ANTERIOR CRUCIATE LIGAMENT RESECTION AND MEDIAL MENISCETOMY RESULT IN MULTIFOCAL CARTILAGE DEGENERATIONS

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1.0 Introduction

The known facts about the mechanism of cartilage erosion that leads to degenerative joint diseases seen in osteoarthritis (OA) remain poor. As the cartilage has very little repair capacity, it poses a major concern to clinicians and medical scientists [1, 2]. OA is defined as a slow progressive structural degeneration in joint tissue which involves biochemical and morphological changes of both cells and matrix. The sequence of degeneration progresses from mild softening,
coarse indentation, cartilage erosion, sclerosis and eburnation, formation of spurs and subchondral cyst [3]. Among the risk factors exposing joints to OA degenerations include accidents and focal injuries to the ligaments, meniscus and cartilage during sports which can cause decrease performance. And if left untreated would probably deteriorate, leading to OA and disabilities in later life [4, 5, 6]. Fast accelerations, decelerations and pivoting movements are among the predisposing factors [7]. After the meniscus and the anterior cruciate ligament (ACL), the articular cartilage of the knee is one of the most frequently damaged structures in athletes. It was reported that meniscal damage alters the pattern of distribution of weight in the knee and most of the time leads to the degeneration of cartilage [8]. Lesions and the necrosis that follow deplete the articular cartilage’s blood supply, hence preventing the normal physiological inflammatory tissue response. The lack of potential for regeneration by the matured chondrocytes and limited presence of undifferentiated cells in the cartilage results in insufficient repair. Furthermore, the constant use of the injured articular joints deteriorates the cartilage, causing degradative enzymes and substance secretions. This in turn, exacerbates the destruction of the articular cartilage collagen ultra structure. These biochemical and metabolic changes mimic the early changes seen in OA [9] however; the progress and the common regions of degenerations remain unclear [10, 11].

The understanding of the onset of OA after a traumatic ligament or meniscal injury is difficult in human, owing to the fact that people would seek medical attention when the pathological changes are advanced [12]. Due to the similarity of the ovine biomechanics to that of humans [13], an important site for osteoarthritis study is the sheep knee joint after meniscectomy in combination with exercise [14,15,16]. An early detection of the focal onset and to which region is greatly affected, is pertinent to the treatment and prevention of further degeneration.

This study aimed to thoroughly investigate the area of greatest impact in reference to the consequential alteration in the maximum force, secondary to resultant gait abnormalities due to traumas as a way of understanding the early onset of osteoarthritis, after a combined excision of anterior cruciate ligament and medial meniscus in a sheep model, mimicking a traumatic knee accident. We hypothesize that the greatest impact and OA will be at the medial side of the stifles joints in line with the medial meniscectomy.

2.0 Materials and Methods

2.1 Study Design

Ethical approval was granted by Universiti Kebangsaan Malaysia (UKM) Animal Ethics Committee (PP/TEC/RUSZYM/25-NOV/342-DEC-2010-JUN-2012) and Universiti Putra Malaysia (UPM) Animal Ethics Committee (RUJ: ACUC 07/R6/JULY 07-DEC 09). A total of 6 healthy un-castrated sheep (Siamese long tail cross) selected at random, aged 1.5±0.5 years and weighing 25±2kg, provided by the animal unit UPM were used. Sheep with pre-existing leg injury, dislocation or non straight legs were excluded from this study. Pre-OA arthrotomy ruled out any pre-existing chondral lesion prior to the OA inductions. This was followed by the surgical resections at the right hind knee. The contra-lateral hind knee joint (un-operated knee joint) was treated as control. After a recovery period of 3 weeks, animal were subjected to exercise regime for another period of 3 weeks. Stocking density was 3 sheep in a 4 x 4 m² pen confined environment. They were fed with a combination of processed sheep pellet and fresh foliages. Post OA induction arthrotomy was done before euthanizing the sheep.

2.2 Surgical Resections

The surgical protocol was conducted according to previous works [17, 18], with modifications. Sheep was sedated with intravenous (IV) xylazine (0.1mg/ kg), IV and induced with Ketamine (7mg/kg). Following intubation it was ventilated and maintained on isoflurane (1.5%) in oxygen. Analgesic consisted of tramadol 2 mg/kg, IV intra-operatively and repeated 6-8 hourly post operatively. Prophylactic antibiotics consisted of amoxicillin 20 mg/kg. 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 were incised and the patella was subluxated laterally to expose the trochlear groove, medial and lateral condyles of the distal femur. Anterior cruciate ligament (ACL) removal was performed by first excising its attachment on the medial aspect of the lateral femoral condyle. The proximal attachment is brought forward and the entire ligament was excised from its tibial attachment. The stifle was moved in a drawer test to ensure that the entire cruciate ligament had been excised. The meniscal meniscus was removed by sharp excision. Working from caudal to lateral, then cranial, the meniscus was excised from its attachments until it was completely removed. The joint was closed using Vicryl sutures. Post operatively, meloxicam was administered 0.2 mg/kg subcutaneous and repeated once daily for 3 days. Following extubation, sheep was left to recover in its pen and was monitored daily for inappetence and wound dehiscence until suture removal, 7 days post-operatively.

2.3 Sheep Exercise

At the end of three weeks recovery period from surgical injuries, the animals underwent rigorous exercises conducted in a confined concrete track of about 25 meters long and 1 meter wide [19]. Sheep was lead to run to and fro within the track twice to complete a 100 meters distance. They were...
exercised for three weeks to increase joint contact stress, and frictional contacts between the femoral condyle and tibia plateau at the operated knee.

2.4 Gross Examination

After 7±1 week post surgery all animal were sacrificed, distal head of the femur and proximal head of tibia plateau of operated and contra-lateral legs were harvested for gross and histological examination. The entire joints were dissected, examined and photographed. Thereafter the patella-femoral groove, femoral condyles and tibia plateaus were sectioned in sagittal plane using the EXAKT cutting machine (Micro Lambda, Malaysia).

2.5 Hematoxylin and Eosin

Sections of 10μm were stained with Haematoxylin and Eosin to assess cell morphology. Samples from the patella-femoral grooves of both the operated and the control knee were deparaffinised with xylene and dehydrated with ethyl alcohol (Essen-Haus Sdn. Bhd). They were washed with tap water and stained with Haematoxylin Harris (DAKO, Glostrup Denmark) for 10mins and counter stained with Eosin (DAKO, Glostrup Denmark) for 3mins. Slides were viewed under the light microscope after dehydration with alcohol and xylene (VWR International LTD).

2.6 Safranin O Stain

Samples were stained with Safranin O to detect proteoglycans. Slides of 10 μm sections were stained with Weigert’s iron haematoxylin (Sigma USA) working solution following washing with tap water and acid alcohol. They were further stained with fast green solution (Dako, Denmark), and counter stained with Safranin O solution (Stain pur TM) before mounting using DPX fluid (Gibco USA) for microscopic examination.

2.7 International Cartilage Repair Society Evaluation (ICRS)

The International Cartilage Repair Society (ICRS) scale for OA assessment [20] was used to evaluate the OA development in both control and the test knee. It has five score grades: Grade 0 – Normal; Grade 1 - Soft indentation superficial fissures and cracks; Grade 2 - Lesions extending down to <50% of cartilage depth; Grade 3 - Cartilage defects extending down >50% of cartilage depth to calcified layer; Grade 4 - Severely Abnormal (extending down through the subchondral bone). The ICRS evaluation was based on the gross and histological examinations of the stifle joints and was done independently by two blinded orthopedic surgeon.

2.8 Statistical Analysis

Data were presented as mean ± standard error of mean (SEM). The means were compared using Mann Whitney U tests for non parametric means. All statistical analysis was performed using the version 17.0 of the SPSS software at 95% confidence limit and p<0.05 was considered statistically significant.

Fig. 1 The arthrotomy images of the knee joints. This shows the Patella, Patello femoral groove, Medial femoral condyle and Medial tibia plateau. White arrow depicts the erosions and the degeneration of cartilage at P, PFG, MFC and MTP; Scale bar: 1.cm
3.0 Results

3.1 Arthrotomy

The arthrotomy (Fig. 1) reveals that the lesions developed are similar with the generalised erosion of cartilage on various regions as seen in OA. A severe degradation reaching to the subchondral is seen at the patella femoral groove (PFG) and the patella (P) while moderate cartilage erosion and mild softening is present at the medial femoral condyle (MFC) and medial tibia plateau (MTP).

3.2 Gross Examination

The gross evaluation of the knee joints shows that the operated knee has varying degrees of cartilage erosion, consistent with generalized cartilage loss found in OA. Considering the different regions of the knee joint examined: the patella (P), patello-femoral groove (PFG), medial (MFC) and lateral femoral condyle (LFC), medial (MTP) and lateral tibia plateau (LTP); OA was prominent at the patella and patello-femoral groove in all the operated knee samples, with relatively severe cartilage degenerations that is dominated by loss of matrix content and cartilage structure, but not on the control (normal) knees (Fig 2a and 2b). The femoral condyle shows mostly mild to moderate cartilage loss prominent on the medial aspect. This is expected because, the medial meniscus was resected (Fig 2c) Examining the tibia plateau, the degradation of cartilage is seen not only on the medial aspect of the operated knee but also on some of the lateral aspect with intact meniscus, inferring an altered pattern of loading in the knee joint (Fig 2d).

3.3 Hematoxylin and Eosin

Histological evaluations using haematoxylin and eosin staining on both knee samples shows signs of cartilage loss and exposure of subchondral bones beneath the calcified cartilage layer on the operated knee; and none on the control knee. In the operated knee joints, there were substantial erosion and fibrillation of the articular surfaces with loss of extracellular matrix compared to contra-lateral knee joints. The degeneration of cartilage is similar to the gross evaluations and scores of ICRS from the 6 regions (P, PFG, MFC, LFC, MTP, and LTP) examined. The control (normal) knee shows lacunae and cartilage isolated cells consistent with the histoarchitectural characteristics of a well distributed cartilage isolated cells within the basophilic ground substances (Fig. 3a).

3.4 Safranin O Stain

The control (normal) knee joints stain positive with Safranin O. They are homogenous in accordance to the histochemical properties seen with the presence of accumulated proteoglycans revealed via Safranin O staining. The operated knee joints reveals varying degrees of subchondral bones exposures as the cartilage degraded; in support of the different mean scores record with the ICRS grades below (Fig. 3b).
3.5 ICRS Evaluation

Examining the various regions of the knee joints, the general ICRS score representation of different regions of all the samples are shown in Table 1. Then, estimating the mean scores of different samples, \( P \) has 2.5±0.42 while the opposite PFG has 3.33±0.17. The MFC has 2.25±0.38 while the LFC has 1.33±0.25. The MTP has 2.0±0.37 and LTP has 1.5±0.22. The control from all the samples at the above regions has 0 with no sign of cartilage degeneration (Fig. 4). Using the Mann Whitney U test which is ideal for a comparison within samples in a non-parametric distribution, the lesions developed at the various regions of the knee are significantly higher compared to control. The lesion at \( P \) is significantly higher to that of LFC and LTP but not PFG, MFC and MTP. The lesion at PFG which has the highest ICRS score is significantly higher compared to all other regions, but not to \( P \) which scored the second highest lesion. The lesion at MFC is significantly higher to that of LFC. Others are only significantly high to the control \( P<0.05 \). PFG has the highest lesion, followed by the opposite side patella, the MFC, then the MTP, LTP and LFC. This demonstrates that the resection of the anterior cruciate ligament and medial meniscus can initiate defects extending down greater than 50% of cartilage depth to calcified layer within a period of 6 weeks.

4.0 Discussion and Conclusion

One of the challenges in inducing osteoarthritis in ovine is mainly the small joint inter-cartilaginous space. It needs meticulous technique in removing the meniscus and the anterior cruciate ligament, as such; aggressive removal will result in widespread damage to the knee. This would in turn cause possible iatrogenic lesion and morbidity. To ensure a
healthy recuperation, post operative injection of pain killers, antibiotics and vitamins must be administered properly as the sheep needed vigorous exercise to induce more frictional and inflammatory changes to the joint. It has been reported that test sheep exercised on hard concrete surfaces had induced degenerative changes in both the articular cartilage and subchondral bone compared to the control, which was exercised on a soft wood platform [21, 22]. From our knowledge, there has not been any study that comparatively examined the induced OA on various regions of ovine stifles joint using this method. One of the previous works on OA progression reported that only the central area of femoral condyle and subjacent subchondral bone were eroded by mechanical force; other portions of the articular cartilages in the joint remained structurally intact [17]. In another study, injury or loss of the meniscus generally leads to degenerative osteoarthritic changes in the knee joint and the locations of new tibiofemoral contact correspond to the early chondral damage in an ovine model [23]. Many other works involving sheep model focused on focal articular defects [24, 25, 26, 27]. In the arthrotomy examination, it reveals that a major change in loading pattern occurred at the knee joints following the resections, hence the degeneration of the cartilages. Considering the extent of lesions, it could be said that the degenerative activities started from the P and PFG contacts, then to the MFC, MTP and other regions. This pattern of lesion is consistent with the other parameters employed, which reflected deterioration of cartilage due to frictional contacts of the opposite surfaces. On gross examination of the operated knee, out of the six regions studied, the major cartilage degeneration is found within the
PFG; followed by the P and MFC, then the MTP, LTP and LFC. This is an unlikely outcome because it was expected that the degenerations should be more at the MFC and MTP in accordance with the medial meniscectomy. Another observation is that the OA affected the LTP which has its meniscus intact. These are consistent with the earlier reports that loss of or damage to the meniscus alters the pattern of loading in the knee joint [9, 28]. It is possible that a diagnosis of osteoarthritis can be missed or delayed because some people have no symptoms, the disease may progress gradually and early symptoms can be mild or assumed to be associated with other conditions. Hence, one of the clinical relevance of this study is to point out the likely area to watch out for onset of degeneration, after such combined trauma. Histologically the observed cartilage loss on the test knee reflects a decrease in cartilage material properties. This provides a qualitative measure of the loss in cartilage function following meniscectomy and reflects a pattern of change that is consistent with damage to the collagen-proteoglycans solid network. This supports the hypothesis that the tensile properties of the surface zone of articular cartilage were altered following total medial meniscectomy [29]. In another study, six month lateral meniscectomy in sheep produced histomorphological and immunohistochemical cartilage changes analogous to those described for early human OA and in other animal models. The severity and type of lesions obtained were dependent on the topographical joint location [30] but in our study, more than these changes were seen within 6 weeks. This reflects a significant joint contact stress and alteration in the loading, supporting that loss of or damage to the meniscus alters the pattern of loading in the knee joint and frequently leads to cartilage degeneration and osteoarthritis. It also indicates a possible obliteration of a function of the patella in cushioning pressure on joint knee. On the ICRS grading [20], the test samples from various regions have mean scores of 1.3 - 3.3 points out of 4 points grade score. These are grades from microscopic softening, fibrillations, and gross cartilage defects extending up to 50% of cartilage depth to the calcified layer. All these show a fast rate of cartilage degradation with meniscal and cruciate ligament injuries which buttressed the earlier report on altered pattern of loading and increased surface contact to the knee joint [10]. There are other grading systems to score the development of OA in animal models like: the Pritzker system [8], Mankin system, Modified Outerbridge system and using Creep Indentation methods [12]. We chose ICRS grading system because this report is part of a greater project of creating OA and treating the cartilage degenerations via cell therapy; hence we use a method that would allow the scoring of both degeneration and the regeneration of cartilages. Uncastrated sheep is used because there is evidence that sex hormones play a role in the development of OA. Specifically, the prevalence of OA increases in women following menopause and rises faster with age in women than men [12]. A limitation to this study is measuring the biomechanical properties of the stifle joints to ascertain the proper pattern of loading and distribution of weight which may have constituted to the specific cartilage losses at different regions. Due to the difficulty in studying OA in humans, owing to their genetic variations and difficulty in clearly identifying the early stages of this disease [8], future investigations should address these aspects highlighted even though there is no animal species with the exact molecular, biochemical, anatomic and physiological characteristics as those of humans.

Medial meniscectomy and ACL resection followed by an exercise regimen could create a multi focal lesion similar to OA in 6 weeks. The changes in joint contact stress due to the resections affected the PFG the most, followed by the P and MFC, then the MTP, LTP and lastly the LFC. This study hopes to open new horizons in the understanding of early onset of knee osteoarthritis following anterior cruciate ligament and medial meniscal trauma.

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