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ULTRASTRUCTURAL OBSERVATIONS OF PERI-IMPLANT MUCOSA MORPHOLOGY AROUND DIFFERENT TYPES OF ABUTMENT IN HUMANS

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Abstract

Scanning electron microscopy (SEM) and transmission electron microscopy (TEM) were used to examine the morphological aspects of peri-implant mucosa around abutments of differing geometry (biconical and cylindrical) and of differing surface micromorphology. The samples were taken from seven patients who had undergone implant surgery at least one year prior to the study. In samples from biconical abutments, SEM of the sulcular epithelium showed that it consisted of flattened polygonal cells with a surface resembling a honeycomb. Superficial desquamation was rarely found. In contrast, in the samples from cylindrical abutments, the sulcular epithelium showed extensive desquamation and surface irregularity, but not the honeycomb structure in its superficial cells. TEM showed in both abutment types a morphologically normal epithelium, with a normal maturation cell pattern. Desquamation of the more superficial layers of the epithelium, associated with thinning in the superficial layer of flattened cells, was more evident around cylindrical abutments. In the transitional area between the sulcular and junctional epithelium, an intra-epithelial leukocyte infiltrate, and a larger amount of keratohyalin granules in the more superficial cells was observed. The morphological differences in peri-implant mucosa between the two abutment types may be related to differences in morphology of the metal surfaces of the abutments themselves.

Key Words: Peri-implant tissues, abutments, scanning electron microscopy, transmission electron microscopy.

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Introduction

The analysis of the implant/host tissue interface has to date focused mainly on the morphological aspects of bone tissue. Numerous studies have clarified the different relationships created between bone and implant (Brånemark, 1983: Brånemark *et al.*, 1977; Adell *et al.*, 1981; Albrektsson *et al.*, 1982, 1983, 1986, 1988; Ericsson *et al.*, 1986; Albrektsson, 1985, 1988; Adell and Eriksson, 1990).

The concept of osseointegration defined by Brånemark has been analyzed in all its aspects, and the formation of new bone on the surface of titanium implants has been examined in the context of many fixtures of differing geometry and surface treatments (Brunette *et al.*, 1983; Taylor and Gibbons, 1983; Inoue *et al.*, 1987; Lowenberg *et al.*, 1987; Brunette, 1988; Smith *et al.*, 1991; Cheroudi *et al.*, 1992; Könönen *et al.*, 1992; Cochran *et al.*, 1994; Daculsi and Delecrin, 1994).

Many studies, both clinical and experimental, have analyzed the implant/soft tissue interface in order to clarify the characteristics of the link that is created between implant and peri-implant mucosa in the junctional area just above the bone crest. The arrangement of the peri-implant fibers would seem to induce the formation of a seal at the edge of the implant. The most widely accepted current hypotheses claim that there is either an epithelial attachment or a connective tissue attachment on the implant, or a combination of both (Schroeder *et al.*, 1981; Gould *et al.*, 1984; Lekholm *et al.*, 1986a,b; Arvidson *et al.*, 1990; Steflik *et al.*, 1990; Berglundh *et al.*, 1991; Listgarten *et al.*, 1991, 1992; Strub *et al.*, 1991; Buser *et al.*, 1992; Weber and Fiorellini, 1992; Bauman *et al.*, 1993; Ruggeri *et al.*, 1994). In any case, this presumed seal has important implications for bacterial infiltration, and, accordingly, for the durability of the implant (Berglundh *et al.*, 1992; Warrer *et al.*, 1995). The importance of the health of soft tissues appears, therefore, to be fundamental to the long-term success of implant treatment. An important factor affecting the condition of the peri-implant mucosa could be the geometry and/or the micromorphology of

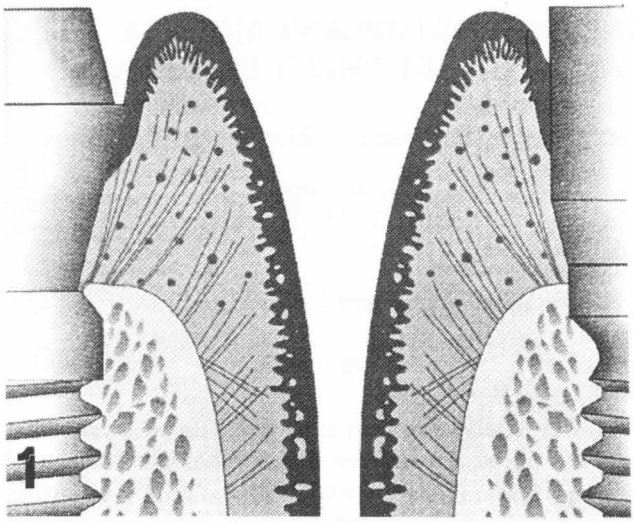

Figure 1. Schematic drawing of the abutments.

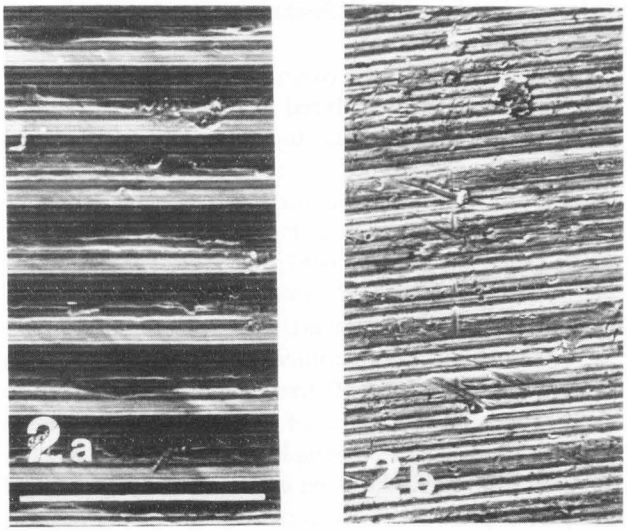

Figure 2. (**a**) Scanning electron micrograph of a surface of abutment A (bar = 0.1 mm). (**b**) Scanning electron micrograph of a surface of abutment B (bar = 0.1 mm).

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the surface of the abutments and/or the actual seal between abutment and fixture. The aim of our research was to analyze the morphology of peri-implant mucosa in the sulcular epithelium around two differing types of abutment (biconical and cylindrical) with differing surface micromorphology.

Materials and Methods

The study enrolled 7 patients with partial edentulism in the premolar and molar zone of the jaw. Each patient received a complete description of, and gave written

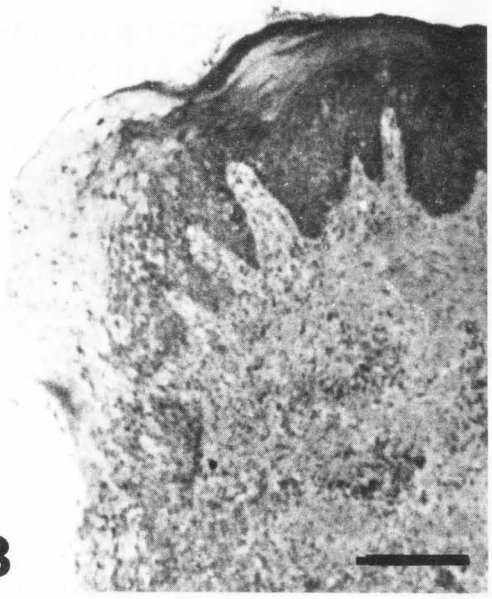

Figure 3. Semi-thin section of peri-implant mucosa showing the sulcular zone where ultrathin sections were cut (toluidine blue) (bar = 100 μm).

Figures 4-9 (*on facing page*). Scanning electron micrographs of A samples.

Figure 4. Surface of the transitional area between oral and sulcular mucosa. Desquamation of the epithelium of the masticatory gingiva (bar = 0.5 mm).

Figure 5. Regular appearance of the more superficial part of the sulcular epithelium made up of flat polygonal cells (bar = 0.1 mm).

Figure 6. Enlargement of the same area: cell edges are well defined and cells well connected (bar = 10 μm).

Figure 7. Fine confluent crests on the cellular surface (bar = 10 μm).

Figure 8. Cells of columnar appearance in the deeper part of the sulcular epithelium (bar = 10 μm).

Figure 9. Cords of collagen fibers parallel to the surface of the abutment with intercalated finer bundles perpendicular to the surface found in the transitional area between sulcular and junctional epithelium (bar = 10 μm).

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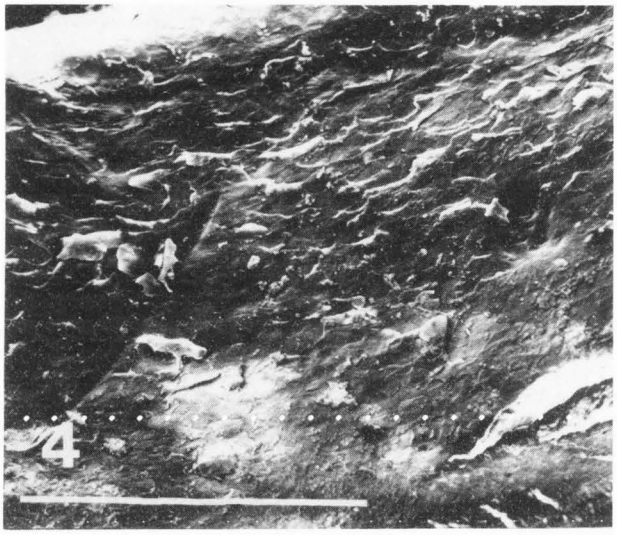
consent to, the proposed surgical procedure. In a two-stage submerged procedure, the first stage consisted in the fitting of each patient with one Standard Astra-Tech (Mölndal, Sweden) 13 mm fixture (Sample A) and one Mk II Nobelpharma 13 mm fixture (Nobel Biocare, Gothenburg, Sweden) (Sample B), both fixtures being located in the same edentulous molar region. Placement was guided by a surgical stent that was constructed on the basis of diagnostic waxing of the final prosthesis. The second stage was performed four months later, with insertion of the abutments (Astra-Tech Uni abutment,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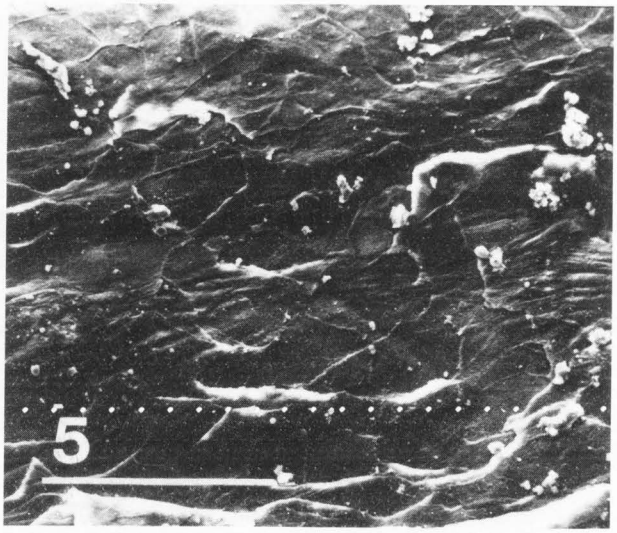
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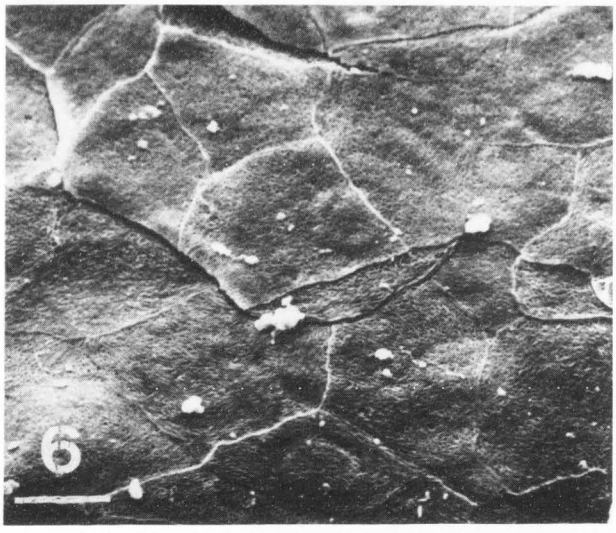
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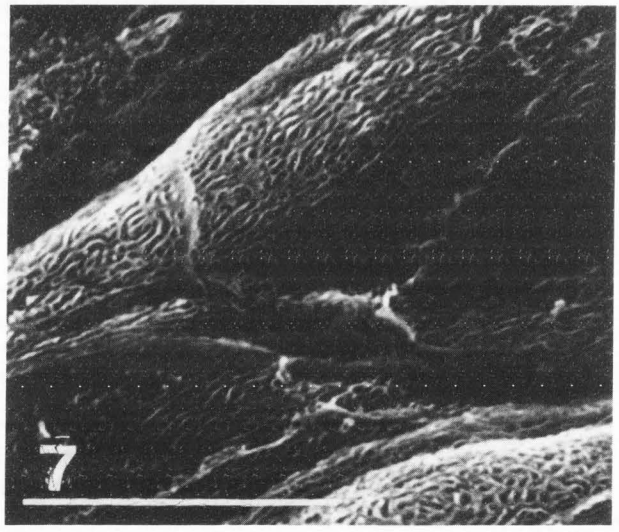
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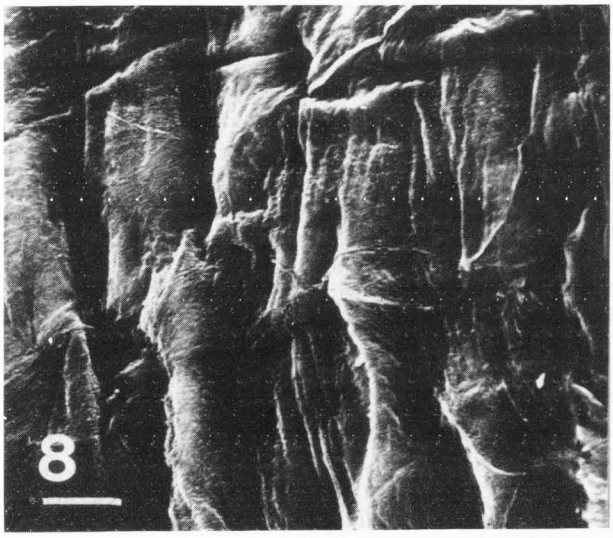
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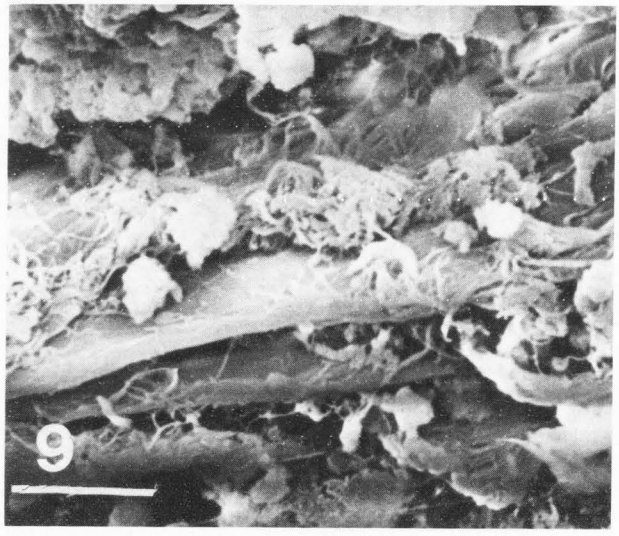
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Ref. 22032 (Sample A) and Nobelpharma Titanium abutment, Ref. SDCA 068 (Sample B)) (Fig. 1).

One month after the second stage, all patients were fitted with twin-component, palladium alloy and ceramic bridges with a closing edge; placement was in the supragingival area, and the bridges were respectively supported by the Astra-Tech and the Nobelpharma implants. The patients were rigorously encouraged to maintain appropriate oral hygiene by means of dental brush, super floss and interdental brushes. The check-up protocol consisted of a visit at 6 months from first stage surgery; at this check-up, the bridges were removed, and the peri-implant mucosa was clinically checked for reddening and edema, and for bacterial plaque and calculus. In all cases, the mucosa showed neither objective signs of inflammation, nor spontaneous bleeding; oral hygiene appeared to be excellent. The same conditions were observed at 12 months from surgery, when biopsies were performed around the abutments by means of circular blades. The diameter of these scalpels was such that the biopsy was one mm wider than the diameter of the abutment. The blade was inserted manually up to the bone crest; subsequently, the abutment and such peri-implant mucosa as appeared to be attached to the titanium were removed together.

After delicate rinsing in physiological solution, the peri-implant mucosa was carefully removed from the abutment and fixed in Karnovsky solution. The samples were then washed in cacodylate buffer and postfixed in 1% osmium tetroxide in the same buffer.

All fixed specimens were divided into two parts, one to be used for scanning electron microscopy (SEM) examination, and the other for transmission electron microscopy (TEM) examination.

The SEM samples were dehydrated in ascending concentrations of acetone, critical point dried with carbon dioxide, sputter coated in gold, and finally examined under a Philips 515 SEM (Philips Electron Optics, Eindhoven, The Netherlands).

The TEM samples were further divided into small fragments, dehydrated in ethanol, and embedded in Epon 812. The resin blocks were appropriately oriented and cut in semi-thin sections for the identification of the exact location of the sulcular epithelium (Fig. 3). Thin sections were then cut with a diamond knife, stained with uranyl acetate and lead citrate, and viewed with a Philips CM 12 STEM electron microscope.

Results

SEM examination of abutment type A revealed an undulating surface made up of remarkably wide peaks and troughs. Ridges perpendicular to the vertical axis of the abutment were visible on the whole surface of the metal (Fig. 2a). In contrast, SEM examination of the surface of abutment type B did not show undulation, but only ridges perpendicular to the vertical axis of the abutment, between which small circular craters could also be seen (Fig. 2b).

SEM of type A samples

Figure 4 shows the transitional zone between oral and sulcular mucosa. In our samples, we found a morphological difference between the two mucosae with, on one hand, an epithelium in desquamation in the masticatory gingiva and, on the other, a more compact and undamaged epithelium in the sulcular mucosa. SEM examination showed that the most superficial part of the sulcular epithelium, near the masticatory gingiva, was regular in appearance, with polygonal flattened cells, as in the covering epithelium. Desquamation was found in only a few areas of the epithelium (Fig. 5).

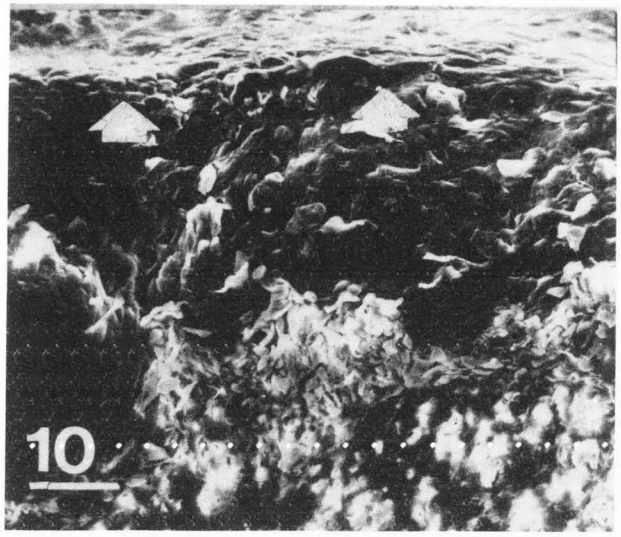
On the surface of the epithelium, at higher magnification, we found polygonal flat cells tightly joined together with well defined cell edges (Fig. 6). The cells had very fine anastomized crests, forming a honeycomb pattern on the cellular surface (Fig. 7). In the deeper part of the sulcular epithelium, the shape of the cells changed, taking on a columnar appearance. The cell edges were still well defined, and the surface of the cells maintained the features previously described (Fig. 8).

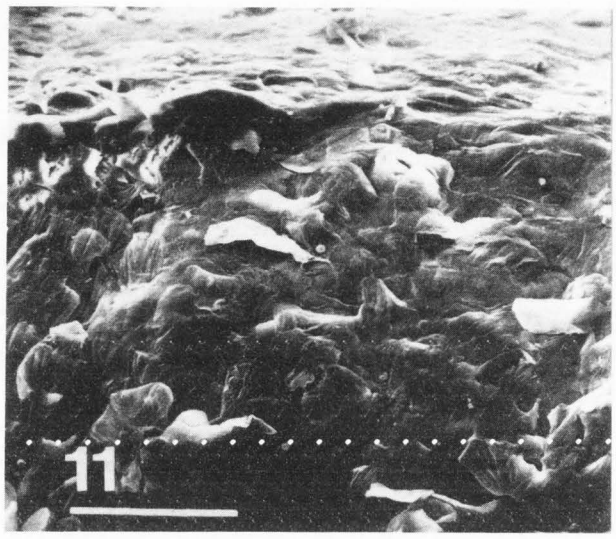
In the deepest part of the sulcular epithelium, on the boundary with the junctional epithelium, the cellular component was increasingly replaced by collagen fibers. We found layers of collagen fibers, some of which were directly parallel to the surface of the abutment, others perpendicular to the vertical axis of the abutment (Fig. 9). The latter seemed to be separated and withdrawn as a result of the traumatic detachment of the soft tissues from the surface of the abutment during biopsy.

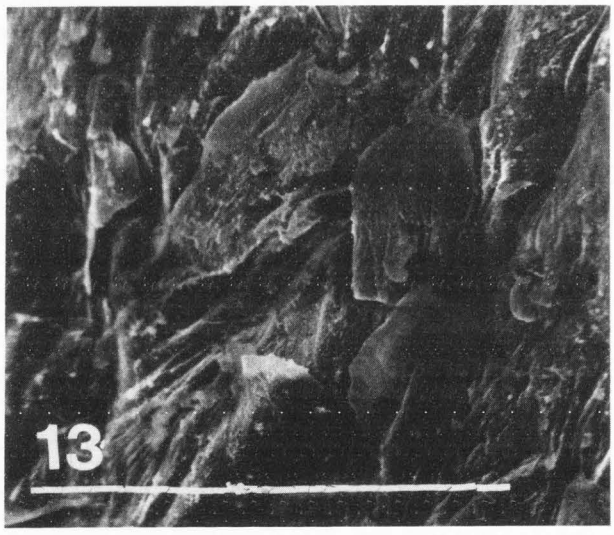
SEM of type B samples

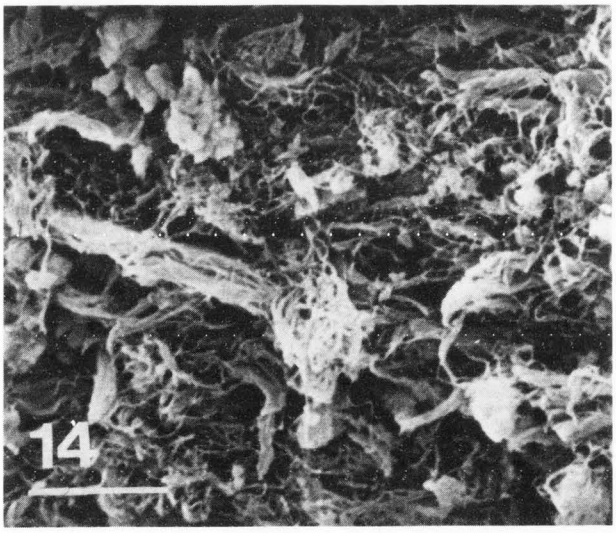
Figure 10 shows the transitional zones between the oral and sulcular mucosa. In these samples too, the sulcular mucosa appeared to be more compact than did the masticatory mucosa. Compared with type A samples, however, the sulcular epithelium was less regular and compact in appearance. We found irregular morphology in the surface of the sulcular epithelium near the masticatory gingiva (Fig. 11). At higher magnification, the cells revealed a more undulating surface and less well defined cell edges than did the type A samples. Desquamation was also more common and more marked (Fig. 12).

Analysis of the deepest part of the sulcular epithelium, close to the junctional area, revealed an appearance that continued to be non-uniform, mainly because of the superimposition of cells in desquamation.









Figures 10-14. Scanning electron micrographs of B samples.

Figure 10. Surface of transitional area between oral and sulcular mucosa (arrows). Sulcular epithelium is not regular or compact in appearance (bar = 0.1 mm).

Figure 11. The more superficial part of the sulcular epithelium has an undulating appearance, the cell edges are difficult to make out, and there is desquamation. (bar = 0.1 mm).

Figure 12. Enlargement shows that the cell edges are not well defined because of the superimposition of various layers of exfoliated material (bar = 10 μm).

Figure 13. Non-uniform appearance of the deeper part of the sulcular epithelium with frequent desquamation (bar = 0.1 mm).

Figure 14. Area between sulcular and junctional epithelium containing isolated or ball-shaped fine bundles of collagen fibers perpendicular to the surface of the abutment (bar = 10 μm).

Alterations in epithelialization were not uniform on all surfaces, but were more prevalent in some areas than in others (Fig. 13). Collagen fibers in the analogous area of type B samples lacked a precise orientation, and were very irregular. There seemed to be many bundles, made up of strips or balls of thinner collagen fibers, directly perpendicular to the surface of the abutment (Fig. 14).

Transmission electron microscopy

In the TEM, the two types of abutment did not reveal great morphological differences. In all samples examined, the covering of the gingival sulcus consisted of a squamous, stratified, non-keratinized epithelium with an underlying layer of connective tissue. The latter was raised in wide papillae and contained in addition to fibroblasts and a few inflammatory cells, bundles of collagen fibers without any definite spatial orientation, as well as some vasular structures (Figs. 15 and 16). The epithelium was, in general, well conserved, showing a normal pattern of cell differentiation from the basal layer to the superficial layer of flat cells. The basal layer was made up of cuboidal or cylindrical cells that were joined to the basement membrane by hemidesmosomes and that possessed a nucleus and the normal content of cytoplasmic organelles. Thin extensions (joined by desmosomes) connected adjacent cells, demarcating wide intercellular spaces that were occasionally full of inflammatory cells, the latter having migrated from the underlying connective tissue (Fig. 17).

In the intermediate layer of the epithelium, the cells were polygonal, and contained a greater number of tonofilaments and desmosomes. Near the surface, the cells tended to have nuclear alterations, to be progressively flatter, and to have lost a large part of their cytoplasmic organelles. Compared with cells in deeper layers, cytoplasm density in the flat cells showed remarkable variability, which was linked to the level of thickening in the fibrillar and granular cytoplasmic components (Fig. 18). Their border was also more irregular because of the great number of short cytoplasmic projections that were thickly interwoven with those of adjacent cells and bound to each other by desmosomes (Fig. 19). Such projections were also visible on the free surface of the epithelium, where they at times came into contact with the bacteria of the oral cavity (Fig. 20).

The only ultrastructural differences between samples A and B that we could detect concerned the more superficial layers of the epithelium, and were more obvious in B samples. In the latter, we noted a reduced thickness in the superficial layer of flat cells (6-8 layers vs. 14-16 layers) associated with greater superficial desquamation (Fig. 21). We also observed intracellular keratohyalin granules and intra-epithelial leukocyte infiltrates in the transitional zone between the sulcular and junctional epithelium (Fig. 22).

Figures 15-20 *(Figures 15-18 on facing page, figures 19 and 20 on page 76).* Transmission electron micrographs of samples A and B.

Figure 15. Bundles of collagen fibers irregularly oriented in the connective tissue of the gingival sulcus. The arrow shows some inflammatory elements (bar = 5 μm).

Figure 16. Ultrastructural aspects of the rare vascular structures found in the connective tissue of the gingival sulcus (bar = 5 μm).

Figure 17. Basal layer of sulcular epithelium containing cylindrical cells bound together by thin cytoplasmic extensions that demarcate extra-cellular spaces full of inflammatory cells (arrow) (bar = 5 μm).

Figure 18. Surface layer of sulcular epithelium. The cells have regressive nuclei and differing cytoplasmic electron-density. Their cellular surroundings are made irregular by numerous digitiform projections that are also visible on the epithelial surface (bar = 5 μm).

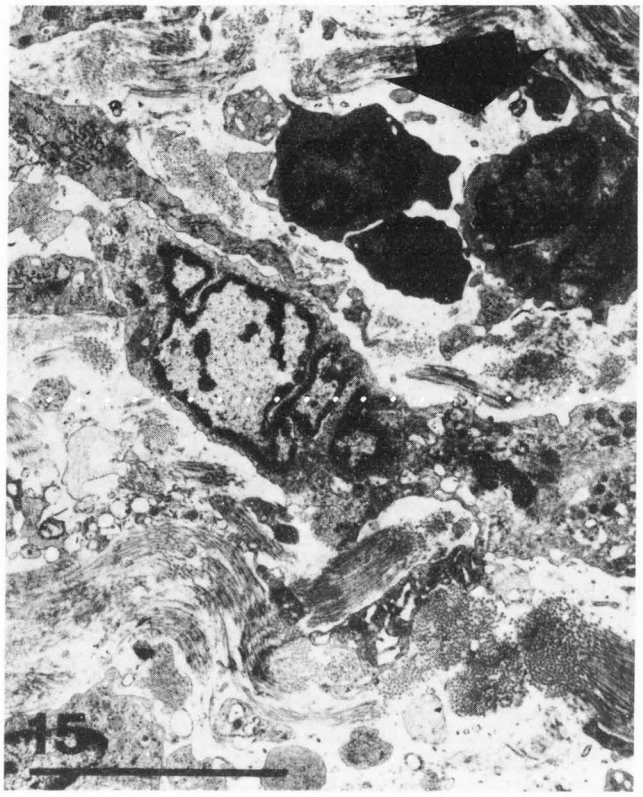
Discussion

The clinical use of endosseous implants is so widespread that research into the bone-implant interface is necessarily thorough. The same thoroughness must now be applied to the examination of soft tissues, since the clinical condition of peri-implant soft tissues may directly influence the success and the longevity of implant therapy.

It is currently believed that the most superficial sulcular epithelium covers the peri-implant sulcus, but that, in the junctional area, a pseudo-epithelial attachment forms on the abutment, or on the implant, through hemidesmosomal structures that are present on the side of the implant. Deeper down, near the bone crest, a collar of collagen fibers is believed to close up at the neck of the implant, or at a lower part of the abutment.

SEM observations made in this study have shown that the morphology of the sulcular epithelium differs on the basis of the geometric form of the abutment used, and/or of the surface characteristics of the abutment. This finding was only partially confirmed by TEM.

Type A abutments observed in SEM showed a higher quality finish in the working on the surface of the metal, and therefore less surface irregularity, than did type B samples. SEM examination showed that the cells of the sulcular epithelium on type A abutments appeared to be very similar to those of the hard palate, in that they were flat, polygonal, and well linked to each other, and had well defined cell edges and a surface of small crests. Desquamation was infrequent and isolated. In the lowest part of the sulcular epithelium, the cells had a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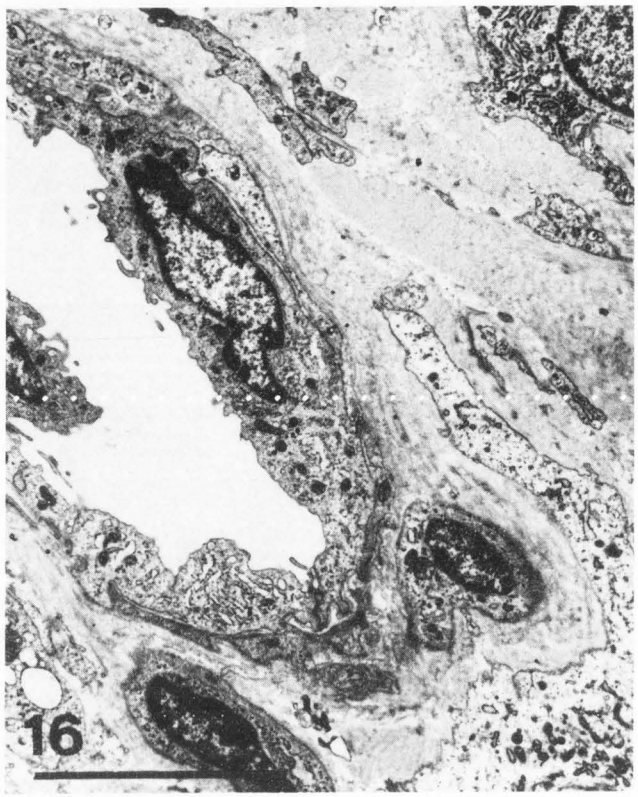
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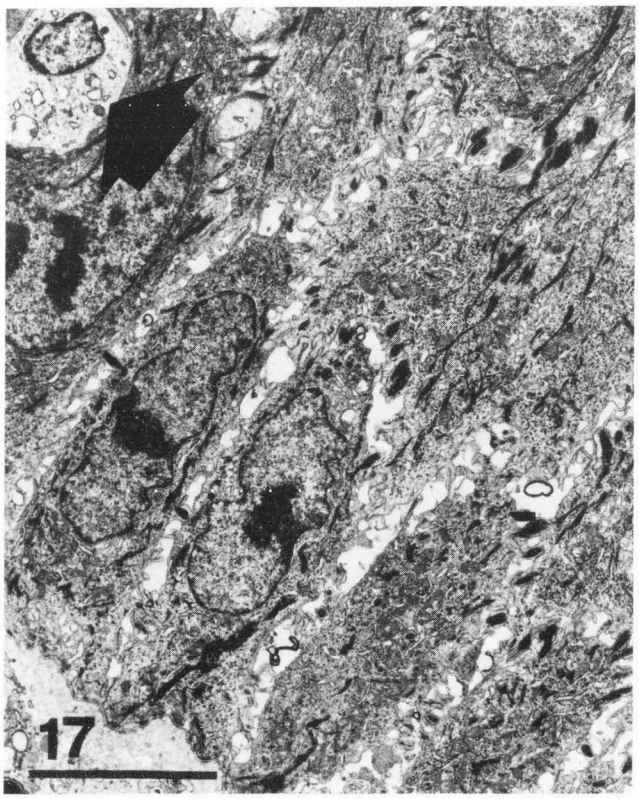
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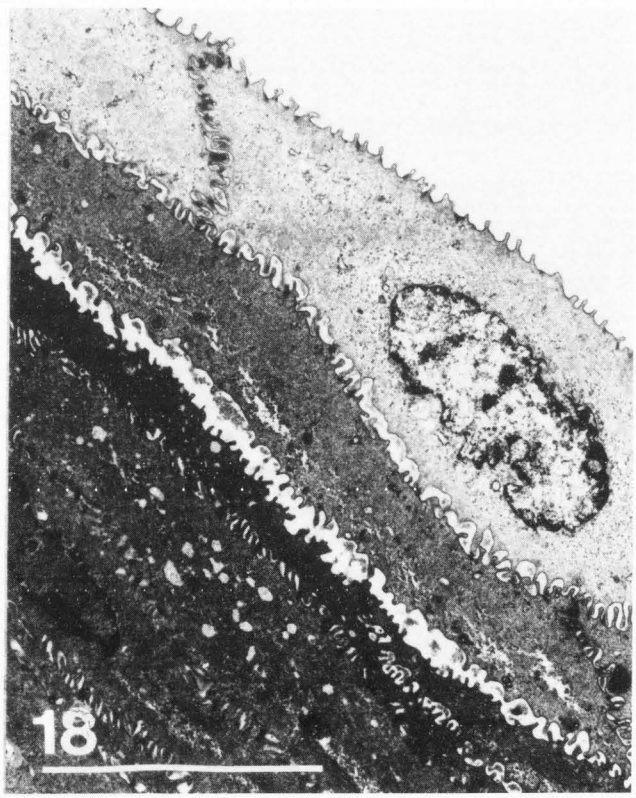
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columnar appearance, but maintained the morphological characteristics of the surfaces near the masticatory gingiva. In the junctional area, bundles of collagen fibers in circular cords were found parallel to the surface of the abutment. Small ball-shaped bundles of collagen fibers, perpendicular to the abutment, lay between the larger cords of collagen fibers.

TEM examination of the same samples showed a well-conserved sulcular epithelium that consisted of three layers: base, intermediary, and surface. Granules of keratohyalin in the cells of the intermediary and surface layers were normally not found, which confirms the observations of other authors who have described the sulcular peri-implant epithelium (Arvidson *et al.*, 1990; Steflik *et al.*, 1990). The surface layer of flat cells was, on average, made up of 14-16 layers of well-compacted cells. This may explain the absence of desquamation on the surface of all type A samples examined.

SEM examination of type B samples, in contrast, showed an epithelium with extensive desquamation. The morphology of the cells was more irregular than that of A samples. The cell walls were not well defined, and were often more raised than those of underlying layers. The connection in the junctional zone appeared, in these samples, to be made up of collagen fibers joined together in partially withdrawn bundles, with an orientation mainly perpendicular to the surface of the abutment. These characteristics were probably linked to the trauma caused by the biopsy of the peri-implant gingiva.

Desquamation observed in the SEM was also visible in TEM, and was consistently associated with a lesser thickness of the superficial cell layer than was the case with A samples (6-8 vs. 14-16 layers). In B samples, we also found a leukocyte infiltrate in the more superficial layers of the sulcular epithelium near the transitional zone contiguous with the junctional area. These infiltrates were not associated with particular structural alterations in, or bacterial infection of, the epithelium.

In contrast with type A, the B samples revealed a certain number of keratohyalin granules in the cells of this zone, but we did not find keratinization of the more superficial layers of the epithelium. If we exclude the influence of the general clinical state of the patients, the only variables in our experiments were the form and micromorphology of the surface of the abutment. We maintain that the geometric design of the abutment might influence the clinical state of the peri-implant mucosa. This, however, was not found in our observations, which revealed mucosa to be in an excellent clinical state under both conditions.

Although surface roughness examination, as demonstrated by Quirynen *et al.* (1994a) showed the two types of abutment to be fairly similar, SEM of the surface of the abutments showed large differences in the working of the metal. The type B abutment had a very irregular micromorphology, with more pointed crests and craters. Although these surface irregularities were important for cell adhesion to titanium in the junctional area (Quirynen *et al.*, 1993; Mc Collum *et al.*, 1992), they might have been the cause of problems in the sulcular epithelium. The greater desquamation and more irregular cell morphology observed in B samples could, however, have been a result of the microtraumatic action of this type of surface-working on the cellular component of the sulcular epithelium.

Similarly, the increased number of keratohyalin granules found in the surface layer of B samples might have been the defensive response to this irritating noxa. The keratinization of an epithelium should always be considered as a protective mechanism. The presence of the leukocyte infiltrate revealed in TEM could also be the sign of trauma and/or local irritants.

From the clinical point of view, the differences in geometric form of the two types of abutment did not influence peri-implant mucosa. However, we did find bacteria in type B samples, and this finding may be the consequence of microbial penetration along the implant components of the type B abutment. It is conceivable that the type A abutment impedes bacterial penetration by virtue of its conical seal between abutment and implant, which prevents microleakage (Quirynen *et al.*, 1994b).

The results obtained from this investigation indicate that a study of a larger number of patients would be worthwile. The results must be assessed in relation to the technical limitations involved in the removal of samples from human gingiva. The impossibility of placing block sections in humans has prevented us from studying the relationships between implant and soft tissues in the transitional zone between sulcular and junctional epithelium.

(Figures on facing page)

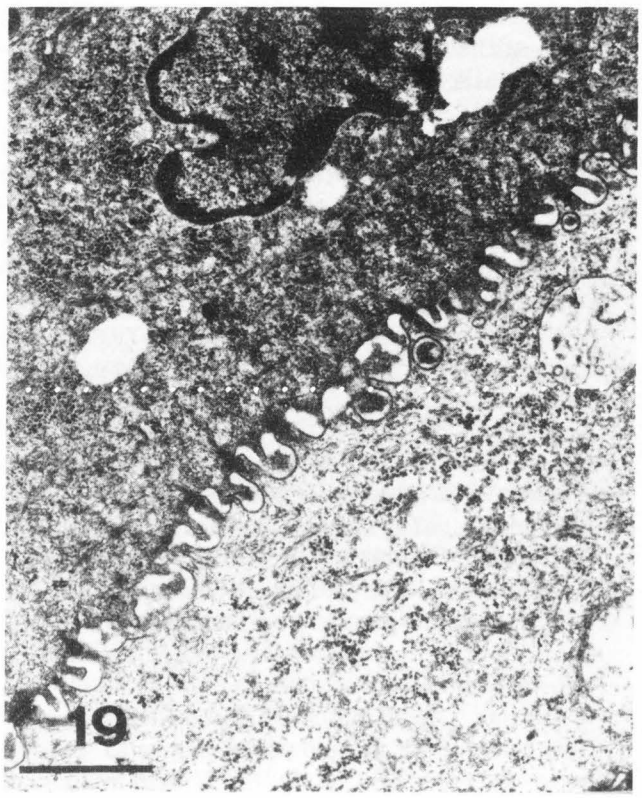
Figure 19. Detail of the juncture that connects the cells of the surface layer to the gingival mucosa (bar = 1 μm).

Figure 20. Bacteria with very fine glycocalyx sticking to the surface of the sulcular epithelium (bar = 1 μm).

Figures 21-22. Transmission electron micrographs of sample B.

Figure 21. Cell desquamation on the surface of the sulcular epithelium (bar = 5 μm).

Figure 22. Intra-epithelial leukocyte in the transitional area between sulcular and junctional epithelium. There are many keratohyalin granules in the epithelial cells (arrows) (bar = 5 μm).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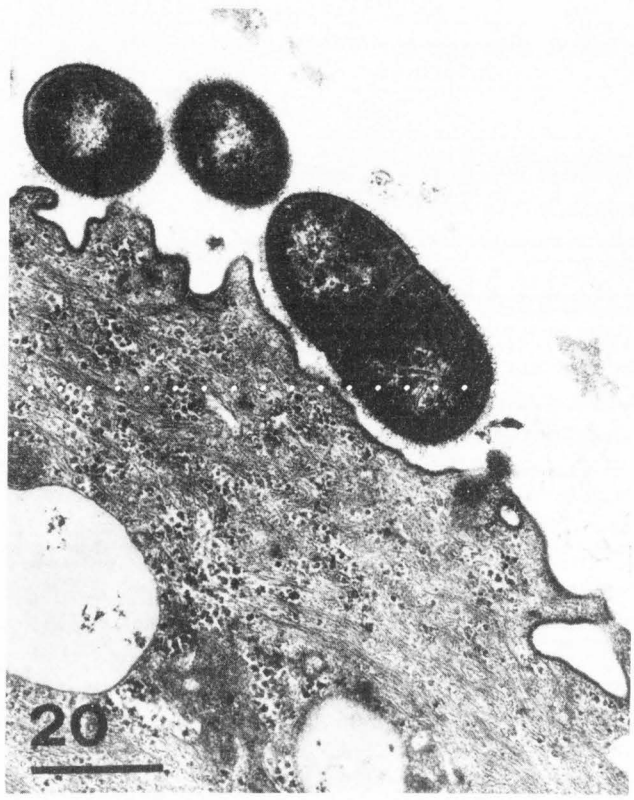
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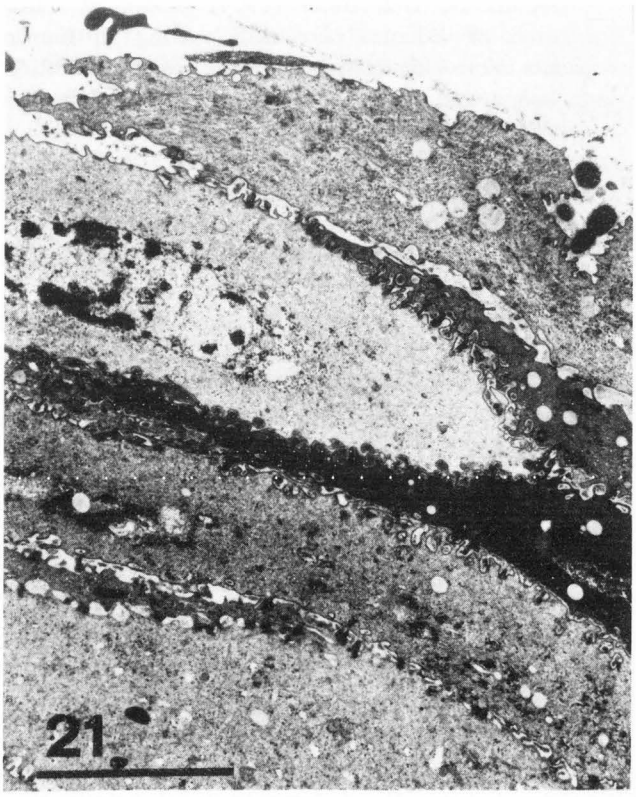
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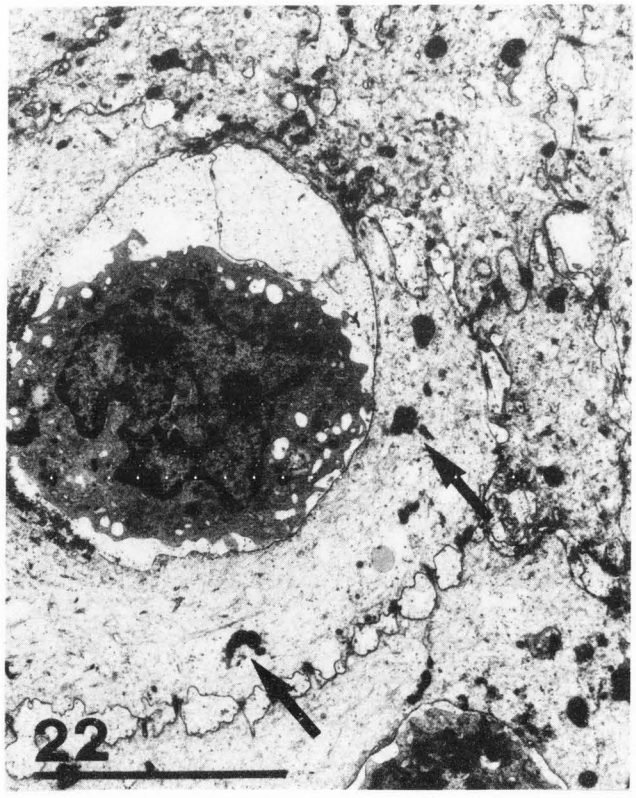
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Acknowledgements

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References

Adell R, Eriksson B (1990) A long term follow-up study of osseointegrated implants in the treatment of the fully edentulous jaws. Int J Oral Maxillofac Implants **5**: 347-357.

Adell R, Lekholm U, Rockler B, Brånemark P-I (1981) A 15-year study of osseointegrated implants in the treatment of the edentulous jaw. Int J Oral Surg **10**: 387-416.

Albrektsson T (1986) The long term efficacy of currently used dental implants: a reviewed and proposed criteria of success. Int J Oral Maxillofac Implants **1**: 11-25.

Albrektsson T (1988) A multicenter report on osseointegrated oral implants. J Prosth Dent **60**: 75-84.

Albrektsson T, Brånemark P-I, Hansson HA, Ivarsson B, Jönsson U (1982) Ultrastructural analysis of the interface zone of titanium and gold implants. In: Clinical Applications of Biomaterials. Lee AJC, Albrektsson T, Brånemark P-I (eds). John Wiley & Sons, New York, 167-177.

Albrektsson T, Brånemark P-I, Hansson HA, Kasemo B, Larsson K, Lindström I, McQueen DH, Skalak R (1983) The interface zone of inorganic implants in vivo. Ann Biomed Eng **11**: 1-27.

Albrektsson T, Zarb GA, Worthington P, Eriksson RA (1986) The long term efficacy of currently used dental implants. A review and proposed criteria of success. Int J Oral Maxillofac Implants **1**, 11-25.

Albrektsson T, Dahl E, Enbom L, Engevall S, Enquist B, Eriksson RA, Feldmann G, Freiberg M, Glantz PO, Kjellman O, Kristersson L, Kvint S, Kondell PA, Palmquist J, Wermdahl L, Åstrand P (1988) Osseointegrated oral implants. A swedish multicenter study of 8.139 consecutively inserted Nobelpharma implants. J Periodontol **59**: 287-296.

Arvidson K, Bystedt H, Ericsson I (1990) Histometric and ultrastructural studies of tissue surrounding Astra dental implants in dogs. Int J Oral Maxillofac Implants **5**: 127-134.

Bauman GR, Rapley JW, Hallmon WW, Mills M (1993) The peri-implant sulcus. Int J Maxillofac Implants **8**: 273-280.

Berglundh T, Lindhe J, Ericsson I, Marinello C, Liljenberg B, Thomsen P (1991) The soft tissue barrier at implants and teeth. Clin Oral Impl Res **2**: 81-90.

Berglundh T, Lindhe J, Marinello C, Ericsson I, Liljenberg B (1992). Soft tissue reaction to de-novo plaque formation on implants and teeth. An experimental study in the dog. Clin Oral Impl Res **3**: 1-8.

Brånemark P-I (1983) Osseointegration and its experimental background. J Prosth Dent **50**: 399-410.

Brånemark P-I, Hansson BO, Adell R, Breine U, Lindström J, Hallén O, Öhman A (1977) Osseointegrated implants in the treatment of the edentulous jaw. Experience from a 10-year period. Scand J Plast Reconstr Surg **Suppl 16**: 1-132.

Brunette DM (1988).The effects of implant surface topography on behaviour of cells. Int J Oral Maxillofac Implants **3**: 231-246.

Brunette DM, Kenner GS, Gould TRL (1983) Grooved titanium surfaces orient growth and migration of cells from human gingival explants. J Dent Res **62**: 1045-1048.

Buser D, Weber HP, Donath K, Fiorellini JP, Paquette DW, Williams RC (1992). Soft reaction to new submerged unloaded titanium implants in Beagle dogs. J Periodont **63**: 225-235.

Cheroudi B, Ratkay J, Brunette DM (1992) The role of implant surface geometry on mineralisation in vivo and in vitro; transmission and scanning electron microscopic study. Cells Mater **2**, 89-104.

Cochran DL, Simpson J, Weber HP, Buser D (1994) Attachment and growth of peridontal cells on smooth and rough titanium. Int J Oral Maxillofac Implants **9**: 289-297.

Daculsi G, Delecrin J (1994) Biological transformation of calcium phosphate coating in human implants micro-characterization using scanning electron microscopy and high resolution transmission electron microscopy. Cells Mater **4**: 63-71.

Ericsson I, Lekholm U, Brånemark P-I, Lindhe J, Glantz P-O, Nyman S (1986) A clinical evaluation of the fixed bridge restorations supported by the combination of the teeth and osseointegrated titanium implants. J Clin Periodontol **13**: 307-312.

Gould TRL, Westbury L, Brunette DM (1984) Ultrastructural study of the attachment of human gingiva to titanium in vivo. J Prosth Dent **52**, 418-402.

Inoue T, Cox JE, Pilliar RM, Melcher AH (1987) Effect of the surface geometry of smooth and porous-coated titanium alloy on the orientation of fibroblasts in vitro. J Biomed Mat Res **21**: 107-126.

Könönen M, Hormia M, Kivilahti J, Hautaniemi J, Thesleff I (1992) Effect of surface processing on the attachment orientation, and proliferation of human gingival fibroblasts on titanium. J Biomed Mat Res **26**, 1325-1341.

Lekholm U, Adell R, Lindhe J, Brånemark P-I, Eriksson B, Rochler B, Lindwall AM, Yoneyama T (1986a) Marginal tissue reactions at osseointegrated titanium fixtures II. A cross-sectional retrospective

study. Int J Oral Surg **15**: 53-61.

Lekholm U, Eriksson B, Adell R, Slots J (1986b) The condition of the soft tissue at tooth and fixture abutments supporting fixed bridges. J Clin Periodontol **13**: 558-562.

Listgarten MA, Lange NP, Schroeder HE, Schroeder A (1991) Periodontal tissues and their counterparts around endosseous implants. Clin Oral Impl Res **2**: 1-19.

Listgarten MA, Buser D, Steinemann SG, Donath K, Lang, Weber HP (1992) Light and transmission electron microscopy of intact interfaces between non-submerged titanium-coated epoxy resin implants and bone or gingiva. J Dent Res **71**: 364-371.

Lowenberg BF, Piiliar RM, Aubin JE, Fernie GR, Melcher AH (1987) Migration, attachment and orientation of human gingival fibroblasts to root slices naked and porous-surface titanium alloy discs and zircalloy 2 discs in vitro. J Dent Res **66**: 1000-1005.

McCollum J, O'Neal RB, Brennan WA, Van Dycke TE, Horner JA (1992) The effect of titanium implant abutment surface irregularities on plaque accumulation in vivo. J Periodontol **63**: 802-805.

Quirynen M, Van der Mei HC, Bollen CML, Schotte A, Marechal M, Doornbusch GI, Naert I, Busscher HJ, Van Steenberghe D (1993) An in vivo study on the influence of the surface roughness of implants on the microbiology of supra and subgingival plaque. J Dent Res **72**: 1304-1309.

Quirynen M, Bollen CML, Willems G, Van Steenberghe D (1994a) Comparison of surface characteristics of six commercialy pure titanium abutments. Int J Oral Maxillofac Implants **9**: 71-75.

Quirynen M, Bollen CML, Eyssen H, Van Steenberghe D (1994b) Microbial penetration along the implant components of the Branemark system. An in vitro study. Clin Oral Res **5**: 239-244.

Ruggeri A, Franchi M, Trisi P, Piattelli A (1994) Histologic and ultrastructural findings of gingival circular ligament surrounding osseointegrated nonsubmerged loaded titanium implants. Int J Oral Maxillofac Implants **9**: 636-643.

Schroeder A, Van der Zypen E, Stich H, Sutter F (1981) The reactions of bone, connective tissue and epithelium to endosteal implants with titanium sprayed surfaces. J Maxillofac Surg **9**: 15-25.

Smith DC, Pillar RM, Chernecky R (1991) Dental implant materials. I. Some effects of preparative procedures on surfaces topography. J Biomed Mater Res **25**, 1045-1068.

Steflik DE, McKinney RV, Sisk AL, Parr GR, Koth DL (1990) Scanning electron microscopic studies of the oral tissue responses to dental implants. Scanning Microscopy **4**: 1021-1038.

Strub JR, Gaberthuel TW, Grunder U (1991) The role of attached gingiva in the health of peri-implant tissue in dogs. 1. Clinical findings. Int J Periodont Restor Dent **11**: 317-333.

Taylor SR, Gibbons DF (1983) Effect of surface texture on the soft tissue response to polymer implants. J Biomed Mater Res **17**, 205-227.

Warrer K, Buser D, Lang NP, Karring T (1995). Plaque-induced peri-implantitis in the presence or absence of keratinized mucosa. Clin Oral Impl Res **6**, 131-138.

Weber HP, Fiorellini JP (1992) The biology and morphology of the implant-tissue interface. Alpha-Omegan **85**, 61-64.

Discussion with Reviewers

Reviewer II: Have the authors considered using a more sensitive way of quantifying and measuring surface irregularities of their implants?

Authors: We did not consider using other assessment methods because the data provided by SEM are sufficient for the purposes of morphological evaluation. We believe that SEM offers greater sensitivity than does surface roughness analysis; for the given two types of abutment, the latter method failed to detect significant differences in surface irregularity (Quirynen *et al.*, 1994a).

Reviewer II: Since the two implants are from different companies, they may have different surface chemistry resulting from the different cleaning and sterilization techniques used. In the author's opinion, how would the possibility of such variation affect tissue response in their biopsies?

Authors: All the given implants and the superstructures were used in accordance with the respective manufacturers' specifications, among other reasons to contain sepsis. The products arrived in sealed packages that bore the "Sterile" sign, which we assume corres-ponds to international standards. Thus, while we concede that cleaning and sterilization procedures may give inadvertently rise to surface pollution, and that this may in turn influence the correlation between implant surface and soft tissues, we are unable to define how this may occur, and we reiterate our belief that each manufacturer follows standard practice.

Reviewer III: There should not be any collagenous elements in contact with the abutment in the region where the sulcular epithelium overlaps the junctional epithelium. The presence of collagenous fibers would be expected apical to the junctional epithelium, unless portions of the junctional epithelium have been torn off,

thereby exposing the collagenous elements.
Authors: Collagen fibers were found in the deepest part of the epithelium, near the bone crest. The finding was constant in all the samples examined.

Reviewer III: Could the rougher appearance of the sulcular epithelium against type B abutments, including the appearance of more desquamating cells, be due to the tearing of junctional epithelium, with some of it left to adhere to the abutment and the torn surface mistakenly identified as the surface of sulcular epithelium?
Authors: We believe tearing of junctional epithelium to be highly unlikely, since if this had occurred, SEM would have revealed polygonal cells at the surface, rather than flat, desquamating cells. We also found bacteria on the epithelial surface, which reinforced our conviction that tearing did not take place.

K. Arvidson: Could you give more information about the clinical parameters and X-ray analysis at base-line and after 1 year *in situ*. What kind of restorations were used on those seven implants?
Authors: Radiography was intraoral with Rinn centering upon implantation. At 6 and at 12 months, the centering mechanism was repositioned by means of a Dura Lay jig. In all cases, initial bone reabsorption was negligible (< 5 mm). We used oro-palladion and ceramic bridges, with a supragingival closure edge. Clinical assessment of peri-implant mucosa consisted of plaque and bleeding evaluation, and of probe. None of the samples examined showed plaque or bleeding.

K. Arvidson: Regarding the biopsies, which part of mucosa was transformed for SEM and TEM? Were all fixtures surrounded by an attached mucosa? If not, please explain if there were any differences.
Authors: The ring of mucosa sampled was cut into two equal parts for SEM and TEM. All the fixtures were surrounded by attached mucosa.

K.Arvidson: It is very easy to distinguish between the sulcular and junctional epithelium surrounding the tooth, but please explain how this is done with regard to implants?
Authors: The distinction between sulcular and junctional epithelium is indeed problematic in peri-implant mucosa, and it would be more appropriate to consider only the structures at the collar of the fixture as junctional.

K. Arvidson: You write that the presumed seal has "important implications for bacterial infiltration, and, accordingly, for the durability of the implant" - has this been proven?
Authors: Certainly. From the microbiological viewpoint, the Nobelpharma system is considered to be an open system, in that its more or less cylindrical abutment provides continuity between the gingival sulcus and the neck of the implant. The conical seal of the Astra-Tech system may create a microbiologically different situation, which could explain the differences in morphology we observed.

H.P. Weber: What was the time sequence of events from implant surgery to second stage surgery with abutment insertion to prosthetic reconstruction?
Authors: At second stage surgery, the implants were uncovered by incision into crest mucosa and by the raising of a strip that was 1 mm thicker than the abutment itself. Any bone spicules found to be covering the screw cover were removed. To assist the healing of the soft tissues, we prepared for the definitive implant by washing the interior of the implant with chlorohexidin and by drying the site of the screw with sterile blotting paper. Replacement took place about 30 days after second stage surgery.

H.P. Weber: What influence may microleakage have as it has been described in the literature for abutment type B in this study?
Authors: As described by Quirynen *et al.* (1994b) and by other authors, microleakage may influence bacterial colonization in the given zone, but neither the mechanism nor the clinical implications of such colonization has been clarified in the literature.

H.P. Weber: How consistent were the findings between the different samples of each abutment type?
Authors: Differences were minimal.

H.P. Weber: How do your findings relate to those of other structural studies in the literature (e.g., Ericsson *et al.*, 1996; Liljenberg *et al.*, 1996)?
Authors: Our findings on peri-implant tissue in the case of the Nobelpharma abutment confirm those reported in the literature. As regards the Astra-Tech Uni abutments, we have not discovered references in the literature.

Additional References

Ericson I, Nilner K, Klinge B, Glantz PO (1996) Radiographical and histological characteristics of submerged and nonsubmerged titanium implants. An experimental study in the labrador dog. Clin Oral Implants Res **7**: 20-26.

Liljenberg B, Gualini F, Berglundh T, Tonetti M, Lindhe J (1996) Some characteristics of the ridge mucosa before and after implant installation. A prospective study in humans. J Clin Periodontol **23**: 1008-1013.