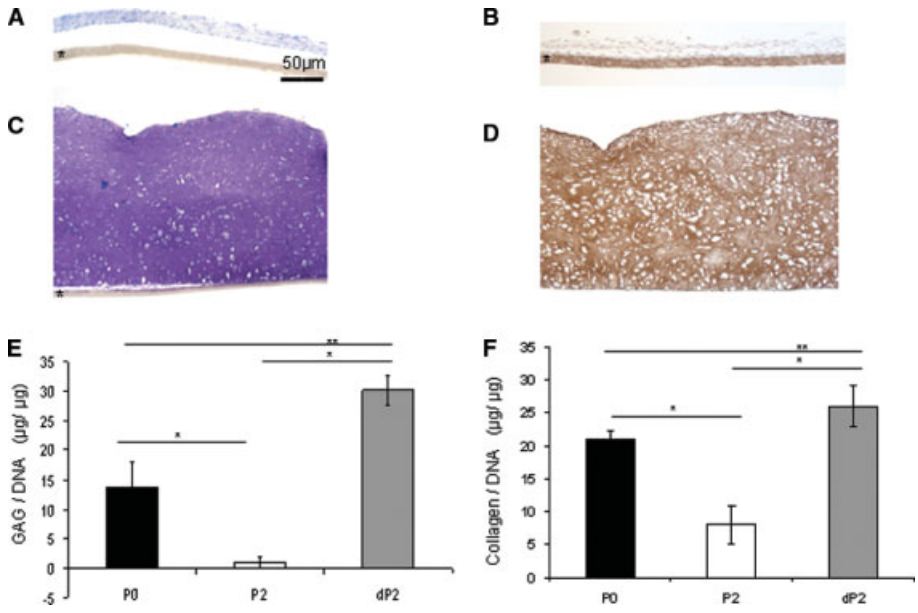

Figure 3. Microscopic and biochemical analysis of tissue formed by redifferentiated dP2 compared with that of P2. After co-culture with bP0 the FACS sorted redifferentiated dP2 were cultured alone (C, D) on filter culture for 4 weeks and compared with the cryopreserved dedifferentiated P2 cells grown alone (A, B). The tissues were processed for histological examination. dP2 cells accumulated proteoglycan and type II collagen-rich matrix, which contrasted to that accumulated by the dedifferentiated P2 culture. *Filter inserts. All photomicrographs are the same magnification; A, C, toluidine blue; B, D, collagen type II immunostaining. Both the proteoglycan (E) and collagen (F) contents of the tissues formed by dP2, P2 and P0 cells were quantified. The results are expressed as the mean ±SEM of four independent experiments, done in triplicate. *$p \leq 0.01$ compared to P2 cells; **$p \leq 0.01$ compared to P0 cells; $n = 12$

cells were unable to form cartilage tissue. The majority of the cells demonstrated full loss of the chondrocytic phenotype, as they had spindled morphology and did not accumulate extracellular matrix (ECM), whereas primary bovine (bP0) chondrocytes produced ECM containing type II collagen and proteoglycan (Figure 2I, J). Human P0 (hP0) chondrocytes were not able to form cartilage tissue when cultured in the same manner (data not shown).

3.2. Redifferentiation of passaged cells to chondrocyte-like cells

As we had shown previously that bP0 can induce redifferentiation of passaged bovine chondrocytes, we examined whether bP0 could induce tissue formation by passaged human chondrocytes (P2). The P2 cells from both non-arthritic and OA cartilage were independently co-cultured with bP0. Tissue was analysed after 4 weeks of culture, as by this time tissue formation was maximal under these culture conditions (data not shown). Histological examination of the resultant tissue showed accumulation of abundant ECM (Figure 2C, D, G, H). The tissue contained sulphated proteoglycans (Figure 2C, G) and type II collagen (Figure 2D, H). Cells obtained from non-arthritic and OA cartilage responded similarly, although the co-culture of bP0 and OA P2 cells appeared to generate slightly thicker tissue. All further experiments were performed using cells from OA cartilage only.

3.3. Analysis of redifferentiated chondrocytes

To assess the stability and extent of redifferentiation P2 cells from human OA cartilage were co-cultured with CFDA-labelled bP0 (ratio 4 : 1). After 4 weeks the *in vitro*-formed tissue was harvested and the cells were isolated by FACS sorting. Two populations were seen and an average of 20.6% ± 5.6 of the cells contained the CFDA dye. The purity of the two separated populations was confirmed by a second FACS analysis, which showed the unlabelled P2 fraction to be 99 ± 4.2% pure ($n = 4$). Henceforth, the sorted P2 cells of human OA tissue origin were designated as dP2 (differentiated P2) cells. To evaluate whether the change in phenotype as a result of co-culture with bP0 was stable, the dP2 cells were independently cultured for 4 more weeks. In contrast to the thin non-cartilaginous layer of tissue formed by P2 cells (Figure 3A, B) the dP2 formed thick cartilage-like tissue rich in proteoglycans (Figure 3C, D). The dP2 cells accumulated significantly more proteoglycan and collagen compared with the P2 cells ($p < 0.01$; Figure 3E, F). Interestingly, more tissue was formed by human dP2 cells than by primary bovine P0 chondrocytes alone.

3.4. Effect of redifferentiated chondrocytes on dedifferentiated cells

To determine whether the redifferentiated dP2 could be used to replace bP0 chondrocytes, dP2 were co-cultured with cryopreserved human P2 cells. Histological

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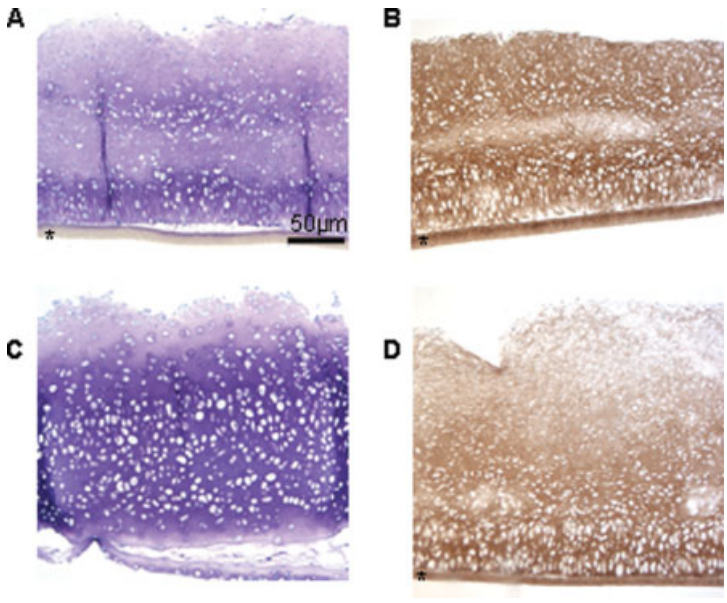

Figure 4. dP2 can induce redifferentiation of P2 cells. FACS sorted redifferentiated dP2 were co-cultured with cryopreserved P2 cells (A, B). As a control bP0 and P2 were grown together (C, D). Tissue formation by the co-culture of dP2 and P2 is comparable to the cartilage formed by co-culture of bP0 and P2, as demonstrated by toluidine blue staining (A, C) and collagen type II immunostaining (B, D). *Filter inserts; $n = 4$

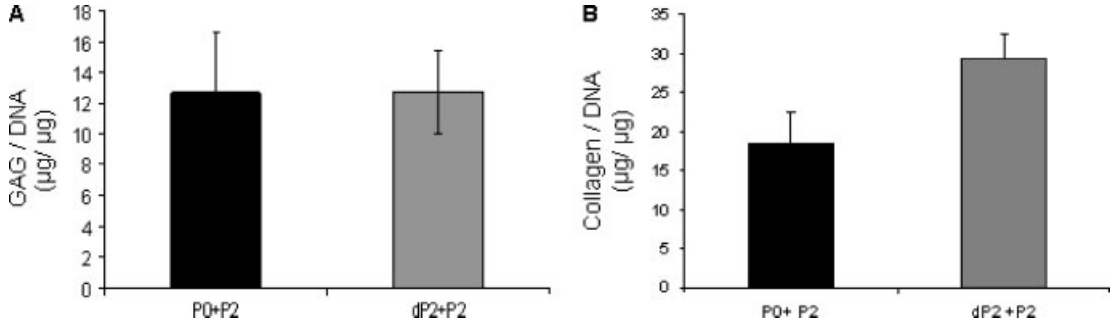

Figure 5. Quantification of matrix accumulation. FACS sorted redifferentiated dP2 were co-cultured with cryopreserved P2 cells. As a control, bP0 and P2 were grown together. The amount of proteoglycans (A) and collagen (B) accumulated in the tissue was quantified and corrected for cellularity. The results are expressed as the mean ±SEM of four independent experiments, each performed in triplicate; $n = 12$

(Figure 4) and biochemical (Figure 5) analysis of the tissue formed by co-culture of dP2 and P2 showed that the effect of dP2 cells on P2 was comparable to the effect of bP0 on P2 cells. There was no significant difference in ECM accumulation between the two types of co-cultures, although the tissue formed by the co-culture of dP2 and P2 appeared thicker than the tissue formed by co-culture of bP0 and P2 cells.

3.5. Analysis of phenotype at the molecular level

Gene expression analysis by real-time PCR showed different mRNA expression levels in passaged P2 and redifferentiated dP2 cells when compared with the primary P0 chondrocytes (Figure 6). dP2 gene expression did not entirely mimic that of P0 cells, which were set as the calibrator for relative quantification. Gene expression of the transcription factor *Sox9* in the dP2 cells appeared slightly higher when compared with P0 but the difference was not significant. Growth factor, *TGFβ-1*, showed significantly higher expression in dP2 cells when compared with P2 but the levels were similar to P0. Importantly, the low *Col2A1* expression observed in P2 cells was reversed in dP2 cells. *COMP* expression was not detected in P2 cells and in dP2 it was observed to be not significantly higher than the primary cells. P2 cells had 10-fold greater expression of *Col1A1* compared with bP0 cells and the dP2 cells showed significantly lower expression than P2 cells and was lower then bP0. dP2 cells showed no significant difference in *ColXA1* expression compared to bP0 cells, which is indicative of a cartilaginous phenotype without induction of hypertrophy. *BMP-2* expression was not significantly different in all three conditions. Gene expression of monocyte chemoattractant protein 1 (*MCP 1*) was significantly higher in dP2 cells when compared to P2 cells and was not significantly different from P0 cells.

DOI: 10.1002/term

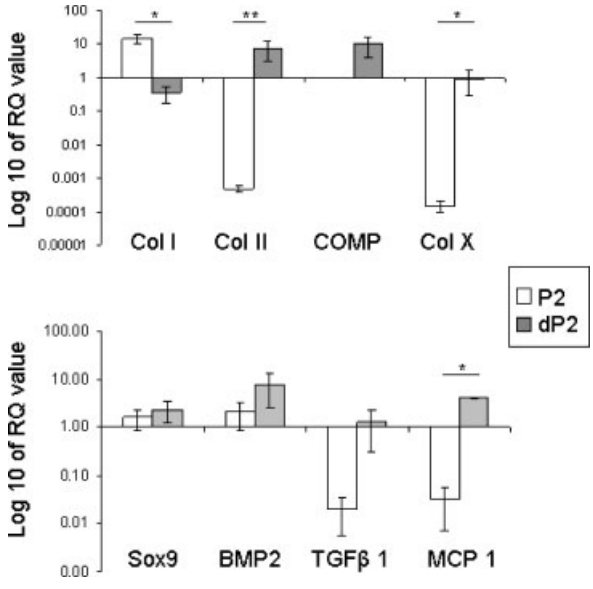

Figure 6. Differential gene expression of cartilage-related genes. Relative quantitative mRNA expression of selected genes was determined by real-time PCR and the ΔΔCt method using 18S RNA as the endogenous control. The results are expressed as the mean ± SEM of four independent experiments, done in triplicate. Expression level of the respective gene in P2 (white bars) and dP2 (grey bars) was compared with that of primary P0 cells, which was used as the calibrator ($n = 12$). *$p \leq 0.01$ and **$p \leq 0.05$ relative to P2; $n = 12$

4. Discussion

It has been shown by us and others that by changing the microenvironment and/or culture conditions chondrocytes can be induced to change their phenotype (Dell'Accio *et al*., 2003; Gan and Kandel, 2007). In this study, we were able to demonstrate that dedifferentiated human chondrocytes, which had lost the capacity to form cartilage-like tissue, redifferentiated in a co-culture system. Small numbers of primary bovine cells induced the passaged human chondrocytes, both osteoarthritic and non-arthritic, to redifferentiate, as indicated by increased gene expression of *COMP* and *Col2A1* and decreased expression of *Col1A1*. These cells accumulated proteoglycans and collagen and formed a continuous layer of cartilage-like tissue when cultured on their own. This change in phenotype was stable, as the cells could be used to redifferentiate other passaged human chondrocytes. The data suggest that it is now possible to develop a tissue-engineering approach for cartilage repair and to generate tissue using small numbers of human chondrocytes obtained from either non-arthritic or OA cartilage; this advance will facilitate the clinical application of this methodology.

Interestingly, both non-arthritic and OA passaged chondrocytes responded similarly to the presence of primary bovine chondrocytes. This observation was surprising because, although some studies have shown these cells to be phenotypically similar, numerous other studies have demonstrated major differences (Aigner *et al*., 2001; Gebhard *et al*., 2003; Lambrecht *et al*., 2008). For example, it has been demonstrated that OA and non-OA chondrocytes under certain conditions can respond differently to culture conditions (Yang *et al*., 2006). They also differ in the number of gap junctions (Marino *et al*., 2004) and the amount of ECM production (Yang *et al*., 2006). Their responses to the microenvironment and mechanical loading is also different (Salter *et al*., 2002). Another major difference between healthy and OA chondrocytes is in the organization of their vimentin cytoskeleton, which regulates focal adhesion formation and plays an integral part in cartilage homeostasis (Lambrecht *et al*., 2008). Yet despite the recognized differences in the chondrocytes in the two types of cartilage (non-arthritic and OA), cells isolated from both of these tissues were able to form tissue in our culture system. The reasons for this have not been elucidated but there are several explanations. It is possible that seeding the cells on type II collagen coated filter inserts may eliminate these phenotypic differences. Alternatively, it may be that the human and OA cells, while retaining their specific characteristics, are able to respond similarly to the signal(s) that induce(s) redifferentiation in this co-culture set-up.

The redifferentiated cells showed differential gene expression of selective molecules when compared with primary chondrocytes. Elevated mRNA levels of the cartilaginous structural molecules, *COL2A1* and *COMP*, as well as increased *COL2A1:COL1A1* ratio in dP2 cells indicate that their phenotype resembles primary chondrocytes (Vats *et al*., 2006). Interestingly, *MCP-1* also known as *CCL-2*, was found to be highly expressed in dP2 cells compared to either P2 or primary bP0 cells, the reason for this difference is not known. Although *MCP-1* function is to recruit monocytes, memory T cells and dendritic cells to the site of injury (Carr *et al*., 1998), it has been detected in MSCs after exposure to a chondrogenically favourable environment (Penolazzi *et al*., 2008). Interestingly, progenitor cells isolated from periosteum have been shown to express receptors for MCP-1 (Stich *et al*., 2008). Chondrocytes can express *MCP-1* mRNA in response to factors such as IL-1 released from synovium (Villiger *et al*., 1992). MCP-1 has been implicated in the repair process by regulating osteophyte development and recruitment of macrophages via hepatocyte growth factor; however, neither of these situations is occurring in this system (Dankbar *et al*., 2007). Our data and the published literature suggest that MCP-1 may have an as-yet unidentified role in cartilage redifferentiation and repair processes.

The mechanism underlying this redifferentiation of passaged chondrocytes has not yet been elucidated. There are several possibilities based on studies reported in the literature. One is that direct cell–cell communication may contribute to this process, as has been observed in co-cultures of osteoprogenitor cells and endothelial cells where connexin 43, a gap junction protein, was shown to be involved in the promotion of osteogenic differentiation of the progenitor cells (Guillotin *et al*., 2004). Alternatively, the matrix produced by the primary

DOI: 10.1002/term

chondrocytes may provide the required cues to the passaged cells. Microenvironment has been shown to play a role in determining cell phenotype for a number of cell types, including chondrocytes (Dell'Accio *et al*., 2003), melanoma cells (Postovit *et al*., 2006) leukaemia cells (Wei *et al*., 2008) or oligodendrocytes (Szuchet *et al*., 2000). Another possibility could be that primary chondrocytes release factors that influence signalling pathways that lead to cell differentiation. It is known that chondrocytes can secrete factors that inhibit the Erk 1/2 pathway in micromass cultures of MSCs (Hwang *et al*., 2007), and a decrease in Erk MAPKinase activity is important for the propagation of chondrogenesis in MSCs (Lee *et al*., 2004). It is likely that a combination of these regulatory mechanisms is critical here. Further investigation to delineate the humoral and/or cellular microenviroment that favours redifferentiation will help in reducing the time required for cell preparation or expansion in culture.

In summary, primary bovine chondrocytes possess the ability to induce conversion to a chondrogenic phenotype in dedifferentiated human cells. Secondly, the effect appears to be stable, because the redifferentiated cells form cartilage tissue similar to primary chondrocytes and in turn they induce the same redifferentiation in other passaged cells. Based on these characteristics, we propose that *in vitro* passaged osteoarthritic chondrocytes are a suitable source of cells for use in cartilage repair.

Acknowledgements

This project was supported by funding from CIHR. NA received fellowship support from the Samuel Lunenfeld Research Institute Special Opportunities Fund. We thank Mr Harry Bojarski and Ryding-Regency Meat Packers for providing the bovine tissues and Dr Yair Gortzak for help in obtaining human tissues. No part of this manuscript has been published elsewhere.

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DOI: 10.1002/term