Osteoarthritis and Cartilage (2009) **17,** 456–463

doi:10.1016/j.joca.2008.09.005

Osteoarthritis and Cartilage

International Cartilage Repair Society

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On new bone formation in the pre-osteoarthritic joint

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Summary

Objective: This study investigated the structural alterations in the osteochondral junction, traversing the intact-to-lesion regions, with the aim of elucidating the way in which the pre-osteoarthritic (pre-OA) state progresses to fully developed osteoarthritis (OA).

Method: Thirty bovine patellae showing varying degrees of degeneration, with lesions located in the distal–lateral quarter, were used for this study. Cartilage-on-bone blocks were cut along the lateral facet to include both the lesion site in the distal end and the intact site in the proximal end. The blocks were formalin-fixed, mildly decalcified and microtomed to obtain 30 μm – thick osteochondral slices. Using differential interference contrast optics, the tissue microstructure was captured at high resolution in its fully hydrated state.

Results: There were structural changes in the osteochondral junction beneath the still-intact articular cartilage adjacent to the lesion site. The changes observed in traversing from the intact to the lesion site exhibited characteristics that were strikingly similar to those associated with *primary* bone formation. The evidence suggests that disruption of the cartilage continuum by a lesion has wider mechanobiological consequences at the osteochondral junction.

Conclusion: The progression of OA appears to involve new bone formation adjacent to lesion sites. We hypothesise that the new bone spicules that appear in regions beneath intact cartilage adjacent to lesion sites provide a snapshot of the elusive pre-OA state.

Key words: Early osteoarthritis, Osteochondral changes, Cones, New bone formation, Mechanobiology.

Introduction

Clinically, osteoarthritis (OA) is the symptomatic loss of cartilage thickness in a habitually loaded region of a joint, and is associated with subchondral sclerosis and osteophyte formation[1]. Together with radiographic joint space narrowing these features present to the physician the defining signs of OA[2,3] and represent a condition well beyond any pre-osteoarthritic (pre-OA) stage. Interestingly, Satku *et al.*[4] have noted the rapidity with which the pre-OA phase develops into symptomatic OA and hence the difficulty of capturing the crucial early stages of this disease.

While pathological changes in OA involve *both* the articular cartilage (AC) and its underlying bone there is still uncertainty as to whether the primary trigger is related to changes occurring initially in the AC or in the underlying subchondral bone region. There is still no consensus as to which precedes which – subchondral bone sclerosis or cartilage thinning[3,5]. Some OA animal model studies have shown that changes in the joint surface occur before subchondral bone thickening and osteophytic bone formation[6–8]. However, other studies have suggested that the fate of the AC is dependent on the mechanical properties of the underlying bone[9–11].

The 'bone-first' hypothesis could be strengthened if early osteochondral changes, suggestive of new bone formation, could be detected in the pre-OA joint in which a fully intact layer of AC is still present. Neuman *et al.*[12] presumed that the new bone formation seen in OA hip joints was preceded by the formation of canals and cavities containing vessels. However, their study had several major limitations. Firstly, their histological data were obtained from late-stage OA joints and thus could not provide the crucial pre-OA structural evidence needed to demonstrate early osteochondral changes. Secondly, the level of resolution of their stained histological images did not provide adequate structural detail, a limitation that would also apply to the autofluorescent images of vascularisation in the OA mice joints reported by Walsh *et al.*[13].

Using scanning electron microscopy and confocal light microscopy Boyde and Firth[14] were better able to show the presence of these vascular canals in the joints of healthy trained and untrained racehorses. They referred to these canals as 'cutting cones', this terminology being similar to that employed by previous bone researchers to describe the early morphological features of normal bone formation and remodelling[15–19].

A more detailed understanding of both the nature and sequence of osteochondral changes will require higher resolution imaging of these changes in tissues that incorporate a graded progression from normal right through to fully developed OA. Data from such a progression should then include the crucial pre-OA state.

Much earlier Walker[20] and Bartels[21] showed that the bovine patella underwent progressive OA-like changes. These changes in this same organ have been documented microscopically and biomechanically[22,23] and are similar histologically to those seen in human OA cartilage[24]. More recently

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Received 2 April 2008; revision accepted 6 September 2008.

Broom *et al.*[25], using the same bovine OA model, showed that the macroscopic appearance of the articular surface consisted of regions largely free of ruptures blending into visible lesions. Alterations in the general matrix microstructure underlying this transition from the intact to the disrupted articular surface were found to correlate generally with the degree of surface disruption. Thus, the mature bovine patella provides a useful means of investigating the cartilage–bone system containing a developing OA lesion within a given joint. In effect, the model provides a snapshot of the transition from the intact or pre-OA state to the fully developed lesion.

The aim of this new study was to use the bovine patella as a model system to investigate progressive bone-related changes occurring across the intact–lesion transition in the early degenerate joint.

Materials and methods

Patellae were obtained from freshly slain mature bovine cows and stored at −20°C. Following thawing in cold running water the articular surface was stained with Indian ink to identify the presence and extent of any fibrillation[26]. Only those patellae exhibiting a Grade-II lesion (Outerbridge classification[27]) on their distal–lateral facets (Fig. 1) were selected for this study. Cartilage–bone samples with *en face* dimensions ≈14 × 45 mm were sawn from the lateral facet of each patella so as to incorporate the intact–lesion transition (Fig. 1). The samples were then equilibrated in 0.15 M saline for 2 h at 4°C, formalin-fixed overnight, washed in cold running water and then decalcified in 8% formic acid solution[23].

Each sample was divided into three areas of interest (Fig. 1), the intact region (I), the transition region (T) and the lesion site (L). These were then snap-frozen and cryo-sectioned to obtain 30–60-μm thin osteochondral sections. These sections were then wet-mounted in 0.15 M saline on a glass slide under a cover slip and examined using differential interference contrast (DIC) optical microscopy and the images digitally recorded. As well as conducting a detailed structural analysis, the distal-most tidemark and the cement line were manually traced out in relevant osteochondral images. The average distance between these two lines was determined using Image J processing software[a]. The percentage change between the lesion region and the intact site was obtained and tested for significance using non-parametric Mann–Whitney statistical analysis. A total of 30 bovine patellae were used in the study.

Results

Figure 2 illustrates at low magnification the morphological changes in both the surface and the osteochondral junction in traversing from the intact to the lesion site. Note how the disruption in the lesion region [Fig. 2(c)] corresponds to the ink-stained rupture site (L) shown *en face* in Fig. 1. The articular surface in the transition is intact apart from some deep clefting [Fig. 2(b)]. Along the osteochondral junction from the intact to the lesion site [Fig. 2(a–c)] there are multiple tidemarks and increasing irregularity of the subchondral bone.

The distal-most tidemark (that furthest from the subchondral bone) was less intense than the deeper proximal ones (Fig. 3). The distance between the distal-most tidemark and the bone cement line was on average 68 μm (±20 μm) in the intact region, reducing to 40 μm (±10 μm) in the lesion region.

Bony spicules were seen to emanate from *Haversian-like* canals within the deeper subchondral region (Fig. 4). In the intact site the spicules contained a clearly defined central canal surrounded by new bone matrix continuous with the deeper subchondral bone (Fig. 5).

[a]Image J 1.31v. NIH, USA (Public Domain http://rsb.info.nih.gov/ij/) and Java 1.3.1_03.

At the lesion site, the spicules were less obvious, obscured instead by more extensive bone formation [Fig. 6(a)] with a well-developed osseous cuff distinguished from the zone of calcified cartilage (ZCC) by a clear cement line [see black arrow in Fig. 6(b)]. That this cuff constituted new bone formation was further evidenced by the characteristic morphology of the embedded osteocytes, with their fine radiating canaliculi (Fig. 7). The spicules advanced towards, but did not breach, the distal-most tidemark (Figs. 5, 6, 8, 9).

Between the intact and lesion regions there were four major differences in the morphology of this new bone. Firstly, spicule frequency and advancement increased towards the lesion region (Fig. 9). Secondly, there was a 40% decrease ($P < 0.05$) in the visible ZCC of the lesion region compared to the intact site. Thirdly, the network of *Haversian-like* canals [see arrows in Fig. 6(a)] was also considerably more developed in the lesion region. Finally, the new bone appears woven and thus significantly different from the original lamellar form (cf. sites A and B in Fig. 10).

To establish whether or not there was any consistency in the pattern of morphological change in the transition from intact to lesion, each transitional sequence from the entire cohort of samples was classified microstructurally with respect to four distinct morphological characteristics, namely articular surface disruption, tidemark-ZCC development, spicule prominence and cement-line advance. Collating the data in terms of these four characteristics then revealed a common graded continuum of structural change defined by five discrete stages (see Fig. 11). From this analysis it was noted that the gradation of change occurring in each patella always spanned only adjacent stages.

Discussion

Not reported in this paper are the changes in matrix structure observed in the increasingly degenerate cartilage matrix. The data relating to this aspect of the study were not included as they have been reported in previous studies from this laboratory[22,25,28,29]. These earlier studies showed how there is an increasing microscopically resolvable '*fibrosity*' arising from '*destructuring*' of the AC general matrix across the intact-to-lesion site but did not include a study of the osteochondral region. However, the new data in the present study suggest that significant structural changes occur in the ZCC even before any significant microscopic changes take place in the overlying articular surface [Figs. 3(a), 5 which also correspond to stages 1 and 2 in Fig. 11].

The ZCC is thought to attenuate the force gradient between the compliant cartilage above and the much stiffer

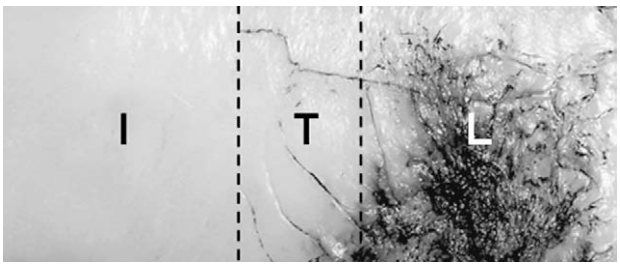

Fig. 1. Indian ink-stained articular surface of the full intact-to-lesion transition of a cartilage-on-bone sample viewed *en face*. The intact (I), transition (T) and lesion (L) regions are indicated. The actual size is ~14 mm by 45 mm.