From Preclinical to Clinical Translation of Intervertebral Disc Cell-based Regeneration - Effects of Species-Specific Scale, Metabolism and Matrix Synthesis Rates

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INTRODUCTION: Despite exciting advances in regenerative medicine, cell-based strategies for treating degenerative disc disease remain in their infancy. After demonstrating safety and efficacy in animal studies, many of these therapies enter a critical period between preclinical validation and clinical evaluation, where they do not appear to work to the same extent in humans. It is commonly believed that the harsh nutrient microenvironment and "hostile" nature of the degenerating intervertebral disc (IVD) capacity is linked to the weak outcomes of prospective studies [1–3]. Therefore, in this work we investigated the species-specific metabolic activity and regenerative capacity of both a small and large animal model in an attempt to ascertain their scientific merit for successful clinical translation. The objectives of this work were, firstly, to geometrically characterize rat, goat and human IVDs and quantify the metabolically active cell density of the animal models. Secondly, we assessed differences in metabolism across the species in terms of nutrient demands and regenerative proteoglycan (sulphated glycosaminoglycan, sGAG) synthesis rates. Finally, experimentally determined parameters were used *in-silico* to elucidate species-specific nutrient microenvironments and predict differences in temporal regeneration between animal models, towards understanding the expectations of cell-based clinical trials.

METHODS: The metabolically active cell density of rat caudal and goat lumbar tissue was determined using methylthiazolyldiphenyl-tetrazolium bromide (MTT) and a 4',6-diamidino-2-phenylindole (DAPI) counterstain as previously described [4]. Nucleus pulposus (NP) and annulus fibrosus (AF) cells were isolated from Wistar rats (~ 6 months old), Saanen goats (4 - 5 years) and patients undergoing discectomy surgery. Rat/goat tissue was obtained from discarded tissue of animals undergoing procedures approved by the Health Products and Regulatory Authority (HPRA) in Ireland; human tissue was collected through informed consent of patients undergoing discectomy procedures and approved by the Mater Misericordiae University Hospital IRB (Ref 1/378/2229) and Trinity College Dublin (TCDFSTEMSREC/15032021/Buckley). Cell spheroids (~20,000 cells) were formed in an agarose microwell array which was fabricated using a positive mold stamp. To create a physiologically relevant local niche [5], spheroids were cultured under low glucose (5.5mM) and 10% external oxygen, informed through *in-silico* models [6]. A Seahorse XFe96 flux analyzer (Agilent Technologies) was used to simultaneously measure in real time the reduction in oxygen level, a measure of the oxygen consumption rate (OCR) and pH level, a measure of extracellular acidification or lactate production rate (LPR), in the medium directly surrounding a single cell spheroid. Spheroids were digested with papain and rates were normalized by DNA content using Quant-iT picogreen dsDNA assay kit. sGAG production was quantified after 14 days of culture using a Blyscan dimethyl methylene blue dye-binding assay, with a chondroitin sulphate standard. 3D *in-silico* models were created from the geometrical analysis and coupled reaction-diffusion kinetics were employed using methods published previously [4-5]. In addition to modelling the nutrient microenvironment, we developed a GAG regeneration model for rat and goat, as employed previously for human [7-8].

RESULTS SECTION:
Geometrical and histological analysis (N=3) was used to create a 3D model for each species, as shown in Figure 1(A). Rat caudal discs have a significantly higher metabolically active cell density than goat lumbar discs ($p<0.0001$, N=6). While no significant difference (ns) was determined between rat NP and AF tissue, goat discs had a significantly higher density in the AF than the NP ($p=0.0264$). Figure 1(B) shows that disc cells formed compact spheroids by day 5, where spheroid diameter dimensions began to plateau, and spheroids retained good viability (N=3). As a result, metabolic rates were determined within 5 – 7 days. Preliminary results in Figure 1(C) show a trend of higher metabolic rates in rat NP cells compared to goat (both N=3) and human (N=1). Synthesis rates were performed up to 14 days to allow for adequate GAG deposition (results not shown).

DISCUSSION: These models address the scale-effect between different species by creating 3D geometries with experimentally determined active cell densities. Results show that rat have a significantly higher cell density, while goat tissue is more comparable with values reported for human [9]. Compared to conventional monolayer, 3D cultures have drawn attention as they more closely mimic the *in vivo* microenvironment. This work established a spheroid culture model which successfully cultures rat, goat and human IVD cells with low size variability and good viability for the assessment of 3D metabolic rates using Seahorse technology and biochemistry assays. As expected for a predominantly glycolytic tissue, this work reported relatively low OCR for disc spheroids, while LPR was greater and comparable to literature values [10]. Preliminary results suggest notochordal rat NP cells are more metabolically active than more chondrocytic-like goat, and human cells which have been long associated with low regenerative capacity. By inputting these experimental parameters into our *in-silico* models, we can demonstrate the predicted rate of regeneration and the period needed for significant extracellular matrix repair, thereby assessing the efficacy of such animal studies for successful clinical translation of cell-based therapies.

SIGNIFICANCE: This work aims to elucidate differences in metabolism between species, providing a path towards understanding "time to regeneration" within commonly used pre-clinical animal models and the stunted success of cell-based clinical trials.

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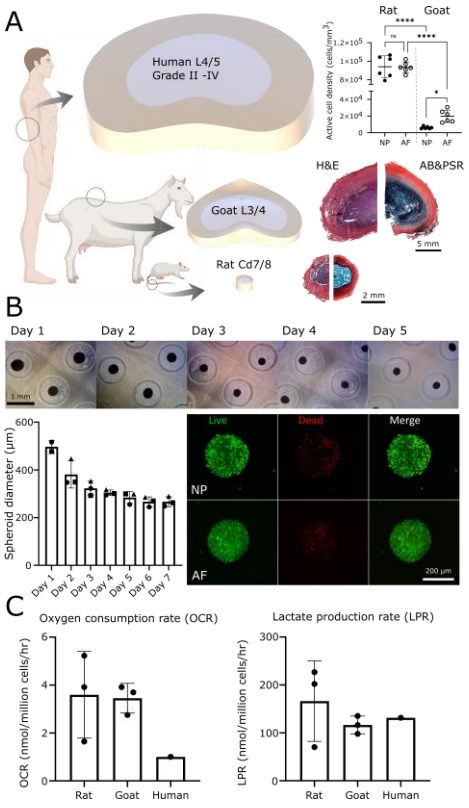

Figure 1. *(A) Establishing 3D models with anatomical geometries and a metabolically active cell density. (B) Temporal assessment of the spheroid cell culture model and qualitative viability of nucleus pulposus (NP) and annulus fibrosus (AF) spheroids undergoing metabolic analysis. (C) Preliminary species-specific oxygen consumption rates and lactate production rates for NP cells.*