**Introduction:**

The biology of the intervertebral disk (IVD) is currently not well understood. Studying the IVD in an *in vitro* setting would be of great value to gain a better understanding of the IVD in both healthy and diseased conditions. There exist few *in vitro* systems that permit whole organ cultures and cell viability assessment without tissue digestions to isolate the nucleus pulposus and annulus fibrosus cells. We aim to develop an *in vitro* IVD organ culture system that is capable of detecting cell viability in the whole IVD organ.

**Methods:**

Isolation of Intervertebral Disks:

Dutch Belt (adolescent) and New Zealand White (NZW) (adult) rabbits were killed using barbiturate overdose under IACUC approval. The lumbar spine was dissected out of the rabbits and the anterior aspect of the intervertebral disk (IVD) was exposed. The IVD organ, composed of (bony) endplate-disk-endplate complex, was removed by fracture through the growth plates proximal and distal to the disk1. The IVDs were washed in Hank’s Buffered Salt Solution containing 500 units/ml penicillin/streptomycin and then placed in six-well culture plates.

Culture of Intervertebral Disks:

Dutch Belt rabbit IVDs were randomly assigned one of five groups (3 per group): 7 days with high (4.5 g/L), low (1 g/L), and no glucose Dulbecco’s Modified Eagle Medium (DMEM) with and without 10% fetal bovine serum (FBS). Control IVDs (3 per group) were included as the following conditions: 0 day, freeze/thaw at 0 day, and freeze/thaw in culture for 7 days in no glucose DMEM without 10% FBS. For the NZW rabbits, the IVDs were randomly assigned to one of eight groups (3 IVDs per group): 7, 14, 21, or 28 days in culture with high glucose DMEM with or without 10% FBS. Control IVDs (4 IVDs) at 0 day were also included.

All samples were cultured with 50 units/ml penicillin/streptomycin and media changes occurred every two days.

Histological Analysis:

After the desired culture time, the IVDs were placed into a 0.75 mg/ml NitroBlue Tetrazolium (NBT) solution in high glucose DMEM with 10% FBS supplemented with 50 µg/ml L-ascorbate. After 24 hours of incubation at 37°C protected from light, the IVDs were fixed, decalcified in EDTA, bisected, and embedded in paraffin. Mid-sagittal 6 µm sections were cut, rehydrated, and mounted with 4’, 6-diamidino-2-phenylindole (DAPI) to label nuclear material. The NBT staining was imaged under transmitted light illumination and the DAPI staining was imaged under ultraviolet (UV) light (approx 358 nm) illumination on a Nikon Eclipse E800 microscope. For one parasagittal section per IVD, 5 µm sections were cut, rehydrated, and mounted with 4’, 6-diamidino-2-phenylindole (DAPI) to label nuclear material. All samples were cultured with 50 units/ml penicillin/streptomycin and media changes occurred every two days.

**Results:**

Dutch Belt Rabbits

Analysis of the NBT and DAPI staining data demonstrated that both high glucose Day 7 culture conditions had similar cell viabilities as the 0 Day control group (p<0.05). The presence of 10% FBS in the Day 7 cultures did not affect cell viability (p<0.05). In the no glucose and low glucose conditions cell viability was significantly decreased and similar to the negative control (Day 7 F/T) (p<0.05). Large STDev for day 0 F/T and Day 7 low glucose without 10% FBS reflects data variability and the small sample sizes of the groups.

New Zealand White Rabbits

Cell viability assessments for NZW IVDs cultured in high glucose DMEM demonstrated no effect of serum at day 7, but better survival at 14, 21, and 28 days when cultured without FBS as compared to those cultured with FBS (p<0.05). High glucose DMEM without 10% FBS, IVDs showed no change in cell viability over the 28 day time course (p>0.05). The IVDs cultured in high glucose DMEM with 10% FBS exhibited a large reduction in cell viability (~50%) after 7 days as compared to the Day 0 control (p<0.05).

Discussion:

The Dutch Belt rabbit data indicate high cell viability in the IVD in the organ culture system for 7 days in high glucose DMEM with and without 10% FBS. Interestingly, the 7 day cultures with low and no glucose concentrations regardless of the presence of 10% FBS, had low viability. This indicates that glucose is a major factor in cell survival.

We expanded on the Dutch Belt rabbit study by applying the organ culture system to the more widely used NZW rabbit model. We were able to successfully maintain high viability for 7 days in the conditions with high glucose DMEM (with and without 10% FBS). However, at 14 days the IVDs cultured with 10% FBS exhibited a remarkably lower viability as compared to their no serum counterparts. This suggests that the serum may inhibit cell survival at long time points. Further studies are in progress to elucidate optimal culture conditions and to examine cell metabolism and gene expression of the nucleus pulposus cells. This organ culture model may also provide an alternative for assessment of IVD treatments (e.g. gene therapy, cytokines, and others) currently only possible using *in vivo* models.

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**References:**