Osteoarthritis and Cartilage 25 (2017) 138–145

Osteoarthritis and Cartilage

OARSI OSTEOARTHRITIS RESEARCH SOCIETY INTERNATIONAL

# Infrapatellar fat pad aggravates degeneration of acute traumatized cartilage: a possible role for interleukin-6

J. He † ‡ [a], Y. Jiang † [a], P.G. Alexander †, V. Ulici † [b], Y. Zhu † [c], S. Wu ‡, R.S. Tuan † [*]

† *Center for Cellular and Molecular Engineering, Department of Orthopaedic Surgery, University of Pittsburgh School of Medicine, Pittsburgh, PA, USA*
‡ *Department of Orthopaedic Surgery, Third Xiangya Hospital of Central South University, Changsha, China*

ARTICLE INFO

*Article history:*
Received 25 February 2016
Accepted 1 September 2016

*Keywords:*
Post-traumatic osteoarthritis
Interleukin-6
Infrapatellar fat pad
Adipocytes
Adipose-derived stromal cells

SUMMARY

*Objectives:* The infrapatellar fat pad (IPFP), which is located underneath the patella, close to cartilage surfaces, functions in distributing mechanical load and has been shown to produce cytokines. This study aims to assess the involvement of the IPFP in the progression of post-traumatic osteoarthritis (OA) through investigating the crosstalk between the IPFP and injured cartilage *in vitro*.
*Methods:* A single blunt impact (36 MPa) on healthy bovine articular cartilage explants was used to generate traumatized cartilage. Conditioned media from IPFP and traumatized cartilage (FP-CM and TC-CM) were prepared separately. After culturing in FP-CM, the posttraumatic cartilage explants were analyzed for expression of cartilage degeneration associated genes and secretion of the interleukin (IL)-6, into the culture medium. The effect of traumatized cartilage on IPFP was studied by treating IPFP-derived adipocytes and IPFP adipose-derived stromal cells (ADSC) with TC-CM followed by analysis of cytokine expression.
*Results:* FP-CM aggravated glycosaminoglycan (GAG) release in traumatized cartilage, but did not significantly affect healthy cartilage. FP-CM raised gene expression of *cyclooxygenase-2*, *inducible nitric oxide synthase*, and *IL-6* in traumatized cartilage explants, and lowered expression of *tissue inhibitor of metalloproteinases-1, 2, 3*, compared to non-conditioned medium. Of particular significance is that medium IL-6 levels increased substantially in both FP-CM and FP-CM treated traumatized cartilage cultures. Extrinsic IL-6 treatment of traumatized cartilage simulated part of the effects of FP-CM. TC-CM elevated levels of *IL-6* expression in IPFP derived adipocytes and ADSCs.
*Conclusions:* IPFP aggravates post-traumatized cartilage degeneration, and IL-6 is a candidate tissue degeneration mediator.
© 2016 Osteoarthritis Research Society International. Published by Elsevier Ltd. All rights reserved.

## Introduction

Osteoarthritis (OA) is the most common form of arthritis characterized by gradual cartilage loss, osteophyte formation and other joint structural changes, with a high prevalence for the knee joint[1]. Mechanical injury on joint cartilage surfaces leads to progressive cartilage degeneration[2]. Acute joint trauma causes tissue injuries in cartilage, bone, ligament, joint capsule and meniscus; the joint responds over the course of the first week after trauma with cell death and inflammation, and later in the second week, with the initiation of biological repair processes, such as matrix formation[2]. The infrapatellar fat pad (IPFP) lies underneath the patellar tendon, in the space between the femoral condyle and tibial plateau, in proximity to the cartilage surfaces and synovial layers. The main function of the IPFP has been thought to be the distribution of mechanical load. Recent studies have focused on the IPFP for its potential role in releasing paracrine inflammatory mediators that reach the articular cartilage as a mediator of OA progression[3–5]. The finding of a thicker and larger IPFP in OA joints compared to healthy joints[6,7] suggests a connection to increased cytokine release[8] and OA pain[7]. However, the interaction of IPFP and articular cartilage in acute trauma has not been studied.

* Address correspondence and reprint requests to: R.S. Tuan, Center for Cellular and Molecular Engineering, Department of Orthopaedic Surgery, University of Pittsburgh School of Medicine, 450 Technology Drive, Pittsburgh, 15219, PA, USA.
*E-mail address:* rst13@pitt.edu (R.S. Tuan).
[a] These authors contributed equally to this work.
[b] Present address: Thurston Arthritis Research Center, University of North Carolina, Durham, NC, USA.
[c] Present address: Department of Orthopaedic Surgery, Shanghai 6th People's Hospital, Shanghai, China.

http://dx.doi.org/10.1016/j.joca.2016.09.001
1063-4584/© 2016 Osteoarthritis Research Society International. Published by Elsevier Ltd. All rights reserved.

Articular cartilage is composed predominantly of an extracellular matrix (ECM) characterized by the presence of sulfated proteoglycans, consisting of aggrecan and sulfated glycosaminoglycans (GAGs), and collagen type II (COL2). Turnover and remodeling of the cartilage matrix is controlled by the resident chondrocytes, via regulation of gene expression of matrix components and matrix-degrading enzymes. COL2 can be cleaved by matrix metalloproteinases (MMPs), including MMP-1, -3, -9, and -13, while aggrecan is cleaved by a disintegrin and metalloproteinase with thrombospondin motifs (ADAMTS) enzymes, most probably ADAMTS-4 and -5[9,10]. Tissue inhibitors of metalloproteinases (TIMPs), including TIMP-1, -2, and -3, act as regulators of MMP activities, and help protect the cartilage from degeneration. In OA pathophysiology, a number of pro-inflammatory molecules are associated with or promote joint articular degeneration. The most important trigger and mediator cytokines are interleukin (IL)-1β, interleukin-6, and tumor necrosis factor alpha (TNFα)[11]; a single injection of such proteins into joint capsule could lead to joint degeneration in animals[12]. Other important molecules include prostaglandin E2 (PGE2)[13], cyclooxygenase-2 (COX2), and inducible nitric oxide synthase (iNOS).

Adipose tissues consisting of adipocytes and stromal cells, secrete numerous cytokines and adipose-derived hormones, including adipokines (e.g., leptin, resistin, and visfatin), to which chondrocytes are known to respond[14,15]. In particular, IPFP has been shown to secrete more inflammatory cytokines than subcutaneous adipose tissue of the same OA patient, and IPFP of late stage OA patient has more inflammatory phenotype than early stage OA patient, suggesting possible functional relationship between the IPFP and joint degeneration, and the involvement of inflammatory cytokines, such as IL-1β and IL-6[3,15,16]. The IPFP is also highly vascularized and innervated and is often infiltrated by immune cells under pathological conditions. For example, Bastiaansen-Jenniskens *et al.*[17] found alternatively activated, $CD206^{+}$, M2 macrophages from the IPFP of end-stage OA patients could contribute to the inhibition of catabolic processes in the cartilage. As most of the IPFP studies have been done with late OA joints, the early stage interaction between the post-traumatic cartilage and IPFP is unknown.

In this study, we have investigated the interaction between traumatized bovine cartilage and the IPFP using an *ex vivo* explant model for a culture period of 7 days post-trauma. Traumatized cartilage explants consisted of healthy bovine articular cartilage explants exposed to a single injury, blunt impact (36 MPa), as established in our recent study[18]. The explants were exposed to medium conditioned by IPFP (FP-CM), and examined for tissue integrity and levels of interleukin (IL)-6 in the medium. In addition, crosstalk between traumatized cartilage and IPFP was assessed by examining the influence of traumatized cartilage-conditioned medium (TC-CM) on IPFP cells, specifically adipocytes and multipotent cells in the stromal vascular fraction (adipose derived stromal cells, ADSCs). Understanding the role of IPFP in early phase post-trauma provides a basis for identifying potential biologic targets for intervention to prevent subsequent joint degeneration.

## Materials and methods

### *Preparation of traumatized cartilage and study design*

Articular cartilage discs (5 mm in diameter, and 1.8–2.2 mm in thickness, and 38–60 mg in wet weight) were obtained from the hind-leg stifle of 2- to 3-year-old cows (J.W. Treuth & Sons Inc., Baltimore, MD), and four qualified cartilage plugs could be harvested from one knee on average. To minimize the variations among biological individuals, we randomly selected and pooled 3 plugs as one sample, and at least 3 samples were included in each group (9 plugs/group, from nine biological donors) for each of the analytical tests, and every experiment was performed independently at least three times with different sample batches.

The single 36 MPa blunt impact to the cartilage surface was applied using a custom-designed impact device to induce trauma to cartilage plugs as described in our recently work[18]. Briefly, the spring-loaded impact device fitted with a hemispherical tip was used to deliver traumatic, injurious impacts of calibrated magnitude and rate into the cartilage piece, resulting in increased catabolic activities and tissue degeneration. The traumatized cartilage developed lesions similar to cartilage lesions seen in OA[18].

The experimental design consisted of three parts. (1) To confirm that the *in vitro* impact model recapitulated trauma-induced cartilage injury, control (non-impact, 0 MPa) and traumatized (impact, 36 MPa) cartilage discs (18 plugs from nine biological donors were randomly distributed into one group) were individually cultured in 1 mL basal medium (BM)[19], consisting of Phenol Red-free Dulbecco's Modified Eagle's Medium (DMEM), ITS (10 mg/L insulin, 55 mg/L transferrin, 6.7 mg/L selenium), antibiotic/antimycotic solution (P/S/F, penicillin/streptomycin/amphotericin), 40 mg/mL proline, 50 mg/L ascorbate, 100 mg/mL sodium pyruvate, 10 mM HEPES (all from Thermo Fisher) and 10 ng/mL recombinant human transforming growth factor-β3 (TGF-β3, PeproTech). Medium was changed and collected on days 1, 2, 4, and 7 and cartilage explants were collected on day 7. Injury induced cartilage surface fibrous network changes were examined by scanning electron microscopy (SEM). Cartilage explant samples were then analyzed (Live/Dead assay, Safranin-O staining), and the medium samples were examined by biochemical assays (GAG, PGE2 assays). (2) Control and traumatized cartilage explants were cultured with BM, IPFP conditioned medium (FP-CM) or BM supplemented with recombinant bovine IL-6 (200 pg/mL, Thermo Fisher) for 2 days. Cartilage explants were then rinsed, snap-frozen in liquid nitrogen and stored at −80°C, while the culture medium was collected and stored at −20°C for further analysis. (3) Adipocytes and ADSCs isolated from IPFP were cultured with TC-CM or control medium for 24 h[17] followed by RNA isolation for quantitative RT-PCR.

### *Preparation of FP-CM and TC-CM*

Explants of IPFP (inner part of IPFP, excluding synovium) from the same batch of bovine knee joints were used to produce FP-CM. The IPFP tissue was cut into small pieces of approximately 10 mg each, and cultured as a suspension (50 mg tissue/mL) for 24 h in Phenol Red-free DMEM containing ITS and P/S/F. After 24 h, the medium (FP-CM) was harvested, centrifuged at 300 g for 8 min and diluted with BM (1:1) and used immediately for Study Part 2 described above.

Cartilage plugs subjected to mechanical impact (36 MPa, $n = 18$ plugs) or control plugs (0 MPa, $n = 18$ plugs) were cultured (1 plug/mL) in BM without TGF-β3 for 2 days. Medium (TC-CM) was collected and centrifuged at 300 g for 8 min and pooled. The pooled TC-CM from unimpacted and 36 MPa impacted cartilage plugs were denoted as TC-CM0 and TC-CM36, respectively, and used for Study Part 3 described above.

### *Isolation and culture of IPFP-derived adipocytes and stromal cells*

Isolation of mature adipocytes from IPFP was done with a modification of the method described previously[20,21]. Briefly, IPFP was minced and digested in 250 U/mL type I collagenase solution (Worthington, USA, diluted with DMEM) at 37°C for 1 h with gentle agitation. After filtration and centrifugation at 135 g for 5 min, the floating top layer containing unilocular adipocytes was collected. After washing with PBS, cells ($5 \times 10^4$) were placed in 25-$cm^2$

Download English Version:

https://daneshyari.com/en/article/5669414

Download Persian Version:

https://daneshyari.com/article/5669414

Daneshyari.com