A SHEAR RIG FOR THE STUDY OF MECHANOTRANSDUCTION IN CHONDROCYTE - AGAROSE CONSTRUCTS: ANALYSIS OF LOCAL STRAIN FIELDS.

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INTRODUCTION

Articular cartilage within synovial joints is exposed to compressive and shear forces during normal activity. These mechanical signals are essential for the health and homeostasis of the tissue. Consequently mechanical conditioning has been proposed within a tissue engineering context for stimulation of isolated cells seeded within 3D scaffolds prior to implantation. However, the mechanotransduction pathways through which cells are able to sense and respond to mechanical loading within 3D scaffolds are poorly understood. A number of research groups have utilised a well characterised chondrocyte-agarose model to examine the influence of compressive strain on the cell metabolism and the associated intracellular signalling pathways [1-2]. By contrast the effect of shear strain has received minimal attention, possible due to the complex test rigs required [3-4]. This study describes the development and characterisation of a new experimental system for the application of static or cyclic shear strain to cell-agarose constructs.

MATERIALS AND METHODS

Specimen Preparation

Chondrocytes were isolated from bovine metacarpophalangeal joint cartilage by sequential enzyme digestion and then seeded in 4% (w/v) agarose (type IX) at $10x10^6$ cell.ml^{-1} as previously described [1]. The cell-agarose suspension was gelled within a specially made perspex mould incorporating sintered porous glass endplates into which the agarose could infiltrate. The resulting cell-agarose specimens (5x5x5mm) with the attached porous glass endplates (Fig. 1) were removed from the mould and cultured in DMEM+20%FCS. Prior to use, the perspex and porous glass components of the mould were sterilised by alcohol or autoclave respectively. The sintered glass was then glued to perspex endplates and the mould assembled under sterile conditions.

Analysis of local strains

After 24 hrs in culture, viable cells within the agarose constructs were stained with Calcein AM (5μM, 1hr). An individual specimen was then mounted in a specially designed test rig placed upon the stage of an inverted microscope. A 10% compressive strain was applied to the construct in the X-axis. Confocal microscopy (Ultra View, Perkin Elmer) with a x10 objective was used to visualise the cells within a field of view of approximately 700 x 600μm. A single image in the X-Y plane was made at the edge of the specimen. A 5% gross shear strain was then applied in the Y axis via a computer controlled stepping linear actuator attached to one of the perspex endplates whilst the other endplate was held stationary. A second image was recorded of the same cells without moving the position of the specimen on the microscope stage. The shear strain was then removed and the procedure repeated for adjacent fields of view along the length of the specimen.

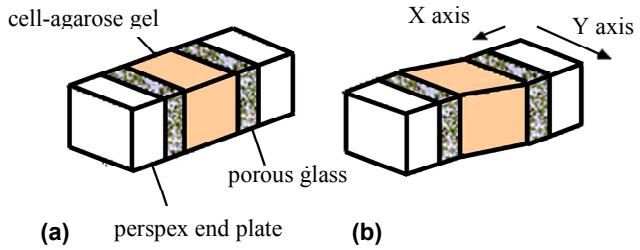

Fig. 1 Schematic diagram showing the cell-agarose specimen gelled between porous glass in the unstrained state (a) and with an applied shear strain in the Y axis (b).

Confocal images were imported into Scion Image analysis software. The X and Y coordinates, perpendicular and parallel to the axis of shear were recorded for individual cells visualised in both the unstrained state and at 5% shear strain. The displacement of each cell in orthogonal directions (X, Y) was calculated and plotted against the X position along the length of the specimen (Fig 2a). The gradient of the Y displacement data represents the local shear strain (Fig 2b). The local compressive strain was determined by subtracting the gradient of

the X displacement data, indicating tensile strain, from the applied compressive strain (10%).

Mechanical characterisation

Separate individual specimens, with and without cells, were mounted in a mechanical testing machine (Instron 4644). A 15% shear strain was applied at a strain rate of 20%.min^{-1}. Shear force was measured with a 2.5N load cell and the shear modulus calculated from the resulting shear stress vs shear strain curves.

RESULTS

Analysis of local strain

Figure 2b indicates that the application of 5% gross shear caused a non uniform shear strain field at the ends of the specimen. However towards the centre of the specimen the shear strain was more uniform with a value of 5.7%. Compressive strain remained constant across the length of the specimen with a mean value of 9.6%.

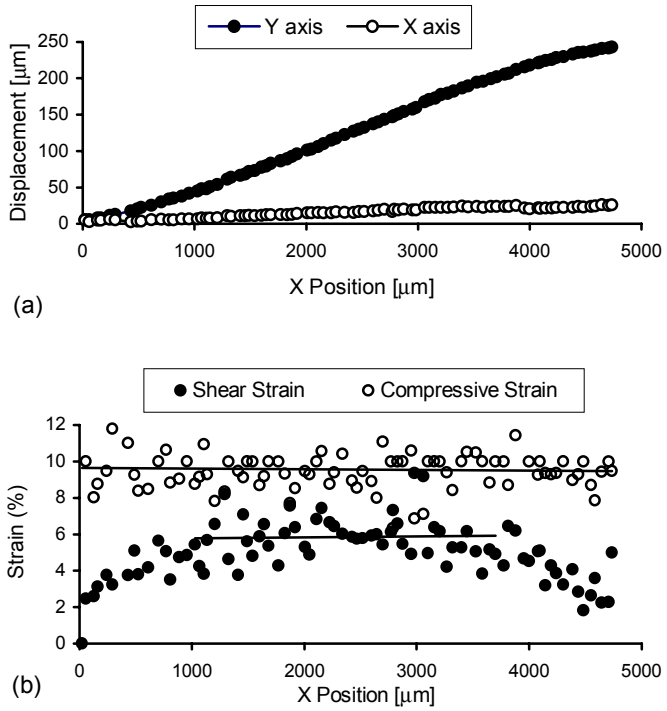

Fig. 2 Displacement of cells parallel (Y) and perpendicular (X) to the axis of shear (a) and the resulting local shear and compressive strains (b) across the specimen length. Linear models have been fitted to the strain data. For shear strain, only points within the central region of the specimen (>1mm from each end) were included.

Mechanical characterisation

Cell-agarose constructs exhibited a linear shear stress strain relationship (Fig. 3) with a mean shear modulus of 15.2kPa (±1.6, n=6). The presence or absence of cells had no statistically significant effect on the shear modulus of the constructs. There was no fracture at the agarose-porous glass interface up to 15% static shear or during 48 hrs of ±5% cyclic shear strain at 1Hz (data not shown).

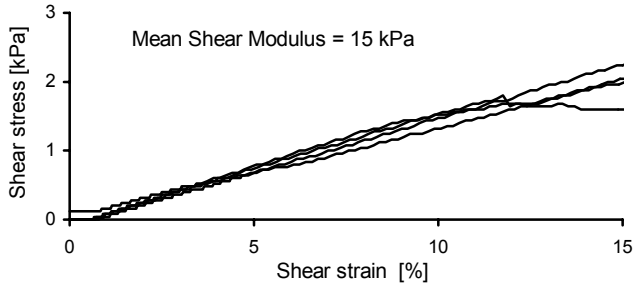

Fig. 3 Shear stress - strain response for 4% cell-agarose constructs.

DISCUSSION

Previous studies have investigated the effect of shear on the metabolism of chondrocytes within cartilage explants [3-4]. However this study is the first to describe a system to apply shear to isolated cells in 3D scaffolds. Cell-agarose constructs have been gelled between porous glass plates enabling the application of shear strain. Mechanical characterisation indicated a shear modulus of 15kPa, significantly lower than the compressive modulus of 100kPa.

A computer controlled test rig has been developed to apply static or cyclic shear strain to individual cell-agarose constructs whilst simultaneously allowing visualisation of fluorescently labelled cells using confocal microscopy. The device is similar to that previously reported for investigating the effect of compressive strain on chondrocytes in agarose [2]. In the present study, individual cells have been used as strain markers to calculate the local shear strains as previously used for measuring the strain distribution within compressed cartilage explants [5]. Results indicate a uniform shear strain field within the central region of the construct with reduced shear strain and localised bending at the ends of the specimen.

The device can now be used to visualise the influence of shear strain on parameters such as cell and nuclear morphology, intracellular calcium signalling and cytoskeletal reorganisation. A similar, multi-station test rig has also been developed to fit within a modified tissue culture incubator. This will enable parallel studies to investigate the effects of prolonged static or cyclic shear on matrix synthesis and cell metabolism within agarose constructs.

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