

## **91] The role of the intervertebral disc niche on embryonic stem cell differentiation and intervertebral disc regeneration**

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The intervertebral disc (IVD) is a highly specialised environment, where cell, water and proteoglycan decrease is strongly associated with disc degeneration, which can lead to back pain. We postulate that the IVD niche is a major factor for IVD cell development and maintenance, and can play a role in progenitor differentiation to become disc cells. The understanding of how stem/progenitor cells develop into IVD cells could potentially facilitate the development of regenerative therapies for degenerated IVD. Here, we aim to introduce human embryonic stem cells (ESCs) into the IVD environment using a model of decellularised bovine disc, and examine how the IVD niche impacts on phenotypic changes *in vitro* using a mechanically loadable bioreactor system.

Fluorescently labelled HES2 cells were seeded onto decellularised bovine nucleus pulposus (NP) tissue or injected into whole bovine caudal discs via the annulus, and cultured in DMEM for 7 or 14 days in standard culturing conditions. Cells injected into the whole disc were cultured with/without mechanical loading in the bioreactor. The samples were then cryosectioned and/or fluorescence staining to determine their viability and phenotypic changes.

The ESCs showed good viability after 7 and 14 days culture on NP tissue and 7 days of culture after injection into the disc. ESCs cultured on NP tissue consisted of mixed populations of clustered star-shaped cells as well as spherical cells in a scattered formation, which was morphologically different to NP cells. On the other hand, ESCs injected into the disc had a more uniform cell morphology than ESCs cultured on NP tissue. Mechanical loading appeared to slightly reduce the cell size. These preliminary results indicate that mechanical loading and culture in a 3D environment impacts on ESC changes. Ongoing and future studies include further characterisation of cells with specific markers and expression of ECM by the cells.