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# Bioadhesion of Polymer/Ceramics Nanocomposite

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Novel bio-adhesive polymer/ceramics nanocomposite composed of acrylic matrix with hydroxyapatite (HA) particles as a filler was developed using 4-methacryloyloxyethyl trimellitate anhydride (4-META) to promote adhesion both to bone and HA. The mechanical strength of the composite did not decrease significantly with increasing HA in the composite by 4-META. However, strength decreased with increasing HA content in the absence of 4-META. Scanning electron micrographic examination of fractured surfaces of the composite clearly showed that the HA particles adhered to the matrix when 4-META was added. Thus, it was important to maintain the original mechanical strengths for 4-META. The HA particles along the surface increased with increased HA content in the composite. The composite adhered to bone with a tensile bond strength was higher than 10 MPa. Biological bone reaction to the composite was studied. The composite was pushed into the femoral canals of dogs and the femurs were taken out on 12 and 24 weeks after operation. The histological sections of femurs, which were undecalcified and reserving, the composite was observed microscopically. In almost all sections composite had contacted with bone directly, and no necrotic and no other adverse reactions were observed on the bone near the composite. The 4-META/MMA-TBB/HA composite has better adhesivity to living bone. A total joint arthroplasty using the 4-META/MMA-TBB/HA composite could have a better result.
Key words: adhesive polymer, nanocomposite, hydroxyapatite, bone cement, orthopedic

## 1 INTRODUCTION

The fixation or attachment of artificial prostheses to hard tissues such as bone and tooth continues to be a major area of interest in orthopedic and dental surgery. The standard method of prosthesis fixation is to use acrylic resin primarily composed of poly(methyl methacrylate) (PMMA)[1,2]. Though immediate fixation of the prosthesis is an advantage in this method, shrinkage of the resin during polymerization could influence loosening of the prosthesis. To minimize gaps between the hard tissues and the prosthesis is important.

Adhesion may be effective in solving this problem. A new resin composed of 4-methacryloyloxyethyl trimellitate anhydride (4-META) and methyl methacrylate (MMA) as monomers, tri-*n*-butyl borane (TBB) as an initiator and PMMA powder (4-META/MMA-TBB resin) has been presented as a dental adhesive[3]. The advantage of the 4-META/MMA-TBB resin is its adhesion to prosthetic materials. The tensile bond strengths of 4-META/MMA-TBB resin to ceramics, hydroxyapatite (HA) and the composite of HA and fluoroapatite are above 15 MPa. The 4-META/MMA-TBB resin also adheres to metals, e.g., Ni-Co alloy, Co-Cr alloy and stainless steel (SUS-304). The bond strength to these metals is above 18 MPa; in the case of SUS-304, it is above 40 MPa[4].

Several types of bioactive ceramic coatings have been placed in order on prosthesis to enhance the attachment of the prosthesis to bone. These coatings have the potential advantage of producing an intermediate region between bone and the prosthesis to enhance the transition of stress between them. Moreover, the bioactive ceramic coating may encourage bone to fill the space, and, perhaps direct attachment of the prosthesis to the bone is possible. However, bone ingrowth requires a longer period to fix the prostheses. If the acrylic resin contained a bone active component, fast fixation of the prostheses and direct bone attachment on the surface might be expected.

Based on the adhesive resin, 4-META/MMA-TBB resin, we introduced HA particles as a bone compatible filler to prepare novel adhesive. In this communication, the mechanical properties, surface characteristics of the composite, and the adhesion to bone were investigated with attention on the effect of 4-META and HA compositions in the composite.

## 2 EXPERIMENTAL

### 2.1 Materials

4-META (Fig.1) was prepared by the condensation between 2-hydroxyethyl methacrylate and trimellitic anhydride chloride in benzene-pyridine mixture, then recrystallized with benzene. HA particles with diameter measuring 15μm on average were supplied from Asahi Pentax Co. Ltd., Tokyo, Japan, and used without further treatment. The MMA, PMMA, and TBB (Sun-Medical Co., Shiga, Japan) were of standard commercial origin.

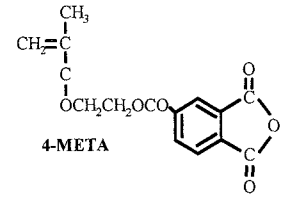


Fig.1. Chemical structure of 4-META

In 4 ml of monomer mixture containing MMA and 4-META, 50 mg of the initiator TBB were added and stirred quickly to oxidize the TBB in air. Ten grams of the powder components of the PMMA or a mixture of PMMA and the desired amount of HA were mixed with the monomer solution and stirred until the mixture became paste like with a high viscosity, similar to conventional bone cement. This viscous mixture was injected and cured in a Teflon® mold to make specimens for testing.

### 2.2 Measurement of Mechanical Strength

The compressive, tensile and bending strength of the composite were measured using an autograph testing machine (DSS-500, Shimadzu, Kyoto, Japan) with a crosshead speed of 2 mm/min. The specimen size was Φ6 mm x 12 mm for compressive measurements and 2 mm x 2 mm x 30 mm for tensile and bending strength measurements. The composite was cured in a Teflon® mold and kept for 60 min at room temperature. The specimens were removed from the mold and dried in vacuo for 15 h to determine the dry weight. One group of specimens was immersed in water at 37 °C for 7 days to evaluate water adsorption. The number of specimens used for each measurement was at least 8. The mean of each mechanical test group was compared by analysis of variance (ANOVA) and the Student *t*-test, with the value of statistical significance set at the $p<0.01$ level. Fractured surfaces were examined with a scanning electron microscope (SEM, JSM 5400, JEOL, Tokyo, Japan). The surface of the composite containing HA particles was analyzed with an X-ray photoelectron spectroscope (XPS, ESCA-200, Scienta, Uppsala, Sweden).

### 2.3 Measurement of Tensile Bond Strength

Human femur was used as the bone sample. The femur was cut with a diamond saw to create adhesive surfaces. The adhesion test was carried out using a vertical cut surface against the femur. The femur surface was covered with an adhesive nylon tape having a round hole of 6.0 mm diameter to regulate the adhesion area.

Monomer (1.0 g) and TBB (50 mg) were stirred well and then mixed with 1.0 g of PMMA powder with or without HA particles to prepare the composite paste. A 5.0 mm diameter PMMA or metal (Ni-Cr, Co-Cr, and SUS-304) rod was fixed perpendicularly to the bone surface with the composite paste. A half-hour later, the joint sample was immersed into water at 37 °C for 24 h. Tensile bond strength was measured using the autograph test frame. The number of specimens used for each measurement was at least 5. The mean of each mechanical test group was compared by ANOVA and the Student t-test.

In the same way, the composite was placed on a SUS-304 rod with a smooth surface 4 mm in diameter and another rod pasted onto it. The tensile bond strength was measured after the specimen was stored in water for 24 h at 37 °C.

### 2.4 Animal Test of 4-META/MMA-TBB/HA Composite

The guidelines for the care and use of laboratory animals were followed in the care of the six adult mongrel dogs (average weight 20 kg) that were used in the present study. Seventy-two (3.0 mm diameter, 7.0 mm length) titanium implant cylinders (Asahi Pentax, Tokyo, Japan) were used as supports and to keep the cement in place. All surgical procedures were conducted under ketamine hydrochloride for intramuscular anesthesia and under thiopental sodium for intravenous anesthesia. Under sterile saline irrigation three cavities for the implantation (diameter 4.0 mm and depth 9.0 mm) were prepared with a trepan bur and hand reamer in the left and right tibial and ischial bones. Each dog had 12 experimental sites, six on each side. Prior to the application of the composite filled with 15 μm HA particles into the bone cavities. The composite paste was delivered into the cavity with a lentulo, and the implant was put in place. After 4, 12, and 24 weeks two dogs were sacrificed by an overdose of thiopental sodium. The implants with the surrounding bone were quickly retrieved and cut into small blocks with a diamond disk. The sections were stained with toluidine blue and examined by optical microscopy (Olympus, Vanox-AHBT, Tokyo Japan).

## 3 RESULTS AND DISCUSSION

### 3.1 Mechanical Properties 4-META/MMA-TBB/HA composite

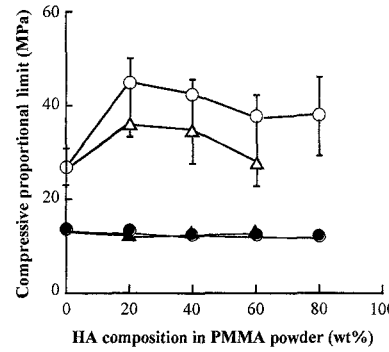


Fig.2. Compressive property of 4-META/MMA-TBB/HA composite. circle: 5 μmHA, triangle: 15 μmHA.

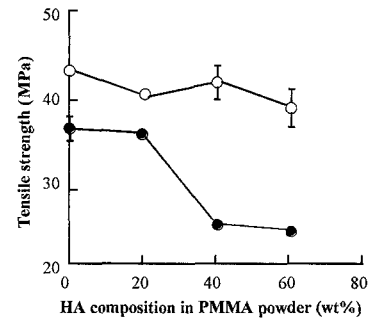


Fig.3. Tensile strength of 4-META/MMA-TBB/HA composite.

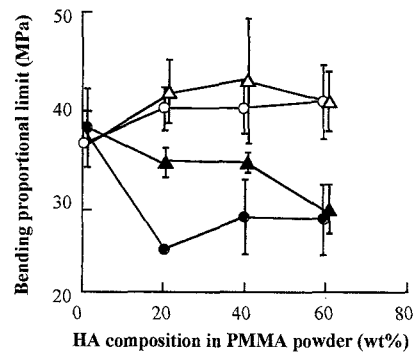


Fig.4. Bending property of 4-META/MMA-TBB/HA composite.

Figs 2 - 4 show effects of HA particles on the mechanical properties of bone cement with (Open plats) or without (Closed plots) 4-META. The values of the compressive proportional limit did not depend on the HA composition of the composite; whereas, the values of the tensile strength and bending proportional limit decreased with increased HA composition in the absence of 4-META. The addition of 4-META improved these mechanical properties significantly. Combination of HA particles in the powder component did not have an adverse effect on the mechanical strength even with a higher HA ingredient composition. These findings clearly showed that 4-META effectively promoted adhesion of HA particles to the matrix acrylic resin and the bonding prevented weakening due to introduction of HA particles.

The SEM examination of fractured surfaces revealed that adhesive failure was visible between the HA particles and PMMA matrix in the absence of 4-META whereas cohesive failure in the PMMA matrix was observed with 4-META resin (Fig.5). A similar state was observed in 40 wt% HA-containing 4-META/MMA-TBB/HA composite. Therefore, the tensile strength of 4-META/MMA-TBB/HA composite was limited by the strength of the original acrylic resin without HA particles. HA particles introduced defects when they were not bonded to the matrix. However, in the case of 4-META/MMA-TBB/HA composite, HA particles did not have an adverse effect on the composite since 4-META created bonding to the HA particles.

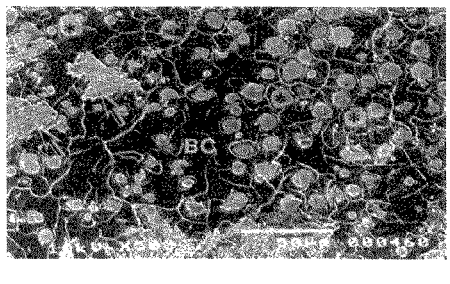


Fig.5. Fracture surface of 4-META/MMA-TBB/HA composite.

When the bending strength of 4-META/MMA-TBB and MMA-TBB resins was measured after immersion in water; the values were lowered compared with those under dry state ($p<0.01$, data was not shown). However the 4-META/MMA-TBB/HA composite maintained at least 30 MPa at all ranges of HA composition. The amounts of water absorbed in the MMA-TBB resin (40 wt% HA) and 4-META/MMA-TBB/HA composite (40 wt% HA) were 3.1 wt% and 0.8 wt%, respectively. Thus, adhesion between HA particles and resin with 4-META suppressed water absorption effectively, and maintained the mechanical properties of the composite even in a wet condition.

### 3.2 Surface Characteristics of the 4-META/MMA-TBB/HA Composite

The surface of 4-META/MMA-TBB/HA composite was analyzed by XPS to estimate the distribution of HA particles on the surface. The value of the calcium (Ca)/carbon (C) ratio increased with an increase in the HA composition. The Ca/C value corresponded to the surface distribution of HA particles. Thus, the HA particles located on the surface of the cured composite which could contact with bone directly.

Boone and coworkers have investigated bone attachment to HA-coated polymers. HA particles molded into the surface of thermoplastic implants or cast into the subsurface of thermoset implants significantly improved the bond strength between the polymers and bone by allowing direct bone apposition and some mechanical interlocking with the bone. However, the SEM picture of the fractured surface after a push-out test from the bone site was very similar to Fig.5. It revealed the existence of gaps between HA particles and polymer implants and the fractures occurred at the interface. This was attributed to a lack of adhesion of the HA particles to the polymer. That is, it was suggested that the adhesion of HA particles to the polymer would improve the bond strength between bone and composite. The 4-META/MMA-TBB resin could be useful for adhering HA particles to the surface of the composite.

A bioactive bone cement composed of bis-phenol-A glycidyl methacrylate (Bis-GMA), triethylene glycol dimethacrylate (TEGDMA) and HAarticles (2 μm in diameter) has been known. It was carried both measurement of the mechanical strength and histological evaluation of the cement implanted into femoral condyle of rabbits. The mechanical strengths of the Bis-GMA/TEGDMA/HA cement were superior to those of a conventional acrylic bone cement. This was due to high level of cross-linking in the cement, though the cement could not adhere the HA particles. The histological evaluation revealed that the HA particles in the cement could attach bone directly. It is believed that the Bis-GMA and TEGDMA starts cross-linking immediately when polymerization starts. This is not good for concentrational applications because the cement becomes viscous quickly and cannot be injected into femoral condyle satisfactorily.

### 3.3 Effect of 4-META on Adhesion of the Composite to Bone

Adhesion between bone and the PMMA rod, it can be seen that the tensile bonding strength using 4-META/MMA-TBB resin was larger than that using MMA-TBB resin with a significant difference. The tensile bond strength of the conventional acrylic bone cement (CMW I) which is polymerized by a benzoyl peroxide (BPO) - amine system, typically N,N-dimethylamino -*p*-toluidine, was quite low.

Metals also adhered strongly to bone with the 4-META/MMA-TBB resin. The bond strength is comparable to that of the PMMA rod. However, the tensile bonding strength of metals by MMA-TBB resin was nearly zero because the MMA-TBB resin cannot adhere to metal. The bond strength was about 14 MPa for PMMA rod and 11 MPa for SUS-304 rod. The 4-META appears to enhance the bonding of bone cement to prosthetic materials.

Fig. 6 shows the effect of the 4-META concentration in MMA of 4-META/MMA-TBB/HA composite containing 40 wt% HA particles on the tensile bond strength to bone. Though the tensile bond strength of the composite without 4-META was about 5 MPa, the value was above 10 MPa in the case of 3 or 5 wt% 4-META. Cohesive fracture of the bone occurred in the 4-META/MMA-TBB composite; on the other

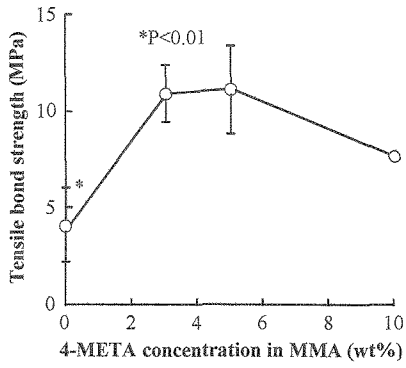


Fig.6. Tensile bond strength of 4-META/MMA-TBB/HA composite to bone.

hand, adhesive failure between bone and MMA-TBB resin was found by SEM examination of the fractured surface. Thus, 4-META could play an important role in improving the bond strength of the composite to bone also ($p<0.01$ vs MMA-TBB resin).

The 4-META/MMA-TBB/HA composite adhered to SUS-304 with a tensile strength of 10 MPa, even if the HA particles composition was 60 wt% in the powder. This result indicates that the 4-META/MMA-TBB/HA composite could be suitable for the intermediate zone between bone and orthopedic prostheses since it can adhere to both components.

From these results, it is clear to say that the HA particles can be contained in 4-META/MMA-TBB/HA composite with no adverse effect on adhesion ability of the composite. Moreover, the surface of the composite possesses an ability to adhere directly to bone.

### 3.4 *In vivo* Characteristics of HA Filled 4-META/MMA-TBB/HA Composite - In Vivo Histologic Evaluation

Optical microscopy revealed that young trabeculae formed toward the HA particles from the original bone at 4 weeks. Bone apposition was observed around the experimental material, which was particularly noticeable in the HA particles as shown in Fig.7. At 12 weeks considerable bone apposition was observed around HA surfaces without intervening fibrous tissue. Bone formation on the composite was quite dense. Areas of direct bone apposition were demonstrated, which were most prevalent in the scattered large particles as well as in the exposed small particles in the composite.

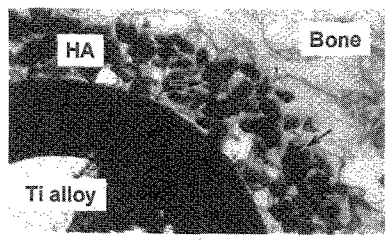


Fig.7. Picture of 4-META/MMA-TBB/HA composite at interfaces between Ti alloy rod and bone after 12 weeks implantation.

Although direct bone- composite contact was minimized by the presence of the large particles, these particles and the exposed smaller HA particles in the cured composite

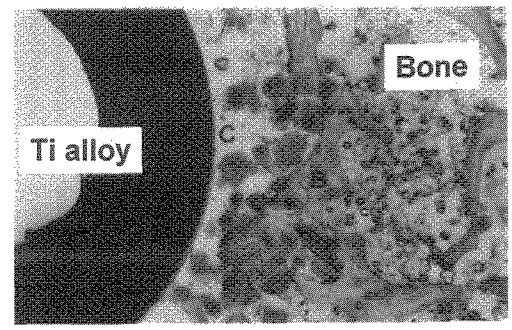


Fig.8. Picture of 4-META/MMA-TBB/HA composite at interfaces between Ti alloy rod and bone after 24 weeks implantation.

encouraged bone attachment. Bone intertwining among the HA particles after 24 weeks and bone tightly attached to the composite are shown in Fig.8. The bone formation was thickened and widened along the cavity.

## 4 CONCLUSIONS

A combination of 4-META and HA particles in the MMA-TBB resin may lessen the polymerization shrinkage, which is one of the common causes of implant prosthetic failure. The rapid initial fixation of the 4-META/MMA-TBB composite stabilized the bonding to bone. HA particles created bone apposition over time with no adverse effect on the mechanical property, thus further contributing to overall composite stability. It is concluded that HA particles can be included in the 4-META/MMA-TBB/HA composite and that they enhance composite -bone bonding.

We concluded that the 4-META/MMA-TBB/HA composite is promising materials for make good adhesion and stability for fixing orthopedic prosthesis.

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