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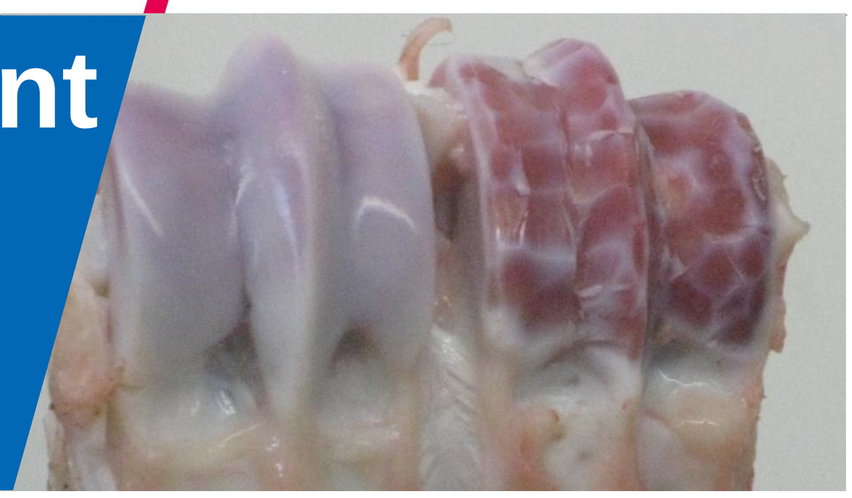
The effect of agarase treatment on chondrocyte-seeded agarose constructs

L.M. Kock, W.A.J. Wouters, C.C. van Donkelaar and K. Ito



Introduction

Agarose is a frequently used scaffold material in cartilage tissue engineering. The unique mechanical and chondrogenic properties of this material are accompanied by a drawback: agarose is not degradable by chondrocytes. This is thought to limit chondrocyte proliferation and ECM production during *in vitro* culturing, due to lack of space and impaired nutrient and waste product diffusion. However, agarose can be enzymatically degraded by the commercially available enzyme agarase.

Hypothesis

Controlled digestion of the scaffold material in engineered chondrocyte-seeded agarose constructs will:

- lower solid fraction of agarose
- improve proliferation and ECM production in time

Methods

- Porcine chondrocytes are seeded in 3% agarose discs

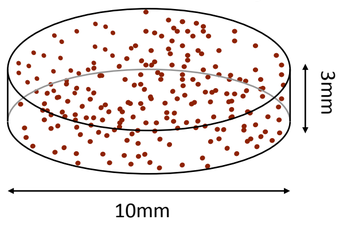
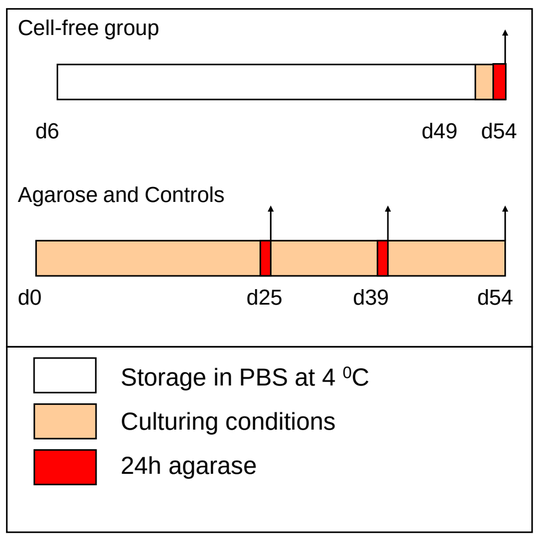


Figure 1: Schematic overview of an agarose construct

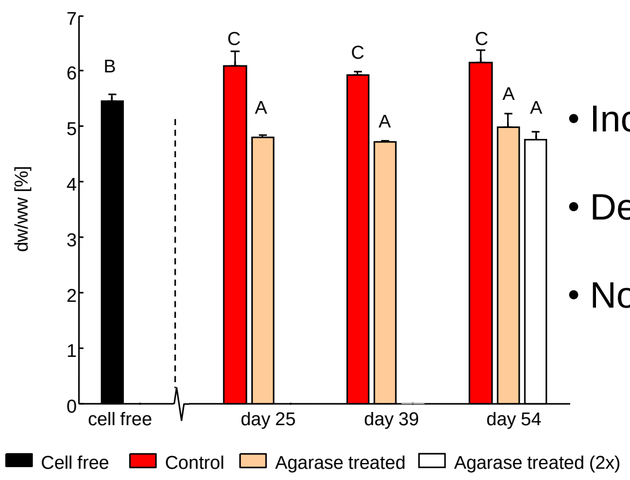


- Four experimental groups:
  1) Cell-free
  2) Untreated control
  3) Agarase treated (day 24)
  4) Agarase treated (day 24&39)

- Solid fraction and DNA, GAG and collagen content were determined using standard techniques at day 25, 39 and 54; distributions of cells and matrix are verified histologically

Results

• Solid fraction



- Increase due to presence of cells
- Decrease due to agarase treatment
- No significant time dependency

Figure 2: Solid fraction of the treatment groups. Significance between treatment groups is indicated by bars not sharing the same letter ($p<0.05$). (A indicates that there is no significant difference between the agarase treated and double treated groups, B and C indicate that there is a significant difference between the cell free, control and both agarase treated groups.)

• Biochemical analyses: GAG, DNA & Collagen

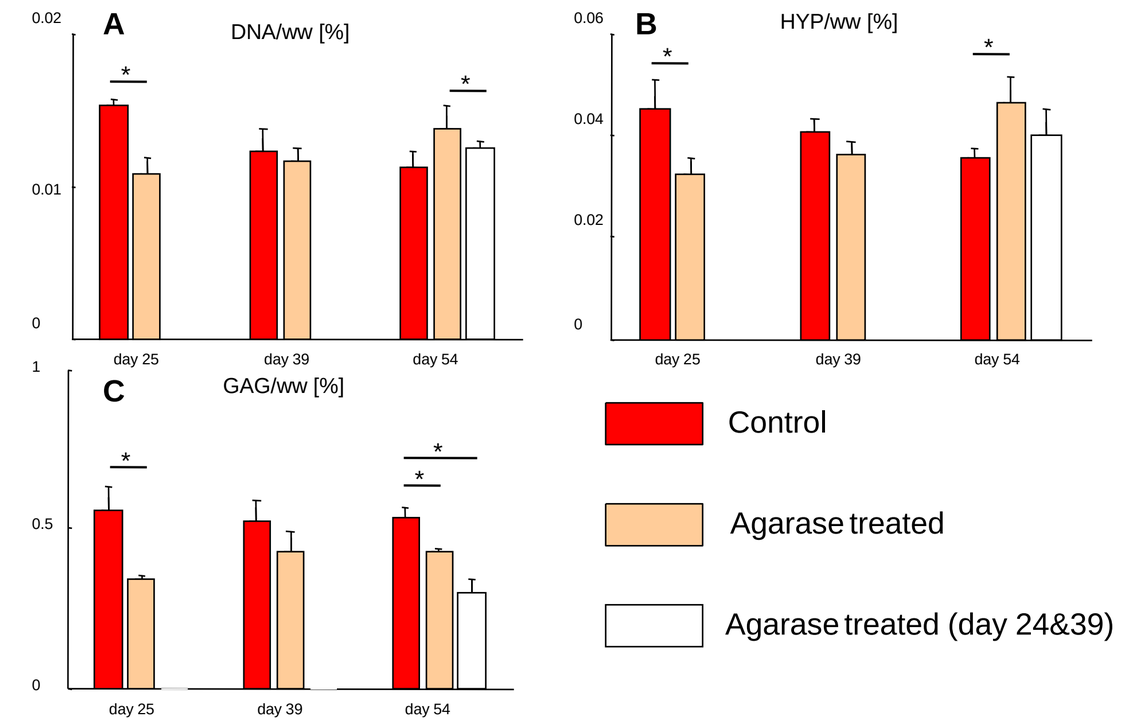


Figure 3: Results of the biochemical analysis: DNA content (A), collagen content (B), and GAG content (C) per wet weight are shown. *$p<0.05$ vs. untreated control

- Agarase treatment lowered DNA, GAG and collagen content initially
- More DNA and collagen content at end of culture period for treated group (day 24) compared to control
- Lower or equal GAG content at end of culture period for treated groups compared to control

• Histology

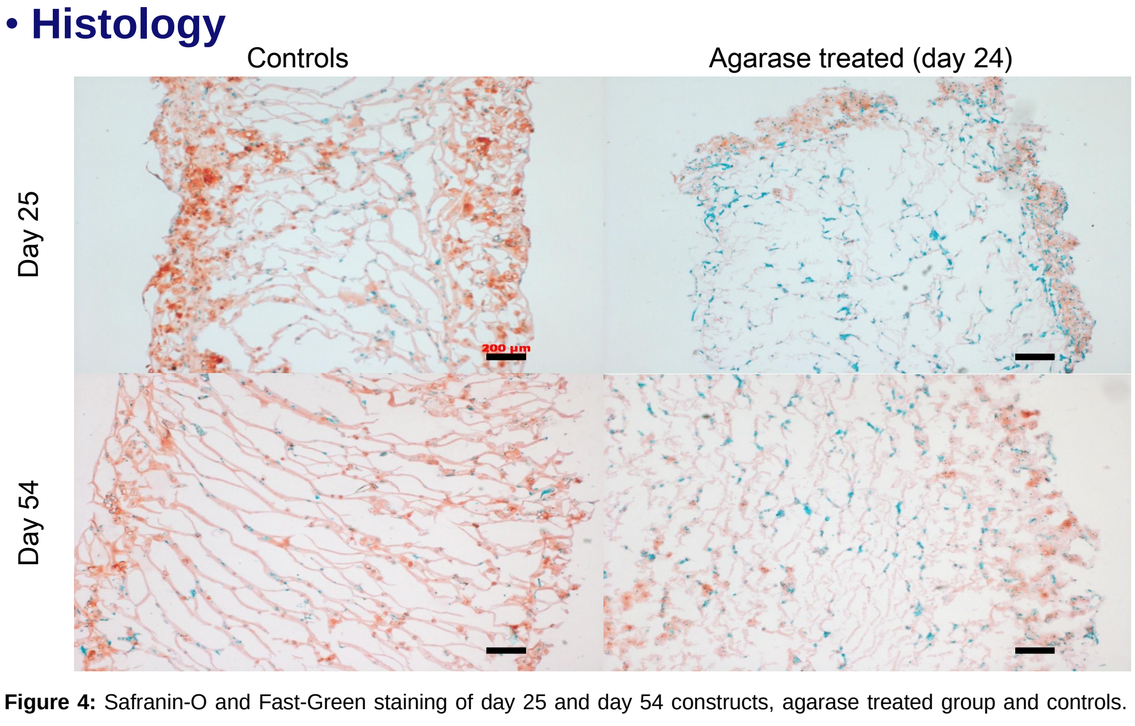


Figure 4: Safranin-O and Fast-Green staining of day 25 and day 54 constructs, agarase treated group and controls. Proteoglycans are stained orange to red and collagens and cytoplasm blue.

- Agarase treatment induced removal of proteoglycans (orange)
- Recovery is visible between day 25 and day 54

Conclusion

It is shown that agarase treatment removes scaffold material and has a positive effect on cell proliferation and collagen content. The mechanism may lie in increased nutrient transport, increased space for collagen fibril formation, and cellular response to the loss of GAG with agarase treatment. This effect of agarase treatment of chondrocyte-seeded agarose constructs is an important step towards the development of a scaffold-free engineered cartilage tissue for clinical implantation.

/ Department of Biomedical Engineering