Development of a Human Intervertebral Disc Organ Culture System to Study Biological Repair

1 Gawri, R; 1Mwale, F; 2Ouellet, J; 1Steffen, T; 1 Roughley, PJ; 1 Antoniou, J; + 1Haglund, L
+1McGill University, Montreal, Canada, 2McGill Scoliosis and Spine Center, Montreal, Canada, 3 Shriners Hospital for Children, Montreal, Canada.

INTRODUCTION

Intervertebral disc (IVD) degeneration is a common cause of back pain. Back pain affects a large portion of the population, and has a negative impact on the quality of life of the patient and is costly to the health care system. Disc degeneration is known to occur early in adult life. The adult human IVD seems incapable of intrinsic repair once injured, and at present there is no medical treatment to reverse or even retard the problem. Experiments in small animals suggest that the early stages of disc degeneration can potentially be retarded or even reversed by the administration of growth factors to promote new extracellular matrix (ECM) synthesis. Many systems use discs from young healthy animals with a high proteoglycan concentration, as well as discs from smaller animals which retain notochordal cells throughout life. However, the early stage of human disc degeneration is characterized by loss of proteoglycans, and adult human discs do not contain notochordal cells. The initial objective of this study was to validate an organ culture system for intact human intervertebral disc (IVD), which could be used to determine whether growth factors or other bioactive substances can stimulate disc cell metabolism and initiate a repair response.

METHODS

Lumbar IVDs, 73 discs from 14 individuals (7 males, 7 females), were obtained through organ donations via Transplant Quebec. The spines were assessed by X-ray to exclude specimens where the majority of the discs were associated with loss of disc height or vertebral osteophyte formation. The discs were prepared for organ culture using 3 different methods: with bony endplates (BEP), without endplates (NEP) or with cartilaginous endplates (CEP). For BEP and CEP, parallel cuts were made through the adjacent vertebral bodies close to the endplates, the CEP discs were then further processed to remove remaining bone and the calcified part of the cartilage endplates using a high-speed bone bur. NEP discs were isolated by parallel cuts using a straight edged microtome blade to fully remove both bone and cartilage endplates. Short term cultures, of 1 week, were performed on NEP and BEP discs to compare cell viability using existing isolation methods to the CEP isolation method. Studies were also performed on the swelling capacity of IVD prepared using the CEP method and compared with discs prepared using the NEP method. DMMB analysis for proteoglycan was done to correlate swelling potential to proteoglycan content. CEP discs were cultured for 4 weeks or 4 months at 37ºC and 5% CO2 with no external load applied. The effect of glucose and FBS concentrations were evaluated. Dulbecco’s Modified Eagle Medium (DMEM) was supplemented with glucose, 4.5g/L or 1g/L, referred to as high and low glucose, and FBS, 5% or 1%, referred to as high and low FBS respectively, giving four different culture conditions. Cell viability in CEP isolated discs was evaluated after 4 weeks and 4 months of organ culture concentration of proteoglycans within the tissue. Cell viability in CEP isolated discs was evaluated after 4 weeks and 4 months of organ culture in order to determine whether cell viability was influenced by nutritional status of the disc. Tissue from nucleus pulposus (NP), inner annulus fibrosus (iAF) and outer annulus fibrosus (oAF) were evaluated. 96-98% live cells were present throughout the discs independent of glucose levels tested (Fig2).

RESULTS

Short term cultures were performed to compare the CEP method to NEP and BEP isolated discs. A drastic drop in cell viability was found by day 7 in BEP discs while viability was maintained in the NEP and CEP discs. When the swelling potential of NEP and CEP discs from the same donor were compared, a two fold increase in swelling was observed in NEP discs (Fig1). The CEP discs retained their shape and morphology whereas the NEP discs were severely deformed due to excessive swelling. In CEP discs cultured under different nutritional conditions, the volume increased by 11-25% after 4 weeks in culture. The swelling potential of the tissue was directly related to the concentration of proteoglycans within the tissue. Cell viability in CEP isolated discs was evaluated after 4 weeks and 4 months of organ culture

DISCUSSION

We have developed a novel technique for isolation and culturing of intact human IVDs with long term cell survival. This CEP system limits excessive swelling and maintains disc conformation in culture, even without loading. In comparison, BEP discs displayed a drastic drop in cell viability already after 1 week of culture, possibly due to impaired nutrient supply via the bony endplates. Although cell survival was maintained in NEP discs, the swelling and deformation of the discs makes them unsuitable for studies of repair in an unloaded system. The discs without endplates show 100% more fluid imbibition and loss of morphology as compared to the discs with endplates. In the CEP discs cultured for four weeks an initial increase of 11-25% was observed that remained unchanged for the culturing period. This variation correlates with proteoglycan concentration of the discs, but the preserved cartilage endplates prevent the tissue from increasing more than 25 %. This increase is within the reported diurnal variation range which may be a major contributor to the retention of cell viability. The CEP system maintained sufficient nutrient supply and high cell survival in all regions of the disc after more than 4 months of culture. The availability of an intact disc organ culture system has considerable advantage over the culture of isolated disc cells, as it maintains the cells in their unique microenvironment, so making any response to catabolic or anabolic agents more physiologically relevant.

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