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Composites of hydroxyapatite and bisphosphonate: properties and alveolar bone response

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A composite was made by adsorption of the bisphosphonate [(3-dimethylamino-1-hydroxypropylidene)-1,1-bisphosphonate; dimethyl-APD] into an hydroxyapatite (HA) tube. Adsorbed dimethyl-APD did not change the bulk properties of the HA tube but the surface properties were altered. The amount of 0.1 mmol/L dimethyl-APD adsorbed into the HA tube was 0.78 ($\pm$ 0.20) μg after 4 weeks. The composite tube of HA and dimethyl-APD placed after extraction of teeth in the premolar regions of dogs were biocompatible, stable and bonded strongly and intimately to the alveolar bone. Although there was no bone resorption around the composite tubes, no conclusion can be drawn yet from this study as far as local inhibition of alveolar bone resorption is concerned.

1. Introduction

In most studies using hydroxyapatite (HA) implants, maintenance of alveolar bone after extraction of teeth was aimed at by plain HA materials [1]. However, clinical results have shown that HA may require biological modification with a bone resorption-inhibiting agent which may be beneficial for local maintenance of alveolar bone [2]. Our approach was to fabricate an HA tube which was capable of adsorption of the agent [(3-dimethylamino-1-hydroxypropylidene)-1,1-bisphosphonate; dimethyl-APD] [3–7].

This communication describes the properties *in vitro* as well as the alveolar bone response in dogs of composite tubes made of HA and adsorbed dimethyl-APD.

2. Materials and methods

2.1. Preparation of the HA tube

The HA tube was designed in the form of a cylinder with a length of 7 mm and a diameter of 3 mm. A hole with a diameter of 2 mm was drilled inside the HA cylinder up to 0.5 mm from the apex, thus leaving the apical portion of the HA tube closed. The open end of the HA tube allowed for bone ingrowth. The HA tube was produced of commercially available HA powder (Merck, Darmstadt, Germany) as described earlier [1]. The morphology of the powder was analysed by scanning electron microscopy (SEM). The specific surface area of the powder was measured by the BET technique on a Quantasorb, Quantachrone instrument Greenvale, NY (USA).

The surface and bulk structure of polished samples of the HA tube were studied by SEM.

2.2. Characterization of the HA of the tube

A complexometric titration method was used for the calcium determination while the phosphorous content was analysed colorimetrically. The Ca/P ratio was determined by quantitative X-ray fluorescence. Trace elements which may influence the biological behaviour of the HA were detected by spectrochemical analysis.

2.3. Stability of the HA tube in different buffer solutions

The stability of the HA tube was tested in a Tris HCl buffered solution (280 mmol/l) at 37 °C at different time intervals. Evaluation was done by means of Auger electron microscopy (AES). The escape depth of secondary electrons was in the order of 200 nm. The experimental conditions were as follows:

1. Corrosion conditions: 0, 1, 22, 97.5 and 238.5 h. The

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sample surface to solution volume was approximately 0.5 cm^{-1}.

2. Measurements:
   (a) background pressure 1.333×10^{-7} ρ_a;
   (b) beam energy 3 keV;
   beam current 5 μA;
   (c) modulation voltage 5 V;
   (d) beam incident angle 45°.

Another stability test was carried out in 0.5% acetic acid solution. The weight loss of the HA tubes in the acid solution was measured at 22 °C over 30 min at pH 3. The weight loss was compared with the total weight of the HA tube and with the total surface area.

2.4. Response of the HA tube to dimethyl-APD

For this study dimethyl-APD was donated by Henkel Kgaa, Düsseldorf, Germany. The response of the HA tube as far as stability is concerned will depend on the concentration of the dimethyl-APD. The stability (or degradability) was measured in terms of weight loss after placement in a 0.9% NaCl solution with the following concentrations of dimethyl-APD: 0.1 mol/l, 0.01 mol/l, 1 mmol/l and 0.1 mmol/l.

2.5. Adsorption of dimethyl-APD into the HA tube

For this study dimethyl-APD was adsorbed into the HA tube under vacuum by placing the tubes in 2.5 ml 0.9% NaCl, 0.1 mmol/l dimethyl-APD solution which was continuously stirred at 37 °C. The solution thus containing 70.3 μg dimethyl-APD. Half the amount of dimethyl-APD was 14C labelled. The adsorption periods were 1, 4, and 8 weeks.

2.6. Bulk and surface changes of the HA tube after adsorption of dimethyl-APD

The bulk changes apply to the changes in the crystallographic structure and crystallinity of the HA. Evaluation of the possible changes was made by X-ray diffraction (XRD) studies. The surface changes concern the possible changes in the chemistry of the HA. The surface changes were determined by infrared reflection spectroscopy (IRRS). Data were obtained on HA tubes after adsorption of different concentrations of dimethyl-APD (0.1 mol/l and 0.1 mmol/l). HA tube blanks were used as controls. For the XRD studies a Philips XRD instrument (PW 1050) was operated at 40 kV and 30 mA, with a scanning rate of 0.25° 2θ/min. A Perkin-Elmer 467 IRRS instrument recorded spectra on 12 mg powdered material, embedded in 300 mg Kbr pellets while operating with a 10 min scanning time for the 4.000 cm^{-1} range.

All measurements and experiments described were performed five-fold.

2.7. Alveolar bone response to composite tubes of HA and dimethyl APD

The HA tubes were cleaned in an acetone-filled ultrasonic cleaner for 30 min and sterilized. Thereafter dimethyl-APD was adsorbed into the HA tubes by placing the tubes in a 0.1 mmol/l dimethyl-APD solution of 0.9% NaCl for 4 weeks. All preparations for the composite tubes before insertion in the alveolar bone were carried out under sterile conditions. Just before implantation the composite tubes were removed from the dimethyl-APD solution and air dried.

2.7.1. Surgical procedure

The experimental animals were two male beagle dogs, over 2 years of age and with an average weight of 18 kg. They were given a soft diet during the week after implantation and were kept in the Central Animal Laboratory of the University of Nijmegen. Anaesthesia was induced with an intravenous injection of Pentothal and the operation was performed under inhalation anaesthesia according to accepted standards. The root sockets of the double-rooted third and fourth premolars were used for implantations. Extraction was performed by separation of the roots with a high-speed drill under water-cooling. Thereafter the roots were gently eased out of their sockets by using elevators and an extraction forceps. The operation site was continuously irrigated with physiological saline and sharp ridges were trimmed.

2.7.2. Implantation

A total of 16 composite tubes were placed in the extraction sites of the premolars in the mandible. Before implantation the extraction sockets were contoured with a cylindrical bone drill with a diameter of 3 mm which corresponded with the diameter of the implants. The composite tubes were gently placed in the prepared sites and a lot of effort was put into primary closure of the wounds thus submerging the tubes.

2.7.3. Radiographic follow-up

An intra-oral technique was used as described earlier [8]. Radiographs were taken of the composite tubes directly after implantation and 3 and 6 months thereafter.

2.7.4. Light microscopy

Composite tubes with surrounding tissues were fixed in buffered formalin solution, dehydrated in aethanol solutions (70–100%), demineralized, embedded in paraffin and cut in 3–5 μm thick sections.

3. Results

3.1. HA tube

3.1.1. HA starting powder

The HA starting powder consisted of agglomerates of powder particles with diameters of up to 123 μm. Individual particles had mean sizes of 1 to 2 μm. The specific surface area was 59 m^2/g. The following trace elements (in wt%) were found to be present in the HA:

Al (0.06); Cu (0.0001); Fe (0.1); Mg (0.2); Mn (0.03); Na (0.3); Pb (0.0004); Si (0.05).

3.1.2. Elemental analysis

Elemental analysis (in wt%) of the HA yielded an average calcium content of 37.50 and an average phosphorus content of 17.30. The Ca/P (w/w) ratio thus being 2.167. The Ca/P (mol/mol) ratio was 1.64. Since most trace elements occupy Ca positions in the lattice it is not surprising that this figure is slightly lower than the normal value, which is 1.67 [9].

3.1.3. SEM

SEM studies of the surface and the bulk of the HA tube are shown in Figs 1 and 2.

3.1.4. Stability

The composition of the near-surface region of the HA tube did not significantly change upon exposure to the Tris HCl buffered solution. The Ca/P ratio, however, differed from that of an HA standard, where it was 6.5. Perhaps the uncorroded surface had already a somewhat increased Ca concentration (Table I). The average weight loss of the HA tube in the 0.5% acetic acid solution was 0.4 mg, which is 4%. This means that 0.003 mg per mm^2 of the surface of the HA tube was lost. The weight loss was very close to the measuring error of the balance used.

3.1.5. Response of HA tube to dimethyl-APD

A concentration of 0.1 mg/l dimethyl-APD caused precipitation of the HA of the tube in the form of a very finely divided powder. Adsorption of a concentration of 0.01 mol/l into the HA tube caused some precipitation and there were particles present in the solution. The weight loss of the HA tube was 4%.

With a concentration of 1 mmol/l dimethyl-APD there was no precipitation and only very few particles were present in the solution. The weight loss of the HA tube was 0.3%. The adsorption period was 1 week. Concentrations of 0.1 mmol/l dimethyl-APD did not cause disintegration of the HA tube after adsorption periods up to 6 months. No weight loss occurred.

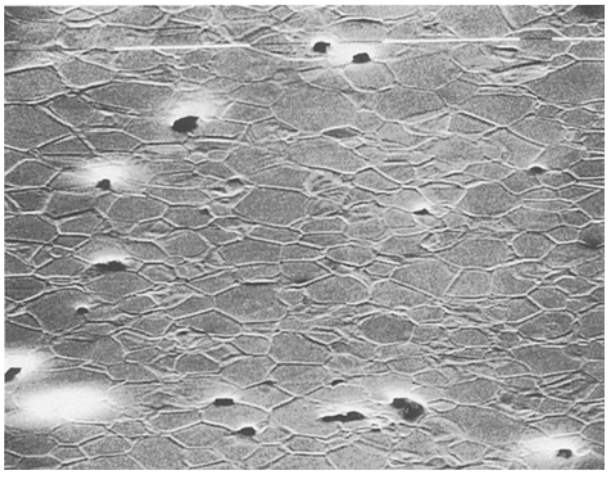

Figure 1 SEM of the surface of an HA tube. Note the boundaries of the HA powder particles and the pores left between the particles due to incomplete fusion of the boundaries (× 2500).

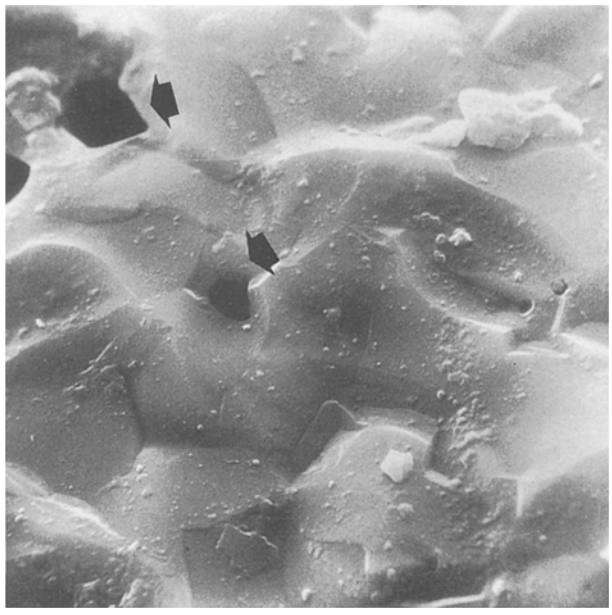

Figure 2 SEM of the bulk of an HA tube showing the pores (arrows) which can have diameters up to 1 μm (× 3600).

TABLE I Stability of the surface of HA tube in a Tris HCl buffered solution

Time (h)	Ca/O	P/O	Ca/P
0	1.22	0.14	8.79
1	1.31	0.15	8.75
22	1.35	0.14	9.93
97.5	1.31	0.13	9.85
238.5	1 27	0.17	7.46

Data obtained with AES were analysed by taking Auger peak-to-peak height ratios Ca/O, P/O and Ca/P.

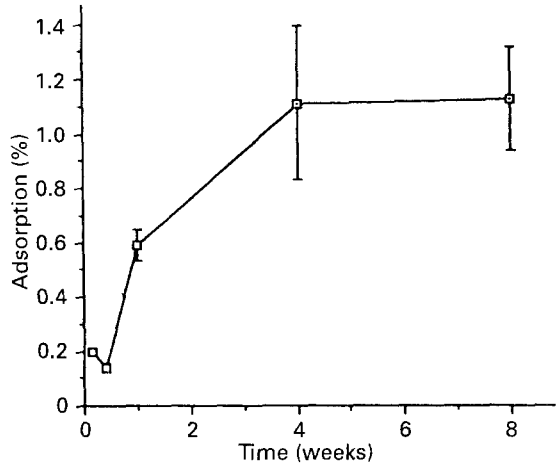

Figure 3 Adsorption of 14C-radiolabelled dimethyl-APD into the HA tube after placement in 2.5 ml 0.1 mmol/l dimethyl-APD The adsorption rate was between 0.83 and 1.39% of the available amount after 4 weeks of incubation. No difference was observed between loading periods of 4 and 8 weeks.

3.2. Adsorption of 0.1 mmol dimethyl-APD into the HA tube

The results of the adsorption study are presented in Fig. 3. The average value of adsorbed dimethyl-APD was 1.11% of the available amount of 70.3 μg, which is 0.78 μg.

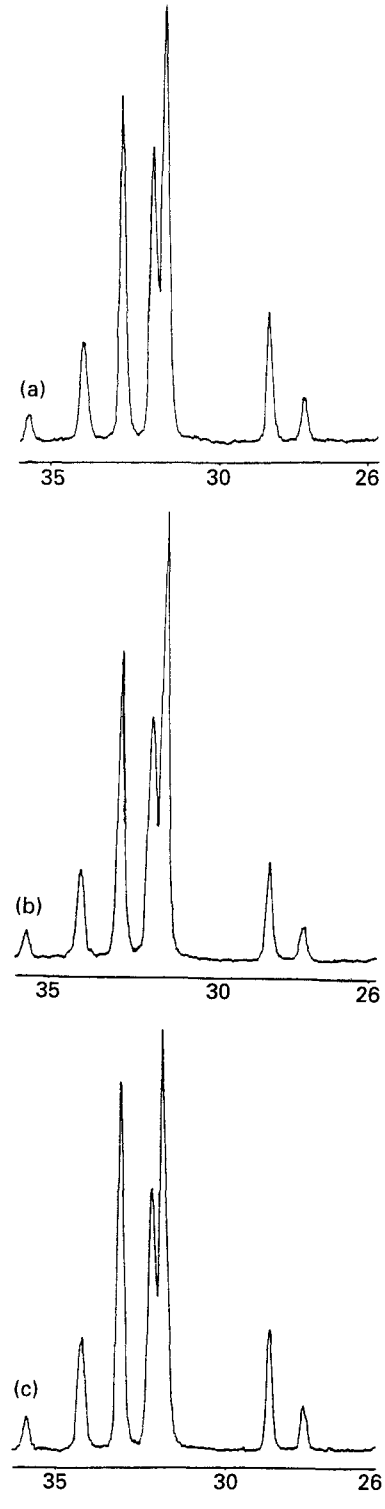

Figure 4 XRD patterns of (a) an HA tube blank; (b) a composite of an HA tube and 0.1 mmol/l dimethyl-APD; and (c) a composite of an HA tube and 0.1 mol/l dimethyl-APD. The typical peaks for HA were present in all patterns. The XRD patterns of HA tube blanks showed that the HA was essentially a one-phase material (> 95.5%) with an HA structure. HA tube particles as deposits after placement in concentrations of 0.1 mol/l dimethyl-APD, as well as intact HA tubes after placement in concentrations of 0.01 mol/l and 0.1 mmol/l dimethyl-APD, showed the same patterns with HA characteristics. Hardly any line-broadening can be seen. This indicates a well-crystallized material in all specimens.

3.3. Bulk and surface changes of the HA tube

After adsorption of dimethyl-APD no changes occurred in the bulk properties of the HA tube according to XRD (Fig. 4). However, the surface characteristics differed from the HA tube blanks after adsorption of dimethyl-APD (Fig. 5).

3.4. Animal experiments

3.4.1. Gross observations

The roots of the premolars were very difficult to remove intact. Consequently the socket walls were often damaged. Therefore the composite tubes initially did not fit in the root sockets. This adverse circumstance did not interfere with the ultimate result. All composite tubes were retained *in situ*, covered with mucosal tissue. Exfoliation during the observation period of 6 months did not occur. The alveolar ridges containing the composite tubes looked healthy. No inflammatory or other adverse processes could be noticed.

3.4.2. Radiographic results

Directly after implantation radiolucencies were present around the composite tubes. However, from the follow-up radiographs it could be deduced that the alveolar bone was deposited in very close proximity to the composite tubes. After 3 months all radiolucencies around the composite tubes had disappeared (Figs 6 and 7). Thereafter no resorption of bone adjacent to the implants could be detected in these intra-oral radiographs.

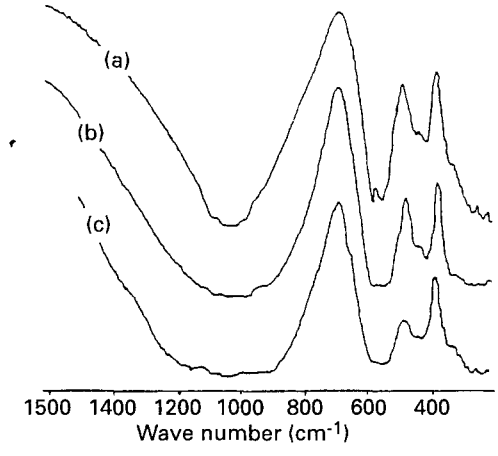

Figure 5 IRRS spectra of (a) an HA tube blank, (b) a composite tube of HA and 0.1 mmol/l dimethyl-APD and (c) a composite tube of HA and 0.1 mol/l dimethyl-APD. The phosphate peaks of the composite tubes have been flattened compared to the HA tube blank. The IRRS spectra show absorption peaks characteristic of HA. However, there is a striking change in the spectra of the composite tubes compared to the HA tubes. The phosphate peaks of all composite tubes were flattened. It was evident that the chemical appearance of the surfaces of the composite tubes had changed.

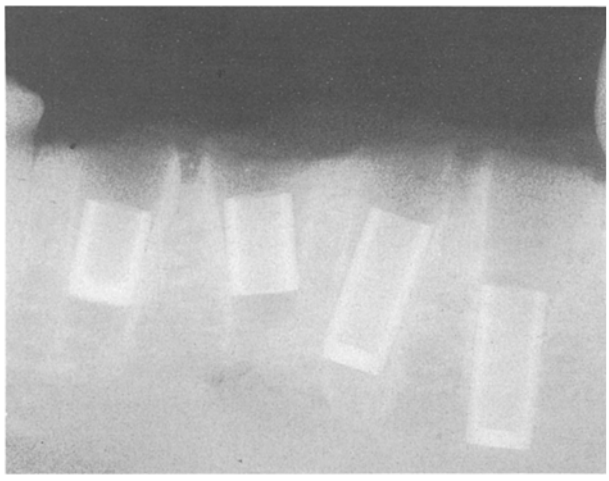

Figure 6 Intraoral radiograph of composite tubes of HA and dimethyl-APD in the third and fourth premolar regions of the mandible directly after implantation. The length of the composite tubes in the third premolar region was sometimes less than the standard length of 7 mm due to the bone availability. Note the radiolucencies around the composite tubes.

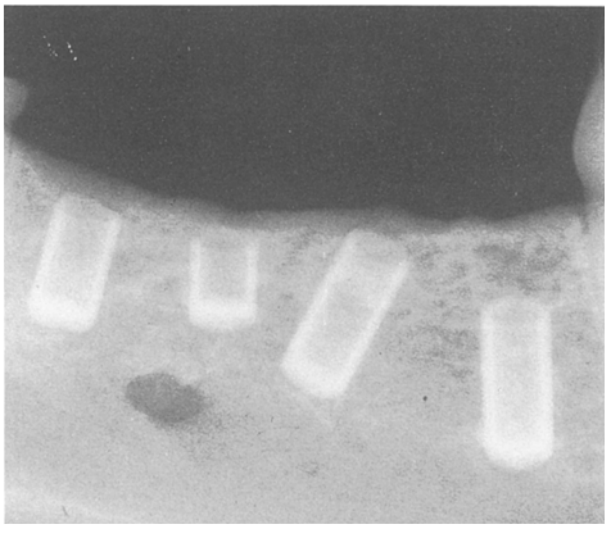

Figure 7 Intraoral radiograph of the composite tubes 3 months after implantation. All radiolucencies had disappeared. The alveolar bone closely encased the composite tubes. The contour of the outline of the composite tubes had been preserved: no degradation of HA of the composite tube was visible. Bone resorption around the composite tubes had not occurred. The compatibility of the composite tubes with alveolar bone was striking.

3.4.3. Macroscopic observations

After 3 months attempts were made to remove the composite tubes from the bone. For this purpose a mucoperiosteal flap was raised and in cases where bone had covered or grown into the tube, this bone was removed as much as possible. Elevators and tweezers were used to dislodge the composite tubes. These attempts, however, always failed because the composite tubes were strongly embedded and bonded to alveolar bone. A microradiograph of a cross-section of a bone-composite tube section is shown in Fig. 8.

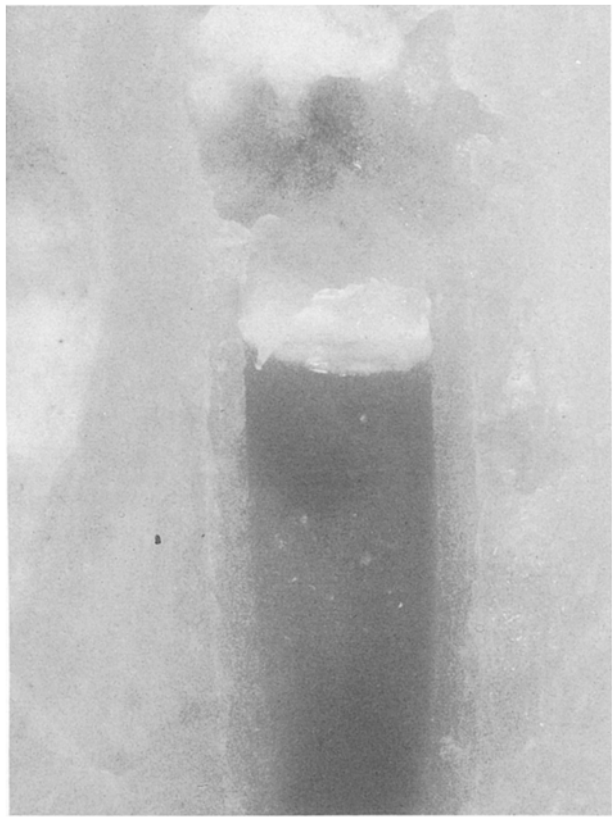

Figure 8 Macrophotograph of cross-section of a composite tube in the mandible of a dog. The tube was completely embedded in bone. Despite the cutting procedure no dislodging of the tube was observed, which indicated a strong bonding between bone and composite tube. Bone ingrowth into the open end of the tube was clearly visible.

3.4.4. Microscopic observations

Two types of interfaces could be observed in the histologic sections. There was either a cortical bone/composite tube or a cancellous bone/composite tube interface. No inflammatory cells, nor cells associated with an immunological response of the organism could be observed (Figs 9 and 10).

4. Discussion

HA bonds strongly to alveolar bone and the bisphosphonate, dimethyl-APD is a bone-seeking agent that adheres to the natural HA of bone [1, 3]. Therefore it was conceivable that a composite system of HA and dimethyl-APD was a promising concept. Data were presented concerning the characterization of HA tubes used for adsorption of dimethyl-APD. The HA tube was stable in different buffer solutions and in solutions of dimethyl-APD $\leqslant 0.1$ mmol/l *in vitro*. Tubes of other materials such as the aluminium–calcium–phosphorus systems, are resorbable, consequently there is a possibility that aluminium is released in the surrounding tissue [10, 11]. Recently tricalcium phosphate implants which can adsorb agents have been described [12, 13]. The long-term stability, however, which we advocate for alveolar bone implants as natural tooth root substitutes after extraction of teeth, is also questionable for these implants [14]. Therefore HA was the material of choice.

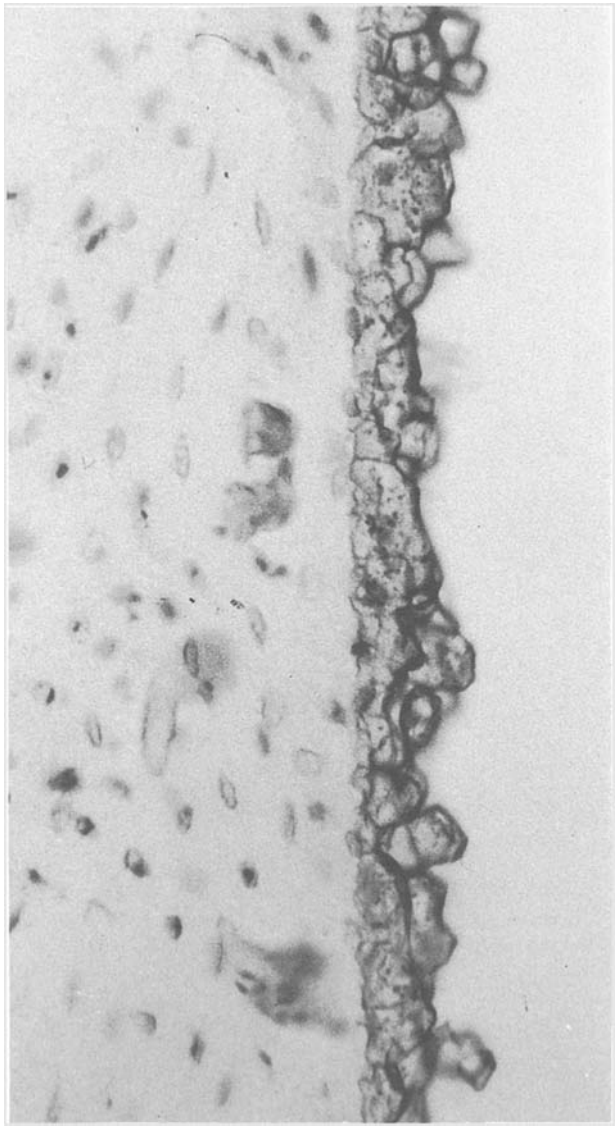

Figure 9 Light micrograph of a section of the mandible of a dog, 6 months after implantation, showing the bone closely following the contour of the outer surface of the composite tube. No encapsulation can be seen along the composite tube wall, nor signs of bone resorption around the composite tube or resorption of the composite tube surface itself (Giemsa, ×40).

The average adsorbed amount of dimethyl-APD was 0.78 μg. Taking the bone-contacting surface of the composite tube into account, we calculate that approximately 0.01 μg dimethyl-APD is in interfacial contact with 1 mm^2 of alveolar bone. From our *in vitro* mice experiments using cultures of fetal long bones we estimate that 0.003 $\mu g/mm^2$ is sufficient to achieve inhibition of bone resorption [15].

Although the compatibility, stability and direct bonding to alveolar bone of the biologically modified HA tube has been proven, the effect of the composite system on alveolar bone resorption cannot be deduced from this study and will be the subject of future investigations. Prevention of alveolar bone loss may have to be accomplished by the release of adsorbed dimethyl-APD from the HA tube to the surrounding alveolar bone, through the modulation of osteoclastic resorption.

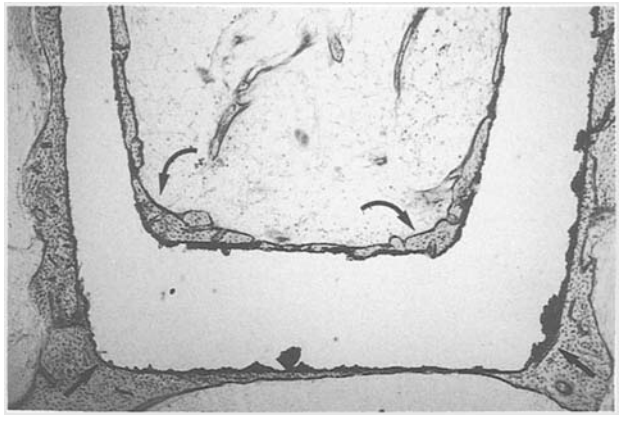

Figure 10 Light micrograph of a section of the mandible of a dog, 6 months after implantation with a cross-section of a composite tube. The (apical) outer surface of the composite tube was completely encaged in bone (straight arrows). The bone had also covered the inner walls of the composite tube due to bone in- and downgrowth all the way from the open upper end of the tube downwards to the closed apical portion (curved arrows) (Giemsa, ×25).

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