Preparation of Calcium Phosphate-Biodegradable Polymer Composites with Ion-Releasing Ability for Enhancing Bone Formation

Akari Kawai\textsuperscript{a2}, Takashi Matsubara, Hirotaka Maeda, Akiko Obata and Toshihiro Kasuga\textsuperscript{a3}

Division of Advanced Ceramics, Nagoya Institute of Technology, Nagoya 466-8555, Japan

An inorganic-organic composite material, consisting of bioresorbable \(\beta\)-tricalcium phosphate (\(\beta\)-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\(_2\)O/MgO 26.9 CaO-2.6 P\(_2\)O\(_5\) (mol\%) glass particles were embedded successfully into the composite material. When the glass particles containing Na\(_2\)O 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.

\[\text{Keywords: composite, tricalcium phosphate, glass, biodegradable polymer, ion release}\]

1. Introduction

\(\beta\)-tricalcium phosphate (\(\beta\)-TCP), that has excellent osteoconductivity and bioresorbability, is widely used as an implant material in bone defects.\(^{1,2}\) Recently, various inorganic-organic composite materials have also been developed.\(^{2,3}\) Composite materials, in whose structure the \(\beta\)-TCP particles are embedded in the polymer as a matrix phase, often have unimproved mechanical brittleness.\(^{3}\) 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 \(\alpha\)-lactides (PDLGGA) 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, \(\beta\)-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.

\[\text{Bioglass}^{\text{®}}\text{ 45S5 developed by Hench et al., with a composition of 46.1 SiO}_2\text{-24.4 Na}_2\text{O-26.9 CaO-2.6 P}_2\text{O}_5\text{ 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.}\(^{1,11}\) By implanting a silicate glass with a low silica content, such as 45S5 into a living body,\(^{1,12}\) 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\(_2\)O 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 \(\beta\)-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\(_2\)O 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.
2. Experimental Procedure

2.1 Preparation of glass particles

4555 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: Xpert-MPD, Malvern Panalytical) was performed. The glass compositions were analyzed by energy dispersive X-ray analysis (EDX: JED-2300, JASCO) 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).

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 μm, Tosoh). A sample concentration of 2 mg/mL, flow rate of 0.6 mL/min, and column temperature of 40°C were used. Polystyrene (PSiQuick™ C, D, Tosoh) was used as the standard sample.

The film-shaped composite material (10 x 10 mm, thickness: 0.63 ± 0.1 mm, weight: 110 ± 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 (Tg), 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.
Advance View 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, $n_i$, 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$^{-1}$, 630, 860–870, and 980 cm$^{-1}$, respectively. The peaks 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, originating from $Q_{Si}^2$ around 860 cm$^{-1}$ and the P–O– peak from $Q_{P}^0$ around 940 cm$^{-1}$. The electric field strength, $F$, proposed by Dietzel is shown in eq. (1).

$$F = \frac{z}{a^2}$$

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 Ca$^{2+}$, and 6-fold-coordinated Mg$^{2+}$ 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 (Mw) and polydispersities (Mw/Mn) of as-received PDLLGA and PDLLGA in the resulting composites. Mn means number-average molecular weights. |
|---|---|---|---|---|
| Sample code | Mw (kDa) | Mw/Mn |
| PDLLGA | 126.7 | 1.86 |
| BG-Na/TCP/PDLLGA | 1.4 | 2.10 |
| BG-Mg/TCP/PDLLGA | 71.3 | 1.95 |

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. Ca$^{2+}$ and Mg$^{2+}$ ions were released almost linearly with increase in soaking time. Judging from the BG-Mg glass composition, the released amount of Si$^{4+}$ ions was controlled at a considerably low level. Almost no P$^{5+}$ ions were measured; this is due to the low P$_2$O$_5$ 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 ~0.5 µm 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.
4. Discussion

It has been reported that, in silicate glasses with low P₂O₅ contents, Na⁺ ions are preferentially coordinated to non-bridging oxygens in the phosphate groups.¹⁹) Meanwhile, when Ca²⁺ and Mg²⁺ ions coexist, it has been reported that Ca²⁺ ions are preferentially coordinated to the non-bridging oxygens in the phosphate groups.²⁰,²¹) These reports insinuate that Ca²⁺ 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 Mg²⁺ ions increased. Depending on the glass composition, some of the intermediate MgO acts as a glass network former.²²) It is possible that some Mg²⁺ 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 Ca²⁺ 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.
Next, we discuss the ion releasing behavior of the composite material containing BG-Mg in TBS. At pH ~ 7, the solubility of β-TCP was considerably lower than that of the glass, and most of the ion release during a short period of 7d 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 ~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. Meanwhile, Mg$^{2+}$ ions enhance cell adhesion and might also enhance calcification. When a trace amount of silicate ions is present, 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. 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$_2$O 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$_2$O$_5$ glass particles are expected to be one of the important candidates to be a source of therapeutic ions for enhancing bone formation.

Acknowledgment

This work was supported, in part, by the NEDO project P15010 and its complementary project.

REFERENCES


