Improved Retention and Bone-to-Implant Contact with Fluoride-Modified Titanium Implants

Jan Eirik Ellingsen, DDS, PhD[1]/Carina B. Johansson, PhD[2]/Ann Wennerberg, DDS, PhD[3]/Anders Holmén, DDS[4]

Purpose: *The purpose of the present study was to investigate whether a fluoride modification of the titanium surface would have an effect on bone response after implantation.* ***Materials and Methods:*** *Titanium-oxide–blasted titanium implants with and without fluoride modification were investigated in a rabbit tibia model. Quantitative analysis of surface roughness, biomechanical interlocking, and in vivo tissue reactions in rabbit bone at 1 and 3 months after placement were compared.* ***Results:*** *The fluoride-modified test implants had a slightly smoother surface (S_a: 0.91 ± 0.14 μm) than the unmodified control implants (S_a: 1.12 ± 0.24 μm). Significantly higher removal torque values (85 ± 16 Ncm vs 54 ± 12 Ncm) and shear strength between bone and implants (23 ± 9 N/mm^2 vs 15 ± 5 N/mm^2) were measured for the fluoride-modified implants after 3 months. The histomorphometric evaluations demonstrated higher bone-to-implant contact for test implants at 1 month (35% ± 14% vs 26% ± 8%) and 3 months (39% ± 11% vs 31% ± 6%) after placement.* ***Discussion:*** *Implant surface modification with fluoride may result in morphologic and physiochemical phenomena that are of significance for the bone response. Another possible explanation for the findings in the present study is that a surface modification changes the surface chemical structures to be more suitable for bone bonding.* ***Conclusion:*** *Based on the biomechanical and histomorphometric data, the fluoride-modified titanium implants demonstrated a firmer bone anchorage than the unmodified titanium implants. These implants achieved greater bone integration than unmodified titanium implants after a shorter healing time.* (More than 50 references.) INT J ORAL MAXILLOFAC IMPLANTS 2004;19:659–666

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Commercially pure titanium has been used for several years as the material of choice for dental and noncemented orthopedic implants. As documented in a number of animal experiments, this material has a high degree of biocompatibility.[1–6] Clinical success has also been reported when treating single and multiple tooth loss with dental implants made of commercially pure titanium.[7–10] In these reports the use of titanium dental implants most often refers to treatment protocols with relatively long healing periods of 3 to 6 months. During the healing period the patient has reduced comfort and, in many cases, severe problems with wearing a temporary prosthesis. For orthopedic use, a 3- to 6-month healing period is unacceptable and limits the potential use of noncemented titanium implants.

To improve the favorable biologic response to titanium, different implant surface modifications have been introduced. These include modifications of the chemistry and topography of the implant surface, such as hydroxyapatite (HA) coating, and modifications of just the surface topography, such as blasting and acid-etching. Significant results have been obtained through these modifications of the surface topography. The great majority of published

[1]Head and Professor, Oral Research Laboratory and Department of Prosthetic Dentistry, Dental Faculty, University of Oslo, Norway.
[2]Professor, Department of Technology, University of Örebro, Sweden; Professor, Department of Biomaterials/Handicap Research, University of Göteborg, Sweden.
[3]Professor, Department of Prosthetic Dentistry/Dental Material Sciences, University of Göteborg, Sweden.
[4]Medical Director, Astra Tech, Mölndal, Sweden.

Correspondence to: Dr Jan Ellingsen, Oral Research Laboratory and Department of Prosthetic Dentistry, Dental Faculty, University of Oslo, Box 1109 Blindern, N 0317 Oslo, Norway. Fax: +47 22 85 23 51. E-mail: janee@odont.uio.no

articles have reported increased bone fixation and increased bone-to-implant contact for rougher implant surfaces compared to polished, milled, or turned surfaces.[11,12] However, very rough surfaces seem to have a negative effect on the bone-to-implant contact, which indicate that there is an optimal surface topography for a favorable bone response.[13–16] Excellent clinical outcomes after 5 years of loading were reported in a study of enlarged implants whose surfaces had been modified by with abrasive blasting with titanium oxide (TiO_2) particles (TiOblast; Astra Tech, Mölndal, Sweden).[10,17–19] Although in vitro and in vivo studies claim improved results with an increased surface roughness, the mechanisms behind these findings still have not been verified.

A tight relation between bone and titanium implant surfaces has been reported.[20–23] Tissue reactions following implantation are influenced by the physiochemical properties of the implant surface. Modifications of the surface oxide layer may thus influence the biologic response following implantation, which may enhance the quality and speed of the bone-to-implant healing process.[24] It has been reported that surface modification with fluoride significantly increased the retention of conical titanium implants in rabbits after 4- and 8-week healing periods.[25] When observed using light microscopy, the fluoride-modified implant surfaces appeared more firmly attached to bone than unmodified surfaces. In an in vitro study, fluoride-modified titanium surfaces adsorbed calcium phosphate (CaP) crystals from a calcium- and phosphate-saturated solution.[26] This phenomenon could not be observed for unmodified surfaces. These observations indicate that fluoride-modified surfaces have properties that may be beneficial for bone healing after implant placement.

The aim of the present study was to compare TiO_2-blasted titanium implants with and without fluoride-modified surfaces with respect to bone-to-implant contact, bone area in threads, and removal torque resistance in rabbit tibia 1 and 3 months after placement.

MATERIALS AND METHODS

Implants

Screw-type implants (n = 80) with an external diameter of 3.5 mm and a total length of 8 mm were turned from commercially pure titanium (grade 4). The implant surfaces were blasted with TiO_2 particles. All implants went through a cleaning process in an ultrasonic bath. The test implants (n = 40) went through an additional cleaning process including diluted hydrofluoric acid.

Surface Analysis

Three test and 3 control implants were subjected to surface analysis with optical profilometry (TopScan3D; Heidelberg Instruments, Heidelberg, Germany). The instrument and evaluation method has previously been described by Wennerberg and colleagues.[27] All measurements were performed on an area 245 × 245 pm, and 9 areas on each implant were analyzed (3 top, 3 valley, and 3 flank areas). Mean values of the measurements performed were calculated for each implant surface. Removal of errors in form and waviness was performed with a 50 × 50-µm Gaussian filter. The parameters evaluated were the average height deviation from the mean plane (S_a), measured in µm; the average distance between surface irregularities (S_{cx}) in spatial direction, measured in µm; and the surface developed area ratio (S_{dr}), ie, 3D/2D, expressed as a ratio or a percentage.

Animals and Anesthesia

The animal ethics committee at Göteborg University approved this study. Twenty male New Zealand white rabbits (mean age 9 months) were included in the study. Anesthesia was administered via intramuscular injections of Hypnorm (Johnson & Johnson/Janssen-Cilag, Saunderton, England) at a dose of 0.5 mL/kg/animal and intraperitoneal injections of 0.5 mL/animal of diazepam (Stesolid Novum, A/S Dumex, København, Denmark). Additional injections were given when needed. Xylocaine (l0 mg/mL, AstraZeneca, Södertälje, Sweden) was administrated for local anesthesia. The animals were euthanized through an intravenous overdose of Pentobarbitalium (Apoteksbolaget, Uppsala, Sweden).

Surgery

Following anesthesia, the skin was shaved and cleaned with chlorhexidine. A local anesthetic agent was then injected. The skin, fascia, and periosteum were opened separately and gently pulled aside to expose the implantation areas. Preparations of monocortical implant sites were made through a sequential drilling procedure according to the Astra Tech protocol. Under aseptic conditions, 2 test implants were placed in the tuberosity of one tibia and 2 control implants were placed in the other. The implants were placed 5 mm apart, with 1 or 2 threads above the bone margin. The fascia was sutured with silk 3-0 and the skin with Vicryl 5-0 (Ethicon, Somerville, NJ). The periosteum was not replaced or sutured.

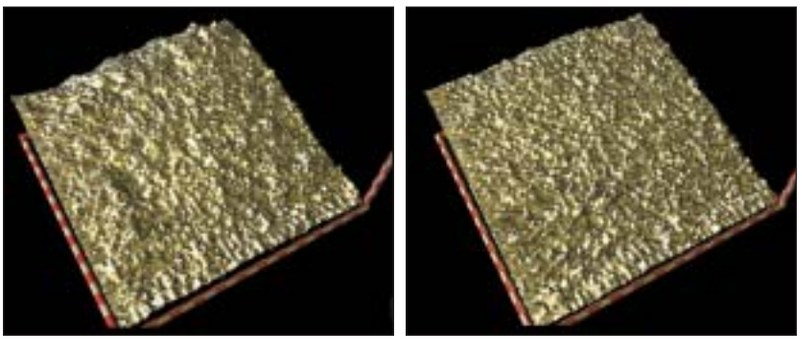

Fig 1 Digital images showing the structures of the flank areas of control *(left)* and test *(right)* titanium implant surfaces. Both the control and the test implants had TiO_2-blasted surfaces; the test surfaces were also modified with fluoride. The images were produced by TopScan 3D. Each red or white section of the bars along the edges represents 10 µm.

The animals were divided into 2 groups of 10, group A and group B. Animals in group A were given a healing period of 1 month following implant placement, whereas animals in group B were given a 3-month healing period.

Quantitative Tests and Specimen Preparation

One implant in each leg was subjected to removal torque tests.[28] The square-headed implants were connected to the removal torque jig via a special adapter, and the torque necessary to loosen the implant was measured in Ncm. The removal torque test was performed with electronically controlled equipment (involving a strain gauge transducer) to limit the influence of human error. Since 1994, this method has been used routinely at the Handicap Research Institute at Göteborg University.[29–33] The remaining implants were retrieved en bloc with surrounding tissue followed by immersion in 4% neutral buffered formaldehyde. After a decalcification process, the samples were cut and ground sections made in the long axis of the implant.[34,35] Ten-µm-thick undecalcified and toluidine blue–stained sections were made.[28,35] Computer-based histomorphometric quantification was carried out in a Leitz Aristoplan light microscope (Wetzlar, Germany) with a 10× objective and a zoom of 2.5×, which allowed the investigator to perform all measurements directly in the eyepiece of the microscope. The quantifications involved bone-to-metal contact and bone area evaluations for all threads as well as for the 3 best consecutive threads in the cortical region (ie, the 3 threads with the most bone-to-implant contact). Based on removal torque value, length of the implant in cortical bone, and the bone-to-metal contact ratio, interfacial shear strength in N/mm^2 was calculated for the 3-month samples.[36,37] Length of the implant in cortical bone was measured on radiographs taken from cut-and-ground sections of the removal torque tested implants, whereas the bone-to-metal contact was deduced from the histomorphometric results of the implants that were not unscrewed. The following formula was used:

Table 1 Surface Characterization of the Implants (Mean ± SD)

Implant	S_a (µm)	S_{cx} (µm)	S_{dr}
Fluoride-modified	0.91 ± 0.14	11.71 ± 0.83	1.21 ± 0.04
Control	1.12 ± 0.24	11.33 ± 1.00	1.34 ± 0.08

S_a = average height deviation from the mean plane; S_{cx} = average distance between surface irregularities; S_{dr} = surface developed area ratio.

$$\text{Interfacial shear strength} = \frac{T}{\pi \times d \times rl \times l \times bmc}$$

where T = loosening torque in Nmm, d = mean diameter of the implant, rl = lever arm, l = length of the implant in cortical bone in mm, and bmc = bone-to-metal contact ratio.

Statistics

The statistical evaluations of biomechanical and histomorphometric data were tested with the Wilcoxon signed rank test. Data were considered significant if P was less than or equal to .05.

RESULTS

Surface Characterization

The fluoride-modified and control surfaces both demonstrated isotropic properties, ie, they lacked any dominant direction of the structure (Fig 1). The fluoride-modified implant surfaces had reduced peak heights compared to the control surfaces, ie, they were slightly smoother (S_a was 1.12 ± 0.24 µm for unmodified implants vs 0.91 ± 0.14 µm for fluoride-modified implants). Furthermore, the control surface had an increased surface area of 34% compared with a totally flat plane; whereas the corresponding value for the test surface was 21%. Furthermore, a slightly longer average wavelength (S_{cx}) was found on the test surface (Table 1).

Table 2 Removal Torque and Shear Strength, Mean ± SD (Range)

Group	Removal torque Ncm	*P*	Shear strength N/mm²	*P*
1-month healing period				
Fluoride-modified	31 ± 11 (14–55)	NS	—	—
Control	27 ± 8.5 (14–42)		—	
3-month healing period				
Fluoride-modified	85 ± 16 (60–114)	.005	23 ± 9 (11–49)	.019
Control	54 ± 12 (41–79)		15 ± 5 (8–23)	

NS = not significant.

Table 3 Percentage of Bone-to-Metal Contact, Mean ± SD (Range)

Group	Fluoride-modified implants	Control implants	*P*
1-month healing period			
All threads	35 ± 14 (15–52)	26 ± 8 (9–36)	.04
3 best	55 ± 15 (34–80)	47 ± 10 (24–59)	NS
3-month healing period			
All threads	39 ± 11 (16–65)	31 ± 6 (22–41)	.05
3 best	70 ± 11 (51–82)	53 ± 10 (34–67)	.005

NS = not significant; 3 best = the 3 consecutive threads with the most bone-to-metal contact.

Table 4 Percentage of Bone Area, Mean ± SD (Range)

Group	Fluoride-modified implants	Control implants	*P*
1-month healing period			
All threads	29 ± 4 (24–36)	29 ± 5 (21–38)	NS
3 best	55 ± 9 (40–67)	56 ± 11 (37–73)	NS
3-month healing period			
All threads	31 ± 8 (20–47)	39 ± 15 (22–69)	.03
3 best	54 ± 13 (35–71)	68 ± 13 (42–84)	.04

NS = not significant; 3 best = the 3 consecutive threads with the most bone-to-metal contact.

Removal Torque and Interface Shear Strength

Implants in group A demonstrated a mean removal torque of 31 ± 11 Ncm for the fluoride-modified implants compared to 27 ± 8.5 Ncm for the control implants. The difference between the 2 groups was not statistically significant. The removal torques for the implants in group B (3 months) demonstrated increased retention. The fluoride-modified implants had a mean removal torque of 85 ± 16 Ncm, which was significantly higher ($P = .005$) than that of the control group (54 ± 12 Ncm).

The mean shear strength calculated for the fluoride-modified group (23 ± 9 N/mm²) was significantly greater than that calculated for the control group (15 ± 5 N/mm²; $P = .019$) (Table 2).

Histomorphometry

Group A. The bone-to-metal contact for all threads following a 1-month healing period was a mean of 35% ± 14% for the fluoride-modified implants and 26% ± 8% for the control implants. This difference was statistically significant ($P = .037$). The mean bone contact for the 3 best consecutive threads in the cortical region was 55% ± 15% for the fluoride-modified implants and 47% ± 10% for the control implants; this difference was not statistically significant (Table 3).

Comparisons of the bone area in all threads, as well as in the 3 best consecutive threads, revealed similar mean percentages (Table 4).

Group B. The bone-to-metal contact for all threads following a 3-month healing period was a

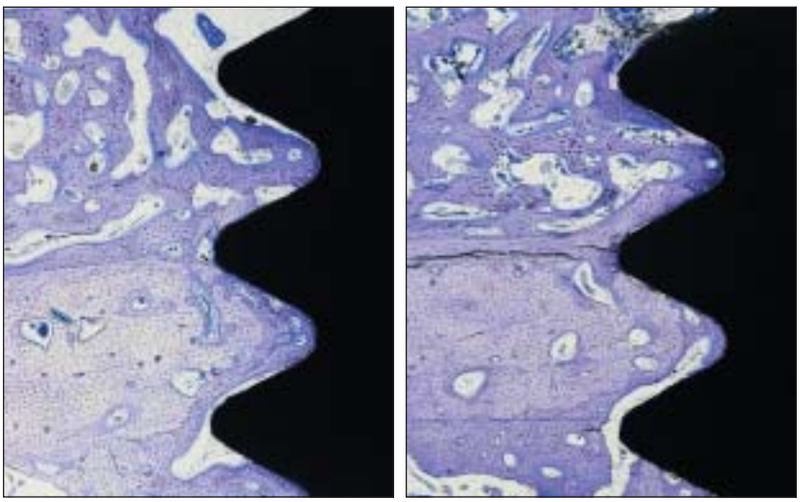

Fig 2 Stained, undecalcified cut-and-ground 10-µm-thick sections of *(left)* an unmodified titanium implant blasted with TiO_2 particles 1 month after placement in rabbit cortical bone and *(right)* an implant with a TiO_2-blasted and fluoride-modified surface 1 month after placement in rabbit cortical bone. The newly formed and immature bone being formed in the periosteal and endosteal areas appears darker than the old cortical bone. Bone remodeling activity can be observed in the bone inside the threads. The distance between the threads is 600 µm (toluidine blue mixed with pyronin G).

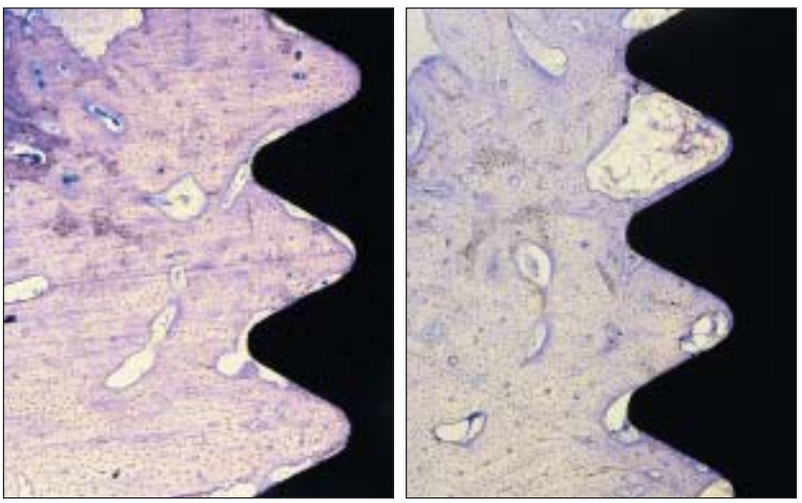

Fig 3 Stained, undecalcified cut-and-ground 10-µm-thick sections of *(left)* an unmodified titanium implant blasted with TiO_2 particles 3 months after placement in rabbit cortical bone and *(right)* an implant with a TiO_2-blasted and fluoride-modified surface 3 months after placement in rabbit cortical bone. The newly formed and immature bone being formed in the periosteal and endosteal areas appears darker than the old cortical bone. Bone remodeling activity can be observed in the bone inside the threads. The distance between the threads is 600 µm (toluidine blue mixed with pyronin G).

mean of 39% ± 11% for the fluoride-modified group, which was significantly greater ($P = .049$) than that of the control group (31% ± 6%). The mean percentage as calculated using the 3 best consecutive threads in the cortical region was 70% ± 11% for the fluoride-modified samples compared to 53% ± 10% for the control group ($P = .005$) (Table 3).

The mean percentage of bone area in all threads was 31% ± 8% for the fluoride-modified samples compared to 39% ± 15% for the control samples ($P = .028$). The mean percentage of bone area in the 3 best consecutive threads in the cortical region was 54% ± 13% for the fluoride-modified samples compared to 68% ± 13% for the control samples ($P = .036$) (Table 4).

Qualitative Observation

For most of the implants with fluoride-modified surfaces in group B, a rather loud clicking sound was heard when the implants were loosened to test removal torque. This was not demonstrated for the control group, nor was it demonstrated after 1 month of healing (ie, for group A).

The 1-month samples revealed a callus formation in the periosteal region, and newly formed bone could be observed in the endosteal part. There was a clear demarcation line between the old cortical bone and the newly remodeled bone inside the threads for both test and control sections. No qualitative differences could be observed.

The 3-month samples revealed the presence of more mature bone compared to the 1-month group (Figs 2 and 3). Periosteal bone tissue formation could be observed on the nonthreaded part toward the implant head on both the fluoride-modified and control implants. The fluoride-modified implants seemed to be covered by a thin collar of bone, outside of which there were marrow spaces, whereas in the control sections a larger amount of the threads were filled with mature bone. Irrespective of the

type of implant surface, one could observe multinucleated giant cells.

DISCUSSION

A significant increase in the retention of implants in bone has been observed in favor of TiO_2 grit–blasted implants compared to turned implants[12,17,38,39] Ellingsen demonstrated improved retention for fluoride-treated turned titanium implants compared to untreated turned implants.[25] Significantly greater retention has also been reported for TiO_2-blasted implants with a fluoride-modified oxide surface layer.[40] In this study, bone was attached to the implant surface after the removal torque of the implants had been tested. In the present study TiO_2-blasted implants, treated in diluted hydrofluoric acid, demonstrated a significantly better bone response than TiO_2-blasted implants not exposed to the diluted hydrofluoric acid treatment. Fluoride-modified implants demonstrated greater shear strength and an increased bone-to-metal contact ratio.

The fluoride-modified implants revealed slightly smoother surfaces with respect to height deviation as compared to the control samples. However, the average wavelength increased for the fluoride-modified implants. This was interesting since for most surfaces, a decrease in S_a is often followed by a decrease in S_{cx}.[13,41] Several experimental studies have shown a positive correlation between an increased height deviation and bone fixation[11,16,42,43]; also within in the range observed in the present study.[27,41,44,45] However, in this study it was not possible to confirm such a finding, indicating that other surface properties of the fluoride-modified implants may play more important roles in bone-to-implant retention than surface roughness as demonstrated by the S_a parameter alone. One possibility may be that the wavelength also is an important factor when characterizing the surface roughness and its role in bone-implant integration. Other surface morphologic and physiochemical phenomena, which were not identified by the methods used in the present study, may also be of significance.

Another possible explanation for the findings in the present study is that surface modification with fluoride changes the surface chemical structures, making them more suitable for bone bonding. The formation of fluoridated HA and fluoroapatite in calcified tissues has been documented.[46,47] An increased seeding rate of apatite crystals, stimulation of osteoprogenitor cells, elevation of the alkaline phosphatase activity, and enhancement of the incorporation of newly formed collagen into the bone matrix are all reported effects of fluoride in calcified tissue.[48–50] These factors may help improve the bone-to-implant interface. Incorporation of fluoride in the surface oxide layer may then aid the bonding of bone cells and calcified tissue to the implant surface.[51] Increased nucleation activity by CaP on the surfaces of titanium implants with a fluoride-modified oxide layer has been demonstrated in vitro; such an effect with increased affinity for calcium and phosphate ions may also be true in an in vivo situation.[26] It could also well be that the small morphologic surface changes that were observed on the fluoride-modified implants compared to the control implants had a beneficial effect on the bone healing. Even though the fluoride-modified surface had lower S_a values, this minor change in morphology, as detected with these techniques, may have had a major biologic input.

Although a significantly higher bone-to-metal contact was recorded after 1 month, this was not reflected by a significant increase in removal torque. This can be explained by the fact that the newly formed bone was not mature and may thus not have enough strength to retain the implants in bone when the torque forces were applied. Three months after placement, the new bone formed around the implants in the test group was more organized and matured, and thus had a higher capacity to withstand the removal torque force. This could explain the significantly higher removal torque values recorded for the fluoride-modified group after a 3-month healing period.

No significant difference in percentage of bone area in the threads was found at 1 month, but after 3 months the control samples had a significantly higher percentage of bone in the threads. Since these implants had less bone-to-implant contact, the increased bone mass at a distance from the implant surface may be explained as a biomechanically regulated compensation in bone support.

CONCLUSION

In the present study, titanium implants with a fluoride-modified TiO_2 surface had improved biomechanical anchorage in bone compared to titanium implants with an unmodified TiO_2 surface. Fluoride-modified implants also achieved greater bone integration than unmodified titanium implants after a shorter healing time. The continuous demand to reduce the period between implant placement and loading, as well as as widened indications for implant operations with regard to bone quality, require improved implants. Fluoride-modified titanium implants have promising prospects for improved clinical results.

REFERENCES

1. Brånemark P-I, Adell R, Breine U, Hansson BO, Lindstrom J, Ohlsson A. Intra-osseous anchorage of dental prostheses. I. Experimental studies. Scand J Plastic Reconstruct Surg 1969;3:81–100.
2. Goodman SB, Fornasier VL, Lee J, Kei J. The effects of bulk versus particulate titanium and cobalt chrome alloy implanted into the rabbit tibia. J Biomed Mater Res 1990; 24:1539–1549.
3. Johansson CB, Han CH, Wennerberg A, Albrektsson T. A quantitative comparison of machined commercially pure titanium and titanium-aluminum-vanadium implants in rabbit bone. Int J Oral Maxillofac Implants 1998;13:315–321.
4. Linder L, Obrant K, Boivin G. Osseointegration of metallic implants. II. Transmission electron microscopy in the rabbit. Acta Orthop Scand 1989;60:135–139.
5. Linder L. Osseointegration of metallic implants. I. Light microscopy in the rabbit. Acta Orthop Scand 1989;60:129–134.
6. Schroeder A, Zypen van der E, Stich H, Sutter F. The reactions of bone, connective tissue, and epithelium to endosteal implants with titanium-sprayed surfaces. Clin Oral Implants Res 1992;3:77–84.
7. Adell R, Eriksson B, Lekholm U, Brånemark P-I, Jemt T. Long-term follow-up study of osseointegrated implants in the treatment of totally edentulous jaws. Int J Oral Maxillofac Implants 1990;5:347–359.
8. Arvidson K, Bystedt H, Frykholm A, von Konow L, Lothigius E. Five-year prospective follow-up report of the Astra Tech Dental Implant System in the treatment of edentulous mandibles. Clin Oral Implants Res 1998;9:225–234.
9. Makkonen TA, Holmberg S, Niemi L, Olsson C, Tammisalo T, Peltola J. A 5-year prospective clinical study of Astra Tech dental implants supporting fixed bridges or overdentures in the edentulous mandible. Clin Oral Implants Res 1997;8: 469–475.
10. Palmer RM, Palmer PJ, Smith BJ. A 5-year prospective study of Astra single tooth implants. Clin Oral Implants Res 2000;11:179–182.
11. Wong M, Eulenberger J, Schenk R, Hunziker E. Effect of surface topology on the osseointegration of implant materials in trabecular bone. J Biomed Mater Res 1995;29:1567–1575.
12. Gotfredsen K, Berglundh T, Lindhe J. Anchorage of titanium implants with different surface characteristics: An experimental study in rabbits. Clin Implant Dent Relat Res 2000;2:120–128.
13. Wennerberg A, Albrektsson T, Johansson C, Andersson B. Experimental study of turned and grit-blasted screw-shaped implants with special emphasis on effects of blasting material and surface topography. Biomaterials 1996;17:15–22.
14. Wennerberg A, Albrektsson T, Andersson B. Bone tissue response to commercially pure titanium implants blasted with fine and coarse particles of aluminum oxide. Int J Oral Maxillofac Implants 1996;11:38–45.
15. Cordioli G, Majzoub Z, Piattelli A, Scarano A. Removal torque and histomorphometric investigation of 4 different titanium surfaces: An experimental study in the rabbit tibia. Int J Oral Maxillofac Implants 2000;15:668–674.
16. Ronold HJ, Ellingsen JE. Effect of micro-roughness produced by TiO_2 blasting—Tensile testing of bone attachment by using coin-shaped implants. Biomaterials 2002;23:4211–4219.
17. Gotfredsen K, Karlsson U. A prospective 5-year study of fixed partial prostheses supported by implants with machined and TiO_2-blasted surface. J Prosthodont 2001;10:2–7.
18. Weibrich G, Buch RS, Wegener J, Wagner W. Five-year prospective follow-up report of the Astra Tech standard dental implant in clinical treatment. Int J Oral Maxillofac Implants 2001;16:557–562.
19. Steveling H, Roos J, Rasmusson L. Maxillary implants loaded at 3 months after insertion: Results with Astra Tech implants after up to 5 years. Clin Implant Dent Relat Res 2001;3:120–124.
20. Sennerby L, Thomsen P, Ericson L. Ultrastructure of the bone-titanium interface in rabbits. J Mater Sci Mater Med 1992;3:262–271.
21. McKee MD, Nanci A. Secretion of Osteopontin by macrophages and its accumulation at tissue surfaces during wound healing in mineralized tissues: A potential requirement for macrophage adhesion and phagocytosis. Anat Rec 1996;245: 394–409.
22. McKee MD, Nanci A. Osteopontin: An interfacial extracellular matrix protein in mineralized tissues. Connect Tissue Res 1996;35:197–205.
23. Nanci A, McCarthy G, Zalzal S, Clokie C, Warshawsky H, McKee M. Tissue response to titanium implants in the rat tibia: Ultrastructural, immunocytochemical, and lectincytochemical characterization of the bone-titanium interface. Cells Mater 1994;4:1–30.
24. Ellingsen JE, Pinholt EM. Pretreatment of titanium implants with lanthanum ions alters alters the bone reaction. J Mater Sci Mater Med 1995;6:125–129.
25. Ellingsen JE. Pre-treatment of titanium implants with fluoride improves their retention in bone. J Mater Sci Mater Med 1995;6:749–753.
26. Ellingsen JE. On the properties of surface-modified titanium. In: Davies JE (ed). Bone Engineering. Toronto: Em Squared, 2000:183–189.
27. Wennerberg A, Albrektsson T, Lausmaa J. Torque and histomorphometric evaluation of c.p. titanium screws blasted with 25- and 75-microns-sized particles of Al_2O_3. J Biomed Mater Res 1996;30:251–260.
28. Johansson CB. On Tissue Reactions to Metal Implants [thesis]. Göteborg: Göteborg Univ, 1991.
29. Gottlander M. On Hard Tissue Reactions to Hydroxyapatite-Coated Titanium Implants [thesis]. Göteborg: Göteborg Univ, 1994.
30. Wennerberg A. On Surface Roughness and Implant Incorporation [thesis]. Göteborg: Göteborg Univ, 1996.
31. Rasmusson L. On Implant Integration in Membrane-Induced and Grafted Bone [thesis]. Göteborg: Göteborg Univ, 1998.
32. Ivanoff C-J. On Surgical and Implant Related Factors Influencing Integration and Function of Titanium Implants. Experimental and Clinical Aspects [thesis]. Göteborg: Göteborg Univ, 1999.
33. Sul Y-T. On the Bone Tissue Response to Oxidized Titanium Implants: The Role of Microporous Structure and Chemical Composition of the Surface Oxide in Enhanced Osseointegration [thesis]. Göteborg: Göteborg Univ, 2002.
34. Donath K, Breuner G. A method for the study of undecalcified bones and teeth with attached soft tissues. The Sage-Schliff (sawing and grinding) technique. J Oral Pathol 1982;11:318–326.
35. Johansson CB, Morberg P. Importance of ground section thickness for reliable histomorphometrical results. Biomaterials 1995;16:91–95.
36. Han CH, Johansson CB, Wennerberg A, Albrektsson T. Quantitative and qualitative investigations of surface enlarged titanium and titanium alloy implants. Clin Oral Implants Res 1998;9:1–10.

37. De Rezende MLR, Johansson CB. Quantitative bone tissue response to commercially pure titanium implants. J Mater Sci Mater Med 1993;4:233–239.
38. Gotfredsen K, Wennerberg A, Johansson C, Skovgaard LT, Hjorting-Hansen E. Anchorage of TiO_2-blasted, HA-coated, and machined implants: An experimental study with rabbits. J Biomed Mater Res 1995;29:1223–1231.
39. Ivanoff CJ, Hallgren C, Widmark G, Sennerby L, Wennerberg A. Histologic evaluation of the bone integration of $TiO_{(2)}$ blasted and turned titanium microimplants in humans. Clin Oral Implants Res 2001;12:128–134.
40. Ellingsen JE, Lyngstadaas SP. Increasing biocompatibility by chemical modification of titanium surfaces. In: Ellingsen JE, Lyngstadaas SP (eds). Bio-Implant Interface: Improving Biomaterials and Tissue Reactions. Boca Raton: CRC Press, 2003:319–336.
41. Wennerberg A, Ektessabi A, Albrektsson T, Johansson C, Andersson B. A 1-year follow-up of implants of differing surface roughness placed in rabbit bone. Int J Oral Maxillofac Implants 1997;12:486–494.
42. Feighan JE, Goldberg VM, Davy D, Parr JA, Stevenson S. The influence of surface-blasting on the incorporation of titanium-alloy implants in a rabbit intramedullary model. J Bone Joint Surg Am 1995;77:1380–1395.
43. Goldberg VM, Stevenson S, Feighan J, Davy D. Biology of grit-blasted titanium alloy implants. Clin Orthop 1995;319:122–129.
44. Wennerberg A, Albrektsson T, Andersson B, Krol JJ. A histomorphometric and removal torque study of screw-shaped titanium implants with three different surface topographies. Clin Oral Implants Res 1995;6:24–30.
45. Wennerberg A, Hallgren C, Johansson C, Danelli S. A histomorphometric evaluation of screw-shaped implants each prepared with two surface roughnesses. Clin Oral Implants Res 1998;9:11–19.
46. Baud CA, Bang S, Very JM. Minor elements in bone mineral and their effects on its solubility. J Biol Buccale 1977;5:195–202.
47. Gedalia I, Zipkin I. The Role of Fluoride in Bone Structure. St Louis: Warren H. Green, 1973.
48. Anderson PA, Copenhaver JC, Tencer AF, Clark JM. Response of cortical bone to local controlled release of sodium fluoride: The effect of implant insertion site. J Orthop Res 1991;9:890–901.
49. Farley JR, Wergedal JE, Baylink DJ. Fluoride directly stimulates proliferation and alkaline phosphatase activity of bone-forming cells. Science 1983;222:330–332.
50. Shteyer A, Liberman R, Simkin A, Gedalia I. Effect of local application of fluoride on healing of experimental bone fractures in rabbits. Calcif Tissue Res 1977;22(3):297–302.
51. Ellingsen JE. Surface configurations of dental implants. Periodontol 2000 1998;17:36–46.