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The development of a six-axis bioreactor for the application of complex physiological loading to the intervertebral disc

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Introduction:

Bioreactor studies for the culture of intervertebral discs (IVDs) have shown that the application of different loading regimes can affect the cell viability in the disc[1-3]. However, to date, the replication of physiological loading in six degrees of freedom within a cell-culture environment is unreported. This study aims to develop the first six-axis bioreactor capable of hosting whole IVD specimens and applying complex physiological loading. This test system will provide a better understanding of the effect of complex physiological loading on cell viability and IVD composition.

Methods:

The six-axis bioreactor was based on the design of a previously developed dynamic six-axis spine simulator[4] with the addition of a custom made biochamber (Figure 1). The simulator has proven able to successfully simulate daily activities[4]. Loading protocols to recreate a 24h activity profile for a UK resident in the 45-64 year group were developed using the Harmonised European Time Use Surveys (HETUS) and the Orthoload database[5]. Intervertebral discs were harvested from bovine tails and maintained for 7 days under standard culture conditions (37 °C, 5% CO2). The cell viability was assessed in the nucleus pulposus (NP) and the annulus fibrosus (AF) using an inverted confocal laser-scanning microscope at day 0 and day 7 (controls). The chemical composition of the disc was assessed using Raman spectroscopy. The six-axis bioreactor, alongside a fully integrated control system (dSPACE Ltd., UK) allowing real-time test capabilities, is currently being used to apply axial diurnal and complex physiological loading to the discs when cultured for 7 days.

Results:

Cell viability at day 0 was 92±9.6 % and 80±14.0% in the NP and AF respectively. This was well maintained in unloaded incubator controls at day 7, with cell viability of 91±7.9% and 76±20% in the NP and AF respectively. Similarly, disc composition in terms of proteoglycans and collagen was similar in both day 0 and day 7 controls. Unloaded and physiological loading is currently being completed to validate the bioreactor system against the control data and provide an initial understanding of how complex loading in the disc affects the cell viability and disc composition.

Discussion:

The specimen preparation and culturing protocol developed has demonstrated good cell viability in control specimens. Current testing will assess cell viability and disc composition using both unloaded and physiological loading protocols with the bioreactor system. This unique test platform will provide the capability to investigate

the effects of physiological loading, and different daily activities load profiles on the IVD, and will provide novel capabilities for the evaluation of medical devices and regenerative therapies to treat degenerative disc disease.

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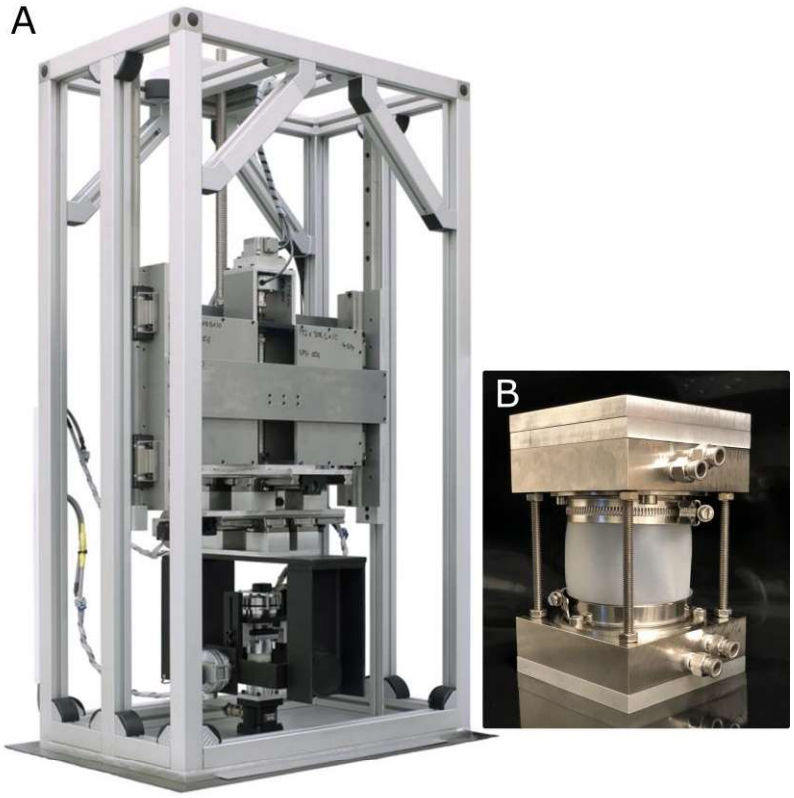

Figure 1. Bath system (A) was redesigned with the integration of a biochamber (B).

Keywords: Intervertebral disc; Bioreactor; IVD biomechanics