INTRODUCTION:

The concept of functional tissue engineering involves the in vitro use of mechanical stimuli to enhance the functional properties of cartilage, i.e. the mechanical properties that enable cartilage to withstand the expected in vivo stress and strain. Application of intermittent mechanical loading such as compression or shear during tissue cultivation has been shown to modulate cartilage formation. In this study we have hypothesized that the combination of compressive and shear forces during cultivation will improve functional tissue properties in engineered cartilage. A novel device that can apply dynamic biaxial loading was designed. Agarose gels were seeded with chondrocytes and cultivated in the presence of uniaxial (compression) or biaxial (compression and shear) mechanical loading. At the end of 30 days biochemical and mechanical properties of engineered cartilage were assessed.

METHODS:

A novel biaxial loading device was designed with two stages used for precision-guided laser applications. The stages along x- and z-axes have a 50 mm travel range and are driven independently by stepper motor actuators. The actuators are controlled by a closed-loop stepper motor driver that enables step sizes of less than 50 nm (Figure 1).

Chondrocytes were freshly isolated from articular cartilage of knee joints of 4-months old female pigs. Agarose (2% w/v in DMEM, low gelling T) was seeded with 20 million cells/ml and cast between parallel glass plates to gel at room temperature. 5 mm diameter and 1.5 mm thick samples were cored using a dermal punch and cultured in 2 ml of defined chondrogenic culture medium in 24-well plates at 37°C, 5% CO2, 10-7M dexamethasone and 10 ng/ml TGF-β1 were supplied for the first 10 days of culture. The samples were loaded for 3h/day between day 10-30. Uniaxial loading consisted of 10% compression peak-to-peak amplitude, 1 Hz and biaxial loading consisted of 10% compression and 5% shear peak-to-peak amplitude, 1 Hz.

DNA content was measured using Quant-iT PicoGreen dsDNA assay (Invitrogen). Glycosaminoglycans (GAG) concentration was measured spectrophotometrically by the DMMB dye assay. Total collagen amount was measured using Sircol Collagen Assay. For histology samples were fixed in 1:1 methanol:acetone, embedded in paraffin, sectioned and stained with alcian blue/nuclear fast red, safranin O/fast green, type I and II collagen immunohistochemistry. The mechanical testing was performed using an EnduraTech 3200 load frame. The samples were positioned between two rigid impermeable loading plates. The mechanical testing was performed using an EnduraTech 3200 load frame. The samples were positioned between two rigid impermeable loading plates. The creep test was performed using a tare load of -2g for 3000 s. Subsequently, a test load of -2g was applied for 3000 s till equilibrium was reached. The equilibrium Young’s modulus was calculated using load and equilibrium strain. Statistics were performed using Instat (GraphPad Software, San Diego, CA) with ANOVA. P<0.05 was considered statistically significant.

RESULTS:

In this study three groups were employed: 1- No loading control, 2- Uniaxial (compressive) loading, 3- Biaxial (compressive and shear) loading. The DNA contents and the wet weights of constructs remained similar in the three groups after 30 days of cultivation (p>0.05). The GAG content was highest in the group that was subjected to biaxial loading (group 3, p<0.001 compared with the control group), followed by the uniaxial loading group (group 2, p<0.05) (Figure 2). The GAG contents of groups 2 and 3 correspond to 48% and 50% of native cartilage, respectively. Group 3 resulted in significantly higher amount of collagen than groups 1 and 2 (p<0.01). Group 2 also had thicker constructs than group 1 (p<0.01). Surprisingly, the equilibrium compressive Young’s modulus was the highest in group 2 (uniaxial loading, p<0.01) and there were no significant differences between group 3 and 1. The Young’s modulus of group 2 corresponded to 60.1% of native porcine cartilage.

The histological analyses indicated positive and homogeneous staining for glycosaminoglycans (alcian blue, safranin O) and type II collagen. All groups stained negative for type I collagen (not shown).

DISCUSSION:

This study demonstrated the use of a biaxial loading device for cartilage tissue engineering. Biaxial loading increased the proteoglycan and collagen deposition and the thickness of the samples but did not seem to significantly influence the mechanical properties of engineered cartilage as we hypothesized. Uniaxial compression increased both the proteoglycan deposition and the Young’s modulus. We believe that the optimum dose of mechanical loading differs with cell and tissue properties. Future studies of collagen architecture and dosimetry of loading are necessary to fully evaluate the effects of biaxial loading on the development of engineered cartilage tissue.

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