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Pre-treatment of titanium implants with fluoride improves their retention in bone

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Fluoride pre-treatment of titanium improved the bone response to this material in the present study. Fluoride pre-treated titanium implants had a four times increased retention in rabbits ulna after four and eight weeks healing periods as measured by a push out technique. Scanning electron microscopic evaluation of the implants revealed that the F-treated implants were partly covered with bone after the push out procedure indicating that an internal fracture had occurred in the bone rather than between the bone and the implant. This was not observed in the titanium control group. It is suggested that the presence of a fluoride coat on the surface of titanium implants stimulates the bone response leading to a connection between titanium and phosphate from tissue fluids. Free fluoride ions will catalyse this reaction and induce the formation of fluoridated hydroxyapatite and fluorapatite in the surrounding bone.

1. Introduction

The use of titanium as dental implant material is based on the light microscopic observation that bone will heal in close contact to the titanium implant surface [1–3]. The result of this healing has been termed osseointegration by some authors [4]. To reach this state of integration between bone and titanium a healing period of six months free from mechanical stress is required for dental implants in the maxillary region. This long resting period is accepted by most patients knowing that this will give stable restorations at the end of the treatment, although the patients may feel uncomfortable during the healing period. In orthopaedic surgery however, a much shorter resting period is advised after implantation of joint prostheses, due to medical as well as economical considerations; cementing is thus still the dominating procedure for fixation of prostheses in orthopaedic surgery [5]. Implantation based on the principles of osseointegration to obtain a tight connection and preferably a bonding between the bone and implant has therefore not been much used in orthopaedic surgery until now [6].

Cementing of the prostheses is a technique that does not take advantage of the ability of bone to adapt to biocompatible or bioactive implant materials. An implant material with an improved and faster bone response could probably be utilized with success as an implant material in orthopaedic surgery.

When titanium implants are exposed to atmospheric oxygen an oxide layer of several Angstrom in thickness forms on the surface [7, 8]. This oxide coat, which mainly consists of titanium(IV) dioxide (TiO_2), is the substance the living tissue is exposed to after implantation with titanium. This ability to form an oxide layer on the titanium surfaces probably explains the biocompatibility of titanium implants *in vivo*. We have previously shown that the titanium oxide surface shares many properties with hydroxyapatite, which is also covered with negatively charged oxide groups from lattice bound phosphate [9]. In another recent study it was demonstrated that the biological response to titanium could be changed by a chemical modification of the surface [10]. Surface treatment with cationic lanthanum increased the serum protein adsorption to the titanium surfaces *in vitro*. Clinically the lanthanum pre-treatment was associated with the formation of a fibrous layer on implanted titanium dioxide and reduced retention of titanium implants as measured by a push out technique.

Fluoride ions have the ability to interact with the hydroxyapatite crystals and form fluoridated hydroxyapatite ($Ca_{10}(PO_4)_6FOH$) or fluorapatite ($Ca_{10}(PO_4)_6\ F_2$). These minerals have greater lattice energy, greater crystallinity and better resistance to dissolution than hydroxyapatite [11]. Fluoride has also been shown to enhance the incorporation of newly formed collagen into the bone matrix and increase the rate of seeding of apatite crystals [12]. Titanium fluoride applied as an aqueous solution is known to form a stable layer, or 'glaze', on hydroxyapatite surfaces [13]. This 'glaze' is assumed to consist of titanium which share the oxygen atoms of phosphate on the surface of hydroxyapatite and thus is covalently bound to the hydroxyapatite surface. The fluoride is essential in this reaction because it is displaced by phosphate and allows this ion to react directly with titanium to form a firm and stable connection.

It was the aim of the present study to investigate whether a similar reaction, or rather inverse reaction,

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would take place on fluoride treated titanium after implantation into bone. If this was the case, a covalent bonding of bone to the titanium surface could be expected.

2. Materials and methods

2.1. Implants

Implants made of commercially pure (c.p.) titanium were used in the study. The implants had a machined surface and were given a conical shape with diameters of 2.0 and 3.0 mm at each end respectively and a length of 5 mm. These conically shaped implants were fabricated to fit exactly into drilled cavities in the rabbits ulnas. The conical shape was made to reduce the influence of friction forces when the bone-titanium interaction was recorded in a push out system.

The implants were separated into two test groups and one control group. The test implants received a pre-treatment with aqueous solutions of either 0.5% NaF pH 3.5, 4% NaF pH 3.5 or 4% NaF pH 3.0 to give a thin layer of fluoride on the titanium surface. The control implants received no treatment. All implants were then washed twice in distilled purified water for 30 s, dried and then autoclaved.

2.2. Animal study

Sixteen rabbits (Chinchilla) were used as test animals in the present 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 before surgery by the use of a combination of fluanozonium 1 mg/kg and fentanylium 0.02 mg/kg (Hypnorm, Janssen Pharmaceuticals, Belgium) and 1.8 ml lidocain 20 mg/ml + adrenaline 12.5 μg/ml (Xylocain/adrenaline, Astra, Sweden) was used for local anaesthesia.

During surgery, two c.p. titanium implants were placed into one ulna, using an atraumatic surgery technique with standardized burs for drilling of a cavity with total fit to the shape of the implants as described above. The implants were placed in the cavities using a titanium tweezer to avoid influence of other metals and given a standardized pressure into the conical cavity of 360 g. Four weeks later during a second operation two implants were placed into the rabbit's other ulna using the same surgical procedure.

The rabbits were sacrificed by intravenous injection with pentobarbital sodium 60 days after the first operation, and the ulnas removed. From each rabbit we thus received two implants with a healing period of four weeks and two implants with a healing time of eight weeks. The forces needed to displace the implants were tested on the same day. During the time between killing of the animals and the push out test, the bones were stored in sterile physiological saline.

2.3. Displacement (push out) procedure

The strength of the bonding between the bone and the implants was tested by pushing the implants out of the bone using an Instron mod. 1121 tensile testing machine (Instron, U.K.). Milling tracks were made in the bone surrounding the implants on the side with the largest diameter of the implant in order to fit the support jig and to give a stable fixation of the bone during the test. A load was then given to the 2 mm diameter end of the implant, longitudinally to the long axis of the implant, and the maximum pressure needed to separate the implants from the bone recorded.

2.4. SEM analysis

The surfaces of the implants were investigated by scanning electron microscopy (Philips SEM 515, Philips, Holland) after the push out procedure as well as those for non-implanted implants. The specimens were sputter coated by gold-palladium and examined at 85 and 500 times magnification at 10.4 kV.

2.5. Histology

After the animals were sacrificed the bones with the implants were removed en bloc. The bones were fixed in formaline followed by dehydration in alcohol and embedded in resin (Technovit, Kulzer & Co., Germany). The specimens were prepared as described by Donath and Breuner [14] by the use of an Exakt sawing machine and an Exakt grinding machine (Exakt Apparatebau, Norderstedt, Germany). The approximately 15 μm thick specimens were stained by toluidine blue. Histomorphometric investigation was performed directly in a Leitz Aristoplan microscope.

3. Results

3.1 Displacement of the implants

The push out test revealed that the implants that had been pre-treated with sodium fluoride had an improved retention in the bone. The fluoride treated implants had 3–4 times greater retention in bone as measured by the push out technique compared to the control implants after eight weeks healing time (Fig. 1). An improved retention could also be observed after four weeks healing time (Fig. 2) although the

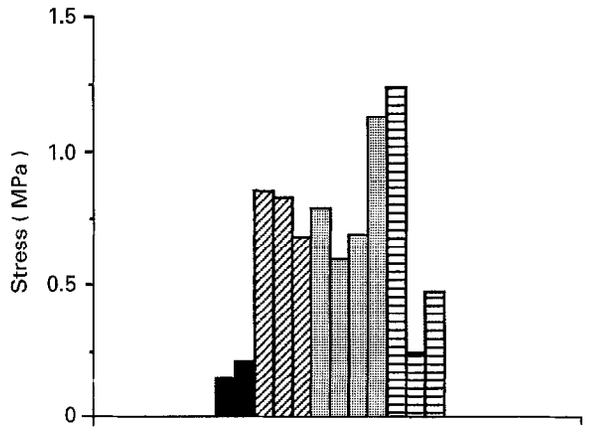

Figure 1 Push out values of the individual conical implants after eight weeks healing period in bone (ulna). A: pure Ti, B: Ti pre-treated with 4% NaF pH 3.5, C: Ti pre-treated with 4% NaF pH 3.0, D. Ti pre-treated with 0.5% NaF pH 3.5. (■), A1–3; (▨), B1–3; (▤), C1–4; (目), D1–3.

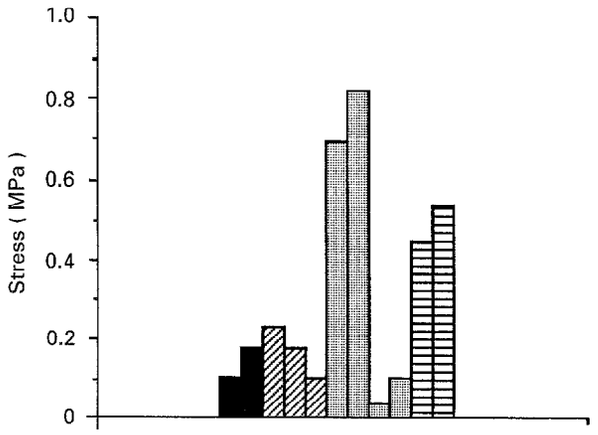

Figure 2 Push out values of the individual conical implants after four weeks healing period in bone (ulna). A: pure Ti, B: Ti pre-treated with 4% NaF pH 3.5, C: Ti pre-treated with 4% NaF pH 3.0, D. Ti pre-treated with 0.5% NaF pH 3.5. (■), A1–3; (▨), B1–3; (▦), C1–4; (目), D1–3.

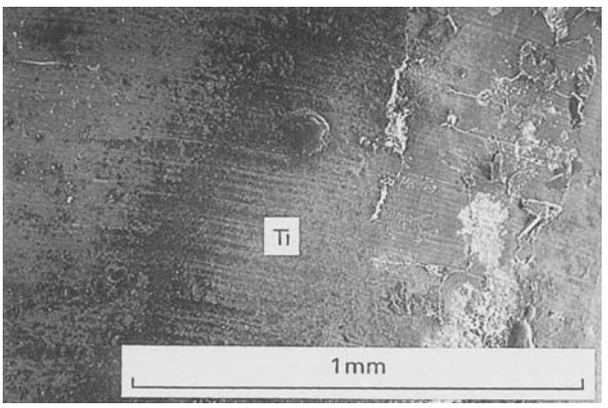

Figure 3 Scanning electron micrograph of the surface of a pure titanium implant after the push out test. The surface is smooth with only few remnants of tissue. The small irregularities from the machining can be identified (× 85).

variation in the push out values was higher in the test group. In the present study the titanium had been exposed to two different concentrations of sodium fluoride, 0.5% NaF and 4% NaF. Both concentrations showed effect, however the most concentrated solution seemed to give the highest retention in bone.

3.2. SEM analysis

SEM analysis of the titanium implants after removal demonstrated a difference between the control and test implants (Figs 3, 4). While the control implants had a smooth metallic surface with few signs of remnants of tissue, the test implants were partly covered by bony tissue. Investigation with high resolution microscopy demonstrated a tight and firm contact between the bone and the implant surface which indicated that a fracture had occurred internally in the bone rather than between the bone and titanium during the push out procedure (Fig. 5).

Histological examination demonstrated that the bone had grown on the test implants and covered them also in the cancellous regions of the bone (Fig. 6).

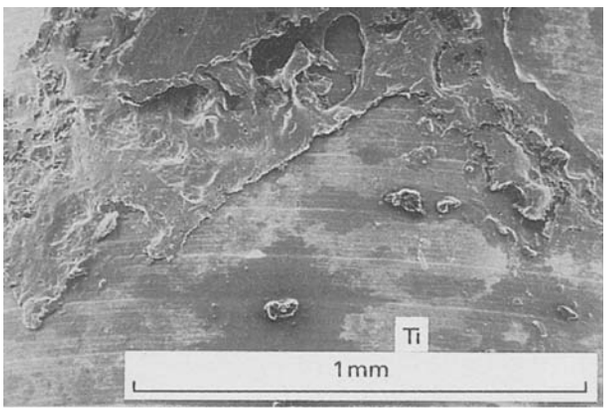

Figure 4 Scanning electron micrograph of a fluoride (4% NaF) pre-treated titanium implant retrieved after the push out test. The surface is partly covered by bone (× 85).

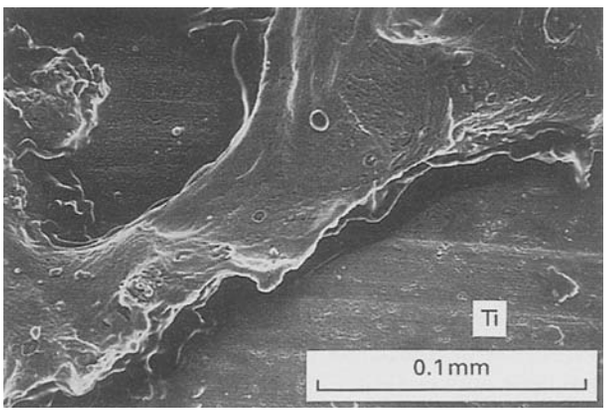

Figure 5 Scanning electron micrograph of a fluoride (4% NaF) pre-treated titanium implant retrieved after the push out test. The bone seems to be firmly attached to the surface (× 500).

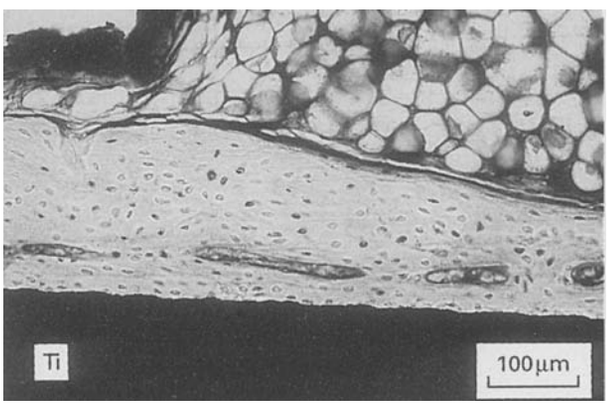

Figure 6 Light micrograph of ground section from the cancellous region of the ulna eight weeks after implant operation. The titanium implant (black) had been pre-treated with 0.5% NaF. New bone has grown in close contact with the implant surface with osteocytes lining the surface.

Bone formation apparently began on each cortical bone lamella and spread along the implant surface into the cancellous areas. High magnifications revealed that the new bone was in close contact with the implant surface and evidence of vitality of the bone was demonstrated by presence of cells in the lacunae. A similar bone growth could not be observed in the

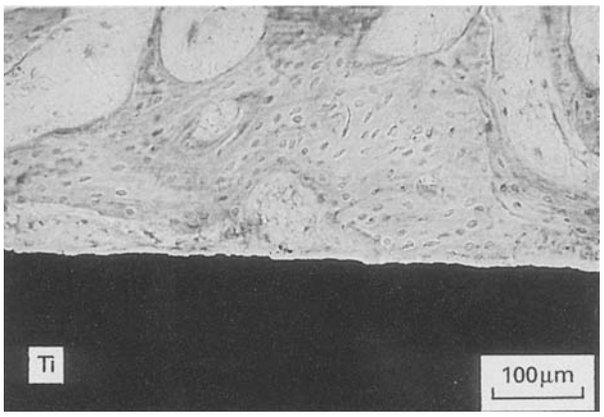

Figure 7 Light micrograph of ground section from the cancellous region of the ulna eight weeks after implant operation with a pure titanium implant (black). New bone has grown into the area, but is only partly in contact with the surface.

control group although new bone was observed in parts of the cancellous regions (Fig. 7). In several areas in the cancellous regions the bone was separated from intimate contact with the control implants by fibrous tissue.

4. Discussion

An improved bone response to titanium implants was observed in the present study when pre-treatment with fluoride was compared to pure titanium implants. Commercially pure titanium, used as control implants in the present study, has been demonstrated by several authors to heal in close contact with bone and induce virtually no inflammatory response [1–3]. This has been attributed to the oxide layer that covers all titanium surfaces. The oxide layer of c.p. titanium implants consists mainly of TiO_2 with minor amounts of Ti_2O_3 and TiO [8].

In a biological situation titanium dioxide surfaces are hydrated and exert anionic properties at physiological pH. These surfaces will therefore attract cations which consequently displace the bound water [15]. Polyvalent cations have higher affinity in this system than monovalent cations. Calcium ions are divalent and are probably the most important cations in the implant bed during bony healing *in vivo*. The calcium ions will presumably be attracted to the titanium dioxide surfaces, as a first step in healing after implantation.

In vitro studies have demonstrated that a calcium phosphate similar to apatite can form on titanium oxide and also that the time needed to precipitate calcium phosphate from supersaturated solutions of calcium and phosphate is reduced by addition of titanium dioxide [16, 17] This ability to induce apatite-like precipitation from calcium and phosphate rich solutions *in vitro* may partly explain the observed good biocompatibility of titanium in bone. Other workers have, however, found that the rate of calcium and phosphate precipitation onto titanium surfaces is much lower compared to precipitation on hydroxyapatite [18]. It might be argued that this difference is due to a difference in number of surface active sites ($-O^-$) between the titanium oxide on metallic titanium and hydroxyapatite.

The 'glaze' observed to form on hydroxyapatite surfaces after titanium tetrafluoride treatment is assumed to consist of titanium which shares the oxygen atoms of phosphate on the surface of hydroxyapatite and is thus covalently bound to the hydroxyapatite surface as discussed above [13]. This reaction occurs because titanium has a high affinity for oxygen and that oxygen available on the hydroxyapatite surface as a part of the phosphate ion thus replaces the fluoride in the titaniumtetrafluoride. Fluoride is essential in this reaction because it allows the phosphate to react directly with titanium by displacing the fluoride.

Fluoride ions have been reported to have the ability to increase trabecular bone density and stimulate osteoprogenitor cell number *in vitro* [19–21]. It has also been speculated that fluoride causes a depression of the osteoclastic activity at cellular level and small doses of fluoride have been demonstrated to induce the calcification of bone in a tissue culture system. Fluoride thus probably also has a catalytic effect on the bone formation. The best known effect of fluoride exposure is the ability of this ion to interact with the hydroxyapatite crystals and form fluoridated hydroxyapatite [22]. This mineral is less soluble than hydroxyapatite and therefore more resistant to osteoclastic resorption.

Fluoride has been shown to increase the proliferation of alkaline phosphatase activity as well as the number of osteoblast cells *in vitro* [23]. Hall [24] found that fluoride induced preosteogenic mesenchyme to become osteogenic in serum-containing medium. Fluoride also supported already induced osteogenic tissue to differentiate in serum-free conditions.

The response to fluoride is dose dependent and administration of high doses of fluoride may cause the formation of poorly mineralized osteoid which has led to reduced use of fluoride in the treatment of osteoporosis [25]. Local release of *low* doses of fluoride has been reported to facilitate bone formation clinically as well as *in vitro* [26, 27]. A slow release of fluoride resulted in a significant increase in bending strength as well as increase in cortical cross-section of rabbits femurs due to new bone deposited on the periosteal surface.

By treating titanium with fluoride, the fluoride will react with the surface titanium dioxide layer and replace titanium bound oxygen to form a titanium–fluoride compound. When this surface comes in contact with tissue fluid during surgical procedures, the oxygen of phosphate in the tissue fluid may replace the fluoride and the phosphate becomes covalently bound to the titanium surface. Such a reaction may induce a bone formation where phosphate in the bone is firmly (covalently) bound to the titanium implant (Fig. 8). The fluoride which is released during this process may catalyse bone formation and facilitate deposition of a particularly well mineralized bone close to the implant surface.

In a clinical situation proteins from biological fluids will adsorb to the implanted surfaces and thus prob-

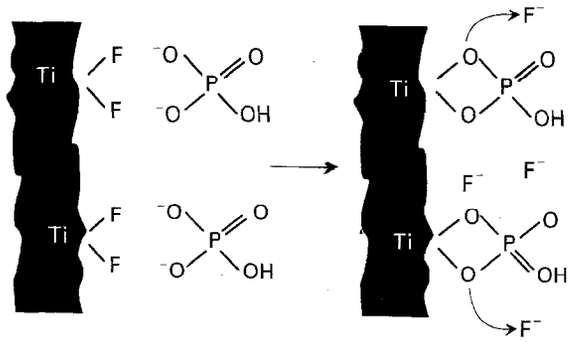

Figure 8 A suggested mechanism for the reaction in bone after implantation of a fluoride pre-treated titanium implant. The oxygen of available phosphate may replace the titanium bound fluoride forming a covalent bonding between titanium and phosphate in bone. The released fluoride may catalyse bone formation by reducing the adsorption of proteoglycans and facilitate deposition of particularly well mineralized bone close to the implant surface.

ably inhibit the apatite formation on the implant surfaces. This initial adsorption of biological molecules is probably determining for the subsequent steps in the healing response. Support for this theory was given in a recent study where an increased protein adsorption *in vitro* following a chemical modification of titanium surfaces with lanthanum coincided with inferior *in vivo* healing response, resulting in fibrous tissue between the titanium and bone and reduced fit as measured by push out testing. This negative healing response observed after lanthanum treatment could be caused by the formation of a thick protein coat on the implant surface which could reduce the possibilities of a firm and tight connection between the implant and the osteoblasts. The suggested covalent interaction between the titanium surface and the oxygen of phosphate in the tissue fluid may eliminate adsorption of macromolecules on the implant surface, which normally occurs. Other studies have shown that the presence of fluoride will reduce the adsorption of proteoglycans and glycosaminoglycans to hydroxyapatite [28, 29]. In a recent *in vitro* study the adsorption of hyaluronan to hydroxyapatite was inhibited by 40% with the presence of 4 ppm fluoride [30].

The bone stimulating effect by fluoride shown in the present study is thought to be caused by a combined effect of fluoride. The protein adsorption is reduced by the covalent binding of phosphate to the surface and the release of free fluoride ions will have the potential to catalyse the bone remodelling with formation of fluoridated hydroxyapatite and fluorapatite. A thin fluoride coat on the titanium implant surfaces may thus reduce the adsorption of mineralization inhibiting proteins and catalyse the bone remodelling close to the implants. This seem to result in a direct connection between the bone and titanium.

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