

Research Article - Histology and Cell Biology

Morpho-structural alterations of sub-chondral bone tissue in patients with osteoarthritis: a scanning electron microscopy study

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Abstract

Osteoarthritis focuses principally on the degeneration of articular cartilage as a primary cause of the disease. The pathophysiological process of osteoarthritis is characterized by alteration of chondrocytes and the increased bone formation by sub-chondral osteoblasts. Infiltration of macrophages and perivascular T and B lymphocytes is observed, and these infiltrates have been demonstrated in both early and advanced disease. The morphological and phenotypic characteristics of osteocytic cells attached to the normal and the osteoarthritic matrix differ from each other, suggesting that specific signalling pathways arise or are altered between matrix and cells. On this basis, we have examined biopsies of bone obtained by normal femur and by femur of subjects affected by osteoarthritis using techniques of scanning electron microscopy in order to identify the morphostructural alterations that occur in the sub-chondral bone. Our results have shown that the bone tissue of subjects not affected by any disease of bone presents a well-organized structure, while the bone tissue obtained by patients affected by osteoarthritis shows a derangement of tissue itself possibly correlated with altered function of the osteoblasts, that during the pathological process produce a less mineralized extracellular matrix with consequent loss of the normal bone structure. In our opinion, during the osteoarthritic process there would be a defective signalling between bone cells leading to the production of an irregular, amorphous extracellular matrix by osteoblasts, characteristic of the pathological condition.

Key words

Osteoarthritis, tissue bone, scanning electron microscopy.

Introduction

The prevailing theory on the development of osteoarthritis (OA) concentrates principally on the degeneration of articular cartilage as a primary cause of the disease, suggesting that instability of the mechanical pressure on the joints leads to progressively more severe damage to the cartilage (Pessler et al., 2008) until a point where the only therapeutic option is the total replacement of the joint (Ayrat et al., 2005; Hill et al., 2007).

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The pathophysiological process of osteoarthritis is progressive, triggered by a change in the microenvironment of the chondrocytes and includes chondrocyte mitosis, increased synthesis of proteoglycan and type II collagen which are the major structural elements of cartilage, and increased bone formation by sub-chondral osteoblasts (Elliot et al., 1993; Nishimoto et al., 2004; McInnes et al., 2005; Baslund et al., 2005). With the increased bone formation in the sub-chondral zone, the physical properties of the bone change: it becomes more rigid with decreased elasticity, hence microfractures occur, followed by callus formation, further rigidity and additional microfractures. At the cellular level, infiltration of macrophages and perivascular T and B lymphocytes is observed, and these infiltrates have been demonstrated in both early and advanced disease (Goldenberg et al., 1983; Oehler et al., 2002; Pearle et al., 2007; Benito et al., 2005).

Cartilage breaks off and the exposed subchondral bone becomes the new joint surface; this leads to the characteristic smooth appearance with increased radio-opacity and sclerosis in the cancellous bone beneath. The typical symptom is pain and, with the progression of osteoarthritis, the joint mobility decreases with limitation of the range of movement. The pain induced in the lower limb joints by load determines a limp of escape, so called because the patient tends to shorten the stance phase on the corresponding foot (Oehler et al., 2002). The observation that the morphological and phenotypic characteristics of osteocytic cells attached to the normal and the OA matrix differ from each other suggests that specific signalling pathways between matrix and cells arise or become altered (Scanzello et al., 2009).

In a healthy joint, processes of growth, modelling, and remodelling occur constantly during the life, but are active to different degrees in the mineralized tissues. Modelling is defined as formation or resorption at a given site: it varies bone mass but also modifies the shape of bone. During remodelling, processes at the cellular level allow for cell recruitment, differentiation, proliferation, and migration to surfaces (Burr, 2004; Portelli et al., 2014). Subsequently, a resorption phase follows and the cells can act more quickly or more slowly and for longer or shorter periods of time. Remodelling does not vary bone volume, therefore remodelling processes cannot account for the sclerosis observed in OA.

Moreover, the process of endochondral ossification occurs throughout life. This causes progression and duplication of the tidemark, and this it is frequently observed in joints affected by OA disease. Advancement of the tidemark leads to make the calcified cartilage thicker and, even though the tidemark advances, remodelling at the osteochondral junction follows more quickly, so that the calcified cartilage may become thinner. In a healthy joint, these processes of endochondral ossification and subchondral remodelling are generally in balance. In an OA joint they go out of balance.

On this basis, in the present experimental study we examined biopsies of bone obtained by femur of subjects not affected by any bone disease and by femur of subjects suffering from OA using techniques of scanning electron microscopy in order to identify morphostructural alterations occurring in the sub-chondral zone, that may have escaped evaluation until now.

Materials and Methods

For this case study, nine male patients 60-71 years old suffering from OA and seven patients 45-70 years old who did not present OA disease but had undergone

surgery for total meniscal traumatic lesions have been selected. Pathological bone tissue biopsies were obtained by articular heads samples who had been cut because of prosthetic substitution. Healthy bone tissue biopsies were extracted through bone coring. Anaesthesia was epidural in all cases. Every patient expressed consensus and the procedures were in conformity with the guide lines from Helsinki declaration of 1975. The present study was approved by the ethical committee.

Biopsies were fixed in 2.5%glutaraldehyde in 0.1 mol/L phosphate buffer, pH 7.4, for 24 hours at room temperature. Samples were dehydrated with ethanol and amyl acetate and critical point dried through liquid CO₂. The fractured surface of bone was then placed on a stub, coated in a Plasma Science CrC Turbo Pumped (Torr International, New Windsor, NY, USA) and was observed in a Phenom G2 scanning electron microscope (Phenom, Eindhoven, The Netherlands).

Results

For the present study we have analyzed, by scanning electron microscopy, bone tissue biopsies taken from subjects not suffering from any disease and biopsies from patients suffering from OA. In particular, we have studied the cortical area and the subcortical area of the femoral condyle.

The fracture surface of femoral condyle in the cortical area of subjects not affected by any bone pathology showed numerous bone lamellae oriented longitudinally and perfectly delineated (Fig. 1A). At high magnification, the lamellae were perfectly delineated and parallel between each other, as is the normal architecture of the bone tissue (Fig. 1B). In another microscopic field of healthy bone tissue there were numerous Havers' canals (Fig. 1C) around which, at higher magnification, it was possible to observe many holes in bone (Fig. 1D).

The fracture surface of femoral condyle in the subcortical area of subjects not affected by any disease of bone showed a lamellar structure more irregular than that seen in the cortical area (Fig. 2A). At high magnification, it was possible to detect bone lamellae irregularly organized and arranged more disorderly than those observed in the cortical area (Fig. 2B). Moreover, a rich fibrillar structure was evident, that appeared constituted by a dense network of collagen fibres (Fig. 2C) which penetrate into bone lamellae (Fig. 2D); such fibres, known as perforating or Sharpey's fibres, are known to firmly anchor, periosteum, joint capsule, ligaments and tendons to the bone tissue.

The fracture surface of femoral condyle in the cortical area of patients with OA showed a generally disorganized structure. At low magnification, the bone was quite uneven (Fig. 3A) and it was no longer possible to recognize bone lamellae regularly oriented and arranged concentrically around Havers' canals (Fig. 3B). Higher magnification revealed the presence of an amorphous structure that made the bone tissue almost unrecognizable (Fig. 3C). In some microscopic fields the structure was most regularly organized for the presence of superimposed layers of bone tissue that may demonstrate new formation of strengthened and thickened tissue (Fig. 3D), indicating osteosclerosis. The observation of other microscopic fields highlighted extensive neoformation of an amorphous matrix similar to roof tiles that thickened the bone tissue (Fig. 4).

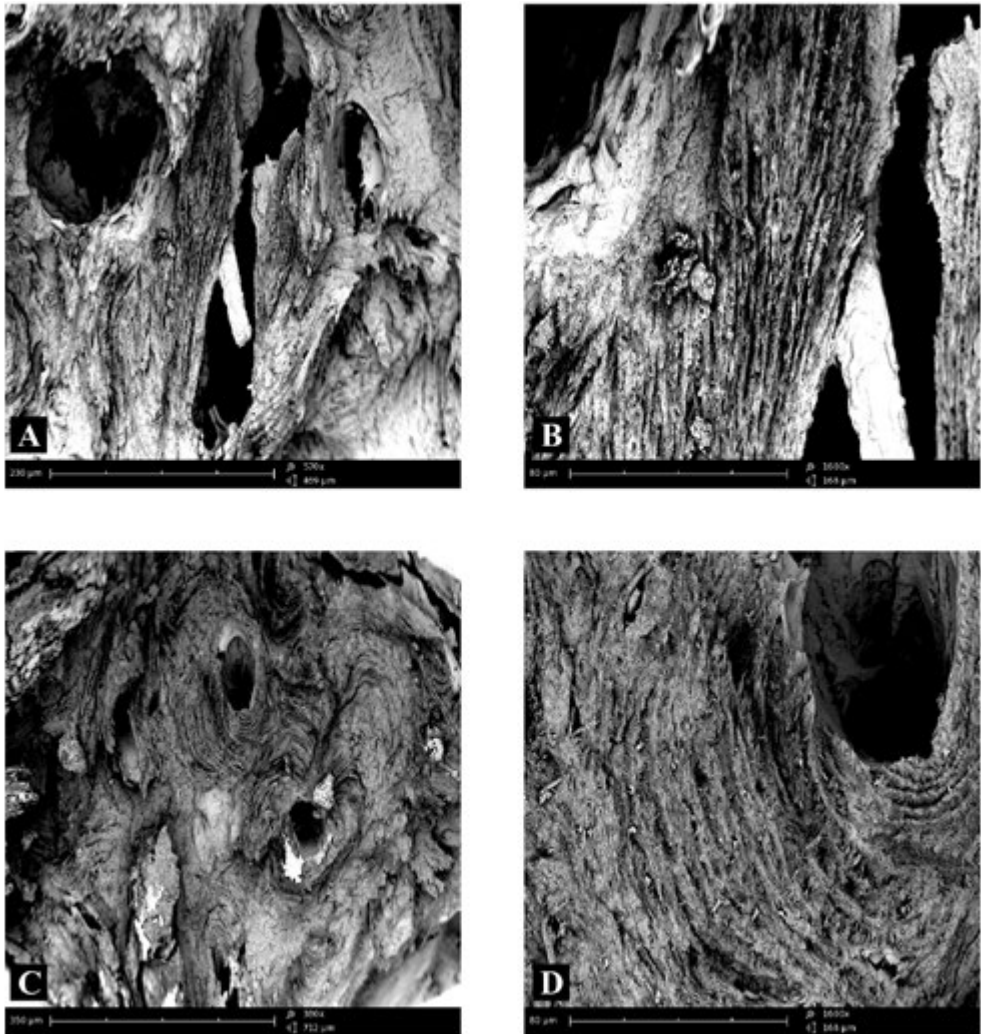


Figure 1 – Compound panel of fracture surfaces of cortical zone of bone tissue from a biopsy of healthy femoral condyle showing bone lamellae oriented longitudinally and perfectly delineated (A, B); the lamellae are perfectly delineated and parallel between them showing a normal lamellar architecture of the bone tissue is shown (B). In another microscopic field, the presence of Havers' canals is evident (C); around these lamellae many holes are visible in bone (D).

The fracture surface of femoral condyle in the subcortical area of patients with OA showed a more organized structure than the cortex. The bone lamellae were relatively well organized and maintained a parallel orientation between each other (Fig. 5A); however, there were areas with diffuse osteosclerosis and areas of erosion of bone tissue, possibly conferring fragility (Fig. 5B). At high magnification of the erosion areas

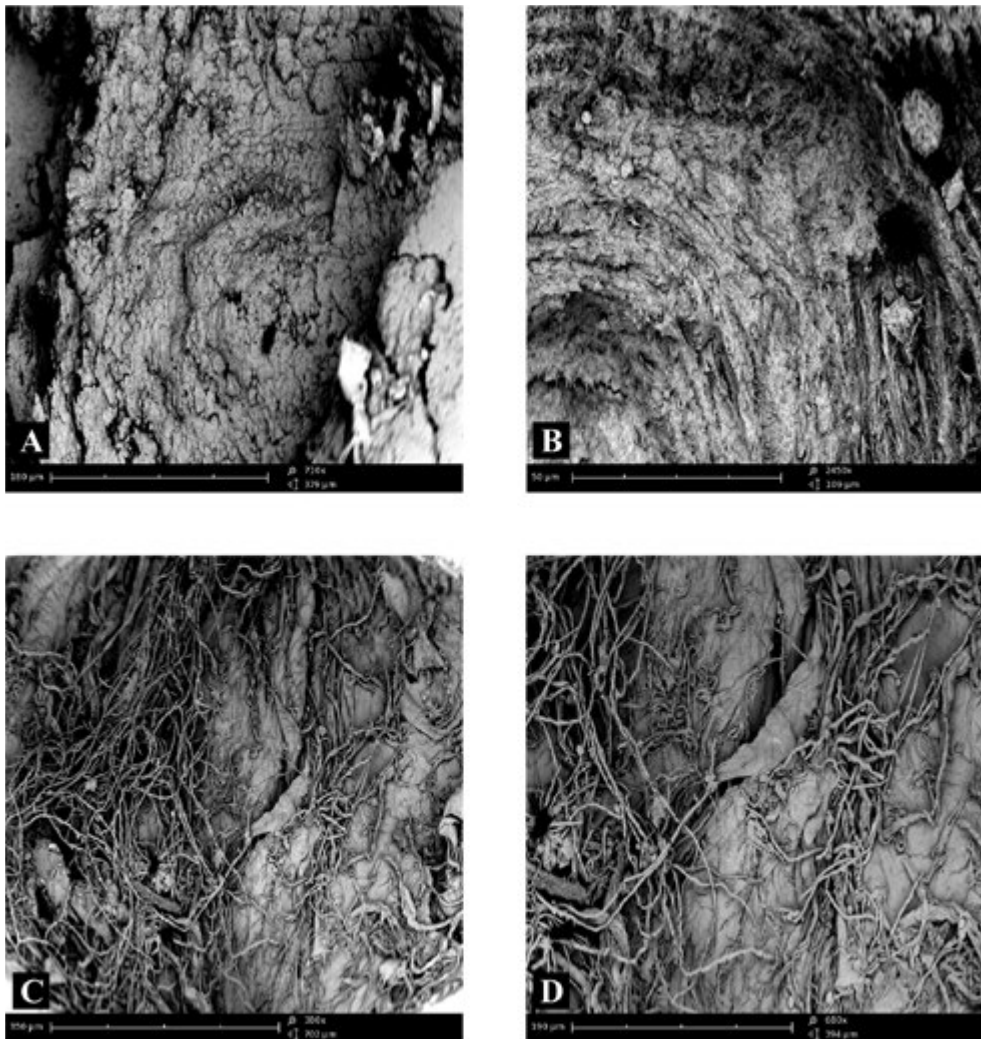


Figure 2 – Compound panel of fracture surfaces of sub-cortical zone of bone tissue from a biopsy of healthy femoral condyle showing more irregular lamellar structure than figure 1 (A); at high magnification, the presence of bone lamellae irregularly organized is clearly visible (B). The observation of another microscopic field allows to see a rich fibrillar structure constituted by a dense network of collagen fibres (C), penetrating into bone lamellae (D).

it was possible to see areas of bone tissue arranged into sheets which showed lesions and signs of bone resorption (Fig. 5C); a further magnification of the same area revealed the presence of numerous small areas of erosion giving an almost speckled appearance to the bone, which perhaps may fuse into large and diffuse areas of erosion (Fig. 5D).

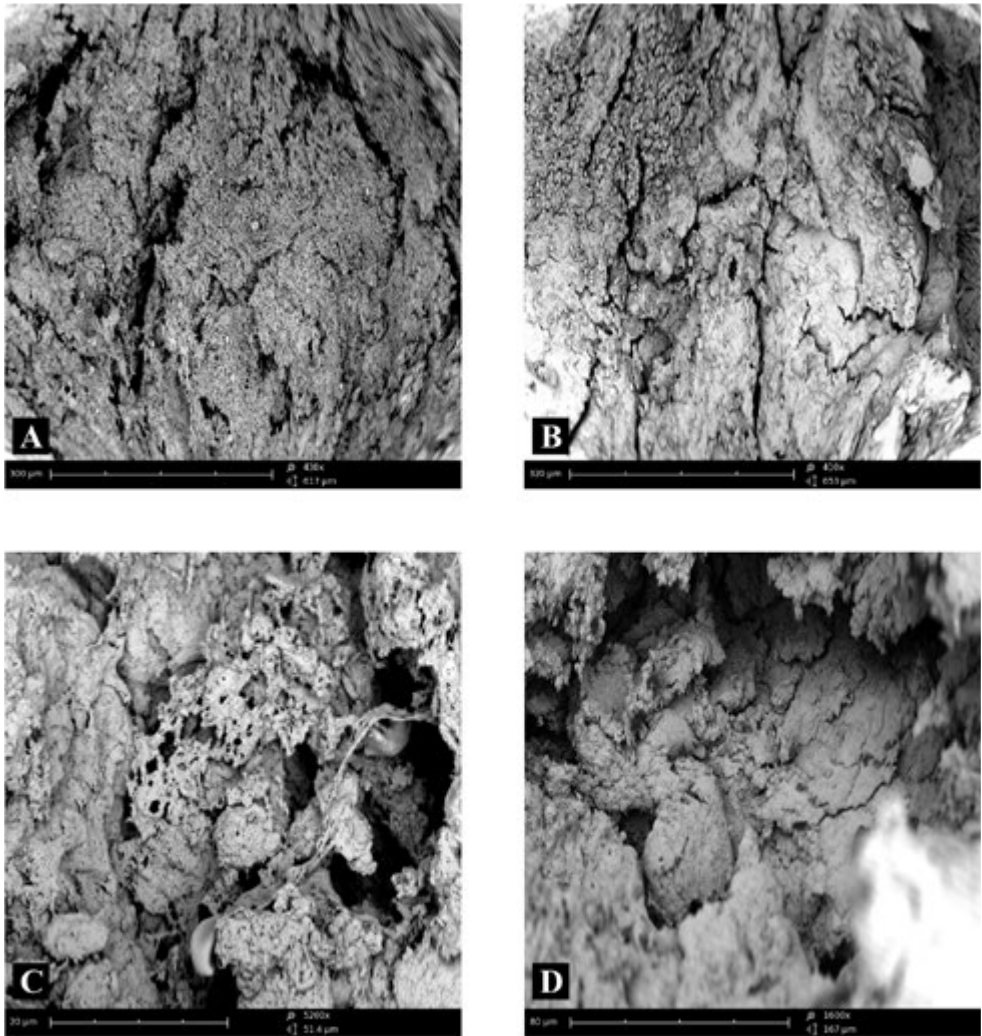


Figure 3 – Compound panel of fracture surfaces of cortical zone of bone tissue from a biopsy of femoral condyle affected by OA, showing a generally disorganized structure (A), in which it is no longer possible to recognize bone lamellae regularly oriented and arranged concentrically around Havers' canals (B). At higher magnification, it is easier to see the amorphous structure, that makes bone tissue almost unrecognizable (C); in other microscopic fields, the structure is more regularly organized for the presence of superimposed layers of bone tissue (D).

Discussion

Osteoarthritis is the most common joint disorder and is characterized by cartilage loss, new bone formation at the margins of joints, changes in subchondral bone, and recurrent synovitis (Fuerst et al., 2009). Skeletal development and homeosta-

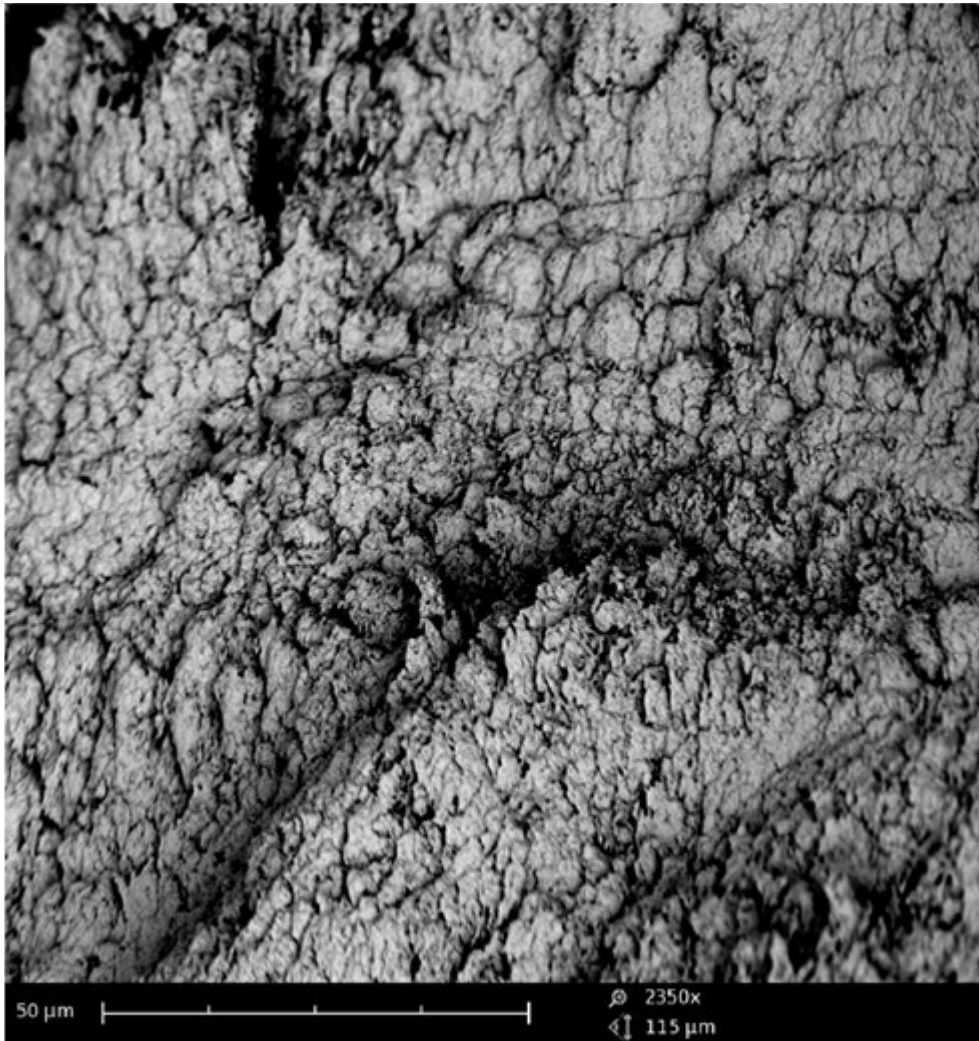


Figure 4 – Fracture surfaces on cortical zone of bone tissue from a biopsy of femoral condyle affected by OA, showing the high neoformation of an amorphous matrix similar to roof tiles that thicken the bone tissue

sis depend on the specific regulation of resorption and new bone formation, which include the degradation and removal of the extracellular matrix and the synthesis and deposition of new constituents (Bord et al., 1997).

Radio-diagnostic techniques show relevant alterations in patients affected by OA such as reduced articular space in 99.5% cases, osteophytes in 98,1% cases and subchondral bone sclerosis in 88,3% cases (Audrey et al., 2014).

Although numerous studies have been carried out on bone tissue affected by OA, most authors have focused on molecular and proteic characteristics of this disease

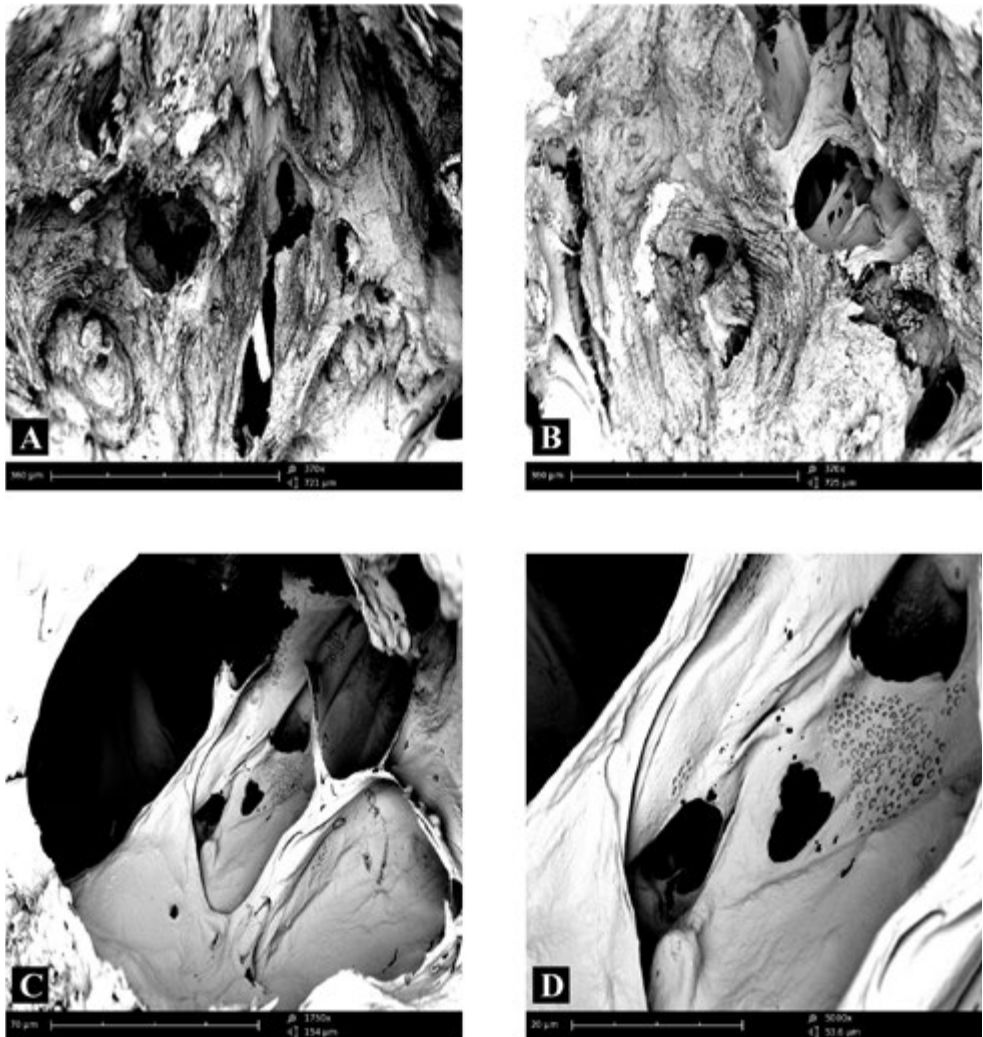


Figure 5 – Compound panel of fracture surfaces of sub-cortical zone of bone tissue from a biopsy of femoral condyle affected by OA, showing relatively well organized bone lamellae which maintain a parallel orientation among them (A); in other microscopic fields erosion areas are detectable in the bone tissue, that may make the tissue fragile (B). A higher magnification of erosion areas allows to recognize bone tissue arranged into sheets showing evident lesions and clear signs of bone resorption (C); an even higher magnification reveals the presence of numerous small areas of erosion damage giving almost a speckled appearance to the bone (D).

demonstrating that a key role in pathogenesis is played by integrins (Prasad et al., 2013). Moreover, it has been demonstrated that matrix metalloproteinases are capable of degrading all components of connective tissue (Murphy and Reynolds, 1993) and are likely to be involved in bone matrix degradation during OA (Everts et al. 1992).

Other studies, carried out through optical and laser scanning confocal microscopy, have highlighted a different morphology of the osteocytes in the arthritic bone tissue as compared with normal tissue: osteocytes from arthritic tissue are round shaped due to increase in cytoplasm volume (Sanchez et al., 2008).

There are few data on morpho-structural aspects of bone tissue affected by OA. The present results have shown a general derangement of bone tissue in patients affected by OA. We observed many alterations in OA bone, such as a important structural alterations of bone lamellae. Moreover, we revealed the absence of cartilage on the articular surface involved in the OA process and a specific increase in underlying bone lacunae and bone tissue, making the tissue almost unrecognizable.

These results, obtained through scanning electron microscopy, underline how bone tissue affected by OA undergoes continuous bone reshaping with consequent loss of normal lamellar architecture typical of healthy bone tissue. Because modeling and remodeling are responsible for adjusting the geometry of the joint to new conditions, many anti-activation agents may prevent the alterations in joint shape that accompany OA.

Our results showed the presence of superimposed layers of bone tissue that demonstrate new formation of strengthened and thickened tissue. In our opinion, these data confirm the highlighted alterations of subchondral bone occurring every time an articulation is affected by arthritic process. In fact, it has been previously demonstrated that during OA disease a higher amount of extracellular matrix proteins is produced by osteoblasts (Prasad et al., 2013). In this respect, bone matrix serves as an organized framework offering mechanical support to and mediating biological activities of bone cells and also mediating signals that maintain bone homeostasis and remodelling (Green et al., 1995).

Bone cells, like most other matrix-associated cells, cannot survive or differentiate without adhesion to their matrix (Popov et al., 2011). Consequently, bone cell morphology and function depend strongly on matrix quality under conditions in which biological signals are constant (Prasad et al., 2013).

The results of our study underline the derangement of the bone tissue affected by OA and its correlation with the altered functionality of osteoblasts that during the pathological process produce a higher mineralized extracellular matrix with consequent derangement of the normal bone structure.

Previous studies have shown that osteocytes in a normal extracellular matrix present a dense network of dendrites allowing important interaction among cells themselves, whereas cells from OA matrix have a wrinkled surface with a small amount of dendrites (Pazzaglia et al., 2012). Thus, during the OA process there would be a lack of signalling between osteocytes, typical of the normal bone tissue, generating an irregular matrix and therefore the typically irregular bone tissue observed in the current study. The results of *in vivo* tests and of analysis of osteocyte morphology from subchondral bone of patients affected by OA and subjected to knee prosthesis surgery have shown alterations in the cytoskeleton (Bord et al., 1997).

In our opinion, the alterations detected in this research may be caused by the constant production of amorphous extracellular matrix produced by osteoblasts due to pathological conditions. Structural alterations that originate from this process inevitably affect the general macroscopic morphology of the joint affected by the pathological process, generating osteophytes and subchondral osteosclerosis that cause the typical symptoms of OA.

Further investigations - in progress - by light, scanning electron, and confocal laser scanning microscopy and using markers for osteoblasts and osteoclasts, could offer support to present results in order to understand the alterations occurring during bone remodelling typical of OA.

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