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Quantitative analysis of the bone–hydroxyapatite coating interface

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The bone–implant interface in stainless steel pins coated with hydroxyapatite, used in a monoaxial fracture external fixation system was examined. The pins were transversally inserted into sheep tibial diaphyses where a defect was created, and they were loaded for 6 weeks. Uncoated pins were implanted as control. The microscopic relation between bone and implant was quantified through image analysis: the residual thickness of the hydroxyapatite coating, pin–bone contact surface and bone ingrowth value in between the threads were measured. The bone tissue at the interface appeared regularly mineralized and viable both in the implants of coated pins and in the control uncoated ones. The ceramic coating showed a slight and not statistically significant increase in thickness. The ceramic coated pins presented contact with bone higher than the uncoated pins (75.6 ± 20.0 versus 47.5 ± 19.4); they also induced a higher bone ingrowth (86.6 ± 22.4 versus 78.7 ± 13.5). Both differences are not statistically significant, but suggestive of a trend. The authors concluded that the hydroxyapatite coating of the pins might improve the performance of external fixators, by favouring bone apposition and reducing rate of loosening.

1. Introduction

The concept of bioactivity was introduced in the late 1960s [1]. In studies on bioactive glasses the hypothesis was proposed that the biocompatibility of implant materials for bone replacement would be optimal if the material elicited the formation of normal tissues at its surface, and in addition, if it could establish an interface capable of supporting the loads which normally occur at the implantation site. A few years later it was reported that, in addition to bioactive glasses, hydroxyapatite elicited a similar reaction pattern [2, 3].

Hydroxyapatite (HA) is a chemical compound with formula $Ca_{10}(PO_4)_6(OH)_2$; it belongs to the calcium phosphate system, and it has long been the subject of intensive investigation. Because of the presence of free calcium and phosphate compound at the surface, it can interact with the surrounding bone; in particular the use of HA is promising in that it has a chemical and crystallographic structure similar to the HA of natural bone, which effectively reduces biocompatibility problems.

There is well-documented proof in the literature that sintered apatite forms very tight bonds with living bone [4, 5].

Unfortunately, some of the mechanical properties of sintered apatite are poor. Although resistance to a compressive force of 250 MPa may be achieved, the resistance to fatigue is very low. Physiological loading in tension will cause fatigue fracture of sintered apatite implants within a few months. One solution to this problem is the use of an apatite coating on a metal substrate. The thickness of such an apatite coating must be a compromise between a number of limiting conditions. The thinner the coating, the better its mechanical properties, but in the first few months of implantation some 10 to 15 μm of an apatite surface may dissolve during the process of acquiring bone. On the other hand, a coating over 100 to 150 μm may suffer from fatigue failure under tensile loading. The required compromise leads to an ideal thickness of approximately 50 μm.

The use of plasma-sprayed HA coating on the implant surface offers the potential advantages of shortening the time needed to achieve adequate fixation strength, increasing the maximum fixation strength that can be attained, as well as the amount of bone–implant apposition or bone ingrowth [6–11]. For these reasons the coating is applied on different devices, from hip prosthesis to dental implants.

Recently the coating procedure was applied also to the pins of external fixators [12]. Such orthopaedic devices, which find several applications in the treatment of fractures or in the lengthening of limbs, can prove ineffective if the pins undergo loosening. The resulting reduced stability can lead to delayed unions or even to non-unions, as well as higher risk of infection [13–15].

Our work aimed at evaluating, from the histological and histomorphometric viewpoints, whether a better relation is established between bone and hydroxyapatite-coated pins as compared to uncoated pins.

2. Materials and methods

Bicylindrical stainless steel external fixation pins were used. The outer thread pin diameters were 4 and 5 mm. The pins were divided into two groups (group A and B). Group A pins were plasma-sprayed with HA (Biocoating, Flamental, Fornovo Taro, Italy) so as to obtain a coating with a thickness ranging from 30 to 60 μm, whereas group B pins remained uncoated. Using a pre-drilling and tapping insertion technique, six pins of the same type were implanted monolaterally in the tibiae of mature sheep, and a monolateral fixator was assembled on the pins.

Thirteen sheep received coated pins, and twelve received uncoated pins. The pins were numbered in proximal-distal order. Then the medial tibial mid-diaphysis was exposed, between pin 3 and 4, and a transverse 5 mm gap osteotomy was performed in order to highly stress the bone pin interface and to obtain an unstable fixation of the fracture. One control animal from each group was sacrificed immediately after surgery (animals I and XIV), from now on referred to as T 0. The other sheep were allowed normal activity and killed 6 weeks after surgery. Only one animal (XV) died 3 weeks post surgery for causes not related to the operation.

The pins 1, 2, 5, 6 were not included in the present study, while pins 3 and 4 from each animal were processed for morphological analysis.

Bone segments containing the pins were isolated and fixed in a 10% formalin solution buffered at pH 7.2. Dehydration was performed with warm methyl alcohol (37–40 °C) under vacuum, impregnation and embedding in methyl methacrylate (Technovit 7200, Kulzer, Germany); polymerization was achieved by exposition to short wavelength light (Histolux, Kluzer, Germany).

Transverse sections of samples were obtained by means of an Exakt cutting system (Kulzer, Germany) with bore nitride saws. The slices were made thin using sandpapers with decreasing granule size to a thickness of about 100 μm, microradiographed and then further ground to a thickness of 30 μm, paying attention not to damage the bone–implant interface. Contact microradiographs were taken by using radiosensitive slides Kodak High Resolution, Type 1A (Eastman Kodak Company, Rochester). The section were exposed for 30 min to an X-ray bundle produced by a Philips generator PW 1729 with copper anode and Ni filter, functioning at voltage of 35 kV and current of 20 mA.

Non-deplastified sections were microwave stained with basic fuchsin-light green in order to distinguish between mineralized bone (green) and osteoid or connective tissue (red-orange). Silver nitrate Von Kossa, Goldner trichromic and solochromocianine staining were also performed.

Histomorphometric evaluations were accomplished both on the microradiographs and on the histologic specimens by means of a light microscope (magnification x 16), connected to an image analyser (Leitz ASM 68 K). Three parameters were evaluated on each sample, as shown in Fig. 1:

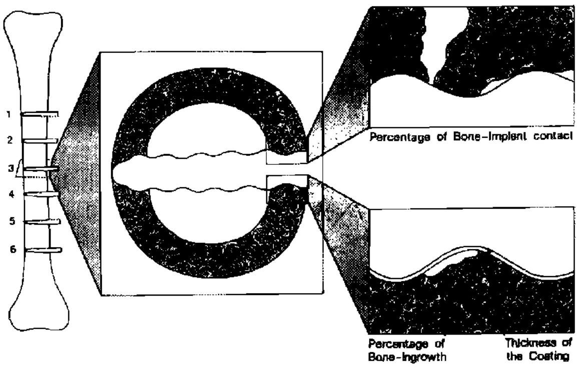

Figure 1 Scheme of pin–bone interface analysis. Pins 3 and 4 were examined. Transverse sections of the tibia are performed; their appearance is shown in the centre of the scheme. Morphometric values considered in the present study are shown on right-hand side.

(a) *residual thickness of the hydroxyapatite coating*, evaluated on 20 equidistant sites along the whole implant profile, 10 on the pin tract inserted inside the cortical bone, and 10 on the pin tract at the medullary canal level;

(b) *percentage of bone apposition* at the implant surface. This was defined as the percentage of implant length at which there was direct bone–implant contact;

(c) *bone ingrowth percentage between the threads* in relation to the area subtended by a line drawn at a distance of 50 μm from the threading tips.

The bone–implant contact percentage and the bone ingrowth percentage were determined on the implant region which crossed the lateral cortical bone, on the fixator side.

3. Results

3.1. Histologic evaluations

Microradiographs showed that the bone density of the perimplant bone tissue was similar to that of the bone tissue far from the implant in all the examined cases.

The observation of histologic specimens demonstrated that the bone tissue close to HA-coated implants was often in direct contact with the implant surface without the presence of an intervening fibrous tissue layer (Fig. 2). In contrast, bone tissue near uncoated pins did not always show a direct contact with the metal (Fig. 3).

In three cases out of 17 uncoated pins (17%) and in one case out of 19 coated pins (5%) a possible subclinical infection took place, proven by the diffuse infiltration of neutrophyl granulocytes and lymphocytes in the perimplant reaction tissue.

The mineralization of the perimplant bone tissue, analysed by means of histochemical techniques, was not altered in any of the two groups. New bone formation, looking like periostal callus, sometimes occurred at the pin insertion site (Fig. 4).

The embedding procedure did not always prove optimal, because of the incomplete resin penetration inside the specimen. The imperfect impregnation gave rise to artefacts during staining: the faulty specimens were therefore excluded from every subsequent evaluation. Slides were not considered for the

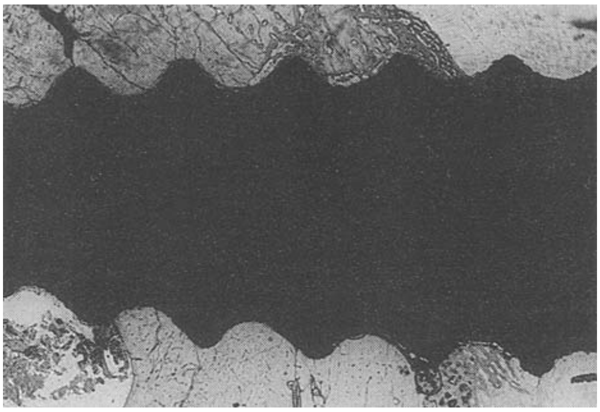

Figure 2 Transverse section of a tibia at the level of a HA-coated pin, retrieved 6 weeks after surgery. Good interface between bone and pin is clearly shown (Trichromic stain; × 1.6).

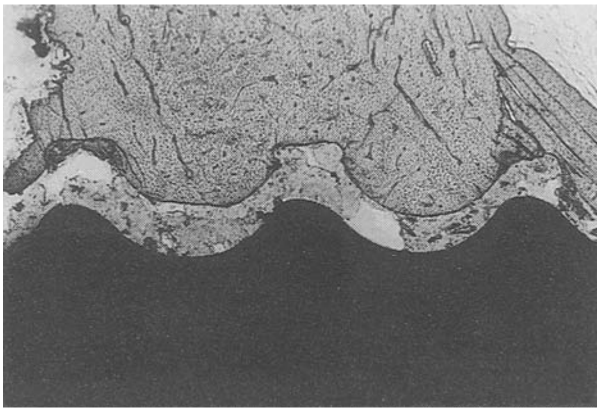

Figure 3 Section similar to the previous one where an uncoated pin has been implanted. No contact between bone and implant can be demonstrated. (Trichromic stain; × 2.5).

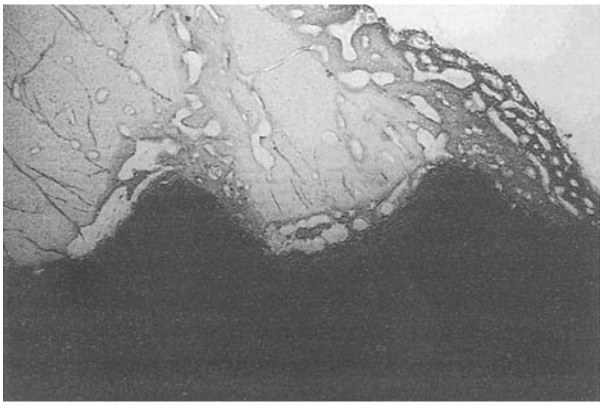

Figure 4 Periostal callus formation at the level of HA-coated pin insertion (Trichromic stain, × 6.3).

histomorphometric study if implant detachment occurred during the grinding procedures.

3.2. Morphometric evaluations

The hydroxyapatite coating appeared less compact after 6 weeks implant if compared to T 0 pins. In some cases a partial disgregation of the coating also occurred and some ceramic particles were found in the newly formed bone. However, these particles seemed not to affect regular tissue growth.

TABLE I Results of the measurements carried out on retrieved uncoated pins

Animal number	Site 3 pin		Site 4 pin	
	Bone–implant contact (%)	Bone ingrowth (%)	Bone–implant contact (%)	Bone ingrowth (%)
XIV	90	66	85	62
XV[a]	22.4	49	57.3	74
XVI	54.2	93	81	97
XVII	51.6	89	40.5	85
XVIII	38.5	71	73	86
XIX	35.9	65	59	75
XX	unsuitable sample		infected	
XXI	unsuitable sample		23.7	60
XXII	infected		infected	
XXIII	unsuitable sample		64.6	76
XXIV	unsuitable sample		35	91
XXV	unsuitable sample		18	56.1

[a] The animal died 3 weeks post-surgery

TABLE II Results of the measurements carried out on retrieved HA coated pins

Animal number	Site 3 pin		Site 4 pin	
	Bone–implant contact (%)	Bone ingrowth (%)	Bone–implant contact (%)	Bone ingrowth (%)
I	86.1	99	83.2	95
II	79.1	95	97.1	93
III	unsuitable sample		91.2	92
IV	21.8	64	46.9	93
V	unsuitable sample		85.0	100
VI	unsuitable sample		89.0	83.6
VII	unsuitable sample		87.4	95
VIII	93.8	87	94.4	100
IX	75.1	90	73.9	98
X	unsuitable sample		69.1	88
XI	infected		83.2	99
XII	85.4	95	86.8	93
XIII	51.6	3	58.2	91

The coating thickness was measured both at the interface with the femoral cortical bone tissue, and at the interface with bone marrow. In both cases the thickness was slightly greater than in unimplanted pins (52.1 μm versus 47.5 μm). Thickness at the medullary level was greater than at the cortical level of the same pin.

In Tables I and II the values of bone–implant contact and bone ingrowth of uncoated and HA coated pins are reported. As it was checked that there was no difference between results obtained on microradiographs and on slides, only the last are reported.

The data from the analyses may be summarized as follows:

- The *coating thickness* increased slightly during the six weeks of implantation; the difference was not statistically significant.
- The *contact percentage at T 0*, i.e. that obtained at the moment of insertion, was similar with coated or uncoated pins.

- The *bone–implant contact percentage at T 42* in the uncoated pins was 47.5 ± 19.4, whereas in the coated pins it was 75.6 ± 20.0: the difference was statistically significant ($p = 0.007$). Both in the case of coated and uncoated pins a decrease occurred in respect to T 0. The contact decrease was remarkable for the uncoated pins, from 87.5 to 47.5, and less significant for the coated pins, from 84.7 to 75.6.
- The *bone ingrowth percentage at T 0* in the uncoated pins was 64 whereas in the coated ones it was 97.5, clearly higher.
- The *bone ingrowth percentage at T 42* in the uncoated pins was 78.7 ± 13.5 whereas in the coated pins it was 86.6 ± 22.4; the difference was not statistically significant.
- The *proximal or distal* position of uncoated pins affected neither bone ingrowth percentage nor contact percentage in a statistically significant way (the bone ingrowth percentage of the proximal pins was 79.5 ± 13.6, and that of the distal pins was 78.2 ± 14.4; the contact percentage of the proximal pins was 44.5 ± 9.3, and 49.1 ± 23 for the distal pins).
- The *proximal or distal* position of the coated pins, in contrast, affected the results. The values obtained from the distal implants were higher than those obtained from the proximal ones (the bone ingrowth percentage of the proximal pins was 69.4 ± 39.2, and that of the distal pins was 93.2 ± 5.3 ($p = 0.03$); the contact percentage of the proximal pins was 62.2 ± 26.4 and that of the distal pins was 80.7 ± 15.2).

4. Discussion and conclusions

Some interesting conclusions can be drawn.

First of all no intense tissue reaction occurred in relation to the insertion of the ceramic coated or uncoated pins: the histological pictures were similar. The occasional presence of tiny fragments of the coating did not interfere with the mineralization of the close tissues, nor it seemed to elicit macrophage or osteoclast reactions.

The slight increase in the coating thickness observed in the pins implanted for 6 weeks with respect to the controls at T 0 is not statistically significant and therefore it is negligible. The residual coating thickness was not constant and was always thinner at the intracortical level of the implant than at the endomedullary level, allegedly because it started being resorbed in the regions undergoing more mechanical stress.

Pin–bone contact percentage recorded at T 0, a measure useful to quantify the implant fixation, was equally high both for coated and uncoated implants; both pins are indeed inserted after burring by means of gauged instruments, being therefore in the same conditions.

At the insertion time the amount of bone occurring between the threads (bone ingrowth at T 0) seemed higher in the coated pins. This datum was justified by the higher capability of the coated pins to fit to the bone they were screwed into, reducing tissue damage, as ascertained by measuring the insertion torque, which was lower in coated pins [15].

After being implanted *in vivo* for 6 weeks, the coated pins presented a higher amount of bone between the threads, even though the difference with respect to the uncoated ones was not statistically significant. The bone ingrowth percentage increased by 18% with respect to T 0 in the uncoated pins, while it decreased in the coated pins, so that the final value reached by the two groups was not statistically different.

The datum that quantifies the implant osteoconductive activity, that is bone–implant contact percentage observed at retrieval after the implant period, was higher in the coated pins.

The better osteointegration that was recorded for pins in position 4 with respect to pins in position 3 cannot be easily justified. It is probably due to the lower density of bone in that position, rather than to interfacial load transfer.

On the whole, the results match those of other authors [16–19], who observed a better bone–implant interface in the case of HA-coated devices with respect to uncoated ones, even though in the comparison between HA and other ceramics the former has not always given the best results from every viewpoint.

Transferring the results obtained in the experimental models to clinical use, the HA pin coating is likely to improve the final result of surgery when external fixators are involved, reducing failures due to pin loosening. Moreover, infective complications could decrease, possibly because of the better interface occurring between bone and coated implants [20–22].

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