

Preparation of bioactive flexible poly(tetramethylene oxide) (PTMO)–CaO–Ta₂O₅ hybrids

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Abstract Poly(tetramethylene oxide) (PTMO)–CaO–Ta₂O₅ hybrids were prepared by hydrolysis and polycondensation of triethoxysilane-functionalized PTMO (Si–PTMO), tantalum ethoxide (Ta(OEt)₅) and CaCl₂. In the system CaO-free PTMO–Ta₂O₅, Si–PTMO/Ta(OEt)₅ weight ratios were 30/70, 40/60 and 50/50 (hybrids PT30Ca0, PT40Ca0 and PT50Ca0, respectively). In the system PTMO–CaO–Ta₂O₅, the Si–PTMO/Ta(OEt)₅ weight ratio was 40/60 and CaCl₂/Ta(OEt)₅ mole ratios were 0.05, 0.10 and 0.15 (hybrids PT40Ca5, PT40Ca10 and PT40Ca15, respectively). Crack-free transparent monolithic hybrids were ob-

tained for all the examined compositions except for PT30Ca0. Even CaO-free hybrids PT40Ca0 and PT50Ca0 formed apatite on their surfaces in a simulated body fluid (SBF) within 14 days. Hybrid PT40Ca0 showed higher mechanical strength, which was increased by soaking in SBF, and larger strain to failure than human cancellous bone. The CaO-containing hybrids showed higher apatite-forming ability than the CaO-free hybrids, and its apatite-forming ability increased with increasing CaO content. Hybrids PT40Ca10 and PT40Ca15 formed apatite within 3 days. The mechanical strength of PT40Ca15 was, however, lower than PT40Ca0 and was decreased by soaking in SBF. Thus obtained flexible bioactive CaO-free PTMO–Ta₂O₅ hybrids are expected to be useful as bone substitutes.

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Introduction

Synthetic materials implanted into bone defects are generally encapsulated by a fibrous tissue isolating them from the surrounding bone. Some ceramics, such as Bioglass[®] [1], sintered hydroxyapatite [2] and glass-ceramic A-W [3], however, form a bone-like apatite layer on their surfaces in the living body and bond to living bone through the apatite layer, i.e. they show bioactivity. Although these bioactive ceramics are used clinically as important bone-repairing materials, they are essentially brittle and hence limited in their applications. It is desirable to develop new types of flexible bioactive materials. Bonfield et al. [4] early

developed hydroxyapatite–polyethylene composites as such materials. These composites, however, lose flexibility when the hydroxyapatite content exceeds 40 vol%. When the hydroxyapatite content is less than 40 vol%, bioactivity of the composite is not high [5].

Currently, organically modified sol–gel-derived metal oxides are paid attention because of their unique properties, such as low elastic modulus and flexibility in addition to the characteristics of the metal oxides [6–8]. In these hybrid materials, organic components are chemically incorporated into inorganic networks at the nanometer level. It has been revealed that some sol–gel-derived metal oxide gels, such as silicon oxide [9–11], titanium oxide [11–13], tantalum oxide [14], zirconium oxide [15, 16] and niobium oxide [17], form a bone-like apatite on their surfaces in a simulated body fluid (SBF) [18]. Therefore, it is expected that if some kind of organic component is incorporated into a network of these metal oxide gels, the obtained hybrids can show not only apatite-forming ability but also flexibility. Tsuru et al. [19] first reported that some polydimethylsiloxane (PDMS)–CaO–SiO₂ hybrids prepared by a sol–gel method form apatite on their surfaces in SBF, although their mechanical properties were not reported. The present authors then revealed that polydimethylsiloxane (PDMS)–CaO–SiO₂ [20, 21], PDMS–CaO–SiO₂–TiO₂ [22–25], poly(tetramethylene oxide) (PTMO)–CaO–SiO₂ [26], PTMO–CaO–SiO₂–TiO₂ [27] and PTMO–CaO–TiO₂ [28] hybrids show high apatite-forming ability in SBF and flexibility. These CaO-containing hybrids, however, showed decreased mechanical strength in SBF [29]. Recently, the present authors revealed that even CaO-free PDMS–TiO₂ [30] and PTMO–TiO₂ [31] hybrids show apatite-forming ability on their surfaces in SBF and flexibility when they precipitated anatase by hot-water treatments. Their mechanical strength, however, decreased as a result of the hot-water treatments. In the present study, PTMO–CaO–Ta₂O₅ hybrids were prepared by a sol–gel method. It has been revealed that tantalum oxide shows high apatite-forming ability in SBF even in an amorphous state as-prepared by a sol–

gel method without a subsequent hot-water treatment. It is, therefore, expected that an organically modified tantalum oxide gel also show high apatite-forming ability without a hot-water treatment. Their apatite-forming ability in SBF and mechanical properties before and after soaking in SBF were examined. The materials which form apatite in SBF are expected to form apatite even in the body and bond to living bone through this apatite layer [5, 32].

Experimental procedure

Preparation of hybrids

Triethoxysilane functionalized poly(tetramethylene oxide) (Si–PTMO) was prepared by the reaction of poly(tetramethylene oxide) (PTMO, HO–(–CH₂CH₂CH₂CH₂O–)_n–H, molecular weight = 1000, Aldrich Chemical Co., Milwaukee, USA) with 3-isocyanatopropyltriethoxysilane (IPTS, (C₂H₅O)₃SiCH₂CH₂CH₂NCO, Aldrich Chemical Co., Milwaukee, USA) according to the method described by Brennan et al. [33]. The mixture of PTMO and IPTS, whose molar ratio was [PTMO/IPTS] = [1/2], was stirred at 70 °C for 5 days under a nitrogen atmosphere.

Solutions whose compositions are given in Table 1 were prepared in glass beakers under ambient conditions. Tantalum ethoxide (Ta(OEt)₅, Aldrich Chemical Co., Milwaukee, USA), Si–PTMO and two third amount of isopropylalcohol (IPA, Nacalai Tesque Inc., Kyoto, Japan) were mixed. The amount of Ta(OEt)₅ was fixed at 5 g for preparing specimens for the evaluation of the apatite-forming ability, and its amount was increased to 7.5 g for preparing specimens for mechanical testing. As soon as a homogeneous solution was obtained, the mixture of CaCl₂ (Nacalai Tesque Inc., Kyoto, Japan), ultra-pure water, HCl (35 wt% aqueous solution, Nacalai Tesque Inc., Kyoto, Japan) and a third amount of IPA was then added slowly to the solution. After stirring for 10 min, the obtained solution was cast into a cylindrical Teflon[®] container 75 mm in diameter and 20 mm in depth

Table 1 Compositions of starting solutions of hybrids

	Notation	Composition				
		Si–PTMO/Ta(OEt) ₅ (weight ratio)	CaCl ₂ /Ta(OEt) ₅ (molar ratio)	H ₂ O/Ta(OEt) ₅ (molar ratio)	HCl/Ta(OEt) ₅ (molar ratio)	IPA/Ta(OEt) ₅ (molar ratio)
Si–PTMO: Triethoxysilane functionalized poly(tetramethylene oxide); Ta(OEt) ₅ : Tantalum ethoxide; IPA: Isopropylalcohol	PT30Ca0	30/70	0	2.5	0.05	30
	PT40Ca0	40/60	0	2.5	0.05	30
	PT50Ca0	50/50	0	2.5	0.05	30
	PT40Ca5	40/60	0.05	2.5	0.05	30
	PT40Ca10	40/60	0.10	2.5	0.05	30
	PT40Ca15	40/60	0.15	2.5	0.05	30

covered with a paraffin film having several pinholes, and it was kept at 40 °C for 3 weeks for gelation and drying. During the drying, the hybrid was turned over repeatedly to obtain a flat bulk.

Soaking in SBF

Specimens about $10 \times 10 \times 2 \text{ mm}^3$ in size were cut from the obtained hybrid and polished with (400 silicon carbide abrasive paper. They were soaked in 30 mL of SBF with pH 7.40 and ion concentrations (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 mM) nearly equal to those of the human blood plasma [18] at 36.5 °C. After various periods, they were removed from SBF, washed with ultra-pure water and then dried at room temperature.

Analysis of the surfaces of the hybrids

The surface structural changes of the specimens due to soaking in SBF were analyzed by thin-film X-ray diffraction (TF-XRD, RINT-2500, Rigaku Co., Tokyo, Japan). In the thin-film X-ray diffraction, the surfaces of the specimens were fixed at an angle of 1° against the direction of the incident beam. The morphological changes of the surfaces of the specimens were observed under a field emission scanning electron microscope (FE-SEM, S-4700, Hitachi Ltd., Tokyo, Japan).

Analysis of SBF

The changes in the element concentrations of SBF due to soaking of the specimens were measured by inductively coupled plasma (ICP) atomic emission spectroscopy (Model SPS1500 VR, Seiko Inst. Inc., Tokyo, Japan).

Evaluation of the mechanical properties of the hybrids

Rectangular specimens $3 \times 4 \times 30 \text{ mm}^3$ in size were cut from the obtained hybrids, as shown in Fig. 1, and polished with (2000 silicon carbide abrasive paper. The 3 mm and 4 mm edges were defined as thickness and width, respectively. They were soaked in 60 mL of SBF or ultra-pure water at 36.5 °C for 28 days. Their mechanical properties before and after soaking in SBF or ultra-pure water were measured with an Instron-type testing machine (Model AGS-10 kNG, Shimadzu Co., Kyoto, Japan) by a three-point bending test over a 25 mm span at a cross-head speed of 0.5 mm min^{-1} . Loading configurations are also shown

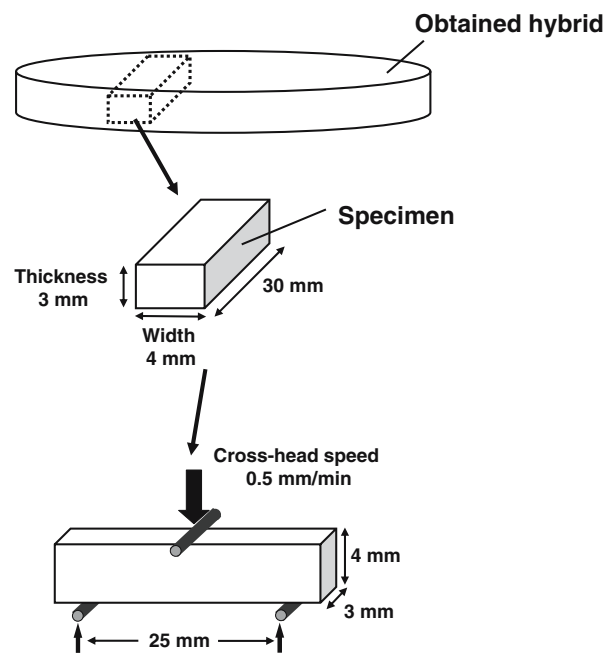


Fig. 1 Preparation of specimens for measurement of mechanical properties and their loading configurations

in Fig. 1. After soaking in SBF or ultra-pure water, the specimens were subjected to mechanical testing under ambient conditions within 5 min of removal from SBF or ultra-pure water. The size of each specimen was measured before and after soaking in SBF or ultra-pure water.

Results

Appearance of the hybrids

Crack-free transparent monolithic discs approximately 40 mm in diameter and 2–4 mm in thickness were obtained for all the compositions except for PT30Ca0, as shown in Fig. 2. Hybrid PT30Ca0 formed many cracks during the drying process.

Calcium-free hybrids, i.e. PT40Ca0 and PT50Ca0, did not change their appearance even after soaking in SBF for 14 days, whereas the calcium-containing hybrids, i.e. PT40Ca5, PT40Ca10 and PT40Ca15, became white and opaque, and formed many small cracks in them after soaking in SBF even for 3 days.

Apatite formation on hybrids in SBF

Figure 3 shows TF-XRD patterns of the surfaces of the hybrids before and after soaking in SBF for various periods. All the as-prepared hybrids were amorphous.

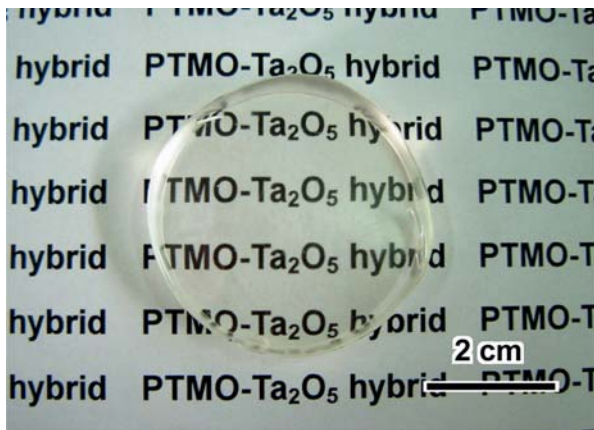
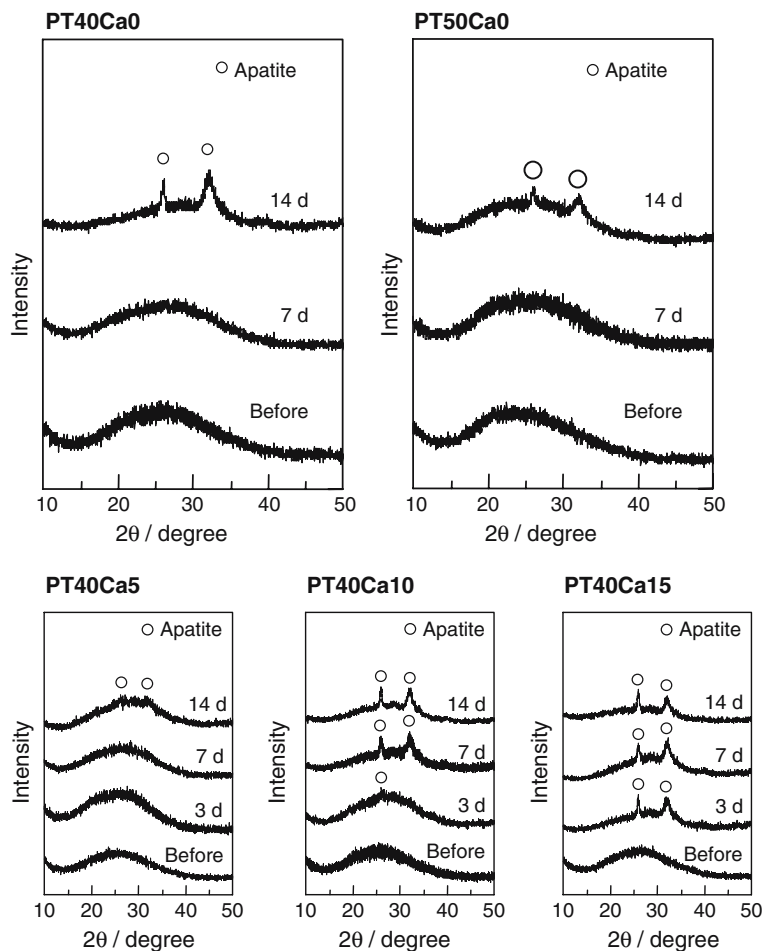


Fig. 2 Appearance of obtained hybrid PT40Ca0

Apatite peaks were newly observed within 3 days for hybrids PT40Ca10 and PT40Ca15, and 14 days for hybrids PT40Ca0, PT50Ca0 and PT40Ca5. Figure 4 shows FE-SEM photographs of the surfaces of the hybrids before and after soaking in SBF for various periods. Precipitation of apatite was observed on the surfaces of the hybrids after soaking in SBF. The

Fig. 3 TF-XRD patterns of the surfaces of the hybrids before and after soaking in SBF for various periods



amount of apatite formed on hybrid PT40Ca0 was larger than that on PT50Ca0, and that on the CaO-containing hybrids increased with increasing CaO content at every period of soaking in SBF.

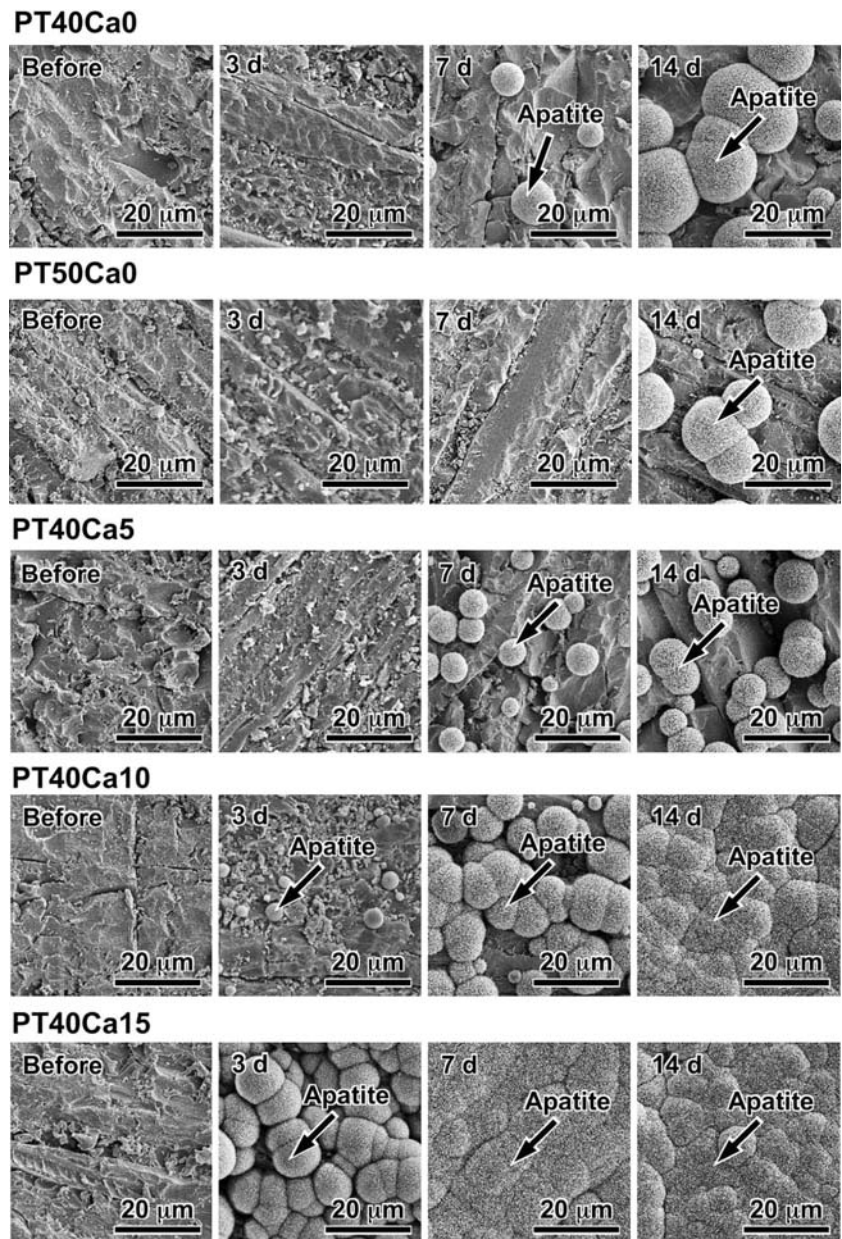
Changes in the element concentrations of SBF

Figure 5 shows changes in the element concentrations of SBF due to soaking of the hybrids. The release of tantalum and silicon from the hybrids was not detected even after 14 days. The CaO-containing hybrids, i.e. PT40Ca5, PT40Ca10 and PT40Ca15, increased the calcium concentration in SBF at the early stage. The hybrid PT40Ca5 and CaO-free hybrids, i.e. PT40Ca0 and PT50Ca0, decreased the calcium concentration in SBF with increasing soaking time at the later stage. All the hybrids decreased the phosphorus concentration in SBF with increasing soaking time.

Mechanical properties of the hybrids

Figure 6 shows the stress–strain curves of hybrids PT40Ca0 and PT40Ca15 before and after soaking in

Fig. 4 FE-SEM photographs of the surfaces of the hybrids before and after soaking in SBF for various periods



SBF or ultra-pure water for 28 days, in comparison with those reported for human cancellous bone [34]. The values of the bending strength, strain to failure and Young's modulus of the hybrid PT40Ca0 and PT40Ca15 before and after soaking in SBF or ultra-pure water for 28 days, in comparison with those reported for human cancellous bone [34], are summarized in Table 2. Hybrid PT40Ca0 showed higher mechanical strength and strain to failure than human cancellous bone, and similar Young's modulus to the latter, before soaking in SBF. Hybrid PT40Ca15 showed a bending strength and Young's modulus similar to those of human cancellous bones and higher

strain to failure before soaking in SBF. The bending strength and Young's modulus increased, and strain to failure decreased after soaking in SBF for hybrid PT40Ca0. Similar phenomena were also observed after soaking in ultra-pure water. On the other hand, the bending strength and strain to failure significantly decreased, and the Young's modulus increased after soaking in SBF for hybrid PT40Ca15.

The changes in size of hybrids PT40Ca0 and PT40Ca15 due to soaking in SBF or ultra-pure water for 28 days are shown in Table 3. Neither the width nor thickness of the specimen significantly changed after soaking in SBF or water for hybrid PT40Ca0. On the

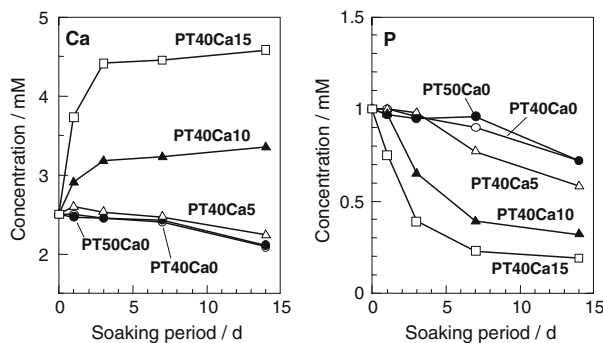


Fig. 5 Changes in element concentrations of SBF due to soaking of the hybrids

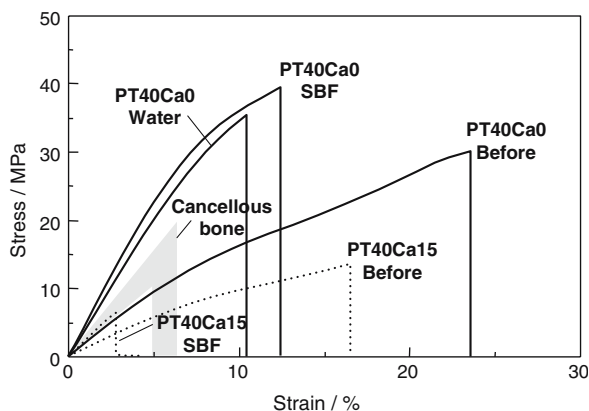


Fig. 6 Stress–strain curves of hybrids PT40Ca0 and PT40Ca15 before and after soaking in SBF or ultra-pure water for 28 days, in comparison to those reported for human cancellous bone [34]

other hand, both the thickness and width of the specimen significantly increased after soaking in SBF for hybrid PT40Ca15.

Discussion

Even the CaO-free PTMO–Ta₂O₅ hybrids showed apatite-forming ability in SBF without any treatment. We previously prepared CaO-free PTMO–SiO₂ [26] and PTMO–TiO₂ hybrids [31], but these hybrids did not form apatite in SBF within 14 days. Although the PTMO–TiO₂ hybrids showed apatite-forming ability when they were treated with hot water, their mechanical strength significantly decreased by the hot-water treatment. The PTMO–Ta₂O₅ hybrids have an advantage that they do not need the hot-water treatment, which induces the decrease in their mechanical strength. Consequently, the mechanical strength of the present PTMO–Ta₂O₅ hybrid (~30 MPa) was higher than that of the PTMO–TiO₂ hybrid with the

hot-water treatment (~10 MPa) [31]. Moreover, the CaO-free PTMO–Ta₂O₅ hybrid increased its mechanical strength in SBF. Such a bioactive hybrid that increases the mechanical strength in the physiological environment has not been reported.

Hybrid PT30Ca0 formed many cracks during preparation. This would be attributed to the insufficient content of the organic component, which relaxes the stress during gelation and drying.

All of the hybrids that were obtained in the present study formed apatite in SBF. The apatite-forming ability of hybrid PT40Ca0 was higher than that of hybrid PT50Ca0. This indicates that the Ta₂O₅ component in the hybrids induced the apatite nucleation on the surfaces of the hybrids. When the ratio of PTMO to Ta₂O₅ was constant, the apatite-forming ability of the hybrids increased with increasing CaO content. This is because incorporated calcium ions were released into the SBF (see Fig. 5) and accelerated apatite nucleation by increasing the ionic activity product of apatite [35]. Once the apatite nuclei are formed, they can grow spontaneously by consuming the calcium and phosphate ions from the surrounding SBF, as shown in Fig. 5, since SBF is already highly supersaturated with respect to the apatite. The obtained hybrids are expected to form the apatite on their surfaces in the body too, and bond to living bone through the apatite layer. The CaO-free PTMO–TiO₂ hybrids did not form apatite in SBF within 14 days when they had not been subjected to a subsequent hot-water treatment [31], whereas the present CaO-free PTMO–Ta₂O₅ hybrids formed it without the subsequent hot-water treatment. It has been revealed that a sol–gel-derived amorphous Ta₂O₅ forms the apatite in SBF [14], whereas a sol–gel-derived amorphous TiO₂ does not form the apatite [13]. The titanium oxide gel formed the apatite only after transformation into anatase or rutile by heat treatment [13]. This might be a reason why the present PTMO–Ta₂O₅ hybrids showed apatite-forming ability without the subsequent hot-water treatment, in contrast with PTMO–TiO₂ hybrids.

For hybrid PT40Ca0, the bending strength and Young's modulus increased, and the strain to failure decreased after soaking in SBF (see Fig. 6 and Table 2). This change was almost identical even in ultra-pure water. This indicates that the increases in mechanical strength and Young's modulus are due to the reaction between the hybrid and water. In the preparation of the present hybrids, unreacted ethoxyl groups of the tantalum ethoxide are speculated to be remained in the hybrid. When the hybrid is soaked in aqueous solution, unreacted ethoxide groups are hydrolyzed and condensation reaction is proceeded.

Table 2 Mechanical properties of hybrids PT40Ca0 and PT40Ca15 before and after soaking in SBF or ultra-pure water for 28 days, in comparison to those reported for human cancellous bone [34]

Hybrid	Bending strength (MPa)	Strain to failure (%)	Young's modulus (MPa)
PT40Ca0-Before ($n = 4$)	30.5 ± 1.8	23.7 ± 7.6	296 ± 29
PT40Ca0-SBF ($n = 3$)	39.8 ± 6.8	12.4 ± 3.6	461 ± 11
PT40Ca0-Water ($n = 3$)	35.6 ± 2.5	10.3 ± 0.3	440 ± 7
PT40Ca15-Before ($n = 7$)	13.6 ± 1.7	16.5 ± 2.4	122 ± 14
PT40Ca15-SBF ($n = 7$)	6.3 ± 1.0	2.7 ± 0.5	268 ± 26
Cancellous bone	10–20	5–7	50–500

Table 3 Changes in size of hybrids PT40Ca0 and PT40Ca15 by soaking in SBF or ultra-pure water for 28 days

Hybrid	Width after soaking Width before soaking	Thickness after soaking Thickness before soaking
PT40Ca0-SBF ($n = 3$)	1.003 ± 0.013	1.014 ± 0.004
PT40Ca0-Water ($n = 3$)	0.996 ± 0.001	1.007 ± 0.003
PT40Ca15-SBF ($n = 7$)	1.067 ± 0.005	1.088 ± 0.014

This condensation reaction increases the number of the bonding in the hybrids and accordingly increases the mechanical strength and Young's modulus. In contrast, when the calcium ions were incorporated into the PTMO–Ta₂O₅ hybrid, the degradation of the hybrids occurred in SBF. The calcium-containing hybrids, i.e. PT40Ca5, PT40Ca10 and PT40Ca15, became opaque and developed many small cracks in them after soaking in SBF. In fact, the bending strength and strain to failure decreased, and hence the strain energies stored until failure significantly decreased after soaking in SBF for hybrid PT40Ca15 (see Fig. 6 and Table 2). It is speculated that the calcium ions inhibit the formation of a continuous three-dimensional network of the hybrid. In such cases, calcium ions are easily released via exchange with hydronium ions in SBF and water molecules also easily enter into the network to break the continuous network structure of the hybrids. The increase in the size of hybrid PT40Ca15 in SBF (see Table 3) would actually indicate that the hybrid incorporated water to be swollen in SBF. It has been revealed that CaO-containing PDMS–CaO–SiO₂–TiO₂ and PTMO–CaO–TiO₂ hybrids show a decrease in mechanical strength in SBF [29]. The degradation mechanism of these hybrids in SBF is considered to be similar to that of the hybrid PT40Ca15.

Although the apatite-forming ability of hybrid PT40Ca0 is lower than that of the calcium containing hybrids, its mechanical strength increased in SBF. A new kind of flexible bioactive material useful as bone substitutes might be developed in the present system.

Summary

A CaO-free PTMO–Ta₂O₅ hybrid which was prepared by a sol–gel method formed apatite on its surface in

SBF within 14 days without any subsequent treatment. Its bending strength and strain to failure were larger than those of human cancellous bone and its Young's modulus was almost equal to that of latter. Its bending strength and Young's modulus increased after soaking in SBF, but its strain to failure decreased. Because of their unique biological and mechanical properties, the present material is expected to find unique applications as bone substitutes.

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