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Electron microscopy of the bone–hydroxylapatite interface from a human dental implant

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A titanium implant coated with hydroxylapatite was observed after removal from a human jaw. The hydroxylapatite coating and bonded bone were observed in the scanning electron microscope. The bone–apatite interface was thinned to electron transparency by a special technique and observed in the transmission electron microscope at high magnification. Bone bonded directly to the apatite coating, and there was no evidence for a foreign-body reaction, fibrous tissue, or any altered structure in bone or ceramic at the bone–apatite interface. There was no transition layer between bone and apatite to atomic resolution. Lattice imaging of the ceramic coating and the direct attachment of bone to it provide evidence that the bone bonds chemically to the apatite. Hydroxylapatite is a most desirable implant material, and coating it on a metal substrate gives a strong, impact-resistant implant.

1. Introduction

Hydroxylapatite (HAP) has excellent potential for a bone implant material because bone bonds strongly to it and the HAP is completely biocompatible [1]. A coating of hydroxylapatite on a metallic substrate takes advantage of the impact resistance of the metal or alloy and the chemical bonding of bone to the apatite, as well as its lack of toxicity and foreign-body reaction. Hydroxylapatite has been coated on titanium [2, 3] and steel [4] by plasma spraying. A well-bonded coating is difficult to make because of the difference in thermal expansion coefficients between the metals and HAP. The ceramic coating can crack, causing inadequate bonding at the HAP–metal interface.

In Ducheyne and Lemmons [5] there is a summary of a meeting on bioceramics, including research articles and reviews. Hanker and Giammara [6, 7] provide extensive background information on implant experiments on many different materials.

Controversy has arisen concerning the strength and stability of the bone–HAP interface in human implants. Most of the implants previously studied were *post mortem*, diseased, had failed, or were from animals. The implants in this study were unusual because they were removed from a healthy patient, for personal reasons, and showed no signs of disease or inflammation.

In this paper we present evidence that bone bonds directly to HAP coated on titanium and implanted in the human jaw. There was no evidence for a foreign-body reaction, fibrous tissue, or altered osteoid structure at the bone–HAP interface. There was no transition layer between bone and HAP to atomic resolution. Regions of the bone–HAP interface were thinned to electron transparency using special techniques developed previously [8]. Direct bonding of the bone to the apatite coating was observed. Lattice images of the ceramic showed it was unaltered at the interface with bone.

2. Experimental procedure

The implants were stored in alcohol after removal from the human jaw. To prepare them for observation they were dried in a dessicator and then vacuum-impregnated with Norcast 32795 epoxy resin. This resin was used because of its superior hardness, which helped to preserve the samples during their preparation for electron microscopic observation.

The samples were prepared for observation in the scanning electron microscope by cross-sectioning them with a diamond wire saw (South Bay Technology, Inc., SBT). This saw has much less friction with the sample than a diamond disc blade on a low-speed saw. The samples were polished successively on 30, 15, 9 and 3 μm polishing paper supplied by PSI Testing Systems, with ethylene glycol as a lubricant. These polishing papers maintained a flat surface across the sample. A final polish of 1 μm Al_2O_3 on nylon cloth was applied. The sample was carbon-coated and examined in a Jeol JSM 840 SEM. Compositionally enhanced micrographs were obtained from a solid-state backscatter detector to achieve greater contrast between the different materials.

The TEM samples were prepared by first cutting thin slices (300–400 μm thick) from the embedded sample with the diamond wire saw. An SBT slurry

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drill with a diamond-coated drill bit produced a 3 mm disc from the sample with the bone–HAP interface in the centre. This disc was mounted on a Pyrex mount with a thick mixture of nitrocellulose in amyl acetate and then ground to 150 μm with a Gatan disc grinder. The sample was then dimpled to 50 μm from one side on a Gatan dimple grinder with a stainless steel wheel, using 5 μm and 1 μm Al_2O_3 powders in ethylene glycol. At this point the sample was removed from the mount. Amyl acetate was selected as the solvent for removal because it did not degrade the epoxy. The disc was placed in an ion-mill holder and a piece of tantalum foil was placed over the bone region to prevent it from being milled away because the bone is softer than the ceramic. The sample was placed in a Gatan Model 600 Duo Mill and was milled in an argon beam at 4 kV and 0.4 mA current on the ion gun, which was positioned at an angle of 20° from the dimpled side of the disc, which was held at liquid nitrogen temperature. After approximately 20 h of milling, the ceramic next to the Ta shield was perforated. The Ta shield was removed and the sample was milled for an additional 30 min with the gun set at a 10° incidence, and 3 kV and 0.3 mA current. Finally the sample was carbon-coated and observed in a Philips CM12 TEM at 120 kV.

3. Results

Figs 1–4 are compositionally enhanced SEM micrographs of the implants. The titanium bone and ceramic regions are represented by T, B and C, respectively.

Fig. 1 shows good bonding of the HAP ceramic to the titanium substrate; bone grew into the rough HAP surface, completely filling it. In Fig. 2 the ceramic coating is quite uneven inside the convex region. Ceramic and bone have separated from the titanium. Fig. 3 shows a higher magnification of another region in which bone has grown right into the irregular HAP surface, and the ceramic–titanium interface is cracked. Fig. 4 again shows the close bonding of bone to the HAP coating. No separation of bone from the HAP was observed over the entire implant surface. Furthermore there was no evidence of a foreign-body reaction, fibrous growth at the bone–HAP interface, or an interfacial layer of altered HAP or bone structure.

Fig. 5 is a transmission electron micrograph of high magnification of a thinned region of bone that has grown into the uneven ceramic surface. The microfibrous organic matrix of the bone is visible; the circular dark regions in the bone are individual bone apatite crystals, about 10–20 nm in diameter. The bonding of bone to the HAP is so intimate that the interface is hard to identify in some regions. Fig. 6 shows another thinned sample in which the (0 0 1) lattice planes (0.82 nm spacing) are imaged (parallel

Figure 1 SEM photomicrograph of a titanium (T) implant with a hydroxylapatite (C) coating after several months implantation in a human jaw. Bone is B; darkest areas are epoxy potting compound.

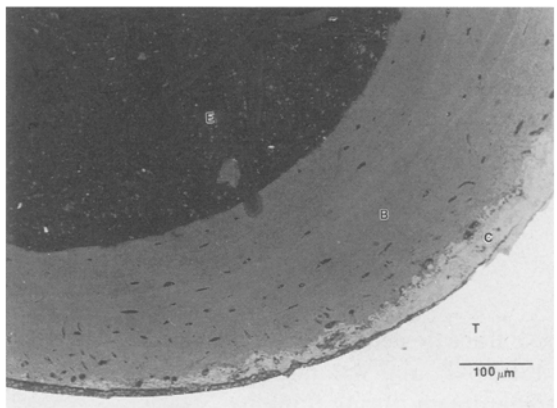

Figure 2 Same as Fig. 1, convex curved region in the implant.

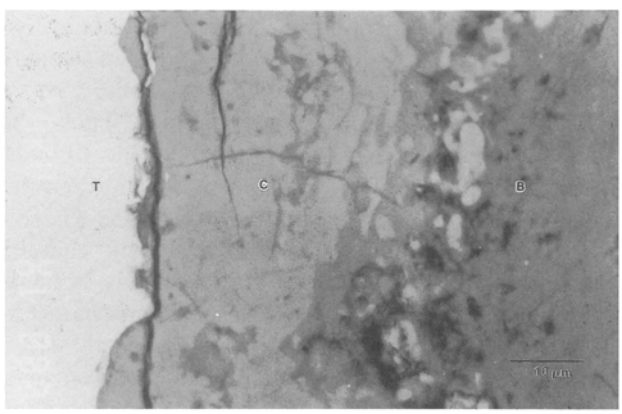

Figure 3 Same as Fig. 1, higher magnification.

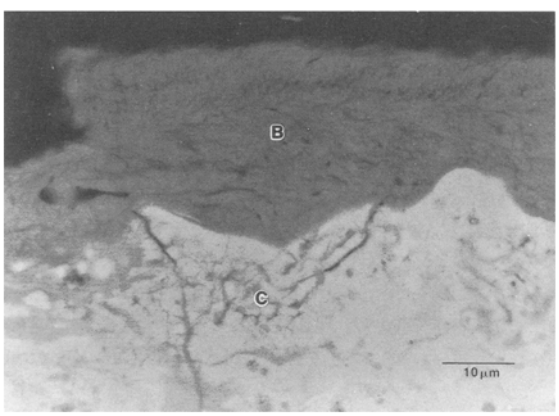

Figure 4 Same as Fig. 1, higher magnification.

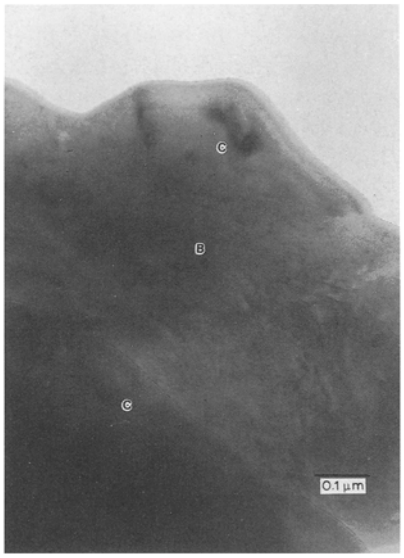

Figure 5 Transmission electron photomicrograph of a thinned region of bone and HAP that was coated on titanium, after several months of implantation in a human jaw. (C) HAP ceramic (B) bone.

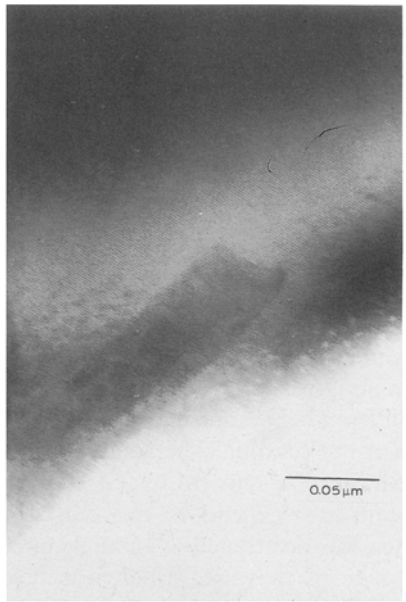

Figure 6 Transmission electron photomicrograph of thinned HAP coating on a titanium implant after several months in a human jaw. Lattice images of (0 0 1) planes in the ceramic are visible; the rectangular area is a strand of collagen.

lines). Bone (lower darker region) has bonded strongly to the HAP, and was not dislodged even by the ion bombardment during thinning.

4. Discussion

The bond between bone and the hydroxylapatite coating was strong enough to survive the rather drastic preparation techniques. The bone bonded strongly and directly to the apatite surface. This bond is not a mechanical hooking or interlocking. Thus the bone–apatite interface must involve chemical bonds. The most likely bonding mechanism is a coordination of negatively charged carboxalate groups on the collagen of the bone to exposed positively charged calcium ions on the apatite surface. This bond is mainly ionic, and can have some covalent character. This strong and direct bonding of bone to the HAP coating is similar to the bonding observed on bulk HAP implants by high-resolution electron microscopy [1, 8].

The scanning electron micrographs show that the hydroxylapatite was coated fairly uniformly on to the titanium substrate by plasma spraying, except where the particle stream did not impinge directly on the titanium (Fig. 2). In most regions, the apatite bonded well to the titanium, but there were cracks in the ceramic and in some places it pulled away from the titanium, probably because of the difference in expansion coefficients between titanium and HAP.

The lattice imaging of the ceramic with the 0.817 nm spacing of the 1 0 0 planes and the direct bonding of bone to the ceramic are strong evidence that the bone attaches directly to the apatite on an atomic scale. There was no evidence of altered structure in either bone or apatite at the interface between them. This structure contrasts with interfacial layers of altered structure in other implant materials [5], even those characterized as "bioactive".

This work reinforces the conclusion that hydroxylapatite is a most desirable implant material from bonding, chemical and biocompatibility viewpoints; coating it on a metal substrate gives a material with high strength and impact resistance as well.

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