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Different response of articular chondrocyte subpopulations to surface motion

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Summary

Objective: To investigate the effect of surface motion on the gene expression of proteoglycan 4 (PRG4), hyaluronan synthases (HAS1, HAS2) and on the hyaluronan (HA) and proteoglycan 4 (PRG4) release of chondrocytes from different zones of bovine articular cartilage.

Design: Superficial zone, deep zone, full thickness, and superficial/deep 1:1 mixed chondrocytes were seeded into 3D polyurethane scaffolds and stimulated using our bioreactor that approximates kinematics and surface motion characteristics of natural joints. One hour of surface motion superimposed on cyclic compression was applied twice a day over 3 consecutive days. Scaffolds were cut into top and bottom sections and analyzed for gene expression of PRG4, HAS1, and HAS2.

Results: Depending on the cell population, the gene expression levels increased within 8 days of culture in unloaded scaffolds, with a stronger increase in the top compared to the bottom sections. Mechanical loading further enhanced the messenger RNA (mRNA) levels in all cell types, with most pronounced up-regulations observed for the PRG4 expression in deep zone and the HAS2 expression in superficial zone cells. The effect of the biochemical and biomechanical environment appeared to be additive, resulting in highest mRNA levels in the top sections of loaded constructs. Bioreactor stimulation also enhanced the HA release in all cell populations. Full thickness chondrocytes experienced the greatest effect on HAS1 mRNA expression and HA release, indicating that the interaction between cell populations may promote HA synthesis compared to subpopulations alone.

Conclusions: Reciprocating sliding can be an efficient tool for generating tissue-engineered constructs from various chondrocyte populations by providing a functional cartilage–synovial interface.

Key words: Chondrocyte subpopulations, Surface motion, Proteoglycan 4, Hyaluronan synthases, Functional tissue engineering.

Introduction

Articular cartilage functions to support and distribute forces generated during joint loading and to provide a lubricating surface to prevent wear or degradation of the joint. In order to meet these requirements, the tissue is organized in distinct zones with differences in structure, composition and mechanical properties. Cell shape, size, metabolic activity, and the composition of the extracellular matrix vary as a function of depth. Accordingly, superficial (tangential), middle (transitional) and deep (radial) zones, each having specialized functional properties, can be distinguished[1–4].

The mechanical functions of the superficial zone (top 10–20% of the total thickness) are to resist shear stresses produced by joint motion and to provide a smooth, nearly frictionless surface. This zone has a relatively low amount of aggregating proteoglycan and a high density of smaller diameter collagen fibers arranged parallel to the joint surface[1–4]. Proteoglycan 4 (PRG4) is secreted predominantly by superficial zone chondrocytes, whereas cells from the middle and deep zone synthesize very low amounts of this protein. Biosynthesis of PRG4 by articular chondrocytes has therefore been used to demarcate the superficial zone of cartilage[5–8]. The PRG4 gene encodes for the homologous proteins superficial zone protein (SZP), lubricin, megakaryocyte-stimulating factor (MSF), and hemangiopoietin (HAPO)[8,9]. During normal joint articulation, expression of this proteoglycan has an important role for both preventing cell attachment to the articular surface as well as maintaining lubrication properties at the cartilage–synovial fluid interface[7,10]. Loss of PRG4 influences the functional properties of synovial joints, and a focal decrease in PRG4 in early OA could have a role in the pathogenesis of cartilage degeneration[10,11]. Hyaluronan (HA) is another macromolecular product with key functions for joint physiology. In cartilaginous matrix, HA serves as a backbone for the assembly of aggrecan, the main matrix proteoglycan, and in synovial fluid, HA is important for providing the level of viscosity necessary to minimize fluid outflow during joint motion. It has been suggested that PRG4 and HA interact to meet the physiological requirements for maintaining proper joint lubrication and providing a protective barrier over the deeper cartilage layers[12].

It is well established that chondrocyte metabolism is influenced by the mechanical environment. The integrity of cartilage might be enhanced by the induction of PRG4 and HA synthesis in response to suitable biophysical stimuli[13,14]. We have previously reported that applied surface motion stimulated the gene expression of PRG4 and hyaluronan synthases (HAS1, HAS2) in chondrocytes cultured in 3D

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porous scaffolds[13]. The exposed cells represented a heterogeneous population of phenotypically different chondrocytes isolated from full thickness articular cartilage. Therefore, it remained unknown whether different chondrocyte subpopulations responded in a similar manner to the applied stimuli, or whether intrinsic differences exist in their responsiveness. To address this question, we investigated the effect of surface motion on the gene expression of PRG4, HAS1, and HAS2, and on the release of HA and PRG4 by chondrocytes from different zones of bovine knee joint cartilage. Superficial zone, deep zone, full thickness, and superficial/deep 1:1 mixed chondrocytes were seeded into hybrid fibrin gel-polyurethane scaffolds[15] and stimulated using our bioreactor that has been designed to approximate the movements and surface motion characteristics of articulating joints[16].

Materials and methods

POLYURETHANE SCAFFOLD

Cylindrical (8 mm $\times$ 4 mm) porous polyurethane scaffolds were prepared as described elsewhere[17]. The scaffolds with interconnected pores had an average pore size of 200–400 μm and a pore-to-volume ratio of 85%. The polymers used for scaffold preparation were synthesized with hexamethylene diisocyanate, poly(ϵ-caprolactone) diol with a molecular mass of 530 Da, and isosorbide diol (1,4:3,6-dianhydro-D-sorbitol) as chain extender[18]. The scaffolds were sterilized in a cold-cycle (37°C) ethylene oxide process, and subsequently evacuated at 45°C and 150 mbar for 3–4 days. Before cell seeding, the scaffolds were evacuated in the presence of growth medium for 1 h, in order to wet the hydrophobic polymer.

CHONDROCYTE ISOLATION, SEEDING AND CULTURE CONDITIONS

Knee joints of ten 2–4 month old calves were obtained directly after slaughter and processed under aseptic conditions. Full thickness cartilage was harvested from the femoral groove. Superficial zone cartilage was harvested by careful abrasion of the joint surface, using a scalpel blade, from the patella, the femoral condyle and the femoral groove. Deep zone cartilage was harvested from the lower third of the remaining cartilage of the femoral condyle and the femoral groove, while the upper two thirds were assigned to the middle zone section. Superficial zone, middle zone, deep zone, and full thickness chondrocytes were isolated from the respective tissue sections using sequential pronase and collagenase digestion[19]. The isolated cells were counted by hemocytometer, and cell viability was assessed by trypan blue exclusion method. In six experiments, primary ($n = 2$), first passage ($n = 2$), and second passage ($n = 2$) superficial and deep chondrocytes were used. In another four experiments, primary superficial, deep, full thickness, and superficial/deep 1:1 mixed chondrocytes were used.

A fibrin–polyurethane hybrid system was used for 3D cell culture. The incorporation of fibrin had previously been shown to improve the cell distribution and extracellular matrix retention within the scaffold and the phenotype expression of the cells as compared to polyurethane without fibrin[15]. Different populations of chondrocytes were suspended in fibrinogen solution and then mixed with thrombin solution immediately prior to seeding them into the polyurethane scaffold at a cell density of 10×10^6 per scaffold. The fibrin components were provided by Baxter Biosurgery (Vienna, Austria). The final concentrations of the fibrin gel were 17 mg/ml fibrinogen, 0.5 U/ml thrombin, and 665 KIU/ml aprotinin[15]. Constructs were incubated for 45 min (37°C, 5% CO_2, 95% humidity) to permit fibrin gel formation before adding growth medium (Dulbecco's modified Eagle's medium) supplemented with antibiotics, 10% fetal calf serum (FCS), 50 μg/ml ascorbic acid, 40 μg/ml L-proline, non-essential amino acids, and 500 KIU/ml aprotinin). Since cells are particularly susceptible to oxidative damage directly after enzymatic treatment for cell isolation[20], the addition of ascorbic acid was delayed until 2 days post-seeding. After 5 days in culture, cell-scaffold constructs were exposed to mechanical loading as described below.

MECHANICAL LOADING

Mechanical conditioning of cell-scaffold constructs was performed using our bioreactor system, which was installed in an incubator at 37°C, 5% CO_2, and 85% humidity (Fig. 1)[16]. Briefly, a commercially available ceramic hip ball (32 mm in diameter) was pressed onto the cell-seeded scaffold. Interface motion was generated by oscillation of the ball about an axis perpendicular to the scaffold axis. Superimposed compressive strain was applied along the cylindrical axis of the scaffold.

For each experiment, samples were assigned in duplicates to one of two groups: the loaded group was exposed to ball oscillation of $\pm 25^\circ$ at 0.1 Hz. Simultaneously, dynamic compression was applied at 0.1 Hz with 10% sinusoidal strain, superimposed on a 10% static offset strain, resulting in an actual strain amplitude of 10–20%. One hour of mechanical loading was performed twice a day over 3 consecutive days. In-between loading cycles, constructs were kept free swelling, without surface contact with the ball. However, they were always kept within the sample holders, in order to assure the maintenance of their orientation. The group of unloaded constructs served as controls.

A subset of loaded and control samples were digested with proteinase K (0.5 mg/ml; overnight at 56°C) and analyzed for total DNA content using Hoechst 33258 dye assay[21].

GENE EXPRESSION ANALYSIS

After 8 days in culture and six loading cycles, constructs were horizontally cut into two sections (top and bottom) of

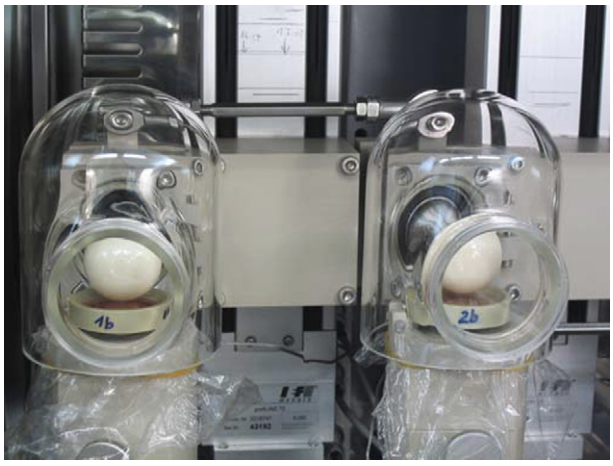

Fig. 1. Two stations of the four-station bioreactor that allows for application of joint specific biomechanical stimuli to cell-seeded scaffolds.