The Knee 18 (2011) 402–406

Contents lists available at ScienceDirect

The Knee

# The pathology of the end-stage osteoarthritic lesion of the knee: Potential role in cartilage repair ☆

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ARTICLE INFO

*Article history:*
Received 22 April 2010
Received in revised form 16 July 2010
Accepted 25 July 2010

*Keywords:*
Pathology
Osteoarthritis
Cartilage repair

ABSTRACT

The purpose was to explore whether there were any pathological characteristics of the end-stage osteoarthritic sclerotic lesion that have potential to participate in cartilage repair.
Specimens harvested following total knee surgery were examined for gross pathology including staining with Safranin O. Multiple small sections of the lesion were placed in tissue culture for 6 weeks. Gross examination and photographic documentation was made at 3 and 6 weeks. At 6 weeks the specimens from culture were subject to histological examination. The pathology of the end-stage osteoarthritic lesion showed sclerotic bone, dead osteons, hypervascularity and scattered cartilaginous aggregates. Additional observations showed multiple pitting on the sclerotic surface, which histologically was related to three events; fragmentation of dead bone, ruptured blood vessels, and eroded aggregates. There were no pathological or biological changes in the specimens following the time in tissue culture.
The in-depth pathological evaluation showed the end-stage osteoarthritic lesion to have certain features with potential to facilitate cartilage repair. The cartilaginous aggregates may be a participant in cartilage repair following surgery. The cartilaginous aggregates remained unchanged in the tissue culture absent the normal synovial joint chemical and physical environment and therefore further testing with a different experimental model would be necessary to establish these aggregates as a source of cartilage regeneration. The multiple small depressions in this lesion may have potential to be a "home" for therapeutics.



## 1. Introduction

The end-stage osteoarthritic articular surface lesion of the knee joint is pathologically characterized by exposed hypervascular dense subchondral bone, dead osteons and focal areas of cartilaginous aggregates [1,2]. Although similar in depth as the traumatic cartilage injury classifications of Outerbridge IV and ICRS IV, it is a different lesion pathologically [3,4]. Some of the contributing factors to the end-stage osteoarthritic lesion are trauma, increased force across the joint as in obesity or angulation deformity about the knee [5]. Little prospect has been held out for clinical attempts for healing such a lesion [6]. Surgery is often recommended for this end-stage condition [7,8]. Biological repair has been reported following arthroscopic surgical methods [1,9]. It was presumed that the biological repair source following these procedures was from bleeding from the lesion's exposed surface vascularity and/or subchondral bone marrow cells. Cartilaginous aggregates have not been considered a source of repair in reports following arthroscopic abrasion arthroplasty or microfracture [1,9].

The end-stage osteoarthritic medial compartment of the knee joint has been reported to have regenerated an articular surface following high tibial osteotomy [10,11]. Similar biological responses have been reported following osteotomy of the proximal femur for severe degenerative arthritis of the hip [12,13].

It is interesting that spontaneous regrowth of the hip joint cartilage has been reported without any specific treatment. This occurred on the contralateral arthritic hip following unilateral total hip arthroplasty [14]. Two patients in that report were followed for seven and 11 years respectively prior to surgery. The surgical

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doi:10.1016/j.knee.2010.07.012

specimens showed histological evidence of cartilage regeneration. These authors offered no explanation for the biological response [10–14]. The common denominator of these reports appears to be an alteration, redirection, and/or reduction of forces across the affected joint surface.

An explanation for these clinical reports of cartilaginous repair may be within the pathological nature of the end stage osteoarthritic lesion. One possible source of the cartilage repair may be the cartilaginous aggregates on and below the surface of the end stage osteoarthritic lesion [1,2]. The nature of the cartilaginous aggregates has been the focus of previous pathologic studies [2]. The cartilaginous aggregates showed intense GAG staining with normal chondrocyte morphology but without columnar arrangement of normal articular cartilage. All cartilaginous aggregates stained positive for type II collagen. Most chondrocytes within aggregates showed positive staining for α-smooth muscle actin. However, an in-depth study of the pathology of the end-stage osteoarthritic lesion has not been available.

The purpose of this study was to examine the pathology of the areas of exposed bone seen on the articular surface in the knee joint having end-stage osteoarthritis in regards to the potential for cartilage repair. The hypothesis was that there were pathological characteristics of this articular lesion of exposed bone that would have the potential to participate in cartilage repair, specifically the cartilaginous aggregates. Our supposition was that cells from these aggregates had the potential to migrate from the aggregates onto the bone surface. The objective of the present study was to evaluate this behavior *in vitro* using samples of end-stage osteoarthritic end stage lesions from human subjects.

## 2. Methods

### 2.1. Selection, classification, and patient demographics

After institutional review board approval, human femoral condyles (ten males, eleven females) from patients with clinically established osteoarthritis were retrieved at the time of total knee arthroplasty surgery. The males were an average of 72.1 years (range 62–84 years) while the female patients were an average of 72.0 years (range 56–86 years).

### 2.2. Preparation of retrieved gross specimens

Medial and lateral femoral condyle specimens identified with exposed bony lesions were grossly stained using 0.1% Safranin O (Fisher Scientific, Pittsburgh, PA) for 3.5 min (Fig. 1A, B). Specimens were then subsequently washed in several changes of deionized water. Gross specimens were then observed under a stereo dissecting microscope (Olympus SZX12) at 10–90× magnification (Fig. 1C). Photomicrographs were taken using an attached digital camera (Optronics MacroFire®, East Muskogee, OK).

One specimen was subjected to a replication of the surgical procedure arthroscopic abrasion arthroplasty to determine the effect of the recommended superficial depth (1–2 mm) of the surgical abrasion on the integrity of the cartilaginous aggregates. The simulated surgical abrasion was accomplished with a motorized rotating burr of 4 mm diameter by a surgeon experienced with this procedure [1]. The specimen was then examined histologically.

### 2.3. Tissue harvest and culture

Under sterile conditions, a 6 mm donor OATS grafting instrument was utilized to remove osteochondral (OC) plugs (Arthrex, Naples, FL) from the region of exposed subchondral bone from the weight-bearing portions of medial femoral condyles (24 plugs from 10 donors). After harvesting, OC specimens were potted in 6% agarose gel within the cap of a 1.5 mL Eppendorf tube flush to the level of the exposed subchondral bone submerging all underlying cancellous bone. The specimens were then stained in a sterile Safranin O solution. Photo documentation of Time 0 gross surface morphology of each OC specimen was taken under a stereo dissecting microscope (Olympus SZX12) at up to 10× magnification using an attached digital camera (Optronics MacroFire®, East Muskogee, OK). OC specimens were cultured in DMEM supplemented with 10% fetal calf serum (FCS) for 3 weeks. After 3 weeks, the specimens were grossly stained in sterile 0.1% Safranin O for 3.5 min, washed, photographed and returned to culture for an additional 3 weeks, for a total of 6 weeks in culture.

### 2.4. Histology

After gross examination, Safranin O stained specimens, taken directly from total knee arthroplasty and from a 6-week culture, were fixed in zinc formalin (Anatech LTD., Battle Creek, MI), decalcified and embedded in paraffin blocks and sectioned at 6 μm thickness. Sections were stained using a standard Safranin O + Fast Green counter-staining protocol. Images were captured using a microscope (Olympus BX60, Olympus, Center Valley, PA ) with a digital camera attached (Optronics MacroFire®, East Muskogee, OK).

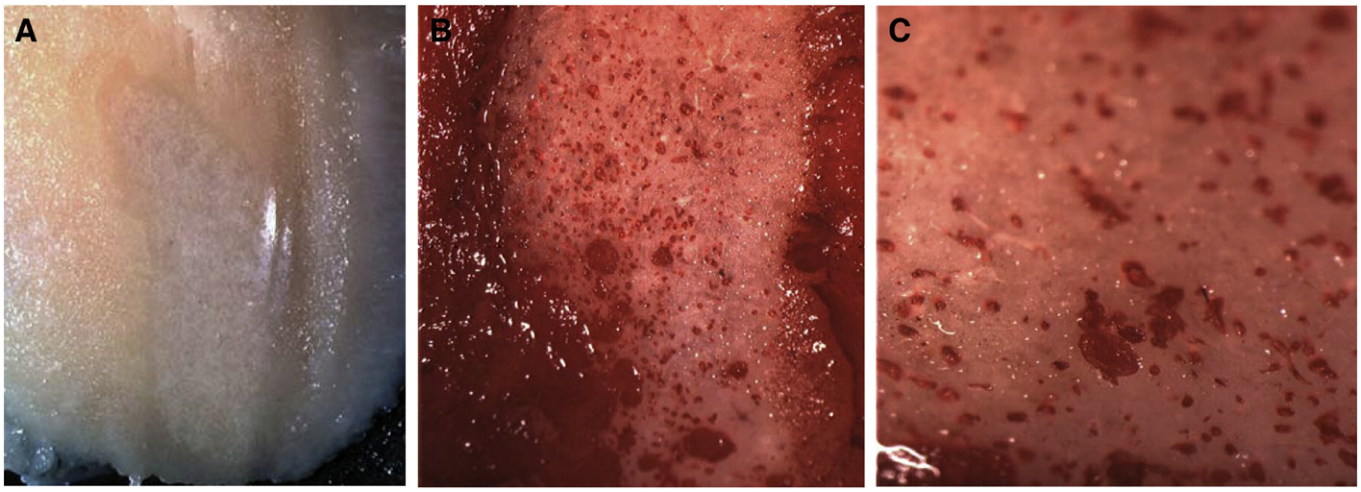


**Fig. 1.** A. Close-up photographs of the gross lesion without Safranin O staining demonstrate the macroscopic findings of such a lesion. The surface is smooth, hard to palpation and readily reflects light. There is little surface definition seen without magnification. There is difficulty in seeing cartilaginous aggregates and the margins of the osteoarthritic end-stage lesion even under magnified photography. B: Photograph of same lesion with staining shows ease of visualization of the cartilaginous aggregates on the bony surface and the margins of the lesion. C. Gross pathologic examination of the osteoarthritic end-stage bony lesions stained with Safranin O reveals cartilage aggregates and the smaller multiple small "pits".