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BS-SEM EVALUATION OF THE TISULAR INTERACTIONS BETWEEN CORTICAL BONE AND CALCIUM-PHOSPHATE COVERED TITANIUM IMPLANTS.

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MOTS CLES: dépôt par ablation laser, interface osseuse, couches de phosphate de calcium, expériments in vivo

RESUME

Dans les derniers temps, se sont succedés les éssais pour obtenir l'amélioration de la fiabilité du contact entre les tissus osseux et les matériaux implantaires, per la méthode de recouvrir les implants métalliques avec des matériaux céramiques, fréquemment des phosphates de calcium. Pour cet étude, les couches de phosphates de calcium ont été déposées grâce a une technique de depôt par pulsations laser. Notre but était d'évaluer les interactions qui s'établissent entre la corticale osseusse et les implants de titanium recouverts par cinq couches différentes dont le degré de cristallinité oscillait entre le phosphate calcique amorphe et l'hydroxyapatite cristalline. Ces différences étant obtenues par des altérations contrôlées des paramètres du procès d'ablation par laser. Le protocole chirurgical consistait en l'implantation simultanée des cinq types d'implants dans la diaphyse tibiale de trois chiens, qui ont été sacrifiés respectivement un, deux et trois mois après l'intervention. Les échantillons ont eté soumis à un procedé standardisé d'inclusion en polymères plastiques sans décalcification préalable, à fin deles soumetre à des études ultra structurales: microscopie éléctronique à balayage à l'aide d'éléctrons secondaires et retrodispersés (BS-SEM). Nos résultats montrent que, pour ce qui fait aux tissus calcifiés qui apparaissent comme réponse à la présence des différentes couches de couverture, aussi que pour le temps de récuperation, les implants recouverts par des couches cristallines obtenues par depôt laser présentent un résultat meilleur que ceux recouverts par phosphate calcique amorphe. Qui plus est, la présence constante de tissu chondroide, en rapport avec l'induction mécanique par les forces appliquées sur l'aire de récuperation, nous mène à suggérer que les mécanismes impliqués en l'ostéointégration sont en rapport avec les processus d'ossification membraneuse, plutôt qu'endochondrale.

ABSTRACT

The improvement of the reliability of the contact between the osseous tissues and the implant materials has been tested by recovering the metallic implants with ceramic materials, usually calcium phosphates. In our study, the calcium phosphate recovering layers were deposited by means of a pulsed-laser deposition technique Our aim was to to evaluate the tissue interactions established between cortical bone and titanium implants covered by five different layers, ranging from amorphous calcium phosphate to crystalline hydroxyapatite, obtained by altering the parameters of the laser ablation process. The surgical protocol of the study consisted in the simultaneous implantation of the five types of implants in both the tibial dyaphisis of three Beagle dogs, sacrificed respectively one, two and three months after the last surgical procedures. After the sacrifice, the samples were submitted to a scheduled procedure of embedding in plastic polymers without prior decalcification, in order to perform the ultrastructural studies: scanning microscopy with secondary and backscattered electrons (BS-SEM). Our observations show that both in terms of the calcified tissues appearing as a response to the presence of the different coatings and of time of recovering, the implants coated with crystalline calcium phosphate layers by laser ablation present a better result than the amorphous-calcium-phosphate-coated implants. Moreover, the constant presence of chondroid tissue, related with the mechanical induction by forces applied on the recovering area, strongly suggests that the mechanisms implied in osteointegration are related to endomembranous, rather than endochondral ossification processes.

INTRODUCTION

The biological and biomechanical phenomena taking place in the bone-implant interface can determine the fate, in terms of success or failure, of the bone implants employed in dentistry. Stability of the implant, the contact surface between bone and implant and the tisular interactions between bony tissues and implant components are some of the determinant factors for the viability of the implant interface.

Numerous attempts have been made in order to improve the reliability of the contact between the osseous tissues and the implant materials by means of recovering the metallic implants with ceramic materials, usually calcium phosphates. A variety of techniques has been proposed for obtaining the deposition of both thick and thin films of calcium phosphates. These include plasma spraying (DeGroot et al. 1990; LeGeros et al. 1995), hot isostatic pressing (Hero et al. 1994), sol-gel deposition (Qiu et al. 1993), biomimetic deposition (Kokubo 1997), high velocity oxygen-fuel spraying (Haman et al. 1995), electrophoretic deposition (Ducheyne et al. 1990), ion beam assisted deposition (Cui et al. 1997), electrochemical deposition (Ban et al. 1994), magnetron (Wolke et al. 1994) or ion beam (Ong et al. 1992) sputtering and pulsed laser deposition (Cotell et al. 1992; Torrisi 1993; Sardin et al. 1994; Singh et al. 1994; Bagrashtavili et al. 1995; Jelínek et al. 1995; Arias et al. 1997).

The plasma spray technique is the most widely applied in the industry. It involves heating a ceramic powder to obtain droplets in a state of partial melting, which are projected onto the metal surface by means of a gas stream. The partially molten droplets adhere to the surface and between them, at the same time that they solidify.

Plasma spraying produces multiphasic calcium phosphate coatings or monophasic calcium phosphate coatings of low crystallinity, with very little control over the composition. In order to increase the crystallinity post deposition thermal treatments have to be performed. Moreover, these calcium phosphate coatings exhibit weak adhesion to the substrate, which could provoke delamination of substantial parts of the coating layer while surgical implantation or once implanted, with the subsequent inflammation of the tissues close to the implant (DeGroot et al. 1995). They also show high porosity (25%), large grain sizes (50-100 μm) and a morphology consisting of distorted molten particles, ceramic chips, randomly distributed pores and an elevated number of cracks (Radin et al. 1992; García-Sanz et al. 1997).

Pulsed laser ablation procedures consist in an excimer laser beam which is focused onto an Hidroxylapatyte pellet inside a vacuum chamber in which water vapor is introduced. Each laser pulse creates a plasma plume comprising evaporated and particulate material. The coating reaches and accumulates on to the titanium alloy substrate, that can be heated to a fixed temperature, as it is placed directly in front of the target.

With the pulsed laser deposition technique most of the major drawbacks of the plasma spray coating techniques can be overcome. The most distinctive difference between the plasma sprayed coatings and those obtained by the pulsed laser deposition technique is their thickness. While with the plasma spray technique thickness in the range of 50-80 μm are obtained, with pulsed laser deposition typical thickness lie between 0.5 and 2 μm Commonly, the coatings obtained by the pulsed laser deposition technique are more dense, do not present great surface irregularities and the deposition process allows the control of the coating phases and their crystallinity (Fernandez-Pradas et al. 1998).

The aim of our study is to evaluate the tisular interactions established between cortical bone and titanium implants covered by five different layers, ranging from amorphous calcium phosphate to cristalline hydroxylapatite. The coating characteristics were obtained by changing the deposition parameters (type of laser, substrate temperature and pressure of water vapor) of the pulsed laser deposition method.

MATERIAL AND METHODS

Titanium alloy (Ti-6Al-4V) foils of 5x10 mm2 were coated with five different calcium phosphate coatings through pulsed laser deposition. Details on the deposition process and on coatings characterisation can be found elsewhere (Fernández-Pradas et al. 1998, 1999, 2000, Clèries et al. 2000a). Basically, deposition was performed in a vacuum chamber under a controlled water vapor atmosphere. A pulsed laser beam was focused onto a hydroxylapatite target inside the chamber. The species ejected after each laser pulse were collected on Ti-6Al-4V substrates placed in front of the target to grow the coatings. The temperature of the substrate was controlled during deposition. Different coating characteristics were obtained by changing the deposition parameters (type of laser, substrate temperature and pressure of water vapor). In this way, four type of monolayer coatings (IAMO, ICRI, IMIX and IYAG) and a bilayer coating (IBIC) were produced. X-ray diffractometry (XRD) was used to verify the phases present in the coatings. Sample characteristics are depicted in Table 1 together with their corresponding deposition parameters.

The surgical protocol of the study constisted in the simultaneous implantation of the five types of implants in the tibial dyaphisis of the experimental animals. Three male Beagle dogs, aged 24 moth, on average 18 Kg, were used. The surgical procedures were scheduled in order to optimize both the postoperatory timing and the number of animals involved. The implants were placed in the medial surface of the tibia by way of the surgical drilling of a made-to-measure cortical bed. Afterwards, the implants were recovered by the periostium. The animals were sacrificed respectively one, two and three months after the last surgical procedures.

After the sacrifice, the samples were submitted to a scheduled procedure of embedding in plastic polymers without prior decalcification, in order to perform the ultrastructural studies: scanning microscopy with secondary and backscattered electrons (BS-SEM), as previously described (Manzanares et al. 1997, Franch et al. 1998, Franch et al. 2000).

RESULTS

At 1 month

The osseous surfaces contacting the implants showed only the evidence of osteoclastic activity, by way of the presence of Howship's lacunae (arrows in Fig. 1) in the cortical surface. As expected, at this stage the osteoclastic activity was present at the cortical surface of in all the samples, unrelated to the coating characteristics or the distance between the implant and the osseous surface.

Tab. 1. *Parameters of deposition for each sample type and coating characteristics. (KrF: KrF excimer laser, Nd:YAG: Nd:YAG laser; ACP: amorphous calcium phosphate, HA: hydroxyapatite, TCP: tricalcium phosphate)*

Sample	Laser/wavelength (nm)	H_2O Pressure (Pa)	Substrate temperature (°C)	Thickness (µm)	Phases (from XRD)
IAMO:	KrF / 248	0.5	20	1.0	ACP
ICRI:	KrF / 248	45	600	1.0	HA
IMIX:	KrF/ 248	150	600	3.0	α–TCP + β-TCP
IYAG:	Nd:YAG / 355	45	600	9.0	HA
IBIC: inner layer	KrF / 248	45	600	1.5	HA
outer layer	KrF / 248	0.5	20	1.0	ACP

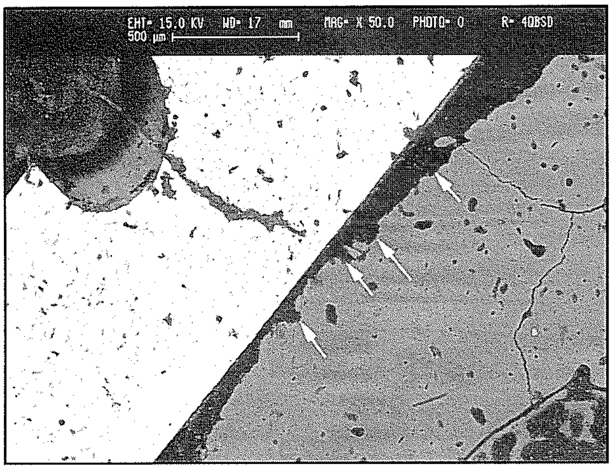


Fig. 1. *(X50) Cortical surface in contact with laser-deposited cristalline hydroxylapatite (ICRI), 1 month after implantation. Arrows: Howship's lacunnae.*

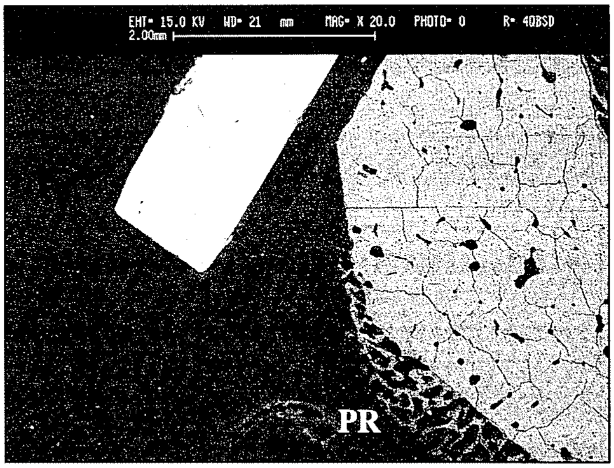


Fig. 2. *(X20) Medial angle of the cortical surface in contact with laser-deposited amorphous hydroxylapatite coating (IAMO), 2 months after implantation. PR: Periosteal reaction.*

At 2 months

The implants covered by amorphous calcium phosphate layers, either unique (IAMO) or double (IMIX), induced a poor response from the calcified tissues involved in the reparative processes. Thin trabeculae, constituted mainly by chondroid tissue and situated in the periphery of the implantation site, were the only reaction to the intervention (Fig. 2) and could be attributed to a periosteal reaction rather than a reparative process.

The implants whose covering layers were of a crystalline characteristic showed a more positive response, evidenced by the presence of denser calcified trabeculae than in Figure 2, facing the implant surface (Figg. 3- 5). The trabeculae differ in orientation: in the ICRI sample (Fig. 3), they are parallel to the implant surface, while those from the IYAG (Fig. 4) and IBIC (Fig. 5) samples are clearly directed towards the implant surface. Those trabeculae shown a core constituted by chondroid tissue, which in some cases appears surrounded by woven bone (Fig. 5).

At 3 months

The differences between the amorphous and the crystalline-coated samples seem to diminish. Even when

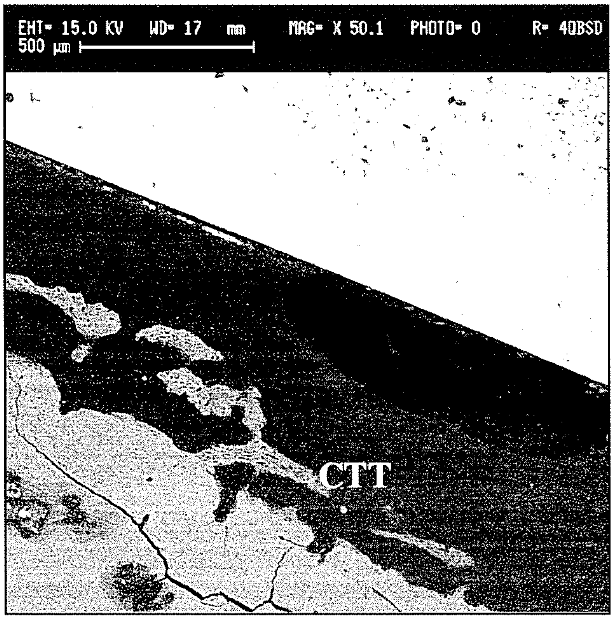


Fig. 3. *(X50) Cortical surface in contact with laser-deposited cristalline hydroxylapatite (ICRI), 2 months after implantation. CTT: Chondroid Tissue Trabeculae.*

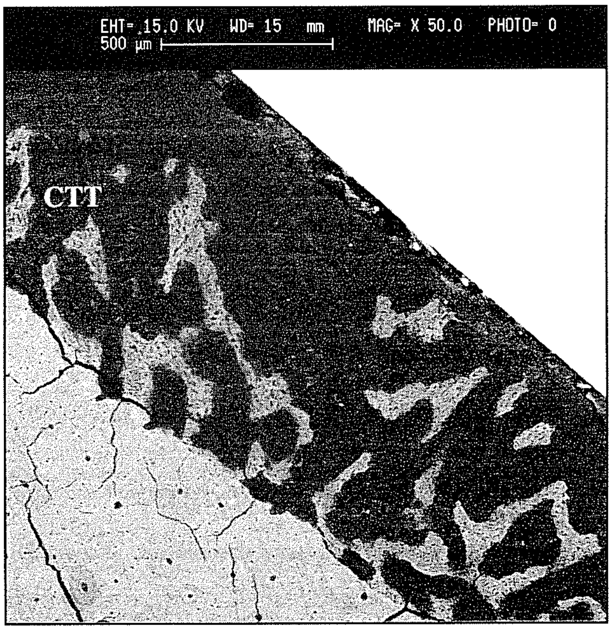


Fig. 4. *(X50) Cortical surface in contact with laser-YAG-deposited cristalline hydroxylapatite (IYAG), 2 months after implantation. CTT: Chondroid Tissue Trabeculae.*

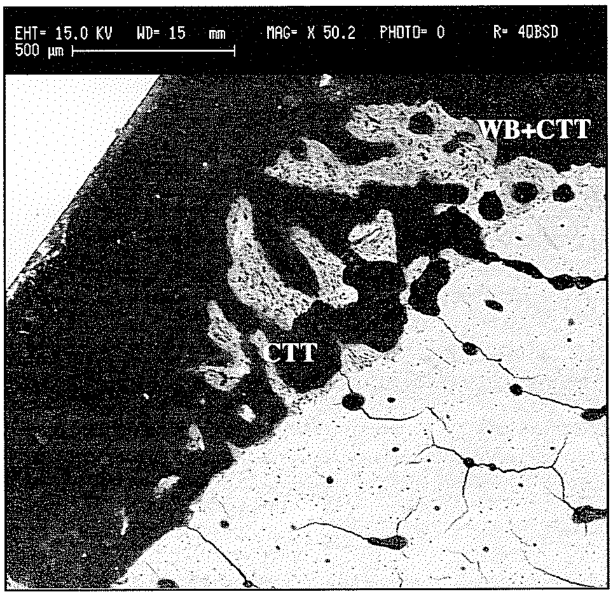


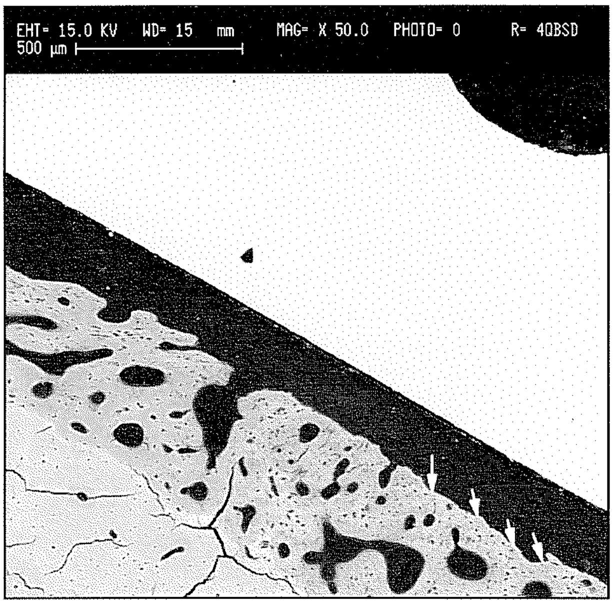
Fig. 5. *(X50) Cortical surface in contact with laser-deposited cristalline hydroxylapatite bilayer (IBIC) 2 months after implantation. CTT: Chondroid Tissue Trabeculae. WB+CTT: Trabeculae constituted by a core of chondroid tissue, covered by woven bone apposition.*

there is a distance between the cortical surface and the implant, a layer of newly formed calcified tissues appear in front of the implant. Both in the implants recovered by amorphous coatings and in those with a crystalline covering, evidences of woven bone and even lamellar bone apposition over the initial chondroid tissue trabeculae are visible (Figg. 6-8). They differ, however, in the orientation of the trabeculae. Those situated upon the cortical facing the layer of amorphous phosphates show a flat surface. Some images compatible with the aspect of Howship's lacunae may even suggest the possibility of an actively maintained distance (arrows in Fig. 6). On the contrary, the trabeculae in front of the surfaces covered by crystalline layers show a marked inclination toward the implant, as is visible in Figure 7.

However, the reactions of the calcified tissues in direct contact with the two types of layers are different. The samples recovered by amorphous layers of calcium phosphates, even when have apparently reached a good amount of contact with the bone surface (Fig 8), present at this moment an intense osteoclastic activity taking place at the contact surface.

On the other hand, Figure 9 clearly shows a high degree of contact with the crystalline-covered surfaces of the calcified tissues constituting the trabeculae. As for the composition of the trabeculae, the chondroid tissue formed initially has been substituted by woven bone, and upon it, lamellar bone apposition is also visible (Fig. 10). Moreover, the direction of the trabeculae, parallel and very near to the implant surface, proves the stability of the implant.

Fig. 6.



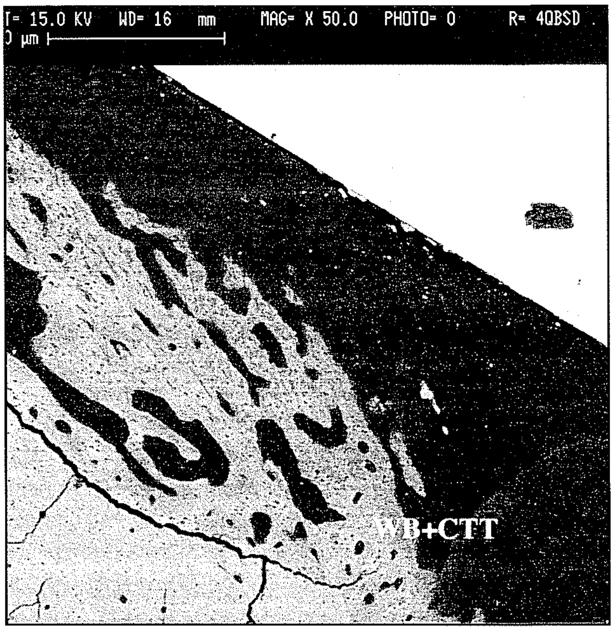
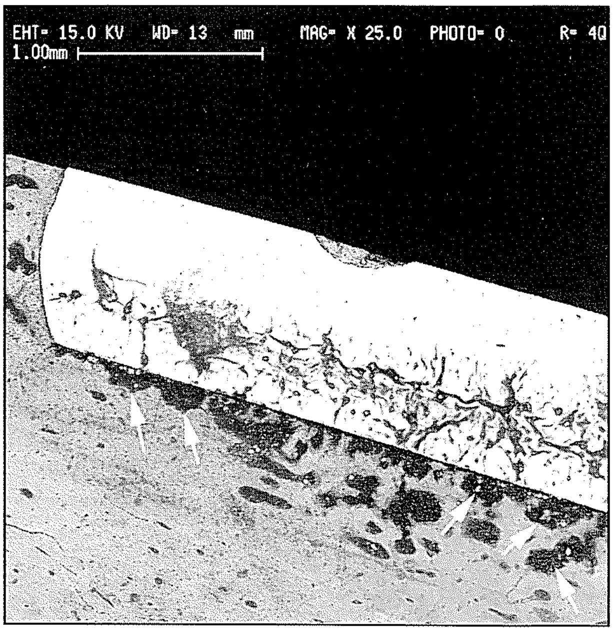


Fig. 7. *(X50) Cortical surface in contact with laser-deposited cristalline hydroxylapatite bilayer (IBIC), 3 months after implantation. WB+CTT: Dense trabeculae constituted by a core of chondroid tissue, covered by woven bone apposition, directed towards the implant surface.*

Fig. 8. *(X25) Medial angle of the cortical surface in contact with laser-deposited amorphous hydroxylapatite double coating (IMIX), 3 months after implantation. Arrows: Howship's lacunnae*



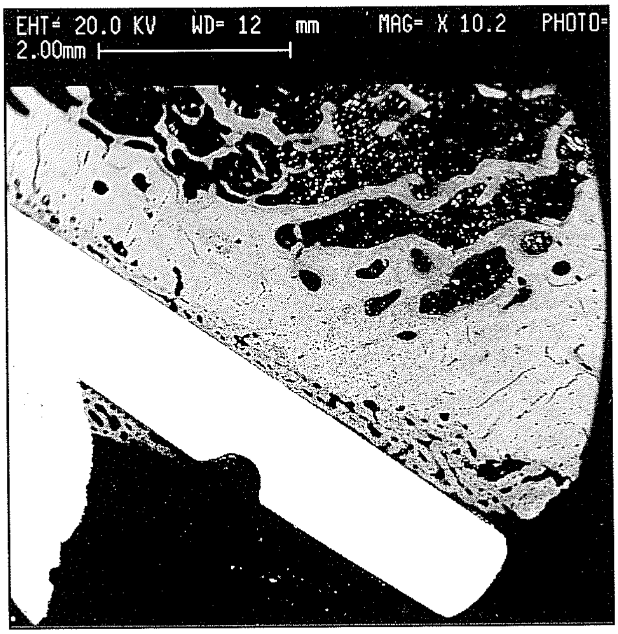
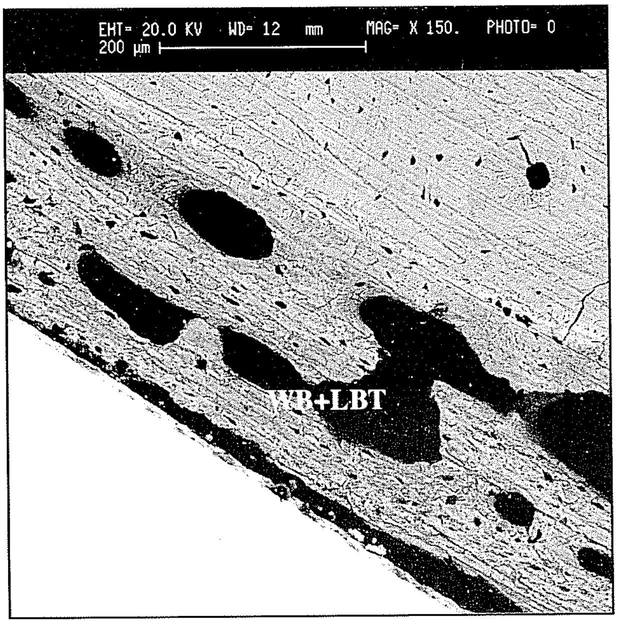


Fig. 9 *(X10) Periimplantary reaction in close contact with laser-deposited cristalline hydroxylapatite bilayer (IBIC), 3 months after implantation. Bar: 2 mm.*

Fig. 10. *(X150) Higher magnification of the central area shown in Figure 9. Newly formed trabeculae, constituted by woven and lamellar bone (WB+LBT), in close contact with laser-deposited cristalline hydroxylapatite bilayer (IBIC), 3 months after implantation.*



DISCUSSION

Osseointegration, as defined by Albrektsson et al. (1981) has been considered as a direct contact between the implant and living bone tissue. Recent studies have shown that the tissue responses to implants can have different patterns of bone formation, related to the nature of the recipient bones and the dimension of the gap between the implant and the osteotomy margin (Futami et al. 2000, Hirai et al. 2001). All those studies, however, stated the presence of osteoclasts in the initial stages of the bone remodeling process around the implant, in agreement with our results.

As for the implants covered with a bioceramic layer, there is no need for a thigh contact with the bone surface, since these materials are known to be osteoconductive (Clèries et al. 2000b, Dostálová et al. 2001). Consequently, they are able to attract the bone to cross the space between the implant material and the bone bed surface (Clemens et al. 1997). Our results are in agreement with the literature data: all the laser-deposited ceramic layers covering the titanium surfaces are osteoconductive, unrelated to their amorphous or cirstalline characteristics. As described by Benhayoune et al. (2000), osteoconductivity allows the bone formation in spaces in which there was no bone present previously.

The degree of osteoconductivity is, however, different between the crystalline and the non-crystalline layers. As is also described by Handschel et al. (in press), in our experiments Tricalcium phosphate (TCP) layers, either unique (IAMO) or double (IMIX) have shown a less favorable response as a covering surface under conditions of non-loading. On the other hand, the implants with a crystalline laser-deposited hidroxylapatite coating (ICRI and IYAG) show a better osteoconductivity, proven by the direction of the trabeculae and the maturity of the calcified tissues constituting the trabeculae. As expected, the best results were those obtained with a double-layer coating (IBIC), probably related to the more adapted response of the different material phases to the subsequent stages of the implant osteointegration (Benhayoune et al. 2000).

At a long term, and also in agreement with the more recent literature (McMillan et al. 2000, Futami et al. 2000, Dostálová et al. 2001, Gotfredsen et al. 2001, Hirai et al. 2001), our samples shown that there is new bone formation around the implants. This new bone formation, which follows the same stages that we previously described for the closure of the skull sutures (Manzanares et al. 1988) and the fracture healing (Franch et al. 1998) is started by the formation of chondroid tissue. The role of chondroid tissue in the first stages of both the fracture healing and the implant osteointegration should be related to its mechanic induction by forces applied upon the areas undergoing growing or reparative processes (Goret-Nicaise 1986, Goret-Nicaise et al. 1988, Manzanares et al 1988, Lengelé 1997, Franch et al 1998). Moreover, the presence of the chondroid tissue as an active participant in the osteintegration strongly indicates that it the ossification mode implicated in this process is intramembranous ossification, rather than endochondral ossification (Goret-Nicaise et al. 1988, Manzanares et al. 1988, Lengelé, 1997, Futami et al. 2000).

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