Biorheology 41 (2004) 335–346
IOS Press

Cyclic, mechanical compression enhances chondrogenesis of mesenchymal progenitor cells in tissue engineering scaffolds

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Abstract. The effects of cyclic, mechanical compression on human bone marrow-derived mesenchymal progenitor cells undergoing chondrogenic differentiation were examined in this study. Mesenchymal progenitor cells were injected into cylindrical biodegradable scaffolds (hyaluronan–gelatin composites), cultured in a defined, serum-free chondrogenic medium and subjected to cyclic, mechanical compression. Scaffolds were loaded for 4 hours daily in the first 7 days of culture. At 1, 7, 14 and 21 days of culture, scaffolds were harvested for reverse transcriptase Polymerase Chain Reaction (RT-PCR), histology, quantitative DNA, proteoglycan and collagen analysis. Scaffolds loaded for 7 days showed a significant upregulation especially of chondrogenic markers (type II collagen, aggrecan; $p < 0.0001$). No significant difference could be found for DNA content between loaded samples and unloaded controls. At day 1 in culture no significant differences in proteoglycan- and collagen contents could be detected between unloaded and loaded samples. After 21 days the proteoglycan ($p < 0.001$) and collagen contents ($p < 0.0001$) were significantly higher in the loaded samples compared to unloaded controls. By histological analysis (toluidine blue) a higher amount of proteoglycan-rich, extracellular matrix production throughout the matrix could be detected for loaded samples compared to unloaded controls. This study indicates that cyclic, mechanical compression enhances the expression of chondrogenic markers in mesenchymal progenitor cells differentiated *in vitro* resulting in an increased cartilaginous matrix formation, and suggests that mechanical forces may play an important role in cartilage repair.

Keywords: Chondrogenesis, stem cell, bone marrow, mechanobiology, compression

1. Introduction

During the *in vivo* repair process of skeletal tissue, the tissue is subject to a variety of mechanical forces that may play an important role in the rate and/or quality of tissue repair. Mesenchymal progenitor cells (MPC) are identified as possible repair cells of various connective tissues, because of their potential to differentiate into a variety of cell types of the mesenchymal lineage [1,22,24–26,35,41,44,45,63]. Incorporation of MPC in suitable tissue engineering scaffolds and culture in chondrogenic medium are described in the literature [1,21,26,42,50,59]. MPCs can be harvested from bone marrow by a small puncture of the iliac crest of patients. In contrast to cartilage based repair this small procedure creates no additional harvest defect in the knee joint of the patients.

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Numerous publications showed the beneficial influence of compression (e.g., mechanical, hydrostatic, osmotic, shear, ultrasonic) on the chondrogenic differentiation of dedifferentiated chondrocytes [6,11, 14,20,33,34,37,46,56,61]. It has been shown that compressive stress is one of the most prominent extracellular factors that regulate biosynthetic and catabolic activities of chondrocytes [62] and that isolated chondrocytes respond to mechanical forces by upregulation of RNA levels for collagen, aggrecan and other matrix molecules [4]. Dynamic loading, which causes intra-tissue fluid flow and dynamic tissue deformation, stimulates the synthesis of matrix molecules at certain amplitudes and frequencies [16,28, 29,32,47].

In the case of mesenchymal progenitor cell mediated repair of skeletal tissue that involves a chondrogenic phase, such as fracture repair or articular cartilage regeneration, it is likely that mesenchymal progenitor cells are subjected to mechanical factors that will influence the quality of repair. Theoretical models have predicted the influence of biomechanical loading on tissue differentiation during skeletal growth and morphogenesis [11–13,53] suggesting that different loading conditions should result in different types of tissue. Hydrostatic pressure may encourage cartilage formation [8–10,52]. *In vivo* application of 2 MPa of hydrostatic pressure to mesenchymal cells in a conduction chamber, implanted in the tibiae of Sprague–Dawley rats was shown to promote cartilage formation [53]. Some forces enhance collagen production (direct compression) whereas others better stimulate proteoglycan deposition (hydrostatic pressure). Mass transfer for constructs under direct compression is expected to be better than for those cultured under hydrostatic pressure or static compression. Dynamic compression helps to alleviate diffusion limitations through a pressure gradient within the scaffold as well as by a secondary mixing effect on the surrounding medium. The compression of the scaffold creates a higher hydrostatic pressure at the center of the constructs than at the surface, which produces different fluid velocities within the construct as the applied load changes [15].

Although much attention has been given to the influences of bioactive factors on mesenchymal progenitor cell differentiation and proliferation, there are few studies that examine the effect of mechanical factors on these cells. A previous study showed the enhancement of chondrogenic differentiation of mesenchymal progenitor cells in aggregate culture by hydrostatic compression [2]. The influence of biomechanical loading on the expression of chondrogenic markers of mesenchymal progenitor cells from post-natal tissues undergoing differentiation in tissue engineering scaffolds is still unclear and should be analysed in this study.

2. Material and methods

Human bone marrow was obtained from the iliac crests of patients undergoing spine fusion. After Percoll gradient fractionation, Dulbecco's modified Eagle's Medium with 10% fetal bovine serum was added to the aspirate and 10×10^6 nucleated cells/100 mm dish were plated and grown at 37°C with 5% CO_2 until the cells reached 80% confluency. Adherent cell colonies were trypsinized, counted, and 2×10^6 synchronized mesenchymal progenitor cells were injected into cylindrical biodegradable scaffolds (hyaluronan–gelatin composites) with defined pore size (250 μm) and volume (70 mm^3) and cultured at 37°C/5% CO_2 in a defined medium, previously shown to induce chondrogenic differentiation of mesenchymal progenitor cells [1,24,63].

To apply cyclic, mechanical compression, scaffolds were transferred under sterile conditions in load definition units (for a total compression of 40%) at the bottom of 15 ml polypropylene tubes (sterile compression chambers) (NeoLab, Germany), which were filled with 4 ml fresh chondrogenic medium

Fig. 1. *In vitro* loading system for the application of cyclic mechanical compression in sterile culture conditions (left half). Sterile compression chambers with sterile bar magnets (right half). Note the electro-magnets below the sterile compression chamber.

(Fig. 1). A sterile bar magnet (NeoLab, Germany) (Fig. 1) with defined weight was inserted under sterile conditions into the tubes. The tubes were placed in a custom-built mechanical compression apparatus (Fig. 1) on electromagnets (RS Components, Germany), located within a temperature-controlled incubator (37°C). Cyclic, mechanical compression was applied to the scaffolds using a computer-controlled interface SIOS and the software Do-it (AK Modul-Bus GmbH Saerbeck, Germany). The samples were subjected to a continuous, uniaxial and unconfined compressive load using a sinusoidal waveform of 0.33 Hz and a peak stress of 7994 Pa over a period of 4 hours/day for the first 7 days. After each daily loading period, the scaffolds were cultured in chondrogenic medium without load application [1,24,63].

The scaffolds were kept in culture for a total of either 1, 7, 14 or 21 days, allowing time for chondrogenic differentiation, matrix formation and biosynthetic response [1,24,63], and then harvested for RT-PCR, biochemical and histological analysis. Each group of cell-scaffold composites subjected to cyclic biomechanical compression was compared with two sets of matched control scaffolds. To investigate the influence of magnetic effects the first set of control scaffolds was manipulated in the same manner as the experimental group, i.e. they were transferred into containers and placed in the loading chamber incubator for the same time period, but was not subjected to cyclic biomechanical compression. The second set of control scaffolds was left in standard culture conditions [1,24,63] without any manipulation. All experiments were performed with three sets of samples.

For RT-PCR RNA was extracted using RNA-Bee (Biozol, Eching, Germany) according to the single-step acid-phenol guanidinium method. cDNA synthesis was performed by using Superscript RNase H^- reverse transcriptase (Invitrogen, Karlsruhe, Germany) in the presence of oligo-dt primers. We performed and monitored RT-PCR by Light Cycler analysis with Light Cycler Kits (Roche, Mannheim, Germany). The level of each target gene was normalized to the reference gene glyceraldehyde phosphate dehydrogenase (GAPDH). The primers were as follows:

GAPDH *Forward* 5′-gAA ggT gAA ggT Cgg AgT C Reverse 5′-gAA gAT ggT gAT ggg ATT TC
Aggrecan *Forward* 5′-ACT TCC gCT ggT CAg ATg gA *Reverse* 5′-TCT CgT gCC AgA TCA TCA CC
Col 1 *Forward* 5′-Agg gCC AAg ACg AAg ACA TC *Reverse* 5′-AgA TCA CgT CAT CgC ACA ACA
Col 2 *Forward* 5′-TTC AgC TAT ggA gAT gAC AAT C *Reverse* 5′-AgA gTC CTA gAg TgA CTg Ag
Col 10 *Forward* 5′-gAg gAA gCT TCA gAA AgC Tg *Reverse* 5′-CTg gTT TCC CTA CAg CTg A.

DNA contents of the scaffolds were assayed using a two-step fluorometric assay. Scaffolds were prepared by individual digestion with Soluene 350 (Packard) for 1.5 hours at 60°C. Aliquots of the digest (100 μl) were mixed with 1 ml of diluted Hoechst 33258 Dye Solution (1 μg/ml). The fluorescence emission of the samples was measured with Cytofluor at $A_{360\ \mathrm{nm}}$ excitation, $A_{460\ \mathrm{nm}}$ emission with a gain of 70. A standard curve was produced with calf thymus DNA (Sigma) and used to determine the DNA content of the experimental samples.

The glycosaminoglycan content of the scaffolds, used as an indicator of proteoglycan production, was quantified with a spectrophotometric assay as described elsewhere [2]. Scaffolds were digested in papain solution overnight at 60°C. After adding 100% ethanol the mixture was incubated at -20°C for 3 hours. After centrifugation (14000 rpm, 10 minutes) the supernatant was discarded and the pellets resuspended in 200 μl distilled water. Samples were allowed to precipitate in Safranin O reagent on nitrocellulose filter (BioRad, USA), which covered wells in a dot-blot apparatus (BioRad, USA). The precipitates were collected on the nitrocellulose filter and then dissolved in 10% cetylpyridinium chloride at 37°C for 20 minutes. The absorbance of the liquid was spectrophotometrically read at 536 nm. For the experimental groups and the unloaded controls the proteoglycan-content of the negative controls (cell-free matrix) was substracted to get a minimal estimation of the produced extracellular proteoglycan even when a complete degradation of the matrices would influence the measurement.

The hydroxyproline content of the scaffolds was measured as described elsewhere [2]. Scaffolds were hydrolyzed with 6 M HCl at 108°C for 24 hours. The hydrolysates were repeatedly dried with resuspension in distilled water in between each drying cycle to wash out the acid. The neutralized samples were resuspended in hydroxyproline buffer. Freshly prepared, chloramine T solution was added and the vortexed suspension incubated for 20 minutes at room temperature. Perchloric acid and freshly prepared P-dimethylaminobenzaldehyde solution was then added and the suspension incubated at 60°C for 20 minutes with intermittent shaking. The absorbance was spectrophotometrically read at 560 nm. Cis-4-hydroxy-d-proline was used to construct a standard curve. The collagen content was calculated by multiplying the total hydroxyproline content by assay with a conversion factor ($\times$7.5). For the experimental groups and the unloaded controls the content of the negative controls (cell-free matrix) was substracted to get a minimal estimation of the produced extracellular collagen even when a complete degradation of the matrices would influence the measurement.

Histological (toluidine blue) and immunohistochemical (type II collagen) analysis was performed on frozen sections of 10% formaldehyde fixed materials as described elsewhere [2]. Serial sections (12 μm) of all samples were cut and sections stained with toluidine blue for histological evaluation. For immunohistochemical analysis, unstained sections were washed in phosphate buffered saline (PBS). Blocking of nonspecific antibody binding was achieved by incubating the slides with bovine serum albumin (BSA) (15 minutes). The sections were digested for 30 minutes with 1% pronase in 1% BSA, PBS and then for 30 minutes with chondroitinase ABC to facilitate the collagen antibody access to the extracellular matrix. The sections were probed with anti-collagen antibody raised against type II collagen (II-II6B3, obtained from the Developmental Studies Hybridoma Bank). Visualization was achieved with a monoclonal fluorescein-conjugated secondary antibody.

The expression of molecular markers, DNA, proteoglycan and collagen contents in the scaffolds with and without application of mechanical compression were compared between the groups at each time point using two way ANOVA with Fisher's post hoc test for multiple comparisons. The tests were performed using Sigma Stat™ Software for Windows Version 2.03, SPSS Inc. Significance was accepted at a level of $p < 0.05$.

3. Results

There were no differences in expression of chondrogenic markers (aggrecan, type II collagen, type X collagen and type I collagen) between the non-loaded control scaffolds and the control scaffolds cultured without any manipulation (data not shown). The non-loaded controls showed a statistically significant increase in chondrogenic marker expression, especially type II collagen, after 21 days in culture compared to unloaded controls after 1 day in culture ($p < 0.0001$) (Fig. 2).

The aggrecan expression (major component of hyaline cartilage) of the 7 days loaded scaffolds showed a 98% increase versus non-loaded control at day 7 ($p < 0.001$), an even higher increase of aggrecan expression (loaded versus non-loaded group) was found at day 14 of culture (+386%; $p < 0.0001$). At day 21 the aggrecan expression of loaded scaffolds showed an increase of 92% over unloaded controls (Fig. 3; $p < 0.001$).

The type II collagen expression (major component of hyaline cartilage) of 7 days loaded versus non loaded scaffolds showed a significant increase at day 14 (687%; $p < 0.0001$) and day 21 in culture (90%; $p < 0.0001$) compared to unloaded samples (Fig. 3).

The type I collagen expression of 7 days loaded scaffolds showed a significant increase at day 14 (221%) and at day 21 in culture (76%) over non-loaded controls (Fig. 2; $p < 0.001$), but did not reach the increased expression levels of hyaline cartilage specific markers.

No statistical significant differences in type X collagen expression were found between any loaded group and its controls at either day 7, 14 or day 21 (Fig. 3; $p = 0.45$).

No statistical significant differences in DNA content were found in unloaded and loaded groups during chondrogenesis (day 1 versus day 21). Also, no differences were found between the non-loaded control scaffolds (those manipulated in the same way as the loaded scaffolds but kept non-loaded for 4 hours per day in the compression chamber incubator) and the control scaffolds cultured without any manipulation (data not shown). There were no significant differences in the DNA contents between the loaded and non-loaded scaffolds (Fig. 4).

The proteoglycan content was significantly higher after 21 days in culture compared to the corresponding samples on day 1 ($p < 0.0001$) (Fig. 4). At day 1 no significant differences in proteoglycan content could be detected between unloaded and loaded samples ($p = 0.23$), however after 21 days in culture a significant increase for loaded samples could be seen ($p < 0.001$) (Fig. 4).

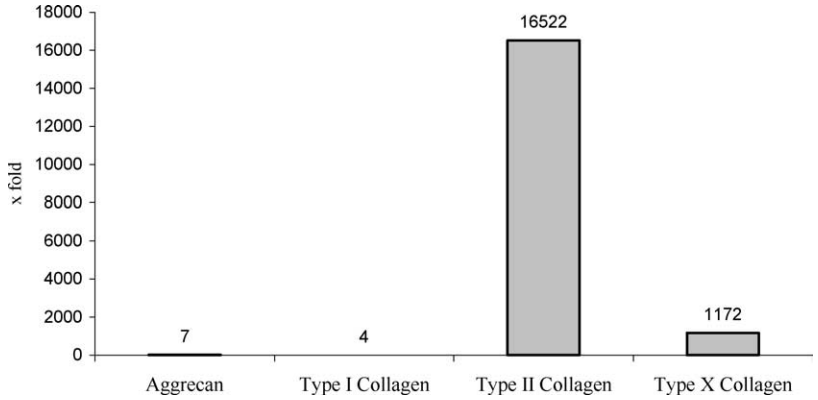

Fig. 2. Increase of relative chondrogenic marker expression (x fold) of unloaded group (day 21) versus unloaded group (day 1).

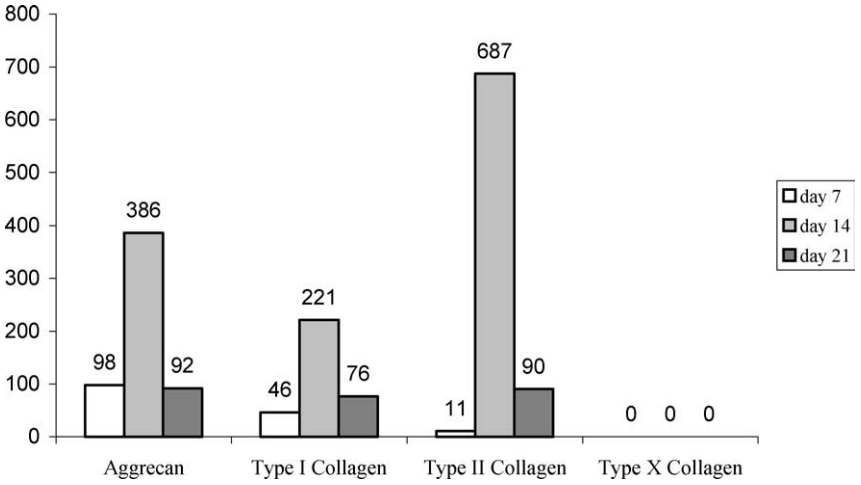

Fig. 3. Increase of relative chondrogenic marker expression of loaded versus unloaded group at days 7/14/21 in percent (%).

The collagen content was significantly higher after 21 days in culture compared to the corresponding samples on day 1 ($p < 0.0001$) (Fig. 4). At day 1 no significant differences in collagen content could be detected between unloaded and loaded samples ($p = 0.12$), however after 21 days in culture a significant increase for loaded samples could be observed ($p < 0.0001$) (Fig. 4).

The histological and immunohistochemical analysis confirmed chondrogenic differentiation after 21 days by extracellular, metachromatic matrix deposition (toluidine blue stained sections) and positive staining for collagen type II in the cell-scaffold constructs (Fig. 5). A comparison of toluidine blue stained sections indicated no qualitative microscopic differences between 14 (not shown) or 21 days in culture. The 7 days loaded scaffolds had a quantitative increase in extracellular matrix production (increased metachromatic, extracellular matrix deposition) throughout the scaffolds, especially in the center of the scaffolds, in comparison with non-loaded controls after 14 days and 21 days in culture (Fig. 5). Immunohistochemical staining of the scaffolds indicated positive expression of collagen type II in all control and experimental groups, confirming successful chondrogenic differentiation of mesenchymal progenitor cells (hyaline cartilage-like appearance) (not shown). No qualitative difference in collagen distribution was found between the controls and the loaded scaffolds by immunohistochemistry.

4. Discussion

This study was performed using a custom-built system (Fig. 1), which allowed application of cyclic mechanical compression to cell – scaffold constructs under sterile culture conditions. The applied mechanical compression was completely transferred to mesenchymal progenitor cells in tissue engineered scaffolds over a pressure range between 0 and 8 kPa. Mesenchymal progenitor cells injected into biodegradable hyaluronan–gelatin-composite scaffolds, which had proven ability to allow the differentiation of mesenchymal progenitor cells to cartilage [1], were used to study the effects of mechanical loading during chondrogenesis. For the first 7 days no extracellular deposition of cartilage-like matrix was detected in the cell-matrix constructs [1]. Therefore this timeframe was used in this study to selectively load mesenchymal progenitor cells and not chondrocytic cells.

In an *in vitro* aggregate chondrogenesis model, the progenitor cells are densely packed as they aggregate and condense prior to cartilaginous matrix production [24,63]. The condensation of progenitor

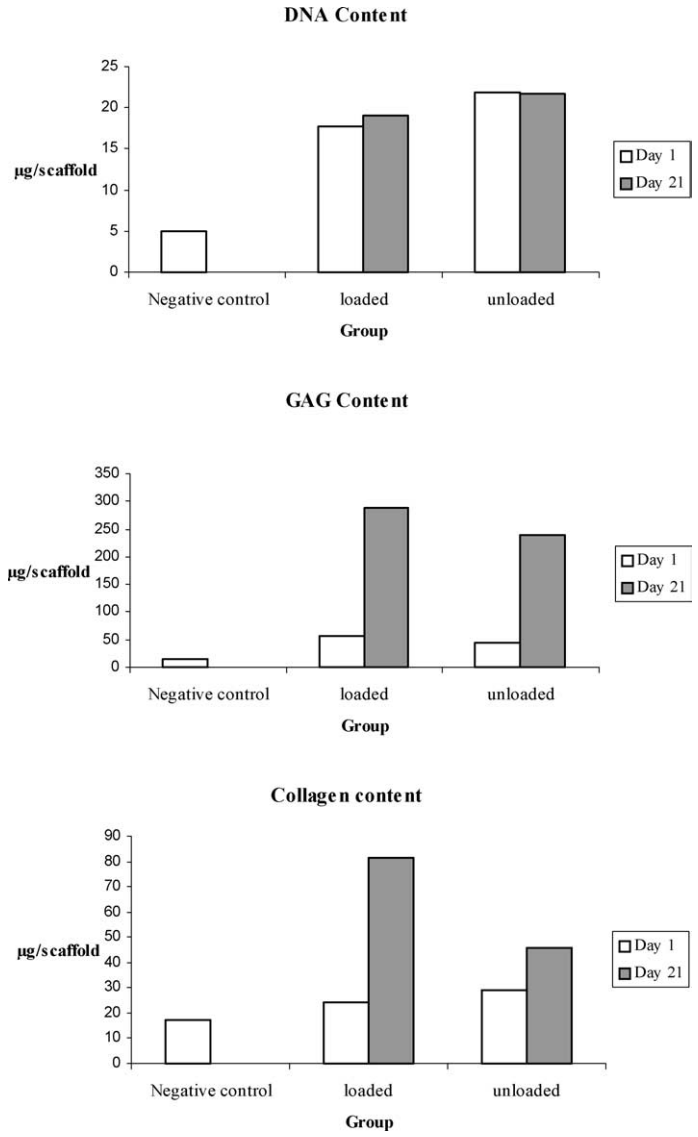

Fig. 4. DNA, proteoglycan and collagen content of loaded and unloaded samples (mean of 3 samples). Negative control represents cell free scaffolds.

cells also occurred when the progenitor cells were injected in biodegradable hyaluronan–gelatin composite matrices as seen in the present study. Densely packed cells were seen early in articular cartilage regeneration, when the subchondral bone is penetrated and marrow cells migrate into the defect [49], during the development of limbs and also in fracture repair [5,23]. The influence of mechanical forces on progenitor cells during cell condensation and chondrogenesis is not well understood. This is partly due to the complex distribution of stresses and strains in each of these situations. For example, the form of mechanical stress in a joint *in vivo* is more complex than simply mechanical compression. According to Mow et al. [38], the typical total stress on the surface of human joints ranges from 2.96 to 9.86 MPa. A complex state of pressure in the interstitial fluid and spatially varying normal and shear stresses in

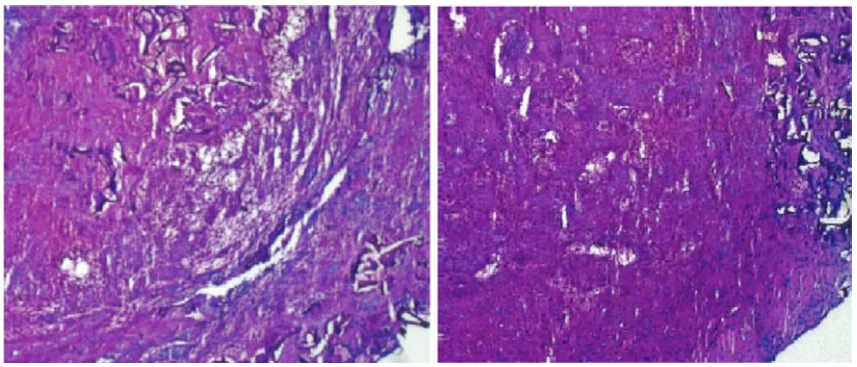

Fig. 5. Toluidine blue stained sections of unloaded (left half) and loaded scaffolds (right half) after 21 days in culture.

the solid matrix are developed in response to the surface stress. According to the mechanical tissue differentiation theory [11,12] an undifferentiated mesenchymal tissue will differentiate into fibrous tissue, cartilage or bone depending on the mechanical load stimulation. Although mechanical compression does not represent the mechanical forces seen by cartilage *in vivo*, compression and its effects on chondrogenic differentiation of mesenchymal progenitor cells were examined selectively in the present study.

More information on the effect of mechanical load exists for chondrocytes and cartilage tissue. Loads below the normal physiological levels appear to stimulate catabolic effects in chondrocytes [3]. In contrast, a load within the physiologic range stimulates production of cartilaginous matrix [36]. Supraphysiologic loads appear to result in tissue damage [19]. An upregulation of aggrecan and type II collagen mRNA expression in bovine chondrocytes by the application of hydrostatic pressure has been described [58]. Convective transport is a critical component of nutrient transfer, especially in the early stages of culture when the constructs are still very porous. In developing soft tissues, the extracellular matrix is soft, so the total hydrostatic stress consists mainly of pressure in the fluid phase [11].

The highly porous tissue engineering scaffolds used in the present study cannot bear physiologic load levels in its initial state. Therefore a lower compression of 8 kPa was applied. A cyclic load of 0.33 Hz for 4 hours per day was used because this rate was previously shown to stimulate extracellular matrix synthesis of articular chondrocytes [48,51]. The results of the present study indicate that with low levels of mechanical compression a similar effect occurred with differentiating cells.

Mechanical influences are also found on the precursor cells of forming limbs [54,55]. Takahashi et al. [54] demonstrated that significant differences exist between undifferentiated mesenchymal cells and differentiated chondrocytes in terms of their responses to compressive forces. They reported enhanced chondrogenesis of mouse embryonic limb bud mesenchymal cells following static compressive force. In contrast, static compressive force was shown to down-regulate cartilage matrix deposition [32] and aggrecan expression [31] in mature articular cartilage. In a separate study of chick limb bud cells, Elder et al. [17,18] found that cyclic compressive loading enhanced chondrogenic differentiation, but they did not find an influence of static loading. They noted an increase in proteoglycan production when differentiating chick limb bud cells were loaded with cyclic compressive displacement. However, they also noted an increase in the total number of cartilaginous nodules in their cultures of cells entrapped in agarose, thus the increase may have been due to increased recruitment of cells into the chondrogenic lineage, rather than to an increase in per cell production. In the present study no significant differences could be seen for DNA-content between loaded samples and unloaded controls, consistent with the liter-

ature for chondrocyte loading, which postulated that mechanical stressed cells may direct their anabolic efforts towards production and maintenance of matrix rather than cell proliferation [40,60].

Hydrostatic pressure in the physiologic range (5.03 MPa) was chosen in a previous study to evaluate its effect on chondrogenesis of mesenchymal progenitor cells [2]. A significant increase in proteoglycan- and collagen-content without change in DNA-content was detected in the loaded groups compared to unloaded controls. In the present study similar results were seen. The mechanical compression applied on multiple days for 4 hours per day significantly increased the chondrogenic marker expression and matrix deposition. The increase in type II collagen and aggrecan expression were noted in the loaded groups after 14 and 21 days in culture. This finding is similar to the effects of cyclical loading on articular cartilage explants or chondrocytes in culture: increased proteoglycan synthesis and overall matrix production [3,7,57]. However, the effect of loading on mature chondrocytes may be more selective. Carver and Heath [14] reported that the matrix produced by mature equine chondrocytes subjected to 6.90 MPa of intermittent loading increased collagen content but there was no significant difference in the glycosaminoglycan content. At a lower level of pressure (3.45 MPa), the collagen concentration did not change but a small increase in glycosaminoglycan concentration was noted. In contrast, the present study had shown that low level compression (8 kPa) resulted in a significant enhancement of chondrogenic differentiation of human mesenchymal progenitor cells, seen by an increase in type II collagen and aggrecan expression and followed by cartilage specific protein production.

In the present study, both the loaded and non-loaded scaffolds were cultured in conditions that promote the chondrogenic differentiation of mesenchymal progenitor cells. Therefore, the influence of mechanical compression on the initiation of chondrogenic differentiation was not directly explored. Nevertheless, the results of this study point to the importance of load during skeletal tissue regeneration, such as is found during the chondrogenic differentiation of mesenchymal cells in fracture repair and articular cartilage regeneration. The study suggested that an appropriate load applied to mesenchymal progenitor cells in the differentiation process enhance the chondrogenic marker expression. The increase in chondrogenic marker expression may facilitate the formation of an appropriate matrix, improving the final quality of either fracture callus or repair cartilage of osteochondral defects.

Direct compression enhances the access of mesenchymal progenitor cells to TGF-β1 (mass transfer) and accelerates the biosynthetic response of the mesenchymal progenitor cells to the growth factor. Because soluble low molecular weight polypeptides such as TGF-β1 tend to be cleared rapidly after intra-articular administration, this ability of dynamic compression to accelerate transport into the tissue is of potential clinical significance [6,30,39,43]. In an *in vitro* system mechanical load might also increase the access of exogenous added TGF-β1 and other supplements to the center of the cell-matrix constructs. This could explain the increase in extracellular matrix deposition especially in the center of the scaffolds seen in the present study.

Mechanical compression was the only component of loading considered in this study. Although mechanical compression is an important component of load seen by cartilage in a joint, additional studies will be needed to evaluate the effects of shear and complex mechanical deformation on progenitor cells undergoing chondrogenic differentiation. The mechanism by which the mechanical loading is sensed by the differentiating cells is presently unknown, as is the mechanism by which the load is transduced into an increase in chondrogenic marker expression and extracellular matrix deposition.

The present study described a system that allows the study of the effects of cyclic mechanical compression on differentiating mesenchymal cells in tissue engineering scaffolds. This system can be used to elicit mechanoreceptors and signal transduction pathways influenced by mechanical forces during chon-

drogenesis of mesenchymal cells. Furthermore the system can be used to optimize tissue engineered implants for cartilage repair.

Acknowledgements

The authors thank Tanja Weinfurtner and Daniela Drenkard for their excellent technical assistance.

References

[1] P. Angele, R. Kujat, M. Nerlich, J. Yoo, V. Goldberg and B. Johnstone, Engineering of osteochondral tissue with bone marrow mesenchymal progenitor cells in a derivatized hyaluronan–gelatin composite sponge, *Tissue Eng.* **5** (1999), 545–554.
[2] P. Angele, J.U. Yoo, C. Smith, J. Mansour, K.J. Jepsen, M. Nerlich and B. Johnstone, Cyclic hydrostatic pressure enhances the chondrogenic phenotype of human mesenchymal progenitor cells differentiated in vitro, *J. Orthop. Res.* **21** (2003), 451–457.
[3] N.M. Bachrach, W.B. Valhmu, E. Stazzone, A. Ratcliffe, W.M. Lai and V.C. Mow, Changes in proteoglycan synthesis of chondrocytes in articular cartilage are associated with the time-dependent changes in their mechanical environment, *J. Biomech.* **28** (1995), 1561–1569.
[4] E.J. Blain, S.J. Gilbert, R.J. Wardale, S.J. Capper, D.J. Mason and V.C. Duance, Up-regulation of matrix metalloproteinase expression and activation following cyclical compressive loading of articular cartilage in vitro, *Arch. Biochem. Biophys.* **396** (2001), 49–55.
[5] M.E. Bolander, Regulation of fracture repair by growth factors, *Proc. Soc. Exp. Biol. Med.* **200** (1992), 165–170.
[6] L.J. Bonassar, A.J. Grodzinsky, E.H. Frank, S.G. Davila, N.R. Bhaktav and S.B. Trippel, The effect of dynamic compression on the response of articular cartilage to insulin-like growth factor-I, *J. Orthop. Res.* **19** (2001), 11–17.
[7] N. Burton-Wurster, M. Vernier-Singer, T. Farquhar and G. Lust, Effect of compressive loading and unloading on the synthesis of total protein, proteoglycan, and fibronectin by canine cartilage explants, *J. Orthop. Res.* **11** (1993), 717–729.
[8] M.D. Buschmann, Y.A. Gluzband, A.J. Grodzinsky and E.B. Hunziker, Mechanical compression modulates matrix biosynthesis in chondrocyte/agarose culture, *J. Cell. Sci.* **108** (1995), 1497–1508.
[9] M.D. Buschmann, E.B. Hunziker, Y.J. Kim and A.J. Grodzinsky, Altered aggrecan synthesis correlates with cell and nucleus structure in statically compressed cartilage, *J. Cell. Sci.* **109** (1996), 499–508.
[10] M.D. Buschmann, Y.J. Kim, M. Wong, E. Frank, E.B. Hunziker and A.J. Grodzinsky, Stimulation of aggrecan synthesis in cartilage explants by cyclic loading is localized to regions of high interstitial fluid flow, *Arch. Biochem. Biophys.* **366** (1999), 1–7.
[11] D.R. Carter, G.S. Beaupre, N.J. Giori and J.A. Helms, Mechanobiology of skeletal regeneration, *Clin. Orthop.* (1998), S41–55.
[12] D.R. Carter, P.R. Blenman and G.S. Beaupre, Correlations between mechanical stress history and tissue differentiation in initial fracture healing, *J. Orthop. Res.* **6** (1988), 736–748.
[13] D.R. Carter and M. Wong, Mechanical stresses and endochondral ossification in the chondroepiphysis, *J. Orthop. Res.* **6** (1988), 148–154.
[14] S.E. Carver and C.A. Heath, Increasing extracellular matrix production in regenerating cartilage with intermittent physiological pressure, *Biotechnol. Bioeng.* **62** (1999), 166–174.
[15] E.M. Darling and K.A. Athanasiou, Articular cartilage bioreactors and bioprocesses, *Tissue Eng.* **9** (2003), 9–26.
[16] T. Davisson, S. Kunig, A. Chen, R. Sah and A. Ratcliffe, Static and dynamic compression modulate matrix metabolism in tissue engineered cartilage, *J. Orthop. Res.* **20** (2002), 842–848.
[17] S.H. Elder, S.A. Goldstein, J.H. Kimura, L.J. Soslowsky and D.M. Spengler, Chondrocyte differentiation is modulated by frequency and duration of cyclic compressive loading, *Ann. Biomed. Eng.* **29** (2001), 476–482.
[18] S.H. Elder, J.H. Kimura, L.J. Soslowsky, M. Lavagnino and S.A. Goldstein, Effect of compressive loading on chondrocyte differentiation in agarose cultures of chick limb-bud cells, *J. Orthop. Res.* **18** (2000), 78–86.
[19] T. Farquhar, Y. Xia, K. Mann, J. Bertram, N. Burton-Wurster, L. Jelinski and G. Lust, Swelling and fibronectin accumulation in articular cartilage explants after cyclical impact, *J. Orthop. Res.* **14** (1996), 417–423.
[20] M.J. Grimshaw and R.M. Mason, Modulation of bovine articular chondrocyte gene expression in vitro by oxygen tension, *Osteoarthritis Cartilage* **9** (2001), 357–364.
[21] N. Jaiswal, S.E. Haynesworth, A.I. Caplan and S.P. Bruder, Osteogenic differentiation of purified, culture-expanded human mesenchymal stem cells in vitro, *J. Cell. Biochem.* **64** (1997), 295–312.

[22] Y. Jiang, B.N. Jahagirdar, R.L. Reinhardt, R.E. Schwartz, C.D. Keene, X.R. Ortiz-Gonzalez, M. Reyes, T. Lenvik, T. Lund, M. Blackstad, J. Du, S. Aldrich, A. Lisberg, W.C. Low, D.A. Largaespada and C.M. Verfaillie, Pluripotency of mesenchymal stem cells derived from adult marrow, *Nature* **418** (2002), 41–49.
[23] S. Jingushi, M.E. Joyce and M.E. Bolander, Genetic expression of extracellular matrix proteins correlates with histologic changes during fracture repair, *J. Bone Miner. Res.* **7** (1992), 1045–1055.
[24] B. Johnstone, T.M. Hering, A.I. Caplan, V.M. Goldberg and J.U. Yoo, In vitro chondrogenesis of bone marrow-derived mesenchymal progenitor cells, *Exp. Cell. Res.* **238** (1998), 265–272.
[25] B. Johnstone and J. Yoo, Bone marrow-derived mesenchymal progenitor cells, *Methods Mol. Biol.* **137** (2000), 313–315.
[26] B. Johnstone and J.U. Yoo, Autologous mesenchymal progenitor cells in articular cartilage repair, *Clin. Orthop.* (1999), S156–162.
[27] K. Kaarniranta, M. Elo, R. Sironen, M.J. Lammi, M.B. Goldring, J.E. Eriksson, L. Sistonen and H.J. Helminen, Hsp70 accumulation in chondrocytic cells exposed to high continuous hydrostatic pressure coincides with mRNA stabilization rather than transcriptional activation, *Proc. Natl. Acad. Sci. USA* **95** (1998), 2319–2324.
[28] K. Kaarniranta, M.A. Elo, R.K. Sironen, H.M. Karjalainen, H.J. Helminen and M.J. Lammi, Stress responses of mammalian cells to high hydrostatic pressure, *Biorheology* **40** (2003), 87–92.
[29] K. Kaarniranta, C.I. Holmberg, M.J. Lammi, J.E. Eriksson, L. Sistonen and H.J. Helminen, Primary chondrocytes resist hydrostatic pressure-induced stress while primary synovial cells and fibroblasts show modified Hsp70 response, *Osteoarthritis Cartilage* **9** (2001), 7–13.
[30] H.M. Karjalainen, R.K. Sironen, M.A. Elo, K. Kaarniranta, M. Takigawa, H.J. Helminen and M.J. Lammi, Gene expression profiles in chondrosarcoma cells subjected to cyclic stretching and hydrostatic pressure. A cDNA array study, *Biorheology* **40** (2003), 93–100.
[31] Y.J. Kim, A.J. Grodzinsky and A.H. Plaas, Compression of cartilage results in differential effects on biosynthetic pathways for aggrecan, link protein, and hyaluronan, *Arch. Biochem. Biophys.* **328** (1996), 331–340.
[32] Y.J. Kim, R.L. Sah, A.J. Grodzinsky, A.H. Plaas and J.D. Sandy, Mechanical regulation of cartilage biosynthetic behavior: physical stimuli, *Arch. Biochem. Biophys.* **311** (1994), 1–12.
[33] J. Klein-Nulend, J.P. Veldhuijzen, R.J. van de Stadt, G.P. van Kampen, R. Kuijer and E.H. Burger, Influence of intermittent compressive force on proteoglycan content in calcifying growth plate cartilage in vitro, *J. Biol. Chem.* **262** (1987), 15490–15495.
[34] H.S. Lee, S.J. Millward-Sadler, M.O. Wright, G. Nuki, R. Al-Jamal and D.M. Salter, Activation of Integrin-RACK1/PKCalpha signalling in human articular chondrocyte mechanotransduction, *Osteoarthritis Cartilage* **10** (2002), 890–897.
[35] D.P. Lennon, S.E. Haynesworth, D.M. Arm, M.A. Baber and A.I. Caplan, Dilution of human mesenchymal stem cells with dermal fibroblasts and the effects on in vitro and in vivo osteochondrogenesis, *Dev. Dyn.* **219** (2000), 50–62.
[36] L. Lippiello, C. Kaye, T. Neumata and H.J. Mankin, In vitro metabolic response of articular cartilage segments to low levels of hydrostatic pressure, *Connect. Tissue Res.* **13** (1985), 99–107.
[37] S.J. Millward-Sadler, M.O. Wright, H. Lee, H. Caldwell, G. Nuki and D.M. Salter, Altered electrophysiological responses to mechanical stimulation and abnormal signalling through alpha5beta1 integrin in chondrocytes from osteoarthritic cartilage, *Osteoarthritis Cartilage* **8** (2000), 272–278.
[38] V.C. Mow, A. Ratcliffe and A.R. Poole, Cartilage and diarthrodial joints as paradigms for hierarchical materials and structures, *Biomaterials* **13** (1992), 67–97.
[39] M. Ohno, J.P. Cooke, V.J. Dzau and G.H. Gibbons, Fluid shear stress induces endothelial transforming growth factor beta-1 transcription and production. Modulation by potassium channel blockade, *J. Clin. Invest.* **95** (1995), 1363–1369.
[40] M.J. Palmoski and K.D. Brandt, Effects of static and cyclic compressive loading on articular cartilage plugs in vitro, *Arthritis Rheum.* **27** (1984), 675–681.
[41] M. Pittenger, A. Mackay, S. Beck, R. Jaiswal, R. Douglas, J. Mosca, M. Moorman, D. Simonetti, S. Craig and D. Marshak, Multilineage potential of adult human mesenchymal stem cells, *Science* **284** (1999), 143–148.
[42] M.S. Ponticiello, R.M. Schinagl, S. Kadiyala and F.P. Barry, Gelatin-based resorbable sponge as a carrier matrix for human mesenchymal stem cells in cartilage regeneration therapy [In Process Citation], *J. Biomed. Mater. Res.* **52** (2000), 246–255.
[43] J.P. Pujol, P. Galera, S. Pronost, K. Boumediene, D. Vivien, M. Macro, W. Min, F. Redini, H. Penfornis, M. Daireaux et al., Transforming growth factor-beta (TGF-beta) and articular chondrocytes, *Ann. Endocrinol.* **55** (1994), 109–120.
[44] M. Reyes and C.M. Verfaillie, Characterization of multipotent adult progenitor cells, a subpopulation of mesenchymal stem cells, *Ann. N. Y. Acad. Sci.* **938** (2001), 231–233; discussion 233–235.
[45] J.J. Rogers, H.E. Young, L.R. Adkison, P.A. Lucas and A.C. Black Jr., Differentiation factors induce expression of muscle, fat, cartilage, and bone in a clone of mouse pluripotent mesenchymal stem cells, *Am. Surg.* **61** (1995), 231–236.
[46] R.L. Sah, J.Y. Doong, A.J. Grodzinsky, A.H. Plaas and J.D. Sandy, Effects of compression on the loss of newly synthesized proteoglycans and proteins from cartilage explants, *Arch. Biochem. Biophys.* **286** (1991), 20–29.

[47] R.L. Sah, Y.J. Kim, J.Y. Doong, A.J. Grodzinsky, A.H. Plaas and J.D. Sandy, Biosynthetic response of cartilage explants to dynamic compression, *J. Orthop. Res.* **7** (1989), 619–636.
[48] D.M. Salter, S.J. Millward-Sadler, G. Nuki and M.O. Wright, Differential responses of chondrocytes from normal and osteoarthritic human articular cartilage to mechanical stimulation, *Biorheology* **39** (2002), 97–108.
[49] F. Shapiro, S. Koide and M.J. Glimcher, Cell origin and differentiation in the repair of full-thickness defects of articular cartilage, *J. Bone Joint Surg. Am.* **75** (1993), 532–553.
[50] M. Sittinger, C. Perka, O. Schultz, T. Haupl and G.R. Burmester, Joint cartilage regeneration by tissue engineering, *Z. Rheumatol.* **58** (1999), 130–135.
[51] R.L. Smith, S.F. Rusk, B.E. Ellison, P. Wessells, K. Tsuchiya, D.R. Carter, W.E. Caler, L.J. Sandell and D.J. Schurman, In vitro stimulation of articular chondrocyte mRNA and extracellular matrix synthesis by hydrostatic pressure, *J. Orthop. Res.* **14** (1996), 53–60.
[52] J.K. Suh, G.H. Baek, A. Aroen, C.M. Malin, C. Niyibizi, C.H. Evans and A. Westerhausen-Larson, Intermittent sub-ambient interstitial hydrostatic pressure as a potential mechanical stimulator for chondrocyte metabolism, *Osteoarthritis Cartilage* **7** (1999), 71–80.
[53] M. Tagil and P. Aspenberg, Cartilage induction by controlled mechanical stimulation in vivo, *J. Orthop. Res.* **17** (1999), 200–204.
[54] I. Takahashi, G.H. Nuckolls, K. Takahashi, O. Tanaka, I. Semba, R. Dashner, L. Shum and H.C. Slavkin, Compressive force promotes sox9, type II collagen and aggrecan and inhibits IL-1beta expression resulting in chondrogenesis in mouse embryonic limb bud mesenchymal cells, *J. Cell Sci.* **111** (1998), 2067–2076.
[55] K. Takahashi, T. Kubo, Y. Arai, I. Kitajima, M. Takigawa, J. Imanishi and Y. Hirasawa, Hydrostatic pressure induces expression of interleukin 6 and tumour necrosis factor alpha mRNAs in a chondrocyte-like cell line, *Ann. Rheum. Dis.* **57** (1998), 231–236.
[56] K. Takahashi, T. Kubo, K. Kobayashi, J. Imanishi, M. Takigawa, Y. Arai and Y. Hirasawa, Hydrostatic pressure influences mRNA expression of transforming growth factor-beta 1 and heat shock protein 70 in chondrocyte-like cell line, *J. Orthop. Res.* **15** (1997), 150–158.
[57] P.A. Torzilli, R. Grigiene, C. Huang, S.M. Friedman, S.B. Doty, A.L. Boskey and G. Lust, Characterization of cartilage metabolic response to static and dynamic stress using a mechanical explant test system, *J. Biomech.* **30** (1997), 1–9.
[58] T. Toyoda, B.B. Seedhom, J. Kirkham and W.A. Bonass, Upregulation of aggrecan and type II collagen mRNA expression in bovine chondrocytes by the application of hydrostatic pressure, *Biorheology* **40** (2003), 79–85.
[59] M.R. Urist, Y. Terashima, M. Nakagawa and C. Stamos, Cartilage tissue differentiation from mesenchymal cells derived from mature muscle in tissue culture, *In Vitro* **14** (1978), 697–706.
[60] G.P. van Kampen, J.P. Veldhuijzen, R. Kuijer, R.J. van de Stadt and C.A. Schipper, Cartilage response to mechanical force in high-density chondrocyte cultures, *Arthritis Rheum.* **28** (1985), 419–424.
[61] M.O. Wright, K. Nishida, C. Bavington, J.L. Godolphin, E. Dunne, S. Walmsley, P. Jobanputra, G. Nuki and D.M. Salter, Hyperpolarisation of cultured human chondrocytes following cyclical pressure-induced strain: evidence of a role for alpha 5 beta 1 integrin as a chondrocyte mechanoreceptor, *J. Orthop. Res.* **15** (1997), 742–747.
[62] Q.Q. Wu and Q. Chen, Mechanoregulation of chondrocyte proliferation, maturation, and hypertrophy: ion-channel dependent transduction of matrix deformation signals, *Exp. Cell. Res.* **256** (2000), 383–391.
[63] J.U. Yoo, T.S. Barthel, K. Nishimura, L. Solchaga, A.I. Caplan, V.M. Goldberg and B. Johnstone, The chondrogenic potential of human bone-marrow-derived mesenchymal progenitor cells, *J. Bone Joint Surg. Am.* **80** (1998), 1745–1757.