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Interface Bone-Implant with an Osteoinductive Treatment

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Introduction: Biomaterials are recognized to lack osteoinductive properties without additional osteoinductive agents, such as bone morphogenetic proteins (BMPs). Recent reports indicate that certain calcium phosphate coated biomaterials have osteoinductive properties when they exhibit a specific porous architecture. These biomaterials promote bone growth at extraskeletal locations without the need for additional osteogenic cells or BMPs.

Titanium is considered a bio-inert material and is used for scaffolds when infused with BMP to stimulate ectopic bone growth. Titanium can be converted into an osteoconductive substance through certain chemical and thermal processes. This bioactive titanium exhibits exceptional apatite formation capacity in vitro and can attach directly to living bone in vivo.

The objective of this study is to analyze the "in vivo" behavior of a titanium alloy implant, subjected to a surface chemical treatment and to determine and evaluate the osteoinduction at the implant-bone interface.

Experimental: The material used for the manufacture of the implantable plates is commercial Ti6Al7Nb that has been subjected to a chemical treatment (immersion in 10M NaOH for 60 hours, dried in air, heated at 600^{a}C with a rate of 5 grades/min and then kept at this temperature for 1 hours and then dried in oven) and subsequently implanted in the tibia of the animal.

The animals that participate in this experiment come from the only national center in charge of breeding and supplying minipigs, the University of Córdoba, where they have a Centralized Service for Experimental Animals. Prior to transfer, the pigs undergo tests, presenting negative serology and the corresponding certificate of animals free of any infectious-contagious disease. These are transferred, in an approved and closed enclosure, to the Bioterium of the Negrín Research Unit after the consent of the Department of Livestock of Las Palmas.

Once the animals have been sacrificed, the implanted bone has been cut and the bone-implant sample has been embedded into resin and analyzed under a FE-SEM microscope (see Fig.1).

Results and discussion: The analysis of the implant-bone interface (osseointegration, osteoinduction, degree of toxicity) after implantation was carried out with the aim of demonstrating that a non-soluble material without Ca and P content, such as the Ti6Al7Nb alloy, can be an osteoinductive material if the surface is treated to have an appropriate microstructure and nanostructure (the most relevant data referring to one of the animals will be presented).

Histological analysis of the bone-implant interface of the samples showed good osseointegration, with close contact between the surface of the implant and the bone, and structures with osteons, osteocytes and Haversian canals typical of bone tissue being observed. It can be seen that the local bone response to the presence of the titanium alloy implants was adequate and rapid, with the differentiation of fibrous tissue into bone tissue beginning from the early stages.

We have generated compositional images in order to observe inorganic materials present in the organic material, that is, the chemical microanalysis performed allows us to obtain the percentage by weight of the elemental chemical composition in a semi-quantitative way. In this way we can also find out if there are elements in the bone-implant interface, not common in the animal organism, that could have a high toxic potential, such as aluminum.

Chemical microanalysis was performed to study the % by weight of the elemental chemical composition of a bone-tissue sample subjected to EDS testing. There are two well-differentiated areas (see Fig.2), a smooth area corresponding to tissue and a more abrupt area that coincides with bone formation.

A random point in the area corresponding to tissue was analyzed and the data reflect the presence of an organic tissue (composition of C and O).

A random point in the area corresponding to bone formation was analyzed and the data reflect a chemical composition that identifies the presence of Ca and P. At this point, it is necessary to identify the mineral phase of hydroxyapatite in the bone, so the Ca/P ratio in the sample must be obtained and compared with that of hydroxyapatite, with a value equal to 1.67.

In this case, the value of the actual ratio is 2.17, higher than the theoretical ratio, so it is within the accepted values. Therefore, there is bone formation at the point analyzed and the presence of c and o is normal because they are the main elements of organic structures.

Studies by Ducheyne and colleagues [1] claim that the stoichiometric value of hydroxyapatite is 1.67. However, more recently Zaichick and Tzaphlidou [2] have attributed to hydroxyapatite a stoichiometric value of 2.16, similar to that obtained in our study, which implies bone growth.

The analysis of the bone-implant interface was carried out in different contact areas, collecting points from the bone part, the bone-metal interface and the metal. Two parts can be clearly distinguished, which correspond to the metal area of the implant and the area corresponding to the bone and organic tissue.

In order to reasonably interpret the results obtained in the scan analysis, three parts must be distinguished: Zone 1: Bone formation (0 mm-0.5 mm), Zone 2: Interface (0.5 mm-0.6 mm), Zone 3: Metal implant (0.6 mm- 1.4 mm). In Fig.3 which corresponds to the scan performed on the sample, the proportion of each microelement in the area where it is located is determined. The high carbon values are due to the degradation that the organic tissue undergoes during the cutting of the sample.

Conclusions:

- The histologies obtained by optical microscopy from the samples of the experimental animals reflect a good osseointegration and osteinduction of the implants with the surface treatment to which they were subjected.
- The analysis by scanning electron microscope and EDS analyzer of the bone-implant interface of the samples indicate that there is no presence of potentially toxic elements (aluminium) belonging to the implant alloy.
- The study of the bone-implant interface of the samples of the experimental animals reflects that elements belonging to the mineral part of the bone tissue (calcium, phosphorus) have been found. The average of the results obtained from the Ca/P ratio that have been analyzed for each animal is higher than that of hydroxyapatite, so we can affirm that there is bone growth.

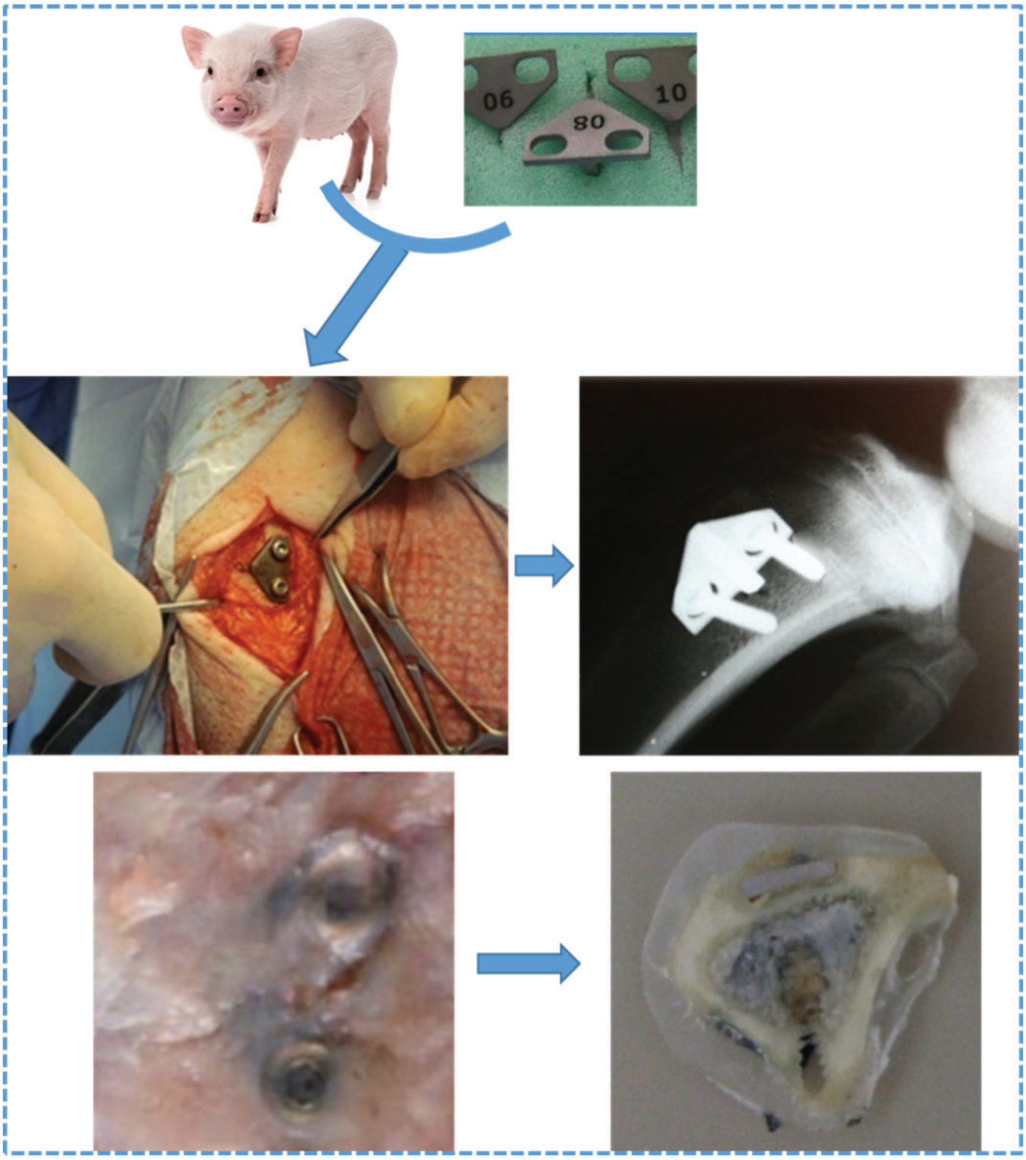
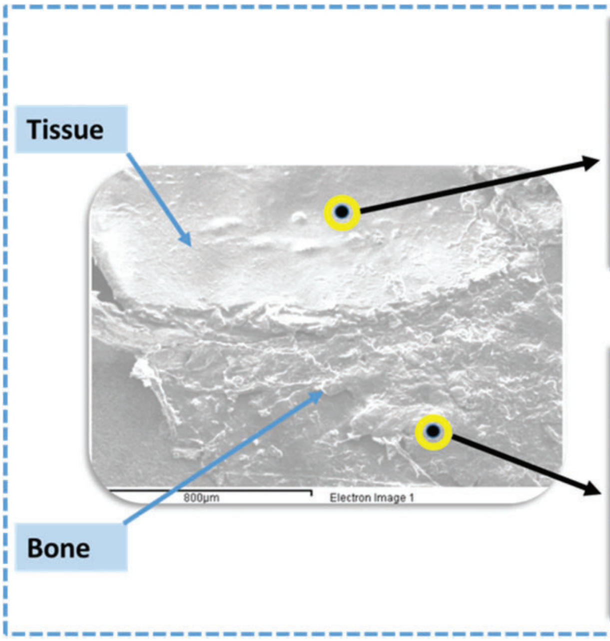


Fig. 1. The pig was implanted with a tibia plate and after 6 months from operation, the samples bone-implant were obtained for analysis. Reparation of the samples for study.



Element	Weight %	Atomic %
C K	77.55	82.92
O K	20.50	16.45
P K	0.00	0.00
Ca K	1.96	0.63
Totals	100.00	

Element	Weight %	Atomic %
C K	30.85	42.97
O K	42.62	44.56
Na K	1.35	0.98
P K	7.93	4.28
Ca K	17.25	7.20
Totals	100.00	

Fig. 2. Tissue-bone interface analysis

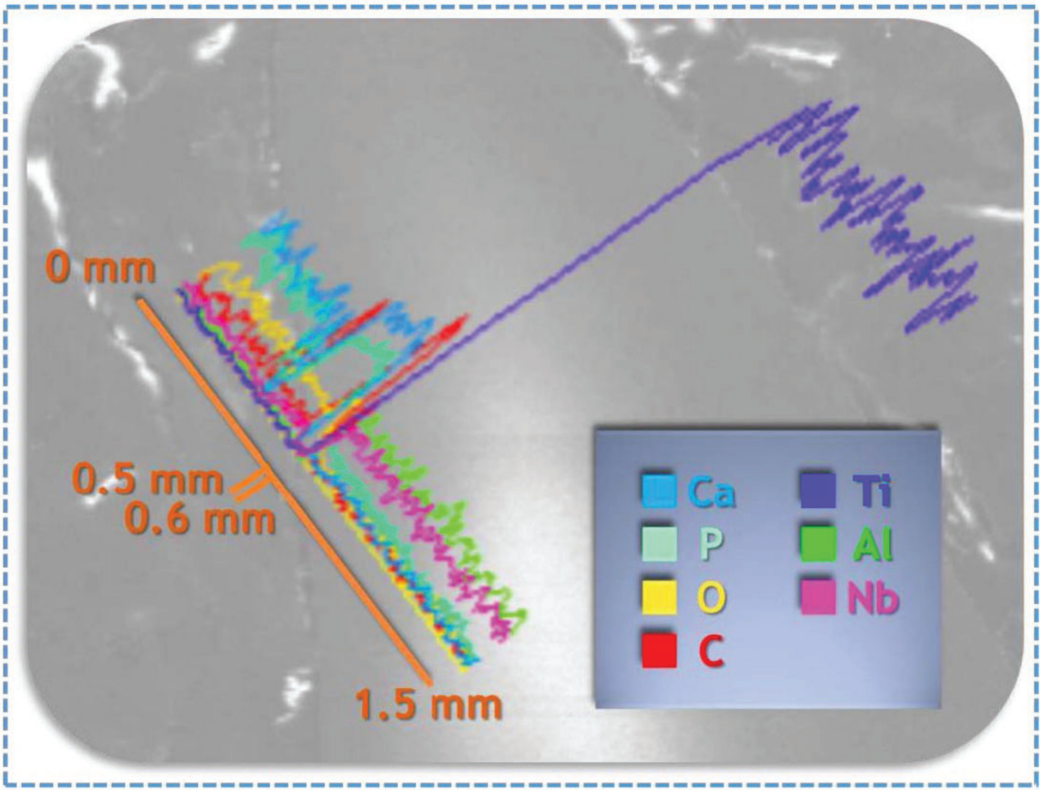


Fig. 3. Tissue-implant interface analysis

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