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Development of a preclinical natural porcine knee simulation model for the tribological assessment of osteochondral grafts *in vitro*

P. Bowland, E. Ingham, J. Fisher, L.M. Jennings *

Institute of Medical & Biological Engineering, School of Mechanical Engineering, University of Leeds, Leeds, UK

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ABSTRACT

In order to pre-clinically evaluate the performance and efficacy of novel osteochondral interventions, physiological and clinically relevant whole joint simulation models, capable of reproducing the complex loading and motions experienced in the natural knee environment are required. The aim of this study was to develop a method for the assessment of tribological performance of osteochondral grafts within an *in vitro* whole natural joint simulation model.

The study assessed the effects of osteochondral allograft implantation (existing surgical intervention for the repair of osteochondral defects) on the wear, deformation and damage of the opposing articular surfaces. Tribological performance of osteochondral grafts was compared to the natural joint (negative control), an injury model (focal cartilage defects) and stainless steel pins (positive controls). A recently developed method using an optical profiler (Alicona Infinite Focus G5, Alicona Imaging GmbH, Austria) was used to quantify and characterise the wear, deformation and damage occurring on the opposing articular surfaces. Allografts inserted flush with the cartilage surface had the lowest levels of wear, deformation and damage following the 2 h test; increased levels of wear, deformation and damage were observed when allografts and stainless steel pins were inserted proud of the articular surface. The method developed will be applied in future studies to assess the tribological performance of novel early stage osteochondral interventions prior to *in vivo* studies, investigate variation in surgical precision and aid in the development of stratified interventions for the patient population.

1. Introduction

There is an increasing clinical need for effective early intervention osteochondral therapies that can restore the structure and function of cartilage and bone tissue. The complex biphasic structure and composition of cartilage provides exceptional functional properties allowing low friction movement under high load bearing conditions. The limitations in current therapies have prompted widespread research in the field of tissue engineering to develop alternative treatment strategies. Tissue engineered osteochondral scaffolds and constructs have the potential to regenerate cartilage and bone tissues that possess the structural, biological, mechanical and tribological properties of native cartilage and bone (Bowland et al., 2015).

The development of such early stage repair interventions in the knee, requires an understanding of how the range of variables in the natural knee environment interact with the design and material properties of the intervention to determine mechanical and tribological performance (Liu et al., 2015). Whole joint experimental simulation models, capable of reproducing the complex physiological loading, motions and interactions in the natural knee, can play a key role in the preclinical testing of early osteochondral interventions in order to determine mechanical and tribological performance prior to *in vivo* studies.

Although it is recognised that ultimately pre-clinical testing should be performed using human cadaveric tissue, the use of animal tissue can be extremely useful for methodology development. The porcine knee joint is particularly suitable as an osteochondral repair model for the following reasons: Joint size, loading, cartilage and trabecular bone thickness that more closely match the human condition than alternative animal models (Chu et al., 2010); easily obtainable from commercial suppliers, where sufficient numbers of samples can be obtained from animals slaughtered at a uniform age and of good health; lower levels of inter-specimen variability and good cartilage and bone quality when compared with donor human tissue; and finally the absence of age related changes and degeneration in porcine cartilage and bone tissue may be more representative of general tissue quality in the younger population

* Corresponding author at: Institute of Medical & Biological Engineering, School of Mechanical Engineering, University of Leeds, Leeds LS2 9JT, UK.

E-mail address: l.m.jennings@leeds.ac.uk (L.M. Jennings).

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in which early intervention therapies are the most clinically relevant.

Currently, there are no published studies reporting the development of an *in vitro* whole joint simulation model, capable of the preclinical tribological assessment of early osteochondral repair interventions. A tribological whole natural porcine tibiofemoral knee joint simulation model has been previously validated and described by Liu et al. (2015). The overall aim of this study was to extend the method developed by Liu et al. (2015) to be able to assess the tribological performance (in terms of wear, deformation and damage) of osteochondral grafts within the *in vitro* whole natural joint simulation model of the porcine tibiofemoral joint. Specifically the aim was to develop a method that showed quantifiable wear, deformation and damage on the opposing surface to the intervention for a positive control osteochondral graft (metal pin) and then apply the method to quantify the resulting wear, deformation and damage on the opposing surface as a result of allograft implantations and focal cartilage defects, and begin to explore the effects of surgical precision in graft implantation on tribological performance through the assessment of proud grafts.

2. Materials and methods

2.1. Study design

A full description of the experimental groups is provided in Table 1 and an overview shown in Fig. 1. All experimental group samples (n = 4 for each group) were tested in the single station knee simulator for a duration of 7200 cycles at 1 Hz (2 h) in a lubricant of 25% (v/v) newborn calf serum (Gibco Life Technologies, Paisley, UK) in PBS.

2.2. Porcine knee joint preparation

Porcine tibiofemoral joints were obtained from the right hind legs of pigs aged 4–6 months old within 24 hrs of slaughter. The porcine tibiofemoral joints were braced in their natural physiological alignment, all tissue and ligaments were dissected away, leaving the meniscus *in situ*.

The centre of rotation of the femoral condyles was determined using a templating methodology (McCann et al., 2008). The femur and tibia were cemented (polymethylmethacrylate (PMMA) cement; WHW plastics, UK) and aligned in custom built test pots, using a purpose built mounting rig that replicated the internal working heights and orientation of the simulator. The axial force was offset 7% of the width of the porcine joint in a medial direction from the tibial axis to ensure more medial loading. The braces were then removed from the tibiofemoral joint and the porcine sample was mounted within a gaiter (holding lubricant) in the natural knee simulator.

Table 1
Description of the experimental groups (n = 4 per group). All experimental tests run for 7200 cycles at 1 Hz (2 h) in the single station knee simulator.

Experimental Group	Description
Negative Control	Articulating surfaces intact (native state).
Cartilage Defects	6 mm diameter cartilage defect to subchondral bone.
Allografts Flush	6 mm diameter porcine osteochondral graft inserted flush with cartilage surface.
Allografts 1 mm Proud	6 mm diameter porcine osteochondral graft inserted 1 mm proud of cartilage surface.
Stainless Steel Pins Flush (Positive Control 1)	6 mm diameter stainless steel pin inserted flush with cartilage surface.
Stainless Steel Pins 1 mm Proud (Positive Control 2)	6 mm diameter stainless steel pin inserted 1 mm proud of cartilage surface.

Osteochondral allografts were harvested using a 6 mm diameter drill aided corer from the contact area of porcine medial femoral condyles. Acufex™ (Smith & Nephew, USA) mosaicplasty surgical drill attachment, drill guide and delivery tamp tools were used for the creation of recipient donor sites and the subsequent implantation of osteochondral allografts and stainless steel pins. Full thickness cartilage defects were created using a 6 mm diameter biopsy punch with the defect extending down to the subchondral bone. For the 1 mm proud grafts, these were 1 mm longer than the standard (flush) grafts, and the implantation procedure was identical (implant hole depth same for all groups). Tissue samples were kept hydrated throughout the preparation procedures using phosphate buffered saline (PBS; MP Biomedicals LLC, UK) and stored until required for testing on PBS soaked tissue paper at −20 °C. Samples were removed from storage prior to testing and thawed at room temperature.

2.3. Single station natural knee simulator

The methods presented in this study were developed from the simulation model as described by Liu et al. (2015). The ProSim electromechanical single station natural knee simulator (Simulation Solutions, Manchester, UK) was used with a standard gait kinematic input profile, which scaled the high kinematic Leeds knee input profiles (McEwen et al., 2005) to the kinematic limits of porcine knee tissue. The simulator had six degrees of freedom and five axis of controlled motion. The axial load (AF) was force controlled; flexion-extension (FE) and tibial rotation (TR) were displacement controlled; the medial-lateral axis was fully constrained; and abduction-adduction (AA) was not controlled and left unconstrained (passive motion). Anterior-posterior (AP) displacement was not driven and was constrained through the use of springs ($k = 2.69\ N.mm^{-1}$). The spring constraints were used to replicate ligament function and achieve rolling and sliding motions of the femur relative to the tibia.

The kinematic input profile applied a peak axial load of 1000 N, a flexion-extension range of 0–21° and tibial rotation of 1.6–1.6° at a frequency of 1 Hz. AP displacement and AP shear force data were recorded for each test. The AP shear force was the force transmitted from the femur to tibia along the AP axis and was used as a measure of friction occurring between the articulating surfaces of the tibiofemoral joint (Liu et al., 2015).

All porcine knees, excluding the negative control, were initially run for 900 cycles at 1 Hz as a reference test to check stability and that each sample could reproduce the AP shear force and displacement outputs demonstrated during the full negative control group tests (7200 cycles at 1 Hz). The porcine knees were then removed from the simulator and allowed to recover for 1 h before either a 6 mm diameter allograft, cartilage defect or stainless steel pin was inserted into the centre of the contact area on the medial femoral condyle. The samples were then mounted back into the simulator and run as the experimental group tests (Table 1) (7200 cycles at 1 Hz).

2.4. Assessment and quantification of articular surface wear, deformation and damage

The surface wear, deformation and damage present on the opposing meniscal surfaces to the medial femoral condyle was assessed and quantified using an Alicona Infinite Focus G5 optical profiler. The Alicona Infinite Focus is an optical device for 3D surface measurement and analysis which operates using focus variation technology. Focus variation combines the small depth of focus of an optical system with vertical scanning in order to provide topographical and full colour information from the variation of focus. The system (Fig. 2) consists of a movable XY-stage above