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Soft tissue healing at titanium implants coated with type I collagen. An experimental study in dogs

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Abstract

Objective: To analyse the soft tissue healing at titanium implants coated with type 1 collagen.

Material and methods: Six dogs were used. The mandibular pre-molars and the three anterior maxillary pre-molars were extracted. Three months later mucoperiosteal flaps were raised and two test and two control implants were installed (3i® TG Osseotite®3.75 × 10 and 2.8 mm transmucosal collar). The test implants were coated with a purified porcine type I collagen. Cover screws were placed and flaps were sutured. The sutures were removed 2 weeks later and a plaque-control programme was initiated. Another 2 weeks later, the procedure was repeated in the contra-lateral mandibular region. Four weeks after the second implant surgery, biopsies were obtained and prepared for histological examination.

Results/Conclusion: The vertical dimensions of the epithelial and connective tissue components as well as the composition of the connective tissue portion facing the implant were similar at collagen-coated and uncoated implants after 4 and 8 weeks of healing. It is suggested that soft tissue healing to implants coated with type I collagen was similar to that at non-coated titanium implants and that no adverse reactions to the collagen-coated implants occurred.

Key words: connective tissue; dental implants; epithelium; histology; peri-implant mucosa; SEM

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Tissue integration to dental implants is a wound-healing process that involves several stages of tissue formation and degradation (for a review see Berglundh et al. 2003, Abrahamsson et al. 2004). The ensuing result of the modelling and remodelling events that occur in the bone tissue following implant installation is osseointegration, while the establishment of the mucosal barrier around the implant is characterized by the gradual shift from a coagulum to granulation tissue followed by the formation of a barrier epithelium and the maturation of the connective tissue (Berglundh et al. 2007).

Conflict of interest and source of funding statement

The authors declare that they have no conflict of interests.

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The soft tissue around implants was described in a series of experimental studies (Berglundh et al. 1991, Buser et al. 1992, Abrahamsson et al. 1996, 1997, 1999, Berglundh & Lindhe 1996, Cochran et al. 1997). Thus, the peri-implant mucosa consisted of a 2 mm long barrier epithelium and a 1–1.5 mm zone of ''connective tissue integration'' (Berglundh et al. 1991). Collagen fibres occured in large proportions and were mainly aligned in direction that was parallel to the implant surface. Further more, the connective tissue integration zone had a low density of blood vessels and a large number of fibroblasts. Ultra-structural analyses using transmission electron microscopy revealed that the fibroblasts in the interface zone appeared to be flat shaped and elongated in the vertical as well as in the horizontal plane (Moon et al. 1999, Abrahamsson et al. 2002).

The titanium/connective tissue interface at implants lacks a mechanical attachment of inserting collagen fibres similar to that of periodontal tissues at teeth. Surface modifications of titanium

implants may improve the ability for connective tissue components in the periimplant mucosa to attach to the implants. Findings from in vitro experiments indicated that cell adhesion to titanium discs that were coated with collagen was enhanced in comparison with un-coated titanium (Roessler et al. 2001, Nagai et al. 2002), while data from in vivo studies demonstrated that healing around collagen-coated implants in relation to un-coated implants resulted in larger bone formation and increased osseointegration (Schliephake et al. 2005). The aim of this study was to analyse the soft tissue healing at titanium implants coated with type 1 collagen.

Material and Methods

Animals

Six Labrador dogs, about 1 year old, were used. The protocol was approved by the regional Ethics Committee for Animal Research, Göteborg, Sweden. All surgical procedures were performed using general anaesthesia induced with propofol (10 mg/ml, 0.6 ml/kg) intravenously and sustained with N_2O:O_2 (1:1,5–2) and isoflurane employing endotracheal intubation.

Implants

The implants used (3i® TG Osseotite®; Implant Innovations Inc., Palm Beach Gardens, FL, USA) had a diameter of 3.75 mm and included a 10 mm long intraosseous portion and a 2.8 mm high transmucosal collar. The marginal 4.7 mm of the implant, i.e. the transmucosal collar, and about 2 mm of the intraosseous portion had a turned surface, while the remaining part of the implant had a dual acid-etched surface.

The test implants were coated with a purified porcine type I collagen using a two-step procedure described by Schliephake et al. (2003). Thus, collagen was integrated partially in an anodically formed oxide layer on the implant surface by an electrochemical process. The implants were subsequently immersed in a collagen solution for 10 min. (pH 7.4, 37°C).

Surgical procedures

The mandibular pre-molars and the first, second and third maxillary pre-molars

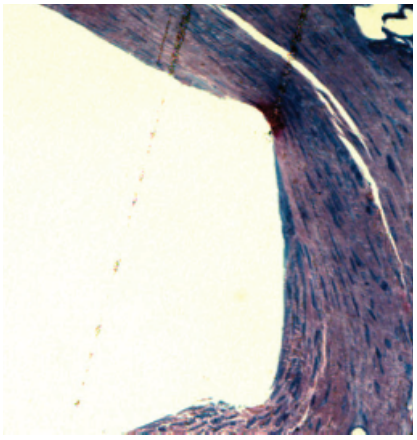

Fig. 2. Control site at 4 weeks of healing. Collagen fibres and fibroblasts are oriented parallel to the implant surface. Epon-embedded section, original magnification × 400.

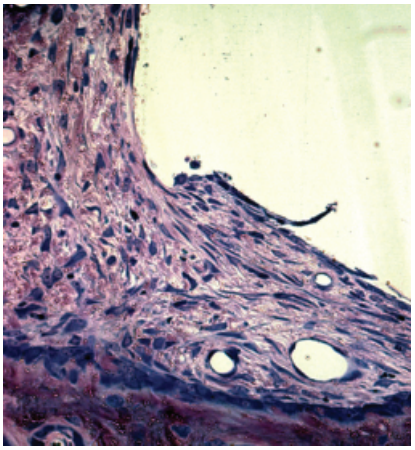

Fig. 3. Test site at 4 weeks of healing. Collagen fibres and fibroblasts are oriented parallel to the implant surface. Epon-embedded section, original magnification × 400.

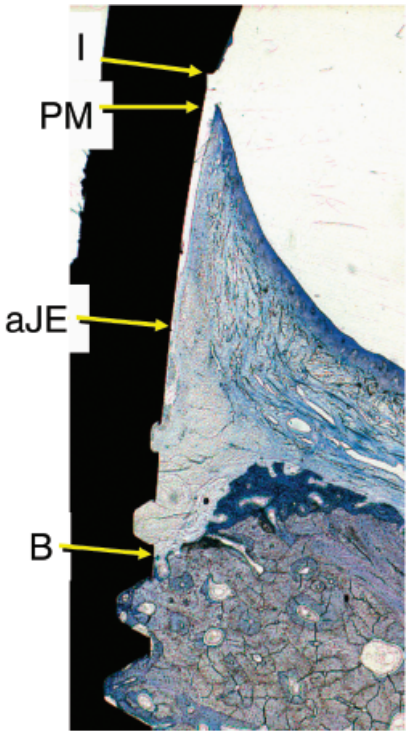

Fig. 1. Ground section showing the landmarks used for the histometric measurements. I, the implant margin; PM, the marginal portion of the peri-implant mucosa; aJE, the apical extension of the barrier epithelium; B, the marginal level of bone to implant contact, original magnification × 50.

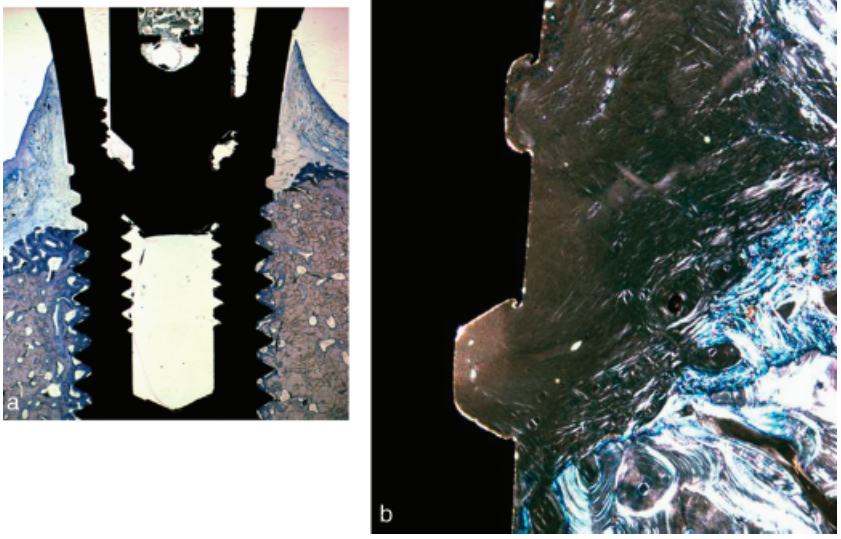

Fig. 4. (a) Ground section of a control implant and surrounding soft- and hard-tissues at 8 weeks of healing, original magnification × 25. (b) Detail of (a) in polarized light. Collagen fibres in different directions, original magnification × 200.

Fig. 5. (a) Ground section of a test implant and surrounding soft- and hard- tissues at 8 weeks of healing. Original magnification × 25. (b) Detail of (a) in polarized light. Collagen fibres orientated towards the implant surface, original magnification × 200.

Table 1. Linear distances between landmarks

mm	Four weeks		Eight weeks	
	control	test	control	test
I–B	3.84 (0.52)	3.81 (0.56)	4.72 (1.15)	4.00 (0.36)
PM–B	2.72 (0.43)	2.88 (0.43)	3.28 (0.63)	2.91 (0.32)
PM–aJE	1.35 (0.42)	1.38 (0.21)	1.47 (0.33)	1.55 (0.22)
aJE–B	1.37 (0.29)	1.49 (0.30)	1.81 (0.87)	1.36 (0.22)

Mean values and (SD).
I, implant margin; B, marginal level of bone to implant contact; PM, marginal portion of the periimplant mucosa; aJE, apical extension of the barrier epithelium; SD, standard deviation.

Table 2. Proportions of connective tissue components

%	Four weeks		Eight weeks	
	control	test	control	test
Co	57.4 (4.7)	55.0 (5.2)	70.2 (6.9)	69.8 (4.0)
V	9.1 (2.1)	11.7 (3.0)	5.7 (3.7)	5.4 (1.4)
Fi	14.8 (1.1)	14.9 (1.6)	13.5 (1.0)	12.7 (1.5)
Mø	2.7 (1.6)	3.3 (1.4)	0.8 (0.5)	0.7 (0.3)
Ly	2.9 (1.3)	3.5 (1.1)	1.8 (0.6)	2.4 (0.8)
Pc	0.2 (0.4)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)
PMN	3.2 (1.5)	3.1 (1.2)	1.0 (0.6)	1.1 (0.6)
R	9.8 (2.5)	8.5 (1.5)	6.8 (2.0)	7.9 (1.9)

Mean values and (SD).
Co, collagen; V, vascular structures; Fi, fibroblasts; Mø, macrophages; Ly, lymphocytes; Pc, plasma cells; PMN, polymorphonuclear leucocytes, R, residual tissue; SD, standard deviation.

were extracted. Three months later a crestal incision was made in the left or right edentulous mandibular pre-molar region. Buccal and lingual mucoperiosteal flaps were raised and in four sites osteotomy preparations to a depth of 10 mm were made. Two test and two control implants were subsequently installed in a randomized order. Cover screws were placed and flaps were adjusted and sutured.

The sutures were removed 2 weeks after implant placement. A plaque-control programme including cleaning of exposed implant surfaces and teeth with toothbrush every second day was

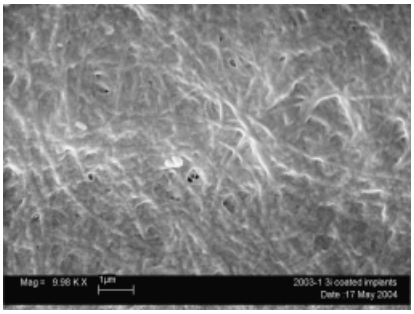

Fig. 6. Scanning electron micrograph from a pristine collagen-coated implant surface (test).

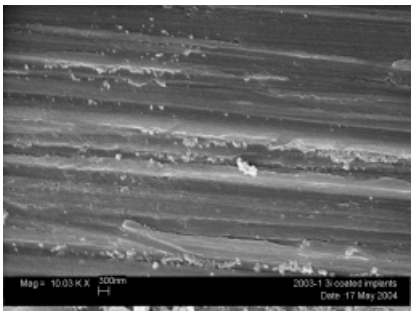

Fig. 7. Scanning electron micrograph from a pristine control implant surface with horizontal grooves and titanium remnants from the machining procedure.

initiated. Another 2 weeks later, i.e. 4 weeks after the first implant surgery, the implant installation procedure was repeated in the contra-lateral mandibular region. Sutures were removed 2 weeks later and the plaque-control procedures were applied to the newly installed implants. Another 2 weeks later, i.e. 8 weeks after the first implant placement and 4 weeks after the second implant surgery, clinical examinations including assessments of plaque and soft tissue inflammation were performed. The animals were euthanized by an overdose of Sodium Pentothal and perfusion through the carotid arteries with a fixative. The fixative consisted of a mixture of 5% glutaraldehyde and 4% formaldehyde buffered to pH 7.2 (Karnovsky 1965). The mandibles were removed and placed in the fixative. Each implant region, was dissected using a diamond saw (Exakt®, Kulzer, Germany). From each pre-molar region two implant units (one control and one test) were processed for ground sectioning (Donath & Breuner 1982, Donath 1988). The remaining biopsies were processed using a modification of the fracture technique (Thomsen & Ericson 1985) as described by Berglundh et al. (1991, 1994).

Ground sections

The tissue blocks selected for ground sectioning were dehydrated in serial steps of alcohol concentrations and subsequently embedded in a methyl–methacrylate resin (Technovit® 7200 VLC, Exakt®). Using a cutting–grinding unit and a micro-grinding system (Exakt®, Apparatebau, Norderstedt, Germany), the blocks were cut in a buccal–lingual plane and two central sections were obtained. The remaining mesial and distal portions of the tissue block were remounted and two central sections in a mesio-distal plane were prepared. All sections were reduced to a final thickness of approximately 20 μm. Thus, from each implant block two mesio-distal and two buccal–lingual ground sections were obtained. The sections were stained in toluidine blue (Donath 1993).

Fracture technique

The tissue samples selected for the ''fracture technique'' were placed in ethylene diamine tetra-acetic acid (EDTA). Incisions that were parallel with the long axis of the implant were made through the peri-implant tissues before the hard tissue was fully decalcified. Four different units (mesio-buccal, disto-buccal, mesio-lingual, disto-lingual) were thereby obtained. Decalcification was completed in EDTA and dehydration performed in serial steps of ethanol concentrations. Following secondary fixation in OsO_4, the specimens were embedded in epoxy resin (EPON® Fluka Chemie GmbH, Buchs, Switzerland) (Schroeder 1969). Sections were produced with the microtome set at 3 μm and stained in PAS and toluidine blue (Schroeder 1969).

Histological examination was performed in a Leica DM-RBE® microscope (Leica, Heidelberg, Germany) equipped with an image system Q-500 MC® (Leica, Heidelberg, Germany).

Histometric measurements

The ground sections were used for the linear measurements and the following landmarks were identified (Fig. 1): the implant margin (I), the marginal portion of the periimplant mucosa (PM), the marginal level of bone to implant contact (B) and the apical extension of the barrier epithelium (aJE). The vertical distances between the landmarks were determined in a direction parallel to the long axis of the implant.

Morphometric analysis

The morphometric measurements were performed in the EPON®-embedded sections using a point-counting procedure. The assessments at ''Level 4'' (Schroeder & Münzel-Pedrazzoli 1973, Berglundh et al. 1991, Abrahamsson et al. 1999) were confined to a 100 μm-wide zone of the connective tissue interposed between aJE and B. A lattice comprising 100 light points was superimposed over the tissue at a magnification of $\times$ 1000 and the relative proportions of the connective tissue occupied by collagen (Co), fibroblasts (Fi), vascular structures (V), macrophages (Mø), lymphocytes (Ly), plasma cells (Pc) polymorphonuclear leucocytes (PMN), and residual tissue (R), e.g. nerves, matrix components and unidentified structures, were determined. The assessments at ''Level 3'' (Schroeder 1973, Berglundh et al. 1992) were restricted to the barrier epithelium. A modified lattice comprising 400 points was superimposed over the epithelium at a magnification of $\times$ 1000 and the percentage of infiltrating leucocytes was evaluated.

Scanning electron microscope (SEM) analysis

The implants prepared according to the fracture technique (retrieved implants) were following the separation of peri-implant tissues examined in a SEM (Leica S420; Leica Microsystems, Heidelberg, Germany, equipped with a LEO Software 15XX). The implants were once again placed in a fixative (Karnovsky 1965) and dehydration was performed using serial steps of ethanol. The prepared implants were air dried and sputtered with gold. In addition, four new (pristine) implants (two test and two control implants) were also analysed in the SEM. In all implants, a 1-mm-high area at the level of the commencement of the treads was identified and analysed at different magnifications (range: $\times$ 50 to $\times$ 10.000)

Data analysis

Mean values were calculated for each implant unit and animal. Differences were analysed using the Student *t*-test for paired observations (the animal was used as the statistical unit; $n = 6$). The null hypothesis was rejected at $p < 0.05$.

Results

Healing was uneventful following implant installation for all 48 implants. At the end of the plaque control period, the implant surfaces and neighbouring teeth were free from visible plaque and the periimplant mucosa showed no signs of inflammation.

While healing at 4 weeks appeared to be incomplete in soft and hard tissues around both test and control implants, the peri-implant tissues at 8 weeks consistently exhibited well-organized structures of connective tissue and bone. Small inflammatory cell lesions in the marginal portion of the periimplant mucosa were occasionally observed in both test and control specimens.

Four weeks

In the 4-week-specimens, osseous remodelling was obvious in areas between threads as well as in crestal portions of the peri-implant bone. The overall morphology of the periimplant mucosa at test and control implants was similar at 4 weeks. In light microscopy utilizing polarized light, collagen fibres adjacent to test implants were aligned in an oblique direction to the implant surface. EPON-embedded sections produced from the fracture technique and representing 4 weeks of healing are illustrated in Figs 2 and 3. In the control specimens, the apical part of the connective tissue integration zone comprised dense bundles of collagen fibres organized in a direction parallel to the implant surface (Fig. 2). Comparable structures with fibroblasts interposed between collagen fibres were found in the corresponding compartments of the test units (Fig. 3).

Eight weeks

Remodelling of the peri-implant bone at 8 weeks was less conspicuous than at 4 weeks of healing. The dimensions of the barrier epithelium and the connective tissue interface to the implant were established and collagen fibres dominated the connective tissue. In the ground sections of the control implants, the soft tissues adhered to the implant surface. In polarized light, the collagen fibres in the supra-crestal connective tissue were organized in different direc-

tions and some of the fibre bundles had a course directed towards the implant surface (Fig. 4). The mucosa around the test implants also adhered to the implant surface. The collagen fibres in the supracrestal connective tissue were organized in different directions. Frequently, the collagen fibres appeared to be orientated towards the surface of the test implant (Fig. 5).

Linear measurements

The results from the histometric measurements are presented in Table 1. The distance I–B was 3.84 ± 0.52 mm for the control implants and 3.81 ± 0.56 mm for the test implants at 4 weeks. The corresponding dimensions of the 8-week specimens were 4.72 ± 1.15 and 4.00 ± 0.36 mm, respectively. The mucosal thickness (PM–B) in the 4-week specimens was 2.72 ± 0.43 mm at the control implants and 2.88 ± 0.43 mm at the test implants, while at 8 weeks the mucosal height was 3.28 ± 0.63 mm (control) and 2.91 ± 0.32 mm (test). An almost similar extension of the barrier epithelium (PM–aJE) at control and test implants was assessed at 4 weeks. At 8 weeks the barrier epithelium extended to a distance of 1.47 ± 0.33 mm (control) and 1.55 ± 0.22 mm (test) apical to the mucosal margin (PM). The dimensions of the connective tissue interface zone in sections representing 4 weeks was 1.37 ± 0.29 mm in the control implants and 1.49 ± 0.30 mm in the test implants. The corresponding dimensions at 8 weeks were 1.81 ± 0.87 and 1.36 ± 0.22 mm, respectively.

Morphometric measurements

The results from the morphometric measurements at Level 4 are presented in Table 2. The overall composition of the connective tissue portion between aJE and B, i.e. the ''connective tissue integration zone'', was similar in the test and control sites at 4 and 8 weeks of healing. Thus, the percentage of collagen (Co) increased from 57.4% (control) and 55.0% (test) at 4 weeks to 70.2% and 69.8% at 8 weeks, while the density of vascular structures at the two healing intervals decreased from 9.1% and 11.7% to 5.7% and 5.4% at the control and test sites, respectively. The fibroblast density remained more or less unchanged between 4 and 8 weeks of healing in the control and test sites. There was also a decrease in the proportions of leucocytes (Mø, Ly, Pc, PMN) between 4 and 8 weeks of healing in both the control and test sites. While plasma cells were virtually absent in all specimens, the densities of the remaining group of leucocytes varied between 2.7% and 3.5% at 4 weeks and between 0.8% and 2.4% at 8 weeks.

The proportion of infiltrating leucocytes in the barrier epithelium in the 4-week specimens was 6.1% in the control implants and 5.9% in the test implants. The corresponding densities at 8 weeks were 5.5% and 7.3%, respectively.

SEM analysis

The coated surface of the pristine test implants was characterized by the presence of dense layer of fibrils, which in large magnification ($\times$ 10,000, Fig. 6) appeared to be organized in a woven pattern. The pristine control implants, however, exhibited horizontal grooves and titanium remnants resulting from the machining procedure (Fig. 7).

Retrieved implants

The implants that were retrieved following the fracture technique preparation were also exposed to SEM analysis. Tissue remnants of epithelium and connective tissue structures were identified in thread-associated areas on both test and control implants at 4 and 8 weeks of healing. The amount and location of the tissue remnants were similar in the test and control groups. Except for the residual tissue structures, there were no signs of remnants from the collagen coating on the test implants. In SEM – analysis, the retrieved test implants exhibited surface characteristics similar to that of the control implants.

Discussion

In this study, the mucosal attachment to collagen-coated titanium implants was analysed. It was demonstrated that the vertical dimensions of the epithelial and connective tissue components of the soft tissue/implant interface as well as the composition of the connective tissue portion facing the implant was similar at coated (test) and uncoated (control) implants after 4 and 8 weeks of healing. It is suggested that soft tissue healing to implants coated with type I collagen was similar to that at non-coated titanium implants and that no adverse reactions to the collagen coating of the test implants occurred in the mucosa.

The current experiment evaluated healing at 4 and 8 weeks following implant placement. In several previous experimental studies on the structure and function of the mucosa around implants, considerably longer healing periods were utilized. Thus, Berglundh et al. (1991) and Abrahamsson et al. (1996, 1997, 1998) examined the peri-implant mucosa in dogs. Implants were placed using a one- or two-stage procedure and biopsies were obtained for histological analyses following healing periods varying between 4 and 6 months. In the studies referred to, it was reported that the dimensions of the components soft tissue interface to titanium, i.e. barrier epithelium and connective tissue zones, were about 2 and 1–1.5 mm, respectively. The data regarding the apical extension of the barrier epithelium and the height of the ''connective tissue integration zone'' presented in this study, however, were different. Thus, at the test and control implants evaluated at 4 and 8 weeks of healing, considerably shorter distances were found between the margin of the mucosa and the apical cells of the epithelium. This variation in results may be related to differences in healing periods but also to differences in the geometry between the implants used in this experiment and in the studies referred to. Recently, the early and later stages of soft tissue healing to implants were described in detail (Berglundh et al. 2007). Berglundh et al. (2007) placed solid screw titanium implants of the ITI–implant system in a one-stage procedure in Labrador dogs and healing was evaluated in periods extending from day 0 (2 h) up to 12 weeks. It was reported that the first signs of epithelial proliferation were observed in specimens representing 1–2 weeks of healing and a mature barrier epithelium with a dimension of about 2 mm occurred after 6–8 weeks of healing. While healing at 4 weeks in the study by Berglundh et al. (2007) was considered incomplete, the findings from the analyses made on sections representing 6, 8 and 12 weeks of healing were judged as representing mature and established characteristics of the periimplant mucosa. The apical extension of the barrier epithelium that was assessed in the 4-week specimens of the current experiment may therefore be considered

as incomplete. On the other hand, the dimensions determined at 8 weeks of healing in the present material, which according to Berglundh et al. (2007) should represent a completed stage of healing, remained smaller than 2 mm in both test and control sections.

The composition of the connective tissue portion of the soft tissue interface to the implant was similar at the test and the control units at both 4 and 8 weeks of healing. It was evident, however, that the proportion of collagen increased, whereas the density of vessels and leucocytes decreased from 4 to 8 weeks of healing. This observation is consistent with data reported in the study by Berglundh et al. (2007) referred above. They found that the collagen content increased, while the proportions of inflammatory cells and vascular structures diminished in the connective tissue interface zones from the early stages of 1–2 weeks up to 6–8 weeks of healing.

In this study, the amount of leucocytes that infiltrated the barrier (junctional) epithelium was analysed. This examination procedure, which was described originally by Schroeder (1973), was applied in previous experiments to illustrate differences in attachment of epithelial cells to the enamel of teeth. Berglundh et al. (1989, 1992) studied the tissue response to plaque formation at deciduous and permanent teeth in beagle dogs and reported that larger fractions of transmigrating leucocytes were found in the junctional epithelium at deciduous than at permanent teeth. It was suggested that these differences reflected varying contact mechanisms between epithelial cells and the enamel of the two dentitions. The results regarding infiltrating leucocytes within the barrier epithelium in this study were similar at test and control implants at 4 weeks of healing. At 8 weeks of healing, however, the proportion of leucocytes at the test implants increased, while at the control implants the density of infiltrating cells slightly decreased. The presence of leucocytes in the barrier epithelium of the test and control units was not associated with inflammatory cell infiltrates in the adjacent connective tissue of the mucosa and, apparently, no differences in the epithelial cell attachment to the test and control implants could be identified.

Despite the lack of differences in the soft tissue dimensions and composition of the connective tissue between the tissues at the collagen-coated and the un-coated implants, the findings in this study indicate that no adverse reactions in terms of inflammatory response to the porcine-derived coating material occurred. The lack of inflammatory reactions to implanted collagen with e.g. porcine origin has also been reported in other animal experiments. Owens & Yukna (2001) evaluated resorption of three different types of collagen membranes in 12 dogs. At 1 month the membranes exhibited slight or moderate degradation, at 2 and 3 months degradation was severe, while at 4 months the membranes were either absent or severely degraded. The authors reported that inflammation was seen in only two out of 42 specimens. Rothamel et al. (2005) examined biodegradation of eight different types of collagen membranes in rats. It was reported that in four out of the eight membranes the degradation process was associated with a foreign body reaction and that only in two cases inflammatory cells seemed to be involved.

The SEM analysis made in this study revealed that the surface characteristics of the pristine test implants were different from those in the retrieved test implants. Thus, the dense layer of fibrils that was found on the surface of the pristine test implants could not be detected on the surface of the retrieved test implants. In the latter case, the titanium surface exhibited features of horizontal grooves similar to that of the control implants. This finding indicates that the collagen coating was integrated to the soft and hard periimplant tissues formed during healing following implant placement. As the collagen coating could not be identified in sections representing 4 and 8 weeks of healing, the observations from the SEM analysis also indicate that the coating may have degraded during the healing periods. Biodegradation of collagen of porcine and bovine origin was examined in the studies by Owens & Yukna (2001) and Rothamel et al. (2005) referred to above. The thickness of the collagen membranes used in the studies referred to was considerably different from the thin layer of collagen of the current test implants. The time required for total or partial degree of biodegradation may therefore not be comparable. Owens & Yukna (2001) in their experimental study in mongrel dogs as well as Rothamel et al. (2005) in the study in rats stated that substantial degradation of the collagen membranes occurred over time. There are reasons therefore to suggest that the thin layer of porcine-derived collagen coating on the test implants in this study became degraded already during 4 weeks of healing.

In summary, collagen coating of titanium implants did not result in a mechanical attachment between the implant surface and the surrounding mucosa.

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Clinical Relevance

Scientific rationale for the study: The soft tissue interface to Ti implants lacks a true mechanical attachment similar to that found in periodontal tissues at teeth. The attempt to provide an organic coating to the titanium surface, e.g. collagen, is made with the intention to improve the contact between the connective tissue and the implant.

Principal findings: Healing at coated and uncoated implants was similar and no mechanical attachment between coated implants and adjacent soft tissue was observed.

Practical implications: Additional research is required to improve the soft tissue contact to titanium implants.