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High-resolution microscopy of the implant-tissue interface

A method is described which permits study of the interface of tissue and bulk implant at the light and electron microscopic level. It is based on epoxy embedding of implant and surrounding tissue with subsequent separation of implant and plastic. The separation takes place at the interface between tissue and implant; neither cells nor matrix are disturbed.

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Electron microscopy is necessary for in-depth analysis of tissue-implant interaction. Sectioning of bone for electron microscopy is technically demanding in itself, and the difficulties involved in obtaining 70 nm sections of an intact bone/bulk implant interface are formidable. These problems were partially circumvented by Linder et al. (1983) by applying titanium as a thin coating on a material which did not preclude processing for electron microscopy. This method is so far only practicable with pure metals and in animal experiments. As most currently used metallic implant materials are alloys, new approaches to the problem are clearly needed.

The method documented here is based on a simple plastic embedding technique permitting electron microscopy of the tissue adjacent to most implant materials used today.

Material and methods

In this documentation, the experimental method developed by Lundskog (1972) has been used. Cylindrical, 3.6 mm implants of pure titanium, cleaned in trichlorethylene and ethanol, were implanted into adult rabbit tibiae and left for 3–4 weeks, by which time the implants are known to be bordered by bone without a soft tissue interlayer. The implants were then removed by use of a trephine with an inner diameter of 6 mm which left a 1–1.5 mm thick collar of bone around the implants. During drilling the bone was cooled by saline irrigation.

The specimen was fixed in 3 per cent glutaraldehyde in cacodylate buffer for about 15 h. After fixation, the bone was rinsed in 0.15 M sodium cacodylate buffer for 2 h and then post-fixed in 2 per cent osmium tetroxide for 2 h. EDTA decalcification was done as an intermediate step in some cases. After post-fixation, the bone was rinsed in sodium cacodylate buffer for 15 min and then taken through a graded series of ethanol (50, 70, 80, 90, 95 per cent and absolute ethanol), allowing 20 min for each step, and then transferred to 100 per cent propylene oxide for 2 × 15 min. It was then kept in a 1:1 mixture of propylene oxide and epoxy resin for 2 h at 4°C and in 100 per cent epoxy resin at 4°C overnight. After about 15 h it was transferred to 100 per cent epoxy resin which was polymerised at 45°C for 15 h and 60°C for 48 h. The epoxy used was Agar Resin 100 (Agar Aids, Stansted, Essex, England).

To separate the tissue from the implant, the entire plastic/implant block was divided in two with a thin rotating saw blade mounted on a dental handpiece. The implant was then gently removed from the plastic, and the interfacing surfaces were processed for scanning electron microscopy by gold sputtering. The gold-covered plastic block was re-embedded in epoxy and the interface was thus buried within the new embedment. One-micron sections were cut and stained with toluidine blue. The plastic embedment was also trimmed down in size, and small areas were sectioned at 70 nm for transmission electron microscopy.

Results

Judging from the shiny metal surface and the scanning electron micrographs, there appeared to be a clean separation of plastic and titanium

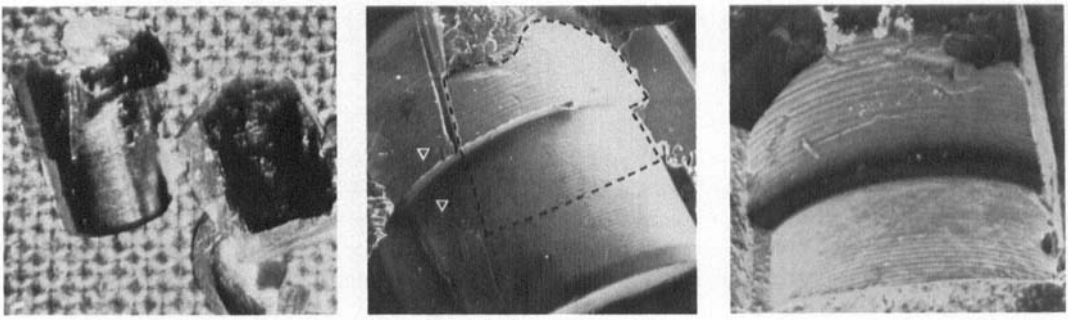

Figure 1. The surfaces of the titanium plug and the plastic embedment containing the interface tissue, after separation. The shiny metal surface suggests a clean separation of metal and plastic (× 6).

Figure 2. Scanning electron micrograph of the same titanium plug after gold sputtering. The area indicated by the dotted line has interfaced against the surface seen in Figure 3. The area indicated by △ has interfaced against the tissue of the electron micrograph seen in Figure 6. Note the clean surface, lacking signs of plastic remnants (× 18).

Figure 3. Scanning electron micrograph of the surface of the plastic embedment seen in Figure 1. The specimen has been divided to fit the microscope. There are no signs of cracks in the plastic surface, nor of lost fragments (× 26).

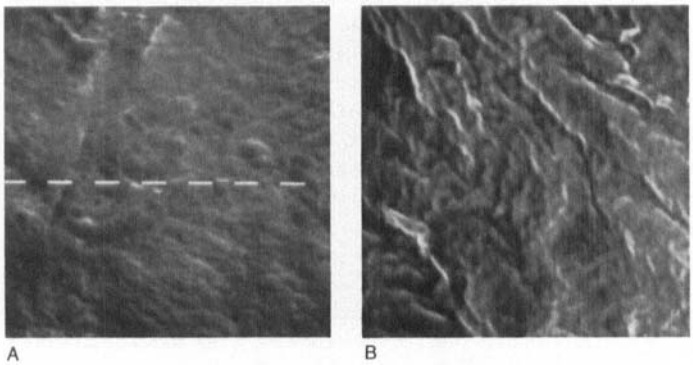

Figure 4. A. The titanium plug at higher magnification. The plane of separation is even and regular (× 5500).
B. The corresponding plastic surface. There is an absence of cracks, crypts and defects (× 5000).

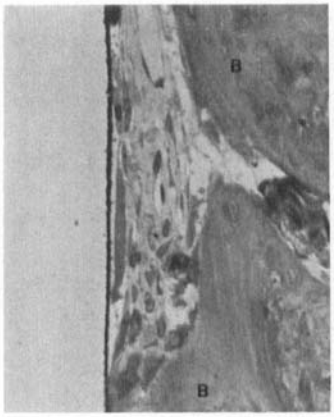

Figure 5

Figure 5. Light micrograph of a 1-μm section of the plastic embedment seen in Figures 1, 3 and 4B. The black line is the layer of gold sputtered on the plastic surface prior to scanning electron microscopy. Its presence proves that no plastic has been lost after separation. The perivascular cells at the interface and the bone (B) surface at the lower part of the picture, are unharmed by the separation procedure (toluidine blue, (× 250).

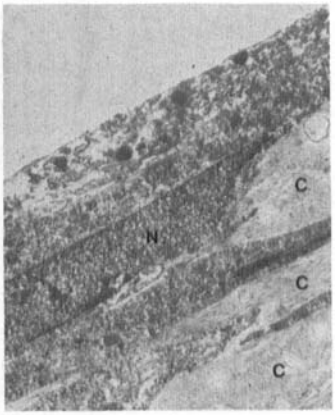

Figure 6

Figure 6. Transmission electron micrograph of a cell adjacent to the area of the titanium implant indicated by (△) in Figure 2. This embedment was not sputtered with gold. The cell surface is unharmed, indicating that the separation of tissue and implant must have taken place within the layer of amorphous substance separating the cell from the implant. Collagen (C) and cell nucleus (N) are indicated (× 6500).

(Figures 1–3). No flakes of plastic were seen on the metal surface.

At higher magnification the metal surface appeared quite even, there were no signs of separation in an irregular fashion, and the plastic surface (Figure 4) did not show cracks or defects. In the light micrograph of Figure 5, the interface tissue, perivascular soft tissue as well as bone, is obviously intact.

The tissue facing another part of the plug of Figure 2 was prepared for TEM (Figure 6). Even though this embedment was not covered with gold, the surface appearance of the plug in this area is identical to that seen in Figures 2 and 4A, and the tissue surface should therefore be identical to that in Figure 5. In the electron micrograph the surface of the cell appears intact, indicating that the plane of separation did not go through the tissue. The same finding has been made with regard to the extracellular matrix, where the filamentous elements are unharmed.

Discussion

The most widely used method for interface tissue analysis has been plastic or paraffin embedding of the tissue after separation from the implant. With this procedure it is not certain whether the entire interface is represented in the specimen or whether some tissue component may have been lost during or after separation. With other methods the tissue and implant have been embedded together in plastic – usually methylmethacrylate or epoxy – and the entire block has either been ground down to a thickness of 40–50 μm, or cut on a cut-all microtome at 5–6 μm (Smith & Karagianes 1974, O'Keefe 1976, Gross & Struntz 1977, Schroeder et al. 1981, Steflik et al. 1983). With yet another method the metal was electrolytically dissolved after plastic embedding (Brown & Simpson 1979).

The reason why the present method was developed was the need for TEM analysis of the tissue surrounding implants which are too hard to cut on a microtome. It was accepted as a fact that such an interface cannot be cut intact, and the crucial point is therefore to demonstrate that the plane of separation coincides with the interface. Demonstration of the interface between bone and pure titanium was chosen because 1) the nature of this interface has been described previously by electron microscopy (Linder et al. 1983, Albrektsson et al. 1984), 2) it was assumed that an interface between a metal and bone without an intervening soft tissue layer would be technically the most difficult situation to handle and therefore a good test of the reliability of the method.

In my experience, a clean separation through the metal/plastic interface is easily distinguished from a fracture through the plastic by viewing the surfaces in a dissecting microscope. As has been shown, a successful separation clearly takes place within the layer of amorphous substance separating the collagen or cells from the implant surface. So far it has not been possible to determine if an extremely thin layer of plastic (of the order of Ångströms) is still present on the implant. For practical purposes, however, the plane of separation obviously does not disturb the fine anatomy of the fibrillar or cellular structure at the interface. Consequently, it would seem that the technique of separating monolayers of cells from culture dishes applies to whole tissue as well (Chang 1971, Elias et al. 1971).

This method is simple because it is based on routine histological embedding techniques. Naturally, depending on the size and density of the tissue sample to be embedded, the times for the various steps of embedding have to be adjusted. In experimental work, therefore, whole-body perfusion of the fixation medium may be the method of choice. The principle of the method also requires that the embedding medium be given enough time for penetration through the tissue. The specimens presented here showed no signs of imperfect or variable infiltration, and obviously the penetration through the bone was good. It is reasonable to assume that if bone can be embedded in this way, soft tissue should be even easier to handle.

This method is suitable for both clinical and experimental research. It can be used in comparative studies of the biocompatibility of various bulk implants and in ultrastructural studies of the events taking place during the healing-in of an implant. In clinical research, it is

already being used for high-resolution microscopy of the tissue surrounding prosthetic replacements.

An obvious limitation of the method is the relative difficulty involved in irregular-surfaced implants. Separation of plastic and implant will have to be made in a dissecting microscope, and then only small segments of the interface can be studied at a time. However, if only the biocompatibility of an implant material is to be studied, the use of a single large tissue specimen is unnecessary. On the other hand, if large specimens are desirable, for example for study of the bony architecture around the implant, tissue embedding in methylmethacrylate is a good alternative (Linder, unpublished data).

In summary, this method seems to be a simple and reliable alternative to earlier methods of interface study as it allows assessment of the tissue at any desired level of microscopy.

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