Evaluation of cell viability in low cell density intervertebral disc (IVD) tissue: tips and technical notes

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Objective:

Little information exists when using cell viability assays to evaluate cells within whole tissue, particularly specific types such as the intervertebral disc (IVD). When comparing the reported methodologies and the protocols issued by manufacturers, the processing, working times, and dye concentrations vary significantly, making the assay's reproducibility a costly and time-consuming trial and error process. This study aims to develop a detailed step-by-step cell viability assay protocol for evaluating IVD tissue.

Methods:

IVDs were harvested from bovine tails (n=8) and processed at day 0 and after 7 days of culture. Nucleus pulposus (NP) and the annulus fibrosus (AF) 3 mm cuts were incubated at room temperature (26˚C) with a Viability/Cytotoxicity Kit containing Calcein AM and Ethidium Ethidium homodimer-1 for 2 hr, followed by flash freezing in liquid nitrogen. Thirty µm sections were placed in glass slides and sealed with nail varnish or Antifade Mounting Medium. The IVD tissue was imaged within the next 4h after freezing using an inverted confocal laser-scanning microscope equipped with 488 and 543 nm laser lines.

Results:

Cell viability at day 0 (NP: 92±9.6 % and AF:80±14.0%) and day 7 (NP: 91±7.9% and AF:76±20%) was successfully maintained and evaluated (Figure 1). The incubation time required is dependent on the working temperatures and tissue thickness. The calcein-AM dye will not be retained in the cells for more than four hours.

Conclusions:

The specimen preparation and culturing protocol have demonstrated good cell viability at day 0 and after seven days of culture. Processing times and sample preparation play an essential role as the cell viability components in most kits hydrolyse or photobleach quickly. A step-by-step replicable protocol for evaluating the cell viability in IVD will facilitate the evaluation of cell and toxicity-related outcomes of biomechanical testing protocols and IVD regenerative therapies.

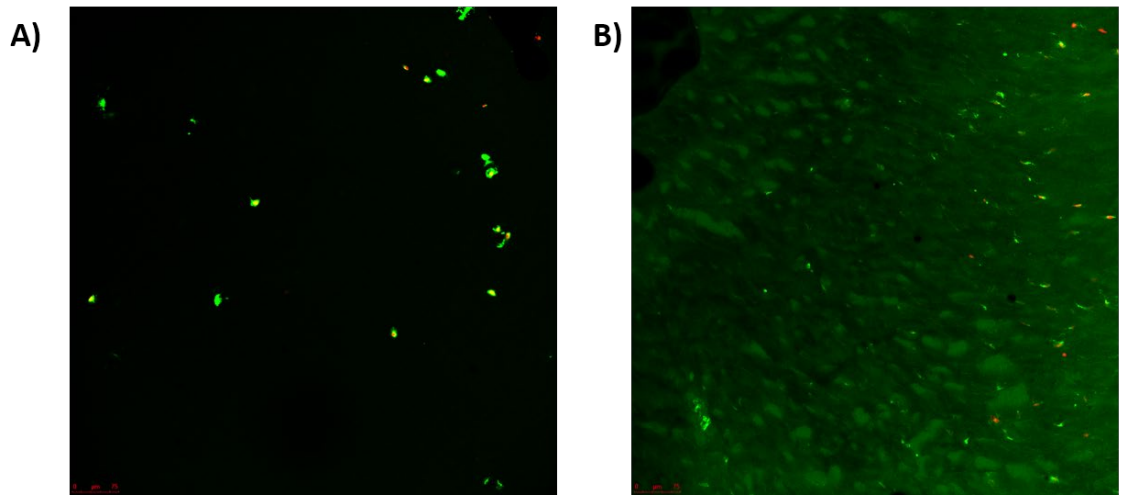

Figure 1. Live (green) and dead (red) cells in A) Nucleus pulposus and B) Annulus fibrosus tissue