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Soft tissue attachment on sol–gel-treated titanium implants in vivo

H. Paldan · S. Areva · T. Tirri · T. Peltola ·
T. C. Lindholm · L. Lassila · L. J. Pelliniemi ·
R.-P. Happonen · T. O. Närhi

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Abstract This study was designed to examine the attachment and reactions of soft tissues to sol–gel-derived TiO_2 coatings. In the first experiment, TiO_2 coated and uncoated titanium cylinders were placed subcutaneously into the backs of rats for 3, 11 and 90 days. Tissue response and implant surfaces were characterized with routine light microscopy and scanning electron microscopic (SEM) analysis. In the second experiment, TiO_2-coated and uncoated discs were implanted subcutaneously into the backs of rats for 14 and 21 days. The discs were pulled out from the implantation sites with a mechanical testing device using a constant speed of 5 mm/min. Rupture force was registered, after which the discs were assigned for SEM and transmission electron microscopic (TEM) analysis. All the coated implants showed immediate contact with the surrounding soft tissues without a clear connective tissue capsule. Significantly better soft tissue response was measured for all the coated compared to the uncoated cylinders ($p < 0.01$). Higher rupture forces were measured for all coated discs, although the differences were not statistically significant. An immediate and tight connection between connective tissue fibroblasts and coatings was noticed in TEM analysis. Our study indicates that TiO_2 coatings improve soft tissue attachment on a titanium surface.

H. Paldan · T. C. Lindholm · R.-P.Happonen
Department of Oral and Maxillofacial Surgery,
Institute of Dentistry, University of Turku,
Lemminkäisenkatu 2, Turku 20520, Finland

S. Areva
Department of Physical Chemistry, Åbo Akademi University,
Porthansgatan 3-5, Abo 20500, Finland

S. Areva · T. Peltola
Turku Centre for Biomaterials, Itäinen Pitkäkatu 4B,
Turku 20520, Finland

T. Tirri · T. Peltola · L. Lassila · T. O. Närhi (✉)
Department of Prosthetic Dentistry and Biomaterials Research,
Institute of Dentistry, University of Turku, Lemminkäisenkatu 2,
Turku 20520, Finland
e-mail: timo.narhi@utu.fi

L. J. Pelliniemi
Laboratory of Electron Microscopy, University of Turku,
Kiinanmyllynkatu 10, Turku 20520, Finland

R.-P.Happonen · T. O. Närhi
Clinic of Oral Diseases, Turku University Central Hospital,
Lemminkäisenkatu 2, Turku 20520, Finland

1 Introduction

Sol–gel-derived TiO_2 coatings have been found to initiate calcium phosphate formation both in vitro and in vivo [1–3]. It has been shown that the calcium phosphate layer starts to form on sol–gel- derived TiO_2-coated commercially pure titanium (c.p.Ti) surfaces within 3–6 days of incubation in simulated body fluid (SBF) [4, 5]. The mechanisms of the calcium phosphate layer formation are not fully understood, but it seems that the negatively charged groups ($Ti–O^-$) on the surface first adsorb calcium (Ca^{2+}) ions, followed by the adsorption of phosphate (PO_4^{3-}), and through a series of solution-induced reactions eventually form a bone-like calcium phosphate layer [6–8]. Surface nanoscale topography has a great influence on the in vitro bioactivity of TiO_2 coatings, which can be controlled by the number of coating layers, sol-ageing times and proper heat treatments [9, 10]. Thus, the coatings can be modified in terms of porosity, adsorption rate and composition.

Good soft tissue attachment is an important prerequisite for many medical devices (e.g. percutaneous devices and oral implants). However, a connective tissue capsule normally forms around subcutaneously implanted devices. The epithelium usually separates the implanted devices that penetrate the skin or mucosa from the soft tissue components that are anatomically normal for the tissues in that area. A smooth surface has been shown to guarantee decent fibroblast attachment on a titanium surface [11]. However, a direct bond between the implant and soft tissues could improve the success of many medical implants.

We have recently reported that sol–gel-derived TiO_2 coating has the potential to mediate direct soft tissue attachment with c.p. titanium [12]. The purpose of this study was to further evaluate the strength of soft tissue attachment on different sol–gel-derived TiO_2 coatings deposited on different sized c.p. titanium implants in vivo.

2 Materials and methods

2.1 Implant materials

Cylindrical (diameter 5 mm and length 7 mm, $n = 72$) and discoid implants (diameter 5 mm and height 1 mm, $n = 56$) were made for the study from commercially pure (c.p.) titanium bar (grade 2). Discoid implants were equipped with a hole (0.8 mm diameter) in the middle of the implant. All the implants were ground with silicon carbide paper of 1,200 grit with R_a value of 0.15 μm, and then washed in acetone (5 min) and ethanol (5 min) before use. The experimental TiO_2 coatings were made using a sol–gel technique [13–15]. Briefly, commercially available tetraisopropyl orthotitanate, $Ti((CH_3)_2CHO)_4$, was dissolved in absolute ethanol (solution I). Ethyleneglycol monoethylether ($C_2H_5OCH_2CH_2OH$), deionised water, and fuming hydrochloric acid (HCl, 37%) were dissolved in ethanol (solution II). Solutions I and II were mixed rapidly and stirred efficiently (>600 rpm) for 3 min. The coating sol with EtOH:Ti(OR)$_4$, H_2O:Ti(OR)$_4$ and HCl:Ti(OR)$_4$ molar ratios of 8.2, 1.0 and 0.018, respectively, was aged at 0 °C for 1 h (A) or 24 h (B) before being used for coating. The coatings were prepared by dipping the titanium implants into the sol and then withdrawing them at a speed of 0.30 mm/s. The sol was kept at 0 °C during the dip-coating process. The coated substrates were heat-treated at 500 °C for 10 min. After heat treatment, the coatings were cleaned ultrasonically in acetone for 5 min and in ethanol for 5 min, and finally dried at the ambient temperature. This dipping, heating, and washing cycle was repeated 5 times to obtain five subsequent layers. Finally, the materials were sterilized in an autoclave (121 °C, 16 min, 1 bar). A third surface treatment was achieved by immersing the coated substrates (B) in simulated body fluid (SBF) for 4 days before implantation (C). Ground and washed titanium implants were used as controls (D). Experimental materials according to the type of coating and shape of implant are shown in Table 1.

2.2 Surgical procedures

Two separate experiments were performed. Altogether 23 (9 + 14) adult male Long Evans rats (weight 300–400 g) were used. In the first experiment, the animals were divided into three groups, three animals in each group: the 3, 11 and 90 day groups. The animals were anesthetized by subcutaneous injection of Hypnorm (Fentanyl citrate 0.315 mg/ml and Fluanisone 10 mg/ml, Janssen-Cilag ltd, Saunderton, England) and Aqua Sterilisata (Orion, Espoo, Finland) at a ratio of 1:3, 0.6 ml/kg. Ultracain D-S (40 mg/ml + 10 μg/ml) was used for local anesthesia. The operation area was shaved and cleaned with 70% ethanol. A total of eight 1 cm long incisions were made through the skin bilaterally on the back of each rat. Eight cylindrical implants, two of each coating (A, B, C and D), were inserted subcutaneously into each animal according to a previously constructed split plot table. Surgical wounds were closed with Dexon (4-0) sutures (Davis and Geck Inc., Manati, P.r., USA). The experimental animals were sacrificed after 3, 11 and 90 days with an overdose of carbon dioxide (CO_2). Implants were removed with a 5 mm margin, fixed in 70% ethanol, and embedded in methylmethacrylate (MMA). The specimens were sectioned longitudinally using a band saw. Half of the specimens were cut and crowned down to 20 μm and stained with haematoxyline eosin (HE) for routine light microscopy and histomorphometric evaluations (MicroScale TC, Digithurst, Royston, UK). The histological evaluation consisted of a description of the observed specimens, and a quantitative scoring analysis of the tissue response [16]. The remaining specimens were prepared for SEM (Model JSM 5500, JEOL ltd., Tokyo, Japan) and X-ray microanalysis.

Table 1 Number of experimental materials according to type of coating

Coating	A	B	C	D
Cylinder	18	18	18	18
Disc		28		28

A = Sol–gel TiO_2 coating after 1 h sol aging time

B = Sol–gel TiO_2 coating after 24 h sol aging time

C = Sol–gel TiO_2 coating after 24 h sol aging time and 4 day immersion in SBF

D = Uncoated control implant

In the second experiment the animals were divided into two groups, with seven animals in each group: the 14 and 21 day groups. The animals were anesthetized and the operation area was cleaned as described in the first experiment. Four titanium discs, two coated (coating B) and two uncoated control discs, were inserted subcutaneously into the back of each rat: altogether 28 implants (14 coated and 14 control) for each time point. After the animals were sacrificed, the implants were removed from the surrounding soft tissues with a 10 mm margin. Half of the samples were used for mechanical testing, while the remaining samples were processed for transmission electron microscopy (TEM; JEM 1200EX, JEOL ltd, Toky, Japan).

During both experiments the animals were kept in cages and fed with standard diet pellets with water ad libitum. The national guidelines for experimental animals were followed. The study was approved by the Ethical Committee of the University of Turku.

2.3 Mechanical testing

Immediately after the animals were sacrificed, half of the harvested implant samples (14 coated and 14 control) were placed in sterile aqua (Aqua Sterilisata) and used for mechanical testing. The samples were fixed in a metallic frame (dorsal side downwards) after which a thin needle was placed through the hole of a titanium disc and connected to the testing device with a wire (LLOYD instruments ltd, LRX 2K5 std. Machine). Perpendicular upwards force was applied to the implants using a constant speed of 5 mm/min. The force required to remove the implant from the soft tissues was registered. All implants ($n = 28$) used for mechanical testing were then fixed in 5% glutaraldehyde in 0.16 mol/L s-colloidin-HCl buffer (pH 7.4), and coated with carbon for SEM-EDX analysis.

2.4 Transmission electron microscopy

The remaining 28 implants (14 coated and 14 controls) were removed from the experimental animals with a 1 mm margin. The tissue slices were cultured for seven days and fixed with freshly prepared 5% glutaraldehyde (Electron Microscopy Sciences, Fort Washington, PA, USA). The specimens were prepared according to standard procedures, as previously described [17]. Orientation sections for light microscopy were cut at 1 μm thickness and stained with toluidine blue. The thin sections (70 nm) were stained with 5% uranyl acetate and 5% lead citrate in Ultrostainer (Leica, Wien, Austria) and examined in a JEM-1200EX (JEOL, Tokyo, Japan) transmission electron microscope.

2.5 Statistical evaluation

Statistical evaluation was carried out by the means of StatView for Windows program (SAS Institute, Cary, NC, USA). Differences among the coatings were tested with Fisher's PLSD test after the p-value of an ANOVA was found to be <0.05.

3 Results

3.1 Physicochemical analysis

On day 3, SEM and EDX analysis revealed some carbonated calcium phosphate formation on the proximal ends of the cylindrical implants with coatings A and B. No calcium phosphate formation was found on coating C or on control implants. No calcium phosphate was detected on any of the samples at later time points.

3.2 Histological analysis

In the first experiment, nine implant cylinders (4 controls and 5 coated) in four animals were lost postoperatively. A total of 63 samples were available for histological and SEM analysis.

On day 3, tissue reaction was characterized by moderate inflammatory cell reaction around both TiO_2-coated and control implants. Formation of a connective tissue capsule had already started around the control implants. No clear capsule was observed around any of the TiO_2-coated implants. The histological picture was similar on all coated implants showing connective tissues and fibroblast-like cells in immediate close contact with both sides and corners of the implants (Fig. 1A). A clear gap was seen between the connective tissue capsule and the implant surface on the control implants (Fig. 1B). Both on TiO_2-coated and on control implants, the connective tissue and connective tissue capsules were loosely attached to the implant surface on both ends of the implants.

On day 11, mild inflammatory cell reaction was observed on the coated implants, whereas only moderate inflammatory reaction was seen on control implants. No clear capsule was seen on any of the coated implants, whereas the connective tissue capsule surrounding the control implants appeared thicker than that on day 3. The connective tissues were in immediate contact with both sides and corners of the coated implants, but a clear gap was seen between the capsule and the surface of control implants.

On day 90, no more inflammatory cells were found in any of the samples. Again the connective tissues appeared to be firmly attached to the surface of all coated implants

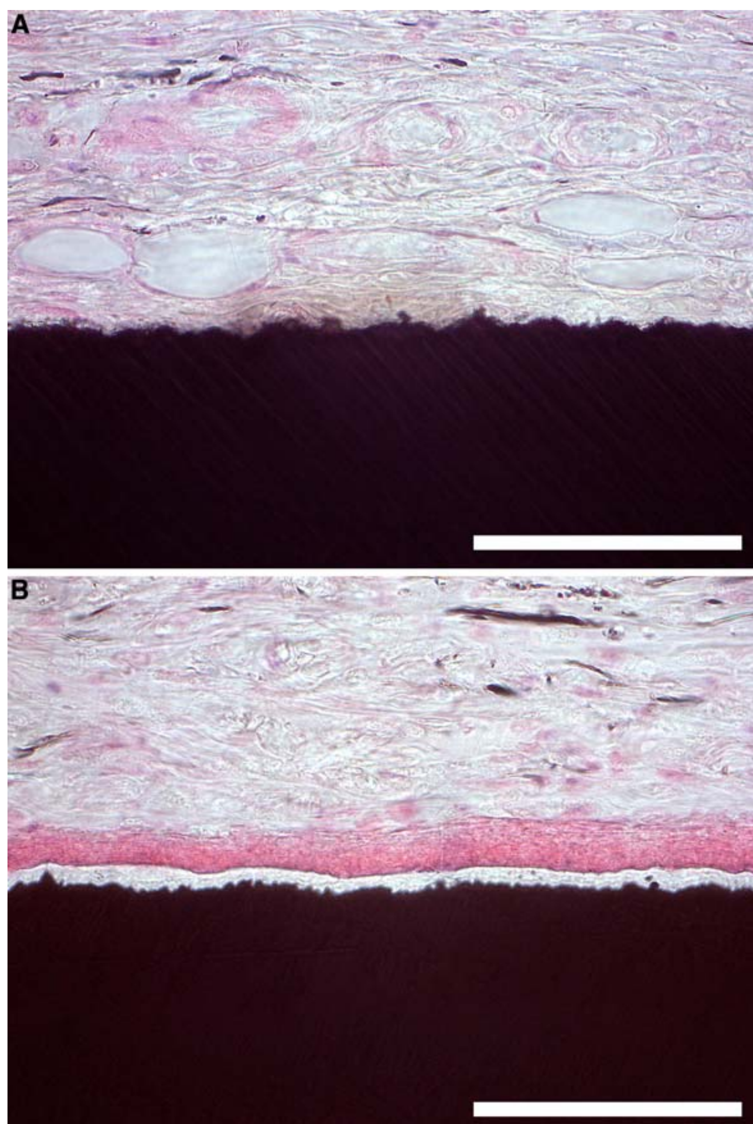

Fig. 1 (**A**) Figure shows subcutaneous connective tissue in immediate contact with TiO_2 –coated titanium cylinder (Coating A) 3 days after implantation. Clear connective tissue capsule cannot be distinguished (scale bar 100 μm). (**B**) Firm connective tissue capsule is formed around uncoated titanium cylinder 3 days after implantation. Cap formation between the capsule and implant surface is clearly visible (scale bar 100 μm)

although the formation of a thin capsule-like structure was noticed. The connective tissue capsule surrounding the control implants had became thicker and a clear gap separated it from the implant surface.

3.3 Histomorphometric evaluation

Data on histomorphometric evaluation are presented in Table 2. All the coated implants scored significantly better in the quantity, quality and interface analyses (p value ranging from <0.05 to <0.001). However, no significant differences were found among the TiO_2 coatings A, B and C in any of the analyses.

3.4 Mechanical test

All the implanted discs were available for pullout measurement. All the coated implants tended to be firmly attached to the surrounding connective tissues. The mechanical test showed higher pullout forces for coated than for uncoated implants (26.8 ± 12.3 N vs 22.5 ± 11.9 N). However, due to the high standard deviation, the difference did not reach statistical significance. After the pullout test, all the implants were prepared for SEM evaluation. At both time points (14 and 21 days), all the coated discs were partly or fully covered with connective tissue components (Fig. 2). No connective tissue was detected in any of the control implants. This indicates that with all coated implants, tissue rupture had occurred in the connective tissue layer and not in the tissue-implant interface, as with the control implants.

3.5 Transmission electron microscopic analysis

The titanium implants were surrounded by fibrocytic cells that formed a connective tissue capsule around the titanium

Table 2 Histomorphometric scores of the titanium cylinder/connective tissue interface

Coating	A	B	C	D
Quantity				
Day 3	1.7 ± 1.4	$4.0 \pm 0.0^{***}$	$4.0 \pm 0.0^{***}$	$3.8 \pm .05^{***}$
Day 11	1.7 ± 0.8	$3.8 \pm 0.5^{***}$	$3.3 \pm 0.6^{**}$	$3.7 \pm .06^{***}$
Day 90	1.5 ± 0.7	$3.0 \pm 0.6^{**}$	$2.8 \pm 0.4^{**}$	$3.2 \pm .04^{***}$
Quality				
Day 3	1.3 ± 1.0	$2.5 \pm 0.6^{*}$	$3.0 \pm 0.0^{***}$	$2.6 \pm 0.5^{**}$
Day 11	2.0 ± 0.6	$3.3 \pm 0.6^{*}$	$3.0 \pm 0.0^{*}$	$3.5 \pm 1.0^{**}$
Day 90	4.0 ± 0.0	3.8 ± 0.4	3.8 ± 0.4	3.7 ± 0.8
Interface				
Day 3	1.7 ± 1.4	$3.3 \pm 0.5^{*}$	$3.4 \pm 0.5^{**}$	$3.2 \pm 0.4^{*}$
Day 11	1.7 ± 1.3	$3.3 \pm 0.6^{*}$	$3.3 \pm 0.6^{*}$	$3.5 \pm 0.6^{**}$
Day 90	3.0 ± 0.0	$3.8 \pm 0.4^{*}$	$4.0 \pm 0.0^{**}$	$3.7 \pm 0.5^{*}$

Difference compared to coating A: $^{***}p < 0.001$; $^{**}p < 0.01$; $^{*}p < 0.05$

disc. The detachment of the cellular capsule from the titanium left an intact cell surface after control and TiO_2-coated implants (Fig. 3A and B). The capsule consisted of typical mature fibrocytes with abundant organelles for protein synthesis. The cells contacting the TiO_2 coating were thicker and they had more and different organelles in the cytoplasm when compared to those in the control implantations (Fig. 3B). A detailed ultrastructural analysis of the contact area with the TiO_2 coating showed the intimate contact between the plasma membrane of the adjacent cell and the oxide layer (Fig. 4). The oxide layer was organized as crystalline rods with their ends facing and making contact with the plasma membrane (Fig. 4).

4 Discussion

The present subcutaneous implantation used to evaluate soft tissue reaction and calcium phosphate formation on TiO_2-coated implants in vivo is a frequently used method to evaluate the biocompatibility and in vivo behaviour of new materials [16, 17]. However, it should be noted that the metabolism of a rat is not comparable to that of a human. Thus, the results of this study cannot be directly extrapolated to humans, although they provide valuable information about the materials' potential in human applications as well.

The reason for postoperative loss of nine cylinder implants (5 coated and 4 controls) due to an infection was most likely the relatively large size of the implant. The large implants were used in the first experiment to obtain a homogenous and smooth coating on all sides of the implants. After withdrawing the implants from the titanium sol a drop may form at the distal end of the coated material [14]. Small cylinder implants can be totally covered with the titanium gel drop, resulting in the formation of cracks and thicker coatings. In the first study, the implants were large enough (7 mm) to avoid this problem.

As is usual after any surgical procedure, the histological evaluation revealed mild (3 days) and moderate (11 days) inflammatory reactions around the TiO_2-coated implants. The inflammatory reaction was stronger around the control implants. Thus, it seems that TiO_2 coatings promote the woundhealing process during the early stage of healing. On day 90, the inflammations had subsided in all implants.

The finding of no clear connective tissue capsules around the coated implants during the experiment, and a clearly visible capsule around the control implants already on day 3 supports our earlier study in which immediate soft

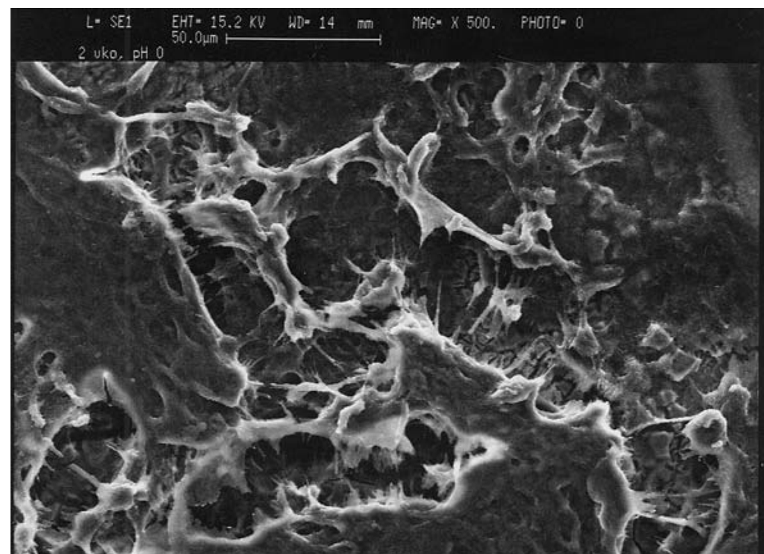

Fig. 2 Scanning electron micrograph of TiO_2-coated titanium disc after mechanical pull-out test. Connective tissues remain firmly attached to the disc surface

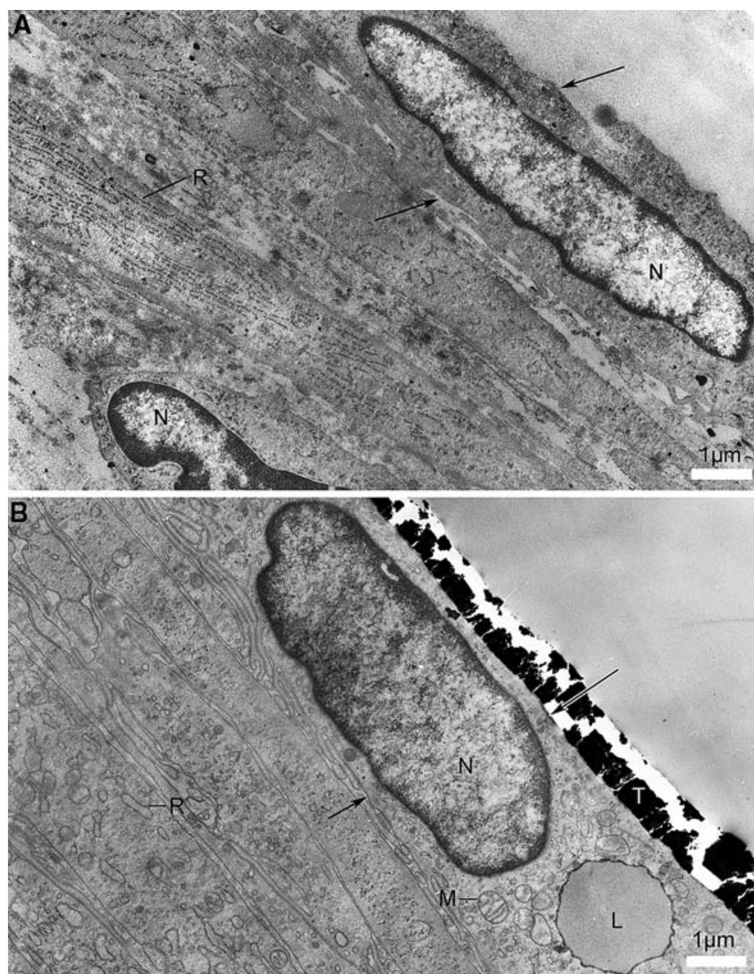

Fig. 3 (**A**, **B**) Survey electron micrographs of connective tissue cells surrounding the titanium implant. The tissue has been detached from the surface of the implant. The thin arrow on the right points at the cell surface that has made contact with the titanium disc and the thin arrow on the left points at the opposite side of the contacting cell (**A**). The cells around the control titanium implant are typical fibrocytes with elongated nuclei (N) and abundant rough endoplasmic reticulum (R). The surface facing the detached implant is similar to that facing other cells (**B**). The cells around the TiO_2-coated implants are firmly attached to the oxide layer (T), which covers the surface of the contacting cell (thin arrow on the right) even after the detachment of the tissue from the implant. The thin arrow on the left points at the opposite surface of the cell. The contacting cell is thicker than in the control (**A**), has a larger nucleus (N) and the cytoplasm contains mitochondria (M), rough endoplasmic reticulum (R), and a lipid droplet (L). The following cells on the left are similar to those in the control (**A**). The TiO_2 coating (T) is partially broken during the process, and in places detached from the plasma membrane (thin arrow on the right)

tissue integration was first noticed [12]. Sol aging time seems to have no influence on connective tissue attachment as no difference was found among the three different surface treatments at any time point. The mechanism of the soft tissue attachment is not known, but it is probably caused by the fact that the TiO_2 coatings attract Ca ions which, in turn, support adsorption of adhesion molecules, such as fibronectin, on the coated surface [12].

The second experiment with titanium discs was performed in order to evaluate soft tissue attachment on the coated implants in less traumatic conditions. Despite the size and shape of the implants, the coating process was uneventful. This study confirmed that a firm connective tissue attachment can be achieved on sol–gel-derived TiO_2-coated surfaces already two weeks after implantation. This experiment utilizing smaller implants also revealed that a connective tissue attachment forms around the entire implant. The forces required to remove the coated implants were higher than those for uncoated control implants. The measured pull-out forces do not indicate actual bond strengths between the implants and the underlying connective tissues, since it was not possible to remove the tissues covering the upper side of the implants before the pull-out measurement. In the case of controls, the measured pull-out forces indicate the toughness of connective tissue capsules rather than real bond strength.

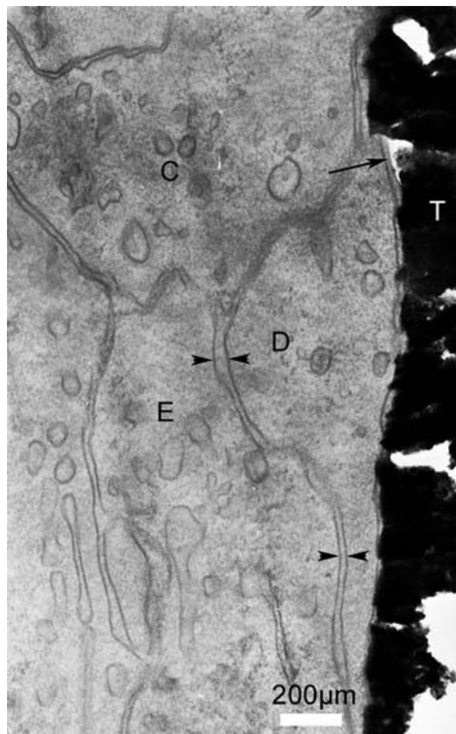

Fig. 4 High power electron micrograph of the contact area between the TiO_2 coating (T) and the contacting cells, (C and D) with a portion of a second-layer cell (E). The plasma membranes of adjacent cells are indicated with arrowheads. At ultrastructural level, the TiO_2 coating (T) is composed of elongated rods oriented perpendicular to the cell surface. The plasma membrane is intact and is in close contact with the TiO_2 coating

SEM examination, carried out immediately after the mechanical testing, showed that with coated implants the rupture occurs within the connective tissue layer, not in the tissue-implant interface as was the case with the control implants. The immediate close contact between the TiO_2 coatings and connective tissue fibroblasts was also verified by TEM. The fact that contacting plasma membrane remained quite intact even after detachment from the metal implant by force during specimen processing, is a sign of physiologically well-tolerated surfaces. The direct attachment of the oxide layer composed of crystal-like rods attaching by their ends to the cell plasma membrane supports the observation that their attachment to the cells is stronger than to the surface of the titanium disc. The thickening of the cell layer in contact with the oxide-coated implants with an increase in cytoplasmic organelles indicates functional activation of the cell. This may by an indication of reactive production of special adhesive molecules to form a firm bond to the oxide layer.

The calcium phosphate formation found in day 3 samples in the first experiment, was seen only at the ends of the implants. This may also result from the relatively large size of the experimental implants, since the connective tissues were not in close contact with the ends of the implants immediately after the implantation. This might have created a niche allowing the exchange of interstitial fluids, thus providing enough Ca^{2+} and PO_4^{3-} ions able to nucleate onto the coated surfaces.

Differences in calcium phosphate formation between the coatings A and B were so small that no conclusions as to the benefit of one over the other could be made. Surprisingly, the only TiO_2 coating that showed no calcium phosphate on day 3 was coating C, which was subjected to SBF immersion for four days prior to implantation. This was done to initiate CaP nucleation. It has been shown that four days is sufficient for the initiation of calciumphosphate formation on TiO_2-coated surfaces, although a longer incubation period improves the quality of the calcium-phosphate coating [4, 5]. In addition, the nucleated calciumphosphate on day 4 is not yet converted into bone like HA, but is rather amorphous, making it more soluble than the thermodynamically stable HA. It might be that, on our samples, calciumphosphate formation had started normally during the SBF treatment, but was resorbed immediately after implantation. This early resorption is not necessarily an unwanted outcome in bone applications because the resorption releases Ca^{2+}ions, which stimulate preosteoblasts to mature and initiate new bone formation [18–20].

In our study, no difference in tissue response was observed between the SBF-treated and non-treated TiO_2 coatings, indicating that the preincubation in SBF is not necessary for the clinical success of TiO_2-coated implants. Rather, preincubation may have disadvantages due to the fact that HA easily forms on the implant surface, which is not necessarily desirable in soft tissue applications. Furthermore, the dissolution of the calciumphosphate layer may delay the bonding of soft tissue to the implant. It was also shown that calcium and phosphate were spontaneously adsorbed from interstitial fluid on the implant surfaces. The sol–gel-derived titanium coating adsorbed more calcium on its surface than did the uncoated titanium implant, i.e. the sol–gel-derived TiO_2 surface is more reactive than the c.p. titanium. However, the adsorption of proteins in the formed calcium phosphate precursor phase probably prevents its transformation to HA as we have recently stated [12].

In conclusion, the sol–gel-derived TiO_2 coatings facilitate direct soft tissue attachment. Neither the aging time of sol nor immersion in SBF seem to have any influence on the rat connective tissue response.

References

1. P. LI, I. KANGASNIEMI and K. de GROOT, *Bioceramics* **6** (1993) 41

2. P. LI, C. OHTSUKI, T. KOKUBO, K. NAKANISHI, N. SOGA and K. de GROOT, *J. Biomed. Mater. Res.* **28** (1994) 7
3. P. LI, I. KANGASNIEMI and K. de GROOT, *J. Am. Ceram. Soc.* **77** (1994) 1307
4. T. PELTOLA, M. JOKINEN, H. RAHIALA, M. PÄTSI, J. HEIKKILÄ, I. KANGASNIEMI and A. YLI-URPO, *J. Biomed. Mater. Res.* **51** (2000) 200
5. T. PELTOLA, M. PÄTSI, H. RAHIALA, I. KANGASNIEMI and A. YLI-URPO, *J. Biomed. Mater. Res.* **41** (1998) 504
6. P. LI, C. OHTSUKI, T. KOKUBO, K. NAKANISHI, N. SOGA, T. KANAMURA and T. YAMAMURO, *J. Am. Ceram. Soc.* **75** (1992) 2094
7. J. K. WEST and L. L. HENCH, *Bioceramics* **5** (1992) 75
8. P. LI, K. de GROOT and T. KOKUBO, *J. Am. Ceram. Soc.* **77** (1994) 1307
9. M. JOKINEN, M. PÄTSI, H. RAHIALA, T. PELTOLA, M. RITALA and J. B. ROSENHOLM, *J. Biomed. Mater. Res.* **42** (1998) 295
10. M. PÄTSI, J. HAUTANIEMI, H. RAHIALA, T. PELTOLA and I. KANGASNIEMI, *J. Sol-Gel. Sci. Tech.* **11** (1998) 55
11. M. KONONEN, M. HORMIA, J. KIVILAHTI, J. HAUTANIEMI and I. THESLEFF, *J. Biomed. Mater. Res.* **26** (1992) 1325
12. S. AREVA, H. PALDÁN, T. PELTOLA, T. NÄRHI, M. JOKINEN and M. LINDÉN, *J. Biomed. Mater. Res.* **70A** (2004) 169
13. C. BRINKER, A. HURD, G. FRYE, P. SCHUNK and C. ASHLEY, in “Chemical Processing of Advanced Materials” (Wiley-Interscience, New York, 1992) p. 395
14. C. BRINKER and A. HURD, *J. Phys.* **4** (1994) 1231
15. S. SAKKA, *J. Sol-Gel Sci. Tech.* **2** (1994) 451
16. J. JANSEN, W. DHERT, J. VAN DER WAERDEN and A. VON RECUM, *J. Invest. Surg.* **7** (1994) 123
17. K. FRÖJDMAN, J. PARANKO, I. VIRTANEN and L. PELLINIEMI, *Differentiation* **50** (1992) 113
18. Y. CHANG, C. STANFORD and J. KELLER, *J. Biomed. Mater. Res.* **52** (2000) 270
19. J. LE HUEC, D. CLEMENT, B. BROUILLAUD, N. BARTHE, B. DUPUY, B. FOLIGUET and B. BASSE-CATHALINAT, *Biomaterials* **19** (1998) 733
20. L. GINESTE, M. GINESTE, X. RANZ, A. ELLEFTERION, A. GUILHEM, N. ROUQUET and P. FRAYSSINET, *J. Biomed. Mater. Res.* **48** (1999) 224