Preparation of Calcium Phosphate-Biodegradable Polymer Composites with Ion-Releasing Ability for Enhancing Bone Formation^{*1}

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An inorganic-organic composite material, consisting of bioresorbable β -tricalcium phosphate (β -TCP) and a biodegradable polymer, is one of the most important materials for use in artificial bone with good shapability. In this work, to activate bone-forming cells immediately after being implanted in a living body, the ability to release therapeutic ions, such as Ca^{2+} , Mg^{2+} , and silicate ions was incorporated in the composite material. A small amount (8 vol%) of 46.1 SiO_2 ·24.4 Na_2O ·26.9 CaO ·2.6 P_2O_5 (mol%) glass particles was embedded successfully into the composite material. When the glass particles containing Na_2O were included in the material, the molecular weight of the polymer in the matrix phase was severely reduced and its hydrolysis in Tris-HCl buffer solution was drastically accelerated. On the other hand, when the glass particles containing MgO were included, therapeutic ions were continuously released with almost no change in the buffer solution pH. The released amount of silicate ions was well controlled, to avoid excessive dissolution, compared to those of Ca^{2+} and Mg^{2+} ions. Therefore, the glass particle is one of the most promising candidates to be a source in the composite for releasing therapeutic ions that enhance bone formation. [doi:10.2320/matertrans.Y-M2020859]

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1. Introduction

β -tricalcium phosphate (β -TCP), that has excellent osteoconductivity and bioresorbability, is widely used as an implant material in bone defects.¹⁾ Recently, various inorganic-organic composite materials have also been developed.^{2,3)} Composite materials, in whose structure the β -TCP particles are embedded in the polymer as a matrix phase, often have unimproved mechanical brittleness.⁴⁾ However, since the composite materials can be easily processed into desired shapes, various applications are expected. Typical biodegradable polymers include poly(lactic acid)⁵⁾ and poly(lactic-co-glycolic acid).⁶⁻⁸⁾ Copolymers containing L- and D-lactides (PDLLGA) are amorphous and highly biodegradable, and may also have water swelling properties.⁹⁾

In recent years, enhancement of the bone-forming ability, for improving therapeutic effects, has become important. One of the concepts is that, when a biomaterial is implanted in a living body, some substances are released to genetically activate the bone-forming cells. These substances also accelerate the bone-forming process through their adhesion, proliferation, differentiation, and mineralization. Examples of these substances, include some proteins and amino acids, or inorganic ions (so-called therapeutic ions; Ca^{2+} , Mg^{2+} , silicate ions, etc.).¹⁰⁾ Inorganic ions are useful since they do no denature during material synthesis nor change over time during storage. Therefore, β -TCP/biodegradable polymer composite material is expected to be one of the most important materials as an artificial bone for a super-aging society. This is because it can be used for activating bone-forming cells by releasing therapeutic ions immediately after implantation in a living body.

Bioglass[®] 45S5 developed by Hench *et al.*, with a composition of 46.1 SiO_2 ·24.4 Na_2O ·26.9 CaO ·2.6 P_2O_5 in mol%, has excellent bioactivity, that is, the ability to chemically bond with bones. When 45S5 is implanted in a living body, it releases inorganic ions, such as Na^+ , Ca^{2+} , silicate and phosphate ions as it gradually dissolves.¹¹⁾ By implanting a silicate glass with a low silica content, such as 45S5 into a living body,¹²⁾ Na^+ and Ca^{2+} ions are released immediately by ion exchange with protons in the body fluid. Subsequently, many silanol (Si-OH) groups are formed along with this reaction and results in a silica gel layer forming on the glass surface through the condensation of the Si-OH groups. Therefore, the release of silicate ions is gradually controlled.

We prepared a PDLLGA composite material containing 40 SiO_2 ·40 MgO ·20 Na_2O glass particles as the source of silicate and Mg^{2+} ions while calcium carbonate (vaterite) particles were used as the Ca^{2+} ions source.¹³⁾ When the material was soaked in a cell culture medium, the surface of the vaterite particles was covered with a silica gel layer, that had originated from the silicate ions released from the glass particles. This gel layer controlled the release of excess Ca^{2+} ions from vaterite immediately after being soaked. As a result, a material with an ability to supply Ca^{2+} ions for a long period of time could be obtained.

Therefore, we aim to develop a material that can activate bone-forming cells earlier after implantation in a living body and then shift smoothly to bone remodeling. We intend to prepare a material in which a small amount of the glass particles is embedded with β -TCP particles in the PDLLGA matrix phase.

In this work, two kinds of composite materials were prepared using 45S5 and a glass in which Na_2O in the 45S5 composition was completely substituted with MgO . The ion releasing behavior, when soaked in a Tris-HCl buffer solution, was investigated, and the behavior of a glass suitable for preparing a composite material was discussed.

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2. Experimental Procedure

2.1 Preparation of glass particles

45S5 glass (46.1 SiO₂·24.4 Na₂O·26.9 CaO·2.6 P₂O₅ in mol%: denoted by BG-Na) and a glass (46.1 SiO₂·24.4 MgO·26.9 CaO·2.6 P₂O₅ in mol%: denoted by BG-Mg), in which Na₂O was substituted with MgO, were prepared. The chemical reagents weighed as a glass yield of 20 g (SiO₂: 99.0%, Na₂CO₃: 99.8%, MgO: 98.0%, CaCO₃: 99.5%, H₃PO₄: 85.0% (liquid), Kishida Chemical) were placed in a Teflon[®] beaker. Then, distilled water was added to make a slurry, that was then stirred to mix. The slurry was then dried overnight under an infrared lamp. The resulting dried mixture was placed in a platinum crucible and melted in an electric furnace at 1500°C for 30 min in air. After melting, the mixture was cast on a stainless-steel plate and the resulting glass was quenched by the iron pressing method. All the resulting glass pieces, 400 g of zirconia balls with a diameter of 3 mm, and 150 mL of methanol (Kishida Chemical) were placed into a zirconia pot, with a volume of 300 mL, and crushed by a uniaxial ball-milling for 24 h. The resulting powder was then dried at 80°C.

To examine whether the resulting powder was amorphous, powder X-ray diffractometry (XRD: X'pert-MPD, Malvern Panalytical) was performed. The glass compositions were analyzed by energy dispersive X-ray analysis (EDX: JED-2300, JEOL) ($n = 5$). Each glass transition temperature, T_g was determined by differential thermal analysis (DTA: Thermoplus TG8120, Rigaku). Laser Raman spectroscopy (LRS: NRS-3300 using Nd: YAG laser, JASCO) was also performed to determine the structure of the glasses.

2.2 Preparation of composite materials

PDLLGA with a (lactic acid):(glycolic acid) ratio of 75:25 was used (Purasorb[®] PDLG7509, Corbin). This was dissolved in hexafluoro-2-propanol, and then β -TCP (β -TCP100 crushed product, Taihei Chemical Industry) and the glass powder (BG-Na or BG-Mg) were mixed to make a slurry. The composition in volume ratio of BG-Na or BG-Mg: β -TCP:PDLLGA was 8:42:50. The slurry was then put in a zirconia ball mill pot with a volume of 45 mL, and then mixed for 5 min with a rotation/revolution-type milling (so-called planetary ball-milling) device without balls. After mixing, the slurry was cast in a Teflon[®] container, with a diameter of 80 mm, and then dried at 80°C for 24 h, resulting in the formation of a film.

Gel permeation chromatography (GPC: HLC-8320GPC, Tosoh) was performed to measure the molecular weight of the polymer in the prepared composite materials. Initially, the material was mixed with chloroform (Reagent Grade, Wako Pure Chemical) to make a slurry, that was then filtered with a Teflon[®] filter (H-13-5, pore size: 0.5 μ m, Tosoh). A sample concentration of 2 mg/mL, flow rate of 0.6 mL/min, and column temperature of 40°C were used. Polystyrene (PStQuick[®] C, D, Tosoh) was used as the standard sample.

The film-shaped composite material (10 $\times$ 10 mm, thickness: 0.63 $\pm$ 0.1 mm, weight: 110 $\pm$ 14 mg) was soaked in 10 ml of Tris-HCl buffer solution (TBS). This was then stirred at 37°C using a shaking incubator (KS4000i, IKA) for a predetermined period ($n = 3$). The mixture was filtered, and

the sample that was retained by the filter paper was then dried at room temperature (22~27°C). The solution pH, after the soaking, was measured. Meanwhile, the concentrations of Ca²⁺, Mg²⁺, silicate, and phosphate ions were measured by inductively coupled plasma optical emission spectrometry (ICP-OES: ICPS-7000, Shimadzu). Silicon and phosphorus existed as silicate and phosphate ions in the solution. In this work, they were measured and presented as Si⁴⁺ and P⁵⁺ for convenience.

The samples before and after 7 days of soaking were sliced by a focused ion beam method (FIB: EM-9320FIB, JEOL) to observe their cross-sections using a transmission electron microscope (TEM: JEM-2100F, JEOL). The compositions of some portions in the images were examined using an incorporated energy dispersive X-ray spectrometer (EDX).

3. Results

3.1 Compositions and structures of glasses

The XRD patterns and LRS spectra of the resulting glasses, and analyzed glass compositions with their glass transition temperatures (T_g s) are shown in Figs. 1(a), (b), and Table 1, respectively. Both BG-Na and BG-Mg showed glass-derived halo peaks; no crystals were included. EDX

Fig. 1 (a) XRD patterns and (b) LRS spectra of BG-Na and BG-Mg.

Table 1 Nominal and analyzed compositions in mol% of BG-Na and BG-Mg and their glass transition temperatures (T_g). The analyzed compositions are shown with standard deviation.

Glass code	Composition (mol%, upper: nominal, lower: analyzed)					T_g (K)
	SiO ₂	Na ₂ O	MgO	CaO	P ₂ O ₅	
BG-Na	46.1	24.4	—	26.9	2.6	804
	47.8 ± 0.2	23.3 ± 0.9	—	25.9 ± 0.8	3.0 ± 0.1	
BG-Mg	46.1	—	24.4	26.9	2.6	983
	48.3 ± 1.1	—	23.6 ± 2.0	25.1 ± 3.9	2.6 ± 0.2	

analysis showed that the glasses, with compositions close to the nominal ones, were obtained.

The positions of the halo peaks shown in the XRD patterns (Fig. 1(a)) were slightly different in both cases, implying that their structures were different. T_g s were also different between the glasses. The numbers, n , of the bridging oxygen around Si and P were expressed as Q_{Si}^n and Q_P^n , respectively, in the LRS spectra (Fig. 1(b)). As shown, the peaks due to Q_{Si}^1 , Q_{Si}^2 , Q_{Si}^3 , and Q_{Si}^n were observed around 680 and 870 cm^{-1} ,^{14,15)} 630, 860–870, and 980 cm^{-1} ,^{15–17)} 1080 cm^{-1} ,¹⁴⁾ and 1040 cm^{-1} ,¹⁴⁾ respectively. The peaks¹⁴⁾ due to Q_P^0 were also observed around 940–960 cm^{-1} . Meanwhile, the spectrum of BG-Mg showed the characteristic shifts of the Si–O[−] peak,¹⁷⁾ originating from Q_{Si}^2 around 860 cm^{-1} and the P–O[−] peak¹⁴⁾ from Q_P^0 around 940 cm^{-1} . The electric field strength, F , proposed by Dietzel is shown in eq. (1).¹⁸⁾

$$F = z/a^2 \quad (1)$$

where z is the valence of positive ions, and a is the distance (Å) between the positive and negative ions. The F s of 6-fold-coordinated Na⁺, 8-fold-coordinated Ca²⁺, and 6-fold-coordinated Mg²⁺ ions were 0.15, 0.31 and 0.44, respectively, showing that there were variation in their bond strengths. The peak shifts mentioned above were due to the difference in the cation coordinated to the non-bridging oxygen.

3.2 Inorganic ion release behaviors from composite materials

Table 2 shows the molecular weights (weight average molecular weight M_w and polydispersity M_w/M_n) of PDLLGA in the resulting composite materials. Compared to as-received PDLLGA, when BG-Na was included, the M_w decreased by two orders of magnitude. Even when BG-Mg was included, the M_w was almost half. Their polydispersities also increased.

Table 2 Weight-average molecular weights (M_w) and polydispersities (M_w/M_n) of as-received PDLLGA and PDLLGA in the resulting composites. M_n means number-average molecular weights.

Sample code	M_w (kDa)	M_w/M_n
PDLLGA	126.7	1.86
BG-Na/TCP/PDLLGA	1.4	2.10
BG-Mg/TCP/PDLLGA	71.3	1.95

Fig. 2 pH after soaking the composites in Tris-HCl buffer solution. Initial pH of the solution is 7.40.

Figure 2 shows the pH values of TBS when the film-shaped composite materials were soaked. When BG-Mg/TCP/PDLLGA was soaked, the pH values were almost unchanged, whereas in the case of BG-Na/TCP/PDLLGA, the pH greatly decreased.

Figure 3 shows the inorganic ion amounts in TBS when the film-shaped BG-Mg/TCP/PDLLGA was soaked. Ca²⁺ and Mg²⁺ ions were released almost linearly with increase in soaking time. Judging from the BG-Mg glass composition, the released amount of Si⁴⁺ ions was controlled at a considerably low level. Almost no P⁵⁺ ions were measured; this is due to the low P₂O₅ content in the glass and the low glass content in the composite.

Figure 4 is a cross-sectional TEM photograph (bright field image) around the surface of the BG-Mg/TCP/PDLLGA film before and after being soaked in TBS. The EDX spectra implied that the sharp and fine particles, portion (a), before the soaking were BG-Mg glass and the relatively large particles, portion (b), were β -TCP. The inorganic particles were embedded in the dense polymer phase; there were no large-sized voids. On the other hand, many voids were observed after 7 d of soaking. Almost no sharp particles were observed, while fine particles of $\sim 0.5 \mu\text{m}$ or less in size, as shown in the portion (c), were observed. The EDX spectrum of portion (c) showed that Si was the major component with almost no Ca and Mg present.

Fig. 3 Ion amounts in Tris-HCl buffer solution after soaking BG-Mg/TCP/PDLLGA.

4. Discussion

It has been reported that, in silicate glasses with low P_2O_5 contents, Na^+ ions are preferentially coordinated to non-bridging oxygens in the phosphate groups.¹⁹⁾ Meanwhile, when Ca^{2+} and Mg^{2+} ions coexist, it has been reported that Ca^{2+} ions are preferentially coordinated to the non-bridging oxygens in the phosphate groups.^{20,21)} These reports insinuate

that Ca^{2+} ions in BG-Mg are coordinated to the phosphate groups, instead of Na^+ ions in BG-Na, and that the amount of silicate groups coordinated with Mg^{2+} ions increased. Depending on the glass composition, some of the intermediate MgO acts as a glass network former.²²⁾ It is possible that some Mg^{2+} ions entered the glass network, resulting in the formation of $Si-O-Mg$ bonds. Due to such difference in glass structure, the durability of BG-Mg against aqueous solutions was supposed to be higher than that of BG-Na.

Practically, BG-Na particles show are highly hygroscopic, therefore careful handling is required to avoid their hydration. During the mixing process in the preparation of the composite materials, the basicity around the surface of the glass particles increased due to the dissolution of Na^+ and Ca^{2+} ions, that had originated from the hydration of the glass particles. The hydrolysis of PDLLGA might have been accelerated by water dissolved in the hexafluoro-2-propanol used for mixing. During this mixing process, the PDLLGA molecular chains were supposed to be broken not only by mechanical impact but also by such chemical reaction. It is, therefore, believed that the molecular weight of PDLLGA became extremely low, and the hydrolysis of the polymer was remarkably rapid in TBS, resulting in a decrease in pH. Preparation of a composite material using BG-Na as the source of therapeutic ions has various advantages and weaknesses, that need to be discussed.

Fig. 4 TEM bright images of FIB-polished cross-sections before and after soaking BG-Mg/TCP/PDLLGA in Tris-HCl buffer solution for 7 d, and EDX spectra collected from positions (a)–(c) shown in the images.

Next, we discuss the ion releasing behavior of the composite material containing BG-Mg in TBS. At pH ~ 7 , the solubility of β -TCP²³⁾ was considerably lower than that of the glass, and most of the ion release during a short period of 7 d originated from the glass dissolution. The TEM observation (Fig. 4) showed that, in the composite material, the inorganic particles were well distributed in the polymer matrix phase, with few aggregations. Therefore, no percolation effect due to the aggregation of inorganic particles and no burst dissolution immediately after soaking occurred. Since many voids were observed after the soaking, PDLLGA was hydrolyzed to penetrate water into the material, and ions were released from the glass particles. Ca^{2+} and Mg^{2+} ions were continuously released for 7 days from the glass particles around the surface and inside the material, through ion exchange with protons. It was estimated from Fig. 3 that approximately 90% of Ca^{2+} and Mg^{2+} ions in the BG-Mg particles in the composite material were released in 7 days. On the other hand, the Si–O–Si bonds forming the glass network were believed to be hydrolyzed by the entry of water, resulting in the formation of Si–OH groups, that were then partially released. The amount of silicate ions released from the material in 7 d was estimated from Fig. 3 to be $\sim 7\%$ of the total SiO_2 content in the BG-Mg particles. In addition, the Si–OH groups partially dehydrated and condensed into a gel, that remained in the material. This is supported by the results of TEM observation (EDX spectrum (c) in Fig. 4) after the soaking. Therefore, the released amount of silicate ions was controlled at a low level.

Ca^{2+} , Mg^{2+} , and silicate ions have been reported as inorganic ions that enhance the growth of osteoblasts and osteoblast-like cells.²⁴⁾ Ca^{2+} and silicate ions enhance the proliferation, differentiation, and calcification of osteoblast-like cells.^{25,26)} Meanwhile, Mg^{2+} ions enhance cell adhesion²⁷⁾ and might also enhance calcification.^{28,29)} When a trace amount of silicate ions is present,^{30,31)} bone-forming ability is reported to be enhanced.

We reported the combined effects of Si–Ca–Mg ions on the mineralization process of osteoblast-like cells.^{32,33)} The presence of Mg^{2+} ions, the combination of three kinds of ions, and the presence of only Ca^{2+} ion or the combination of Ca^{2+} and silicate ions at the adhesion, proliferation, and calcification stages, respectively, were reported to induce excellent cell activation effects. To further enhance bone formation, it is important to optimize the inorganic ions releasing behavior. In this work, BG-Mg was one of the candidates for the ion source. Optimization of the ion-releasing amount for cell activation is currently in progress, through the investigation of the appropriate glass content in the composite material.

5. Conclusion

In this work, β -TCP/PDLLGA composite materials containing a small amount of glass particles were prepared, to obtain a material that can activate bone-forming cells at the initial stage after implantation in a living body. During preparing the composite materials, blending Bioglass® 45S5 glass particles significantly reduced the molecular weight of

PDLLGA. The resulting composite material showed high degradability and acidified the aqueous solution, TBS, in which it was soaked. On the other hand, when a composite material, containing glass particles with a composition in which Na_2O in 45S5 was substituted with MgO , was soaked in TBS, Ca^{2+} , Mg^{2+} and silicate ions were released continuously. The released amount of silicate ions was controlled compared to those of Ca^{2+} and Mg^{2+} ions. 46.1 SiO_2 ·24.4 MgO ·26.9 CaO ·2.6 P_2O_5 glass particles are expected to be one of the important candidates to be a source of therapeutic ions for enhancing bone formation.

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REFERENCES

- 1) D. Buser, B. Hoffmann, J.P. Bernard, A. Lussi, D. Mettler and R.K. Schenk: *Clin. Oral Implants Res.* **9** (1998) 137–150.
- 2) K. Rezwan, Q.Z. Chen, J.J. Blaker and A.R. Boccaccini: *Biomaterials* **27** (2006) 3413–3431.
- 3) S.J. Peter, L. Lu, D.J. Kim and A.G. Mikos: *Biomaterials* **21** (2000) 1207–1213.
- 4) Y.M. Khan, D.S. Katti and C.T. Laurencin: *J. Biomed. Mater. Res. A* **69** (2004) 728–737.
- 5) F.A.D. Santos and M.I.B. Tavares: *Polímeros* **24** (2014) 561–566.
- 6) P. Gentile, V. Chiono, I. Carmagnola and P.V. Hattori: *Int. J. Mol. Sci.* **15** (2014) 3640–3659.
- 7) D.K. Gilding and A.M. Reed: *Polymer* **20** (1979) 1459–1464.
- 8) R.A. Miller, J.M. Brady and D.E. Cutright: *J. Biomed. Mater. Res.* **11** (1977) 711–719.
- 9) A.G. Mikos, M.D. Lyman, L.E. Freed and R. Langer: *Biomaterials* **15** (1994) 55–58.
- 10) M.M. Stevens: *Mater. Today* **11** (2008) 18–25.
- 11) L.L. Hench, R.J. Splinter, W.C. Allen and T.K. Greenlee: *J. Biomed. Mater. Res.* **5** (1971) 117–141.
- 12) D.S. Brauer: *Angew. Chem. Int. Ed.* **54** (2015) 4160–4181.
- 13) N. Osada, A. Terada, H. Maeda, A. Obata, Y. Nishikawa and T. Kasuga: *R. Soc. Open Sci.* **6** (2019) 190612.
- 14) C.-C. Lin, S.-F. Chen, K.S. Leung and P. Shen: *J. Mater. Sci.: Mater. Med.* **23** (2012) 245–258.
- 15) N. Trcera, S. Rossano and M. Tarrida: *J. Raman Spectrosc.* **42** (2011) 765–772.
- 16) H. Yamashita, H. Yoshino, K. Nagata, I. Yamaguchi, M. Okawa and T. Maekawa: *J. Ceram. Soc. Jpn.* **106** (1998) 539–544.
- 17) H. Aguiar, E.L. Solla, J. Serra, P. González, B. León, F. Malz and C. Jäger: *J. Non-Cryst. Solids* **354** (2008) 5004–5008.
- 18) A. Dietzel: *Z. Elektrochem* **48** (1942) 9–23.
- 19) A. Pedone, T. Charpentier, G. Malavasi and M.C. Menziani: *Chem. Mater.* **22** (2010) 5644–5652.
- 20) M.A. Karakassides, A. Saranti and I. Koutselas: *J. Non-Cryst. Solids* **347** (2004) 69–79.
- 21) I. Wacławski and M. Szumera: *J. Therm. Anal. Calorim.* **84** (2006) 185–190.
- 22) S.J. Watts, R.G. Hill, M.D. O'Donnell and R.V. Law: *J. Non-Cryst. Solids* **356** (2010) 517–524.
- 23) L.C. Chow: *J. Ceram. Soc. Jpn.* **99** (1991) 954–964.
- 24) A. Hoppe, N.S. Güldal and A.R. Boccaccini: *Biomaterials* **32** (2011) 2757–2774.
- 25) S. Maeno, Y. Niki, H. Matsumoto, H. Morioka, T. Yatabe, A. Funayama, Y. Toyama, T. Tagushi and J. Tanaka: *Biomaterials* **26** (2005) 4847–4855.
- 26) P. Han, C. Wu and Y. Xiao: *Biomater. Sci.* **1** (2013) 379–392.
- 27) H. Zreiqat, C.R. Howlett, A. Zannettino, P. Evans, G. Schulze-Tanzil, C. Knabe and M. Shakibaei: *J. Biomed. Mater. Res.* **62** (2002) 175–184.

- 28) S. Yamada, A. Obata, H. Maeda, Y. Ota and T. Kasuga: [Front. Bioeng. Biotech. 3 \(2015\) 195](#).
- 29) S. Yamada, Y. Ota, A. Obata and T. Kasuga: [Biomed. Mater. Eng. 28 \(2017\) 47–56](#).
- 30) R. Jugdaohsingh, K.L. Tucker, N. Qiao, L.A. Cupples, D.P. Kiel and J.J. Powell: [J. Bone Miner. Res. 19 \(2004\) 297–307](#).
- 31) A. Obata, N. Iwanaga, A. Terada, G. Jell and T. Kasuga: [J. Mater. Sci. 52 \(2017\) 8942–8956](#).
- 32) A. Obata, T. Ogasawara, S. Yamada and T. Kasuga: *Ceramic Engineering and Science Proceedings* **37** (2017) pp. 107–112 (Proceedings of the 40th International Conference on Advanced Ceramics and Composites (ICACC), ed. by J.C. LaSalvia, R. Narayan, P. Colombo, M. Fukushima and A. Gyekenyesi, The American Ceramic Society, OH, 2017).
- 33) A. Obata, T. Ogasawara and T. Kasuga: [J. Biomed. Mater. Res. A 107 \(2019\) 1042–1051](#).