DESIGN AND VALIDATION OF AN IN VITRO LOADING SYSTEM FOR THE APPLICATION OF CYCLIC COMPRESSION AND SHEAR TO CHONDROCYTES-SEEDED CONSTRUCTS

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Introduction

Tissue engineering of cartilage requires appropriate cues to persuade cells to produce functional extracellular matrix competent over a long term. Several groups have shown that cyclic compression up-regulates GAG in chondrocyte-seeded constructs. By contrast fewer have indicated synthesis of type II collagen [Fraser et al., 2003; Ng et al., 2009]. In normal joint activities cartilage is subjected to shear superimposed on compressive forces [Sawae et al., 2004]. Thus, this study describes the design of a biaxial loading system, applying both cyclic compression and shear to chondrocytes seeded in 3D agarose constructs.

Methods

The bioreactor utilises a controlled loading system in a sterile culture chamber. Shear strain is delivered by a linear positioning motorised stage (Zaber Thechnologies, UK), and compressive strain via a commercial system (BioDynamic® test system, BOSE, US). This arrangement permits the simultaneous testing of 12 constructs, each in a separate plate well (Fig. 1). The horizontal and vertical displacements in the bioreactor are controlled by dedicated programmes. The system was validated by monitoring temporal movement in vertical and horizontal planes.

An optimised design was produced, which enabled compressive and shear strains to be cyclically applied to a cylindrical sample held using customised nylon endplates.

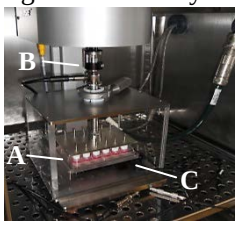

Figure 1. Bi-axial bioreactor within incubator. A: Cell seeded constructs; B: Vertical actuator; C: Motorised stage.

Finite element analysis (FEA) was performed to optimize the sample profile. This ensured the design of an experimental model with uniform stress distribution to minimise the risk of premature failure of the samples during loading. The sample integrity under dynamic shear was estimated by testing six constructs under 15% static compressive strain and 10% cyclic shear strain using a sinusoidal waveform at a frequency of 1 Hz for 48 hrs. Storage (G') and loss moduli (G'') were determined over 40 cycles at three different times during the conditioning period.

The viability of bovine chondrocytes seeded in agarose gel constructs over 48 hrs was estimated for a range of loading regimens.

Results

The synchronisation between orthogonal displacements revealed a minimal mean time delay of 0.014 ± 0.013 sec over 48 hrs. Based on the predicted stress distribution, samples were produced with chamfered incline of 121° at each end. Results indicated no evidence of structural failure on such sample profiles when tested under cyclic shear (Fig. 2). The dynamic mechanical tests revealed values for storage and loss moduli of 46.2 $\pm$3.8 kPa and 17.9 $\pm$2.3 kPa, after equilibration at 2 hrs. These values decreased by 6.9% at 48 hrs. Nonetheless, the load-bearing capacity of the sample was maintained during the entire duration of the test, as illustrated (Fig. 2).

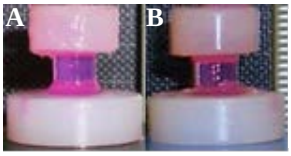

Figure 2: Construct a) before and b) after shear fatigue test.

Mean chondrocyte viability was maintained above 90% for all test conditions. No statistically significant differences in viability were observed when compared to control constructs, defined as free swelling constructs cultured for 24 hrs.

Discussion

The biaxial bioreactor incorporated with biomechanical conditioning of cell-seeded agarose constructs with chamfered ends matched the design brief. In particular, it enabled the constructs to be loaded with a combined compressive and shear loading regimen at 1 Hz for up to 48 hrs with no appreciable loss of cell viability or mechanical integrity. Accordingly, it is ideally suited for the systematic investigation of the biosynthetic response of chondrocytes to dynamic compression and shear deformation separately or simultaneously using a wide range of strain and frequency regimes. In particular, we can examine the temporal nature of the synthesised ECM components and its organisation and how it matches the functionality of native cartilage tissue.

References

Fraser et al, Arthrit Rheumat, 48:3085-95, 2003.
Ng et al, Osteoart Cartilage, 17:220-7, 2009.
Sawae et al, Annals of Biomed Eng 32:860-70, 2004.