‘Rumours of my death may have been greatly exaggerated’: a brief review of cell death in human intervertebral disc disease and implications for cell transplantation therapy

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Abstract
The avascular nature of the human intervertebral disc is thought to reduce the ability of resident disc cells to maintain their extracellular matrix, rendering the tissue susceptible to degeneration. It has also been suggested that the lack of a blood supply may result in disc cell death via nutrient deprivation. Therefore, transplanting new cells into the disc to promote tissue regeneration would be akin to ‘putting cells in a coffin’ and doomed to failure. This review considers the available evidence for cell death in the human intervertebral disc, describing briefly the methods used to assay such death, and concludes that further analysis is required to ascertain whether extensive cell death truly is a marked feature of human intervertebral discs and whether it bears any relationship to disc degeneration and hence regenerative strategies.

The intervertebral disc is the largest avascular tissue in the human body (Figure 1). This lack of a blood supply, allied with the unique biomechanical requirements of human posture, has long been considered to play a major role in the susceptibility of the human disc to undergo degenerative changes relatively early in adult life. Thus one of the principal reasons the disc is thought to fail is because disc cells inhabit such a harsh environment that few are able to survive, let alone maintain or replace their extracellular matrix [1]. In other connective tissues, notably articular cartilage, focal areas of tissue damage have been treated by autologous cell transplantation, the rationale being that the transplanted cells replace the lost tissue [2]. This treatment appears to be beneficial [3], with long-term trials in place. However, a similar cell transplantation strategy for treatment of intervertebral disc degeneration has been suggested as inappropriate as it would be akin to “putting cells into a coffin” (S.M. Eisenstein, personal communication). Coffins, of course, are for the dead. This review briefly outlines available evidence of cell death within the human intervertebral disc in relation to disc disease and considers the implications of this evidence for potential cell transplantation therapies.

In the late 1970s and 1980s, a series of studies demonstrated the avascular nature of the adult human intervertebral disc [4] while also suggesting that nutrients such as glucose and oxygen reached cells in the centre of the disc by diffusing from peripheral blood vessels in the vertebrae and through the cartilage endplates [5]. It was later shown that age-related calcification of the endplate region occurred, which could reduce solute transport and consequently a decrease in the provision of nutrients to disc cells [6]. Support for this hypothesis, and the first definitive report of disc cell death, was published in 1982 by Trout et al. [7], who used TEM (transmission electron microscopy) to examine the presence of cells with a necrotic appearance [NB to date, there have been very few (if any) convincing reports of classical apoptotic morphology in human disc cells in vivo]. In foetal discs, fewer than 2% of cells were necrotic and this was seen to increase to over 50% of cells by adulthood. However, no correlation between the incidence of disc cell death and degree of disc degeneration was noted. Furthermore, assessing cell death by TEM is time-consuming and rarely extensive; for example, in this study fewer than 50 cells (per sample) were scored in 9 of the 12 autopsy samples studied.

Subsequently, Gruber and Hanley [8] examined levels of disc cell death by using the TUNEL (terminal deoxynucleotidyl transferase-mediated dUTP nick-end labelling) technique, which detects breaks in double-stranded DNA that arise through apoptotic endonuclease activity. Compared with other tissues, including tissue in which large numbers of cells are deleted by triggering apoptosis (such as reactive lymph nodes), very high levels of TUNEL-positive disc cells were reported, although, again no classical apoptotic morphologic morphology was shown. While approx. 73% of cells in the control discs, i.e. removed from cadavers, were TUNEL-positive, only 54% of cells in discs surgically removed to treat low back pain were positive. This difference between the control and the diseased group was suggested to relate to...
Figure 1 | The blood supply in the spine of a 65-year-old male showing lack of vascular supply to the intervertebral disc, but an abundant supply to the vertebral bodies. Reproduced with permission from [4] © 1976 Lippincott Williams and Wilkins (http://lww.com).

Recently, we have utilized a different technique to assess cell death in our laboratory. We have examined samples of herniated disc following incubation of freshly isolated tissue (i.e. obtained within 15 min of surgical excision) with the fluorescent probes, calcein-AM (acetoxymethyl ester) and ethidium homodimer-1 (Live/Dead viability/cytotoxicity assay; Molecular Probes; 30 min incubation period). This assay results in the intracellular release (through esterase activity) of green fluorescent calcein in viable cells only, while dying cells, which have disrupted membranes, fluoresce red as the ethidium homodimer-1 passes into the cell and binds to nuclear DNA. We have found that greater than 90% of cells are viable in most of the disc samples examined (15 of 17 to date; Figure 2), with no clear relationship between cell viability and patient age. This level of observed disc cell viability is in sharp contrast with previous reports that have used TUNEL to measure disc cell death. However, they are closer to those reported by Bibby et al. [12] who also used the calcein-AM/ethidium homodimer-1 system to measure cell viability in discs obtained from patients with scoliosis, and in non-pathological (control) discs. They found that in the control discs ~85–90% of disc cells were viable (age range: 18–56 years), while in scoliotic discs ~60–85% of cells were viable (age range 12–65) [12]. Interestingly, these workers also found decreased cell viability on the convex side of the disc, compared with the concave side, and this difference was most pronounced at the disc level that was apical to the spinal curvature. Finally, a recent study of scoliosis using TUNEL to detect apoptosis also reported similar overall levels of cell viability (i.e. TUNEL-negative cells) to those seen by Bibby et al. [12], with no clear relationship between cell viability and age (range 10–48 years) [13]. However, no significant differences were seen in the overall incidence of cell death between apical or non-apical discs.

Suffice to say, and in conclusion, early indications that most cells present in the mature adult intervertebral disc are dead cells, using TEM or the TUNEL technique, have not been consistently borne out by several repeated studies or studies using alternative techniques. Furthermore, there does not appear to be a general consensus regarding the relationship between cell viability and either age or pathological conditions of the disc. Therefore we would emphasize the need for more research into the incidence of cell death in the human intervertebral disc and its potential role in the aetopathogenesis of disc diseases, including disc degeneration and scoliosis. In light of this, it remains a moot point to consider whether transplanting cells (of any source) into the human intervertebral disc will be a sentence to death. For example, cell transplantation procedures have been investigated into the discs of animal models that are of sufficient size to replicate the likely nutritional environment present in the human disc and at least some of these cells remained viable and capable of matrix synthesis [14]. Finally, it should be noted that clinical transplantation of disc cells in humans has begun and in early follow-up studies these procedures are reported to be beneficial [15]. Perhaps the degenerate disc is not the coffin it has been suggested to be.
Figure 2 | Live/dead staining of freshly isolated samples of human intervertebral disc
Fluorescently labelled cells (using calcein-AM and ethidium homodimer-1) demonstrate few dead cells (red) but many viable cells (green) in herniated human discs. (a–c) L4-5 disc, 50 years old; (d) C5-6 disc, 41 years old; (e, f) L5-S1 disc, 50 years old. Original magnifications: ×100 (a–c), ×200 (d) and ×400 (e, f). All tissue was examined with informed consent and local research ethics committee approval.

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References

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