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The mechanical and tribological performance of decellularised porcine osteochondral allografts in an *in vitro* porcine tibiofemoral joint model

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Introduction

Osteochondral autografting and allografting are two surgical techniques used to repair articular cartilage in the knee joint. However, both interventions have existing issues[1][3], including:

- Quality and availability of replacement tissue
- Donor-site morbidity (autografting)
- Immunological complications (allografting)

Decellularised (dCell) osteochondral constructs (Figure 1) are being developed to provide off-the-shelf solutions. However, there is often a lack of *in vitro* evidence to demonstrate the mechanical and tribological performance of novel osteochondral interventions before clinical use.

Figure 1: A decellularised osteochondral allograft.

Robust preclinical assessment can establish efficacy and enable predictions about in vivo performance, reducing risk prior to clinical use.

Aim: To compare the mechanical and tribological performance of decellularised porcine osteochondral allografts with porcine osteochondral allografts during extended duration (48-hour) simulations of activities of daily living. The hypothesis for this study was decellularised allografts would provide comparable mechanical and tribological performance to the gold standard allografts due to the similarity between their structures.

Methods

Samples and groups: Grafts/pin of Ø6.5mm, were implanted into the medial femoral condyle of porcine tibiofemoral joints within the contact region (Figure 2), the experimental groups included:

- Porcine osteochondral allografts (n=3)
- Decellularised porcine osteochondral allografts (n=3)
- Stainless-steel pins (positive control, n=3)
- Untreated negative controls: lateral compartment of knees (n=3, one from each group)

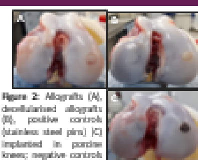

Figure 2: Allografts (A), decellularised allografts (B), positive controls (stainless steel pins) (C) implanted in porcine knees; negative controls = lateral compartments.

Knee Simulation: Tibiofemoral joints were mounted into a single station knee simulator[3] (Simulation Solutions) (Figure 3) and subjected to 48-hour experimental simulations incorporating activities of daily living (47-hours walking gait (WG) and 1-hour stair ascent (SA)); all simulations used a 25% new-born calf serum in Ringer's Solution lubricant

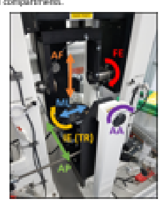

Figure 3: Axes of motion in single station knee simulator: axial force (superior-inferior) (orange), anterior-posterior displacement (green), internal-external (tibial) rotation (yellow), flexion-extension rotation (red), abduction-adduction rotation (purple), medial-lateral displacement (blue).

Wear, Damage and Deformation Assessment:

- Sirius Red and H&E stained graft and femoral sections were used to assess structural changes and identify occult damage post-test
- ICRS/OARSI grading 0-4 (0=normal, 4=severely abnormal) of cartilage surfaces was performed pre walking gait (t=0), post walking gait (t=47 hours) and post stair ascent (t=48-hours)
- Femoral and tibial surfaces were divided into 9 regions and menisci into 3 regions. Values reported were the mean total score for each articulating surface (femoral and tibial 0-36, meniscal 0-12)
- *Statistics:* Kruskal-Wallis test and post hoc Dunn's test with Bonferroni correction ($p < 0.05$)

Graft/Pin Stability in Recipient Site :

- Silicone replicas of the graft/pin and surrounding femoral cartilage were created after each visual inspection period then scanned using an optical profiler (Alicona Infinite Focus) to calculate the mean relative height difference between graft/pin surfaces and femoral condyle surfaces at eight locations around graft/pin (Figure 4)
- *Statistics:* one-way ANOVA and post hoc Tukey *HSD* test ($p < 0.05$)

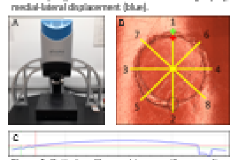

Figure 4: Optical profiler used to scan silicone replicas of cartilage surfaces (A). Traces (yellow lines) drawn across the graft/pin replica surface to enable relative height difference between the femoral condyle and graft/pin to be measured (B). Resultant trace on sample surface with red and green crosshairs placed to measure height difference (C).

Results

Graft Stability:

- Decellularised allografts subsided from the initial position after 47-hours walking gait, then subsided beneath femoral cartilage after an additional 1 hour of stair-ascent (Figure 5)
- Allografts and positive controls showed minimal movement during simulations
- There was a statistically significant difference ($p<0.05$) in relative height between the decellularised allograft group and the other two experimental groups post stair ascent
- No significant differences were observed between the three experimental groups pre walking gait or post walking gait

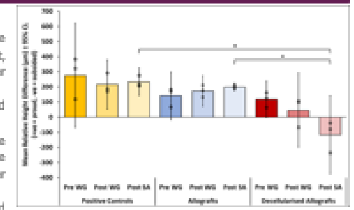

Figure 5: Relative height difference between graft/pin surface and femoral condyle surface pre-walking gait (pre WG), after 47-hours of walking gait (post WG) and after 1-hour of stair ascent (post SA) for positive controls (orange), allografts (blue) and decellularised allografts (red). Bars are the mean of n=3 with 95% confidence limits; points on the bars represent height difference for individual samples within each group. Statistical analysis was performed using a one-way ANOVA with post hoc Tukey HSD test (* indicates statistical significance, $p<0.05$).

Wear, damage and deformation:

- Histological analysis was inconclusive showing regions without damage as well as evidence of cartilage delamination of the graft and condyle for both experimental groups (Figure 6). Positive controls damaged the meniscus/tibia
- No clear differences between decellularised allografts and allografts were observed
- ICRS/OARSI grading showed significant differences ($p<0.05$) between allografts and negative controls for the femur post walking gait and between positive and negative controls for meniscus post walking gait and post stair ascent. No significant differences were observed between allografts and decellularised allografts (Figure 7).

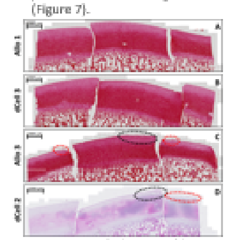

Figure 6: Sirius Red (A-C) and H&E (D) stained osteochondral graft and medial femoral condyle sections highlighting similarities between the allograft and decellularised allograft groups. Intact cartilage on both the graft and femoral condyle (A and B), evidence of delamination on both the graft (black dotted circles) and femoral condyles (red dotted circles).

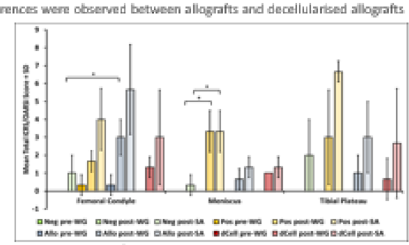

Figure 7: Mean total ICRS/OARSI score assigned to each articulating surface + standard deviation pre walking gait, after 47 hours walking gait and after an additional 1 hour of stair ascent (total=48hrs). Neg = negative controls, Pos = positive controls, Allo = allografts, dCell = decellularised allografts, WG = walking gait, SA = stair ascent. Femoral condyles and tibial plateau scored 0-36, menisci scored 0-12. Statistical analysis for each articulating surface was performed using a Kruskal-Wallis test with post hoc Dunn-Bonferroni correction (* indicates statistical significance between: Neg post-WG and Allo post-WG for the femoral condyle ($p=0.017$), Neg post-WG and Pos post-WG for meniscus, ($p=0.013$), Neg post-SA and Pos post-SA for meniscus, ($p=0.020$)).

Discussion

- No obvious difference in tribological performance was observed between the allograft and decellularised allograft groups based on ICRS/OARSI scoring or histological staining, this indicates decellularised grafts are potentially a viable alternative
- For graft stability, decellularised allografts showed subsidence, whereas allografts did not. This could be disadvantageous, however the observed deficiencies are potentially due to experimental limitations:
  - The conditions used represent the immediate post-implantation situation before any host tissue ingrowth has occurred. Ingrowth of the host tissue into the decellularised scaffold *in vivo* could potentially stabilize the graft and prevent subsidence from occurring
  - Changes in relative height could have been due to changes to graft cartilage or femoral cartilage not just movement of the graft within the recipient site
  - A small sample size (n=3) for each group was used during the current study

SIGNIFICANCE/CLINICAL RELEVANCE: Decellularised allografts show potential as a viable alternative to existing cartilage repair interventions; but further investigation is required. Larger sample size and translation of this study into human knees is necessary to confirm findings related to wear, damage and deformation and graft subsidence.

References:

[1] Dowland P.C., et al. 2015. Proc Inst Mech Eng H, 229(12): 879–888.

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[3] Dowland P.C., et al. 2018. Journal of Biomechanics, 77, pp.91-98.

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