

# The Reactions of Bone, Connective Tissue, and Epithelium to Endosteal Implants with Titanium-Sprayed Surfaces

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## 1. Introduction

For a long time research on dental implants was mainly concerned with the macroscopic form of the implants and therefore concentrated on the clinical aspects and on questions of surgical technique. Recently, however, it has become apparent that the success of an endosteal implant is largely – if not totally – determined by the biocompatibility of the material used. This biocompatibility seems in turn to be determined not only by the chemical nature of the material but also by the micromorphological surface properties of the implant. This is certainly true for biopassive materials such as tantalum, titanium, and aluminium oxide (Klawitter 1970, Hahn and Palich 1970, Hirschhorn et al. 1971, Taylor and Smith 1972, Karagianes 1973, Vitredent Corp. 1973/75, Jameson 1974, Klawitter and Weinstein 1974, Mühlmann 1975, Karagianes et al. 1976, Klawitter et al. 1977, Kresch and Young 1977, Weiss 1977, Young et al. 1979). However, the surface properties of the material are unimportant in the case of bioactive or “bioreactive” materials such as bioglass or apatite (Hench and Paschall 1973, 1974, Strunz et al. 1976, Eulenberger et al. 1978, Newesely 1979).

The results reported here are derived from a series of animal experiments begun 7 years ago in which the reactions of bone, connective tissue, and epithelium to certain modern implant materials were investigated. The materials of primary interest were:

- titanium (polished, sandblasted, or with a titanium-sprayed surface)
- vitreous carbon
- aluminium oxide
- bioglass.

Preliminary experiments showed that the titanium/titanium-sprayed surfaces was the material to which our investigative efforts should primarily be directed. Although the other materials were tested in parallel this report deals mainly with the tissue reactions to titanium with a titanium-sprayed surface.

## Summary

The results of a research project which has been under way for the last 7 years are reported. The project has been centred around a series of animal (monkey) experiments designed to test the suitability of cylindrical titanium implants with titanium-sprayed surfaces. The following results have been obtained: 1. The bone applies itself “ankylostatically” (i.e. without an intervening layer of connective tissue) to the surface of the implant and, as revealed by observation over periods of up to 1<sup>3</sup>/<sub>4</sub> years, even remains attached to the implant surface when placed under load. 2. The fibres of the connective tissue between the bone and the epithelium insert into the titanium-sprayed surface and appear to be functionally orientated. 3. If the post of the implant is situated in a region of immobile, keratinized mucosa signs of adhesion of the epithelial cells to the titanium-sprayed surface become apparent. 4. The clinical success of the implant is clearly dependent on (a) the presence of tethered, immobile mucosa (“gingiva”) around the post of the implant, and (b) perfect oral hygiene.

## Key-Words

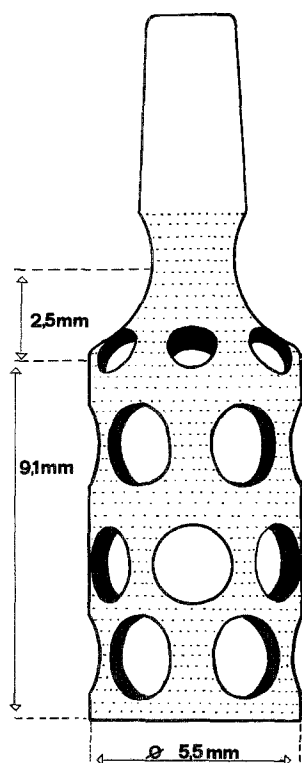
Hollow cylinder endosteal titanium/titanium-flashed implant; Animal experiment; Histological findings.

## 2. Methods

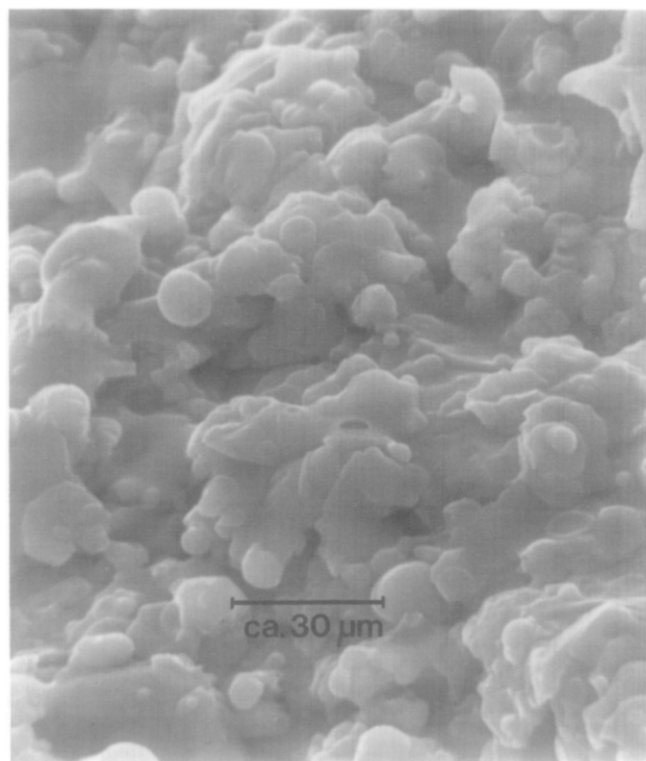
The experimental animal was the primate *Macaca speciosa*. Animals were used which were also being employed for other (e.g. endodontic) investigations. The experience of the authors has shown that the reactions of this species to a variety of surgical procedures are much more pronounced than those of the human. If the results of primate surgery are satisfactory then one may assume that the human will react as well – if not better – to the same procedure. In biocompatibility experiments which are focussed mainly on the implant material and its surface the implant shape is important inasmuch as it is one of the determinants of the initial stability of the implant. The tissue reacts to relative movement and, therefore, when histologically examining specimens from a biocompatibility experiment – particularly specimens containing bone – it is not always possible to differentiate a reaction to instability from a reaction to the surface of the foreign body. Implant stability is therefore an important prerequisite of a biocompatibility test procedure (Schroeder et al. 1976). For this reason an implant shape was developed which provides an optimum adaptation between the implant surface and that of the tissue bed and thus ensures stable macroanchorage immediately following implantation.

### 2.1. Implants and Surgical Technique

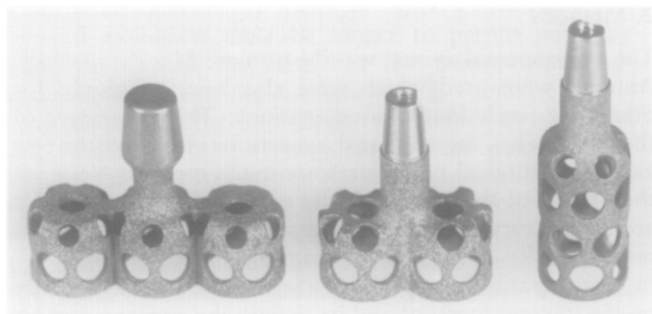
The basic shape of the implant was that of a perforated hollow cylinder (based on the idea of Greenfield, 1913). The length, diameter, and degree of fenestration could be varied (Fig. 1) and single-, double-, or triple-bodied versions could be constructed (Fig. 2). The implant was made



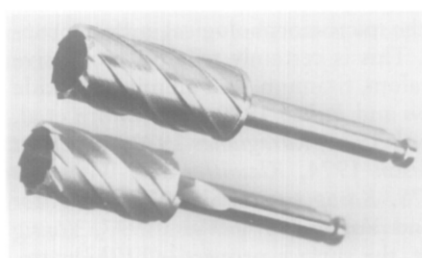
**Fig. 1** Basic shape and dimensions of the first experimental cylindrical implant.



**Fig. 2** Various types of cylindrical implant.



**Fig. 3** Implant surface, showing the rough and porous layer of titanium-sprayed powder (SEM, magnification approx. 1500 x).



**Fig. 4** Hollow cutter for preparation of the implant socket.

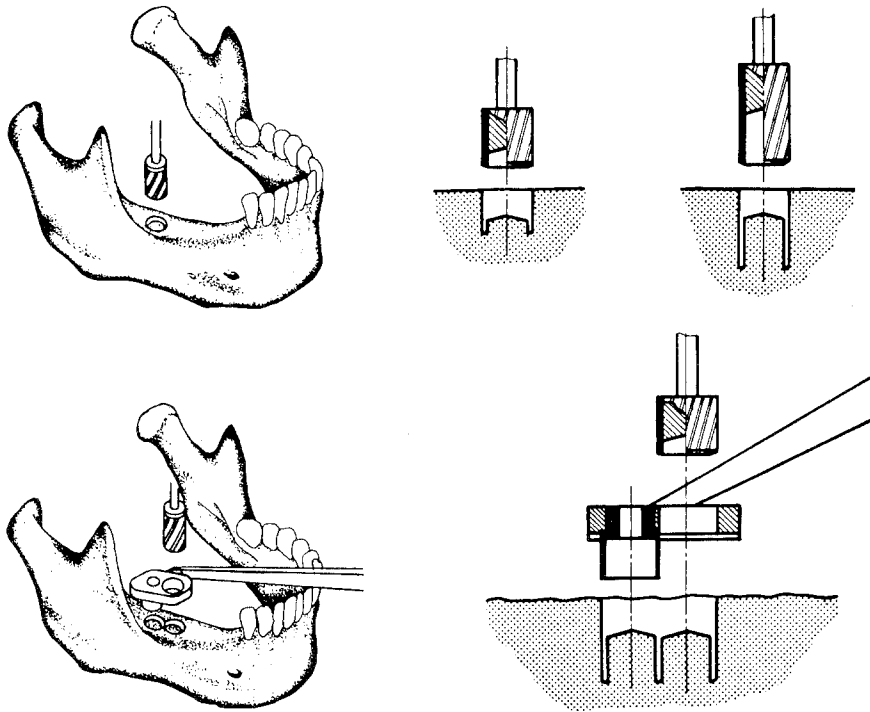
of titanium and was coated by flame-spraying with titanium powder at a high temperature. During this process the particles of the powder weld to each other and to the implant, producing a rough surface with crevices and pores. The cavities were in the order of 25–100  $\mu\text{m}$  in size (Fig. 3).

Orthopaedic surgical experience has shown that – at least as far as bone is concerned – the adhesion of tissue to a surface of this type is several orders of magnitude greater than that to a smooth titanium surface (Claes et al. 1976). The implant bed was cut with suitable hollow cutters which we attached with an angled drive to a power drill whose maximum speed did not exceed 1000 r.p.m. (Fig. 4). Special cutting jigs were used when preparing the sockets for multi-cylinder implants (Fig. 5).

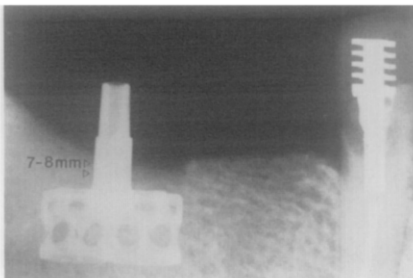
In the course of the experiments it was found that there were 3 aspects of implantation which require special attention, i.e.

1. The bone core should be left in situ whenever possible rather than be broken off at its base and removed with the hollow cutter.
2. In order to ensure total incorporation of the cylinder into the bone following implantation the shoulder of the implant should lie 3–8 mm. below the surface of the cortex immediately following insertion (Fig. 6).
3. It is essential that the implant posts come to lie in the taut, keratinised, i.e. non-mobile, mucosa.

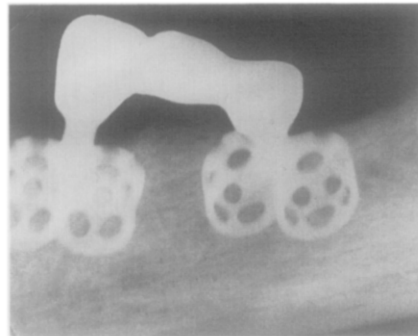
The primates received a total of 30 implants. In the initial phase only single cylinders were implanted but later experi-



**Fig. 5** Surgical procedure for the implantation of a double-cylinder implant. A cutting-jig is used for drilling the second hole.



**Fig. 7** Example of an animal experiment (*Macaca speciosa*) with loaded implants. Two double-cylinder implants are joined by a bridge which occludes well with the opposing teeth in the upper jaw.



**Fig. 6** Countersinking of the shoulder of the implant 3–8 mm below the surface of the cortex.

ments were carried out with double- and triple-cylinder implants. Implants were inserted into the upper jaw in only two cases.

Following solution of a number of problems of instrumentation and surgical technique the routine insertion of the implants presented no difficulties.

Two cylindrical implants were inserted in each of five cases and, after a preliminary 8–12 week period of healing and incorporation into the host tissues, were joined by simple well-occluding gold bridges (Fig. 7). In no case was the implant stably anchored to the natural canine tooth. Thus the implant anchorage was subjected to extreme loads which one would not normally risk in clinical practice. As a result the functional stresses partially resulted in lateral tilting of the whole construction. However, this did not lead to complete loss of occlusion between the bridge and

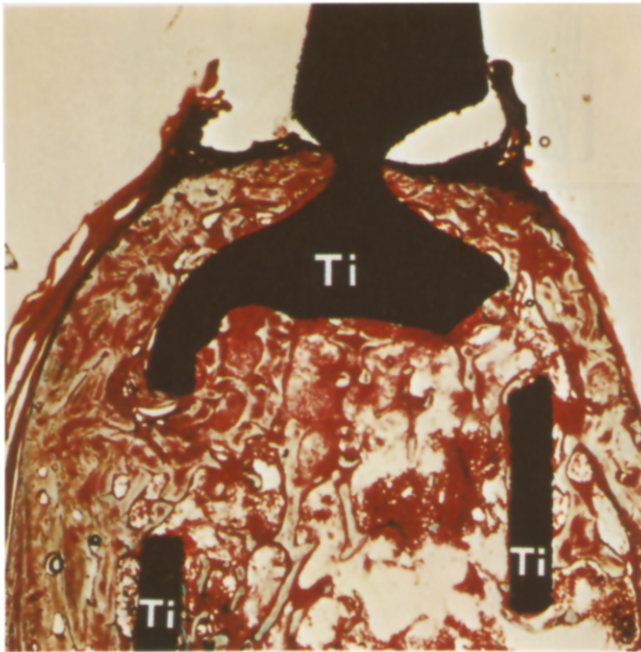
the opposing elements of the upper jaw. The implants remained in situ for 6–20 months and were then excised in vivo with the surrounding bone or – following sacrifice of the animal – removed in situ with the mandible.

### 3. Results

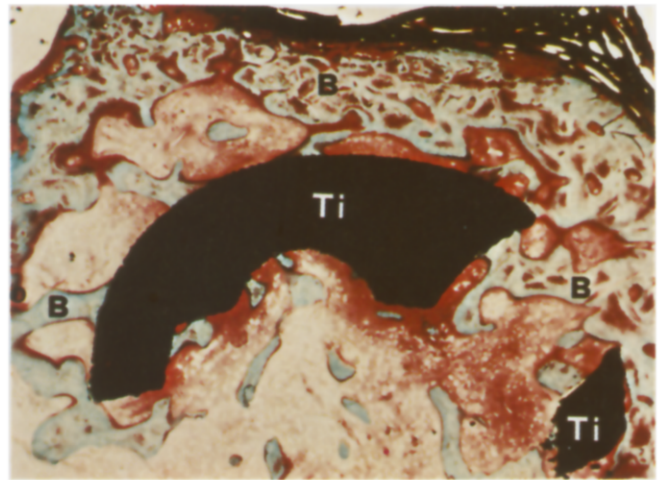
#### 3.1. Light Microscopy Findings

The specimens were prepared in the following manner:

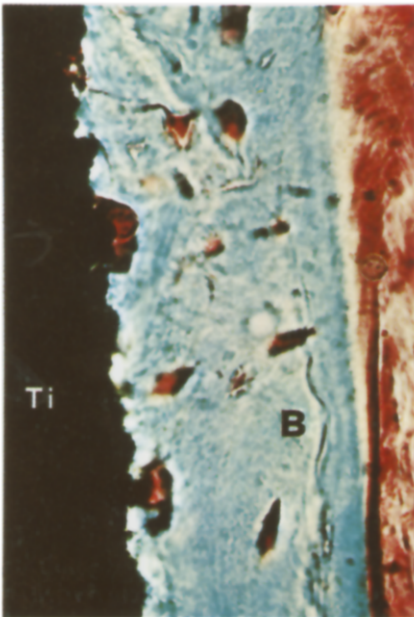
- Vital fixation of the anaesthetised animal by injection of glutaraldehyde into the left ventricle.
- Storage of the specimen in neutral buffered formalin solution, 10%.
- Dehydration with alcohol and acetone, with addition of fuchsin to the alcohol.
- Storage in pure MME monomer (elution of the solvent).



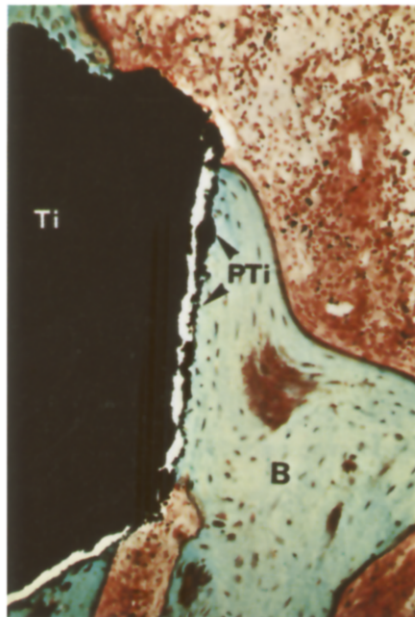
**Fig. 8a** Overview: the titanium implant (Ti) is completely surrounded and permeated by bone. (6 x)



**Fig. 8b** Shoulder of the implant. Bone (B) has been laid down directly on the rough titanium surface (Ti). (10 x)



**Fig. 8c** Bone (B) in contact with the powdered titanium surface (Ti). Live osteoblasts are in direct contact with the metal. (300 x)



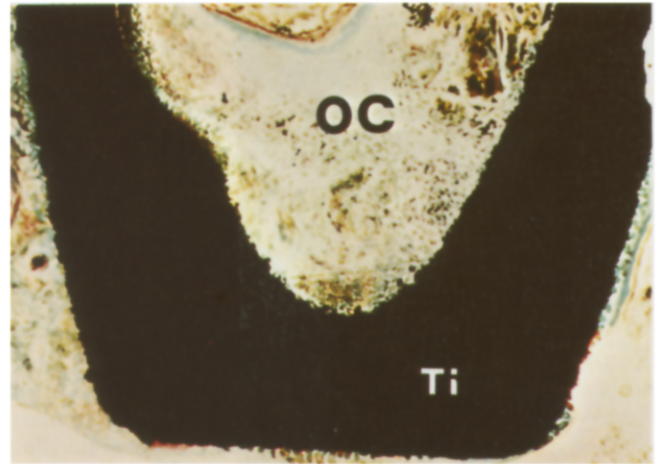
**Fig. 8d** Normally-structured osteons are attached to the layer of powdered titanium (PTi). Stresses which arise during histological preparation do not lead to separation of the bone (B) from the metal but avulse the powdered titanium (PTi) from the body of the implant. (45 x)

- Embedding in fresh MME monomer/benzoyl peroxide/Plastoid N at room temperature.
- Slicing of the hardened specimens with an Isomet diamond-surfaced circular saw under paraffin oil (section thickness approximately 0.2 mm.).

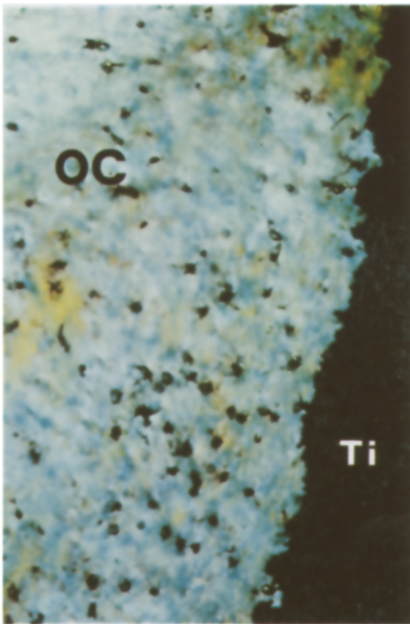
- Thinning of the slices by hand grinding, followed by brief immersion in a solution of Light Green and acetic acid.
- Differentiation in alcohol and, finally, surface protection with lacquer.



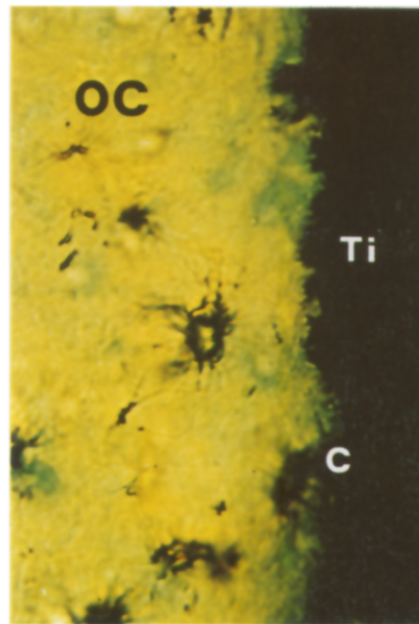
**Fig. 9a** Cylindrical implant which has been fully stressed for 12 months. As in Fig. 8, normal bone (B) has formed on the rough surface of the implant (Ti) and has remained in immediate contact despite the loads to which it has been subjected. (6 x).



**Fig. 9b** Lowest part of a cylindrical implant which has been under full load for 16 months and which is completely embedded in compact hard tissue (overview, 4 x). Ti = titanium, OC = osteocementum.



**Fig. 9c** Part of Fig. 9, showing the inner surface of the implant (160 x). The compact hard tissue is shown to be true cellular cementum (OC) which is deposited in all the crevices of the titanium-sprayed surface.



**Fig. 9d** Magnified (400 x) section of Fig. 9c showing the ankylotic junction between the osteocementum (OC) and the titanium-sprayed surface. Classical cementocytes (C) are visible in the immediate vicinity of the implant surface.

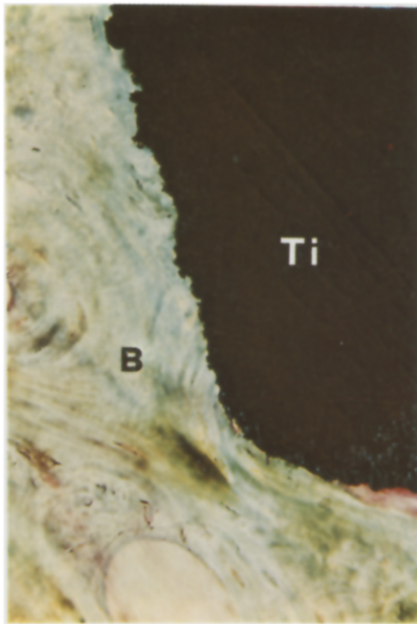
This procedure stains connective tissue red, cells dark red, and bone and dentine green (see colour plates).

### 3.1.1. Bone, Non-Loaded Implants

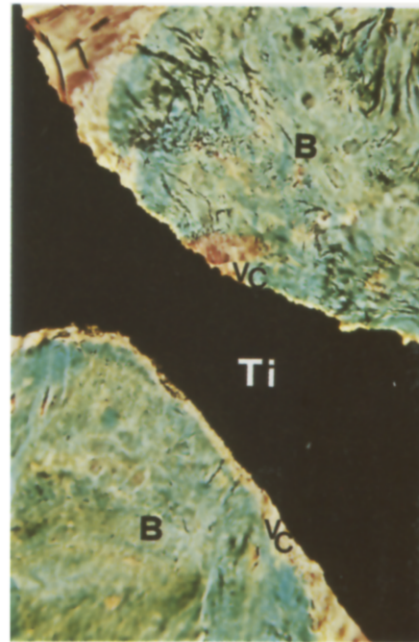
The following description is of one particular preparation

but examination of other specimens showed that the findings were common to all preparations of this type.

The low-power view (Fig. 8a) revealed that the bone not only overgrew the shoulder of the implant in close contact with the metal but also grew through the holes in the

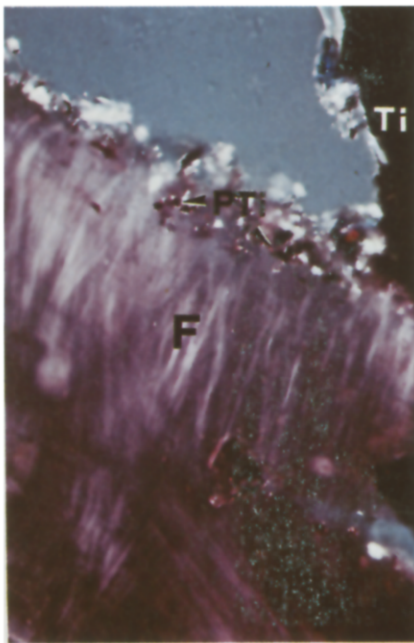


b) Surfacing with vitreous carbon: The gap between metal and bone corresponds approximately to the thickness of the carbon coating. It can therefore be supposed that the bone is in direct contact with the surface of the implant. (180 x)

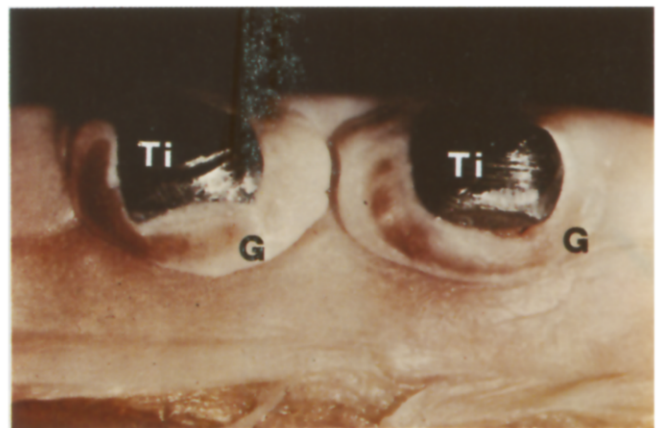


**Fig. 10a and b** Interfaces with various types of implant surface (loaded implants).

a) Surfacing with powdered titanium: ankylosis fixation of bone (B) with a regular lamellar structure as described above. (180 x)



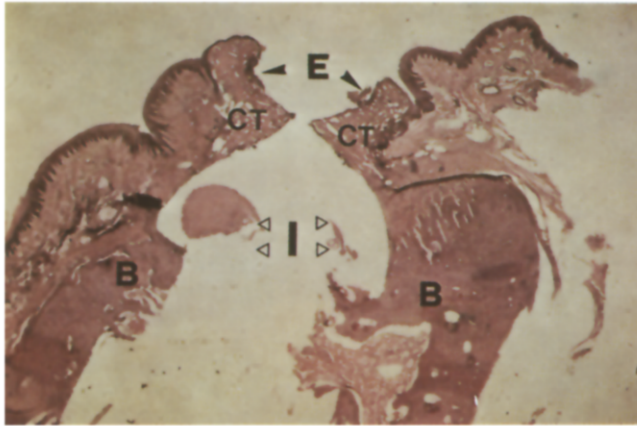
**Fig. 11** Light microscopy appearance of the marginal area of connective tissue between the bone and the epithelium. Fibres (F) insert perpendicularly into the titanium-sprayed surface. The adherence is sufficiently strong for stress to result in avulsion of particles of powdered titanium (PTi) from the body of the implant (Ti). (180 x)



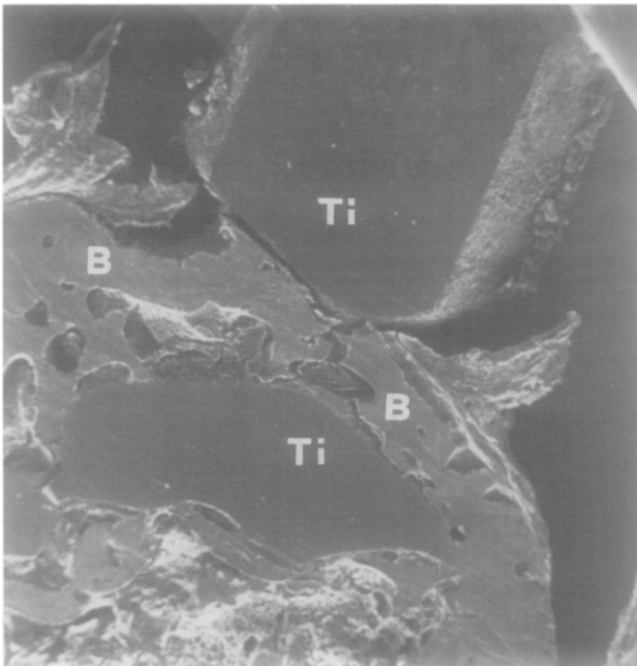
**Fig. 12** Macroscopic appearance of the "gingival" area of a well-incorporated implant. The epithelium of the "gingiva" (G) seems to be firmly adherent to the post of the implant (Ti) and there is no sulcus.

implant. At higher magnification (Fig. 8b) a section through the shoulder of the implant at a distance from the axis of its stub revealed direct contact between the bone trabeculae and the metal. There were no signs of inflamma-

tion in the medullary cavity. The views showing the close ("ankylosis") adaptation of the bone to the rough surface of the implant are particularly interesting (Fig. 8c). The bone (green) adapted to all the irregularities and gaps in the



**Fig. 13** Light microscopy overview of one of the two implants shown in Fig. 12. Like the bone (B) and connective tissue (CT), the epithelium (E) seems to be closely applied to the (extracted) body of the implant, which is indicated by a line and arrows. However, nothing can be deduced about the nature of the epithelial attachment.



**Fig. 14** Non-loaded implant (SEM overview). The shoulder of the implant has been overgrown by bone. Ti = implant, B = bone. (14 x)

titanium-sprayed surface and living osteocytes were in direct contact with the metal. In one part of the specimen which was apparently subjected to considerable stress during histological preparation a section of the powdered titanium had broken away from the body of the implant but the bond between the powdered titanium and the bone had remained intact (Fig. 8d). It is therefore apparent that the attachment between the bone and the powdered titanium is unusually strong.

### 3.1.2. Bone, Loaded Implant

The histological appearances of stressed implants and of the surrounding bone also differed little between specimens so that, here too, it suffices to describe one or two preparations.

It is significant that the histological appearance was identical with that of the unstressed implant and its surrounding bone, i.e. the bone was closely applied to the surface of the implant and remained in intimate contact with it despite heavy loading over a period of several months (Fig. 9a).

A particularly interesting and unexpected finding was the accretion of large masses of typical, genuine osteocementum (cellular cementum) around one of the implants. On the roentgenogram the area concerned did not appear particularly opaque or sclerotic, but on microscopic examination of the preparation it was apparent that the hard tissue in which the lowest part of the implant cylinder was embedded could not be normal bone. It appeared unusually pale, compact, and even glassy, and in contrast to the neighbouring tissue it did not readily take up the fuchsin on staining of the whole specimens. It can be seen histologically (Fig. 9b) that the cell-bearing cementum was in extremely close contact with the rough surface of the implant and had been deposited in the lacunae and pores of the latter. Classical cementocytes with cytoplasmic processes were distributed throughout the thickness of this hard tissue (Fig. 9c) and were also to be seen in the immediate vicinity of, or in direct contact with, the titanium-sprayed surface (Fig. 9d). Cementocytes which were in the process of being incorporated into the cementum which they were forming were organising themselves into rows or palisades on the surface of the cementum or cementoid.

The second case in this series of stressed implants deserves particular mention since it allowed direct comparison between the reaction to a titanium-sprayed implant and the reaction to a vitrified carbon implant (Reedy et al. 1973, Vitredent Corp. 1973/75, Shim 1977) which had been implanted in the same quadrant. For 20 months the two implants supported a bridge which, because the quadrant in question was totally edentulous, was subjected to extremely high stresses. As they supported the same bridge they were sustaining identical loads. The implant with the titanium-sprayed surface was firmly anchored in closely applied lamellar bone (Fig. 10a), whereas the bone surrounding the implant with the vitreous carbon surface (Fig. 10b) was separated from the metal by a gap. This gap may well correspond to the 10–20  $\mu$ m carbon layer on the surface of the implant, suggesting that, here too, the bone was laid down in contact with the bio-inert surface. The contours of the bone surface matched those of the smooth carbon surface and the bone itself tended to have a woven structure.

### 3.1.3. Connective Tissue

In those cases in which we succeeded in positioning the implant with its post in the region of the tethered, immobile mucosa and in which macroscopically apparent marginal inflammation did not occur, microscopic examination of the connective tissue between the bone and epithelium confirmed that it was rich in fibres and largely free from inflammation. The collagen fibres inserted into the neck of the implant and were obviously so firmly attached to the latter that – analogous to the insertion of a tendon into bone – the application of tensile stress tended to tear out

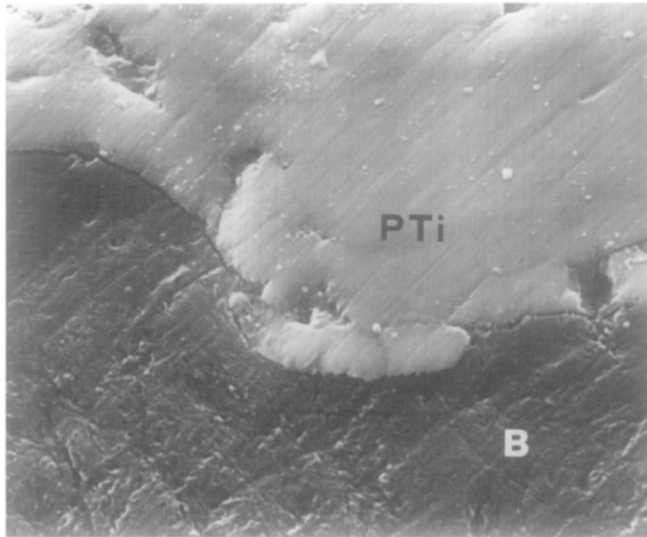


Fig. 15

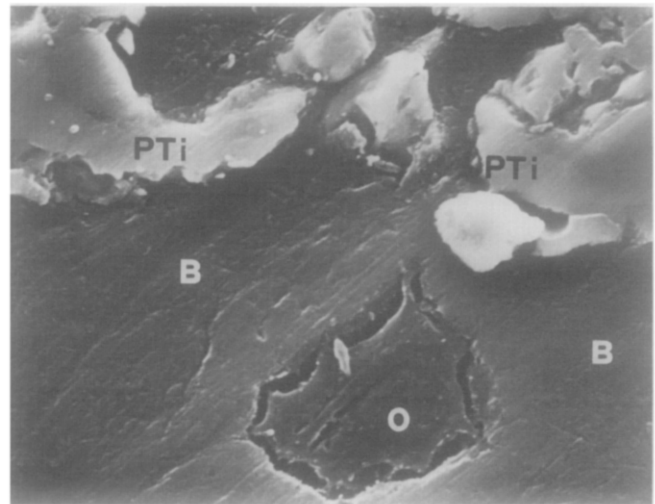


Fig. 16

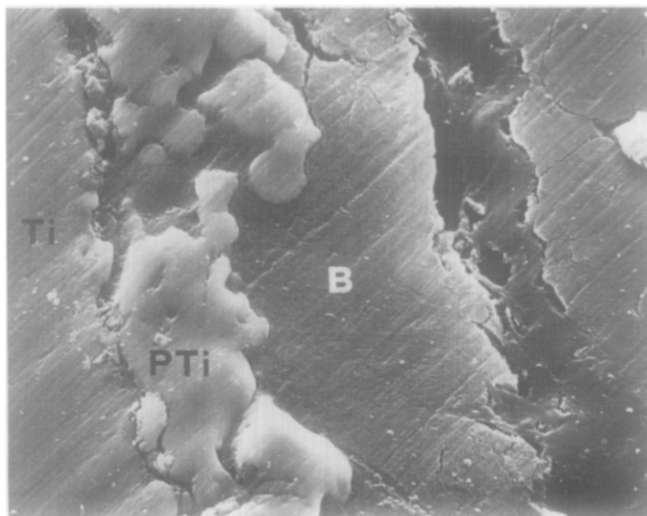


Fig. 17

**Fig. 15–17** At higher magnification the SE-micrographs also show the ankylotic bond between the bone (B) and the titanium-sprayed layer (PTi). This feature is present whether or not the implant is loaded. Extreme loads tend to cause fissures in the bone rather than separation of the bone from the titanium-sprayed layer (Fig. 17). Magnifications: Fig. 15: approx. 4000 x; Fig. 16: approx. 22 x; (O = osteocyte) Fig. 17: approx. 1800 x

the particles of the sprayed surface layer rather than detach the fibres from the rough surface (Fig. 11). Furthermore, as in the case of the natural tooth, the intimate contact between the mucosa and the post of the implant seemed to be the result of functional orientation and arrangement of the fibres.

#### 3.1.4. Epithelium

In individual cases there was macroscopic evidence of true adhesion of the epithelium to the surface of the implant, i.e. there was no recognizable sulcus and the tissue could not be detached from the metal with, for example, a periodontal probe. The epithelium seemed to have crept forwards over the foreign body (Fig. 12).

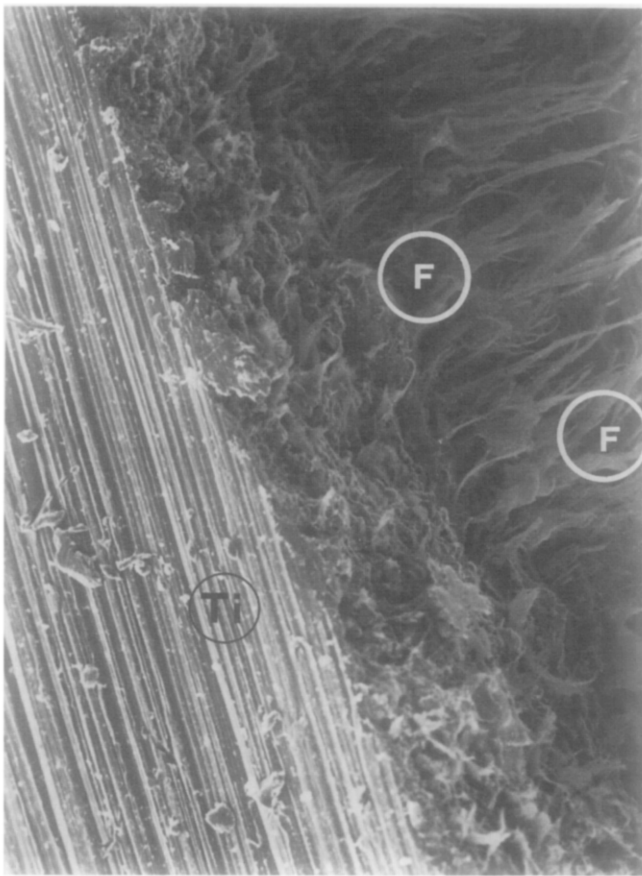
However, it was difficult to confirm these apparently clear findings by microscopy, mainly because it has been hitherto impossible to obtain histologically evaluable specimens

with the implant in situ. In those cases in which the implant was extricated in order to allow the preparation of very thin sections the presumed contour of the neck of the extracted implant was delineated by a fine layer of epithelium (Fig. 13). However, it was impossible to determine whether the epithelium actually adhered to the neck of the implant and, if so, in what manner. In order to clarify this point it will be necessary to use more suitable histological techniques.

### 3.2. Electron Microscopy Findings

#### 3.2.1. Bone and Connective Tissue

The images obtained by scanning electron microscopy (SEM) fully confirmed the light microscopy findings in that they also showed intimate contact between the bone and



**Fig. 18** SE-micrograph of an area of connective tissue between the bone and the epithelium. Here too, the insertion of perpendicularly-arranged bundles of fibres (F) into the surface of the implant is clearly visible. Magnification approx. 220 x.

the titanium-sprayed surface of the implant, whether or not the latter was under stress (Figs. 14, 15, 16). If the stress was sufficient to cause tearing the line of separation passed through the tissue (Fig. 17). This is further evidence of the firm microanchorage of the bone to the titanium-sprayed surface.

In the region between the bone and the epithelium the rough titanium-sprayed surface was covered with a dense fibrous layer of tissue whose interwoven bundles of fibres were even more clearly evident than in the light micrographs. They were firmly adherent to the surface of the implant and appeared to be functionally orientated (Fig. 18).

### 3.2.2. Epithelium

For transmission electron microscopy (TEM) a small piece of the neck of an implant with attached, macroscopically healthy gingival epithelium was used. Despite the technical difficulties encountered in visualizing the interface between the epithelial cells and the titanium-sprayed surface a number of features were revealed which were sufficiently interesting to warrant somewhat more detailed description. At the site of implantation the regenerated gingival epithelium had a loose structure. The wide intercellular spaces were traversed by the long branched microvilli of adjacent cells. At irregular intervals these microvilli – or, in

some places, plump cell processes – met and formed intercellular junctions. The desmosomes were generally orientated at an angle to the elongated body of the cell.

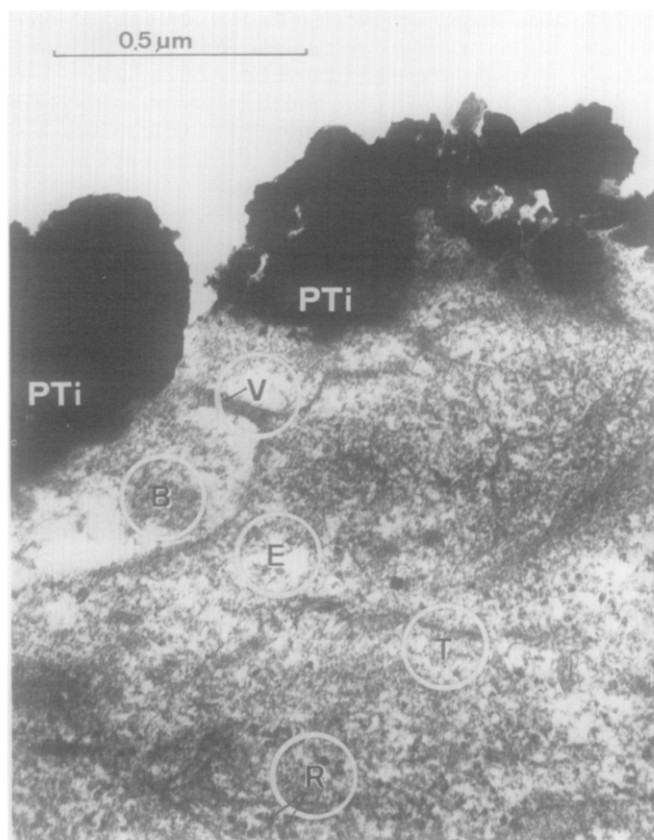
During extraction of the metal implant variously sized fragments of the titanium-sprayed surface remained attached to the tissue. This metal is electron-opaque and therefore appears black in the micrograph.

The particles of powdered titanium were embedded in an amorphous, finely granular substance whose ultrastructure corresponded to that of a thickened basal lamina (Fig. 19a). Microvilli of the adjacent epithelial cells projected into this basal lamina. It has been hitherto impossible to obtain evidence of direct contact between microvilli and titanium particles, and hemidesmosomes were not seen. The epithelial cells occasionally formed pseudopod-like processes which partially surrounded titanium particles (Fig. 19b). However, even at these sites there was no direct contact between cell membrane and metal; there was always a residual gap – however narrow – which was filled with granular substance.

The fine ultrastructure of the epithelial cells adjacent to the implant did not differ significantly from that of other epithelial cells. This implies that the cells were not influenced by the titanium. In the regions in which the powdered-titanium had sunk deeply into the basal lamina the intracytoplasmic filaments, which were usually aligned parallel to the axis of the cell, changed direction. These tonofilaments, which were concentrated in these areas of increased proximity, seemed to converge on the points of close contact (Fig. 19c). In terms of function this might imply that the mechanical loads imposed by the implant are partially taken up by the epithelial cells.

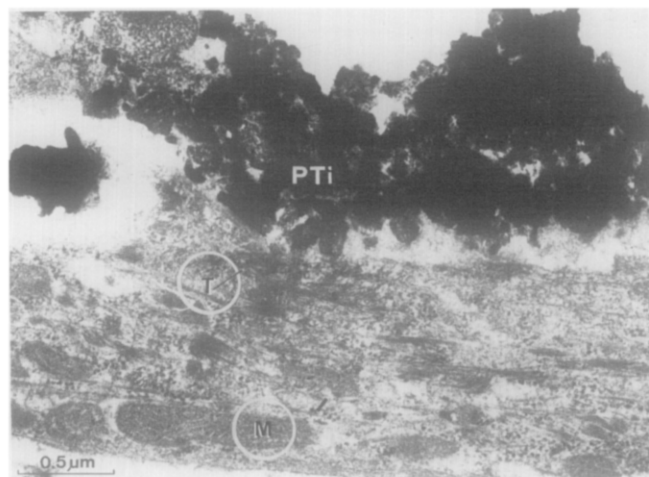
## 4. Discussion

The findings summarized here (the bone and connective tissue features of which were first reported by *Schroeder et al.* in 1976) clearly show that titanium implants with titanium-sprayed surfaces become attached to the bone by an “ankyrotic” type of microanchorage. The bone is joined to the surface of the metal by extremely fine fibrous bridges. This histological feature is also regularly seen in implants which have been under load for nearly 2 years. It seems, therefore, that a fibrous structure such as that which surrounds the natural tooth is not needed to absorb the loads imposed by chewing. In other words, the bone itself apparently possesses sufficient elasticity to prevent the functional stresses from loosening the ankyrotic bond between the bone and the surface of the implant. The biocompatibility and corrosion resistance of the titanium is mediated by a passive layer on the surface of the metal ( $\text{TiO}_2$ ) which forms spontaneously in a moist environment and which can be reinforced by anodic oxidation (*Newesely, 1979*). The ingrowth of bone into rough, pitted, or porous titanium surfaces and the establishment of intimate contact between bone and metal has been described by several authors (*Klawitter 1970, Karagianes et al. 1976, Juillerat and Kueffer 1977, Weiss 1977*). As revealed by our own investigations, prolonged loading leads to an increase in the density of the bone which, with the passage of time, takes on an increasingly regular, lamellar structure with formation of osteons in the classical manner. The enclosure of the apical part of the implant in osteocementum (described above)



**Fig. 19a** Powdered titanium in contact with regenerated epithelial tissue 83 days after implantation of a single-cylinder implant in a *Macaca speciosa* monkey. A finely granular substance which corresponds ultrastructurally to a basal lamina (B) is situated between powdered titanium (PTi) and an epithelial cell (E). Microvilli (V) of the cell are buried in the basal lamina. R = ribosomes, T = tonofilaments. 20000 x

**Fig. 19** Transmission electron micrographs of the epithelial attachment.



**Fig. 19c** Magnified view of part of Fig. 19b showing increased density and orientation of the tonofilaments (T) in the region of relative contact between the metal and the cell membrane. M = mitochondrion. 7000 x



**Fig. 19b** Powdered titanium in contact with regenerated epithelium 83 days after implantation of a single-cylinder implant. Pseudopod-like cell processes (T) are partially enclosing the metal particles (PTi). The ultrastructure of the epithelial cells does not appear to be influenced by the titanium. 3500 x

can be regarded as an extreme form of sclerosis (Schroeder et al. 1978). Clinically, the establishment of this type of bone anchorage implies the complete absence of relative movement between the implant and its socket. This is true even if an osteolytic marginal infection erodes the bone to such an extent that it encloses only one third of the implant. However, human patients will have to be followed up clinically and radiologically for very long periods in order to determine whether the ankylotic bone anchorage which is elicited by a rough titanium surface does indeed offer decisive advantages.

The long term success of an implant is by no means exclusively determined by its anchorage in the bone. A further prime determinant is the ability of the tissues to form pseudo-gingiva around the part of the implant post which projects into the oral cavity. The animal experiments and observations in humans show that close adherence of the mucosa is basically unlikely to occur if the post comes to lie within the mobile, non-keratinized mucosa. On the other hand, our histological investigations show that if it is situated in a region of immobile, highly fibrous, keratinized

mucosa then collagen fibres will insert into the rough titanium surface at approximately 90° to it. Thus there is adequate evidence of a fibrous structure which is able to resist the mechanical loads around the post of the implant. It remains to be seen whether the surface of the post becomes covered with a marginal epithelium which – as with the natural tooth – would maintain the continuity of the epithelial surface coverage and prevent the ingress of bacteria or other harmful agents. To date our histological investigations have simply shown normal epithelial cells which are separated from the sprayed surface by nothing more than basal lamina. True desmosomes could not be seen. This possibly provides an adequate seal, particularly as, in view of the constant turnover in the region of the epithelial insertion, it may improve with time. However, further research will be needed to determine whether the titanium-sprayed surface provides better conditions for epithelial adhesion than other (i.e. non-metallic) materials such as tricalcium phosphate, bioglass, or vitreous carbon.

### Conclusions

This paper demonstrated in animals the type of union which forms between bone and a hollow cylinder of titanium flashed with titanium powder. The ankylotic type of connection, which was formed between the bone and the surface of the implant, remained unchanged under functional loading for an experimental period of two years. It is yet to be determined whether such a union is advantageous for long term implant therapy.

It was shown that the gingival tissues are tightly adapted to the post of the implant; however, the nature of the adaptation has yet to be clarified.

The results of this experiment indicate that in order to be successful it is necessary to have: (1) the post of the implant located in an area of attached gingiva; (2) the shoulder of the implant sunk at least 3 mm. below the cortical layer of bone; and (3) the patient practice, scrupulous oral hygiene. Studies on humans, already in progress, should provide additional valuable information.

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