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Microhardness evaluations of the bone growing into porous implants

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Small cylinders of a new composite porous material consisting of a dense alumina core coated with two layers of beads of the same material, bonded to each other and to the underlying surface by a high-temperature melting glass have been implanted in the proximal femurs of rabbits. The explants were carried out 1, 4, 6, 8, and 18 weeks after surgery. The bone fragment containing the implant was embedded in methyl methacrylate without performing decalcification, and morphological observations were carried out. These showed that four weeks after surgery it is already possible to observe the development of bone spicules in the implant porosities. Along with these studies, microhardness measurements were carried out by using a microhardness tester connected to an image analyser. The mineralized tissue in close contact with the implant showed, one month after surgery, a compression strength similar to that of healthy bone.

1. Introduction

The fixation of orthopaedic implants in bone by tissue ingrowth presents a possible solution to the problem of long-term implant loosening. To reach this objective, several types of porous prosthetic surfaces made of metal, as well as plastic, have been proposed. The first present some disadvantages due to the fretting corrosion and ionic dispersion, [1–4], while the second still lack final experimental confirmation with regard to mechanical strength and biocompatibility. Due to their high degree of biocompatibility, ceramic surfaces could represent an alternative solution.

We have therefore studied and developed a ceramic alumina-bioglass composite, a new porous coating for prosthetic devices, that couples the advantages of the ceramic–ceramic bearing surfaces with the improved biocompatibility of porous materials comprising of only ceramics [5, 6].

Porosity has been carefully studied both in animals and in humans by many authors; the optimal range of diameters for interconnecting pores appears to be 250–450 μm [1, 7–12].

The material, called PORAL®, was implanted into rabbit femura and at 1, 4, 6, 8 and 18 weeks the bone ingrowth was evaluated both from the morphological and the mechanical point of view, by measuring the microhardness of the tissue ingrowth.

We believe that microhardness of bone is important because it should be an accurate and reliable measure of the degree of mineralization. Moreover, the progression of mineralization that accompanies the maturation of the tissue ingrowth should be reflected by an increase of microhardness.

This could be explained by the fact that a lower mineral content should be associated with a large free space in the ultrastructure of the bone, which permits greater displacement of the microcrystallites. The resultant permanent deformation will be extensive if the bone is immature or decalcified [13–16].

2. Materials and methods

The alumina-bioglass porous composite was obtained as previously described [6]. Briefly, on an alumina core (Ostalox®) two layers of small beads of alumina (Ostalox®) 99.7% pure were applied at 1400 °C with the fusion of a high-temperature melting bioglass.

The structure thus obtained is characterized by a range of porosity from 23 to 31% (average 27%). The diameter of the surface pore varies from 290 to 510 μm. The average diameter of the interconnecting pores facing the bone in the area of the porosity is equal to 350 μm.

An experimental study of the bone ingrowth into porous alumina-bioglass composite was performed by implanting 4 mm cylinders in the rabbit distal femoral metaphyses. The surgical procedures were carried out under general anaesthesia. After surgery, tetracycline was administered every 15 days to label new bone formation. The specimens were then retrieved at intervals of 1, 4, 6, 8 and 18 weeks (two rabbits for each group). The specimens were embedded in methyl methacrylate, cut into slices approximately 20 μm thick with a circular diamond saw (Leitz 1600), and Paragon stained. A transmitted light microscope evaluation was carried out. Due to the brittleness of

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the ceramic, the porous composite was sometimes lost during specimen cutting, but this fact did not compromise the following histological study.

After preparing the histologic sections, the residual block, about 0.5 cm thick, was embedded in methyl methacrylate and then prepared for the microhardness test. The surface to be tested was ground with carborundum paper of increasing fineness from 400 grit to 1200 grit. The final polishing was carried out by pads, using abrasive alumina in a moist medium. All the preparations were carried out on a rotary wheel. In this way perfectly smoothed surfaces were obtained and the bone was microscopically visualized with reflected light [17].

At this stage no alterations in the surface structure of the bone tissue were noticed.

To assess microhardness the Durimet–Leitz device was used, which has a pyramidal diamond point, with a square base with 136° angles between the opposite sides, which produces on the surface of the material under analysis shallow imprints, whose height is about 1/7 the length of the base diagonals. In these experiments a load of 25 g was used and the imprints were precisely measured, by means of an image analyser (ASM 64 K), connected with the microhardness tester through Varioscan V 16 camera.

This method of measurement proved to be perfectly superimposable with the classical precision ocular micrometer technique. The higher speed of execution of the former makes it preferable to the traditional method.

Once the average length of the diagonals is assessed, the microhardness value expressed in Vickers degrees is obtained by the following equation:

$$H_V = (2P/d^2) \sin \tau = (P/d^2) \times 1.854 \text{ kg mm}^{-2}$$

where P is the mass applied on the pyramid expressed in kilograms, τ is one half of the included angle of the pyramid (68°) and d is the mean length of the diagonal of indentation in millimetres [18].

The imprints were performed on the tissue which grows inside the porosities of the implant at various levels, from the surface towards the inner part of the pore. Every imprint covers an area of about 900 μm^2, that is why in every pore no more than two or three measurements were taken. By comparison, other imprints were carried out on the bone tissue on the femoral area far from the implant.

For each examined specimen 40 imprints were carried out.

3. Results

3.1. Morphological examination

One week after surgery the pores were invaded by mesenchymal tissue rich in cells, blood vessels and a loose fibrillar network. Abundant connective tissue was present around the alumina beads. In a few areas small centres of ossification were seen. Residual elements of fully organized clots still remained in a few areas around the implant.

Four weeks after surgery bone ingrowth was proceeding well and was clearly seen in the specimens from rabbits marked with tetracycline. Nearly all the pores contained new bone spicules, and trabeculae were still immature and roughly organized. The extracellular matrix appeared to be still poorly mineralized. The situation at 8 weeks is revealed in Fig. 1.

Eighteen weeks after surgery, the bony spicules were remodelled into mature lamellar bone even inside the pores. Moreover, the bone surrounding the implant appeared to be oriented so that the trabeculae circumscribed the ceramic beads. Haversian systems are formed at this time.

3.2. Microhardness test

The results obtained in this test are summarized in Table I.

One week after surgery, when from a morphologic point of view there was no evidence of new bony ingrowth inside the pores, the hardness of the growing tissue was 23.5 Vickers degrees, clearly lower than that of the bone far from the implant, which averaged 57.5.

Four weeks after intervention, on the other hand, an increase in the hardness of the newly grown bone was assessed, reaching the values of the control bone far from the implant. It was impossible to predict this result in advance as, under the microscope, the newly formed trabeculae appeared relatively immature.

The hardness then remained constant with time up to the last observations 18 weeks after surgery.

4. Discussion and conclusion

The studies conducted till now on the bone ingrowth inside the porosities of orthopaedic or dental implants were mainly aimed at the morphologic observation of the tissue growth, to assess the times and, if possible, the patterns [8, 19].

Other authors [20, 21] have performed pushout tests to define the fixation strength of the implant to the bone. The ingrowth rate that is identified in this way could, however, be influenced by various factors, such as the position of the implant in respect to the

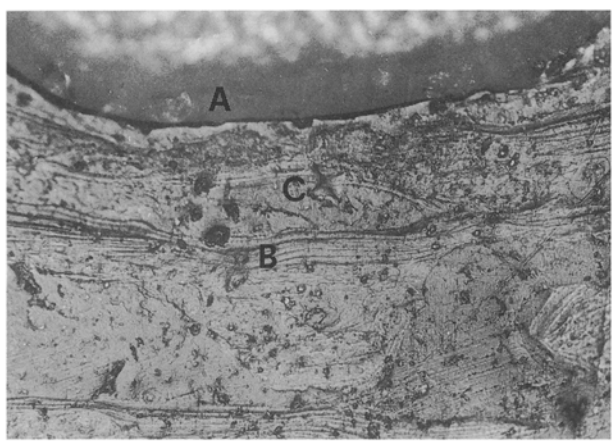

Figure 1 Rabbit femur transverse section of a porous composite specimen 8 weeks after surgery: (A) implant, (B) bone, (C) imprint carried out by the microhardness tester. (Reflecting microscope 5.75×.)

TABLE I Bone tissue hardness diameter of the imprints and Vickers degrees

Implant duration (Weeks)	Inside the pores Length of diagonals (μm)	Bone microhard.	Far from the implant Length of diagonals (μm)	Bone microhard.
1	44.4 ± 8.2	23.5	29.2 ± 2.5	54.3
4	28.0 ± 4.5	59.3	28.8 ± 2.9	55.8
6	28.2 ± 3.0	58.3	28.6 ± 4.0	57.3
8	28.5 ± 2.9	57.0	27.6 ± 3.7	60.7
18	27.4 ± 2.4	61.6	28.0 ± 3.4	59.2

bone and the correct preservation of the bone specimen, until the test is carried out.

To avoid these drawbacks and to establish a quantification of the bone-ingrowth phenomenon, we performed microhardness studies on the bony trabeculae which penetrate the porosities of an experimental implant made of a composite based on ceramic material.

The results are important not only as absolute values, but also because they allow a comparison with the hardness of the bone tissue which was identically embedded but which lies far from the implant. The rate of bone growth – verified through the traditional morphologic observation on slices of non-decalcified bone tissue – has a close correlation with the results of microhardness studies.

These studies also showed that the compression strength of the newly formed tissue four weeks after intervention is already comparable to that of the normal bony tissue, far from the implant. These data are the only ones which seem to contrast with the results of morphologic observations, which showed how the newly formed bony trabeculae still appeared slightly immature.

On the other hand, a perfect correspondence can be found in the data for the implant which has remained *in situ* for only one week, where the tissue that under morphological examination appeared as a mesenchymal tissue rich in cells, blood vessels and a loose fibrillar network, presents a hardness of only 23.5 Vickers degrees, about one third of the bony tissue hardness.

No significant increase in compression strength was demonstrated after the fourth week. These data make it possible to state that, at least under the described experimental conditions, one month after surgery the bony tissue growing inside the porosities of an implant under these conditions has not only the aspect but also the mechanical and physiological requisites of a mature bone.

In conclusion, the method we proposed for the evaluation of the "quality" of the tissue that takes part in the phenomenon of tissue ingrowth seems to be reliable and reproducible, and it can moreover be useful in the study and design of implants or porous coatings.

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