Journal of Musculoskeletal Research, Vol. 15, No. 1 (2012) 1250006 (9 pages)

DOI: 10.1142/S0218957712500066

World Scientific
www.worldscientific.com

CHONDROCYTE RESPONSE TO TENSILE AND COMPRESSIVE CYCLIC LOADING MODALITIES

Srinath Kamineni

Elbow Shoulder Research Centre
Department of Orthopaedics and Sports, Kentucky Clinic
740 South Limestone Lexington, Kentucky 40502, USA
srinathkamineni@gmail.com

Zubair Wani

Faculty of Life Sciences, Stopford Building
Oxford Road Manchester, M13 9PL
zubair.wani13@gmail.com

Zong-Ping Luo

Department of Orthopedic Surgery, The First Affiliated Hospital
Soochow University, Suzhou, Jiangsu, 215007, China
zongping_luo@yahoo.com

Yoshida Ruriko

Assistant Professor in the Department of Statistics
University of Kentucky, 805A Patterson Office Tower Lexington, KY 40506-0027
Ruriko.yoshida@uky.edu

Kai-Nan An

Biomechanics Laboratory, Division of Orthopedic Research
Mayo Clinic, Rochester, Minnesota 55905 USA
An.kainan@mayo.edu

Received 14 November 2011
Accepted 12 March 2012
Published 9 May 2012

Correspondence to: Srinath Kamineni Department of Orthopaedics and Sports, Kentucky Clinic, 740 South Limestone, Lexington, Kentucky 40502, USA.

ABSTRACT

There is very little data addressing cartilage response to tensile forces, and no literature attempts to correlate compressive with tensile modalities. Our hypothesis was that the cyclic compression and tension modulate chondrocyte matrix proteoglycan synthetic response differently. Porcine chondrocytes cultured to confluence on a flexible membrane were subjected to cyclic compression (Group A: 13 KPa at 1 Hz) or tension (Group C: 10% strain at 1 Hz) for 16 or 32 h; while controls not subjected to any force were kept (Group B). The chondrocytes were then stained with alcian blue and stained areas quantified with confocal microscopy and image processing software. Two-factor ANOVA with post-hoc tests (Scheffe and Bonferroni) statistical analysis were used. Proteoglycan staining covered 46% (range 28%−61%) and 39% (range 26%−49%) of the surface area following 32 and 16 h of compression respectively, 23% (range 15%−49%) for control, and 19% (range 10%−29%) and 16% (range 9%−25%) following 16 and 32 h tension respectively. Proteoglycan content following all compressions was significantly greater than with cyclic tension or control ($p < 0.0001$). Our data demonstrate that chondrocytes cultured *in vitro* respond to compression distinctly different from tension and that it is highly sensitive to mechanical loading, with rapid adaptation to its mechanical environment. These results imply that cartilage grown in culture, with the intention of transplantation, may structurally benefit from an environment of cyclic loading at higher frequencies.

Keywords: Porcine cartilage; Cyclic loading; Synthetic response.

INTRODUCTION

One function of articular cartilage is to withstand and distribute mechanical loads in diarthrodial joints, and its structure is uniquely designed for this purpose. Articular cartilage is a heterogenous tissue consisting of a large volume of extracellular matrix, predominantly consistent of proteoglycan and collagen, with a sparsely interspersed population of specialized cells, the chondrocytes. Human articular cartilage is known to undergo numerous sequential structural and compositional changes during its natural history.[19] Osteoarthritis is the result of one such sequence of changes that appears to culminate in a dysfunctional joint that causes significant morbidity. Although the cause of osteoarthritis is not fully understood, a greater understanding of its effect on cartilage is emerging. One such effect of the osteoarthritic process is that the cartilage structure, mainly due to the extra-cellular matrix component, is less able to resist mechanical forces. This change in behavior has been characterized as decreases in stiffness or modulus of elasticity of the osteoarthritic cartilage under these loading conditions, and a propensity to lose its morphological integrity and for its cells to become swollen.[15] The relative importance of these different loading modalities in the genesis and propagation of the pathological process is unclear. However, normal, non-arthritic, articular cartilage experiences the same forces of similar magnitudes yet its structure allows normal function to continue.

Thus far, the vast majority of investigations have focused on compressive mechanisms, either static or dynamic, because of the dominant compressive loading in the physiological articular joints at the gross level.[6,7,11,16,18] However, it is arguable that at the cellular level, due to non-homogeneity and anisotropy of cartilage matrix, embedded chondrocytes are likely to be loaded by compression in one direction while by tension in another direction. Compression and tension may have profound differences in regulating the metabolic activity of chondrocytes. To date, scant data have been reported addressing cartilage

response to tensile forces,[6,7,12] and no reports attempt to correlate the two loading modalities, within the same experiment.

The purpose of this study was to examine whether or not the cartilage matrix and chondrocytes are regulated differently by compression and tension. Our hypothesis was that cyclic compression and tension would result in a different chondrocyte response in the downstream synthesis of matrix proteoglycan, a major extracellular matrix molecule in resisting compression.

MATERIALS AND METHODS

Porcine chondrocytes were harvested under sterile conditions from the patellofemoral joints of six 3-month-old piglets. The cartilage was sharply separated from the underlying femoral condyle, and divided into 1 mm^3 blocks, which were rinsed three times in refrigerated calcium magnesium free saline (CMFS) solution. The cartilage was then immersed in a collagenase II-trypsin in EDTA solution (2 mg/ml collagenase II [Sigma Chemical] and 2 mg/ml trypsin [Gibco BRL, Grand Island, NY, USA]), diluted into a calcium magnesium free saline solution with added glucose (CMFSG) for 1 h. The suspension was centrifuged and the remaining pellet of cells and cartilage was resuspended, for a final digestion stage, in a solution of collagenase II (0.5 mg/ml of CMFSG) for 4 h. The suspension was constantly and gently agitated by pipetting, until it became cloudy. At this stage, the suspension was visualized under light microscopy, to confirm that a significant cell suspension was achieved. The solution volume was doubled by adding 50 ml of Dulbecco's Modified Eagle's Medium (DMEM−BioWhittaker, Wlakersville, Maryland), in order to reduce the enzymatic reaction, and the solution was centrifuged for 10 minutes at 1000 revolutions per minute, at 5°C. The pellet of cells deposited at the bottom of the centrifuge tube was then resuspended in a culture medium (DMEM solution with high glucose and L-glutamine; 10% Fetal Bovine Serum [FBS], 1% Fungizone [Gibco BRL, Grand Island, NY, USA], Penicillin 100 U/ml [Sigma St. Louis, MO], Streptomycin 100 μg/ml [Sigma St. Louis, MO], and ascorbic acid 30 μg/ml [Sigma St. Louis, MO]), by gentle pipetting. Cell density within the re-suspension was measured with a hematocytometer and 4×10^6 cells were placed onto each culture membrane (Bioflex, Flexcell International Coporation). The membranes were pronectin coated and had a total growth surface area of 57.75 cm^2. The total volume of culture medium on each membrane was standardized to 5 ml, and the suspension fluid was topped with a DMEM solution with added 10% FBS, 1% Fungizone, 1% Penicillin, and 50 μg ascorbic acid.

The culture, following a single passage, was undisturbed for 5 days in a 37°C incubator, at which point there was microscopic evidence demonstrating a significant proportion of cell attachment to the underlying membrane, (> 50 cells per 100 times magnified viewing field). Pilot studies with fluid change at times between one and four days resulted in a vast majority of cells removed with the elutant. Fluid was subsequently changed three times each week with that of the same composition described above. Cells were allowed to grow until there was a demonstrable monolayered cartilage sheet, as viewed with light-microscopy, which took an average of 12 weeks (range 10−14 weeks). Samples of the multi-layered cell sheets were stained with eosin and thickness measured with a Zeiss Axiopan 100 fluorescent microscope. Once a multi-layered sheet was achieved, testing and staining were performed.

Testing in compression and tension was conducted utilizing previously validated techniques.[14,17] Dynamic compression of the gas phase above the fluid covering the cell sheet was applied

as described by Saris *et al.*[14] with a square wave-form. The chondrocyte sheet was exposed to dynamic pressurization contained in a rigid container, at 1.3 Kpa and at a rate of 1 Hz square wave form. Cyclic compression was conducted for 16 h, or 32 h. Following testing the culture medium was changed and the cartilage sheets of all test specimens were left undisturbed for 16 h in a 37°C incubator, prior to staining. Cyclic tensile testing was conducted utilizing the experimental device described by Stroetz *et al.*[17] Pilot studies were performed with well membrane tensile strains of 10%, 15%, and 25%. The former two strain conditions resulted in higher than normal numbers of detached/dead cells compared to equivalent control specimens, with the former also sustaining observable areas of delamination from the underlying membrane. Only the 10% strain value, with a square wave-form, allowed testing to be completed for 16 h and 32 h without visible cell death beyond that seen with control wells. Each strain condition was repeated with three membranes from the same animal, and from each animal.

Immediately prior to staining, the specimens were rinsed twice with refrigerated PBS, fixed for 10 minutes in Kahle fixative, and rinsed twice with refrigerated PBS. Each well was filled with 5 ml of filtered pH1 Alcian blue solution for 16 h. The cultures were then rinsed with double distilled water and allowed to air dry. Once fully dry the samples were stored in a darkened chamber (temperature 30°C), for between 8 and 16 h, until the whole specimen cohort was ready to be viewed under the microscope at the same occasion.

Microscopy of the specimens was performed with an Axioplan 2 microscope (Carl Zeiss, Oberkochen, Germany) at a magnification of 100 times. Images were captured with an AxioCam digital camera (Carl Zeiss, Oberkochen, Germany) and stained area measurements were performed with a KS400 image analysis software (Carl Zeiss, Oberkochen, Germany). Statisical analysis was performed with a two factor ANOVA, which, if significance was present, was followed by Scheffe and Bonferroni post-hoc tests to detect differences between the timing and loading conditions. The person responsible for the strain application and the person responsible for the subsequent staining and area measurement were blinded to each others' work.

RESULTS

A mechanically confluent tissue was produced in 12 weeks. The tissue thickness was constant with little variation between different wells and between different areas within each well, 90 μm ($\pm$10%).

Control cell sheets produced distinct and consistent areas stained for proteoglycan, in the form of small islands stain (Fig. 1). Areas of stain appeared to become more darkly stained, confluent, and larger in area with compression, when compared with the control specimens, and became more lightly stained with tension. However this was a qualitative observation and no statistical significance was achieved for staining density.

The area of cell sheet that was covered by staining was measured by a commercially available product. Alcian blue staining covered 46% (range 28%–61%) of the membrane surface area following 32 h of compression testing (Fig. 1), with large tracts having become confluent. Following 16 h of compression 39% (range 26%–49%) of the surface area was covered with staining, which resembled islands, seen in the control specimens, having become confluent into small tracts of staining (Fig. 1). Control specimens had an average of 23% (range 15%–49%) of the surface area covered by stain (Fig. 1). 16 h of tension resulted in an average of 19% (range 10%–29%) (Fig. 1), and 32 h of tension resulted in an average of 16% (range 9%–25%) of surface area staining (Fig. 1). Proteoglycan content following cyclic

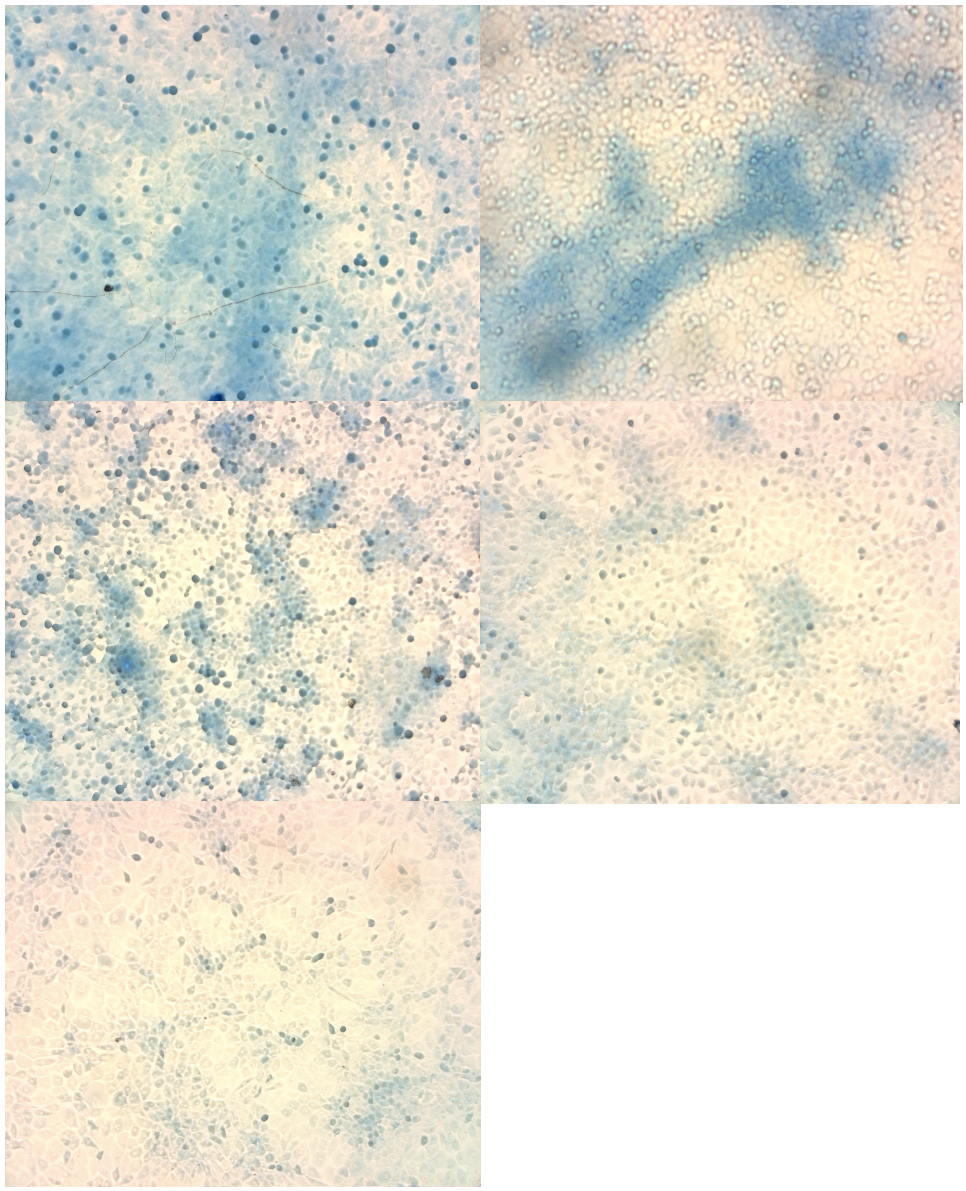

Fig. 1 Upper left: Alcian blue staining after 32 h compression — 13 KPa, 1 Hz, square wave (×100); Upper right: Alcian blue staining after 16 h compression — 13 KPa, 1 Hz, square wave (×100); Middle left: Alcian blue staining in control specimen (×100), Middle right: Alcian blue staining after 16 h tension — 10%, 1 Hz, square wave (×100); Lower left: Alcian blue staining after 32 h tension — 10%, 1 Hz, square wave (×100).

compression, both 16 and 32 h, was statistically significantly greater than with cyclic tension or control ($p < 0.0001$). No statistically significant increase was observed between the two time periods in compression. The observation between the two time intervals suggested that the islands of staining enlarged and, in some cases, became confluent in some areas. No statistical differences were found between the control and tension groups, although there was a visible decrease in the staining density and the size of stained areas between the control and tension groups and between the two time periods of tension. These results are summarized in Fig. 2.

Group	Area of cell sheet covered by Alcian Blue stain (%)		
	Mean	Range (lower limit)	Range (upper limit)
32 hours Compression	46	28	61
16 hours Compression	39	26	49
Control	23	15	49
16 hours Tension	19	10	29
32 hours Tension	16	9	25

Fig. 2 Table summarizing the percentage cell sheet stained by Alcian blue stain measured in compressive, tensile and control groups in the study.

DISCUSSION

Precise knowledge of the effects of an altered biomechanical environment is needed to understand the biological response of the chondrocytes. In our study we measured the synthetic activity of chondrocytes, experiencing either tensile or compressive forces of similar order of magnitudes. This study demonstrates that cartilage cultured *in vitro* responds to cyclic compression distinctly differently from cyclic tension. Compressive forces at a high frequency increased while tensile forces did not change proteoglycan synthesis as compared to controls. Differences in experimental conditions, loading frequency, and force magnitudes reported in different studies makes a direct comparison of results difficult from existent literature.[1,3,6–9,11]

Chondrocyte response to compressive stimulus has been variously investigated in the reported literature. Cyclic compression of chondrocytes of frequencies of 0.1 Hz has been shown to inhibit proteoglycan synthesis, whereas frequencies of 0.01 Hz increased the proteoglycan synthesis.[3] This finding is in marked contrast to this current study in which we used an even higher frequency of 1 Hz, in a square wave-form mode, which showed an increased proteoglycan synthesis compared to control specimens. Since we focused on the differences between tensile and compressive loads, we did not test the frequency dependence. However, our findings agree with previous results that predict a time dependent effect of compressive loading. A shorter duration of static compressive force has been associated with increased synthetic activity of chondrocytes as opposed to longer periods of compressive force, which has resulted in reduced proteoglycan synthesis.[1] This phenomenon has been explained by an initial phase, in which loading pressure dominates. With increasing loading duration, fluid exudation from the cell causes a transfer of the load to the solid component of the cell resulting in increased cell strains. In our experiment pressure presumably did not lead to fluid exudation due to the high frequency used, which may explain the increased synthetic activity observed.[1]

High frequency cyclic tensile forces [30 cycles per minute] have been shown to lead to an increased proteoglycan destruction as opposed to low frequency cyclic tension force [1 cycle/4min].[6] These findings do not correlate with our results, showing a proteoglycan content with both tensile loading conditions that did not differ from the controls statistically, although our visual observations tend to support these data. A clinical correlate is that ageing[19] and osteoarthritic cartilage[5] lose the ability to remain avascular and also lose proteoglycans and other glycosaminoglycans. In addition to a decrease in proteoglycan

synthesis with tension, there appears to be a trend towards decreasing synthetic activity with increased duration of cyclic tensile loading. However, this latter observation was not found to be statistically significant but an observable trend was present.

There are some limitations to our study. Due to our experimental set-up we were restricted to the use of compressive stresses of 1.3 kPa, which do not correspond to the 5–10 Mpa that have been estimated for the lower limb joints (hip/knee/ankle) during standing.[10] However, in our experiment we were looking at the effect of changing the mechanical environment in terms of tensile and compressive forces, as opposed to attempting a direct physiological correlate. Furthermore, 1.3 kPa of compression does not markedly differ from previously published studies.[6] Tension was tested at a 10% strain due to pilot data that showed that at this level of strain, compared to 15% and 25%, the quantity of cell death did not vary from that normally observed in control specimens. A 25% strain was also noted to cause focal areas of delamination from the underlying membrane. Additionally, 10% tensile strain has been utilized and corroborated as a safe experimental testing value in previous studies,[6] although the tensile forces experienced *in vivo* have yet to be elucidated.

The majority of literature reports cartilage models using rabbit chondrocytes, a smaller biological system, the results of which are more difficult to transfer to human cartilage. We elected to use porcine chondrocytes since they represent a biological system of cartilage more relevant to humans. In addition, the current environment of donor shortages has lead to a resurgent interest in xenografts, with porcine grafts already in clinical use.[20]

Chondrocytes grown in monolayers have a tendency for dedifferentiation and for the production of abnormal matrix components, such as Type I and Type III collagen,[2] as is the case with osteoarthritic cartilage in which types I and X collagen are produced.[5] However, this process of dedifferentiation has been demonstrated in cells grown following multiple passages at low densities.[2] We followed a single passage protocol that has previously been demonstrated to produce a proteoglycan and collagen density comparable to that of replacement cartilage.[4]

Hence, in essence, this study does not attempt to equate the changes in synthetic product, proteoglycan, to physiological values of compression or tension. We do, however, attempt to correlate sub-physiological loads in compression and tension, of similar magnitudes, with a direct comparison between these loading modalities possible with our results. Our findings have significant clinical implications. Since cartilage appears to have a high sensitivity to its mechanical environment, subtle changes of this environment may alter the cartilage matrix structure and function. Since poor proteoglycan content has been considered detrimental to the functional integrity of cartilage, cartilage degradation and the possible formation of osteoarthritis may be the consequence of loading patterns switching from compression to tension. However, we recognize that pure applied forces, in compression and tension, are likely to be interpreted at the cellular level as a combination of both forces. These subjects are currently under investigation for further details.

The correlation between compressive and tensile loading in proteoglycan synthesis of chondrocytes supports the hypothesis that compression and tension are two distinct stimuli which can potentially modulate the chondrocyte response independently. Although it was out of the scope of our study to consider the question of how cells sense and transduce mechanical signals, it is conceivable that changes in cell shape or size due to mechanical loading initiate the

production of intracellular second messengers, either through stretch activated and/or stretch inactivated ion channels, on the plasma membrane.[9,13] The findings of this study may help in further studies of mechanical behavior and biological response of chondrocytes to compressive and tensile loads. Furthermore, our results imply that cartilage grown in culture, with the intention of transplantation, may structurally benefit from an environment of cyclic loading at higher frequencies.

CONFLICT OF INTEREST

None reported.

AUTHOR DISCLOSURE

All authors have made substantial contributions to the conception and design of the study, acquisition, analysis and interpretation of data, drafting and critical revision and final approval of the manuscript prior to submission.

ACKNOWLEDGMENTS

We thank S. O'Driscoll, and R. Hubermeyer for allowing this study to be conducted using their cyclic compression and tensile equipment, respectively M. Schroeder, G. Wang, J. Fitzsimmons, and M. Mello for their technical assistance, N. Mura for statistical analysis, J. Tarara for technical assistance with image processing quantification.

References

1. Bachrach NM, Wilmot BV, Stazzone E, Ratcliffe A, Lai M, Mow VC. Changes in proteoglycan synthesis of chondrocyts in articular cartilage are associated with the time dependent changes in their mechanical environment. *J Biomech* **28**: 1561–1569, 1995.
2. Benya PD, Brown PD. Modulation of the chondrocyte phenotype *in vitro*, in Kuettner KE, Schleyerbach R, Hascall VC (eds.) *Articular Cartilage Biochemistry*. Raven Press, New York, 1986, pp. 219–233.
3. Buschmann MD, Yehezkiel AG, Grodzinsky AJ, Hunziker EB. Mechanical compression modulates matrix biosynthesis in chondrocyte/agarose culture. *J Cell Sci* **108**: 1497–1508, 1995.
4. Fedewa MM, Oegema TR, Schwartz MH, Adrienne M, Lewis JL. Chondrocytes in culture produce a mechanically functional tissue. *J Orthop Res* **16**(2): 227–236, 1998.
5. Fenwick SA, Gregg PJ, Rooney P. Osteoarthritic cartilage loses its ability to remain avascular. *Osteoarthr Cartil* **7**(5): 441–452, 1999.
6. Fujisawa T, Hattori T, Takahashi K, Kuboki T, Yamashita A, Takigawa M. Cyclic mechanical stress induces extracellular matrix degradation in cultured chondrocytes via gene expression of matrix metalloproteinases and interleukin-1. *J Biochem* **125**: 966–975, 1999.
7. Fukuda K, Asada S, Kumano F, Saitoh M, Otani K, Tanaka S. Cyclic tensile stretch on bovine articular chondrocytes inhibits protein kinase C activity. *J Lab Clin Med* **130**: 209–215, 1997.
8. Guilak F, Mow VC. The mechanical environment of the chondrocyte: a biphasic finite element model of cell-matrix interactions in articular cartilage. *J Biomech* **33**(12): 1663–1673, 2000.
9. Guiliak F, Myers BC, Ratcliffe A, Mow VC. The effect of matrix compression on proteoglycan metabolism in articular cartilage explants. *Osteoarthr Cartil* **2**: 91–101, 1994.
10. Kurosawa H, Fukubayashi T, Nakajima H. Load bearing mode of the knee joint: Physical behavior of the knee joint with and without menisci. *Clin Orthop* **149**: 283–290, 1980.
11. Larrson T, Aspden RM, Heinegard D. Effects of mechanical load on cartilage matrix biosynthesis. *in vitro. Matrix* **11**: 388–394, 1991.
12. Mawatari T *et al.* Effects on tensile strain and fluid flow on osteoarthritic human cartilage metabolism *in vitro*. *J Orthop Res* **28**(7): 907–913, 2010.
13. Sachs F. Mechanical transduction by memebrane ion channels: A mini review. *Mol Cell Biochem* **104**: 57–60, 1991.
14. Saris D, Mukherjee N, Berglund L, Schultz F, An K, O'Driscoll S. Dynamic Pressure transmission through agarose gels. *Tissue Eng* **6**(5): 531–537, 2000.
15. Setton LA, Elliott DM, Mow VC. Altered mechanics of cartilage with osteoarthritis: Human osteoarthritis and an experimental, model of joint degeneration. *Osteoarthr Cartil* **7**(1): 2–14, 1999.
16. Steinmeyer J, Torzillli PA, Burton-Wurster N, Lust G. A new pressure chamber to study the biosynthetic

response of articular cartilage to mechanical loading. *Res Exp Med* **193**: 137–142, 1993.

17. Stroetz R, Vlahakis N, Walters B, Schroeder M, Hubmayr, R. Validation of a new live cell strain system: Characterization of plasma membrane stress failure. *J Appl Physiol* **90**: 2361–2370, 2001.
18. Torzilli PA, Grigiene R, Huang C, Friedman SM, Doty SB, Boskey AL, Lust G. Characterization of cartilage metabolic response to static and dynamic stress using a mechanical explant test system. *J Biomech* **30**(1): 1–9, 1997.
19. Verbruggen G, Cornellissen M, Almqvist KF, Wang L Elewaut D, Broddelez C, de Ridder L, Veys EM. Influence of aging on the synthesis and morphology of the aggrecans synthesized by differentiated human articular chondrocytes. *Osteoarthr Cartil* **8**(4): 288–293, 2000.
20. Zhang Z, Bedard E, Luo Y, Wang H, Deng S, Kelvin D, Zhong R. Animal models in xenotransplantation. *Expert Opin Investig Drugs* **9**(9): 2051–2068, 2000.