Institute of Medical & Biological Engineering

Development of an Ex-Vivo Organ Culture Model of the Femoral-Tibial Joint

UNIVERSITY OF LEEDS

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Background

- The capacity for pre-clinical evaluation in viable physiological biotribiological models, such as the femoral-tibial joint would enhance the development of cartilage substitution therapies.
- We have previously developed methods for organ culture of femoral osteochondral plugs.
- The aims of this study were to explore the feasibility of maintaining whole femoral condylar and tibial-osteochondral tissues in organ culture.

Materials & Methods

- ***Osteochondral (OC) plugs***: 9 mm diameter removed from porcine condyles.
- ***"Whole joint" tissues***: femoral condyle and tibial plateau from porcine knees (within 4h of slaughter) dissected aseptically. Majority of cancellous bone reamed leaving intact cartilage and layer of cortical bone (~5 mm). Blood and bone marrow removed by dental water flossing (Waterpik) and incubated overnight in HBSS (12.5 $U.ml^{-1}$ heparin and antibiotics).
- ***Culture conditions***: defined medium DMEM with 2 mM L-glutamine, 1 mM sodium pyruvate, 0.1% (v/v) ITS Pre-mix (BD Biosciences), 50 $mg.ml^{-1}$ ascorbic acid, 0.1 µM dexamethasone and antibiotics. OC plugs cultured in 24-well plates and "whole joint tissues" in 250 ml pots , bubbled with 5% (v/v) CO_2 in air at 37°C.
- ***Cartilage viability:*** determined at 0, 8 and 14 days of culture by XTT and LIVE/DEAD staining.
- ***Histology:*** Standard histological techniques. Sections stained with haematoxylin & eosin (H&E) and alcian blue.
- ***Glycosaminoglycan (GAG) levels*** quantified using dimethylene blue assay (DMB).

Results

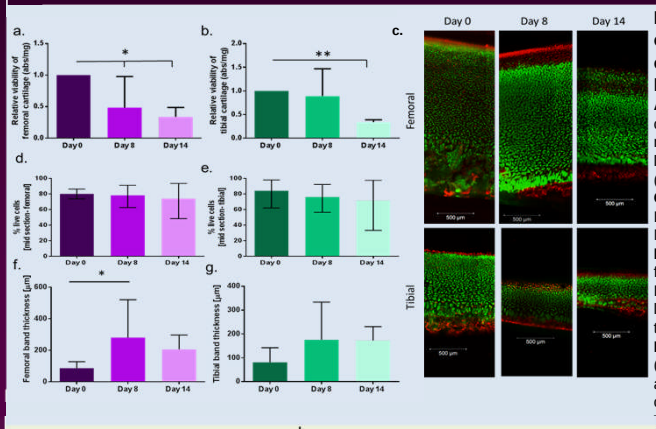

Figure 1: Cartilage viability at 0, 8 and 14 days of whole femoral condyle and tibial plateau organ culture; assessed by XTT and LIVE/DEAD staining.
A & B: XTT Conversion in femoral (A) and tibial (B) cartilage at day 8 and day 14. Data are expressed as mean relative to matched day 0 levels with 95% confidence limits. Raw data analysed by student t-test (* $p < 0.05$, ** $p < 0.01$).
C: Confocal imaging of LIVE/DEAD stained cartilage. Live cells are green and dead cells red (Zeiss LSM510 Meta)
D & E: Proportion of live cells within the mid portion of femoral (D) and tibial (E) cartilage. Analysed by Image J. Data are expressed as the mean with 95% confidence limits and analysed by one-way ANOVA of arcsine transformed data which showed no significant variation
F & G: Depth of non-viable surface zone of cartilage (apical portion of cartilage with dead cells) in femoral (F) and tibial (G) cartilage. Data expressed as mean with 95% confidence limits, analysed by one-way ANOVA with Tukey's multiple comparison (* $p < 0.05$).

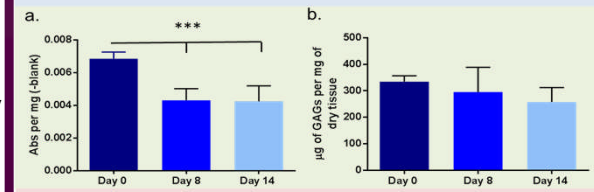

Figure 2: Viability and GAG content of cartilage from 9mm femoral OC plugs
A: XTT conversion of OC plugs at day 8 and day 14 of culture.
B: GAG content of OC plugs at day 8 and day 14 of culture. Data expressed as mean with 95% confidence limits and analysed by one-way ANOVA with Tukey's multiple comparison test (*** $p < 0.001$) .

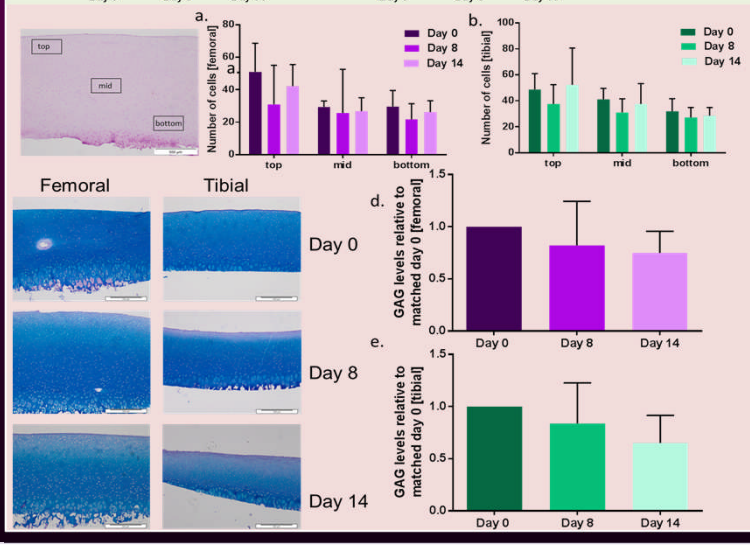

Figure 3: Histological assessment of cartilage and quantification of G AG content at 0, 8 and 14 days of "whole joint" organ culture.
A & B: Quantification of cell number following H&E staining of cartilage. Sections were stratified into the top, mid and apical portion in femoral (A) and tibial (B) cartilage and cell number counted using image J. Data expressed as mean with 95% confidence limits and analysed by two-way ANOVA with Sidak's multiple comparison which showed no significant variation.
C: Visualisation of GAG content of cartilage with Alcian Blue staining (counter stained with periodic acid- Schiff)
D & E: : GAG content of tissue quantified by DMB assay in femoral (D) and tibial (E) cartilage at day 8 and 14 relative to matched 0 cartilage; Data expressed as mean with 95% confidence limits and analysed by student t-test which showed no significant difference.

Results

- No change in XTT conversion in tibial cartilage after 8 days of "whole joint" culture. Reduced XTT conversion in femoral condylar cartilage after 8 days and femoral and tibial cartilage following 14 days in culture (Fig 1a & b).
- Majority of chondrocytes in the mid and deep cartilage zones were viable (LIVE/DEAD staining) (Fig 1c) with no significant reduction in viable proportion during culture (Fig1 d&e).
- Depth of non-viable surface zone significantly increased following 8 days of femoral condyle culture from 86 mm at day 0 to 280 mm at day 8 (Fig 1 f & g) but no further change after 14 days. The increase did not reach the level of significance in tibial cartilage.
- Conversion of XTT in OC plugs reduced significantly between day 0 and both 8 and 14 days in culture, but no further reductions between days 8 and 14 (Fig 2a).
- GAG levels in OC plug cartilage did not significantly change throughout the culture period (Fig 2b).
- No change in chondrocyte number at any depth following "whole joint" culture (Fig 3 a&b).
- No significant loss of GAGs from the whole joint cultures after 8 or 14 days in culture (Fig 3 d&e). Supported by alcian blue staining of tissue sections. (Fig 3c).

Conclusions

- Large femoral and tibial osteochondral cuts were maintained in organ culture for extended periods.
- Whole joint cultures behaved in a similar manner to OC plugs, with reductions in viability during culture (assessed by XTT conversion) but no change in cartilage GAG content.
- Chondrocytes in mid- and deep zones remained viable. Chondrocytes in the surface zone lost membrane integrity rapidly, with further loss of viability during organ culture.
- Future studies will focus on physiological loading in a novel physically interactive bioreactor with a view to maintaining the viability of surface zone chondrocytes and maintain GAG levels.