Scanning Microscopy

Volume 4 | Number 4 Article 15

9-8-1990

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Scanning Microscopy, Vol. 4, No. 4, 1990 (Pages 1039-1048)
Scanning Microscopy International, Chicago (AMF O'Hare), IL 60666 USA

0891-7035/90$3.00+.00

ULTRASTRUCTURAL INVESTIGATIONS OF THE BONE AND FIBROUS CONNECTIVE TISSUE INTERFACE WITH ENDOSTEAL DENTAL IMPLANTS

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(Received for publication February 6, 1990, and in revised form September 8, 1990)

Abstract

The interface between the tissues of the oral cavity and ceramic and titanium cylindrical endosteal dental implants was investigated with correlated light microscopy, transmission electron microscopy and scanning electron microscopy. This study suggested that mandibular bone can directly interface and form an intimate association with one-stage endosteal dental implants. This potential attachment matrix is composed of a composite of calcified bone, and an osteoid unmineralized matrix in association with an apparent osteogenic connective tissue. Further, results from this study suggested that at a level inferior to the junctional epithelium, and superior to the level of crestal bone, fibrous connective tissue can attach to the dental implant. This non-loadbearing attachment of gingival connective tissue could, by contact inhibition, prevent apical epithelial migration. In association with previously documented epithelial attachment, such apical support and connective tissue attachment appears to suggest that endosteal dental implants can be adequately maintained in the oral cavity.

KEY WORDS: Dental Implants, Bone, Morphology, Attachment Complex, Oral Tissue Interfaces, Surface-Etching, Cryofracture, Gingival Connective Tissue, Contact Inhibition

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Introduction

The field of oral implantology has undergone a dynamic increase in interest in the past fifteen years. Concurrent with this heightened interest has been a desire on the part of dental scientists and clinicians to more fully understand the supporting tissue interfaces with endosteal dental implants. These supporting tissues include primarily calcified bone, but also varied amounts of soft, fibrous connective tissue. The mechanism of how these tissues actually interface the implanted biomaterial is the subject of this investigation.

Endosteal dental implants must exist in two environments. The implant is supported in the jaw bone and extends into the bacteria-rich environment of the oral cavity. The implant acts as the root of a natural tooth would, that is, it acts as a support mechanism for a prosthesis which takes the place of the lost tooth or teeth. To protect the underlying apical support tissues for the implant, the gingiva (or gum tissue) forms a biological seal to the implant. Previous studies from our laboratory have shown that this attachment complex consists of hemidesmosomes attaching the junctional epithelium to the implant via an external basal lamina (Steflik et al, 1984a; McKinney et al, 1985a; Steflik et al, 1988).

Separating the underlying crestal bone from the level of junctional epithelium appears to be a viable gingival connective tissue layer (Schroeder et al, 1981; Steflik et al, 1989c). Previous studies have not been able to suggest any attachment mechanism of this layer of connective tissue to the implant.

Beneath the layer of the gingival connective tissue, the support system to endosteal dental implants has been shown to include cortical bone, trabecular bone, osteoid, soft fibrous connective tissue and marrow space (Cook et al, 1983; Albrektsson et al, 1981; McKinney et al, 1985b; Roberts et al, 1984; Steflik et al,

1989a). Light microscopic studies utilizing implants of various physical characteristics have shown that implants can maintain a direct bone to implant interface at some point along the implant circumference (Hipp and Brunski, 1987; Deporter et al, 1986). Any potential attachment complex between bone and implant has not, however, been elucidated. Meenaghan et al (1974) suggests that a triple layer of osseous tissue interfaced serviceable titanium alloy blade implants. Other investigators suggest that a ground substance of between 20 and 2000 Angstroms in thickness exists interfacing titanium implants and the calcified bone (Hansson et al, 1983; Albrektsson et al, 1983; Linder et al, 1983). DeLange and associates (1988) suggest that a thin electron dense layer, of approximately 50 Angstroms in thickness, exists between the bone and hydroxylapatite implants. They further suggest that collagen fibers of the mineralized bone were within 500 Angstroms of the implant surface.

It is the purpose of this paper to report correlated light microscopic, scanning electron microscopic and transmission electron microscopic observations of two specific oral tissue interfaces to endosteal dental implants. The first interface is the layer of connective tissue inferior to the junctional epithelium and superior to the level of crestal bone -- the gingival connective tissue interface. The second interface is the level of bone association to the endosteal implant. This investigation utilized 32 ceramic and titanium one-stage implants placed into the mandibles of 8 adult mongrel dogs.

Materials and Methods

Implants And Surgical Protocol

For this investigation sixteen cylindrical alpha alumina oxide ceramic endosteal dental implants and sixteen identically prepared commercially pure titanium implants were inserted into the mandibles of eight adult mongrel dogs after bilateral extractions of all premolars (Fig. 1). Following a healing period of two months, two ceramic implants were inserted in the right premolar region and two titanium implants were inserted in the left premolar region of each dog. Copious external irrigation was utilized in all drilling protocols and implant receptor sites were hand tapped to minimize any detrimental local heating of these sites. In four of the animals, the implants supported a fixed bridge. The implants were autoclaved prior to insertion with the titanium implants passivated by routine preparation. The surface characteristics of the implants were of a smooth texture. The surface

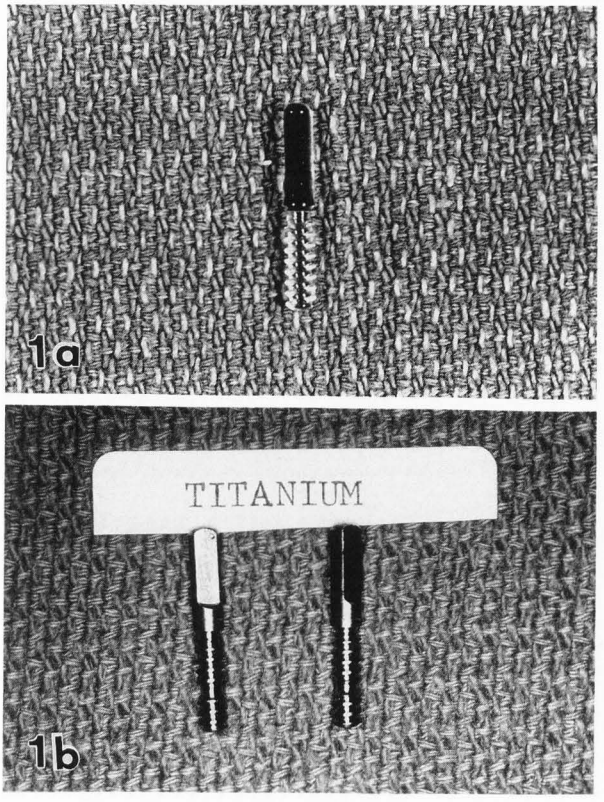


Figure 1. Photographs of the ceramic (a) and titanium (b) implants utilized in this study.

texture of the titanium implants was kept at a minimum to correlate as closely as possible to the smooth fire-polished nature of the ceramic implants. All implants were inserted 10 millimeters into the mandibular bone. At all times the 9 millimeters of the threaded radicular portion were placed 1 mm below the initial alveolar bone crest.

The animals whose implants remained freestanding were euthanized at one, two, three and five months post-implantation. The animals whose implants supported fixed bridgework were euthanized at two, three, four and six months after luting of the bridges. Bridges were luted one month after implantation.

At the time of euthanasia, the jaws of the animals were fixed by vascular perfusion via a carotid artery cutdown as per our previous reports (Steflik et al, 1984a; Steflik et al, 1988; McKinney, Steflik and Koth, 1985a). Vascular perfusion employed 3% phosphate buffered glutaraldehyde for approximately 45 minutes. Mandibular block samples containing the entire implant were removed with a Stryker bone saw and the samples were immediately immersed into fresh 3% glutaraldehyde for an additional 24 hours.

Randomly selected implant block samples were then processed for electron microscopy via three protocols. First, fixed samples were hemisected using a Buehler Isomet low speed saw while immersed in saline. These 2 resulting hemisectioned samples were critical point dried from absolute ethanol after dehydration in ascending concentrations of ethanol. Carbon dioxide was used as the transitional solvent. Second, entire block samples were routinely dehydrated through ethanols and critical point dried for scanning electron microscopy (SEM). Third, remaining glutaraldehyde block samples were washed with phosphate buffer and underwent secondary fixation with 1% phosphate buffered Osmium Tetroxide for two hours. After dehydration through ethanols, the block samples were embedded in Maraglass 655. The plastic embedded blocks were then sectioned via the Isomet saw with the sections ranging in thickness from one to two millimeters. Half of these resultant thick sections were then immersed into first liquid nitrogen followed immediately by immersion in boiling water. This cryofracture technique cleanly separates the implant from the interfacing oral tissues. The oral tissues are then reembedded in Maraglass for transmission electron microscopic analysis. The other half of the thick sections were surface etched with oxygen plasma via our previously reported protocol (Steflik et al, 1983, Steflik, McKinney and Koth, 1984a; Steflik et al, 1984b). Specimens were placed into a vacuum chamber into which oxygen gas was introduced. Using a radiofrequency generator contained in the plasma etching unit, oxygen plasma was produced. This plasma surface etched the sample by removing some of the superficial plastic embediment, thereby exposing surface topography for SEM analysis.

Scanning electron microscopic samples were mounted on standard mounts and shadowed by vacuum evaporation of Platinum/Palladium wire. The samples were also lightly sputter coated with gold prior to analysis using an AMR 1000A scanning electron microscope. Transmission electron microscopy (TEM) blocks were sectioned with both glass and diamond knives. Resultant ultrathin sections were stained with uranyl acetate and lead citrate, and examined with a JEOL 100 C transmission electron microscope.

Results

General orientation of the oral tissues to a cylindrical endosteal dental implant is diagrammed in figure 2. Immediately inferior to the level of junctional epithelium is the layer of gingival connective tissue. In serviceable dental implants this layer separates the epithelium from the level of cortical bone. Subsequent micrographs originate from this level of connective tissue and from the bone interface to the implant beneath the gingival connective tissue.

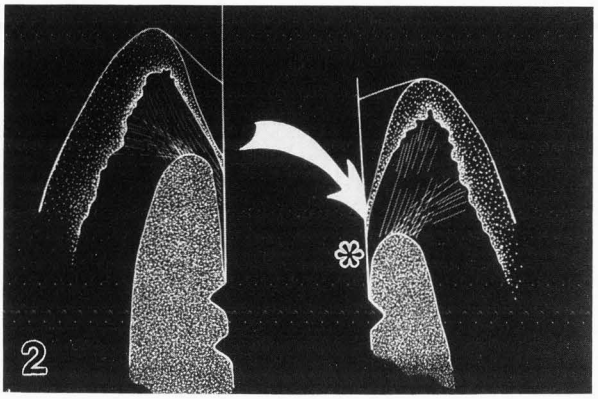
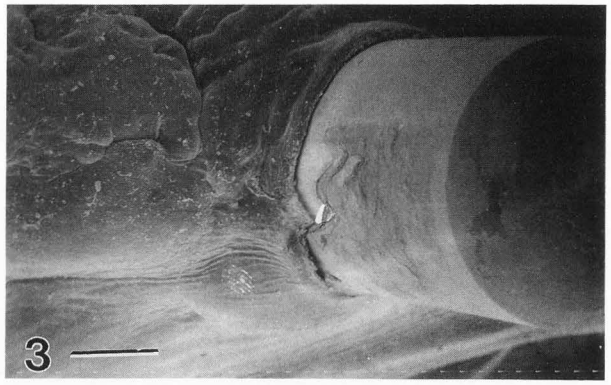


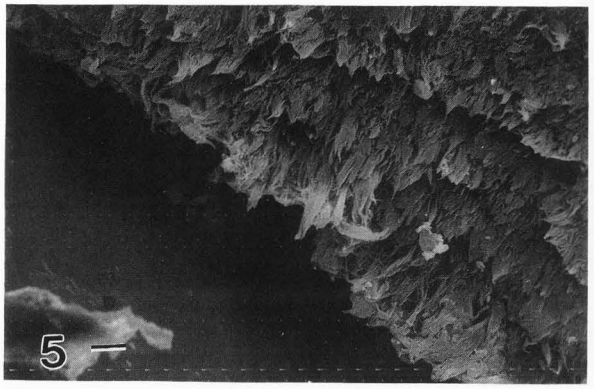
Figure 2. Diagram displaying the orientation of the oral tissues to the implant. The arrow points out the level of the junctional epithelium. The shaded area represents the mandibular cortical bone. The asterisk marks the area of gingival connective tissue.

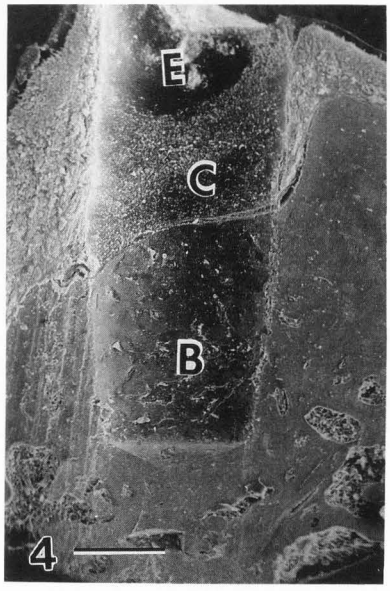
SEM of block implant specimens which did not support fixed bridgework displayed normal appearing gingival collars interfacing the implant (Fig. 3). This was seen for both titanium and ceramic implants. Examination of the level inferior to the gingival margin required hemisectioning the implant specimens.

After hemisectioning one implant sample, the implant came free. SEM of the crypt previously occupied by a portion of the ceramic dental implant (which was in situ for 6 months and supported a prosthesis) demonstrated the orientation of tissues in vivo (Fig 4). Three distinct locations can be identified and clearly demonstrates the interpositioning of a layer of gingival connective tissue between the epithelium and the cortical bone. Examination of other SEM samples suggest that the orientation of these connective tissue fibers to be at times perpendicular to the implant surface (Fig. 5). These fibers appeared to appose the implant surface and terminate into an amorphous association with the implant (Fig. 6). Further, slender bands of connective tissue fibers extended to the implant surface (Fig. 7). Transmission electron microscopy of such gingival connective tissue fibers showed that the collagen fibers approach the implant and may embed into a matrix comprised of longitudinally and cross-sectionally oriented fibers within an amorphous matrix (Fig 8).

Beneath the layer of gingival







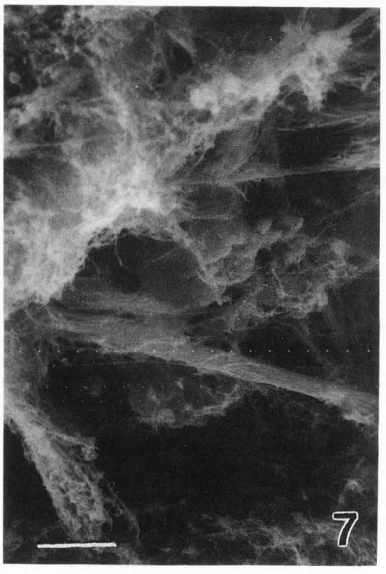


Figure 3. Scanning electron micrograph showing a free standing (not supporting any fixed bridgework) titanium implant in situ in the mandible after block resection. Bar = 1000μm.

Figure 4. SEM of the crypt previously occupied by a ceramic dental implant in situ for 6 months which supported fixed bridgework. The following three distinct locations were identified: E= Junctional Epithelium; C= Gingival Connective Tissue; B=Cortical Bone. Note how the gingival connective tissue separates the inferior aspect of the junctional epithelium and the superior aspect of the crestal bone. Bar = 1000μm.

Figure 5. SEM of the gingival connective tissue interface to a ceramic dental implant showing the apparent perpendicular arrangement of the fibers to the implant surface. Bar = 10μm.

Figure 6. SEM of the interface between the gingival connective tissue and a titanium dental implant. The fibers again appear to be oriented perpendicularly to the implant surface and terminate into an amorphous association with the implant. Bar = 10μm.

Figure 7. SEM of a similar gingival connective tissue region showing a slender band of connective tissue extending to the implant. Bar = 5μm.

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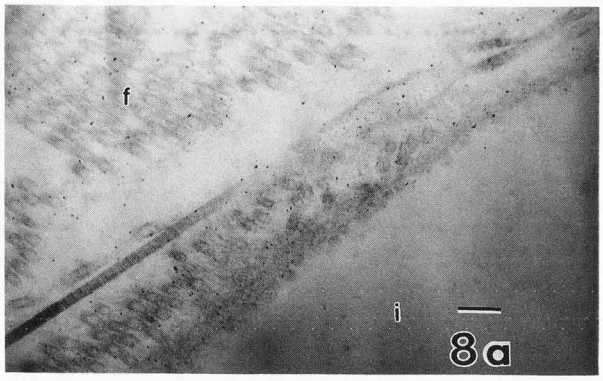
connective tissue, both the titanium and ceramic implants were proportionally interfaced directly by cortical bone (Fig. 9). Since the emphasis of this report is the existence of a potential attachment complex between bone and the implant, results will be restricted to this region.



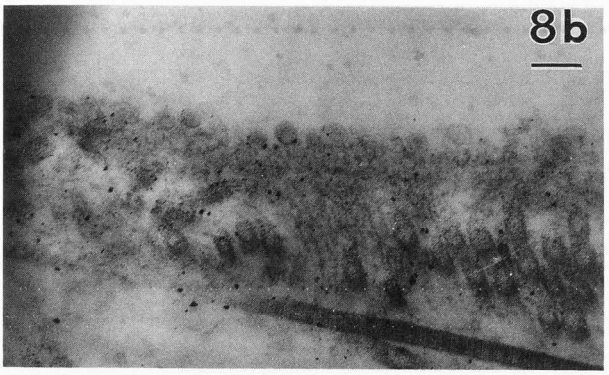


Figure 8. Transmission electron micrographs showing the interface of the connective tissue association to the implant; similar to that seen by SEM in figure 6. Figure 8a shows the connective tissue fibers (f) tangentially approached the space previously occupied by the implant (i) which was removed by the cryofracture technique. The fibers appeared to embed into a matrix comprised of a fiber network, with fibers oriented in two dimensions, associated with an amorphous material. Bar = 200nm. Figure 8b shows the interface in more detail and displays the connective tissue fibers associated with the amorphous material. Bar = 100 nm.

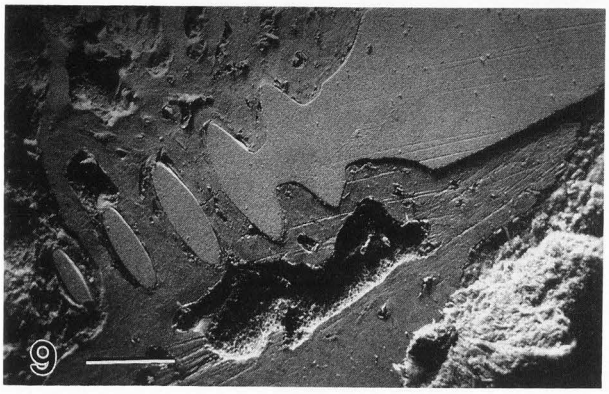


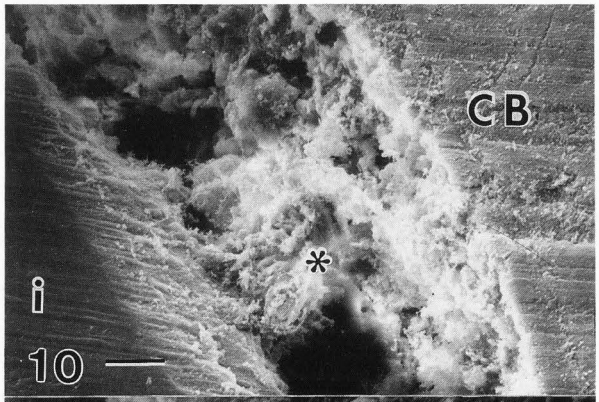
Figure 9. Backscattered electron scanning electron micrograph showing the close adaptation of cortical bone to a ceramic implant which supported a fixed bridge for 4 months. Note the close congruency of the mandibular bone to the implant surface. Bar = 1000μm.

Along the three dimensional interface to the implant, there were considerable areas of intimate bone-to-implant association. One such region (Fig.10) demonstrated that the implant surface and the calcified bone surface was separated by a bridging millieu of approximately 25 micrometers. The consistency of the material is evident as it extends from the titanium implant surface to the calcified bone front. By examining the bone front, the bridging material is intimately associated with this calcified bone, and extends from it (Fig. 11). The morphology of the material away from the bone appears similar to the actual bone-material complex. By examining the implant surface, it can be seen that the titanium surface is coated with a similar material as observed in the bridging complex (Fig 12). In fact, the material is intimately associated with the implant surface.

Histologically, this region of the implant appeared to be directly apposed by healthy bone. By histologically examining retrieved cryofractured specimens, hemotoxylin and eosin staining showed the osteocytes to be in close proximity to the implant surface (Fig 13). Van Gieson staining disclosed a differentially staining region within an area of connective tissue interposed between the bone and the implant (Fig. 14). This staining suggested that this area was an area of calcification within the interfacing connective tissue.

Similar regions of such potential osteogenic attachment were observed consistently around the circumference of the titanium implants. At the interface of the support system and the endosteal dental implant, areas of mature bone was observed interfacing the implant within this described matrix. Figure 15 shows regions of calcification incorporated within the matrix.

Even though ceramic implants were closely adapted by bone (Fig. 16), there apparently was a different appearance of the bone matrix association to the identically prepared ceramic implants. At increasing magnifications the close juxtapositioning of bone was apparent to this ceramic implant, however the potential attachment matrix was not as clearly identifiable. At some regions the ceramic implant was coated with an amorphous material and fibers may have attached to this interface (Fig. 17).



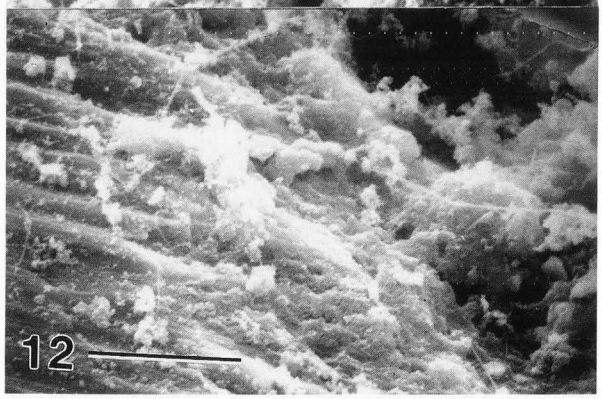


Figure 10. Secondary electron scanning electron micrograph displaying the bridging millieu (asterick) extending from the calcified bone surface (CB) to the titanium implant surface (i). The implant supported fixed bridgework for 4 months. Bar = 10 μm.

Figure 11. SEM of the calcified bone front associated with the dental implant seen in figure 10. The bridging millieu is intimately associated with the calcified bone. Bar = 5μm.

Figure 12. Higher magnification SEM of the titanium implant associated with the bridging complex shown in figures 10 and 11. The implant surface is obscured by the bridging complex which appears to coat the implant surface. Bar = 5μm.

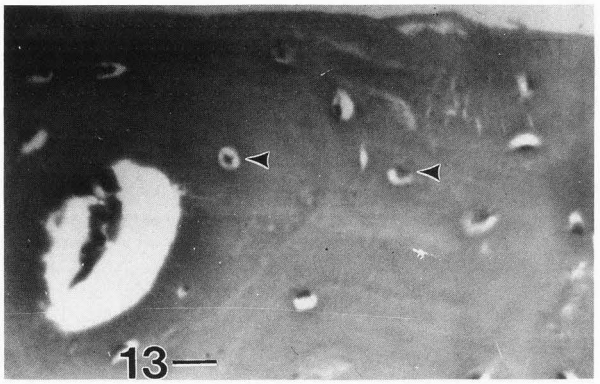


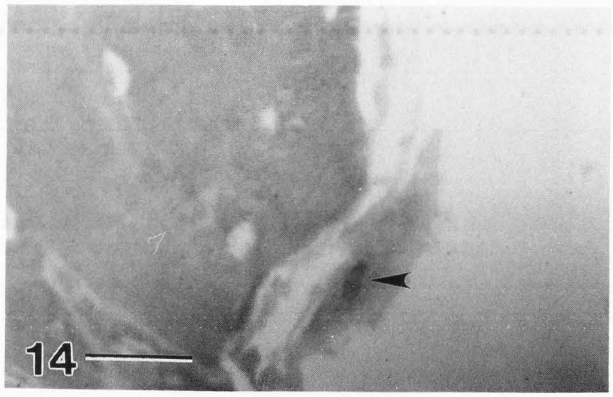
Figure 13. Light micrograph (hematoxylin/eosin staining) of the support tissues previously apposing a titanium implant which had been cryofractured away from the tissue. The cryofracture protocol has been shown to leave intact those tissues associated to the implant. This light micrograph showed the implant to be directly apposed by healthy bone replete with osteocytes (arrowheads). Bar = 50μm.

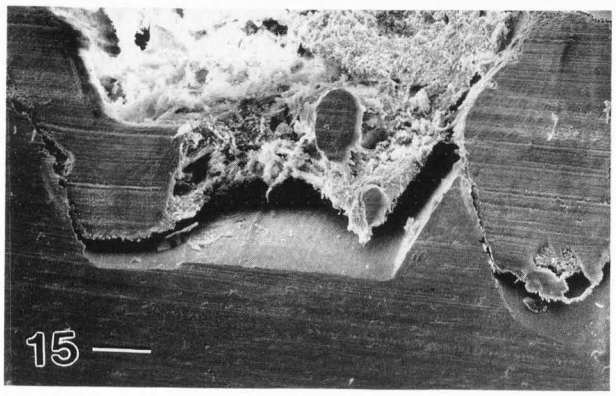
Discussion

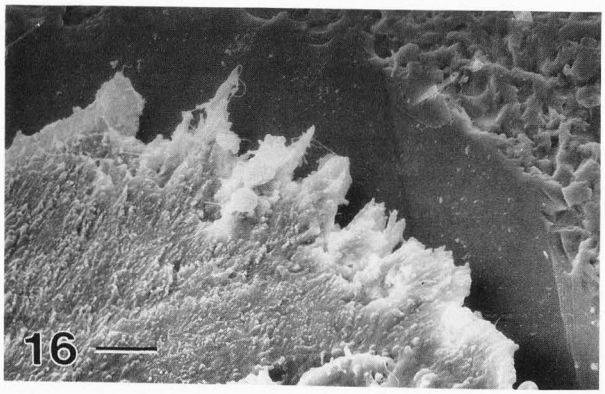
This experimental study suggests that the oral tissues can form attachment complexes to one-stage, cylindrical endosteal dental implants. Attachment complexes were observed for junctional epithelium; gingival connective tissue; and for mandibular bone. Such attachment complexes continue to document the biocompatibility of ceramic and titanium dental implants.

We have previously described the attachment mechanism of junctional epithelium to ceramic dental implants (Steflik et al, 1984a; McKinney et al, 1985a; Steflik et al, 1988); and to a lesser extent, with titanium dental implants. This complex consists of hemidesmosomes attaching to an external basal lamina which is complexed to the implant. The basal lamina is comprised of glycosaminoglycans and the basal lamina glue-like template has both a lamina lucida and lamina densa component (Steflik et al, 1988; Steflik et al, 1989b). The epithelial attachment is critical for the generation of a biological seal, protecting the apical support system.

There now appears to be evidence suggesting that connective tissue can attach to dental implants at a level inferior to the junctional epithelium and superior to the level of crestal bone. In this region, connective tissue fibers extend perpendicularly to the implant surface and attach to an amorphous







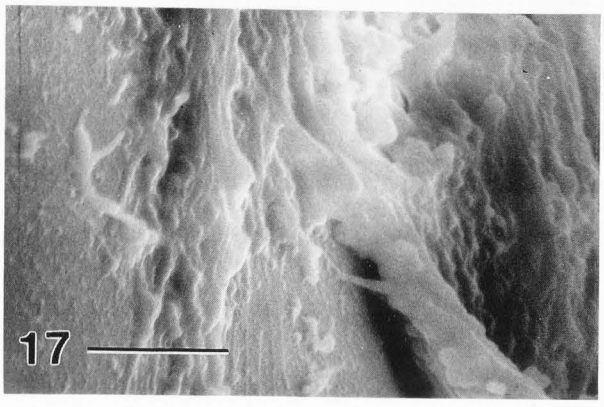


Figure 14. Light micrograph of the support region to a titanium implant which was removed by cryofracture. Here connective tissue was interposed between the implant and the bone. Differential staining by the van Giesa method showed an orange staining inclusion (arrowhead) within the yellow staining connective tissue. Bone has been documented to stain orange via this method. Bar = 50μm.

Figure 15. SEM of the uncalcified connective tissue matrix apposing a titanium dental implant which supported fixed bridgework. Observed within this osteoid matrix were spicules of calcified bone. Bar = 100μm.

Figure 16. SEM of the interface of mandibular bone to a ceramic dental implant which supported fixed bridgework for 4 months. Close bone-implant congruency is observed, however, little of the attachment matrix is apparent. Bar = 10μm.

Figure 17. Higher magnification SEM of the interface of cortical bone with a ceramic dental implant. Note that fibers apparently attach to the coated implant. However, the bridging complex as seen with titanium implants was not as apparent. Bar = 5μm.

material suggestive of a glycosaminoglycan template. Brunette and associates (Chehroudi et al, 1989) suggest epithelial migration or downgrowth may be inhibited by some mechanism. They suggest this mechanism could be the physical characteristic of the implant and have provided evidence that groove size and shape affect cellular migration. This is their proposed hypothesis of contact inhibition for cellular apical migration. We suggest that this non-loadbearing connective tissue attachment may be this mechanism. This gingival connective tissue fiber association to the implant could provide for the contact inhibition preventing junctional epithelium downgrowth. However, this association does not contribute to the actual support of the implant -- it is nonload bearing.

This study has now presented evidence that mandibular bone can directly interface and form an intimate association with one-stage endosteal dental implants. A similar amorphous material was consistently observed on the implant surface at the bone level, as was seen at the gingival connective tissue level. Such a template could provide the matrix for direct and viable bone attachment to implants.

This intimate association involves calcified bone and an unmineralized matrix comprised of osteoid and osteogenic connective tissue. A direct bridging between implants and the bone support was observed. Histochemical analyses of this bridging area demonstrated the incorporation of calcified bone within the bridging complex. The particular bridging complex demonstrated in this report was on the order of 25 micrometers in thickness. This complex was intimately associated with the titanium implant surface and the calcified bone front. Other complexes were much thinner. In fact, evaluation of other implants (Steflik et al, 1989a) documented the close proximity of bone to the implants within a micrometers distance to the implant. Even though this complex was observed more readily with titanium implants, it does not preclude the possibility of a similar complex to ceramic implants. This concept requires further research. However, this study did appear to show a difference in the bone attachment appearance between ceramic and titanium implants.

It should also be noted that the apical support system to serviceable, clinically immobile dental implants involves areas of intervening fibrous connective tissue. Brunski (Hipp and Brunski, 1987) has reported that approximately 50% of apparently osseointegrated Nobelpharma implants are interfaced directly by calcified bone; with the remainder interfaced by soft tissues. Deporter and associates (1986) also report that calcified bone directly interfaces approximately the same percentage of the surface area of apparently osseointegrated porous rooted implants. Various percentages of the remainder of the implant are in close proximity to bone, but some intervening soft tissue was apparent. We (McKinney et al, 1985b) have previously reported similar histomorphometric results concerning one-stage ceramic implants. It does appear that these regions are far thinner than the dimensions of the periodontal ligament and are not load bearing. Further, this connective tissue segment must not be confused with those of the gingival connective tissue discussed above. These are two distinct areas of interest. In the dynamic interface region of dental implants, these areas change. Bone remodels and, perhaps, the connective tissue at the level of mandibular bone may be osteogenic, similar to those described by Meenaghan et al (1974). Also, these minor regions of interfacing connective tissue must be distinguished from wider areas of interfacing and encapsulating connective tissue which are suggestive of implant failure and mobility.

It should also be remembered that histomorphometic analyses represent a static, two-dimensional photograph in time. These interfaces should change over time and areas once interfaced by bone may, a month later, be interfaced with thin interposed layers on connective tissue; and areas interface by thin layers of, perhaps, osteogenic connective tissue may later be interfaced directly by calcified bone.

Summary

This study appears to show that bone can attach to endosteal dental implants. This attachment complex consists of bone bridging to the implant via an osteogenic connective tissue matrix. This attachment complex is one of three apparent apical support mechanisms. These three mechanisms of acceptable apical support to the same implant are: 1. A direct bone contact; 2. An intimate bone attachment; 3. Thin areas of intervening fibrous connective tissue. Together, these three components represent a dynamic interface of the oral tissues with the apical portion of serviceable dental implants. Further, in association with epithelial attachment and connective tissue attachment, such apical support should prognosticate continued implant serviceability.

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Discussion with Reviewers

H.A. Hansson: Is it of importance to use "atraumatic surgical technique" for the insertion of implants, of titanium, ceramics or other material, to improve the attachment, healing, and possible future osseointegration?

Authors: Probably the greatest advancement of clinical oral implantology in the past decade has been the development of atraumatic surgery protocols for implant placement. Oral implantology surgery is extremely technique sensitive and, further, individual techniques are required for individual implant systems. The advent of slow speed drilling, tapping implant receptor sites by hand, and the use of external and internal irrigation during implant surgery are examples of attempts to minimize the damage done to the bone of the receptor site. Once damage occurs (generally by overheating of the bone or by over aggressive bone removal) the implant can probably never be truly oseointegrated. For endosteal root form implants, the lack of osseointegration primarily reflects implant failure.

J. P. Waterhouse: Under what circumstances can morphological appearances, which show apposition of a regular fine structural tissue surface made up of the ends of fibrils oriented at right angles to it together with amorphous material, or a fine structural surface of healthy bone, to the smooth surface of the material of an endosteal dental implant, be interpreted as evidence that the tissues are specifically attached to the implant?

Authors: Attachment of biological tissues to inert biomaterials can be described by morphological analyses; especially if concurrent physical tests document that force is needed to separate the tissues from the implant. In our case, we could not remove the epithelium, the connective tissue, or the mandibular bone from the implant without the use of the cryofracture technique. Any attempt to physically dislodge the implant from the interfacial tissues resulted with tissue remaining on the implant. However, by cryofracturing the tissues away from the implant we were able to obtain those interfacing tissues which contained any potential attachment complexes. SEM analyses of the implant surface

demonstrated the lack of adhering tissue at that resolution level. Previous reports from our laboratories demonstrated that the junctional epithelium formed an attachment structure to implants similar to that shown by epithelium to natural teeth. Therefore interpretation of like structures was possible to document attachment. However, the lack of a periodontal ligament precludes similar correlational interpretation at the bone and connective tissue levels to implants. Therefore interpretation is critical. Since the tissues which previously adhered to non-cryofractured implants were retained with the cryofractured samples, analysis of the carefully oriented tissue samples provide descriptive data as to these potential attachment complexes.

M. A. Meenaghan: Are techniques and/or methods available for identifying the glycosaminoglycans present at the implant/tissue interfaces?
Authors: The interface complex is a complicated millieu of glycosaminoglycans and proteoglycans. Most morphological techniques offer only descriptive data of this area. However, the improvement of cryoultramicrotomy protocols offer the potential for adequate ultrastuctural resolution of immunological markers to tag specific components of the interface. This is an exciting avenue we intend to approach in future research.

R.E. Baier: Could you please clarify the results from the cryofracture technique that is cited to "clearly separate" implants from interfacing tissues? What does SEM, or perhaps ESCA (Electron Spectroscopy for Chemical Analysis), inspection of these removed implants show in terms of residual attached organic material (cells, cell fragments, etc.)?
Authors: By placing a 1mm embedded section containing the implant and associated tissues into liquid nitrogen and boiling water, we are able to create a thermal fracture plane along the implant surface. The implants then just fall away from the associated tissues. The appearance of the tissue interface is smooth and glistening whereas the tissue obtained by mechanical disruption without cryofracture is dull and rough. All TEM analyses are accomplished from samples apposing areas of the implant where no cellular debris can be identified. If any tissue remains adherent to the implant it is easily identified with SEM and the area is not used for TEM. The resolution limits of the SEM allows us to identify cells and parts of cells, however we have not utilized ESCA or related protocols in this current study to positively identify the lack of any extremely thin residual organic material. Subsequently we shall.

R.M. Pilliar: Is the amorphous substance a result of a protein adsorption layer and therefore influenced by implant surface characteristics? How typical are the figures of overall structures?
Authors: It does appear that this amorphous material may be a result of a protein adsorption layer. It appears similar to the basal lamina complex that exists at the junctional epithelial level. It will be critical to positively identify the protein component of this material (perhaps by immunological markers) as well as the existent glycosaminoglycans. With such identification hypotheses could be formulated as to the intracellular or extracellular origin of these materials. With such understanding it may be possible to alter the implant surface to enhance implant success due to these adherent proteins. The micrographs of the osteogenic bridging complex are fairly typical, especially of loaded titanium implants. All implants appeared to show such regions.