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SCANNING ELECTRON MICROSCOPIC STUDIES OF THE ORAL TISSUE RESPONSES TO DENTAL IMPLANTS

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Abstract

Scanning electron microscopy (SEM) and its associated technologies have proven invaluable in elucidating the interfacial oral tissue responses to dental implants. Since the dental implant must extend from the mandibular or maxillary jaw, through the oral mucosa, and into the oral cavity, these tissue responses include epithelium, connective tissue and bone. The continual occlusal forces acting upon these tissues reinforce the dynamic character of these tissue responses. Immediately upon implantation, a healing phase begins as a response to the implanted biomaterial. Following this immediate response a longer healing phase occurs, beginning approximately 1 week after implantation, resulting in the modeling of bone to the implant as well as the formation of epithelial attachment to the implant. This later, delayed healing continues throughout the lifetime of the implant since these tissues must die and be replaced by similar tissues. Current dental research employing scanning electron microscopy is now documenting these tissue responses. This paper reviews, in detail, SEM observations of these tissue responses.

KEY WORDS: Dental Implants, Tissue Interface, Scanning Electron Microscopy, Epithelial Attachment, Implant Support Tissues

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Introduction

The decay and traumatic loss of natural teeth has plagued man throughout history. Concurrent with this dilemma has been the desire to find replacements for this lost dentition. These replacements have included transplantation of natural teeth and natural materials, and the implantation of artificial biomaterials. The repugnance of replacing lost teeth with natural teeth obtained from donors or cadavers, as well as the potential spread of disease, provided the dental profession with the impetus to find artificial sources for tooth replacements. In fact, in 1913, Greenfield suggested that implantology was the missing link in dentistry due to the imperfections of natural tooth replacements. However, even implants are not a modern invention. Pre-Columbian South American Indian cultures were shown to utilize natural materials such as shells, wood and gems as implanted materials to replace lost teeth. Therefore, dental practitioners have a storied history in finding the proper artificial replacement for loss dentition.

This historical search has culminated in the recent explosion of interest in dental implants. The past quarter century has witnessed an unparalleled growth of clinical oral implantology. Unfortunately, basic biological investigations of how the tissues of the oral cavity react to these implanted biomaterials have, for the most part, only been reported in the past decade. Therefore, the first purpose of this paper is to review the research data, as provided by scanning electron microscopy, concerning the oral tissue responses to dental implants. Following this review, the second purpose of this paper will be to report recent scanning electron microscopic results from our laboratories concerning comprehensive and correlative analyses of the epithelial, connective tissue and bone response to ceramic and titanium endosteal dental implants.

As Albrektsson et al (1983) suggested, the interface zone between oral tissues and implants needs to be considered at the macroscopic, microscopic and submicroscopic (molecular) resolution levels. The scanning electron microscope provides the unique opportunity to examine all these levels in some manner. Babbush and Staikoff (1974) delineated the opportunities of using scanning electron microscopy as a research tool to evaluate endosteal implants as early as 1974. They suggested that SEM was particularly effective for implantology research because of the following criteria:

1. Specimens can be viewed without elaborate preparation;
2. Specimens can be viewed through a wide magnification range;
3. There is true imaging since specimens are displayed with a three dimensional quality;
4. Large samples can be studied.

Even though SEM sample preparation can be complex, such preparation is not as difficult as related to ultramicrotomy for transmission electron microscopy (TEM). Since dental implants are fairly large samples, the large specimen chamber of the SEM offers the ability to examine the entire sample and obtain three dimensional data throughout the entire area of the implant-oral tissue interface.

Therefore, we have the opportunity to make whole sample evaluations of entire implants with the SEM. These obtained whole sample observations can be analyzed in conjunction with preliminary macroscopic clinical impressions of the implant prior to removal. Following these important overt observations, the extended magnification range of the SEM permits intermediate magnification observations of the tissue and cellular responses to the implanted biomaterial. This is especially important since the implant can remain in situ with the encasement of oral tissues. Such in situ observations are critical advantages over TEM observations for which metallic and dense ceramic implants must be removed by some technique. Since orientation of appositional tissues for transmission electron microscopic ultrastructural observations is critical, these in situ SEM observations act as an important intermediary between macroscopic interpretation and actual intracellular data obtained by TEM. Such correlational morphological studies now appear mandatory to critically examine the specific tissue responses to dental implants.

The third level of interface study is the molecular and/or subcellular level. In addition to cellular morphological data, transmission electron microscopy necessarily provides subcellular morphological observations. However, as Baier (1988) suggests, various modalities of SEM provide critical data related to the bonding capabilities of implant surfaces. He suggests that routine SEM is required for surface morphology studies and that scanning auger microprobe examinations provide elemental and chemical state data for the outermost 0.005 micrometer layer of the implant. Further, by using EDAX (energy dispersive X-ray analysis) in association with SEM, elemental analysis of the outermost micrometer of the surface can be analyzed. Electron spectroscopy for chemical analysis (ESCA) can also provide intermediate elemental and chemical bonding data for the outermost 0.01 micrometer of the surface. Such analytical evaluations of implant surfaces are critical for understanding the actual tissue to implant interface (Baier and Meyer, 1988). It has to be understood that tissue adaptation begins with the surface activity of the implant. Baier et al (1984; 1988) suggest that the cleanliness, sterility and surface morphology may affect the rate of wound healing of the prepared tissue site. Smith (1988) discussed the need for scientific studies to identify and characterize new and modified materials at the 1988 NIH Consensus Conference concerning dental implants. He suggested that SEM and related techniques must be employed to validate surface characterization procedures and to measure trace element levels in animal and human tissues.

Scanning electron microscopy thereby provides a wide dispersion of characterization opportunities ranging from surface morphology, through tissue morphology, and ultmately morphological and analytical observations related to surface activity. A review of the literature discloses various uses of each of these three general activities.

Scanning electron microscopy has greatly facilitated examination of the gross implant surface morphology. Weinstein et al (1981) examined the dimensions of microgrooves of a cylindrical ion textured aluminum oxide implant; Kirsch (1983) presented the plasma sprayed titanium surface of the IMZ implant; Grafelman (1983) examined the textured surface of blade-vent implants; Sutter et al (1983) examined the surface of titanium plasma sprayed (TPS) hollow basket implants; Yamagami et al (1988) examined the surface of porous rooted ceramic implants; and we have examined the surface of single crystal ceramic implants (McKinney et al, 1982). Such observations have permitted elucidation of the nature of bone adaptation to smooth and textured apical implant designs.

Such surface examination is also important for elucidation of the mechanism of implant mechanical failure (Hart, 1986; Pearsall, 1986). Consequences of corrosion fatigue and corrosion assisted cracking (Jur, 1986) events can be disclosed by SEM analysis. Further, examination of broken transmucosal abutments or posts (Grafelman, 1983) and correlations between cracks in implants and teeth (Stanley et al, 1976) can be observed. Scanning electron microscopy provides extensive opportunities to evaluate such failed dental implants (Aguero et al, 1989). Such studies are now becoming increasingly required by the scientific community (Lemons, 1988).

In regards to clinical implant failure, an early report by Klawitter et al (1977) suggested that the coronal or permucosal surface of one stage implants can not be of a porous nature. It was shown that any surface microporosity adjacent to the gingival cuff results in inflammation and prevents the development of an adequate biological seal. Clinical failure of all 6 implants in this study was reported. These and other studies have concluded that porous rooted implants are of great potential, but the porosity must be kept well below the soft tissue level and within the prepared bony receptor site.

Currently, more emphasis is now being placed upon using the SEM to examine the intact implant - tissue interface. Albrektsson et al (1981) have documented a close spatial relationship of bone to titanium implants. Further, they examined the packing of gingival cells and connective tissue fibers on the titanium implant surface. However, interpretation that such packing suggests epithelial or connective tissue attachment through a ground substance layer may be liberal interpretation of surface morphological observations (Albrektsson et al, 1981; Linder et al, 1983). Attempts were also made to estimate the thickness of this ground substance with surface morphological SEM protocols. Since it is impossible to evaluate thickness of such a layer with surface observations the reader must be concerned with such conclusions. The reader must be concerned with the overinterpretation of any ultrastructural observation. This is particularly evident with SEM observations of the implant-tissue interface, and the difficult interpretation of cell type with such SEM observations. SEM identification of cells as osteoblasts or fibroblasts (Branemark, 1983; Albrektsson et al, 1981) is difficult, as is the similar identification of various blood cells (Steflik, 1978). Interpretation is critical, and care must be exercised when reviewing such observations. Such over-interpretation does not, however, supercede the immense wealth of data from this research group. Their extensive use of SEM, in association with TEM, has shown that bone can form a close interface with titanium implants in vivo. This finding provided much of the early research support for the clinical utilization of endosteal dental implants. It is when the interpretation of the data leads to speculative opinions interpreted as dogmatic conclusions (James et al, 1986) that overinterpretation of data becomes a concern. It must be remembered that there is the opportunity for such overinterpretation from every research group.

Even though it has been shown histomorphometrically that the actual direct bone to implant interface of apparently osseointegrated implants ranges from 40 to 60 % of the implant circumference (Hipp and Brunski, 1987; Deporter et al, 1986), the direct interface of bone to implant surfaces is a major concern of dental implant research. Stanley et al (1976), Gross and Stunz (1985) and Hench (1980) have shown a direct association of bone to a bioactive glass ceramic. This glass ceramic is coated upon a metallic core to enhance bone association. SEM and concurrent spot compositional analysis showed that there apparently was a continuity between the core metal, the reactive glass ceramic (Bioglass), and the bone. Therefore, there may be a biologically reactive hydroxylapatite and silica rich layer on the glass surface. Currently, this glass ceramic is the center of much interest in the biomaterials research community. In fact, Gross (Gross et al, 1986; Gross, 1988) and Roggendorf et al (1986) have shown SEM and TEM results of osteocytes interacting with glass ceramics in vitro and in vivo. In related SEM studies, Von St Kohler et al (1980) appeared to show that bone adhered in a closer relationship to "bio-vitroceramic" implants than to titanium, tantalum or teflon.

The direct bone to implant interface without any apparent interfacing fibrous connective tissue was also shown in vivo to single crystal alpha alumina oxide ceramic implants (Kawahara, 1978; Steflik et al, 1988b; Steflik et al 1989). This implant is a one stage endosteal root form implant and was shown to be proportionally interfaced by bone even after periods of occlusal loading. A porous rooted version of the one-stage ceramic implant has recently been introduced and has been shown to be supported well by mandibular bone (Yamagami et al, 1988; Nakagawa et al, 1987; Fukuyama & Sugimoto, 1987).

SEM was also used to evaluate the tissue interface to a macroporous one-piece permucosal metallic implant in dogs (Zak et al, 1977). SEM examined the lateral surface porosity and showed penetration of blood vessels into the pores. Appositional bone growth was also apparent over exposed areas of the implant and evidence was presented of trabecular bone remodeling. SEM of the opposing mandibular surface showed adherence of the implant metal suggesting a certain amount of bone "tenacity" adhering to the implant. Maniatopoulos et al (1986) examined threaded versus porous rooted endodontic implants and concluded that the porous rooted implant was superior since the porous nature of the implant appeared to enhance the amount of bone adaptation. This study has led to the development of a porous rooted endosteal root form implant (Deporter et al, 1986).

Hydroxylapatite used as atrophic jaw augmentation material and as coatings on implants has also been a principal emphasis of dental scientific inquiry. With SEM of in vivo dog studies Block et al (1987) showed that in order to develop any bone in the augmented area, ridges needed to be augmented not only with hydroxylapatite (HA), but via a mixture of HA with either demineralized bone powder or autogenous bone. With HA augmentation alone, there occured only a fibrous connective tissue interface. This and earlier works initiated the clinical utilization of HA with organic additives. Using SEM, EDAX and electron microprobe studies, Ducheyne et al (1980) examined the effects of an HA lining on fibers. The mineral lining was shown to stimulate infiltration and bone formation within pores. Bone forms at the mineral lining first, leaving uncalcified tissue at the center of the pore. Osteoid activity was noted and a high cellular population was evident suggesting a high remodeling activity.

Schroeder et al (1981) have also shown close congruency of bone to the apical portion of TPS basket type implants. Further, by demonstrating the relationship of fibers to an in situ implant, their SEM observations were critical in examining the orientation of gingival connective tissue fibers to this implant. This concept of orientation of the connective tissue inferior to the junctional epithelium and superior to the level of bone is of immense current interest in the oral implantology research community. If the fibers are oriented perpendicular to the implant surface rather than parallel to the surface, such an arrangement could suggest a mechanism for stopping apical epithelial migration. Such epithelial downgrowth primarily indicates implant failure. SEM provides exciting opportunities to investigate this research aim. This orientation will be addressed later in this paper during the discussion of recent advances in our laboratories. This concept was first addressed by James and Kelln (1974) and also by Steinberg (1978), and Mishima et al (1984). The possibility may exist for a composite of fibers oriented perpendicularly and in parallel. This gingival connective tissue region, even though not load bearing, can quite tenaciously adhere to the implant. James and associates showed an abrupt termination of the apical migration of bacterial plaque at this region. This termination could be a result of gingival attachment to the implant (James & Schultz, 1974), connective tissue attachment (James & Kelln, 1974), or a combination of the two. It should again be noted that this area of connective tissue association is probably non-load bearing. There is little evidence of any load bearing and functionally oriented connective tissue providing the apical support for endosteal root form implants. The only evidence for any firm attachment of connective tissue fibers to an implant material is that provided by Hench (1980) and Hench & Wilson (1984) for Bioglass. However, this is not a dental implant proper, but a glass ceramic coating. (It should be noted that Bioglass is used for inner ear replacement devices). However, blade-type implants and particularly subperiosteal implants (Russell & Kapur, 1977) may be supported by such oriented tissues (James, 1986). Steinberg (1978) documented with SEM that two layers of connective tissue fibers apposed blade type implants. The layer closest to the implant ran parallel to the blade implant and the second extended perpendicularly from the bone to the parallel fibers. The limited amount of research concerning connective tissue interfaces to blade implants somewhat suggests that fibers may extend to the implant and perhaps through the vents of the implant to the contralateral bone plate in a type of hammock arrangement (James, 1986; Steinberg 1978). Future SEM studies need to explore this concept of alternative apical tissue responses in more detail. Goldberg (1982) has also used SEM to suggest that certain endodontic implants may have such a tissue support.

Therefore, scanning electron microscopy has been widely employed by dental scientists to examine various aspects of the dental implant to oral tissue interface. SEM has proven critical in characterizing the actual surface of the implants; the surface which initiates the initial healing phenomena of the oral tissues to the implants. Further, SEM has provided exciting images and insights into the elucidation of the healed tissues to

successful implants; and to the interface of failed dental implants. Research still has not determined the ideal tissue interface to dental implants, however, extensive amounts of data are now becoming available to aid in the elucidation of this interface. Since more dental scientists are now employing the powerful tool of the scanning electron microscope and its associated technologies, the next era of morphological research will extend from the knowledge now presented to explore this critical interfacial area. The ultimate goal of this basic experimental research is to provide clues as to how we can clinically improve the chances to achieve the ideal healed state. Such is the role of all basic experimental research. That is, to provide the opportunities for application to the applied clinical sciences.

The use of scanning electron microscopy must, however, be tempered with the knowledge of the shortcomings of this investigative tool. Primarily SEM data presents surface morphological observations. Such observations often fail to positively identify cell type. Further, dehydration techniques (primarily critical point drying of large samples after alcohol dehydration) can lead to extensive shrinkage artifacts. Also, the accumulation of electrons on the surface of large biological samples which are hard to entirely coat with conductive materials, often leads to charging artifacts which masks the true morphology of the specimen. These are but a few of the problems which can lead to interpretation dilemmas. For this reason, scanning electron microscopic observations need to be considered in conjunction with correlational transmission electron microscopic and light microscopic observations. Only in this way can cell type be identified and tissue morphology be described. Such correlational studies have been described by Hansson et al (1983); Albrektsson et al (1981); and by research from our laboratories.

Based upon this extended literature review, the second purpose of this paper is to present recent correlated observations from our laboratories concerning the oral tissue interfaces to dental implants. This report will be restricted to observations made with conventional and alternative SEM protocols.

Materials And Methods

Data presented here originate from two similar investigations from our laboratories. Forty-eight cylindrical single crystal alpha alumina oxide root form endosteal dental implants and sixteen similar commercially pure titanium root form endosteal dental implants were utilized for these studies. Following a healing period of two months after bilateral extractions of all premolars, the implants were inserted into mandibles of 24 adult mongrel dogs. The animals were sacrificed at periodic intervals up to 24 months post implantation.

At euthanasia, the head and necks of the animals were fixed by vascular perfusion via a carotid artery cutdown procedure (Steflik et al, 1989). The perfusate used was 3% phosphate bufferred glutaraldehyde for 45 minutes. After initial fixation, implant samples were block resected from the mandible and fixed by immersion in glutaraldehyde for an additional 24 hours. Samples were post-fixed in 1% phosphate bufferred osmium tetroxide for 2 hours. Samples were washed three times with phosphate buffer and prepared as follows.

Randomly selected implant samples were processed for scanning electron microscopy via six protocols. First, fixed implant samples en block with associated mandibular tissues were routinely dehydrated in ascending concentrations of ethanol and critical point dried with carbon dioxide. Second, while immersed in saline, fixed samples were hemisected using a diamond wafering blade on a Buehler Isomet saw. The two resulting hemisected samples were then critical point dried after dehydration as above. Third, in two samples, a gingival flap was carefully dissected from the in situ implant block sample and immersed into fixative. After washing in buffer, the samples were dehydrated and critical point dried as above. Fourth, random glutaraldehyde and osmium tetroxide fixed block samples were dehydrated through ethanols and embedded in either Maraglass 655 or Epon 812. Alternatively, samples were also embedded in polymethyl methacrylate. Sections were then cut at thicknesses of 1 to 2 mm. These sections were then processed in one of two ways. First, the sections were subjected to our cryofracture technique which is a modification of the technique first developed by James and Schultz (1974). Briefly, the sections are immersed into liquid nitrogen, followed immediately by immersion into boiling water. This creates a thermal fracture plane and the implant is cleanly removed from the associated oral tissues. The lack of any adhering oral tissues to the implant was confirmed by SEM analysis. The resulting tissue sample was then reembedded into the same embedding media. TEM ultramicrotomy then ensued as per normal techniques resulting in both 1 micrometer thick sections and normal 700 Angstrom thin sections. Second, the 1 to 2 micrometer in situ implant plastic embedded sections (as well as

random 1 micrometer TEM orientation thick sections) were subjected to the plasma etching protocol of Steflik et al (1983; 1984a). Briefly, specimens were placed in a vacuum chamber into which oxygen gas was introduced. A radiofrequency generator was used to produce an excited oxygen plasma which surface etched the plastic embedded specimens permitting SEM analysis by exposing surface topography. Specimens were then shadowed by vacuum evaporation of platinum/ palladium wire at an angle of 45 degrees at a distance of 10 cm.

All samples to be used for SEM were then mounted on standard AMR mounts, sputter coated with gold and viewed with an AMR 1000A scanning electron microscope. Images were recorded with both secondary and backscattered detectors.

Results

Composite Results

Forty-eight ceramic and sixteen titanium cylindrical implants from two specific investigations are included in this report.

Study One: Thirty two ceramic cylindrical endosteal implants were inserted into the edentulated mandibles of sixteen adult mongrel dogs. One implant was placed in the left premolar region of the mandible and one implant was placed in the right premolar region. Prior to animal euthanasia, 8 implants were in situ for 24 months; 8 implants were in situ for 18 months; 8 implants were in situ for 12 months; and 8 implants were in situ for 3 months. None of these implants supported any fixed bridgework. Of these implants 3 were considered as failures due to clinical mobility and excessive radiolucency around the implants as observed by clinical evaluation radiographs.

Study Two: Sixteen ceramic and sixteen titanium cylindrical endosteal implants were bilaterally inserted into the mandibles of 8 adult mongrel dogs. Two ceramic implants were placed into the right premolar region and two titanium implants were placed in the contralateral region. In four dogs, the 16 implants did not support fixed bridgework. In these dogs, 2 ceramic and 2 titanium implants were in situ for the following time periods: 5 months, 3 months, 2 months, and 1 month. In four other dogs the 16 implants did receieve fixed bridgework one month after implantation with the implants acting as anterior abutments and the first molar acting as the posterior abutment. In these dogs two ceramic and two titanium implants were in situ for each of the following time periods: 2 months, 3 months 4 months and 6 months. Of these implants 2 ceramic implants which supported bridgework for 2 months and two titanium implants which supported bridgework for two months were considered as failures due to the same criteria as in study one. This result of clinical failures may suggest that adjacent implants may have influenced the serviceability of one another.

Morphological Results

SEM of in situ implant block mandibular samples showed that both ceramic (Fig. 1) and titanium (Fig. 2) one stage endosteal implants appeared to be well tolerated by mandibular tissues. A regenerated gingival cuff was apparent with an intact gingival sulcus. Closer examination showed the progression from the outer squamous free gingival cells to the more bulbous cells at the crest of the

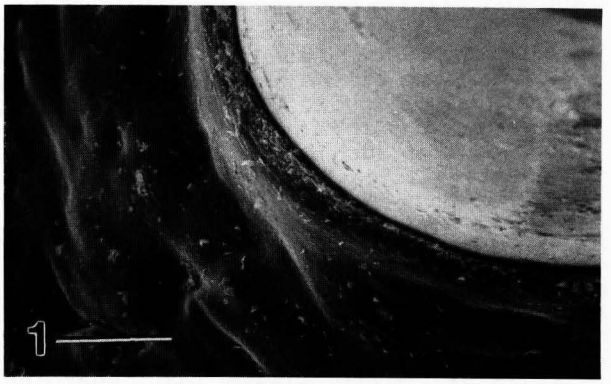


Figure 1. Scanning electron micrograph showing acceptable mandibular tissue response to an in situ ceramic implant. Bar = 500μm.

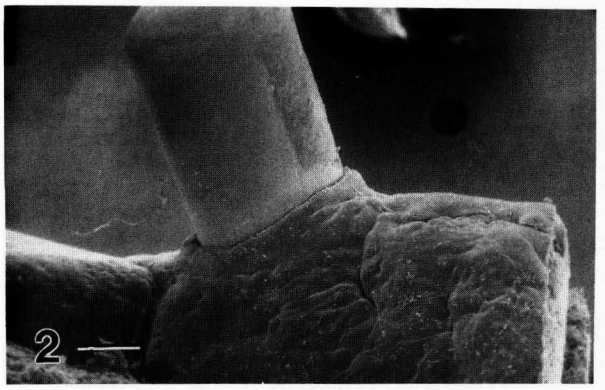
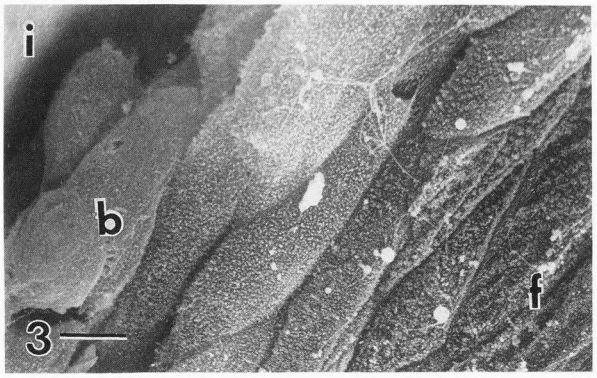


Figure 2. Scanning electron micrograph showing acceptable mandibular tissue response to an in situ titanium implant. Bar = 1mm.



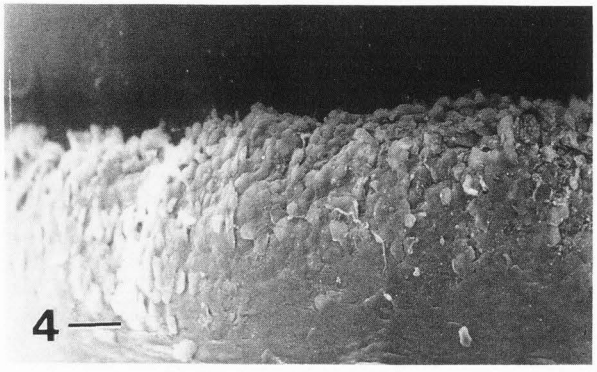
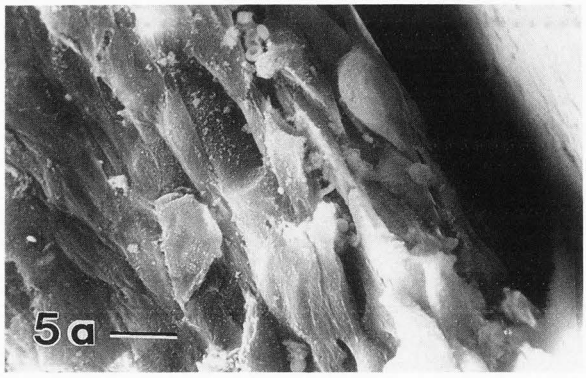


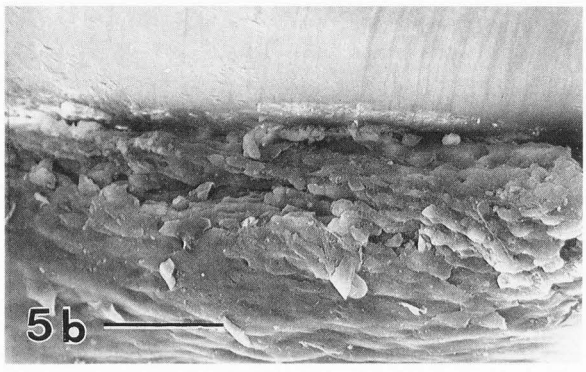
Figure 3. Scanning electron micrograph demonstrating the normal gingival progression to a ceramic dental implant. This progression extends from the flattened squames of the free gingival margin (f); to the more bulbous cells (b) of the crestal gingival cells at the gingival margin which interfaces the implant (i). Bar = 10 μm.

Figure 4. SEM of a more lateral view demonstrating the cellular makeup of the gingival cuff adjacent to a ceramic implant.
Bar = 100 μm.

gingival margin (Fig. 3). This was similar to earlier results from our laboratories (McKinney et al, 1984a; 1984b). Lateral views (Fig 4) showed the cellular makeup of the gingival cuff and higher magnification from a more superior view disclosed that these elongated cells were aligned to the titanium (Fig 5a) and ceramic (Fig 5b) implant surfaces at the crestal margin. At an area of an implant which was clinically evaluated as eliciting a slightly hyperemic response, erythrocytes were observed between the more loosely arranged epithelial cells (Fig 6).

Some implant samples were hemisected without any prior embedding. A hemisected ceramic implant was shown to be well supported by mandibular bone (Fig 7), with bone regenerating over the implant





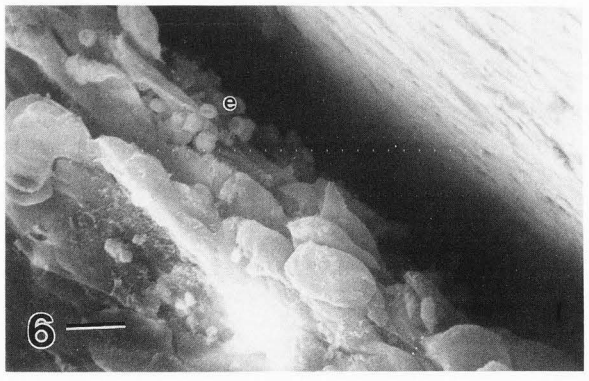
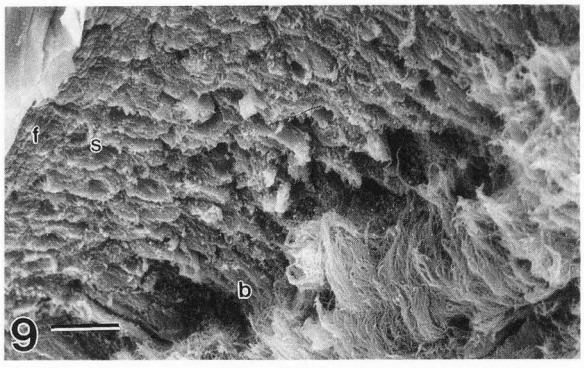
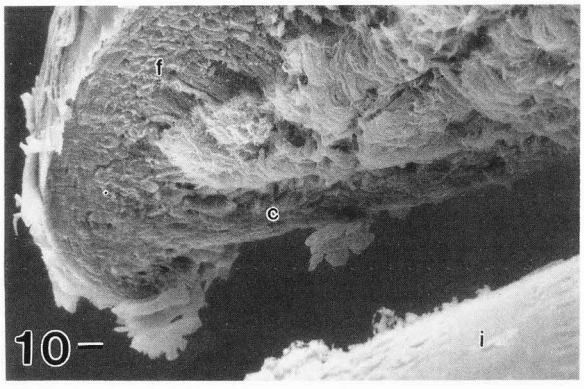


Figure 5. Scanning electron micrographs taken from a more superior aspect disclosing the alignment of the crevicular epithelial cells to a titanium (Fig. 5a) and to a ceramic (Fig. 5b) implant.
Fig. 5a bar = 10 μm; Fig. 5b bar = 100 μm.

Figure 6. SEM demonstrating the appearence of the gingival response to a titanium implant which was clinically rated as slightly hyperemic. Note the erythrocytes (e) at the crestal margin and the looser arrangement of the epithelial cells. Bar = 10 μm.





shoulder (the bone above the shoulder had to be removed at surgery in order to place the implant). A second sample (Fig.8) confirms this close bone association. Similar to previous reports, normal appearing crevicular and junctional epithelium interfaced these ceramic implants in the permucosal region. Normal maturation patterns of the free gingival region (Fig 9) were demonstrated with basal cells eminating from an internal basal lamina region associated with gingival connective tissue. Stratum spinosum epithelial cells were apparent, as were the keratinized squames of the free gingival margin. The crevicular epithelium (Fig 10) was shown to be nonkeratinizing epithelium, lacking any stratum corneum. Upon examination of the narrowing layer of crevicular epithelium as it extended to the actual implant interface, the junctional epithelium could be examined. The junctional epithelium (Fig 11) was shown to interface the implant as a ridge of cells, often extending short cellular projections to the implant.

Figure 7. SEM of a ceramic implant hemisectioned without any prior embedding. Bone was closely apposed to the implant, and extended over the shoulder of the implant. At surgery, the bone had to be removed at this region in order for the implant to be placed.
Bar = 1 mm.

Figure 8. SEM of a second ceramic implant processed as in figure 7. Bone is closely apposed to the implant; however a region of connective tissue is noted at the implant shoulder margin (arrowhead). Further, an area of uncalcified tissue (*) is noted within the bone and interfacing a portion of the inferior aspect of the implant shoulder. Bar = 100 μm.

Figure 9. Examination of the free gingival margin by SEM disclosed the normal epithelial maturation patterns. Basal cells (b) eminated from the internal basal lamina region and the intermediate stratum spinosum cells (s) were apparent. Keratinized squames of the outermost differentiated free gingival cells (f) were identified.
Bar = 10 μm.

Figure 10. The crevicular epithelium (c) was shown by SEM to have similar differentiation patterns; however the differentiated cells were not keritinized; they lacked a stratum corneum. The free gingival cells (f) are also identified. The crevicular epithelium forms a sulcus to the implant (i). Bar = 10 μm.

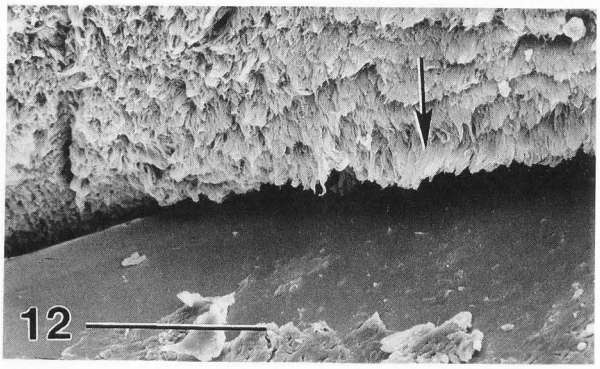
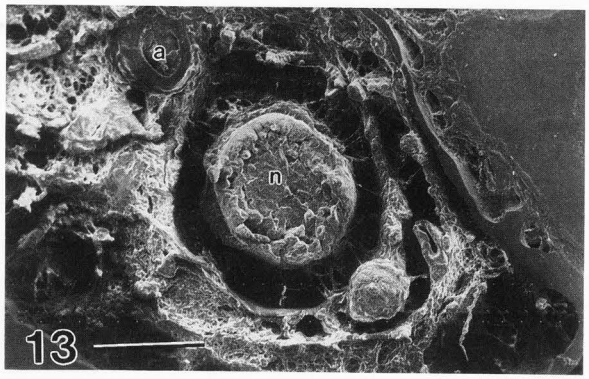


Figure 11. SEM of the region where the epithelium abutted the ceramic implant. The junctional epithelium formed a ridge of epithelial attachment (r) to the implant with short cellular projections (arrowhead) also extending from that ridge to the implant. Bar = 1 μm.

Figure 12. SEM of the gingival connective tissue located inferior to the junctional epithelium and superior to the level of crestal bone. Notice that the connective tissue fibers appear to be oriented perpendicularly to the implant surface (arrow).
Bar = 100 μm.

Inferior to the junctional epithelium and superior to the level of crestal bone, gingival connective tissue was apparent (Fig 12). At this region, the connective tissue fibers appeared to be oriented perpendicular to the ceramic implant surface.

At the most apical region, the implant was primarily interfaced by fibrofatty marrow space. At a distance from the implant, the mandibular nerve and artery were apparent (Fig. 13). The mandibular nerve and artery were supported by a fibrous connective tissue sheath.



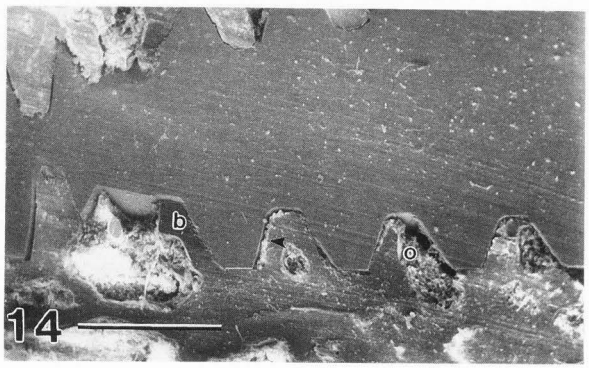
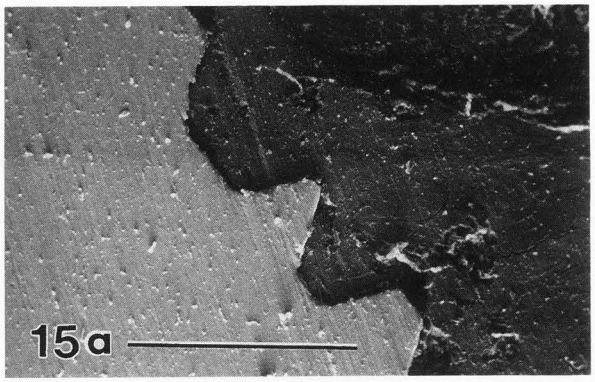


Figure 13. SEM of the mandibular nerve (n) and artery (a) found at the most apical region associated with an implant. The artery and nerve were supported by a connective tissue sheath. Bar = 1mm.

Figure 14. Low magnification SEM of a hemisectioned titanium implant without any prior embedding. The implant was proportionally interfaced either by bone (b), connective tissue (arrowhead), or by a fibrocellular osteoid (o). Bar = 1mm.

Successful titanium implants were also shown to be proportionally interfaced by mandibular bone. The implant was either directly interfaced by bone, separated from the bone by narrow interposed layers of fibrous connective tissue, or was interfaced by fibrocellular osteoid tissue (Fig. 14). Alternative electron imaging was used to identify the close approximation of mandibular bone to the implant and the nature of the osteoid material. Backscattered imaging (Fig. 15a) disclosed the bone association while secondary imaging (Fig. 15b) provided superior osteoid determination. The calcified bone front was readily apparent interfacing the titanium surface (Fig. 16). A closer examination of the osteoid region showed the fibrocellular morphology of this material as it adhered to the titanium implant surface (Fig. 17).



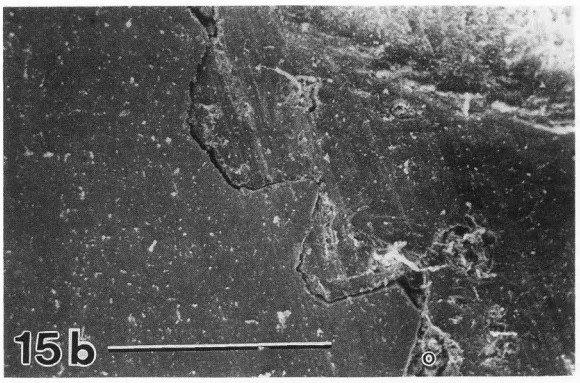


Figure 15. Alternative electron imaging of a region of mandibular bone association to a titanium implant processed as in figure 14. Backscattered SEM imaging (15a) demonstrated the bone appearence to the implant, while secondary electron imaging (15b) more clearly disclosed the fibrocellular morphology of the osteoid material (o). Bar = 1000 μm.

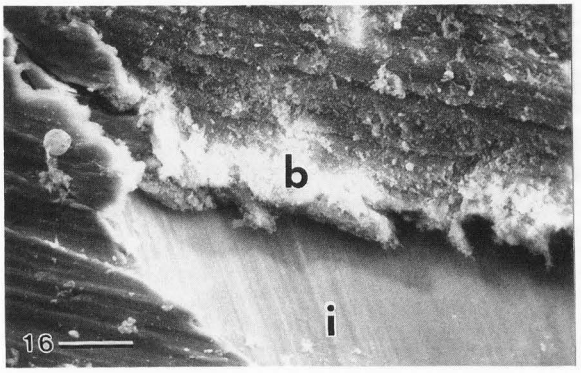


Figure 16. Intermediate magnification SEM of the calcified bone front (b) interfacing a hemisectioned titanium implant (i). Bar = 10 μm.

Figure 17. Higher magnification SEM of the osteoid interface to a titanium implant. The fibrocellular stroma is apparent and adheres to the titanium surface. Bar = 5 μm.

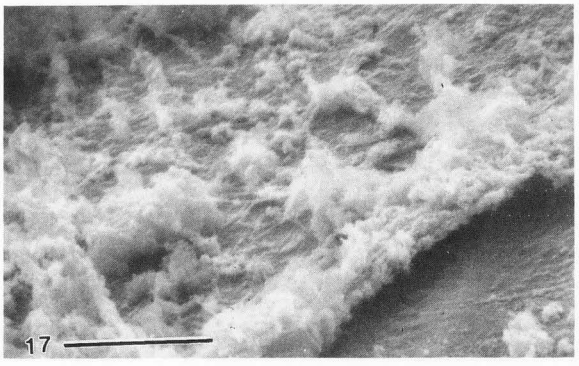
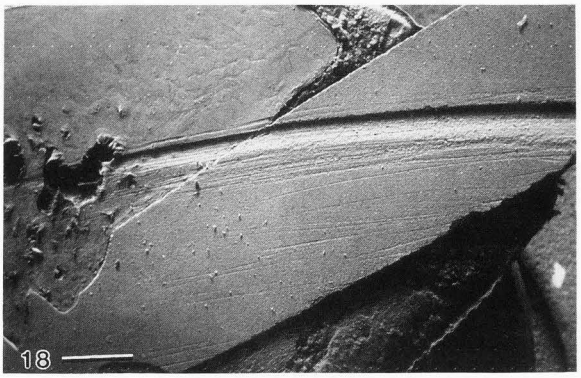
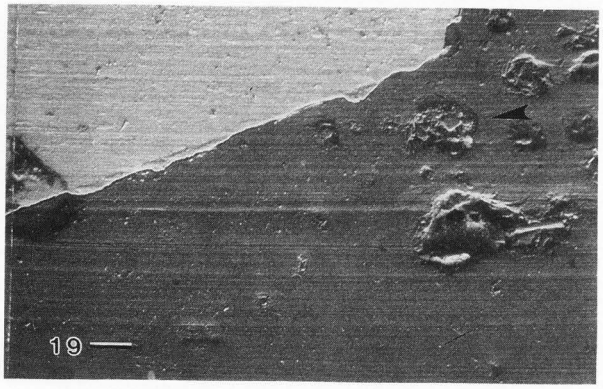


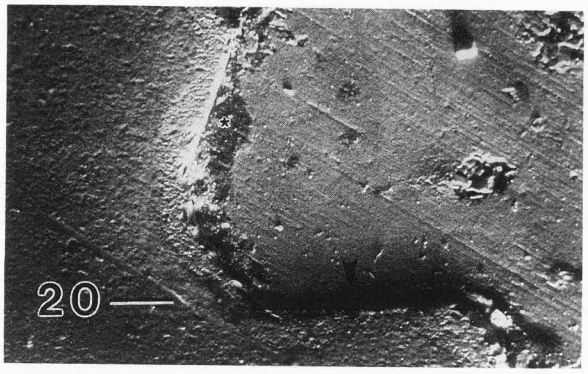
Figure 18. SEM of a ceramic implant plastic embedded, hemisectioned, and then plasma etched. Backscattered imaging disclosed that the mandibular bone was closely apposed to the coronal third of the implant. Further, the normal but differing buccal and lingual heights of the crestal bone was apparent.
Bar = 1000 μm.

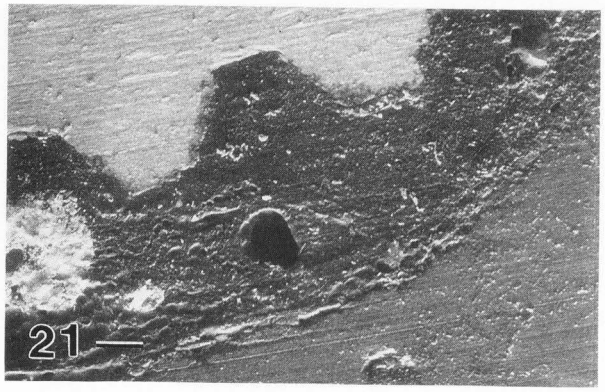


Implant samples which were sectioned after plastic embedding and then surface etched with oxygen plasma also showed acceptable apical bone support to both ceramic and titanium implants. The ceramic implant was closely apposed by dense cortical bone (Fig. 18). As can be seen in Figure 18, the normal but differing levels of bone height on the buccal and lingual aspects of the mandible supporting the implant was apparent. The coronal level of crestal bone was also closely apposed to the titanium implant with an haversion canal apparent in these embedded, plasma-etched samples (Fig. 19).

Neither ceramic nor titanium implants were apposed 100% by bone along their entire circumference. At some point,







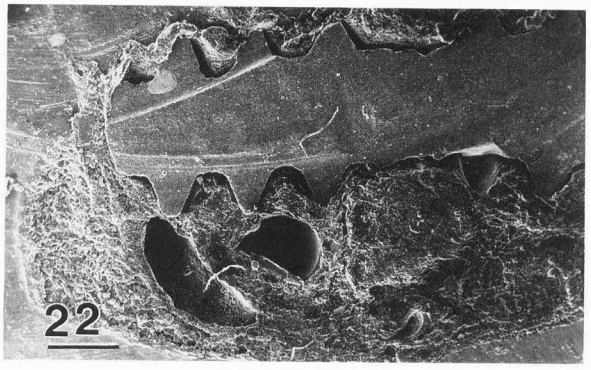


Figure 19. SEM of a similarly processed titanium implant showing the close bone interface with Haversian systems (arrowhead) apparent. Bar = 100 μm.

Figure 20. Backscattered SEM of an embedded, hemisectioned and plasma etched ceramic implant sample. At the thread apex thin areas of noncalcified tissue (*) were apparent interfacing the implant as well as calcified bone without any intervening connective tissue (arrowhead). Bar = 100 μm.

Figure 21. Backscattered SEM of the fibrofatty marrow space interfacing the most apical region of a titanium implant and separating the implant from the inferior border of the mandible. Bar = 100 μm.

Figure 22. SEM of an unembedded ceramic implant clinically rated as mobile and failing. The implant was encapsulated within a wide expanse of fibrous connective tissue. Bar = 1 mm.

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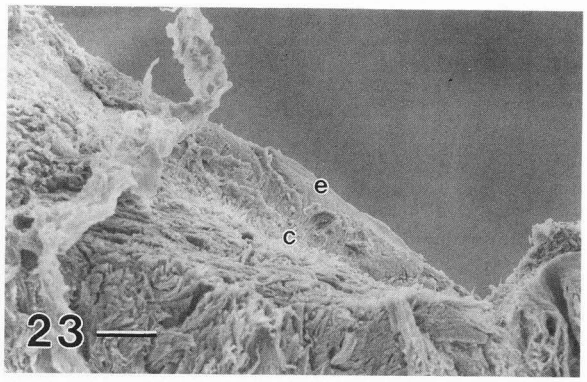
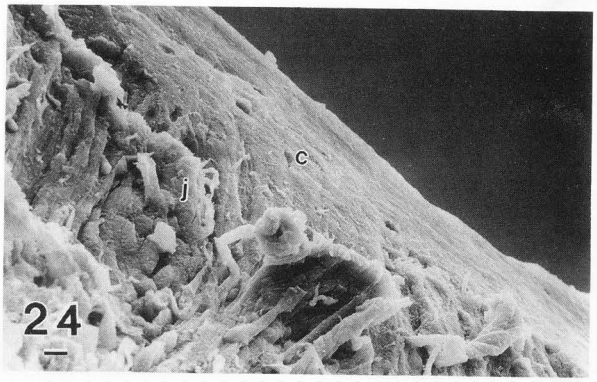
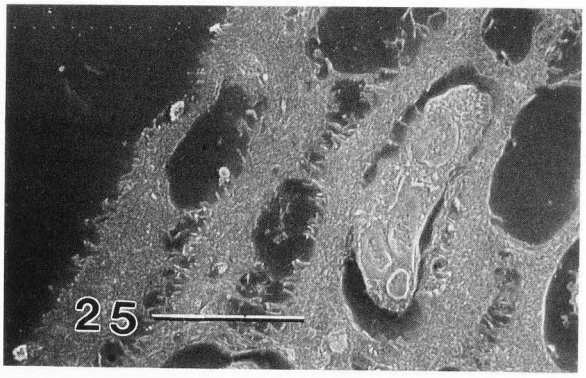
narrow layers of soft connective tissue interfaced the implant. This was especially apparent at the thread apices of these threaded cylindrical implants (Fig. 20). Also, the most apical aspects of the implants were often encased by soft tissues in the fibrofatty marrow space. Backscattered electron imaging was particularly useful in identifying this soft tissue encasement and its vasculature (Fig. 21). This region was easily distinguishable from the mandibular bone.

This proportional bone, connective tissue, marrow space interface to successful endosteal dental implants is markedly different to that observed with failed endosteal implants. As is apparent in figure 22, a failed ceramic implant was encapsulated within a wide expanse of fibrous connective tissue. This interface provided minimal support for this implant which was rated clinically as failed and exhibited excessive mobility. Failed titanium implants also were interfaced by a similar fibrous connective tissue encapsulation.

Returning to the epithelial interface to ceramic implants, upon removal of two implants, gingival biopsies were microdissected away from the permucosal aspect of the implant. SEM of this region disclosed the crevicular and junctional epithelial response to the serviceable dental implant (Fig. 23). The underlying gingival connective tissue region was also apparent. Upon closer examination (Fig. 24), the smooth surface of the crevicular epithelium was apparent terminating into a ridge of junctional epithelium which







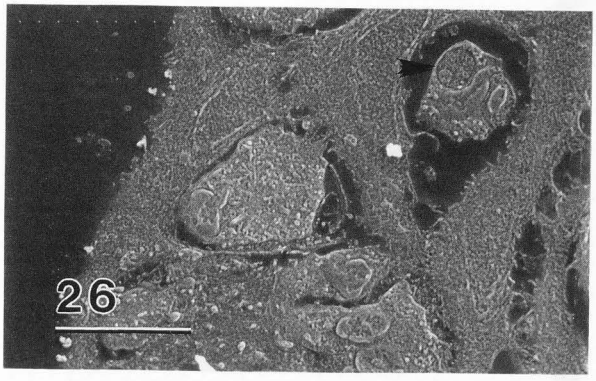


Figure 23. SEM of a mucosal biopsy microdissected away from a ceramic implant. This view from the more apical aspect of this biopsy showed the level of gingival connective tissue (c). Further, the termination of the crevicular epithelium (e) was apparent. Bar = 100 μm.

Figure 24. Higher magnification SEM of the epithelial aspect of this biopsy. The crevicular epithelium (c) of the gingival sulcus terminated into a ridge of junctional epithelial cells (j). These junctional epithelial cells at times appeared to extend short cellular projections in the direction previously occupied by the implant. Bar = 10 μm.

Figure 25. SEM of a plasma etched 1 um TEM orientation section of the crevicular epithelium adjacent to a ceramic implant. The crevicular epithelial cells were elongated with a smooth surface with some short projections. A leucocyte was apparent within an intercellular space. Bar = 5 μm.

Figure 26. SEM of a similar section disclosing the appearance of the crevicular epithelial cells. Nuclear morphology is retained. Leucocytes exist in intercellular crypts, with one such cell containing an apparent phagocytic vacuole (arrowhead). Bar = 5 μm.

appeared to, at times, extend short cellular projections toward the implant surface (note: the implant was removed in these samples). This appearence was similar to the <u>in situ</u> interface seen in figure 11.

Plasma etching the 1 micrometer TEM orientation sections and ultrathin TEM sections disclosed intracellular detail with SEM observations of the crevicular and junctional epithelial cells. SEM of 1 micrometer sections showed that the crevicular epithelium was elongated and exhibited primarily a smooth outer surface with some cellular projections (Fig 25). Polymorphonuclear leucocytes were apparent within intercellular crypts (Figs. 25 & 26). Membrane and nucleus morphology was retained by the surface etching protocol. Further, the closely interdigitating intercellular bridges between the layers of epithelial cells was apparent (Fig. 27) suggesting the healthy tight intercellular junctions. Plasma etching of ultrathin sections of the junctional epithelium disclosed cellular differentiation of these cells (Fig. 28). The junctional epithelial tissue ranged from six to two cells in thickness for successful implants. Closer examination disclosed that the outermost cell was also limited by an extracellular structure (Fig. 29). This structure was shown to be of similar dimensions to the external basal lamina

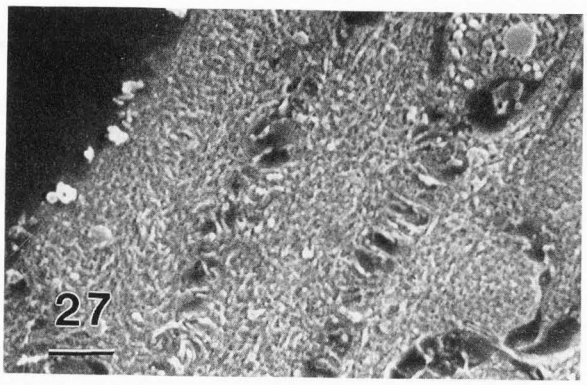


Figure 27. SEM of a similarly processed section demonstrating the tightly interdigitating intercellular bridges connecting healthy gingival cells. Bar = 1μm.

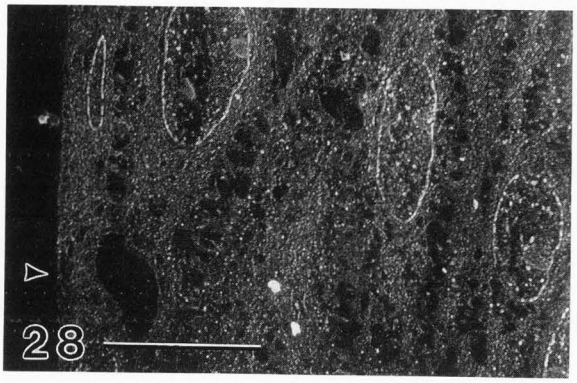


Figure 28. SEM of a plasma etched ultrathin TEM section demonstrating the epithelial maturation of junctional epithelial cells. The outermost cell layer (arrowhead) appears alligned to the space previously occupied by the implant. Bar = 5 μm.

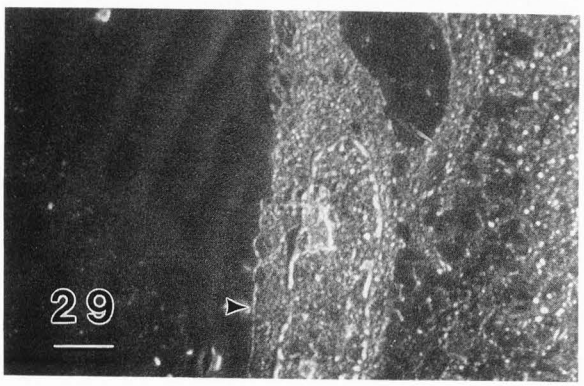


Figure 29. SEM of a similarly processed section showing that the outermost cell was limited by an extracellular organelle (arrowhead). Bar = 1μm.

identified by our laboratories in previous investigations. Since serial sections were cut, TEM of the next section confirmed the existence of the basal lamina at this region.

Discussion

From the extended literature review presented here, and from ongoing investigations in our laboratories, it becomes apparent that the oral tissue interface with endosteal dental implants is multifacited and complex. These tissue interfaces can be classified into three general categories. These categories are the epithelial response; the gingival connective tissue response; and the apical support system response. It must be understood that a dental implant must exist in two unique environments. It must exist within an internal milieu composed of the tissues of the oral cavity. The implant must also protrude from the oral tissues, through epithelium and into the oral cavity. It is in the oral cavity that the implant serves its purpose; that is, it provides the support for a fixed prosthesis. The oral tissues provide the anchoring for the implant to provide this service.

Since the implant must penetrate the covering oral mucosa to enter the oral cavity, it appears justified to suggest that the epithelium must form a permucosal seal to the implant. Such a biological seal would prevent oral bacteria and oral debris from penetrating into and disrupting the apical support system. Evidence for the formation of a biological seal has been reported here and in previous reports from our laboratories (Steflik et al, 1988a; Steflik et al, 1984b; McKinney, Steflik & Koth, 1985) and others (Karagianes et al, 1982; James and Schultz, 1974). The role of the crevicular epithelium must also not be forgotten. As shown here, leucocytes were apparent within intercellular crypts in the crevicular epithelium layer. Such cells permit the crevicular epithelium to perform its function. This function is to serve as a filter to eliminate the destructive influences of the oral cavity before they can enter the support tissues. The junctional epithelium attaches to the implant and provides the seal preventing any remaining bacteria from infiltrating into the support complex, and producing any toxic response damaging the bony support. This attachment complex consists of hemidesmosomes and an associated external basal lamina (Steflik et al, 1988a). The basal lamina was also

demonstrated in alternative studies reported in this paper.

Junctional epithelium provides for the beneficial sealing of the implant as described. However, apical epithelial migration or downgrowth is also a detrimental condition for dental implants (Meenaghan, 1974). This epithelial downgrowth usually coincides with wider intercellular junctions and if left unchecked will lead to the failure of dental implants. The level of connective tissue inferior to the junctional epithelium and superior to the level of bone (the gingival connective tissue) is currently gaining wide interest. As Brunette et al (1990) have described, contact inhibition can influence and prevent epithelial downgrowth. Evidence has been accumulating (Shroeder et al, 1981; Steflik et al, 1989) that the gingival connective tissue may at times be oriented perpendicularly to the implant surface. If data continues to accumulate supporting this hypothesis, this connective tissue may represent a contact inhibition mechanism to prevent epithelial downgrowth. This is a key concern of oral implantology research, and an area where SEM has provided critical morphological data. It must be recalled that this connective tissue is non loadbearing and provides no apical support for the implant.

The third, and arguably the most important region of oral tissue responses to dental implants is the apical support tissue for the implant. Bone is critical to support endosteal root form implants. In clinically and radiographically appearing osseointegrated implants, approximately 40 to 60 % of the implant surface is interfaced by calcified bone. The rest is composed of fibrous connective tissue and osteoid. However, this soft connective tissue is not a wide expanse separating the implant from supporting bone. Rather, it primarily represents narrow regions of soft tissue on the order of approximately 40 micrometers. Therefore, calcified bone is still in close proximity to the implant. Of course, in anatomical regions where bone does not naturally occur, soft tissues will predominate. These regions include the marrow space and some regions of cancellous bone.

Returning to the bone surrounding dental implants, this bone does appear to intimately contact the implant. The bulk of current evidence suggests that successful implants are proportionally apposed directly by some percentage of bone. This is the meaning of osseointegration of implants -- the term developed by Branemark (Branemark, 1983). Evidence is growing that some sort of attachment complex may also exist (Albrektsson et al, 1981; De Lange et al 1988; Steflik et al, 1990). An osteogenic connective tissue and osteoid may be indicated in this complex, as may a glycosaminoglycan basal lamina type organelle. Correlated scanning and transmission electron microscopic analysis will be required to evaluate this hypothesis.

Even though bone is mandatory to support endosteal root form implants, other implants can be supported by soft fibrous connective tissues. Subperiosteal implants are primarily maintained by a connective tissue support. Thin blade-type implants have also been shown to provide acceptable function with a fibrous connective tissue interface. In this case, the connective tissue does not attach into the implant but penetrates through the vents or slots of the blade and attaches into the contralateral side. This hammock or sling support for blades and subperiosteal implants has been reported by James (1986). But it must be remembered that these oriented fibers attach into bone on both ends, not to the implant itself. The implant is then supported by this sling. This alternative tissue response is of interest and should be examined in more detail with SEM analysis.

Summary

Alternative and dynamic tissue responses occur during the healing events following oral implantology procedures. After the surgical protocol epithelium must regenerate and reform a biological seal to the implanted biomaterial. It has to be understood that this event continues throughout the lifetime of the implant. Epithelium regenerates, reattaches, sloughs and dies; only to be replaced with new gingiva. That is why the junctional epithelium initially extends short cellular processes to the implant surface; it is growing to that surface. This is followed by the more strongly adhering ridge of epithelial adhesion forming the protective permucosal biological seal. This attachment may also be assisted by the attachment of gingival connective tissue. This connective tissue attachment may not only play a role in the contact inhibition of epithelial downgrowth, but also in the further sealing off of the apical support system from the destructive influences of the oral cavity.

The apical support system is also a dynamic, regenerating tissue which actively responds to the actions of the biomaterial. Occlusal forces acting upon the implant also act upon the interfacial tissues supporting the implant. These forces can cause active and beneficial bone remodeling if the forces are properly maintained. However, over stressing of the implant can lead to bone destruction and stress shielding can lead to bone necrosis

and resorption. Proper surgical technique and implant alignment, and excellent prosthodonic restoration assure that occlusal forces are beneficial to the active remodeling process. Scanning electron microscopy and correlated morphological protocols are currently elucidating the most beneficial tissue interfaces which successfully support dental implants in the applied clinical setting.

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Discussion with Reviewers

H.A Hansson: Why wait several months after the extraction of teeth before insertion of the implant? Have the authors any experience of trying a one-step procedure?

Authors: At the time of these investigations, as we do now, we thought it was critical to allow the extraction sites to heal for a period of two months prior to implantation. Since we also allow extended periods of healing after extraction for our human clinical implantation cases, we considered it a proper research protocol. Since we desire to examine the behavior of various implants placed in healthy edentulated jaws, we wanted to eliminate any extraneous variables that could exist by placing implants into extraction sites that often require various amounts of surgical intervention during the extraction process. We have found that two months of post-extraction healing in the dog is satisfactory. Currently, we are investigating the effects of placing implants immediately into fresh tooth extraction sites in dogs. However, data is incomplete and we will continue to permit a healing phase prior to implantation.

L.B. Heffez: Were the implant failures due to deliberate poor surgical technique or an incedental finding?

Authors: The implant failures were not the result of deliberate poor surgical technique. However, it does appear that unintentional poor surgical technique was the cause for implant failure. Retrospective review of our records showed that radiographic radiolucency was noted one month after implant insertion for four of the failures. This would suggest that the implant receptor sites may not have been properly prepared, resulting in the implant failures noted. We have also investigated failed implants received from human patients. It does appear that a correlation exists between the obtained animal research data for failed implants and for that obtained from failed implants in patients. Similar failure scenarios occured which resulted from improper surgical technique, improper prosthodontic technique, or inadequate oral hygiene and implant maintenence. These resulted in chronic and acute inflamatory responses and eventual osteoclastic disruption of the apical support for the implant. Therefore, it does appear that the SEM/TEM findings may be representative of clinical failures.

H.A. Hansson: How many weeks ought to elapse after insertion of an implant before true conclusions can be stated about its biocompatibility, including mechanical stability and osseointegration?
Authors: It appears that in the dog model it takes approximately 3 weeks for the reformation of a strong junctional epithelium attachment to the implant and at least 8 weeks for bone to heal after surgical intervention. Biocompatibility of implant material as described by histological assessments of an inflammatory response, elongated junctional epithelium, sulcus depth, and bone loss can be initially assessed to that point, especially since that is adequate time for the reformation of the epithelial attachment to the implant. It appears that if an implant is to fail due to surgical insertion the manifestation of the failure will be seen by 8 to 12 weeks. When an implant supports fixed bridgework, we believe that the effects of improper occlusion or prosthodontic technique should be manifested by 6 months after loading. Therefore the time period of 6 to 12 months is critical for loaded implants in the dog model to assess adequate osseointegration. Long term effects for up to 24 months also need to be incorporated as to conclusions regarding prolonged and maintained osseointegration. Obviously these time periods need to be extended for assessment of osseointegration and clinical serviceability in humans.

Editor: Where can the references from Proc. Southeast. Ele. Microsc. Soc. be obtained?
Authors: The references from the Proceedings of the Southeastern Electron Microscopy Society can be obtained from the following address:
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