A Histomorphometric Study of Tissue Interface by Laminar Implant Test in Rats

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Study of the implant-tissue interface is one of the fundamental issues in implantology, both odontologic and orthopedic. The characteristics of this interface will influence the success or failure of an implant. The aim of the present study was to evaluate histomorphometrically the capacity of different metals to osseointegrate employing laminar implants of zirconium, titanium, aluminum, and zirconium coated with diamond-like carbon. The experimental model herein allowed for the quantitative evaluation of the tissue-implant interface for different metals. The implants were placed in the tibiae of Wistar rats under anesthesia and allowed to remain in situ for a 30-day period. The interfaces of the zirconium and diamond-like coated zirconium implants exhibited better responses than the interface of titanium implants. Aluminum produced a local toxic effect, evidenced by osteoid formation.
(Int J Oral Maxillofac Implants 1999;14:565–570)

Key words: biocompatibility, experimental implants, histomorphometry, interface, osseointegration

One of the most important features of an implant is that it will be in contact with the living tissues of the body, thus creating an interface between itself and the tissue. This interface is of vital importance, since it will determine the success or failure of the implant. When implants are placed into the body a reparative process begins. During the healing phase, several local and/or systemic factors may influence the degree of bone apposition on the implant (osseointegration), eg, implant biocompatibility, surface condition, implant material, cleanliness, surface activation, surface topography, and systemic alterations, among others.[1–11]

In a previous experimental study,[12] a standardized method was developed to obtain numerical values indicative of histocompatibility of metal implants as a function of time during the healing stage. The aim of the present study was to comparatively analyze the biocompatibility and the biomineralization capacity of unloaded metallic implants of zirconium (Zr), titanium (Ti), aluminium (Al), and zirconium coated with diamond-like carbon (DLC) during the healing period. Aluminium implants were used to evaluate the possibility of local toxicity. Toxicity is considered possible on the grounds that Al is deposited on the ossification fronts, and that the Al ions are toxic for osteoblasts, as occurs, for example, in renal osteodystrophy and, as a result of dialysis, aluminum-containing water.[13]

Evans et al[14] demonstrated that diamond-like carbon coating of Ti alloy surfaces made them resistant to corrosion. The biocompatibility of DLC coating was investigated in vitro by cell culture techniques.[15] However, in vivo investigation of the osseointegration capacity of this coating has not been performed to date.

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Materials and Methods

Implants. The implants used in the study measured 7 mm in length, 1 mm in width, and 0.1 mm in thickness. The Zr implants were exposed to the following preparatory procedures: *(1)* washing with a solution of nitric acid, fluorhidric acid, and distilled water (4.5 mL, 0.3 mL, and 5.2 mL, respectively); *(2)* rinsing with distilled water; and *(3)* air drying.

The Ti (commercially pure grade 2) implants were treated according to Implant-Vel (Buenos Aires, Argentina). Briefly, the procedure consists of washing with acid solutions, followed by rinsing with distilled water, air drying, and sterilization with gamma radiation.

The Al metallic implants were washed in alcohol and air dried.

The DLC implants were treated as follows: *(1)* the amorphous films of the diamond-like carbon coating were approximately 1 μm in thickness; *(2)* thin DLC films were deposited using mass separation employing a vacuum chamber (ISOL 100 kV isotope separator of the Scandinavian type in the NAVE line of Buenos Aires Tandem TANDAR Accelerator) and CH^{+4} ion beams of energies between 1 and 30 keV; and *(3)* the films were characterized by Raman Spectrometry (Jarrel-Ash 25-300 spectrometer, Fisher, USA).[16]

Surgical Procedure. Forty male Wistar rats 30 days old and weighing 90 g were grouped into 4 sets of 10 animals. Each set was implanted with Zr, Ti, Al, and DLC implants, respectively. Under intraperitoneal ethyl urethane anesthesia (1 g per kg of body weight), the implants were placed in the tibiae, following the atraumatic surgical technique previously described.[12]

The animals were sacrificed 30 days postimplantation by ether overdose. The tibiae were resected and fixed in 20% formalin solution. Radiographs were taken and the tibiae were then processed for embedding in methyl methacrylate resin. With a fine saw, 3 slices were cut perpendicular to the major axis of the tibia at the middle of the implant and at 2 points equidistant from the middle. The cross sections were ground, first using a grinding machine and then manually with sandpaper to obtain sections about 30 to 50 μm thick; they were then stained with Von Kossa and Masson's trichrome stains.

Histomorphometric Analysis. Histomorphometric measurements based on standard stereologic methods[17] were performed using a semiautomatic image analysis system (Kontron MOP AM 03, Carl Zeiss, Jena, Germany) on tracings obtained from projections of the sections. The following histometric determinations were carried out: *(1)* osseointegrated tissue thickness (OTTh), *(2)* percentage of direct bone-to-implant contact, and *(3)* osseointegrated tissue volume (OTV).[12] To obtain OTTh, 6 values were obtained for each projection and averaged to provide a single value of OTTh per section. Finally, the mean value was obtained for the 3 sections of each tibia. To evaluate percentage of direct bone-to-implant contact, the percentage of the total perimeter of the implant in direct bone-to-implant contact was determined. To measure OTV, the local volume of osseointegrated tissue was evaluated.

Statistical significance was determined by Student's *t* test. Statistical significance was considered to have been reached if $P < .05$.

Results

At the end of the experimental period, macroscopic observation revealed that wound healing was occurring satisfactorily in all instances and that all implants remained in situ in the diaphyseal area as revealed by roentgenographic observation. Analysis of the histologic sections revealed that at 30 days postimplantation, bone formation could be seen on the surface of the metal strip in all the animals (Figs 1 to 3) except in those implanted with aluminum. Analysis of the histologic sections of Al implants revealed that in some cases, osteoid deposition (Masson's stain and Von Kossa stain) was present on the surface of the metal and that in others, only bone marrow was in contact with the metal. There was no occurrence of macrophages or related inflammatory cells in any interface region.

Histomorphometric Analysis. Osseointegrated tissue thickness was greater in Zr (3.3 ± 0.3 mm) and DLC (3.8 ± 0.5 mm) implants than in Ti (2.46 ± 0.46 mm) and Al (2 ± 0.4 mm) implants ($P < .001$) (Table 1). The percentage of direct bone-to-implant contact did not show differences among Zr, Ti, and DLC implants, but with Al implants it was significantly lower (Table 1). Osseointegrated tissue volume was greater with Zr and DLC implants than Ti and Al implants ($P < .001$) (Table 1).

Discussion

The laminar implant test used in this project offers a simple, quantitative, and inexpensive method for objectively evaluating the first stage of bone healing in contact with different implant materials. Bone tissue responds to the action of local and systemic toxicity, fundamentally altering osteogenesis

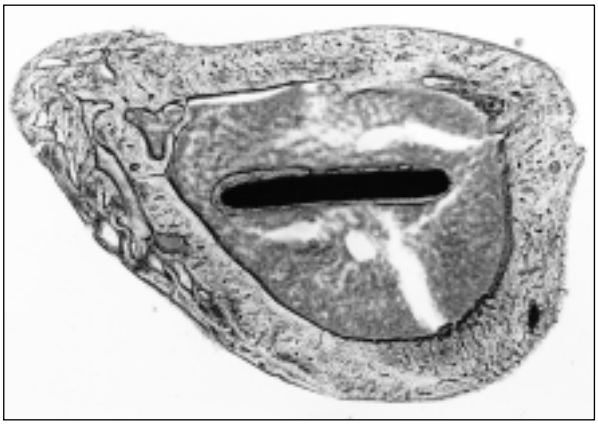

Fig 1a Low-power magnification of a titanium implant showing apparent osseointegration through the marrow space of rat tibia (original magnification ×25).

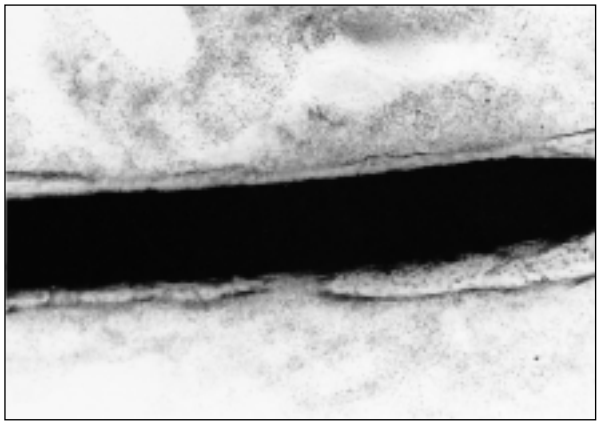

Fig 1b Ground section showing close bone apposition to titanium implant (original magnification ×100).

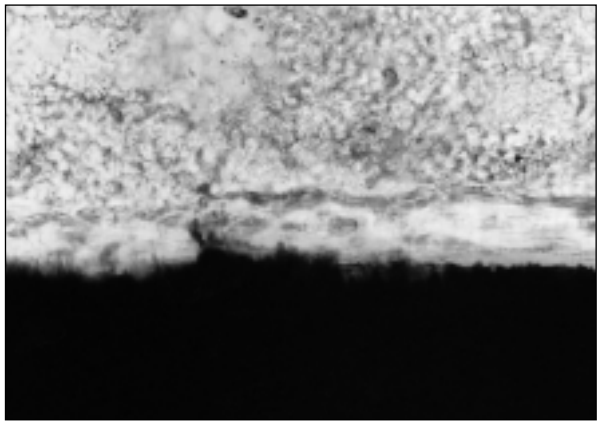

Fig 1c Ground section showing close bone apposition to titanium implant (original magnification ×400).

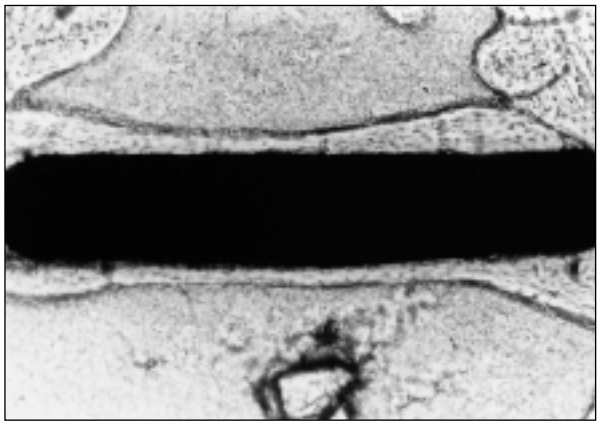

Fig 2a Zirconium implant is tightly surrounded by newly formed bone (original magnification ×100).

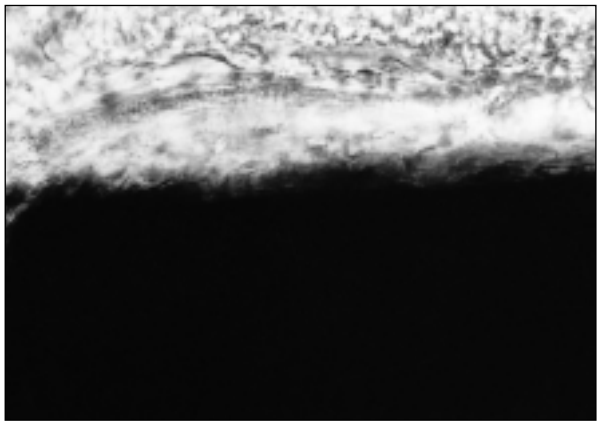

Fig 2b Notice the close bone apposition to the zirconium implant (original magnification ×400).

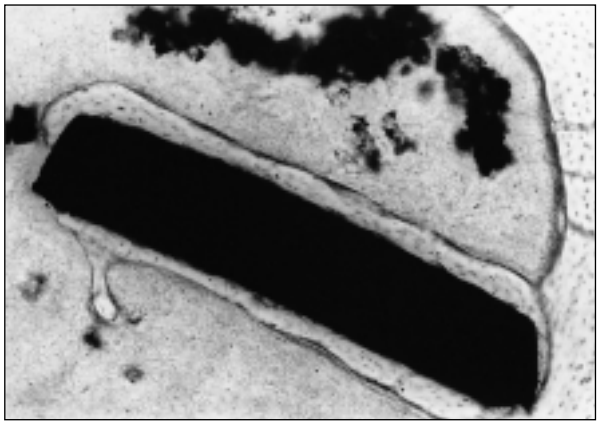

Fig 3a Zirconium implant coated with diamond-like carbon showing close bone formation on the surface (original magnification ×100).

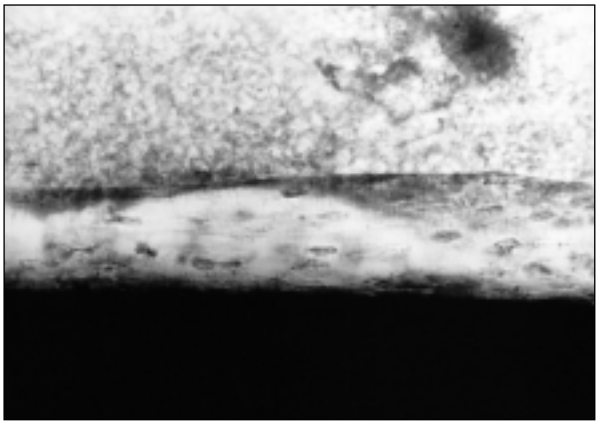

Fig 3b At higher magnification the intimate contact and the full congruency at the level of the bone-implant interface is evident (original magnification ×400).

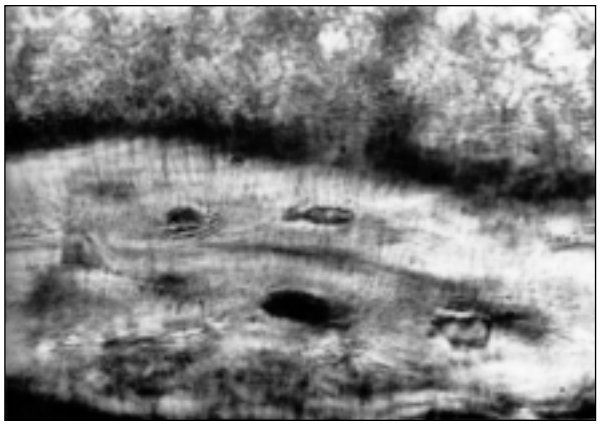

Fig 3c Higher magnification, showing intimate contact between bone and implant (original magnification ×1000).

Table 1 Histomorphometric Analysis of Zr, Ti, Al, and DLC Implants 30 Days Postinsertion

Type of Implant	Osseointegrated tissue thickness (mm)		Percentage of direct bone-to-implant contact		Osseointegrated tissue volume (mm^2)	
Zr (n = 10)	3.3 ± 0.3	B	91	B	1200 ± 70	B B
Ti (n = 10)	2.46 ± 0.46	A A B	90	A B	820 ± 213	A B
Al (n = 10)	2.0 ± 0.4*	B B	44*	A B	420 ± 120*	B B
DLC (n = 10)	3.8 ± 0.5		91		1200 ± 220	

A = not significant; B = $P < .001$.
*Osteoid tissue.

and mineralization.[18–23] Albrektsson[24] reported finding significant differences between Ti and titanium-aluminum-vanadium alloy when attempting to remove implants after 3 months. Implants made of commercially pure titanium were always more difficult to remove. Johansson et al[25] demonstrated that leaked Al may compete with calcium during the calcification process, resulting in a local type of "osteomalacia."

In the present study, the response of bone marrow with osteogenic capacity in contact with an Al implant was evaluated. Aluminium might have a local toxic action, inhibiting mineralization, and in other situations it might produce a direct toxic effect on the preosteoblasts, impairing osteoid deposition. This experimental model of aluminium implants could be used to evaluate the protective or anticorrosive effect of coating surfaces, such as DLC. Another significant feature of this study involves the proportion of aluminium in alumina coating implants. Stea et al[26] examined the behavior of bone tissue close to the alumina coating in cementless hip prostheses that were removed because of pain. The presence of osteoid deposition parallel to the prosthesis profile was detected. This phenomenon was attributed to the presence of Al ions, similar to what happens in osteomalatic osteodystrophy in nephropatic dialysed patients.

The present experiment demonstrated that the formation of bone around Zr, Ti, and DLC implants was similar. However, the Zr and DLC implants showed increased osseointegrated tissue volume and osseointegrated tissue thickness, as compared to Ti implants. It would be important to determine whether the difference in the osseointegration response persists over longer periods of time. Albrektsson et al[27] have previously indicated that Ti and Zr were well accepted in the body, as indicated by the lack of adverse tissue reactions to the implants.

The interface between living tissues and different biomaterials used for metallic implants is complex because of the variable concentrations of liquids, tissues, gases, and, eventually foreign substances. These factors, alone or associated, could determine metal corrosion, one of the major causes of failure of metal implants.

Conclusion

The results described here indicate that, in this model, the DLC coating does not interfere with osseointegration, providing an increase in resistance to corrosion of the material and leading to barrier formation, which would circumvent different types of ion interactions. The strength of adhesion of DLC to the various substrates is of prime importance. Diamond-like carbon tends to adhere strongly to various metals used in bioengineering. This coating could protect such materials from attack by the biologic environment and could protect that environment from products that would leach from implanted materials.[14] The results of this research indicate that the laminar implant test may constitute an appropriate model to quantitatively evaluate the biologic response to different implant materials.

Acknowledgments

This research was supported by a grant from the University of Buenos Aires (Grant OD-09). Our thanks are extended to H. Mendoza of the Department of Oral Pathology, Faculty of Dentistry, University of Buenos Aires, for his technical assistance and to M. A. R. de Benjacar, H. Huck, A. Jech, and E. B. Halac of the Department of Physic, TANDAR, National Atomic Energy Commission, for preparing the diamond-like carbon coating.

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