Institute of Medical & Biological Engineering

# Internalisation of micron and nano-particles by cells of the dura mater

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## INTRODUCTION

Low back pain is one of the most common health problems in industrialised countries (Lambeek *et al.*, 2010). Conservative interventions are the first line of treatment. When conservative treatment fails, patients and health providers may consider surgery (Luk *et al.*, 2008).

For many years, lumbar fusion has been the gold standard surgical treatment for disc degeneration. The long term results of this procedure, however, are poor with complications including restriction of movement and adjacent segment degeneration. These complications together with advances in surgical techniques and increasing demand and expectations from patients lead to the development of total disc replacement (TDR) or spine arthroplasty (Mayer, 2005).

Wear particles generated by TDR have the potential to give rise to inflammation, toxicity and bone loss as has been well documented for hip replacements. Additionally, these adverse effects have the potential to be manifest in the para-spinal tissues specifically the dura mater of the meninges.

*The aims of this study were to utilize a porcine model system to isolate, identify and characterise dural cells of the meninges for studies of the biological effects of model particles in vitro. More specifically this study is focused on the permeability of the dural cells and whether they acted as a barrier to foreign body infiltration.*

## MATERIALS-METHODS

- Dural epithelial and dural fibroblasts were isolated from the porcine dural membrane.
- The cells were expanded in culture and characterised by immunocytochemistry
- The cells were cultured with micron-sized (1µm) and nanometre sized (40nm) fluorescent polystyrene particles for 24h, 2 and 3 days.
- Cells were then stained with rhodamine-phalloidin [actin filaments] and Hoechst 33342 [nuclei] and visualised using confocal and deconvolution microscopy.
- Two hundred of cells of each type were randomly selected and assessed for particle uptake.

## RESULTS

### *Cell phenotyping by indirect immunofluorecence*

Dural epithelial cels and fibroblasts expressed fibronectin, tenascin, collagen I & III (Fig 1).

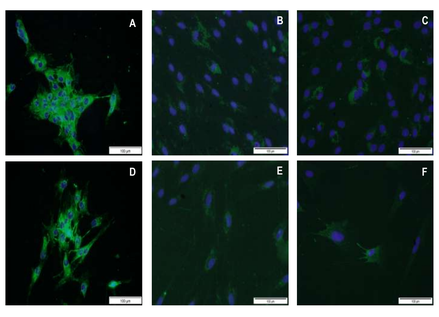


**Figure 1.** Immunocytochemical staining of dural epithelial cells (A-C) and dural fibroblasts (D-F) with antibodies specific for fibronectin (A,D), collagen I(B,E) and collagen III (C, F)

## RESULTS

Dural epithelial cells also had characteristics of endothelial cells. They expressed von Willebrand factor (Fig 2A) and CD31 (Fig 2B). Von Willebrand factor was localised in small intracellular granules and CD-31 was localised in the cellular membrane. The dural epithelial also expressed E-cadherin (Fig-2C), which is a transmembrane protein with a role in cell-cell adhesion and maintenance of tissue structure and function. The dural fibroblasts did not express these markers. (Fig 2 F, G, H)

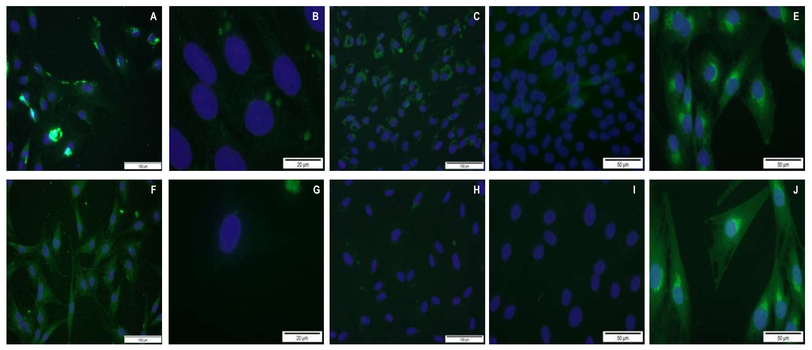


**Figure 2.** Immunocytochemical staining of dural epithelial cells (A-E) and dural fibroblasts (F-HJ) with antibodies specific for von Willebrand factor(A,F), CD-31(B,G), E-cadherin (C, H), desmoplakin I&II (D,I) and glucose transporter I (E,J)

In order to identify proteins associated with barrier function both cell types were stained with antibodies to Desmoplakin I & II and glucose transporter (Glut1). Dural epithelial cells expressed Desmoplakin I & II in culture which was enhanced at high confluent density regions supporting their role in barrier formation (Fig.2D). The dural fibroblasts failed to express Desmoplakin I & II (Fig.2I). However, both cell types were positive for Glut 1 suggesting a role for the glycoprotein in glucose transport in the cells (Fig. 2E&J).

Both dural cell types were free from smooth muscle contamination, and failed to express smooth muscle myosin (heavy chain), smoothelin and desmin.

### *Cellular uptake of the particles*

Particle uptake by both cell types was assessed over 3 days.

With regard to micron sized particle uptake, the dural epithelial cells failed to uptake significant numbers over a period of 24 hours. However, for the same exposure period the fibroblasts engulfed large numbers of the particles. The inability of the epithelial cells to engulf micron sized particles was a temporal phenomenon and after 2 days, particles were taken up. By day 3 of exposure both types of cells were full of micron-size fluorescent polyethylene particles.

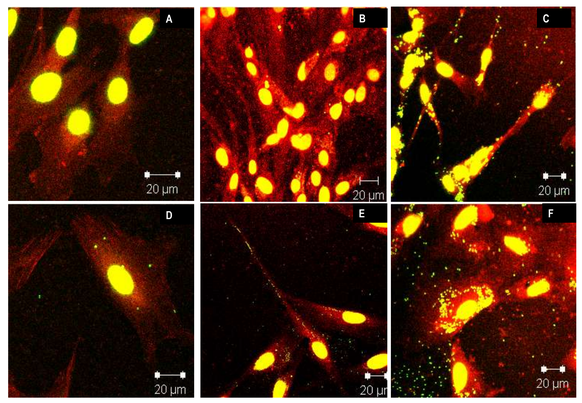


**Figure 3.** Confocal microscopy images of cells exposed to fluorescent microspheres (1µm in diameter). (A-C) Dural epithelial cells. (D-F) Dural fibroblasts. (A,D) Cells exposed for 1 day. (B,E) Cells exposed for 2 days. (C-F) Cells exposed for 3 days. The actin filaments were stained with rhodamine phalloidin and the nucleus with sytox green. All the images were taken at 630 magnification.

This pattern of particle engulfment was not repeated for the nano-sized particles. Both epithelial and fibroblast cells contained high numbers of nano-particles following 24h exposure (Fig. 4A&D) and after 2 days (Fig. 4B&E) and 3 days (Fig. 4C&F) exposure both the epithelial and fibroblast cells were full of nanoparticles. Particle uptake into cells was confirmed by deconvolution microscopy.

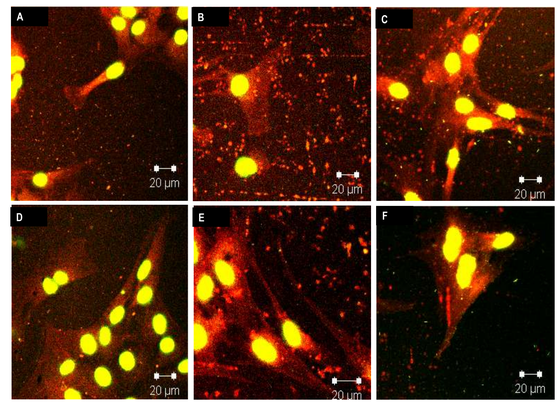


**Figure 4**. Confocal microscopy images of cells exposed to fluorescent nano-particles (40nm in diameter). (A-C) Dural epithelial cells. (D-F) Dural fibroblasts. (A,D) Cells exposed for 1 day. (B,E) Cells exposed for 2 days. (C-F) Cells exposed for 3 days. The actin filaments were stained with rhodamine phalloidin and the nucleus with sytox green. All the images were taken at 630x magnification.

To further assess this difference between the dural epithelial and fibroblast cells uptake of micron sized particles, the particle per cell number was determined (Fig. 5A). Dural epithelial cells exposed for 24 hours to micron size particles had engulfed low numbers of no particles. However, the dural fibroblasts exposed to particles for 24h had taken up greater than 10 particles per cell.

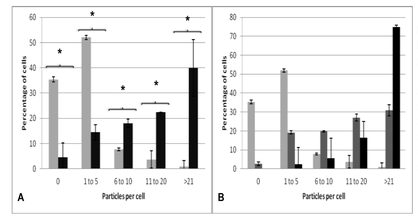


**Figure 5**. Cellular uptake of fluorescent microspheres. (A) Percentage of dural fibroblasts and dural epithelial cells that had phagocytosed a given number of particles after 24 h exposure. Epithelial cells, Fibroblast cells. The results were analysed by one-way ANOVA test and the MSD calculated using the T-method. A statistical difference ($p<0.05$) in particle uptake between the 2 group of cells, is indicated by an asterisk (*)(B) Percentage of dural epithelial cells that had phagocytosed a given number of particles after 24h, 2 and 3 day exposure. Day1, Day 2, Day 3. Data is expressed as the mean (n=3) +/- 95% confidence limits.

After 2 and 3 days of exposure of the dural epithelial cells to micron sized particles they began to internalize increasing numbers of particles (Fig 5B).

## DISCUSSION

- In this study for the first time, two types of cells in the porcine dura mater were isolated, characterized and shown to be epithelial cells with endothelial cell characteristics and barrier functionality, and fibroblast-type cells
- This study represents the first attempt to understand particle interactions with the isolated dural cells in vitro and has revealed that the capacity of dural cells to take up model particles was dependent on the size of the particles.
- Nanometer size particles were able to readily penetrate both types of cells. However, dural fibroblasts engulfed micron-sized particles at a much higher rate than dural epithelial cells. This suggests that dural epithelial cells may offer some barrier action to the penetration of larger particles.

**References**

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