An experimental study in X-ray spectroscopy of the zirconium (Ca-PSZ) – bone interface. Microanalytic evaluation of the osteogenetic response

M. MATTIOLI-BELMONTE, P. MENGUCCI*, N. SPECCHIA#, G. GOBBI*, S. DUBINI, L. SIMONELLI**, F. GRECO‡, G. MAJNI*, G. BIAGINI and C. RIZZOLI**
Institute of Normal Human Morphology, Faculty of Medicine, Via Ranieri-Montedago, 60131 Ancona, Italy
** Department of Material Science and ‡ Orthopaedic Clinic, University of Ancona*
 # Orthopaedic Clinic, Catholic University of Rome, Italy
*** Institute of Histology, University of Bologna, Italy*

Ultrastructural difractometric and chemical evaluations of calcium partially stabilized zirconium (Ca-PSZ) implants were performed in an *in vivo* study on animals in order to evaluate its biological behaviour. The chemical–morphological investigations demonstrated the presence of an osteogenetic activity at the bone–biomaterial interface. The new-osteogenesis was preceded by the formation of a loose connective tissue around the implants. This mesenchymal-type tissue without a capsular organization, allowing modulation of the mechanical forces to which the implant is subject, could be considered a positive event in the osteogenetic process and not a sign of future failure of the implant. Finally, microanalytical investigations carried out on non-implanted and implanted Ca-PSZ tools suggested that the surface of this ceramic material does not undergo modification once it has been inserted in the biological environment (12 months).

1. Introduction

Technological innovation and the availability of new materials have encouraged the widespread use of osteosynthetic and prosthesic supports. The materials used in the orthopaedic field have mostly been metals and polymers, and the problems encountered have been of two main types: mobilization of the implant and sensitivity to the material used. These side effects have led researchers to develop new classes of biomaterials.

The use of ceramic materials in orthopaedics appears to be relatively recent, yet these materials have been used for some time in dentistry [1, 2], ear, nose and throat surgery [3, 4], ophtalmology and for vascular prostheses [5]. The choice of these materials for orthopaedic applications is mainly due to the fact that they release very few ions into the physiological environment and that they suffer very little wear and tear and have a very low friction coefficient compared to that of ultra-high molecular weight polyethylene. (UHMWPE), for example [6].

The most commonly used ceramic in orthopaedics has been alumina, due to its tried and tested biocompatibility [7], and at present it is widely employed in load-bearing prostheses (knee and hip replacement). However, in spite of the fact that the industry is capable of producing alumina implants with a high degree of purity, there are still numerous failures related to the poor mechanical properties of this material [8, 9].

Garvie *et al.* [10] therefore proposed the use of partially stabilized zirconium oxide (PSZ) because of its superior mechanical qualities. For example, zirconium oxide partially stabilized with yttrium oxide (Y-PSZ) presents an inferior Young's module, greater flexibility [11], and greater resistance to fracture with a lower level of wear and tear [12]. These factors make it mechanically suitable for orthopaedic applications [13]. There is, however, a degree of controversy about the possible deterioration in its mechanical properties *in vivo* with time; this is almost entirely related to a transition phase [13, 14]. Zirconium oxide exists in three crystalline phases: monoclinic, tetragonal and cubic, where the cubic one is stable but extremely fragile. The tetragonal crystalline phase is very resistant but unstable and tends to transform into the monoclinic phase. The transformation can be induced by pressure and/or tension and causes a release of energy and the formation of a transitional state whose mechanical properties are superior to those of the tetragonal phase, but which is only stable for a short time [15, 16]. These factors raise doubts as to the

0957–4530

suitability of zirconium oxide for use in orthopaedics, especially as regards its use as a material for load-bearing prostheses. We therefore decided to study the biological behaviour of a zirconium oxide partially stabilized with calcium oxide (Ca-PSZ) using *in vivo* research on experimental animals aimed at evaluating local reactions through ultrastructural, diffractometric and chemical assays.

2. Materials and methods

2.1. Animal experiments

Small cylinders of zirconium oxide (Ca-PSZ) were cleaned in an ethanol filled ultrasonic cleaner, sterilized by autoclave and implanted under general anaethesia in the methaphyseal bone of male New Zeland rabbits in sterile conditions. The animals were divided into four groups and sacrified at 1, 6 and 12 months after implant operation.

2.2. Light microscopy

The samples were fixed in 4% formalin in 0.1 phosphate buffer, pH 7.4 for 12 h. Specimens were then decalcified in 4N formic acid for 4 weeks, embedded in paraffin, cut into 6 μm thick sections and stained with Azan-Mallory's tricromic stain.

2.3. Scanning electron microscopy (SEM) and microanalysis

The specimens were cut into 400 μm thick sections using a diamond-bladed circular saw (Buehler, Germany), and the surface was cleaned using abrasive papers. The samples were then fixed in 2% glutaraldehyde in a cacodilate buffer, post-fixed in osmium tetroxide in the same buffer and dehydrated in an ascending series of alcohols and using critical point drying (CPD). The specimens were then mounted on stubs with colloidal graphite, covered with gold and observed using a SEM, Philips XL20.

For microanalysis the sections were treated with an analogous process to that for SEM. They were then coated with carbon for vacuum evaporation and observed using a SEM Philips XL20 equipped for X-ray microanalysis (EDS). The following parameters were kept constant during the semiquantitative analysis: voltage 25 KV; magnification $\times 400$, counts per second (cps) not less than 2000, tilt angle 15°, count time 600 s. The values realting to K_α of each elements were considered and the calculation was made using the ZAF (Z = atomic number; A = absorbtion; F = fluorescence) method. The centre of the implant, the centre of the bone and the interface zone were all investigated in each sample analysed. Furthermore, a bar of pure Ca-PSZ which had not been implanted was submitted to an analogous investigation.

2.4. Chemical analysis

The joint was extracted mechanically from the portion of the sample not used for the ultrastructural investigations. The specimens were then incinerated in a platinum crucible until they arrived at a constant weight at 600 °C. The ashes were then weighed and dissolved in 10 ml of pure water until no more fumes were given off. After adding 0.5 ml of HF at 60% and checking that there was no undissolved material left, all the samples were brought up to standard volume in 25 ml flasks in FEP. The solutions obtained were analysed at two different ICP wavelengths ($\lambda = 343.82$ nm and $\lambda = 349.82$ nm) to avoid, as far as possible, the risk of spectral interference caused by the presence of iron. Analyses at the spectral level carried out before the quantitative assays showed an almost total absence of interference.

The calibration curve was obtained using standard solution of pure zirconium at 0.5, 1 and 1.5 gm/l.

3. Results

3.1. Light microscopy (at 1 year after surgery)

As already described, the light microscope analysis showed active remodelling of bone around the implant. The newly formed osseous tissue presented a homogeneous distribution around the implant and had the characteristic of compact lamellar bone (Fig. 1).

3.2. Ultrastructural analysis

Morphological investigations carried out with the scanning electron microscope (SEM) showed how, at *1 month*, the growth of the bone around the ceramic material was preceded by the appearance of a tissue of mesenchymal origin at the bone–implant interface, however, without a capsular organization (Fig. 2). This tissue was still found, in smaller quantities, in the sample taken *6 months* after surgery. At that time the bone surrounding the implant appeared to be well structured morphologically, in the presence of areas of bone marrow (Fig. 3). *One year* after surgery, the Ca-PSZ surface was in continuity with the bone surface at several points. By this time the bone surface had

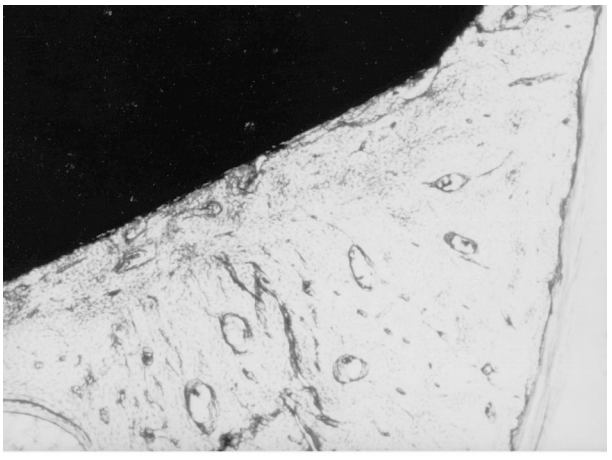


Figure 1 Light micrograph of Ca–PSZ inserted in rabbit tibia. At 1 year bone growth, integration of the ceramic and active bone remodelling can be seen around the implant (original magnification $\times 250$).

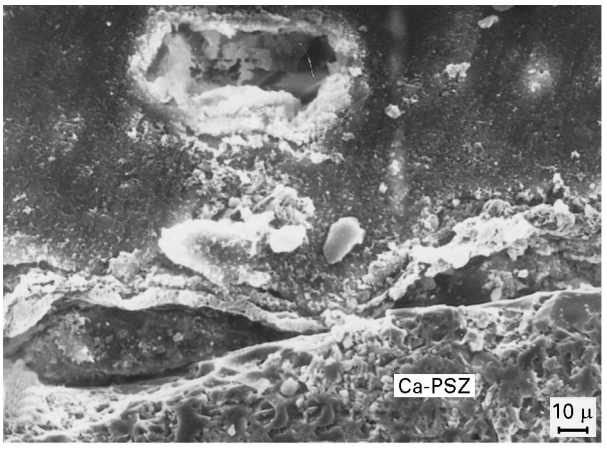


Figure 2 Scanning electron micrograph of bone–implant (Ca–PSZ) interface 1 month after surgery.

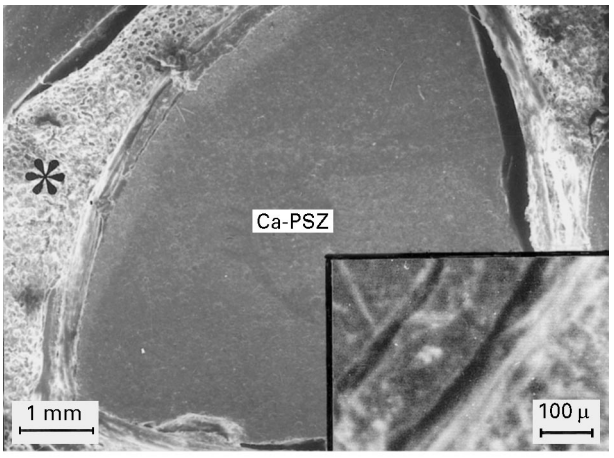


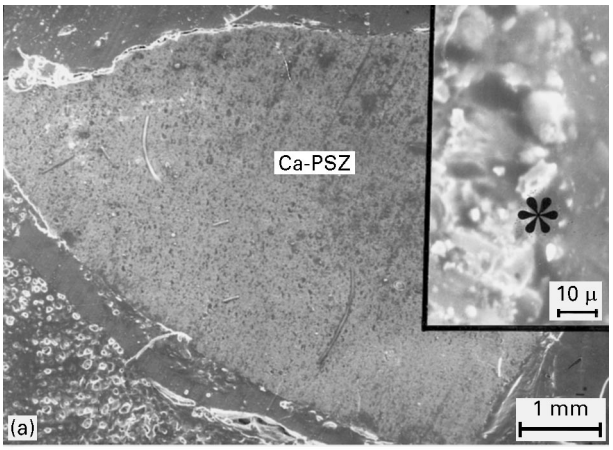
Figure 3 Scanning electron micrograph of Ca–PSZ inserted in rabbit tibia 6 months after surgery. Note the presence of bone marrow (∗) around the implant. Inset: higher magnification of bone–biomaterial interface.

already assumed the morphological characteristics of compact bone. In the zones where morpho-structural contiguities between the bone and the implant were observed, the presence of non-mineralized mesenchymal tissue was no longer evident (Fig. 4).

3.3. Microanalysis and chemical analysis

The results of the microanalytical and chemical investigations of the samples taken at 1 and 12 months are show in Tables I and II, respectively.

The X-ray spectroscopic analysis demonstrated how the central area of the Ca-PSZ implant presents a composition which is not dissimilar to that of pure non-implanted Ca-PSZ, at both a short and a long period after the operation (Fig. 5). At the bone–implant interface level there are changes in the relative percentages of the three elements considered (calcium, phosphorous and zirconium) (Fig. 6). The discrimination between phosphorous (P) and zirconium (Zr) at the interface level has been possible by taking into account the K_α transition of P (2.02 keV) and the L_α transition of Zr (2.05 keV), as well as by emphasizing the LL transition of Zr (1.79 keV) (Fig. 7). The



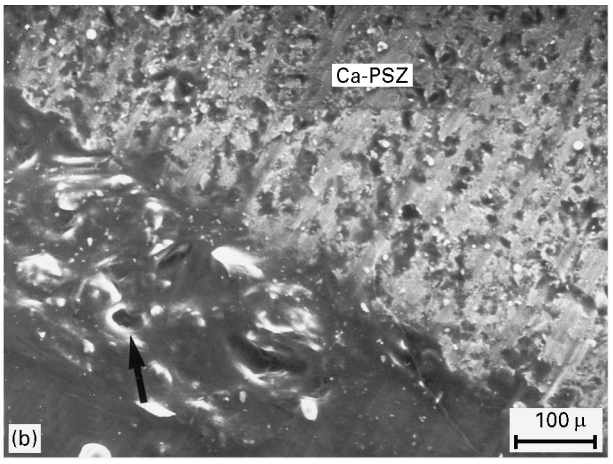


Figure 4 Scanning electron micrograph of (a) Ca–PSZ inserted in rabbit tibia 1 year after surgery. Inset: higher magnification of bone–biomaterial interface showing osteointegration (∗): (b) bone–implant (Ca–PSZ) interface with some periosteocytic laucunae (arrow).

TABLE I X-ray analysis of bone–Ca–PSZ implants

	Percentage content of Zr	Ca	P
Pure Ca-PSZ	96.75	3.25	
Implant after 1 month			
Ca-PSZ	92.46	7.24	
Bone	/	65.06	34.94
Interface	18.89	73.54	7.54
Implant after 1 year			
Ca-PSZ	91.83	8.17	
Bone	/	67.83	32.17
Interface	42.14	33.95	23.91

TABLE II Chemical bulk analysis of bone–Ca–PSZ implants

	Ash content at 600 °C (mg)	Dehydrated samples (mg)	Percentage of ash content
Implant after 1 month	240.7	466.8	0.00645 +/− 0.01141*
Implant after 1 year	203.1	325.5	0.00571 +/− 0.06257*

* Standard deviations were calculated on five consecutive measures.

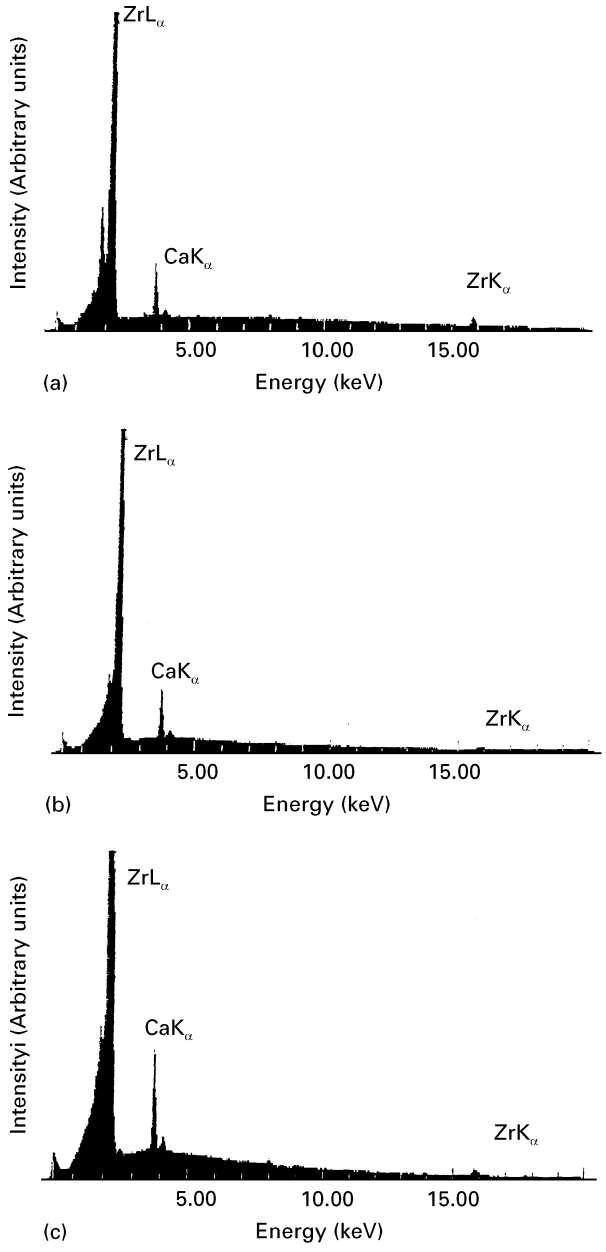


Figure 5 Electron energy dispersive spectra obtained form pure Ca–PSZ (a) and after implantation in rabbit tibia for (b) 1 month; (c) 1 year. Ca–PSZ is not affected by the implantation.

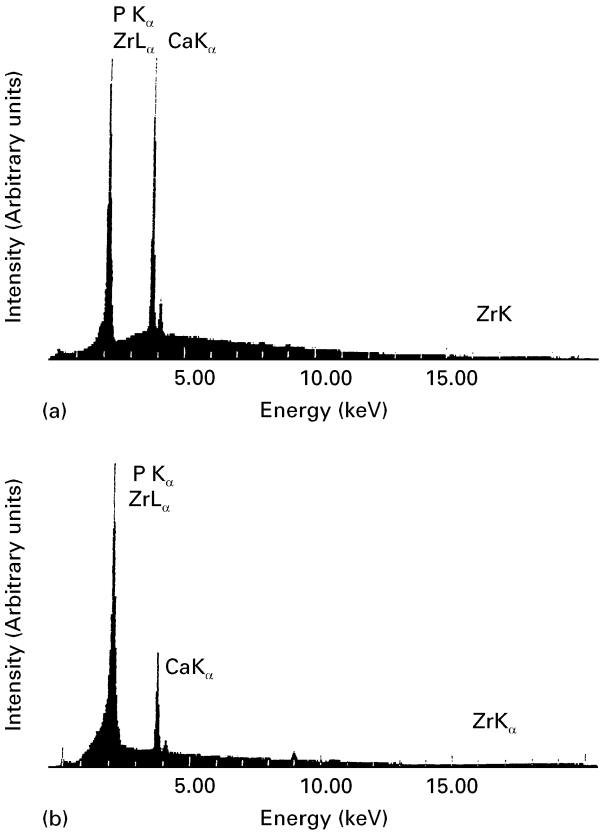


Figure 6 Electron energy dispersive spectra obtained at the bone–implant interface (a) 1 month and (b) 1 year after implantation showing the component (Ca, P, Zr) changes.

error in estimating the P reading at the interface level has been calculated as being about 3%.

Chemical analysis of the bone mass after the implant extraction showed no difference in the zirconium concentration between the samples taken at 1 month and those taken at 12 months.

4. Conclusions

The chemical-morphological investigations carried out demonstrated the presence of osteogenetic activity at the bone–implant interface level after the operation. An overall analysis of the data, in agreement with the literature [17, 18], shows that neo-osteogenesis is preceded by the formation of a connective tissue around the implant. This mesenchimal material is then progressively replaced by osseous tissue. In fact, the newly formed bone initially presents the characteristics of immature bone with scarcely orientated trabeculae. In the successive remodelling that occurs in the continuing direct ossification, normal osseous tissue is formed with trabeculae orientated along the major axis of the implant. The microanalytic analyses together with the chemical analyses [19, 20] suggest the presence of diffusion of calcium ions at the bone–implant interface. In fact, the bone is not a static deposit of mineral tissue, but is maintained in a state of continuous dynamic equilibrium by two-way movement of calcium and phosphate ions (but also other ions) produced by osteoblastic and osteoclaste activities. The synthesis and demolition of the structure is known as remodelling and is controlled by numerous, mainly hormonal growth-factors [21]. In the light of this therefore, the phenomenon of calcium diffusion could be connected to the active bone remodelling observed around the implant [22]. As regards possible difficulties in the microanalytic evaluation of zirconium in the presence of phosphorous at the interface level [23], observation of the shift in the zirconium energy peak at this level compared to that detected in the centre of the implant, appeared to be a valid tool for distinguishing the presence of zirconium from that of phosphorous.

There is a great deal of controversy surrounding the possible deterioration in mechanical properties of PSZ with time [15]. This damage could be connected to degenerative phenomena found at the bone–implant interface, with consequent dissolution and/or hydrolitic decomposition of the material. For

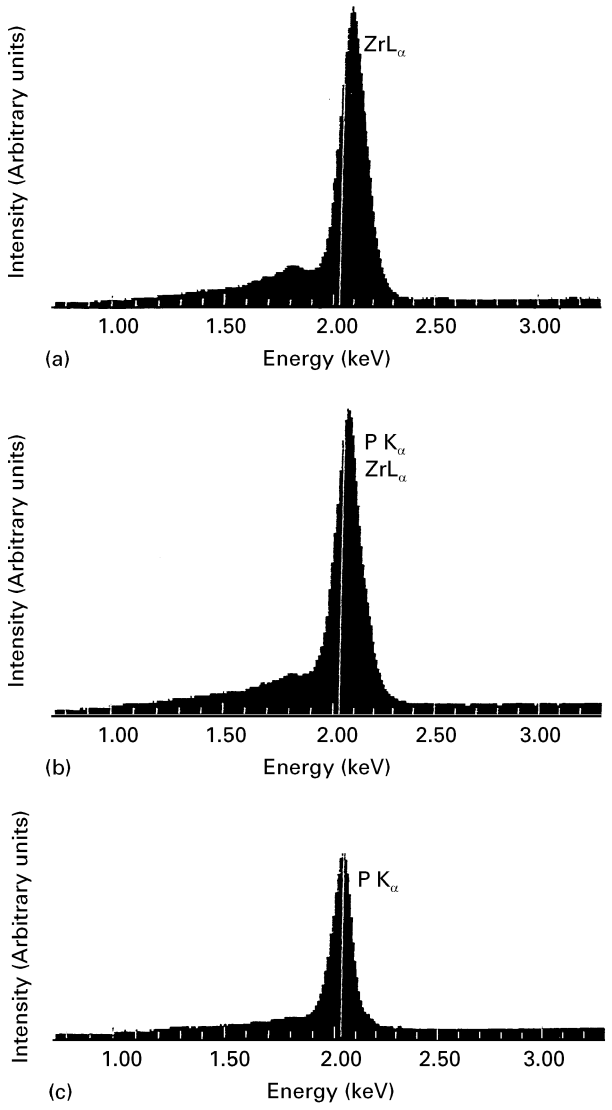


Figure 7 Electron energy dispersive spectra of Zr and P. Note that the peak of Zr (a) shows modification at the bone–implant interface (b) due to the interference of P (c). The white line indicates the cursor position.

example, the degrading events could be tied to the inflammatory response which develops after the insertion of the implant [24], and the release of ions at that level could generate phenomena of local or systemic hypersensitivity and/or toxicity [25, 26]. In any case, the comparison between microanalytical investigations carried out on non-implanted and implanted Ca-PSZ suggest that the surface of this ceramic does not undergo modification once it has been inserted in a biological environment, at least in the time span of 12 months. This demonstrates a similarity between the properties of Ca-PSZ and those already demonstrated for Y-PSZ [23, 27].

The data obtained showed that Ca-PSZ is capable of providing an osteogenetic response with direct ossification, as in the case of hydroxylapatite [28]. We can hypothesize that, in the presence of Ca-PSZ, the reconstitutive processes of bone which occur at the interface depend not only on phenomena of biocompatibility of the material in contact with the bone but also, in an important manner, on its mechanical stability. The osteogenesis is of the cartilaginous type, in fact, under particular conditions of tension [29] which may occur, for example, in the presence of micromovements at the bone–implant level.

Several authors have demonstrated how the presence of a load can generate a fibrous capsule around the implant [30, 31]. However, in a study on experimental animals subjected to load, PSZ was found not to be encapsulated, but about 70% of the implant was in direct contact with the osseous tissue [32]. In our study we did not find any evidence for the presence of a fibrous capsule but rather the transitory appearance of a soft connective tissue reaction at the interface level. In that case, in agreement with the findings of other authors [18], it can be supposed that the initial production of this mesenchymal tissue at the interface allows modulation of the mechanical forces to which the implant is subject, so guaranteeing that the bone–implant unit has a good degree of stability during the very early phases of bone repair. The initial formation of a connective tissue can therefore be considered a positive event in the osteogenetic process and not a sign of any future failure of the implant. Finally, according to some authors [33, 34] the reconstitutive processes of bone depend directly on the ties that are established at the interface level (bone–implant continuum): the greater the stability, the greater the metabolic–functional equilibrium achieved by the implant. The mechanisms regulating bone repair at this interface are certainly complex and depend not only on the initial stability of the implant, but also on the physical–chemical interactions which are established between the surface of the material and the surrounding tissue. These interactions in fact determine the transfer of loading force through the interface and therefore influence functional processes and bone remodelling [35].

Thus, our study appears to confirm the importance of establishing efficacious and preferential physical–chemical interactions between the bone and the prosthetic biomaterial, even if their long–term efficaciousness requires to be further assayed.

References

1. S. SANDAHUS, ed. CBS in "Noveaoux aspects de l'implantologie", Stuttgart Media (1969).
2. J. G. N. SWAT and K. DE GROOT, in "Dental implants", edited by G. Heimke (Carl Hansen Verlag, Berlin, 1980) p. 97.
3. R. RECK, *Clin. Otolaryngol.* **6** (1981) 63.
4. J. J. GROTE, *Arch. Otolaryng.* **110** (1984) 197.
5. F. M. POLACK and G. HEIMKE, *Opthalmology* **87** (1980) 693.
6. K. KAWAUCHI, S. KUROKI, S. SAITO, H. OHGIYA, S. SATO, S. KONDO, I. HIROSE, S. OBARA, H. ASO, K. YAMANO, S. SASADA and S. NOROSE, in "Orthopaedic ceramic implants", edited by H. Onoishi and Y. Ooi (1984) p. 253.
7. C. J. KIRKPATRICK and C. MITTERMAYER, *J. Mater. Sci. Mater. Med.* **1** (1990) 9.
8. L. SEDEL, A. MEUNIER, R. S. WIZARD and J. WITVOET, in "Bioceramic 4", edited by W. Bonfield, G. W. Hastings, K. E. Tanner (Butterworth, London, 1991) p. 27.
9. P. CHRISTEL, A. MEUNIER, M. HELLER, J. P. TORRE and C. N. PEILLE, *J. Biomed. Mater. Res.* **23** (1989) 45.
10. R. C. GARVIE, C. URABANI and D. R. KENNEDI, *J. Mater. Science* **19** (1984) 3224.

11. J. L. DRUMMOND, *J. Amer. Ceram. Soc.* **72** (1989) 675.
12. B. DERBISHYRE, J. FISHER and D. DOWSON, *Med. Eng. Phys.* **6** (1994) 299.
13. B. CALES, Y. STEFANI and E. LILLEY, *J. Biomed. Mater. Res.* **28** (1994) 619.
14. T. MATSUHASHI, M. ICHINORA and U. TSASUHE, *J. Amer. Ceram. Soc.* **60** (1974) 97.
15. D. L. PORTER and H. HENER, *ibid.* **60** (1977) 280.
16. K. KOBAYASHI, H. KUWAIJAMA and T. MASAKI, *Solid State Ionics* **11** (1981) 489.
17. R. G. T. GEESINK, K. DE GROOT and C. P. A. T. KLEIN, *Clin. Orthop.* **225** (1987) 147.
18. R. R. RICHARDS, T. MINAS and D. W. C. JOHNSTON, *ibid.* **150** (1980) 263.
19. T. AOBA, E. C. MORENO and S. SHIMODA, *Calcif. Tissue Int.* **5** (1992) 143.
20. I. M. SKURIKHIN, *J. AOAC Int.* **72** (1993) 257.
21. L. G. RAISZ and G. A. RODAN, in "Metabolic bone disease", 2nd Edn, edited by Aviali and Krore, USA (1992) p. 1.
22. S. BARTIN, *Orthop. Res.* **12** (1994) 375.
23. B. CALES and Y. STEFANI, *J. Mater. Sci. Mater. Med.* **5** (1994) 376.
24. P. TOMSEN and L. E. ERICSON, in "Bone biomaterial interface", edited by J. E. Davies (Univ. of Toronto Press, Toronto, 1993) p. 153.
25. K. K. LIPP, S. L. ATTILA and O. TAINIKE AHO, *Amer. Rev. Respir. Dis.* **148** (1993) 1089.
26. S. HATANAKA, S. OHEDA, S. OKASAKI *et al.*, *in vivo* **7** (1993) 11.
27. K. SHIMIZU, M. OKA and P. KUMAR, *J. Biomed. Mater. Res.* **27** (1993) 729.
28. D. C. R. HARDY, P. FRASSYNET, A. GUILHELM, M. A. LAFONTAINE and P. E. DELINCE, *J. Bone. Joint. Surg.* **73B** (1991) 732.
29. T. M. SKERRY, R. SUSVILL, A. J. EL HAI, N. N. ALI', R. A. DODDS and L. E. LANYON, *Calcif. Tissue Int.* **46** (1990) 318.
30. J. B. BRUNSKI, A. F. MOCCIA Jr, S. R. POLLACK and R. SKOLOCK, *J. Dent. Res.* **58** (1979) 1953.
31. L. B. LUM, O. R. BEIRNE and D. A. CURTIS, *J. Oral Maxillofac. Implants* **6** (1991) 456.
32. Y. AGAWAKA, Y. ICHINAWA, H. NIKAI and H. TSURU, *J. Prosthet. Dent.* **69** (1993) 599.
33. R. HUISKES, *Proceeding of the Symposium* "*The use of hydroxyapatite coated prostheses*" (The Furlong Research Foundation, London, 1991).
34. G. W. HASTINGS, in "Imaging techniques in biomaterials", edited by M. A. Barbosa and A. Camilho (Elsevier, 1994) p. 11.
35. D. C. SMITH, *Int. J. Prosthodontics* (USA) **2** (1993) 106.

Received 12 May 1995
and accepted 10 January 1996