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Effect of titania-based surface modification of polyethylene terephthalate on bone–implant bonding and peri-implant tissue reaction

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ABSTRACT

Organic polymers can be uniformly surface-modified with bioactive TiO_2 by using a sol–gel method. Titania-based surface-modified polyethylene terephthalate (TiPET) plates and fabric have shown apatite-forming ability in simulated body fluid.

Here, we first investigated the bone-bonding ability and mechanical bonding strength between the surface-modified layer and the base material (PET) of TiPET plates in vivo. For clinical applicability, we also examined the bone-bonding ability of TiPET fabric and the effect of titania-based surface modification on peri-implant tissue reactions (e.g. connective tissue capsule formation) in bone in vivo. Solid PET plates and PET fabric were prepared. Test plates and fabric were surface-modified with titania solution by using a sol–gel method. Histological examinations of the plates implanted into rabbit tibiae revealed direct contact between the TiPET plate and the bone. After the detaching test, a considerable amount of bone residue was observed on the surface of the TiPET plate. This result suggests that the mechanical bond strength between surface-modified layer and the base material is stronger than that between newly generated bone and tibia, and indirectly ensures the mechanical stability of the surface-modified layer. Pulling tests and histological examinations of the TiPET fabric revealed its excellent bone-bonding ability and micro-computed tomographic images showed excellent osteoconductive ability of TiPET fabric. The connective tissue capsule was much thinner, with less inflammatory tissue around the TiPET implants than around the control samples. These results indicate that TiPET fabric possesses a mechanically stable surface-modified layer, excellent bone-bonding ability, osteoconductive ability, and biocompatibility in bone.

1. Introduction

Nondegradable synthetic polymers such as polyethylene (PE), polyamides (e.g. nylon), and polyethylene terephthalate (PET) have been used clinically for making artificial ligaments [1,2], suture threads [3,4], weight-bearing materials in artificial joint prostheses [5], and synthetic vascular prostheses [6] because of their stability in the human body, flexibility with low elastic modulus, and durability. Although the end use of polymer biomaterials varies with each application, several surface-modification techniques can alter the interaction between the biomaterial surface and the biological environment while preserving the mechanical properties of the base materials. For example, in cardiovascular applications such as vascular prostheses, many researchers have attempted to reduce thrombogenicity by modifying the material surface of a vascular graft with antithrombogenic materials [7,8]. Reportedly, grafting of poly(2-methacryloyloxyethyl phosphorylcholine) (PMPC) on the PE liner surface of artificial joint prostheses dramatically decreases wear production; PE particles grafted with PMPC are biologically inert and do not cause bone resorptive responses [5]. Polyester fabric surface-modified with apatite and chitosan has recently been developed for artificial tendons and ligaments: the chitin/chitosan coating effectively induces bone formation in the spaces between the fabric fibers and enhances biological fixation of the fabric polymer materials to bone [9].

In 1994, titania gel was found to form bone-like apatite on its surface in simulated body fluid (SBF), and was considered to have bone-bonding ability through this apatite layer formed in the body fluid [10]. Since then, titania-based surface modification has been applied to various biomaterials because of the notion that a nonresorbable bioactive coating may guarantee direct, immediate, and long-standing contact between such implants and bone tissue, unlike the use of bioresorbable bioactive coatings, wherein the

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underlying inert substrates eventually become exposed during long-term implantation [11–14].

Recently, we have shown that organic polymers including PET can be uniformly surface-modified with bioactive titania by using a sol–gel method without a silane-coupling agent [14]. We have reported the in vitro apatite-forming ability of titania-based surface-modified PET (TiPET) plates and fabric in SBF and the mechanical bonding strength between the apatite layer and the PET plate [11]. In the current study, we first investigated the in vivo bone-bonding ability of TiPET plates and the mechanical bonding strength between the surface-modified layer and the base material (PET) because the mechanical bonding mechanism between them has been unresolved. Further, to determine the clinical applicability, we examined the in vivo bone-bonding ability of TiPET fabric and investigated the effect of titania-based surface modification on peri-implant tissue reactions such as connective tissue capsule formation surrounding such implants in bone.

2. Materials and methods

2.1. Preparation of PET specimens

PET plates (15 × 10 × 2 mm) and fabric (10 × 300 mm; PET fiber diameter: 24 μm) were supplied by Unitika Ltd (Osaka, Japan). One set of plates and fabric was surface-modified with titania solution, and the other was used as the control set.

TiPET plates were manufactured as described previously [11]. In brief, the PET samples were subjected to UV–ozone treatment for 30 min. Subsequently, the samples were quickly immersed in titania solution containing Ti $(OiC_3H_3)_4$, H_2O, HNO_3, and C_2H_5OH in the molar ratio 1.0:1.0:0.1:9.25 at 25 °C for 24 h. The samples were removed from the solution at a rate of 12 mm min^{-1} and air-dried at 80 °C for 24 h. They were then immersed in a 0.1 M aqueous solution of HCl at 80 °C for 24 h. Finally, they were removed from the solution, washed with ultrapure water, and air-dried.

TiPET fabric was manufactured using an identical procedure, except for the molar ratio of the titania solution and the method of modification. The molar ratio of the titania solution was 1.0:1.0:0.1:100 for Ti $(OiC_3H_7)_4$:H_2O:HNO_3:C_2H_5OH. The PET fabric was surface-modified with the titania solution as follows. PET samples were placed on polyethylene film, 1 ml titania solution was dropped onto the samples, and they were covered with another polyethylene film for 10 min.

2.2. Characterization of surface treatment

To observe the PET plate and fabric surfaces, a scanning electron microscope (S-4700; Hitachi Ltd, Tokyo, Japan) and an energy dispersive X-ray microanalyzer connected to the SEM (SEM-EDX, EMAX-7000; Horiba Ltd, Kyoto, Japan) were used. SEM-EDX data were quantified using the standardless ZAF method, and recalculated to 100% for the three elements of Ca, P, and Ti. The acceleration voltage was 15 kV, working distance was 12 mm, and EDX collection time was 500 s. The spectral recordings were controlled using a software package provided by the manufacturer of the EDX unit. The samples were observed before and after the sol–gel treatment.

The apatite-forming ability of the samples was examined by soaking them in SBF [15,16] at pH 7.40 for 3 days at 36.5 °C. The ion concentrations (in mmol l^{-1}) were as follows: Na^+, 142.0; K^+, 5.0; Ca^{2+}, 2.5; Mg^{2+}, 1.5; Cl^-, 147.8; HCO^{3-}, 4.2; $HPO_4{}^{2-}$, 1.0; $SO_4{}^{2-}$, 0.5. The samples were removed from the SBF, washed with distilled water, and dried on a clean bench. Their surfaces were examined with SEM and SEM-EDX, and apatite formation was determined by the presence of spherulites consisting of tiny flake-like crystals, which are the characteristic morphologies of apatite deposited from SBF [15,16].

2.3. Animal experiments

The animal study was approved by the Animal Research Committee, Graduate School of Medicine, Kyoto University, Japan.

2.3.1. In vivo detaching test using PET plates

The implants were conventionally sterilized with ethylene oxide gas and implanted into the tibial metaphysis of mature male Japanese white rabbits weighing 2.8–3.5 kg. The surgical methods used have been described previously [17,18]. In brief, the rabbits were anesthetized with an intravenous injection of pentobarbital sodium (0.5 ml kg^{-1}), an intramuscular injection of ketamine hydrochloride (10 mg kg^{-1}), and local administration of 1% lidocaine solution. A 3 cm longitudinal skin incision was made on the medial side of the right knee, and the fascia and the periosteum were incised and retracted to expose the tibial cortex. A 16 × 2 mm hole was made using a dental bur from the medial to the lateral cortex parallel to the longitudinal axis of the tibial metaphysis (Fig. 1A). After the hole was irrigated with saline, a control PET plate was implanted in the frontal direction as tightly as possible, perforating the tibia and protruding from the medial to the lateral cortex (Fig. 1B). After irrigation, the fascia and the skin were sutured layer by layer. The same surgical procedures were repeated contralaterally (left side), and the TiPET plate was implanted. The animals were housed individually in standard rabbit cages and fed standard rabbit food and water ad libitum. They were sacrificed with an overdose of intravenous pentobarbital sodium at either 4 or 8 weeks after implantation. In total, nine rabbits were used at each time point in this experiment.

2.3.1.1. Measurement of failure load by a detaching test. The mechanical test condition [17,18] was first established. Following euthanasia, segments of the proximal tibial metaphyses containing the implanted plates from six rabbits at each time point were cut and prepared for a detaching test (Fig. 1C). All the specimens were kept moist after harvesting. To remove periosteal bone growth, the bone tissue surrounding the plates was carefully removed from both sides and the ends by using a dental bur (Fig. 1D). Traction was applied vertically to the implant surface at a cross-head speed of 35 mm min^{-1} by using an Instron-type autograph (Model 1011; Aikoh Engineering Co. Ltd., Nagoya, Japan) with specially designed hooks to hold the bone–plate–bone construct (Fig. 1E). The detaching failure load was measured when the plate detached from the bone. If the plate detached before the test, the failure load was defined as 0 N.

2.3.1.2. Surface observations after the detaching test. After the detaching test, to observe onto which side (plate or tibia) the newly formed bone attached, specimens were prepared for surface analysis with SEM and SEM-EDX. The specimens were washed in sodium hypochlorite solution to remove soft tissue, fixed in 10% phosphate-buffered formalin (pH 7.25) for 3 days, dehydrated in serial concentrations of ethanol (70%, 80%, 90%, 99%, 100%, and 100% [v/v]) for 1 day each. They were soaked in isopentyl acetate solution for 1 day, dried in a critical-point drying apparatus (hcp-2; Hitachi Ltd., Tokyo, Japan), and coated with carbon. All surfaces of the plate and tibia were then examined by SEM and SEM-EDX analyses.

2.3.1.3. Histological examination. Following explantation, the implant sites from the remaining three rabbits at each time point, which were not used in the detaching test, were removed and prepared for histological examination. Parts of the specimens were