Development of decellularized intervertebral disc scaffold for tissue engineering

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INTRODUCTION:
One of the aims of tissue engineering is the production of functional cell-scaffold composites for the replacement of diseased or damaged tissues. A composite which closely mimics the native extracellular matrix (ECM) would facilitate cell interaction with the composite, thereby promote the maintenance of the cell phenotype [1-2]. Even though current studies using 3D culture methods which include alginate beads, agarose, collagen sponge and chitosan have reported some success in maintaining cell phenotypes and proliferation, they do not provide a 3D pattern that can be perfectly suited to the specific types of cultured cells [3]. A way to circumvent the problems is the development of a scaffold that allows for disc cells to be cultured in a physiological 3D microenvironment.

Decellularization is a process that removes the cellular contents from a tissue or an organ whilst minimizing adverse effects on the composition, biological activity and mechanical integrity of the ECM. Biological scaffolds derived from decellularized tissues and organs have been commonly and successfully used in both animal studies and in human clinical applications [4]. We hypothesize that a proper combination of physical and chemical methods can be effective in removing the cellular content in bovine IVDs whilst maintaining the ECM structure. This study aims to develop a decellularized disc scaffold with preserved ECM content for the culture of disc cells by using bovine IVD as a model.

MATERIALS AND METHODS:
Bovine caudal discs of sizes ranging between 18 to 23 mm were harvested with the flanking endplates attached. Whole discs were decellularised with 25 mL of phosphate buffer solution (PBS) containing 0.1% sodium dodecyl sulphate (SDS) and protease inhibitor with constant agitation. The decellularization was performed using 4 different protocols (protocols A to D) which tested variations of the duration of IVD washing, washing temperature, number of solution changes at regular intervals, and the number of snap-freezing cycles to identify the optimum conditions for maximal removal of cells from the bovine IVDs.

After treatment, the annulus fibrosus (AF) and nucleus pulposus (NP) were harvested from the disc and then pieces of 4 x 4 mm were extracted from both regions. Live/Dead staining and the Alamar blue assay were used for analysing the number of remaining viable cells and the metabolic activity after decellularization. Numbers of live or dead cells were quantified from the photos of Live/Dead staining and compared with the fresh control to obtain the percentage of cells removed from the tissue. The Alamar blue readouts were normalized with the fresh control for comparisons.

RESULTS:
Among the 4 protocols, protocol D was the most effective in removing the cells from the bovine IVD, where 69% of the cells were removed from the AF (Fig. 1a), and that 73% of the cells were removed from the NP (Fig. 1b). Under this condition, the number of cells remaining inside the tissue decreased as the washing duration gradually increased (Fig. 2.). The majority of the cells were dead (red) from the staining which was in agreement with the results of the Alamar Blue assay. Increase of the duration of washing, regular replacement of the solution, and using an increased number of snap-freezing cycles decreased the cell viability and facilitated removal of cellular contents, however, after 60% of the cells were removed, the rate of cellular content removal did not greatly vary.

DISCUSSION:
Decellularization was achieved using 2 common methods including physical cell removal (snap-freezing cycles) and chemical washing (SDS). The freeze-thaw cycles helped to expose the matrix of the IVD and kill the cells, in addition to disruption of the cell membrane by the formation of intracellular ice crystals that allowed the internal content of the cells to be washed out. Snap-freezing cycles also helped destruct the DNA content of the cells into smaller fragments, which aided in their diffusion out of the tissues and subsequently removed more cells. SDS, which is an ionic detergent, solubilized the cytoplasmic and nuclear membranes which further aided in removing the cell remnants. In a previous study by Bodnar et al [5], 1% SDS was used for decellularization of porcine heart valves, but resulted in a negative impact on the tissue ECM. In this study, a lower concentration of SDS was used to minimize damage to the ECM. Regular solution replacement was associated with the successful removal of more cells at the end point, where changing of the solution may have improved the diffusion of chemicals and cell remnants out of the tissues.

The rate of cell removal after 60% of the cells had been removed, did not vary greatly despite changing the temperature, the number of solution replacements, nor washing duration. This suggested that other barriers existed in limiting the efficiency and extent of decellularization which may be due to the existence of strong bonds between the cells and the ECM. Overall, this study shows that a combination of physical and chemical manipulation can effectively eliminate the majority of disc cells from the IVD.

REFERENCES: