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Morphology and ultrastructure of the interface between hydroxyapatite-polyhydroxybutyrate composite implant and bone

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A composite of polyhydroxybutyrate (PHB) polymer, reinforced with synthetic hydroxyapatite (HA) particles, with potential as a bone-analogue material, was examined microscopically using scanning electron microscopy and transmission electron microscopy. These imaging techniques provide the means of understanding and monitoring the morphological and structural behaviour of retrieved implants. Scanning electron microscopy was used to assess the overall mechanism of new bone formation at the implant interface after up to 6 months implantation. This procedure was followed by a detailed ultrastructural examination at lattice plane resolution level, using high resolution electron microscopy and selected area diffraction of the regions showing bone apposition. Fine hydroxyapatite crystallites were found to form at the interface after *in vivo* implantation into cortical bone.

1. Introduction

Hydroxyapatite (HA)/polyhydroxybutyrate (PHB) composite has been reported to have favourable bioactive properties and therefore could be potentially considered for applications as a degradable bone substitute material [1, 2]. The composite in a natural body environment retains its bioactive properties, while undergoing continuous physico-chemical degradation [3, 4]. In addition the mechanical properties of the composite were found to match closely the properties of cortical bone [5].

When an implant is used to replace a bone, the overall success of creating a long-lasting function depends on the associated biological events, with the interface between the implant and the new tissue being continuously formed and remodelled. Overall, the bone–implant interface can be considered to be a product of combined chemical, biological and physiological interactions. A strong and continuous interface normally can secure stable functioning of the implant in a body. Large interfacial contact areas, which develop after the implantation of a biodegradable material, can ensure better new tissue ingrowth into spaces left by degrading material and therefore lead to a mechanically stronger interface. Under normal physiological conditions, the formation of a physical anchorage at the interface affects the rate and the strength of the bone–implant assimilation.

Previous research, which investigated the microstructure and ultrastructure of the interface between hydroxyapatite–filled high density polyethylene composite and bone in an animal model found a coherent and epitaxial interface developed up to 6 months post-implantation [6]. This composite has been successfully used in patients for orbital reconstruction surgery [7]. Other research has produced evidence that apatite material attaches directly and epitaxially to bone [8, 9]. The mechanism of bone bonding to implants is still not clear and appears to depend on the nature and properties of the implant, as well as on the host environment. Consequently, there is a need to understand the bone bonding mechanism to a variety of a bone analogue materials and this study reports a detailed microstructural analysis of the HA/PHB composite/bone interface developed *in vivo*.

2. Experimental method

A composite of polydroxybutyrate (PHB) reinforced with 40% (by volume) of hydroxyapatite (HA) particles ($Ca_{10}(PO_4)_6(OH)_2$) was the subject of the investigation. PHB is a naturally occurring polyester which degrades slowly in a biological environment. The rate of degradation depends on the polymer molecular weight and pH value of the system. The HA component is bioactive and shows strong osteogenic properties. The overall biological and mechanical properties of the composite based on the PHB matrix were found to be distinctive [2, 5]. On average, greater than 90% of bone apposition was observed for PHB polymer, with or without the HA reinforcement, at 6 months after *in vivo* implantation. The Young's modulus of the composite was found to match the lower band of values for cortical bone range. Small pins from previous experiments were used in this study as implants [2]. The implants, 5 mm long and 2.4 mm in diameter

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(as shown in Fig. 1), were sterilized using γ-radiation before implantation into condyles of skeletally mature New Zealand white rabbits. At 1, 3 and 6 months, the implants with surrounding tissue were retrieved. The retrieved samples were fixed, dehydrated and finally vacuum infiltrated in LR White Resin. Initially, the cross-sections containing the implant in bone, were polished using 6 μm and then 1 μm diamond pastes. Scanning secondary electron beam imaging was performed at 10 or 20 keV after the samples were coated with gold or carbon. A JEOL, JEM 35CF SEM instrument was used. The implanted composite samples, as well as control samples composed of unfilled PHB, were examined and the areas showing close contact between the implant and bone selected for further microstructural and ultrastructural examination on a transmission electron microscope (TEM), JEOL, JEM 200CX fitted with high resolution pole piece and operated at 200 keV. The thin sections for TEM were cut with a Reichert ultramicrotome fitted with a diamond knife. The sections were collected on 400 mesh uncoated copper grids and unstained as well as stained sections were examined. Staining was performed by exposing the thin sections on TEM grids to a vapour of osmium tetraoxide acid for 30 min in an air tight dish placed in a fume cupboard. Selected area diffraction mode was also used in order to obtain the right orientation conditions for lattice imaging and to examine the structural state of the samples.

3. Results and discussion

3.1. Scanning electron microscopy (SEM)

After 1 month of implantation, bone apposition was found to occur along the whole length of the implant interface, as illustrated in Fig. 1. The irregular boundary and the interface developed between the implant and the bone was characterized by the presence of an intermediate region containing exposed and partly loose parent HA particles of the composite material as a result of PHB matrix degradation (Fig. 2). At low magnification, two regions of different contrast

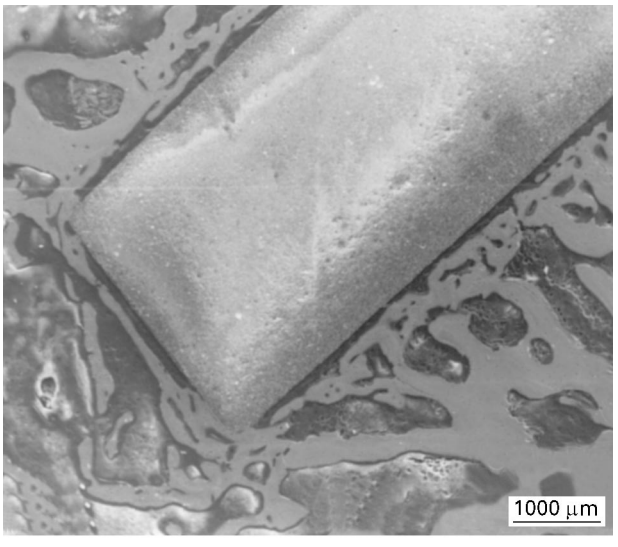

Figure 1 SEM micrograph showing a cross- section of the implant in bone at 1 month.

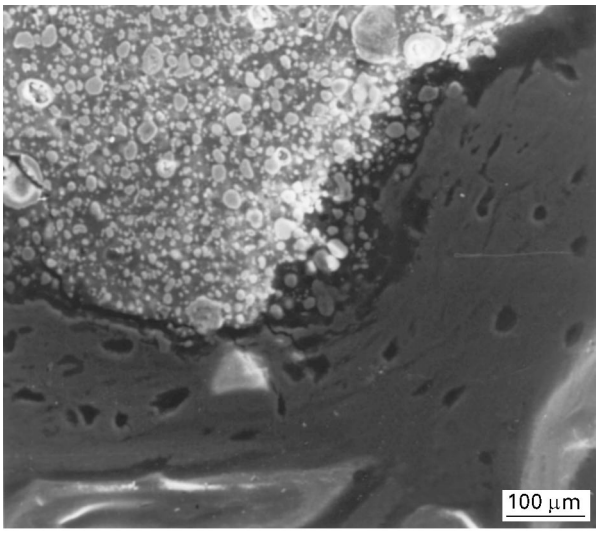

Figure 2 SEM micrograph of the interface at 1 month showing irregular boundary and loose HA particles.

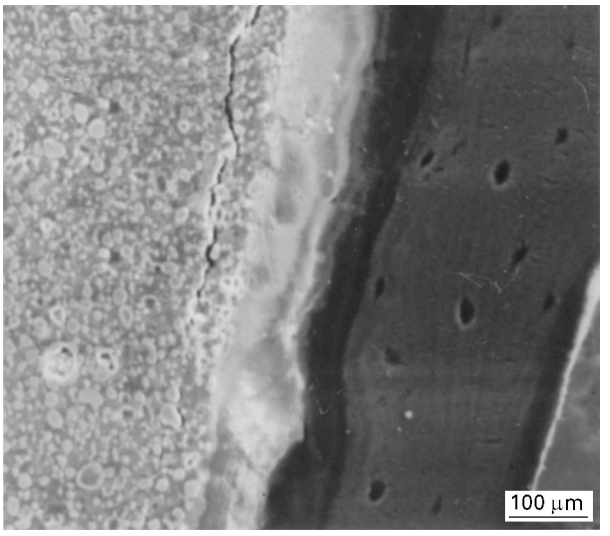

Figure 3 SEM micrograph of the interface at 1 month with an active region in the implant and new bone growing along the implant.

separating the implant and the new bone were observed (Fig. 3). The region giving rise to the lighter contrast at the implant side of the interface corresponds to an active region where the degradation process of the composite matrix had taken place. Cracks had also developed near the implant interface zone. These observations lead to the conclusion that the interfacial activities at this time were already well in progress, resulting in a remodelling of the interface in terms of its morphology.

At 3 months, the bone was seen advancing into the spaces between the exposed parent HA particles in the composites, forming a characteristic interlocking pattern at the interface as the interface moved further into the implant (Fig. 4). After another period of 3 months (6 months of implantation in total), dense bone formed at the interface as composite degradation continued inside the pin-implant in a radial fashion as indicated by the light contrast zones observed in the implant. The arrows in Fig. 5 point to these areas.

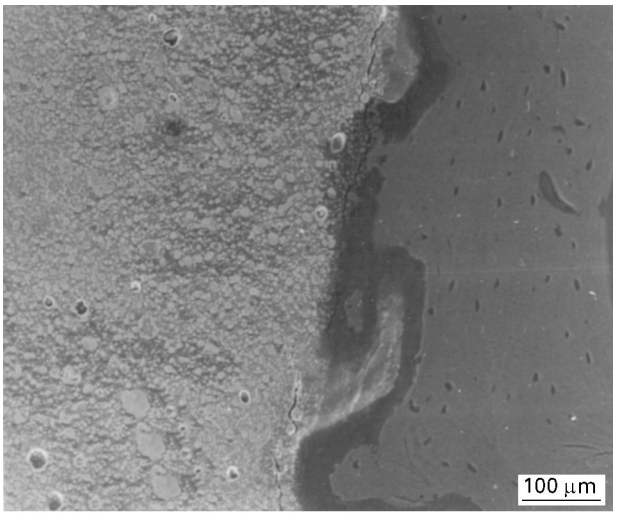

Figure 4 SEM micrograph of the interface at 3 months showing the characteristic interlocking system developed at the interface.

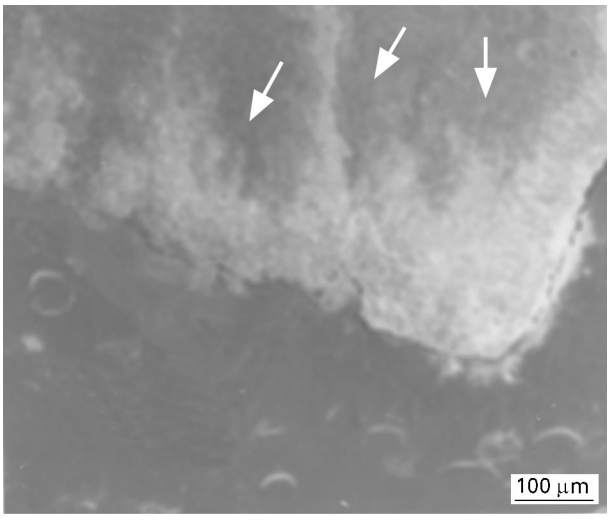

Figure 5 SEM micrograph of the interface at 6 months. The arrows indicate the light contrast zones present in the implant.

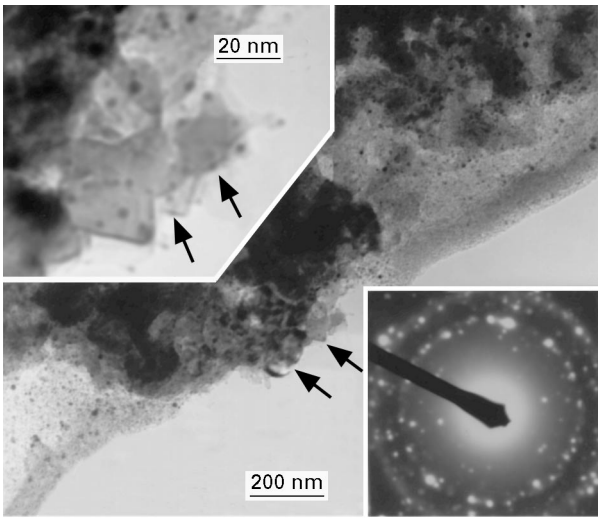

Figure 6 TEM micrograph of a thin section of the control implant–bone interface (bone part only), stained. Arrows indicate the new HA particles formed at the interface. SAD pattern shows polycrystalline material.

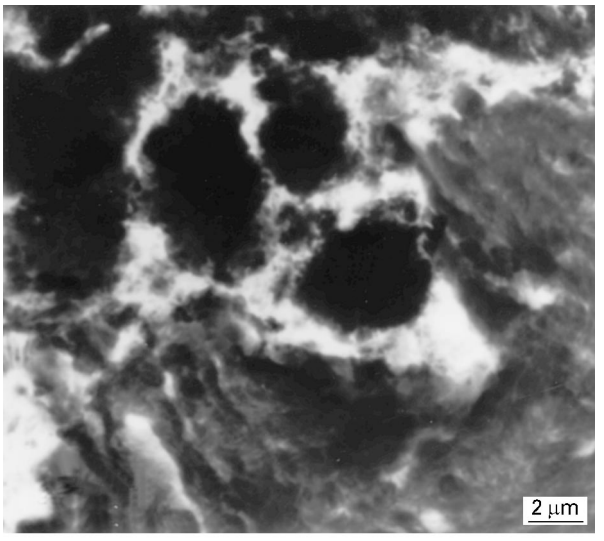

Figure 7 TEM micrograph of a thin section of the composite interface at 6 months showing bone advancing into the degrading matrix of the composite.

In contrast to the composite pins, the control pins (composed of PHB polymer only) at 6 months produced a smooth bone surface at the bone–implant contact area.

3.2. Transmission electron microscopy (TEM)

In all control specimens implanted for up to 6 months, the implants separated from the bone during thin section preparation. It would therefore appear that the interfaces were mechanically weak, with little or no chemical or physical bonding across the interface. The observations made after 6 months of implantation on stained thin sections of bone which separated from the implant showed that the bone was surrounded by a degrading PHB phase. The bone after staining gave rise to a darker contrast, while the polymer appeared of a light grey colour (Fig. 6). The insert in Fig. 6 shows a close-up of the interface at 6 months. The arrows on the main micrograph in Fig. 6 indicate small and thin crystallites present at the interface. The selected area diffraction pattern of the area confirmed the polycrystalline state of the interface. The overall observation suggests a bone–implant failure within the polymer matrix, rather than at the interface.

The interfaces formed between the bone and the composite implants were comparatively stronger and none of the pins detached from the adjacent bone during thin sectioning. Examination of the interface with the implant composed of 40% HA in PHB at 6 months showed the bone advancing into the degrading polymer matrix and also in between the parent HA particles in the composite (Fig. 7). New crystallites were found within the composite matrix as well as at the surface of the parent HA particles in the composite, as shown in Fig. 8. A close-up of the particles is

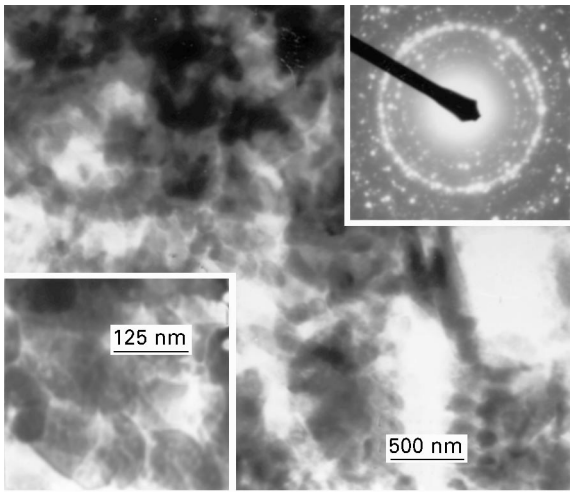

Figure 8 TEM micrograph of the composite–bone interface at 6 months. N-HA are the new crystallites. SAD identified the crystallites as HA.

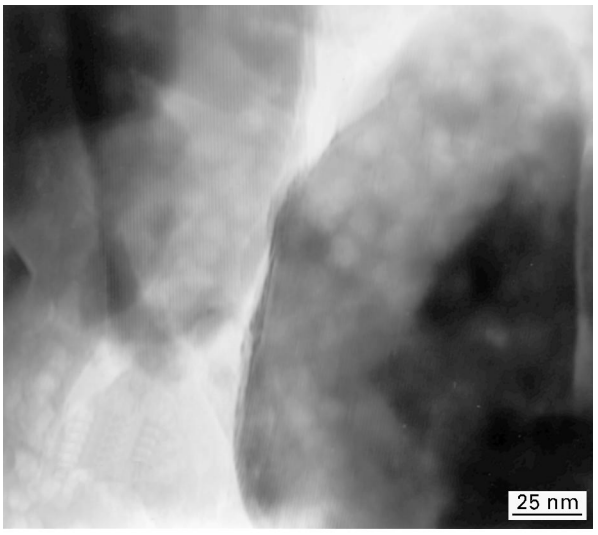

Figure 9 TEM micrograph of the new crystallites at 1 month.

showed in the insert. Selected area diffraction patterns taken from this area confirmed the hydroxyapatite composition of the particles.

High resolution electron microscopy technique was used to identify the fine crystallites. The individual crystals varied in size to about 450 nm in length and 100 nm in width. The larger crystals were usually found at the surface of the parent HA particles in the composite. The crystallites within the composite matrix were relatively smaller, growing close to each other. The situation after 1 month of implantation is shown in Fig. 9. In some of the crystallites of the correct orientation in relation to the electron beam, the lattice planes are resolvable. High resolution images in two neighbouring particles are clearly displayed in Fig. 10. The resolved lattices were identified as $\{1\,0\,0\}$ HA type with 0.82 nm lattice spacing. The lattice planes of the individual crystals were shown to be defect-free. However, at the interface of the two adjacent crystals, dislocations were observed, as indicated by the arrows in Fig. 10. High resolution imaging performed on the crystallites at 6 months also resolved undisturbed $\{1\,0\,0\}$ lattice planes of HA particles.

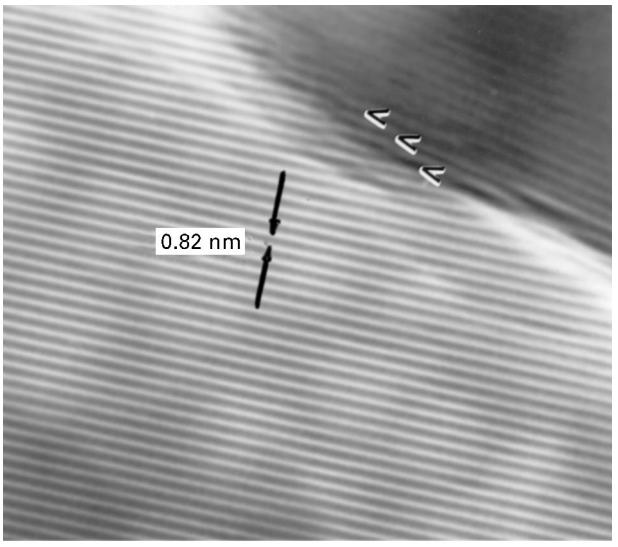

Figure 10 High resolution TEM micrograph showing the $\{1\,0\,0\}$ HA lattice planes of the new crystallites at 1 month. The arrows indicate dislocations between the two particles.

The overall observations point to a combined morphological and structural mechanism of bone bonding to a PHB/HA composite due to the bioactive properties of the PHB and HA components of the composite. The HA component of the implant appeared to play an important role in the process of implant integration with the bone.

The PHB/HA composite interface with the bone was physically and biochemically active over a 6-month period *in vivo*. The degrading polymer was found to be replaced by small HA crystallites independently of the new crystallites formed at the surface of parent HA particles, which were included in the original composition of the implant. The bioactive behaviour of the composite makes the material potentially suitable for applications in bone reconstruction surgery.

4. Conclusions

The morphology and crystallography of a PHB/HA composite bone–implant interface, over a 6-month implantation period *in vivo* was established. It was found that the mechanism of bone bonding to the implant occurred by degradation of the PHB matrix, which led to the formation of new crystallites between the parent HA particles in the PHB/HA composite, as well as at the surface of the HA particles. Selected area diffraction and high resolution imaging identified the newly formed crystallites as hydroxyapatite, indicating the bioactive properties of the composite in a body environment.

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