Osteoarthritis and Cartilage 20 (2012) 719–726

Osteoarthritis and Cartilage

Ultrasonic evaluation of acute impact injury of articular cartilage *in vitro*

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ARTICLE INFO

Article history:
Received 2 July 2011
Accepted 22 March 2012

Keywords:
Diagnosis
High frequency ultrasound
Cartilage injury

SUMMARY

Objective: The aim of the study was to investigate whether high frequency ultrasound technique, originally designed for arthroscopic use can be utilized to detect traumatic cartilage injuries.
Methods: A total of four intact osteochondral plugs were prepared from eight patellas for parallel comparison (total of 32 plugs). The plugs were injured by dropping an impactor on them from heights of 2.5 cm, 5.0 cm, 10.0 cm and 15.0 cm (corresponding to impact energies of 0.12, 0.25 0.50 and 0.74 J, respectively), in a custom made dropping tower. The samples were imaged with a high frequency (40 MHz) ultrasound device before and after the injury. Reflection coefficient (*R*), integrated reflection coefficient (*IRC*), apparent integrated backscattering (*AIB*) and ultrasound roughness index (*URI*) were determined for each sample.
Results: Injuries invisible to the naked eye could be sensitively detected *via* the decreased values of the ultrasound reflection parameters ($P < 0.05$). Furthermore, a decreasing trend was detected in the values of *R* and *IRC* as the momentum of the impactor increased. The values of *AIB* were significantly lower for samples injured by dropping the impactor on the cartilage from heights of 2.5 cm and 15 cm but the *URI* values were similar in intact and injured cartilage. Histological analysis of the cartilage samples revealed that the injured cartilage exhibited depletion of the cartilage surface proteoglycans but the structure of collagen network was almost normal.
Conclusions: Quantitative ultrasound imaging enables the detection of minor visually non-detectable cartilage injuries. As the present technique is feasible for arthroscopic use it might have clinical value in the evaluation of cartilage lesions during arthroscopy e.g., after tear of the anterior cruciate ligament.

Introduction

Osteoarthritis (OA) is a degenerative joint disease characterized by progressive degeneration of the articular surfaces and remodeling of subchondral bone, leading to pain and loss of joint function[1]. Although the pathogenesis of OA is still unclear, several factors that affect the development of OA have been identified. Impact injuries of articular cartilage generated by joint traumas are a known cause of degeneration of articular cartilage and the appearance of posttraumatic OA[2]. Thus, for effective treatment as well as prevention of the degeneration of articular surfaces, it is important to make an early diagnosis of cartilage damage. However, detection of non-macroscopic cartilage surface damage is challenging with current clinical imaging techniques. Articular cartilage is not visible in X-ray images and the high costs and limited resolution of magnetic resonances imaging (MRI) limit their application in the microscopic assessment of cartilage. Currently, arthroscopic examination of the joint is a routine procedure for evaluation of the severity of cartilage injury. However, arthroscopy enables only a visual evaluation of the cartilage surface although it can be combined with subjective palpation of the cartilage stiffness. A recent study reported that the majority of experienced arthroscopists considered that the differentiation between low-grade and high-grade cartilage lesions is challenging and in need of improvement[3]. In the same study, 75% of the arthroscopists stated that a more objective measurement would be very useful or somewhat useful[3]. Furthermore, inter-observer reliability of arthroscopic grading has been reported to be poor[4]. Thus, more sensitive imaging methods are needed to detect acute cartilage lesions.

Ultrasound is a widely used medical imaging modality. It is based on measurements of reflection and scattering of ultrasound that occurs at the interfaces between tissues with different acoustic

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doi:10.1016/j.joca.2012.03.018

impedances; these are determined by tissue density and mechanical properties[5]. By measuring the pressure of an ultrasound pulse reflected at the interface, the difference in the acoustic properties of the materials can be approximated[5].

Numerous ultrasound techniques have been described in the literature for quantification of the integrity of articular cartilage[6–11]. The techniques are based on the measurements of ultrasound velocity[12], attenuation and scattering inside cartilage[6,11] reflection and scattering from the cartilage surface[6,7] and measurements of cartilage thickness[10]. With ultrasound mechanical or enzymatic degradation as well as spontaneous degeneration of the cartilage surface can be detected[6–8,13]. Furthermore, ultrasound backscattering from the inner structures of cartilage has been reported to be sensitive to changes in the collagen network in cartilage[9,14]. Importantly, quantitative ultrasound imaging has already been applied during arthroscopy of human knee[15].

We have recently introduced an arthroscopic ultrasound technique for evaluation of the integrity of articular cartilage[16]. The technique is based on the use of a clinical high frequency intravascular ultrasound (IVUS) device and catheters (Boston Scientific, San Jose, CA, USA). This novel arthroscopic ultrasound technique can assess in a sensitive manner cartilage thickness, artificial degeneration of the cartilage surface as well as abnormal structures in the collagen network inside surgically repaired cartilage[16–18]. Furthermore, with the arthroscopic ultrasound technique, the severity of mechanical degradation and depth of the mechanically induced cartilage lesion in a bovine knee joint could be sensitively evaluated during arthroscopy *ex vivo*[19]. Importantly, with the arthroscopic ultrasound technique spontaneous degeneration of human knee cartilage could be detected under arthroscopy *in vivo*[20]. Since ultrasound allows an evaluation not only of articular surfaces but also of cartilage inner structures and subchondral bone (i.e., not seen in the traditional arthroscopic examination) we postulate that arthroscopic ultrasound examination could improve the diagnosis of cartilage damage.

The aim of this study was to investigate the ability of the arthroscopic ultrasound technique to detect acute injury of articular cartilage after mechanical impact. We hypothesized that mechanical impact would damage the cartilage collagen network of superficial cartilage and that this could be detected in the ultrasound measurement as a decrease in cartilage surface reflection, an increase of cartilage surface roughness and an increase of scattering inside the cartilage.

Materials and methods

Intact bovine (18-months-old) knee joints (number of joints = 8) were obtained from a local slaughterhouse (Atria Oyj, Kuopio, Finland) and stored at +4°C until the experiment. The joints originated from eight different animals and were treated as independent specimens in the statistical analysis. The knees were opened within two days' post mortem and osteochondral plugs (*diam.* = 25.4 mm) were prepared from lateral proximal patellae. Subsequently, the discs were divided into four pieces and a smaller osteochondral plug (*diam.* = 6.1 mm) was punched from each piece. Since the samples were prepared from an area with a diameter of 25.4 mm, the tissue characteristics of samples prepared from each patella were assumed to be similar. One sample from each patella was included in each sample group. The tissue adjacent to the smaller plug was used as intact control tissue in the histological analysis. All samples were immersed in phosphate buffered saline (PBS), containing inhibitors of proteolytic enzymes (5 mM disodium Ethylenediaminetetraacetic acid (EDTA) and 5 mM benzamidine HCl), during the measurements. Disodium EDTA inhibits activity of enzymes dissolving collagen and benzamidine HCl specifically retains the proteoglycans.

Impact injury was created by dropping an impactor (weight 500 g) onto the osteochondral plug from different heights 2.5 cm, 5.0 cm, 10 cm, or 15 cm (corresponding to the energies of 0.12 J, 0.25 J, 0.50 J and 0.74 J at the instant of the impact, respectively) in a custom made drop tower (Fig. 1). The impactor was lifted from the sample within 1 s after the impact to prevent any major creep deformation. After the measurements samples were prepared for histological analysis. Samples were first chemically fixed in 4% (weight/volume, w/v) formaldehyde buffered to pH 7.0 for 48 h and then decalcified in 10% (w/v) EDTA in 4% (w/v) formaldehyde, buffered to pH 7.4 for 2-weeks. Subsequently, the samples were dehydrated in alcohol, filtered and embedded in Tissue-Tek III embedding wax (polymer added) (Sakura Fintek Europe, Zoeterwoude, Netherlands).

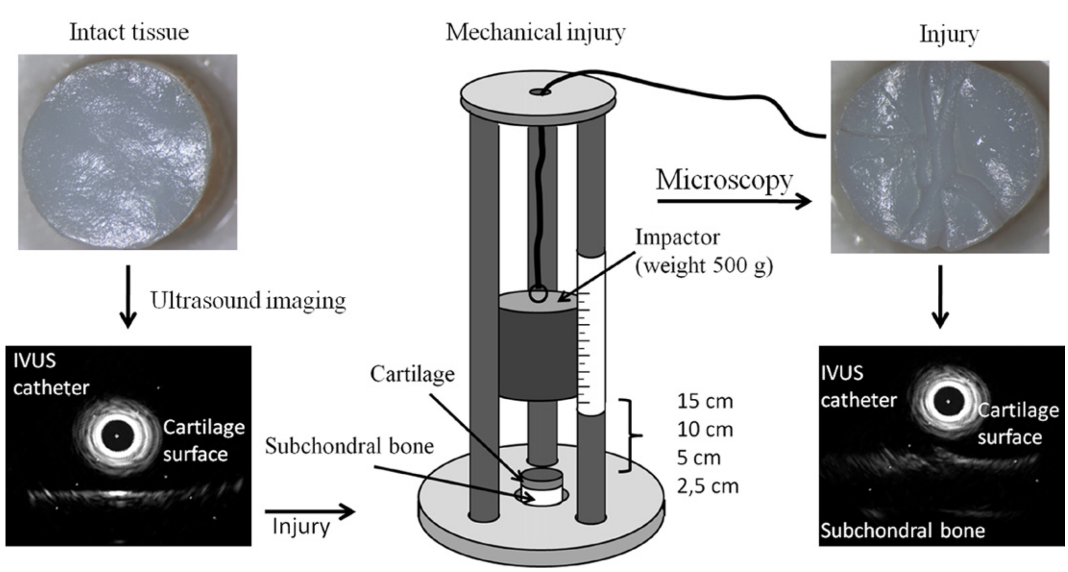

Fig. 1. Intact osteochondral samples were imaged with ultrasound and a light microscope. Subsequently, an impact injury was created on cartilage samples with a custom made dropping tower. The weight of 500 g was dropped on the cartilage samples from heights of 2.5 cm, 5.0 cm, 10 cm and 15 cm. In order to prevent any major creep deformation of the cartilage the weight was lifted from the sample within 1 s. After the injury, samples were imaged again with ultrasound and a light microscope.