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INTRODUCTION

New approaches to osteoarthritis treatment have focused on early intervention, with the development of methods to repair initial cartilage defects to prevent or delay disease onset. However, current therapies have shown variable success.

The aim of this research is to develop an immunocompatible acellular xenogenic osteochondral matrix with the same structural, biochemical and mechanical properties as natural tissues.

CHARACTERISATION

Porcine (6 month), bovine (18 month) and ovine (~1yr and >4 yr) tissues were characterised to identify the best starting material for decellularisation. Cartilage was stained with haematoxylin and eosin to visualise cartilage structure.

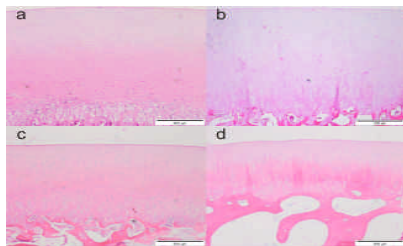


Figure 1. H&E staining of cartilage from the medial condyle. a) porcine, b) bovine, c) ovine (~1 yr), d) ovine (>4 yr)

CHARACTERISATION

Thickness of cartilage was measured using image analysis software from the resultant images

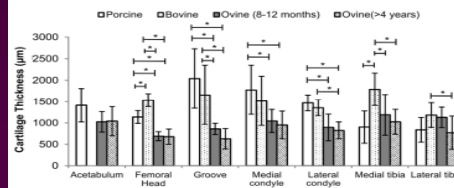


Figure 2. Cartilage thickness. Data is displayed as the mean (n=5) ± 95% CL. *Significant p < 0.05 (ANOVA).

Glycosaminoglycan (GAG) content was quantified

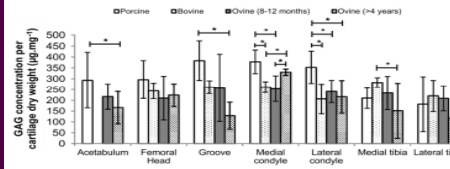


Figure 3. GAG content. Data is displayed as the mean (n=5) ± 95% CL. *Significant p < 0.05 (ANOVA).

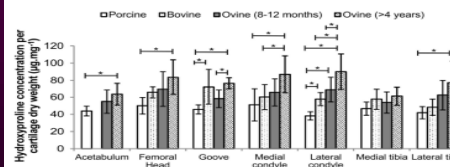


Figure 4. Cartilage hydroxyproline content. Data is displayed as the mean (n=5) ± 95% CL. *Significant p < 0.05 (ANOVA).

CHARACTERISATION

Osteochondral pins (9 mm ø, 12 mm deep) were compressed using a hemispherical impermeable indenter with a load of 0.8N to measure deformation, this was normalised to cartilage thickness. From the deformation data, the permeability and equilibrium elastic modulus of cartilage was determined using a finite element method¹.

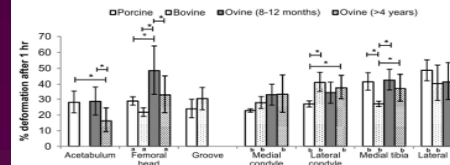


Figure 5. Cartilage percentage deformation. Data is displayed as the mean (n=5) ± 95% CL. *Significant p < 0.05 (ANOVA).

Figure 6. Equilibrium elastic modulus and permeability of cartilage. Table shows mean (n=5) and R² value for closeness of fit between experimental and FE modelled deformation curves. a) data from ref (2) b) data from ref (3).

DECELLULARISATION

Bovine tissue was selected for decellularisation. A method was developed, based on a patented process⁴, to decellularise bovine osteochondral tissues. Freeze/thaw cycles, hypotonic washes, low concentration SDS and nucleases were employed to remove DNA and cellular debris and the scaffolds sterilised using peracetic acid.

DECELLULARISATION

Cartilage was stained with DAPI to identify nuclear material.

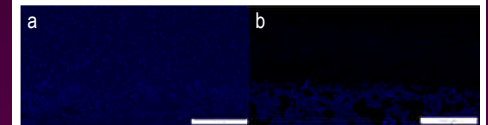


Figure 7. DAPI staining of cartilage. a) Fresh bovine medial groove b) decellularised cartilage.

DNA was extracted from tissues and isolated using Qiagen DNeasy kits and quantified using a nano spectrophotometer. GAG content was measured using dimethylmethene blue.



Figure 8. Cartilage DNA content. Data is displayed as the mean (n=3) ± 95% CL. *Significant p < 0.05 (ANOVA).



Figure 9. Cartilage GAG content. Data is displayed as the mean (n=3) ± 95% CL. *Significant p < 0.05 (ANOVA).

Percentage deformation of cartilage was calculated following unconfined compression testing and hydroxyproline content measured using dimethylaminobenzaldehyde.

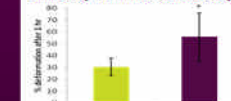


Figure 10. Cartilage percentage deformation. Data is displayed as the mean (n=3) ± 95% CL. *Significant p < 0.05 (ANOVA).



Figure 11. Cartilage hydroxyproline content. Data is displayed as the mean (n=3) ± 95% CL. *Significant p < 0.05 (ANOVA).

Bovine osteochondral plugs from the medial groove have been successfully decellularised. However, the process has led to a loss of GAGs and subsequent loss of mechanical properties.



References

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