

Apatite formation abilities and mechanical properties of hydroxyethylmethacrylate-based organic–inorganic hybrids incorporated with sulfonic groups and calcium ions

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Abstract Apatite formation in the living body is an essential requirement for artificial materials to exhibit bone-bonding bioactivity. It has been recently revealed that sulfonic groups trigger apatite nucleation in a body environment. Organic–inorganic hybrids consisting of organic polymers and the sulfonic groups are therefore expected to be useful for preparation of novel bone-repairing materials exhibiting flexibility as well as bioactivity. In the present study, organic–inorganic hybrids were prepared from hydroxyethylmethacrylate (HEMA) in the presence of vinylsulfonic acid sodium salt (VSAS) and calcium chloride (CaCl_2). The bioactivities of the hybrids were assessed *in vitro* by examining the apatite formation in simulated body fluid (SBF, Kokubo solution). The hybrids deposited on the apatite after soaking in SBF within 7 days. Tensile strength measurements showed a tendency to increase with increases in VSAS and CaCl_2 content. It was assumed that this phenomenon was attributed to the formation of cross-linking in the hybrids.

1 Introduction

Artificial materials implanted in bony defects generally become encapsulated by collagen fibrous tissues so that they are isolated from surrounding bony tissues. This is a normal reaction of the body to protect us from foreign substances. On the other hand, several kinds of ceramics are known to bind to living bone without initiating a foreign body reaction [1, 2]. They are called bioactive ceramics and are currently used in cases where bone substitution is medically necessary. However, bioactive ceramics have some features which limit their clinical applications to areas under low load and include higher brittleness and lower flexibility than natural bone. Therefore, novel materials exhibiting both better bioactivity and mechanical properties are required in medical fields.

Bioceramics achieve bone integration via the formation of bone-like apatite on their surfaces in the body environment. This apatite formation can be observed even in acellular simulated body fluid (SBF, Kokubo solution) with inorganic ion concentrations analogous to those found in human extracellular fluid [3, 4]. Apatite nucleation is triggered by specific functional groups such as Si–OH [3, 5], Ti–OH [5, 6], Zr–OH [7], Ta–OH [8], Nb–OH [9], COOH [10, 11], phosphate ($-\text{PO}_3\text{H}_2$) [12] and sulfonic groups ($-\text{SO}_3\text{H}$) [13, 14]. This is significantly accelerated by the release of calcium ions (Ca^{2+}) from the material surfaces [15]. These findings indicate that organic modification of the substances that provide these functional groups in the presence of Ca^{2+} may be an effective approach for designing novel bioactive materials abundant in flexibility. Based on this concept, several bioactive organic–inorganic hybrids have been developed using poly(dimethylsiloxane) (PDMS) [16], chitin [17], chitosan [18], starch [19], polycaprolactone [20], and poly(vinyl alcohol) (PVA) [21].

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Table 1 Composition of the specimens synthesized in this study

Sample	Composition/mol				
	VSAS	HEMA	CaCl ₂	TEGDMA	BPO
S10H90Ca05	0.01	0.09	0.005 in EtOH	0.003	0.001
S10H90Ca10	0.01	0.09	0.01 in EtOH	0.003	0.001
S20H80Ca05	0.02	0.08	0.005 in EtOH	0.003	0.001
S30H70Ca05	0.03	0.07	0.005 in EtOH	0.003	0.001

EtOH: Ethanol

Hydroxyethylmethacrylate (HEMA, (CH₂=C (CH₃)COOCH₂CH₂OH)) is an attractive candidate for organic component of the bioactive organic–inorganic hybrids, as it has high hydrophilicity and high biological affinity. Its polymer is already in use for medical applications such as contact lenses and as the coating agent on artificial blood vessels [22]. The present authors previously synthesized bioactive organic–inorganic hybrids from HEMA by modifications with functional groups such as Si–OH [23] and Ti–OH [24] as well as Ca²⁺. The relationship between various functional groups and the properties of the hybrids gives important fundamental information for designing novel biomaterials with different biological and mechanical characteristics.

In this study, the present authors attempted to synthesize the bioactive organic–inorganic hybrids from HEMA by modification with sulfonic groups and Ca²⁺. Generally, Si–OH and Ti–OH groups incorporate into a hydrogel structure through dehydration and polycondensation. The formed gel may increase the brittleness and decrease the flexibility of the hybrids. It is expected that organic–inorganic hybrids containing sulfonic groups will maintain high flexibility, since, they do not form such a gel structure. The extent of apatite deposition on the synthesized hybrids was examined in SBF. In addition, the mechanical properties of the hybrids were investigated with respect to their composition.

2 Materials and methods

The chemical reagents vinylsulfonic acid sodium salt (VSAS, CH₂=CHSO₃Na, Tokyo Chemical Industry Co., Ltd., Japan), HEMA (Wako Pure Chemical Industries, Ltd., Japan) and triethyleneglycol dimethacrylate (TEGDMA, CH₂=C(CH₃)COO(CH₂CH₂O)₃COC(CH₃)=CH₂, Wako Pure Chemical Industries, Ltd., Japan) were dissolved in ethanol. TEGDMA was used as the cross-linking agent to improve the chemical durability of the synthesized hybrids. The mixture was radically polymerized by heat treatment at 75°C for 3 h using benzoylperoxide (BPO, (C₆H₅CO)₂O₂, Nacalai Tesque, Inc., Japan) as the polymerization initiator. Then 100 cm³ of the obtained polymer solution was mixed with 20 cm³ of ethanol solution containing calcium chloride

(CaCl₂). The mixed solution was cast in a polypropylene mold of 120 × 160 mm² in size and dried for 2 weeks at room temperature. After a bulk gel was formed, a rectangular specimen of 10 × 10 × 1 mm³ in size was cut from the gel. The compositions of the specimens are shown in Table 1.

All specimens were subsequently soaked in 30 cm³ of SBF (Na⁺ 142.0, K⁺ 5.0, Mg²⁺ 1.5, Ca²⁺ 2.5, Cl⁻ 147.8, HCO₃⁻ 4.2, HPO₄²⁻ 1.0 and SO₄²⁻ 0.5 mol m⁻³) [3, 4]. The fluid was prepared by dissolving reagent-grade chemicals of NaCl, NaHCO₃, KCl, K₂HPO₄ · 3H₂O, MgCl₂ · 6H₂O, CaCl₂ and Na₂SO₄ in ultrapure water and buffering at pH 7.4 with tris (hydroxymethyl) amino-methane ((CH₂OH)₃CNH₂) and the appropriate volume of hydrochloric acid. All the reagents to prepare SBF were supplied from Nacalai Tesque, Inc., Japan. The temperature of the solution was kept at 36.5°C for various periods up to 7 days. After soaking in SBF, the specimens were removed from the fluid, washed gently with ultrapure water and dried at room temperature.

The surface structural features of the specimens before and after soaking in SBF were examined by scanning electron microscopy (SEM; S-3500N, Hitachi Co., Japan) and thin-film X-ray diffraction (TF-XRD; MXP3V, Mac Science Ltd., Japan). In the TF-XRD, the angle of the incident beam was fixed at 1° against the surface of the specimen, and the measurements were performed using a step scanning mode with 0.02° steps with 1 s at each step. For the SEM observation, a thin film of gold was coated on the surfaces of the specimens using a sputtering method. Changes in element concentration of SBF due to soaking of the specimens were measured by inductively coupled plasma (ICP) atomic emission spectroscopy (Model Optima 4300DVCYCLON, Perkin-Elmer Co., England).

Mechanical properties of the hybrids were assessed by a tensile test. Dumbbell-shaped specimens 60 mm in length and 10 mm in width at the center were prepared by the method described above. The specimens were subjected to the tensile test using an Instron-type material testing machine (Model AG-I, Shimadzu Co., Japan) according to Japanese Industrial Standards (JIS) 7127. The cross-head speed was fixed at 1.0 mm/min. Five specimens at each composition were tested and the average tensile strength and Young's modulus were calculated.

3 Results

Figure 1 shows SEM photographs of the surfaces of the specimens before and after soaking in SBF for 7 days. The new deposition of fine spherical particles was observed on the surfaces of all the specimens after 7 days. Figure 2 shows TF-XRD patterns on the surfaces of the specimens before and after soaking in SBF for various periods. Broad peaks assigned to the apatite were observed at 26° and 32° in 2θ for S10H90Ca10 after 3 days and the other specimens after 7 days. This indicates that the fine particles formed on the specimens in SBF are low-crystalline apatite. Figure 3 shows element concentrations of SBF after the specimens have been soaked, which was measured by ICP analysis. The Ca concentration once increased at the initial stage and then gradually decreased, whereas the P concentration gradually decreased. The S concentration slightly increased at the initial stage and became almost constant after 1 day.

Figure 4 shows a representative stress–strain curve of the specimens. S10H90Ca05 showed strain at failure as high as 100%. The tensile strength increased and the strain steeply decreased with an increase in CaCl_2 and VSAS content. Almost specimens showed a tendency to fracture

after plastic deformation. The measured mechanical properties were summarized in Table 2.

4 Discussion

Organic–inorganic hybrids synthesized from HEMA by modification with sulfonic groups and Ca^{2+} have potential to form apatite in SBF. This indicates that the prepared hybrids can achieve direct bone-bonding after implantation in living bodies. Sulfonic groups in the hybrids are assumed to induce heterogeneous apatite nucleation. The increase in Ca^{2+} content in the hybrids shortens the induction period for the apatite formation in SBF. However, the apatite-forming abilities of the hybrids were not significantly enhanced by the increase in the numbers of sulfonic groups. This may be because when the sulfonic group and Ca^{2+} form a complex such as $-\text{SO}_3\text{Ca}^+$ or $(-\text{SO}_3)_2\text{Ca}$ via ion–ion interactions, the initial release of Ca^{2+} into SBF is suppressed. In addition, acidity of the dissolved VSAS would decrease the degree of supersaturation of the fluid with respect to the apatite. The results shown in Fig. 3 support these assumptions.

Fig. 1 SEM photographs of the surfaces of the hybrids before and after soaking in SBF for 7 days

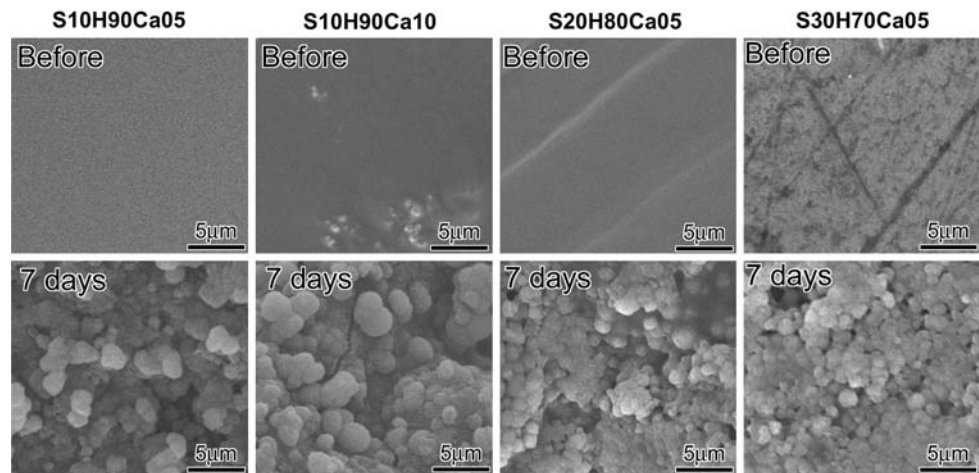


Fig. 2 TF-XRD patterns of the surfaces of the hybrids before and after soaking in SBF for various periods

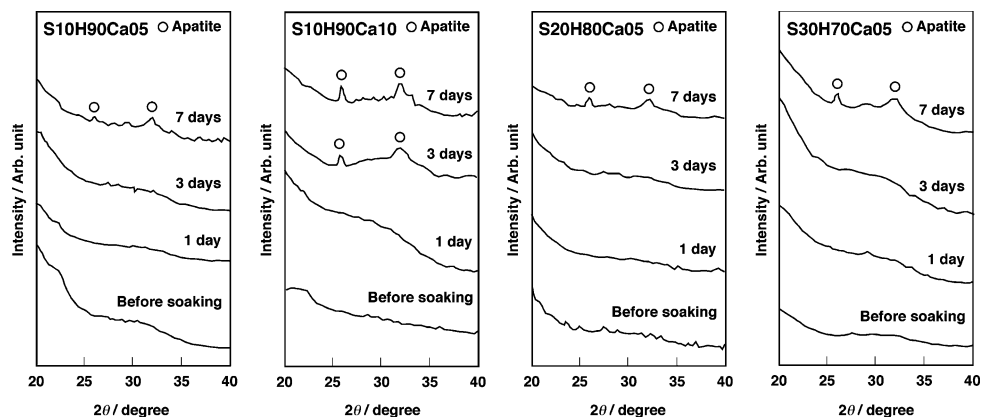


Fig. 3 Changes in element concentrations of SBF after soaking of the hybrids measured by ICP analysis

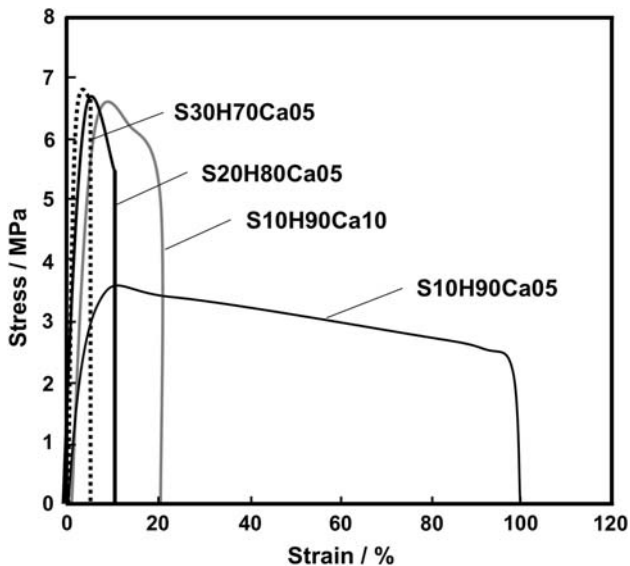
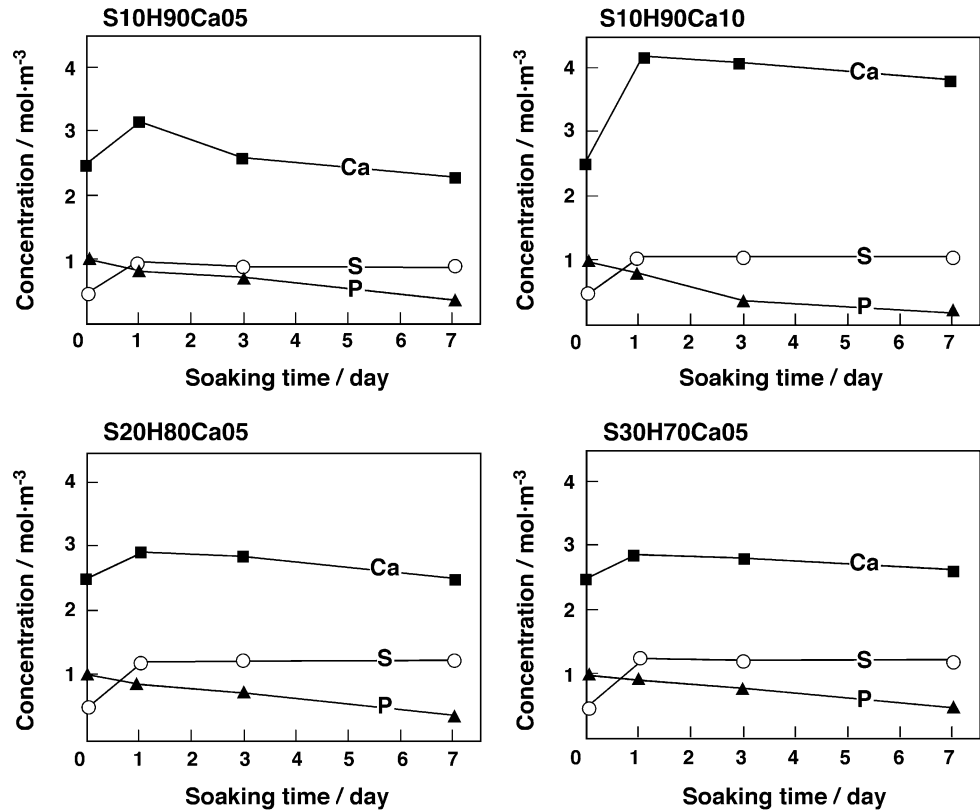


Fig. 4 Representative stress–strain curves of the hybrids

The results of the tensile test revealed that the hybrids obtained had highly flexible characteristics at a low content of VSAS and CaCl_2 . Almost all of them fracture after plastic deformation, whereas HEMA-based organic–inorganic hybrids combined with silicon and titanium alkoxides show brittle fracture [24, 25]. This may be attributed to the formation of domains of silica and titania gels through a sol–gel process which includes hydrolysis and polycondensation of

Table 2 Mechanical properties of the hybrids

Sample	Tensile strength/ MPa	Strain at failure/%	Young's modulus/ GPa
S10H90Ca05	3.58 ± 0.08	100.3 ± 0.9	93.6 ± 1.7
S10H90Ca10	6.80 ± 0.7	21.5 ± 0.3	180.7 ± 2.1
S20H80Ca05	6.82 ± 0.9	9.1 ± 0.5	223.4 ± 3.8
S30H70Ca05	6.95 ± 0.3	4.1 ± 1.2	338.1 ± 5.2

the alkoxides. On the other hand, sulfonic groups do not form such a brittle gel structure. Generally swelling property is improved at low cross-linking. It is also known that the solvent contained in the swelled gels can play a role as a plasticizer. Therefore, S10H90Ca05 would show significant plastic deformation. However, tensile stress and Young's modulus is steeply increased and strain at failure is steeply decreased with an increase in VSAS and CaCl_2 . The added Ca^{2+} ions would increase ionic cross-linking through the formation of $(-\text{SO}_3)_2\text{Ca}$. In addition, the sulfonic groups would make hydrogen bonds to the hydroxyl groups to form cross-links between VSAS and HEMA.

5 Conclusions

Organic–inorganic hybrids were synthesized from HEMA by modification with sulfonic groups and Ca^{2+} . They

formed apatite in SBF within 7 days. They showed significant plastic deformation at low content of sulfonic group. Incorporation of large amount of sulfonic group and Ca^{2+} drastically increased the brittleness of the hybrids.

References

1. L.L. Hench, J. Am. Ceram. Soc. **81**, 1705 (1998)
2. T. Kokubo, H.-M. Kim, M. Kawashita, Biomaterials **24**, 2161 (2003). doi:10.1016/S0142-9612(03)00044-9
3. S.B. Cho, K. Nakanishi, T. Kokubo, N. Soga, C. Ohtsuki, T. Nakamura, T. Kitsugi, T. Yamamuro, J. Am. Ceram. Soc. **78**, 1769 (1995). doi:10.1111/j.1151-2916.1995.tb08887.x
4. T. Kokubo, H. Takadama, Biomaterials **27**, 2907 (2006). doi:10.1016/j.biomaterials.2006.01.017
5. P. Li, C. Ohtsuki, T. Kokubo, K. Nakanishi, N. Soga, K. de Groot, J. Biomed. Mater. Res. **28**, 7 (1994). doi:10.1002/jbm.820280103
6. M. Uchida, H.-M. Kim, T. Kokubo, S. Fujibayashi, T. Nakamura, J. Biomed. Mater. Res. A **64**, 164 (2003). doi:10.1002/jbm.a.10414
7. M. Uchida, H.-M. Kim, T. Kokubo, T. Nakamura, J. Am. Ceram. Soc. **84**, 2041 (2001)
8. T. Miyazaki, H.-M. Kim, T. Kokubo, H. Kato, T. Nakamura, J. Sol-gel Sci. Tech. **21**, 83 (2001)
9. T. Miyazaki, H.-M. Kim, T. Kokubo, C. Ohtsuki, T. Nakamura, J. Ceram. Soc. Japan **109**, 934 (2001)
10. T. Miyazaki, C. Ohtsuki, Y. Akioka, M. Tanihara, J. Nakao, Y. Sakaguchi, S. Konagaya, J. Mater. Sci.: Mater. Med. **14**, 569 (2003). doi:10.1023/A:1024000821368
11. A. Sugino, T. Miyazaki, C. Ohtsuki, J. Mater. Sci. Mater. Med. **19**, 2269 (2008). doi:10.1007/s10856-007-3327-8
12. M. Tanahashi, T. Matsuda, J. Biomed. Mater. Res. **34**, 305 (1997). doi:10.1002/(SICI)1097-4636(19970305)34:3<305::AID-IBM5>3.0.CO;2-O
13. T. Kawai, C. Ohtsuki, M. Kamitakahara, T. Miyazaki, M. Tanihara, Y. Sakaguchi, S. Konagaya, Biomaterials **25**, 4529 (2004). doi:10.1016/j.biomaterials.2003.11.039
14. I.B. Leonor, F. Balas, M. Kawashita, R.L. Reis, T. Kokubo, T. Nakamura, J. Mater. Sci. Mater. Med. **18**, 1923 (2007). doi:10.1007/s10856-007-3106-6
15. C. Ohtsuki, T. Kokubo, T. Yamamuro, J. Non-Cryst. Solids. **143**, 84 (1992). doi:10.1016/S0022-3093(05)80556-3
16. K. Tsuru, S. Hayakawa, C. Ohtsuki, A. Osaka, J. Mater. Sci.: Mater. Med. **9**, 479 (1998). doi:10.1023/A:1008875502451
17. T. Miyazaki, C. Ohtsuki, M. Ashizuka, J. Biomater. Appl. **22**, 71 (2007). doi:10.1177/0885328206070447
18. A. Yamada, K. Tsuru, S. Hayakawa, A. Osaka, in *Abstract of the 3rd Asian International Symposium on Biomaterials and Drug Delivery Systems*, ed. by G.-H. Hsiue (Hung Shin Co., Ltd., Taipei, 2002) p. 279
19. T. Miyazaki, S. Yasunaga, E. Ishida, M. Ashizuka, C. Ohtsuki, Mater. Trans. **48**, 317 (2007). doi:10.2320/matertrans.48.317
20. S.H. Rhee, Biomaterials **25**, 1167 (2004). doi:10.1016/j.biomaterials.2003.08.004
21. C. You, T. Miyazaki, E. Ishida, M. Ashizuka, C. Ohtsuki, M. Tanihara, J. Eur. Ceram. Soc. **27**, 1585 (2007). doi:10.1016/j.jeurceramsoc.2006.04.055
22. B.D. Ratner, A.S. Hoffman, F.J. Schoen, J.E. Lemons, *Biomaterials Science*, 2nd edn. (Elsevier Academic Press, Amsterdam, 2004), p. 104
23. T. Miyazaki, C. Ohtsuki, M. Tanihara, J. Nanosci. Nanotech. **3**, 511 (2003)
24. T. Miyazaki, H. Akita, E. Ishida, M. Ashizuka, C. Ohtsuki, J. Ceram. Soc. Japan **114**, 87 (2006)
25. T. Uchino, C. Ohtsuki, K. Kikuta, M. Kamitakahara, M. Tanihara, S. Hayakawa, A. Osaka, in *Archives of BioCeramics Research*, vol 6, (Organizing Committee of Asian BioCeramics 2006, Thailand, 2006), p. 138