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Long-term evaluation of titania-based ceramics compared with commercially pure titanium *in vivo*

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Fifty-four cylinders (2.8 mm in diameter) machined from hot isostatically pressed titania (TI) and titania-hydroxyapatite (TI/HA-15 vol%) sintered at 925°C, as well as commercially pure titanium (c.p.Ti), were implanted in the femoral cortical bone of New Zealand white rabbits for 1, 3 and 12 months. The shear strength between bone and implant was measured by a push-out test. The TI/HA composite showed a significantly higher bonding strength to bone compared to c.p. Ti at all times, while no differences were observed between TI and c. p. Ti at 1 and 3 months after implantation. Titania-based materials had a significantly higher bonding strength than that of c.p.Ti one year after implantation. The results indicate that bioactivity of HA in TI/HA composite contributes to the early bone apposition reflected by high bonding strength, while the stability of the oxide, determines the development of long-term bonding strength. Both effects may be explained by the level and type of ions released from the ceramic implant. HA has a positive conduction to bone ingrowth while TI has a limited interaction to the bone apposition due to the extraordinary low ion release *in vivo*. Under light microscopy, similar patterns of bone–implant interfaces were seen from titania-based materials and c.p.Ti in 3-month samples, indicating high biocompatibility of these materials. However, histological evaluation by light microscope cannot identify the differences between physical contact and chemical bonding of implant–bone interface, and thus does not give information on bonding mechanism and the level of shear stresses developed.

1. Introduction

Hydroxyapatite (HA) and titanium are two well-established implant materials used in the medical field [1, 2]. The successful clinical performance of these materials is due to the chemical bonding ability of HA to bone and the chemical stability of titanium *in vivo* [3, 4]. The bioactivity of HA is believed to be related to its constitutional similarity to natural bone, while the outmost layer of titanium dioxide contributes to the chemical stability of titanium metal implants. Although titanium-based materials are extensively used clinically, the role of this oxide layer in the biocompatibility of titanium is not well documented.

The passive surface of titanium due to oxidation may absorb calcium phosphate. However, the clinical significance of this absoption is still unclear [5]. In a review article, Tengvall and Lundström [6] claimed that the advantageous properties of the oxide covering the metal are low inherent toxicity, low solubility in water, small $Ti(IV)_{(aq)}$ reactivity with biomolecules, and advantageous peroxide chemistry with apparent anti-inflammatory action. The dielectric constant of TiO_2 is similar to that of water and the isoelectric point is not much different from physiological pH which gives rise to a weakly negative charged oxide. Therin *et al.* [7] studied TiO_2-coated titanium (0.2 μm thick, by anodization) and concluded that the tissue reaction was lower than for c.p. Ti without coating. The outmost layer of titanium dioxide seems to play the main role in the tissue response to titanium metal implants. Therefore, attempts have been made to create a TiO_2 layer on titanium using various methods, e.g. TiO_2 coatings, thermal treatment or anodization.

However, limited information is available regarding the biological response to pure titania bulk materials. TI exists in three different crystal forms, namely as anatase, brookite and rutile. Rutile is the most stable modification. It is thus of interest to study this oxide layer in a defined form. It will contribute to the understanding of the underlying mechanism of so-called osseointegration, particularly the role of the outmost layer on the titanium implant. In this study, TI and TI/HA ceramics, densified using a glass-encapsulated hot isostatic pressing process, were compared to c.p. Ti *in vivo*. The mechanism of biocompatibility of titania-based materials is discussed.

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2. Materials and methods

2.1. Material preparation

Cylinders (2.8 mm in diameter) were ultrasonically machined from dense TI (rutile, Tioxide Ltd. England) and TI/HA ($Ca_{10}(PO_4)_6(OH)_2$, Merck, Germany) as well as c.p. Ti. The ceramic materials were sintered by glass-encapsulated hot isostatic pressing at 925 °C at a pressure of 200 MPa for 2 h. The surface roughness of the cylinders was measured and R_a ranged between 0.4 and 0.9 μm. The cylinders were thoroughly washed in 70 % ethanol solution in an ultrasonic bath and subsequently in deionized water. The cylinders were sterilized by autoclaving.

2.2. Surgical operation

Three cylinders of different materials were implanted in one femur of 18 New Zealand White rabbits, weighing 3.5–4.5 kg. The animals were anesthetized with i.m. injection of Hypnorm^R, 0.3 mg/kg (a mixture of fluanisone and fertanyl, Janssen), and Stesolid^R, 0.5 mg/kg (diazepam, Dumex, Denmark). The surgery was performed under sterile conditions. In order to avoid initial bone–impact direct contact, oversized holes, 2.9 mm in diameter (0.1 mm larger than the implant cylinder) were prepared in one lateral cortices of the femur in each animal using special drills under saline irrigation to avoid heating, according to the principle described by Eriksson [8]. Implants were at least 10 mm from each other and the top implant was approximately 15 mm from the growth plate. The implants were implanted in one femur of each animal.

2.3. Pushout testing

Twelve of the 18 rabbits were used for push-out testing. Four of them were killed at 1, 3 and 12 months after implantation with an overdose of Mebumal^R (NordVacc, Sweden). The femur with implants was separated from the soft tissue and divided into three parts with one implant in each. The overgrowth bone on the periosteum and endosteum was carefully removed by a low speed dental burr to create a free end and to facilitate the measurement of bone thickness. The sample was further divided along the long axis of the bone. The prepared bone with the implant was fixed in a setup as shown in Fig. 1. The maximum force to loosen the implant was measured in universal strength testing machine (Alwetron, T50) at a crosshead speed of 0.5 mm/min. The shear strength was calculated by dividing the force by the actual measured bone–implant contact area, which was determined by use of a stereo microscope. Four samples were used for each measurement at each condition.

2.4. Histology preparation

Six of the 18 animals were used for histological evaluation, two of them were sacrificed at 1, 3 and 12 months. Thin slices of femur with one implant were fixed in phosphate-buffered 4% aldehyde solution and dehydrated in 70 % and 99.5 % ethanol for 24 h, respectively. The specimens were then transformed to chloroform twice, for 2 h each time to improve the penetration of acrylic resin into the tissue. The specimens were kept in 50/50 vol/vol ethanol/LR white resin (LR white, Bio Rad, Polaron Equipment Ltd., England) overnight. Finally each specimen was placed in a mould filled with LR white resin. The specimens were placed on a rotatory device during the processes. Polymerization of the resin occurred in an oven at 60 °C overnight. Thin (50–70 μm), paralleled slices along the long axes of the implants, were obtained using an Exakt-Cutting-Grinding and Mikro-Grinding system (EXAKT-Apparatebau, Norderstedt, Germany). The sections were then stained with toluidine blue for light microscopic observation.

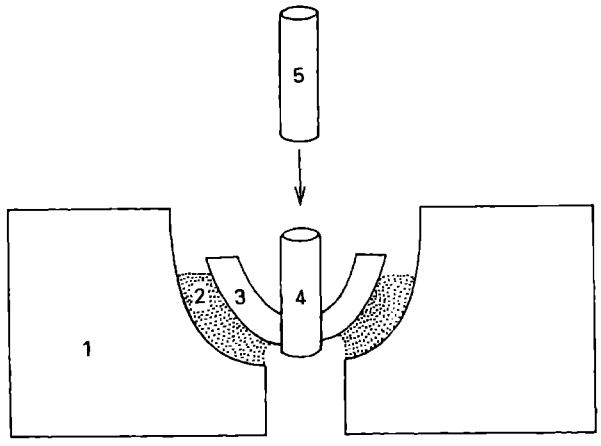

Figure 1 Schematic of the setup for push-out tests. (1. Metal holder; 2. Self-cured resin; 3. Rabbit femuer; 4. Implant; 5. Crosshead)

2.5. Ion release measurement

Titania and c.p.Ti plates, with surface areas of 5 cm^2, were immersed in 5 ml 0.1 M HCl solution at 37 °C. 1 ml samples were taken periodically, from 5 to 180 days, and the sample cells were refilled with stock solution to maintain the same volume. The samples were analysed in an atomic absorption spectrometer (EYE, UNICAM SP9, Philips). Ten specimens of each material were used for the measurements.

2.6. Statistics

The data were statistically evaluated using ANOVA and students' *t*-test. The 95 % confidence limit was used as the significant level.

3. Results

The push-out strength data after different implant times are presented in Fig. 2. The TI/HA composite showed a significantly higher shear strength compared to c.p.Ti at all implantation times. No difference can be measured between TI and c.p.Ti at 1 and 3 months after implanation, while a significantly higher strength was obtained for TI compared to c.p.Ti at 1 year after implantation. Pure TI ceramic showed somewhat

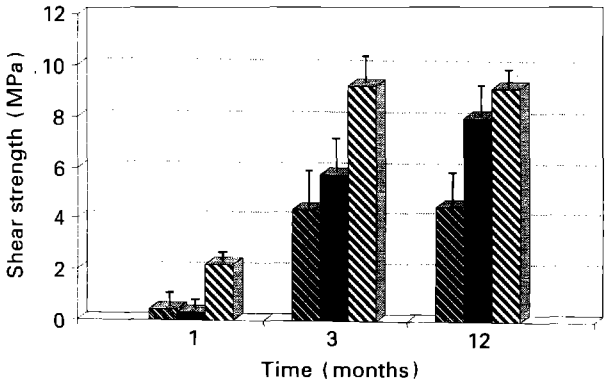

Figure 2 Shear strengths of different materials to bone developed at 1, 3 and 12 months after implantation (▨ Titanium, ■ Titania; ▨ T/HA composite).

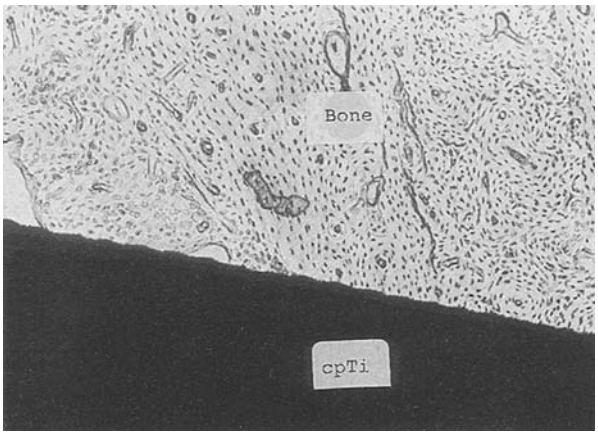

Figure 3 Optical micrograph of TI–bone interface of rabbit femur after 3 months implantation. Magnification ×25.

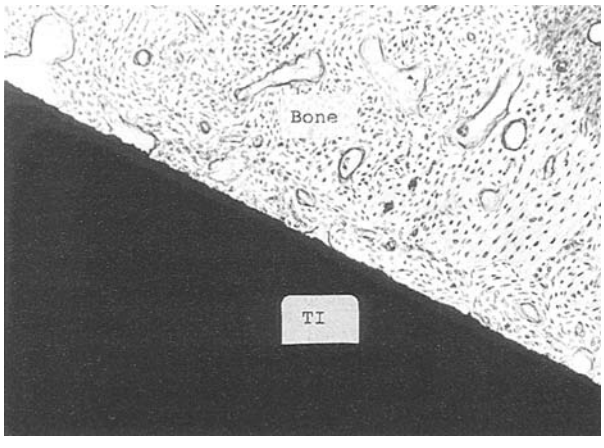

Figure 4 Optical micrograph of c.p.Ti–bone interface of rabbit femur after 3 months implantation. Magnification ×25.

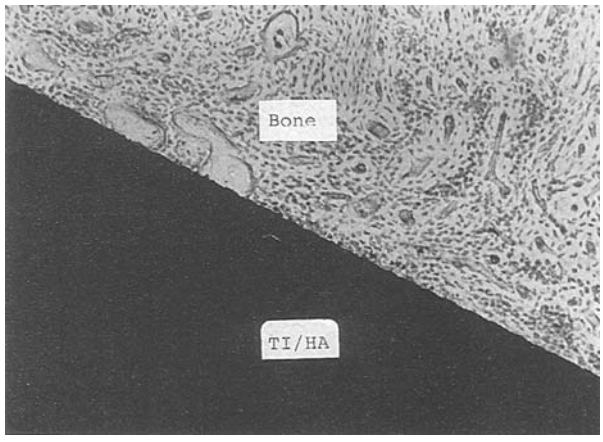

Figure 5 Optical micrograph of TI/HA–bone interface of rabbit femur after 3 months implantation. Magnification ×25.

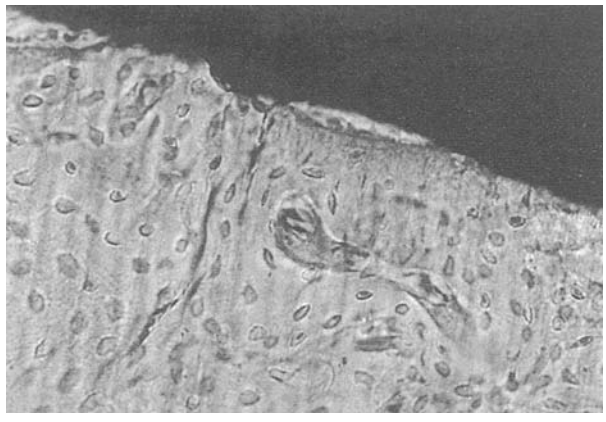

Figure 6 Optical micrograph of TI–bone interface of rabbit femur after 3 months implantation. Magnification ×50.

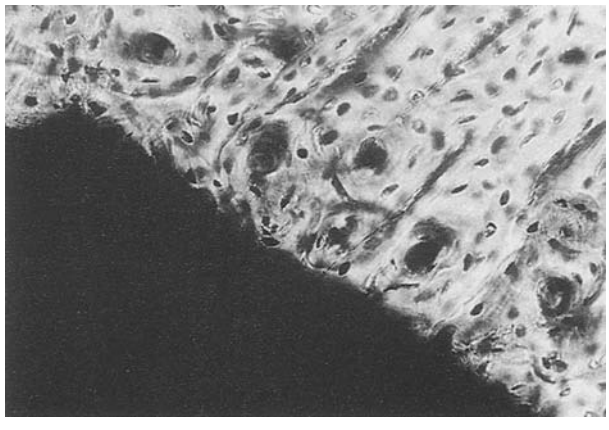

Figure 7 Optical micrograph of TI–bone interface of rabbit femur after 12 months implantation. Magnification ×50.

lower shear strength than TI/HA composite at 1 year after implantation, but the difference was not significant.

Figs 3,4 and 5 show the bone–implant interface of c.p. Ti, TI and TI/HA 3 months after implantation. Bone has direct contact with all the materials (at light microscope level) around the implants. A similar bone ongrowth pattern for all the materials was observed. Implants were covered by new ongrowth bone on the periosteum and endosteum side. The new bone has a normal bone structure. Fig 6 and 7 show the interface of TI–bone after 3 and 12 months implantation. More mature bone is seen after 12 months.

In Table I the concentration of titanium ions in a 0.1 M HCl solution released from titania and c.p.Ti at different times from 5 to 180 days are compared. A continuous release of titanium ion was detected for c.p.Ti, while no detectable titanium ions were recorded for TI in the testing period.

TABLE I. Titanium concentration ($\mu g\,cm^2$) in 0.1 M HCl solution from c.p.Ti and TiO_2

Time (days)	Concentration	
	c.p.Ti (μ/cm^2)	TiO_2 (Mg/cm^2)
5	8.21 ± 1.05	< 0.5[a]
15	11.41 ± 0.81	< 0.5
30	13.06 ± 0.86	< 0.5
180	20.46 ± 2.09	< 0.5

[a] Under the detectable level

4. Discussion

The characteristics of the interface between bone and different implant materials in the nanometre to millimetre range reflects the different interaction behaviour between tissue and material after implantation [9]. Basically, three types of bone–implant interface can be observed according to the tissue at the interface: (1) soft tissue separation; (2) physical contact; and (3) chemical bonding. Type 2 and 3 behaviour are desired and lead to high retention of the implants. The signal or the mediator which activates the fibrous tissue formation is complicated, however, tissue formation seems to be related to the ions (or small molecules) released from the implant materials; in the case of metallic implants, increased ion release seems to favour a thicker fibrous interface [10]. In the case of bioactive ceramics, the activation of fibrous cells appears to be suppressed and bone-forming cells are activated instead. The underlying mechanism of the ion interaction with tissue requires further study. However, the high push-out strength of titania materials indicates that titania has no negative influence on bone formation, probably due to its high chemical stability. The high chemical stability of titania is demonstrated in Table I, and high push-out strength seems to reflect the long-term stability of titania ceramics. Thus, further oxidation of titanium to obtain dense and somewhat thicker and well-defined titanium dioxide (rutile) will likely contribute to the long-term stability of titanium-based implants. Coating of titania on metals is another way to use the chemical stability of this ceramic [11].

Under light microscopy, the similar patterns of bone response to titania-based materials and c.p.Ti indicate similar biocompatibility of these materials. The concept of osseointegration, defined by Brånemark and used for describing the bone–titanium interface [12], can also be applied to titania-based materials. However, the potential difference of bone response to these materials could not be identified according to this concept. Furthermore, even bioactive ceramics (e.g. HA) show similar bone–implant interface under light microscopy. Therefore, it seems that the information obtained under light microscopy observation has limited use in the design of an implant to assure high retention of the implant clinically when new bone is in direct contact with the implant, i.e. no thick soft tissue intervention as with aluminia and polyethelene. It appears that mechanical testing combined with electron microscopy will provide the necessary information with regard to clinical significance and mechanistic aspects.

The significantly higher push-out strength of TI/HA (compared with that of TI and c.p.Ti) after 1 and 3 months of implantation shows the function of the HA phase in the initial healing of bony tissue. However, the function of HA becomes less important with time (Fig. 2).

The increase of push-out strength with time for TI indicates ongoing changes in the interface. This may be due to maturation of the surrounding bone and the new bone ingrowth. Figs 6 and 7 show the direct bone apposition to TI at 3 and 12 months, and the bone is more mature at 12 months than at 3 months. In this case, the long-term stability of the implant becomes more clinically significant to the retention of the implants. The surface roughness of the implant in the micrometre scale starts to play an important role in the retention of the implant, particularly in the long term, for these chemically stable ceramics, e.g. TI. The increase of shear strength of titania after 1 year of implantation is probably a reflection of the contribution of this microretention. Michaels *et al.* [13] observed a difference in cell attachment and spreading when they were comparing different surface preparations of titanium implants. Therefore, the status of the oxide layer of the metallic implants must be taken into account when evaluating the biocompatibility of such implants.

In conclusion, the high chemical stability of TI contributes to long-term bone apposition, and HA results in early bonding strength to bone.

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