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# Pretreatment of titanium implants with lanthanum ions alters the bone reaction

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The experiment was designed to study the effect of chemical modification of titanium surfaces on the response from body fluids and tissues after implantation. By treatment of titanium dioxide and titanium with lanthanum ions, which have high affinity for calcium binding sites, an increased protein adsorption was observed. The *in vivo* effect was also studied both on rats and rabbits, with an inhibition of osseointegration as a result. The observations in the present study indicated that it is possible to change the healing response of titanium by chemically changing its surface properties.

## 1. Introduction

Titanium implants have been shown to induce a positive healing response when inserted into living tissues [1–3]. The growth of bone in close contact with titanium is in contrast to the bone reaction observed with most other metals. Fibrous encapsulation is often the healing response when metals are implanted into bone [4–6]. Titanium has been used for more than two decades as a dental implant material and good results have been obtained in clinical trials [7, 8]. In spite of this, little information is available concerning the chemical and biological mechanisms that are involved in the observed healing response.

During exposure to atmospheric oxygen an oxide layer a fraction of a nanometre in thickness forms rapidly on a pure titanium surface [9, 10]. The titanium implants are reported to be coated mainly by titanium(IV) dioxide ($TiO_2$), which subsequently is the substance the living tissue is exposed to. The oxide layer places titanium in a special category as an implant material, compared with other metals used for the same purpose. During a clinical implantation procedure proteins and macromolecules from the body fluids will adsorb to the implant. This adsorption process is probably governed by the chemical properties of the oxide layer on the implant surface. At physiological pH the hydrated titanium dioxide surface has anionic properties and will therefore attract cations which then displaces the bound water [11]. Polyvalent cations have higher affinity than monovalent cations. Calcium is the most important cation encountered in biological fluids during bony healing. The ability of titanium to bind cations is similar to that of hydroxyapatite when this mineral is exposed to tissue fluids. The similar properties of titanium dioxide and hydroxyapatite surfaces was demonstrated in a recent study which showed that titanium dioxide and hydroxyapatite attracted selectively identical proteins when exposed to human serum [12]. These proteins could be solubilized by ethylene-diamine-tetraacetic acid (EDTA), a reaction which indicated calcium bridging between the surface oxide and the proteins. Titanium dioxides have also been shown to have a capacity for function as a nucleator, and to induce calcium phosphate precipitation from solutions *in vitro*. In a study by Damen *et al.* [13] addition of autoclaved titanium powder into a supersaturated solution of calcium and phosphate reduced the induction time of calcium phosphate precipitation by 66%, whereas titanium only briefly exposed to air had no such effect. Pure titanium dioxide powder induced an even stronger reduction of the induction time in this system. Calcium-phosphate crystal precipitates were observed by the use of scanning electron microscopy and identified as poorly crystalline hydroxyapatite. The authors suggested that this ability of $TiO_2$ to promote nucleation of calcium-phosphate crystals might be one important aspect of the biological response on Ti implants.

Calcium ions from tissue fluids are probably adsorbed to the implant surface before any macromolecule (because of their small size), a process which will favour the adsorption of calcium-binding proteins (mostly negatively charged) and proteoglycans. The significance of calcium for the binding of macromolecules is demonstrated by the effect of EDTA, which rapidly desorbs all macromolecules adsorbed to titanium surfaces, as discussed above [12].

It seems interesting to consider the significance of a change in the surface charge of titanium on its biological properties (i.e. osseointegration). The present study was designed to examine to what extent charge and chemical properties of counter ions adsorbed to a titanium implant would affect its biocompatibility.

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Lanthanum ($La^{3+}$), which is known to have high affinity for binding sites usually occupied by calcium, was chosen; pretreatment of the titanium surface with lanthanum preceded its insertion into bone. The bone reaction to this treatment was then examined *in vitro* and *in vivo*.

## 2. Materials and methods

### 2.1. *In vitro* reactions

#### *2.1.1. Adsorption of albumin*

50 mg of titanium(IV)dioxide ($TiO_2$) powder, anatase (density, 3.90 g cm$^{-3}$) (Aldride Chemical Company Milwaukee, USA) was treated with 3.0 ml 300 mM lanthanum chloride ($LaCl_3$) dissolved in distilled water. Distilled water was used as a control solution. The mixture was shaken for 20 min and the suspension centrifuged, washed twice with 3.0 ml distilled water and the supernatant discarded. 50 mg of the treated or non-treated $TiO_2$ powder was exposed to 3 ml of albumin solutions containing from 1.0 mg ml$^{-1}$ to 5.0 mg ml$^{-1}$ for 2 h and the uptake of albumin was calculated from loss of albumin from the solution analysed by the use of spectrophotometry (Shimadzu OV 240, Kyoto, Japan).

#### *2.1.2. Adsorption of human serum proteins*

Either 100 mg of lanthanum-treated or untreated $TiO_2$ was added to 1 ml of human serum. The mixture was incubated for 5 min, the $TiO_2$ powder washed with distilled water and the serum proteins desorbed with ethylene-diamine-tetraacetic acid (EDTA). The amount of desorbed proteins was analysed by rocket immunoelectrophoresis(RIE) using a Pharmacia PhastSystem (Pharmacia, Sweden).

### 2.2. Healing response *in vivo*

#### *2.2.1. Implant study in rats*

A total of 28 rats (Wistar: Kyoto) were used as test animals in the *in vivo* part of this study where titanium dioxide powders were implanted on the rats' skulls. Titanium(IV)dioxide powder, 1 g, anatase (density 3.90 g cm$^{-3}$) (Aldride Chemical Company, Milwaukee, USA) was treated with 10 ml 300 mM $LaCl_3$ dissolved in distilled water for 1 min in the same way as described above. Untreated $TiO_2$ was used as control. A skin-incision was made medially to the rats' right ears and a subperiostal tunnel was established upon the rats' skulls. The $TiO_2$ powder was placed subperiostally in this tunnel and thereafter the periosteum and the skin were sutured in layers.

The animals were sacrificed by the use of carbon dioxide after 2, 4 or 10 weeks, and the skulls dissected and embedded in 10% formaldehyde. The bones were decalcified by the use of EDTA and embedded in paraffin before sectioning. The sections were stained by Harris hematoxillin/eosin and analysed by light microscopy.

#### *2.2.2. Implant study in rabbits*

Six rabbits (Wistar: Chinchilla) were used as test animals in this part of the study. The rabbits were randomly distributed regarding sex, but all rabbits had a weight of 2.5 kg at the start of the study. The animals were sedated by using a combination of fluanozonium 1 mg kg$^{-1}$ and fentanylium 0.02 mg kg$^{-1}$ (Hypnorm, Janssen Pharmaceuticals, Belgium) and Xylocain/adrenalin (Astra, Sweden) was used for local anesthesia. During surgery, c.p. titanium implants were placed into the rabbits' right ulnae, using an atraumatic surgery technique with standardized burs for drilling a cavity with total fit to the shape of the implants. The implants were placed in the cavities using a titanium tweezer to avoid influence of other metals and given a standardized pressure of 360 g. The implants used in this study had a conical shape and a diameter of 2.0 and 3.0 mm at each end, respectively. The length of the implants was 5 mm. The conical shape was made to reduce the influence of friction forces when tested in a push-out system. Before the surgical treatment four implants were pretreated with 300 mM $LaCl_3$ for 10 min followed by a rinsing in distilled water for 30 s. Two untreated titanium implants were used as control.

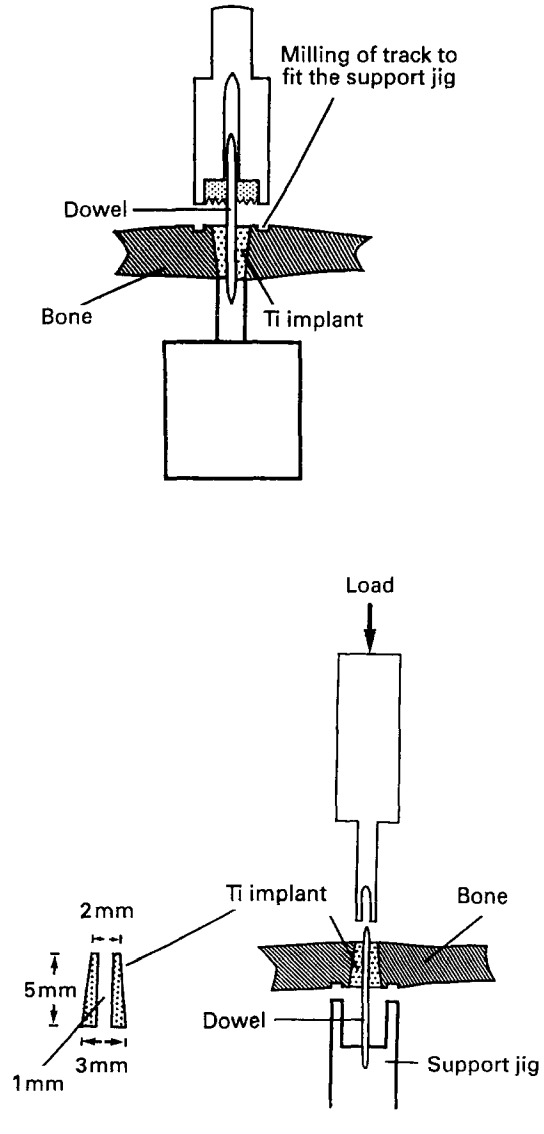


*Figure 1* Push-out test equipment.

The rabbits were sacrificed by intravenous injection with pentobarbital natrium after 60 days and the ulnae removed. The force needed to displace the implants were tested the same day. During the time between killing the animals and the push-out test, the bones were stored in sterile physiological saline.

### 2.2.3. *Displacement (push-out) procedure*

Milling tracks were made in the bone surrounding the implants on the side with the largest diameter of the implant to fit the support jig. The implants were then pushed out of the bone by the use of an Instron mod.1121 tensile testing machine (Instron, UK) and the maximum pressure needed to separate the implants from the bone was recorded (Fig. 1).

## 3. Results

### 3.1. *In vitro* studies

The pretreatment of $TiO_2$ with $LaCl_3$ resulted in an increased adsorption of albumin compared with the untreated titanium dioxide (Fig. 2). The capacity of La-treated $TiO_2$ to adsorb albumin was more than five times the capacity of untreated $TiO_2$.

A similar effect of the pretreatment with $LaCl_3$ was also observed when $TiO_2$ was exposed to human serum. By the use of rocket immunoelectrophoresis it was demonstrated that the La treatment increased the uptake of human serum proteins markedly compared to the untreated control (Fig. 3).

### 3.2. *In vivo* healing response

Implantation of pure $TiO_2$ subperiostally on the rats' skulls resulted in no adverse reaction from the tissues. The histological examination revealed a tight connection between the titanium dioxide powder and the bone and no indication of bone resorption was observed (Figs 4–6). Two weeks after the implantation new bone formation was observed and a concentration of osteoblasts was found in the area with new bone formation. After 4 and 10 weeks new bone formation was observed in the area under the $TiO_2$ powder.

Following the implantation of La-pretreated $TiO_2$ a totally different histological picture was observed. A layer of fibrous tissue and mononuclear cells was separating the bone and the $TiO_2$ powder. After 10 weeks massive ingrowth of blood vessels in this fibrous tissue was observed (Fig. 7). On the bone surface several resorption lacunae were apparent.

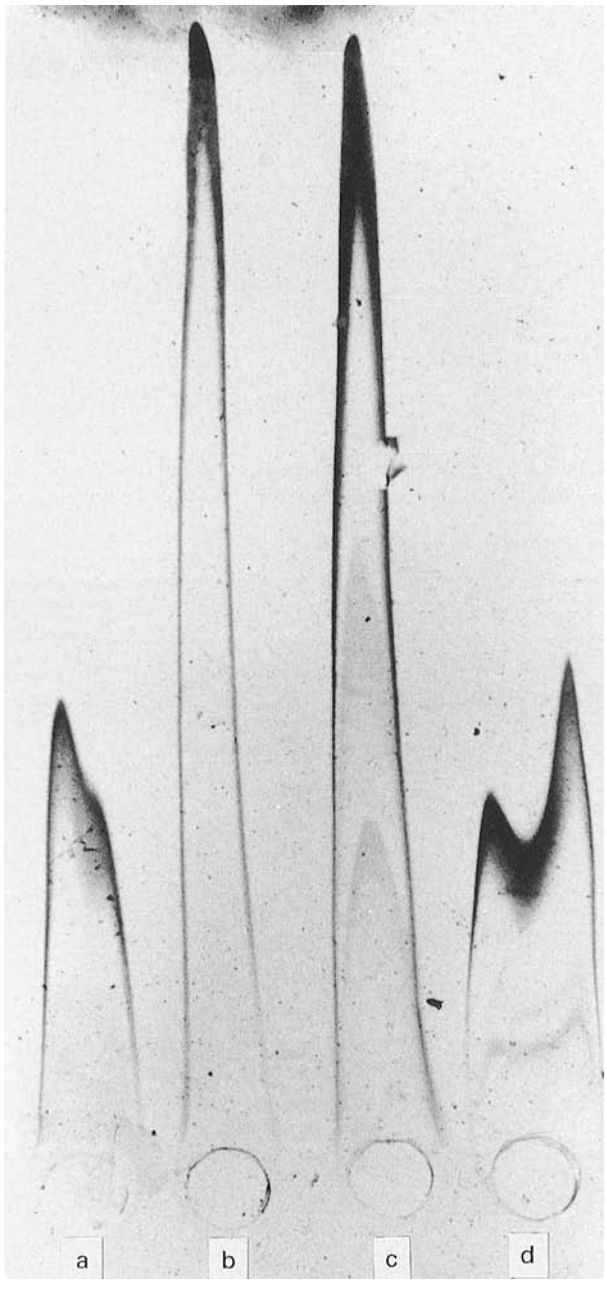


*Figure 3* Rocket immuno electrophoresis (RIE) of human serum proteins adsorbed to untreated (a, d) and $La^{3+}$-treated (b, c) $TiO_2$.

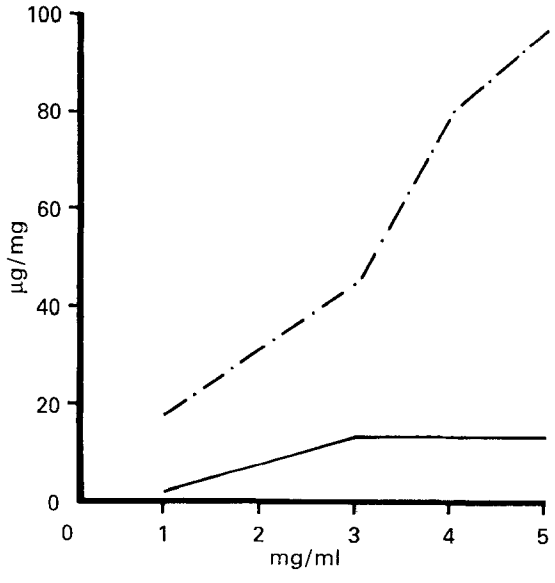


*Figure 2* Uptake of albumin by untreated (——) and $La^{3+}$-treated (–.–.–) titanium dioxide ($TiO_2$) in µg per mg $TiO_2$.50 mg of $TiO_2$ was added to 3 ml solution of albumin at different concentrations. The uptake was calculated from loss of albumin from the solutions and analysed by spectrophotometry.

### 3.3. Implant study

The fit of the test implants that were implanted in the rabbits' ulnae were tested in a push-out system. In this

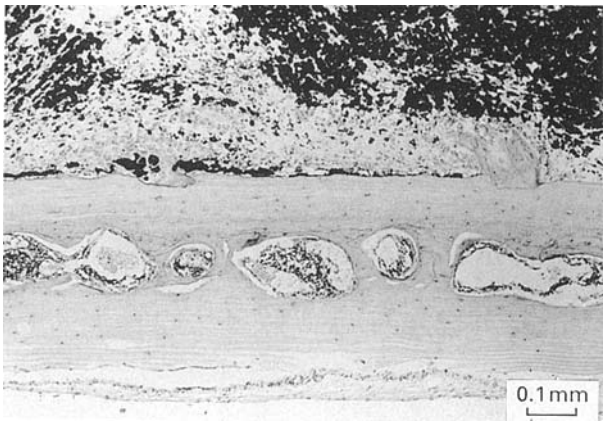


*Figure 4* Rat skull (os parietale) 2 weeks after implantation of $TiO_2$ powder(black). The $TiO_2$ is in close contact with the bone.

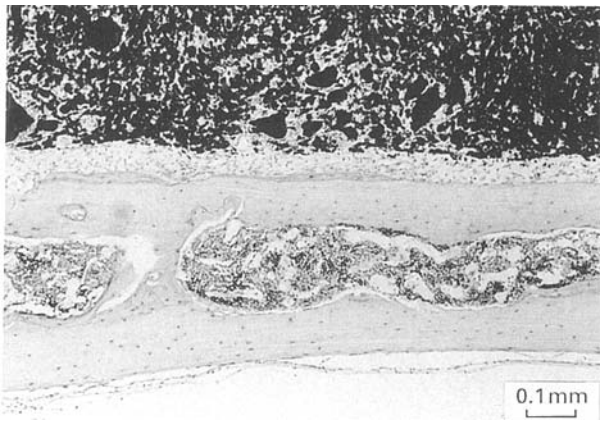


*Figure 5* Rat skull (os parietale) 4 weeks after implantation of $TiO_2$ powder(black). New bone is formed.

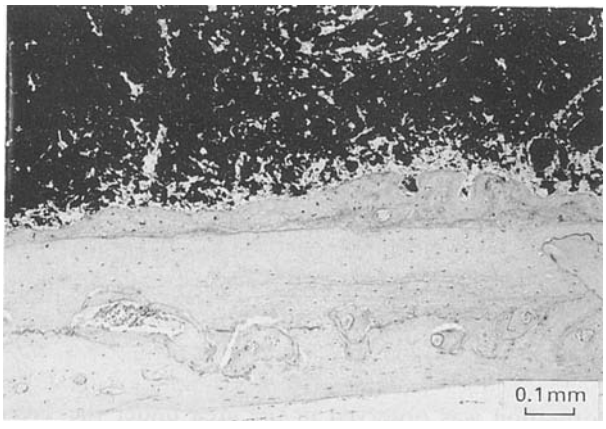


*Figure 6* Rat skull (os parietale) 10 weeks after implantation of $TiO_2$ powder(black). The $TiO_2$ is in close contact with the new bone formed.

TABLE I Push-out values for control and $La^{3+}$-pretreated implants after 2 months' healing period

| Push-out values (N) | | | | | |
|---|---|---|---|---|---|
| Control implants | | $La^{3+}$-pretreated implants | | | |
| C1 | C2 | T1 | T2 | T3 | T4 |
| 53 | 72 | 29 | 0 | 40 | 0 |

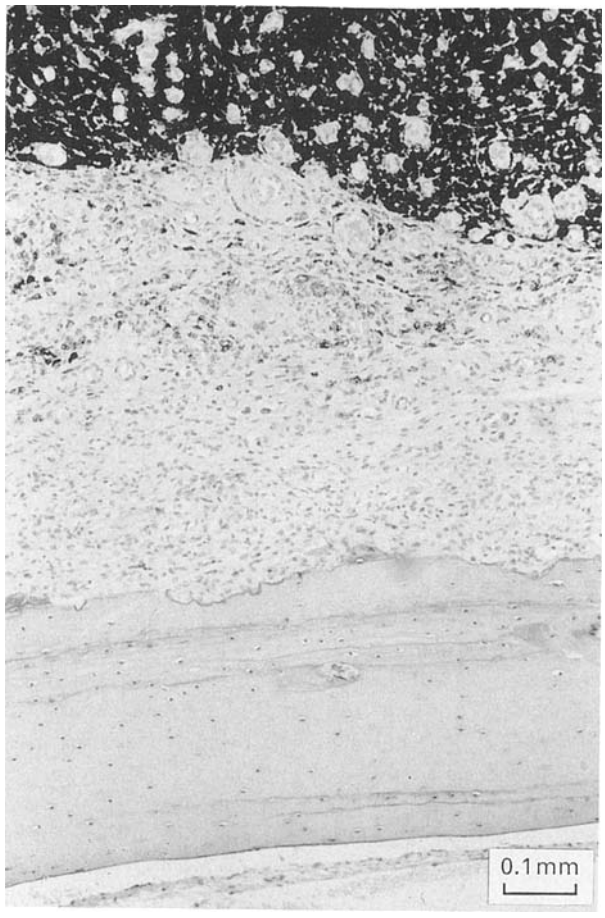


*Figure 7* Rat skull (os parietale) 10 weeks after implantation of $La^{3+}$-pretreated $TiO_2$ (black). A layer of mononuclear cells and fibrous tissue are separating the $TiO_2$ implanted powder from the bone and resorption activity can be seen on the bone surface.

test system the conically shaped titanium implants that had been pretreated with lanthanum had a significantly looser fit to bone than the non-treated titanium implants (Table I).

## 4. Discussion

In the process of bone healing after an implantation, a large number of macromolecules, cells and ions will be present in the implant bed and the surrounding tissue and fluids. Among them both nucleation-promoting and nucleation-inhibiting substances presumably are present, and participate in a process that may or may not lead to osseointegration. There is probably a 'race' towards the surface of the implant where the first molecules or ions that reach the implant surface will govern the subsequent sequence of events in the healing process [14]. Osseointegration is dependent on osteoblasts having access to the surface. If an implant surface is covered by a thick protein layer, the migration of the osteoblasts towards the implant surface may be inhibited. Nucleation inhibitors, e.g. proteoglycans and certain glycoproteins, may well inhibit the formation of new bone around the implant [15].

The reported favourable clinical effect of titanium implants is probably due to the coating by an oxide layer, which takes place on the titanium surface only

seconds after its exposure to oxygen, as mentioned above. In a previous study we observed a chemical similarity between hydroxyapatite and titanium dioxide surfaces in their ability to selectively adsorb serum proteins [12]. This further supported the concept, stressing the observed importance of titanium dioxide in the healing response of titanium and giving a rational basis for this observation. The charge of the implant surface is one aspect which may be of importance for the tissue response. In the present study we modified the titanium surface chemically by adsorption of trivalent lanthanum ions ($La^{3+}$), this ion thus replacing calcium as counter-ion on the surface. By this treatment the surface becomes more cationic, compared with adsorbed calcium ions, which presumably are present on the untreated control implants under "normal" conditions. This was experimentally confirmed by the finding that an increased adsorption of albumin occurred at the lanthanum-treated surfaces compared with untreated $TiO_2$ (Fig. 2). Albumin is anionic and is thus attracted to positively charged surfaces. In the *in vitro* part of the present study it was demonstrated that the changes in the surface properties subsequent to the $La^{3+}$ treatment resulted in increased adsorption of human serum proteins (Fig. 3). This increased protein adsorption *in vitro* coincided with changed tissue responses *in vivo* after implantation. The treated titanium dioxide caused a completely different tissue reaction. The $TiO_2$ was separated from the bone by fibrous tissue and invasion of mononuclear cells and vessels was observed. The increased protein adsorption observed *in vitro* in the present study may well be the cause of the negative response on bone healing. A thick protein coat on the implant surface probably reduces the possibilities of a firm and tight connection between the implant material and the osteoblasts.

Untreated titanium implants are coated with a thin layer of unidentified proteins or proteoglycans when exposed to tissue fluids. The interface layer probably also contains inhibitors of mineralization. The increased adsorption of proteins to $La^{+++}$-treated $TiO_2$ may well contain more of the inhibitors as mentioned above and thus reduce the mineralization. Lanthanum and calcium have both been shown to enhance the proliferative activity of cultured fibroblasts [16]. This indicates that $La^{+++}$ and $Ca^{++}$ have comparable biological properties and that a possible toxic effect of $La^{+++}$ on the tissue cannot explain the present observation.

The present study showed that an introduction of a different counter-ion on the implant surface affected the material's biocompatibility to a major degree. It appears possible that counter ions also may lend beneficial properties to the titanium surface.

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## References

1. T. ALBREKTSSON, P-I. BRÅNEMARK, H. A. HANSSON, B. IVARSSON and U. JÖNSSON, in "Clinical applications of biomaterials" (John Wiley, London, 1982) p. 167.
2. T. ALBREKTSSON, P-I. BRÅNEMARK, H. A. HANSSON, B. KASEMO, K. LARSSON, I. LUNDSTRÖM, D. MCQUEEN and R. SKALAK, *Ann. Biomed. Eng.* **11** (1983) 1.
3. T. ALBREKTSSON and M. JACOBSSON, *J. Prosth. Dent.* **57** (1987) 597.
4. S. B. GOODMAN, V. L. FORNASIER, J. LEE and J. KEI, *J. Biomed. Mater. Res.* **24** (1990) 1539.
5. T. ALBREKTSSON, P-I. BRÅNEMARK, H. A. HANSSON, B. IVARSSON and U. JÖNSSON, *Adv. Biomater.* **4** (1982) 167.
6. G. ELLENDER and K. N. HAM, *J. Oral Implant.* **15** (1989) 47.
7. R. ADELL, B. ERIKSSON, U. LEKHOLM, P-I. BRÅNEMARK and T. JEMT, Int *J. Oral Maxillofac. Implants* **5** (1990) 347.
8. D. VAN STEENBERGHE, U. LEKHOLM, C. BOLENDER, T. FOLMER, P. HENRY, I. HERRMAN, K. HIGUCHI, W. LANEY, U. LINDÉN and P. ÅSTRAND, *ibid.* **5** (1990) 272.
9. B. KASEMO, *J. Prosth. Dent.* **49** (1983) 832.
10. B. KASEMO and J. LAUSMAA, *J. Biomed. Mater. Res.: Appl. Biomater.* **22** (1988) 145.
11. M. ABE, in "Inorganic ion exchange materials" (CRC Press, Boca Raton, 1982) p. 161.
12. J. E. ELLINGSEN, *Biomaterials* **12** (1991) 593.
13. J. DAMEN, J. M. TEN CATE and J. E. ELLINGSEN, *J. Dent. Res.* **70** (1991) 1346.
14. A. G. GRISTINA, P. T. NAYLOR and Q. MYRVIK, in "Molecular mechanisms of microbial adhesion" (Springer Verlag, New York, 1988) p. 176.
15. G. EMBERY and G. RØLLA, *Acta. Odontologica Scand.* **38** (1980) 105.
16. F. C. PRAEGER and B. A. GILCHREST, *Proc. Soc. Exp. Biol. Med.* **190** (1989) 28.

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