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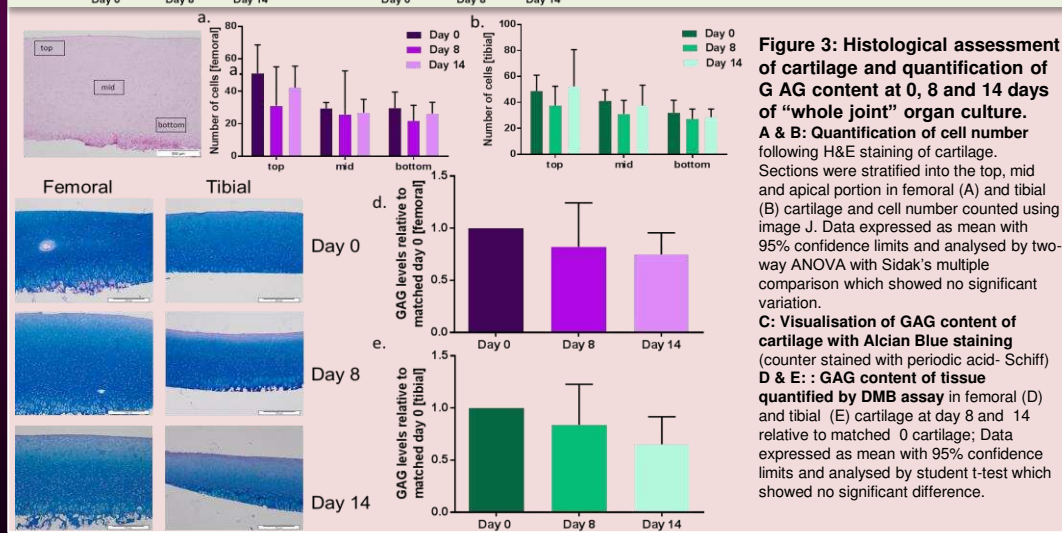
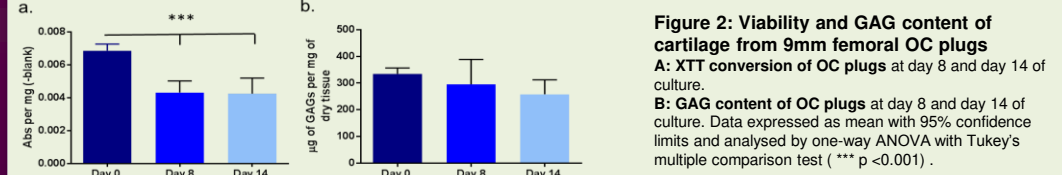
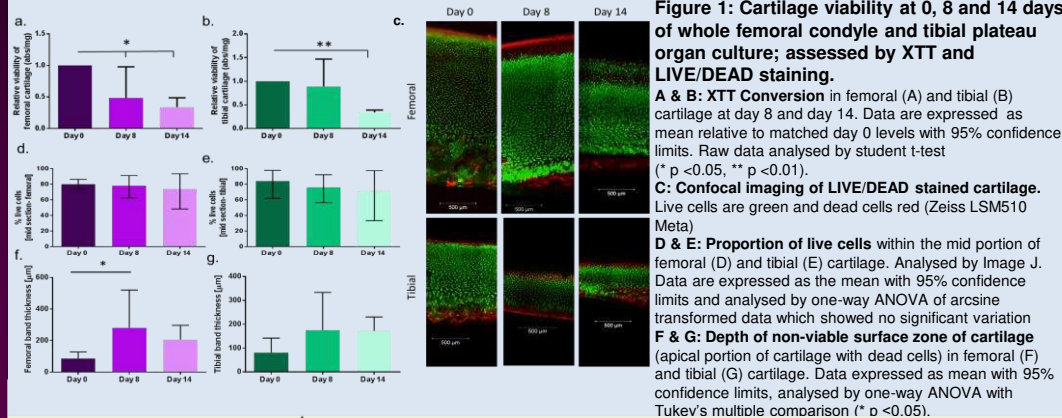
Background

- The capacity for pre-clinical evaluation in viable physiological biotribological 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) CO₂ 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



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 (Fig 1d&e).
- Depth of non-viable surface zone significantly increased following 8 days of femoral condyle culture from 86 μm at day 0 to 280 μm at day 8 (Fig 1f & 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.