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Improved functional assessment of osteoarthritic knee joint after chondrogenically induced cell treatment

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SUMMARY

Objectives: Our previous studies on osteoarthritis (OA) revealed positive outcome after chondrogenically induced cells treatment. Presently, the functional improvements of these treated OA knee joints were quantified followed by evaluation of the mechanical properties of the engineered cartilages.
Methods: Baseline electromyogram (EMGs) were conducted at week 0 (pre-OA), on the locomotory muscles of nine un-castrated male sheep (*Siamese long tail cross*) divided into controls, adipose-derived stem cells (ADSCs) and bone marrow stem cells (BMSCs), before OA inductions. Subsequent recordings were performed at week 7 and week 31 which were post-OA and post-treatments. Afterwards, the compression tests of the regenerated cartilage were performed.
Results: Post-treatment EMG analysis revealed that the control sheep retained significant reductions in amplitudes at the right medial gluteus, vastus lateralis and bicep femoris, whereas BMSCs and ADSCs samples had no further significant reductions ($P < 0.05$). Grossly and histologically, the treated knee joints demonstrated the presence of regenerated neo cartilages evidenced by the fluorescence of PKH26 tracker. Based on the International Cartilage Repair Society scores (ICRS), they had significantly lower grades than the controls ($P < 0.05$). The compression moduli of the native cartilages and the engineered cartilages differed significantly at the tibia plateau, patella femoral groove and the patella; whereas at the medial femoral condyle, they had similar moduli of 0.69 MPa and 0.40–0.64 MPa respectively. Their compression strengths at all four regions were within ±10 MPa.
Conclusion: The tissue engineered cartilages provided evidence of functional recoveries associated to the structural regenerations, and their mechanical properties were comparable with the native cartilage.

Introduction

It has been well established that abnormal deviations from normal kinematics, kinetics, or electromyogram (EMG) patterns are used to diagnose specific conditions and to predict the outcome of treatments[1–4]. Several studies have been conducted over the past two decades with the aim of engineering cartilage constructs for repairing or restoring damaged cartilage[5–9]. It is desired that an engineered neo-cartilage should possess mechanical properties

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that match those of the native cartilage[10,11]; thus meeting the criteria for a translation of therapy to humans includes a proof of concept that the engineered product possessed properties matching the native tissue as well as the ability to provide functional recovery[12].

Among the major methods for determining the mechanical properties of cartilage are tensile, compressive and shear testing[13]. Tensile testing measures a force applied at a constant strain rate[13]. Compressive testing often measures the compressive strength of a material such as cracking or pitting[14], while shear testing determines the dynamic characteristics[15]. During the clinical follow up of osteoarthritis (OA) treatment, a major degenerative joint disorder, various labs including our orthopaedic department and physiotherapy units use surface EMG to evaluate the contribution of the affected limb to gait[1,16–19]. EMG studies revealed that after the development of OA, the muscle activation pattern decreases in the affected limb compared to the normal, due to atrophy which ensues with time, because of muscle inactivation[16,17,20].

In our previous studies, we optimised a procedure for the surgical induction of osteoarthritis in sheep, which was a combined technique of anterior cruciate ligament resection, medial meniscectomy and an exercise regimen[21–23]. These osteoarthritis-like sub chondral lesions were treated with autologous chondrogenically induced ADSCs and BMSCs based therapy, and the gross images revealed good structural regeneration in the treated animals[24]. The homing of the injected cells was traced to the lesions; and the histology and the protein analysis of the regenerated cartilage were similar to the native[8,24]. In the present work, we considered investigating this probable gained functional improvement and quantifying the mechanical strength of the regenerated cartilage after OA treatments.

Thus, our objectives included: (1) quantifying the functional improvements of OA knee joints following chondrogenically induced cell treatments using EMG; (2) measuring the compression-related mechanical properties of the tissue-engineered cartilage compared to those of the native cartilage of the same animal. We hypothesised that there would be a significant functional improvement in the knee joints after cell therapy treatments.

Materials and methods

Experimental design

Ethics approval was granted by the University Kebangsaan Malaysia Animal Ethics Committee (PP/TEC/RUSZYMAH/25-NOV/342-DEC-2010-JUN-2012) and the Universiti Putra Malaysia Animal Ethics Committee (RUJ: ACUC 07R6/JULY 07-DEC 09). A total of nine randomly selected, healthy un-castrated male sheep (*Siamese long tail cross*) aged 2.5 ± 0.5 years and weighing 25 ± 2 kg provided by Veterinary Faculty University Putra Malaysia were used. EMG measurements were performed on three separate occasions on the study groups: 3Controls; 3BMSCs and 3ADSCs. The first measurement was taken before the surgical induction of OA (week 0); the second was taken after the OA induction in the right knee joint of each sheep (week 7). ADSCs and BMSCs harvested during the OA inductions were concurrently cultured, labelled with PKH26 and induced into the chondrogenic lineage before autologous injection to the knee joint (week 7). The third EMG was taken at the end of cell treatments (week 31). As it was impossible to obtain a maximum voluntary contraction of muscles in sheep, EMG measurements before surgery were considered the baseline amplitude (pre-osteoarthritic). The pre-osteoarthritic, post-osteoarthritic and the post-cell treatment recordings were compared to quantify the variation in activation patterns that commonly occurs in OA-affected knees due to muscle atrophy. After sacrifice of sheep (week 32), the regenerated cartilage biopsies were tested mechanically in comparison with the native cartilage.

Preparation for EMG

For easy carriage and safety of instruments, a jacket with a pouch to hold the portable data logger (ME6000 Measurement System, Finland) was designed [Fig. 1(A) and (B)]. To improve the adaptability and consistency of EMG recordings, sheep were trained to walk in a straight motion along a slated wooden corridor every day for 12 weeks (before week 0). Two days prior to the EMG measurement, the area around the muscles of interest was shaved with clippers (Shears, USA) and cleaned with soap to increase electrode pad's adhesion.

Muscles of interest

EMG activities of four major muscles for locomotion were measured to determine their contractions at a normal walking pace [Fig 1(C) and (D)]. These muscles include the medial gluteus muscle, which helps to extend the hip and stifle joints[25,26]; the lateral vastus, which are the major knee extensors in mammalian quadrupeds; and the bicep femoris, which work in conjunction with the vastus muscles[27,28]. The infraspinatus, which assist in the extension and flexion of the fore limbs[29], were also measured to explore the compensatory reactions in the forelimbs for the osteoarthritic right hind limb. Both sides of the limbs were evaluated.

Electromyography measurements (surface EMG)

A portable EMG machine (ME6000 Measurement System, Finland) was used. This has four socket units and eight channels. Each of the channels has three electrode pad points (two live conductors and one neutral) for attachment to a particular muscle. The protocol was ME6000-T8 Neuro Stimulus, and the activities of the muscles were recorded with Raw Free data. Sheep were led to walk to and fro within the corridor while carrying the gadgets [Fig.1(E)]. The mean amplitudes from the Root Mean Square (RMS) were analysed in a single spectrum using the MegaWin PC-software by a blinded Neurologist. Each sample had an average of six separate recordings for a measurement.

Osteoarthritis induction

All sheep from different groups underwent an arthroscopy evaluation to rule out any impending chondral lesion[24] and thereafter OA induction was conducted according to our optimized protocol[21,22]. Briefly, the sheep were sedated with intravenous (IV) xylazine (0.1 mg/kg) and induced with IV ketamine (7 mg/kg). Following intubation, they were ventilated and maintained on isoflurane (1.5%) in oxygen. All sheep received long acting amoxicillin 20 mg/kg, IM, once, as prophylactic antibiotic. Analgesia consisted of tramadol 2 mg/kg, IV, intra-articular and peri-incisional bupivacaine, and meloxicam 0.2 mg/kg, SC. Tramadol and meloxicam were repeated at every 6 and 24 h, respectively. Thereafter the anaesthetization, a medial parapatellar skin incision was made beginning at a level 2 cm proximal to the patella and extending to the level of the tibial plateau. The joint capsule was incised and the patella was subluxated laterally to expose the trochlear groove and the medial and lateral condyles of the distal femur. Anterior cruciate ligament removal was performed by excising the attachment on the medial aspect of the lateral femoral condyle and the proximal aspect from its tibial attachment. The medial meniscus was removed by excision of the caudal horn and

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