Intervertebral disc regeneration with notochordal cells

Orthopaedic Biomechanics

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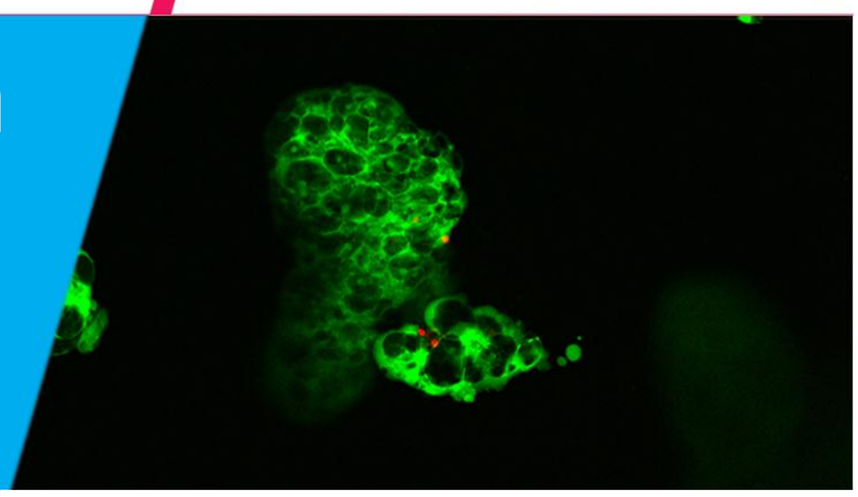

Introduction

The nucleus pulposus (NP), the gelatinous core of the intervertebral disc, resists compression but allows flexibility of the spine. At birth, the NP contains two distinct cell types: chondrocyte-like nucleus pulposus cells (NPCs) and notochordal cells (NCs, *fig 1*). The ratio between NPCs and NCs differs per species and age [1].

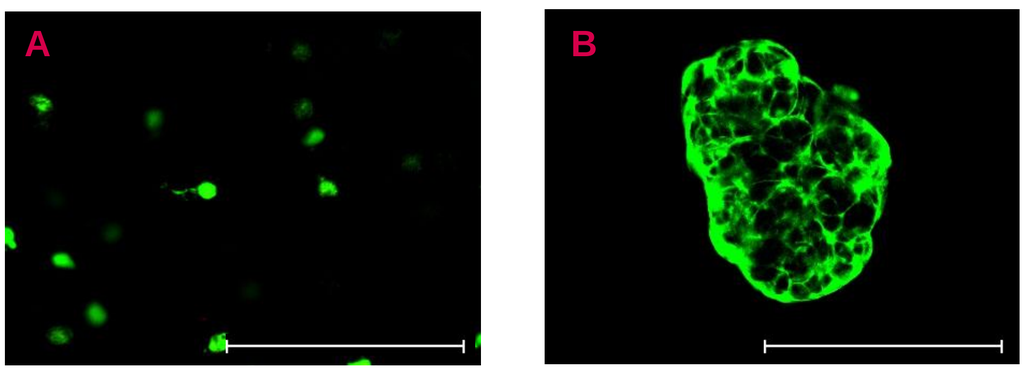

Figure 1: a. NPCs from a 24-months old cow, b. clustered NCs from a 3-months old pig. Green=cell cytoplasm (calcein-AM), scale bar=200 µm

Certain species maintain their NCs to an advanced age and their NPs remain healthy. Humans lose their NCs before adolescence and the NP starts to degenerate. Therefore, it is believed that NCs play a vital role in maintaining a healthy disc and that they may constitute good candidates to regenerate non-notochordal NPs.

Objective

The goal of this study is to evaluate the *in vitro* regenerative potential of NCs in a non-notochordal NP explant.

Study design

Tissue culture:

- **NP tissue from caudal discs of a 24-months old cows**
- **NC clusters from discs of a 3-months old pig, NCs are stained with long-term cell tracker CFSE (green)**
- **NPs are injected with 10 µL I) PBS (sham), II) 250.000 NCs in PBS or III) TGF-β3 in PBS**
- **Injected NP explants are cultured in dialysis tubing in hypertonic PEG medium [2] at 5% O_2 for 42 days**

Samples at day 42 are compared to day 0 regarding:

- **Cell viability (calcein blue-AM, PI, CFSE – NCs only)**
- **Water content**
- **DNA content (Hoechst dye assay)**
- **Glycosaminoglycan (GAG) content (DMMB assay)**
- **Hydroxyproline (HYP) content (Cloramin-T assay)**
- **Matrix distribution (hematoxylin, safranin-O, fast green)**
- **Gene expression (aggrecan, collagen type II and other NP markers)**

Preliminary results

Injected NCs remain viable during the 42 days of culture, according to cell viability images. Bovine NPC viability is similar in all groups. The measured DNA content (*fig 2C*) confirms these findings, as only the DNA content in the NC injected group is significantly higher.

No differences in water content (*fig 2A*) can be observed between the different injection groups after 42 days of culture. The same holds for the collagen content (*fig 2D*). The GAG content (*fig 2B*) is significantly decreased in the TGF-β3 group compared to that of day 0.

In conclusion, the NC injection increases the DNA content but not the amount of extracellular matrix proteins.

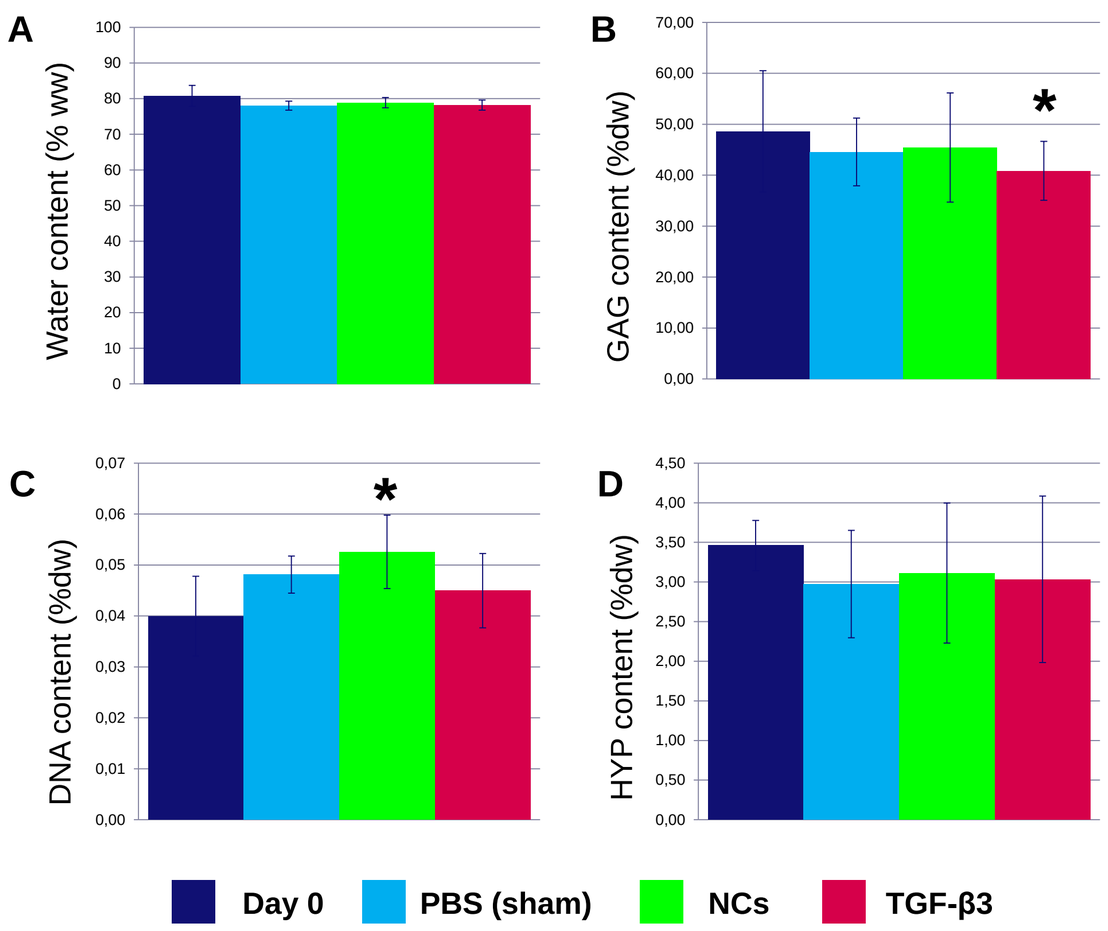

Figure 2: a. water, b. proteoglycan (GAG), c. DNA and d. collagen (HYP) content. Values are mean ± St Dev, n = 6, *p < 0.05 compared to day 0.

Continuation

The next step will be to measure if the NCs have an effect on the gene expression profiles of typical NP markers.

/ Department of Biomedical Engineering

References

[1] Hunter et al., *J. of anatomy,* 205 (2004), 357
[2] van Dijk et al. *Tissue Eng Part C*, 17 (2011),1089