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4 Poly(L-lactic acid)/Vaterite Composite Coatings on Metallic Magnesium

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21

1 Abstract

2

3 Poly(L-lactic acid)/vaterite composite materials were coated onto metallic magnesium substrates
4 to control rapid degradation and to improve biocompatibility. Two types of composites were prepared
5 by adding 30 and 60 wt % of vaterite to poly(L-lactic acid) (PLLA). The composite coating layer that
6 contained 30 wt % vaterite in the PLLA matrix had almost no pores on the surface and suppressed
7 the initial rapid degradation of the Mg substrate. After immersion in a culture medium for 7 days,
8 pores of 0.5–1.0 μm in diameter formed on the surface. The composite coating layer that contained
9 60 wt % vaterite with pores of 1.0–2.0 μm in diameter on the surface did not suppress the
10 degradation of the Mg substrate. During immersion, the pH of the media near the composite coating
11 surfaces was maintained at 7.4–7.5 because of the degradation of PLLA and because the vaterite
12 particles dissolved in the solution. Proliferation of murine osteoblast-like cells (MC3T3-E1) on the
13 substrates was improved using composite coatings. Cells on the coating that contained 60 wt %
14 vaterite had significantly higher proliferation than those on a bare Mg substrate. Our coating provides
15 the optimum combination to suppress the initial Mg degradation and to promote cell growth on the
16 coating surface by adjusting the vaterite content in the composite.

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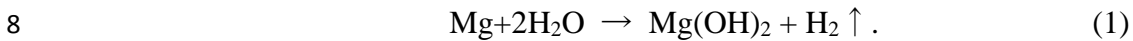
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1 **1. Introduction**

2 Recently, magnesium and its alloys have attracted attention in the metallic
3 biomaterials field because of their biodegradability and good mechanical properties
4 [1-6]. They have a low Young's modulus of 41–45 GPa comparable to human cortical
5 bone [1]. When Mg or Mg alloys are used in materials for implants, bone resorption
6 should be avoided near the alloy.

7 After implantation of Mg into the living body, the following reaction follows:



9 This reaction proceeds rapidly since it contacts body fluids. Because of this corrosion
10 reaction, the pH of the fluid near the Mg sample surface increases, generating H₂. The
11 accumulation of H₂ gas in the soft tissue surrounding the Mg sample was observed
12 within a week after implantation [2]. The local pH increase near the sample surface is
13 harmful to the surrounding tissue. Following Mg corrosion, the sample surface was
14 covered with corrosion products such as magnesium hydroxide, magnesium phosphate
15 and magnesium carbonate. Precipitation of calcium phosphate was also reported
16 because of the increase in the pH of the body fluid surrounding the implanted sample
17 [2]. Formation of these insoluble salts at the sample surface retards the corrosion of the
18 Mg sample.

19 The suppression of Mg corrosion at the initial stage of implantation plays an
20 important role for biomedical applications. To control Mg corrosion, the Mg sample has
21 been coated with a biodegradable polymer [7, 8]. Films of poly(L-lactic acid) (PLLA)
22 and poly(ε-caprolactone) (PCL) less than 1 μm thick and without pores were effective in
23 reducing the initial degradation rate of Mg and to improve its cytocompatibility [7].
24 However, detachment of the PCL film from the Mg substrate was observed. This was

1 probably because of H₂ gas generation between the polymer film and the substrate [7].
2 The porosity of the biodegradable polymer coating is important for controlling the
3 degradation of the Mg substrate [8].

4 Besides control of the degradation, biodegradable Mg devices used as substitute
5 materials for bone must be bioactive to stimulate bone growth [9, 10]. Surface coating
6 techniques using calcium phosphate ceramics such as hydroxyapatite have been used to
7 prepare bioactive layers on the Mg surface [11, 12]. Biodegradable polymer-based
8 materials are likely candidates for coating the Mg substrate for this purpose. One way to
9 form an ideal coating of Mg and its alloys is to form a layer consisting of biodegradable
10 PLLA-based materials that are bioactive and can control the degradation of the Mg
11 substrate.

12 Bioactive poly(L-lactic acid)-based composites containing calcium carbonate
13 (vaterite) particles (PVCs) have been developed [13-15]. Vaterite is a polymorph of
14 calcium carbonate and can be precipitated as submicrometer-sized secondary particles
15 comprising several tens of nanometer-sized primary particles using a carbonation
16 process. Vaterite particles with these shapes are expected to be highly soluble, releasing
17 calcium ions to induce bone formation. When a PVC-coated Mg substrate is implanted
18 into the body, vaterite can dissolve in the body fluid, making micrometer-sized pores on
19 the coating surface after a certain period of time. This would aid in controlling the
20 degradation of the Mg substrate. The composite coating could initially suppress the
21 substrate degradation to improve the cytocompatibility. The pores of the coating also
22 maintain the degradation of the substrate to prevent H₂ gas accumulation beneath the
23 coating. Here, PVC coatings were applied to a Mg substrate to control the initial rapid
24 degradation and improve the cytocompatibility.

1

2 **2. Materials and methods**

3 **2.1. Sample preparation**

4 A Mg rod (99.95%, 9.5 mm in diameter, Nilaco Corp., Japan) was employed in the
5 present study. The impurities in the rod were 0.005 wt % of Zn, 0.032 wt % of Al, 0.016
6 wt % of Si, 0.001 wt % of Cu, 0.015 wt % of Mn, 0.006 wt % of Fe and 0.001 wt % of
7 Ni. The rod was cut into 2.5 mm thick disks for use as substrates and polished with a
8 #1200 (15 μ m) SiC abrasive paper (Noritake Coated Abrasive Co. Ltd, Japan). The
9 disks were washed in an ultrasonic bath in acetone for 10 min. All of the Mg samples
10 were embedded with methyl methacrylate resin (Technovit 4004, Heraeus Kulzer,
11 Germany) except for the top surfaces. The specimens obtained were 15 mm in diameter
12 and 5 mm in thickness.

13 Two types of PVCs, containing 30 and 60 wt % vaterite, were prepared by a melt
14 blending method at 180 °C for 10 min using poly(L-lactic acid) (PLLA) (LACEA[®],
15 molecular weight; 120 kDa, crystallinity; ~40 %, Mitsui Chemicals Inc., Japan) and
16 vaterite particles (0.5 μ m in diameter, Yabashi Industries Co. Ltd., Japan) as starting
17 materials. The prepared materials are referred to as PVC_x ($x=30, 60$). The molecular
18 weights of the PLLA components in the composites were measured using gel
19 permeation chromatography (GPC; LC-20, Shimadzu Corp., Japan). The crystallinity of
20 the composites was determined using differential scanning calorimetry (DSC;
21 DSC-8230, Rigaku, Japan) [16].

22 Each PVC sample was dissolved in chloroform to obtain a solution for spin-coating.
23 The PLLA component of PVC in each solution was 4 wt %. One hundred μ L of PVC
24 solution was dropped onto the top surface of the Mg disk in the resin and the disk was

1 spin-coated at 5000 rpm for 90 s. It was confirmed prior to coating that the resin did not
2 dissolve in chloroform. The uncoated Mg disk in the resin, referred to as “uncoated”,
3 was used as a control sample. The disk in resin, coated with PLLA by the same melt
4 blending method without vaterite, was also prepared.

5 The top surface and cross section of the samples were observed using a scanning
6 electron microscope (FE-SEM, JSM-6301F, JEOL, Japan) equipped with an energy
7 dispersive X-ray spectrometer (EDX). The average surface roughness (Ra) of the coated
8 surface was examined using a surface roughness tester (SURFCOM 1400-D, Tokyo
9 Seimitsu Co. Ltd., Japan). The bonding strength between the PVC-coating layer and the
10 Mg substrate was measured using a tensile testing machine (AGS-H, Shimadzu Corp.,
11 Japan). Aluminum shafts (diameter: 6.0 mm) were glued with an epoxy adhesive
12 (Araldite[®] Rapid, Huntsman Advanced Materials, Japan) on the top and bottom surfaces
13 of the Mg disks that were not embedded in the resin. After resting for 24 h for
14 solidification of the adhesive, the supporting shaft glued on the top surface was pulled
15 up at a tensile rate of 1.0 mm/min. At least five samples were tested for each coating.

16

17 **2.2. Immersion in a cell culture medium**

18 The uncoated Mg disks and the PLLA or PVC-coated samples embedded in resin
19 were sterilized using ethylene oxide gas (EOG). Each sample was immersed in 27.5 mL
20 of α -modified minimum essential medium (α -MEM, 1031120, Wako Pure Chemical
21 Industries, Ltd., Japan) supplemented with 10 % v/v fetal bovine serum (FBS),
22 abbreviated here as α -MEM+FBS. The α -MEM+FBS simulates human body fluid as
23 previously reported [17]. An average adult human has about 2.75 L of blood plasma,
24 and for this experiment the scale was reduced to 1/100 of the total capacity of an adult

1 human. The samples were then incubated in 5 % v/v CO₂ at 37 °C for 1, 3 or 7 days. To
2 estimate the degradation rate of the substrate and the coating layer, the concentration of
3 Mg²⁺, Ca²⁺ and P⁵⁺ ions in the α-MEM+FBS were measured using inductively coupled
4 plasma atomic emission spectroscopy (ICP-AES; ICPS-500, Shimadzu Corp., Japan).
5 The experiment was performed in triplicate. The change in the amount of ions was
6 given by the following equation:

$$7 \quad R_x = [(C_{xs} - C_{x0}) \times 27.5] / A, \quad (2)$$

8 where R_x is the amount of ion x released from the sample, C_{x0} and C_{xs} are the
9 concentrations of the ion x in α-MEM+FBS before and after immersion, respectively,
10 and A is the sample top surface area.

11 The top surface of each sample was observed using an optical microscope
12 (BIOREVO BZ-9000, Keyence Corp., Japan) and FE-SEM after immersion in
13 α-MEM+FBS. To examine the amount of precipitate on the samples, EDX analysis was
14 also performed over an area of 100 × 100 μm² for the samples immersed for 1, 3 or 7
15 days in α-MEM+FBS. Samples were analyzed in at least five different positions for
16 each type of coating.

17

18 **2.3. Cell proliferation assay**

19 Murine osteoblast-like cells MC3T3-E1 (passage 18) were seeded on the samples
20 sterilized with EOG in a culture dish with 27.5 mL of α-MEM+FBS with a density of
21 6,000 cells/mL, which equates to 16.5 × 10⁴ cells/dish. The dishes were placed in a CO₂
22 incubator for 1, 3 or 7 days. A 1 mL portion of the supernatant was then poured into a 24
23 well microplate and each sample was transferred from the culture dish to the well of the
24 microplate. Cell proliferation was determined using the WST-8 assay with Cell

1 Counting Kit-8 (CCK-8, Dojindo Laboratories, Japan). The CCK-8 solution is a
2 tetrazolium compound, which is reduced by living cells into a colored formazan product.
3 A 100 μL portion of the prepackaged CCK-8 solution was poured into each of the wells.
4 The microplate was then placed in a CO_2 incubator for 2 h. The number of viable cells
5 on the samples was estimated by measuring the absorbance of the resulting media at
6 450 nm with a microplate scanning spectrophotometer (SUNRISE Remote, Tecan Japan
7 Co. Ltd., Japan). The strength of the absorbance signal of formazan product measured at
8 450 nm was directly proportional to the number of living cells. Cell culture tests were
9 conducted in triplicate. The effect on the cytocompatibility of resin samples without a
10 Mg disk was also examined.

11

12 **2.4. pH measurements**

13 Each sample was soaked in 27.5 mL of α -MEM+FBS and kept in a CO_2 incubator
14 for 4 days after sterilization. During incubation, the pH of the medium near the sample
15 surface was measured using a pH meter (TPX-999i, Toko Kagaku Kenkyusho Co. Ltd.,
16 Japan). The pH electrode was set to 1 mm from the top surface of the sample.

17

18 **3. Results and Discussion**

19

20 **3.1. Properties of PVC_x-coating layers**

21 The thickness of the coating layers was estimated from cross-sectional SEM images
22 of the samples. PLLA-, PVC₃₀-, and PVC₆₀-coating layers were determined to be 1.8,
23 2.0, and 3.0 μm thick, respectively. The thickness of the coating layers prepared by
24 spin-coating is related to the concentration and the viscosity of the coating solution [18].

1 The vaterite content in the PVC solution was proportional to the thickness of the coating
2 layer. The viscosity of the PVC solutions increased with increasing vaterite content in
3 the PVC.

4 Figure 1 shows the SEM images of the coated surfaces. Almost no micrometer sized
5 pores can be seen on the PLLA-coated or PVC₃₀-coated surfaces. However, some 0.5–
6 1- μ m diameter pores were observed on the PVC₆₀-coated surface. This may be because
7 of the relatively low amount of PLLA matrix for the high content of vaterite in PVC₆₀.

8 The molecular weights of the PLLA component in the PLLA, PVC₃₀ and PVC₆₀
9 samples after melt blending were estimated to be 90, 76 and 46 kDa, respectively. In
10 addition, the PLLA polydispersity in the PLLA, PVC₃₀ and PVC₆₀ samples after melt
11 blending was estimated to be 1.7, 2.1 and 2.8, respectively. The amount of crystallinity
12 in the PLLA, PVC₃₀ and PVC₆₀ samples after melt blending was 15, 13 and 11%,
13 respectively. Melt blending reduced the molecular weight of the polymer component
14 and increased its polydispersity. The higher vaterite content also gave a lower molecular
15 weight. Our previous report [14], suggested that the calcium ions in the vaterite
16 coordinated with the carboxy groups in PLLA and formed a coordination bond. The
17 cleavage of the PLLA chain might occur by melt blending, and subsequently form a
18 coordination bond. A higher vaterite content may result in a lower molecular weight.
19 The difference in the molecular weights may influence the degradation of the materials
20 used for coating and therefore that of the Mg substrate.

21 The average surface roughness of the uncoated, PLLA-, PVC₃₀- and PVC₆₀-coated
22 surfaces was 0.12, 0.08, 0.15 and 0.20 μ m, respectively. PVC₃₀ and PVC₆₀ tended to
23 have a slightly higher surface roughness; however there were no significant differences
24 between the samples.

1 The bonding strengths of the coating layers and Mg substrate were approximately
2 0.8–1.0 MPa. There was no clear dependence on molecular weight, coating layer
3 thickness or vaterite content. These values are slightly lower than the 2.5–4.0 MPa
4 previously obtained for the PLLA and PCL coating layers prepared by spin-coating [7].
5 The free ends of the polymer chain are believed to provide free carboxy groups and
6 form electrostatic intermolecular interactions with the Mg substrate surface. The
7 difference in the bonding strength between the previous report and our results is
8 probably due to the difference in molecular weights of the polymers used, and the
9 polishing condition of Mg substrate resulting in a difference in the substrate surface
10 roughness.

11

12 **3.2. Degradation of uncoated and coated samples**

13 Figure 2 shows optical micrographs of the sample surfaces after immersion in
14 α -MEM+FBS for 7 days. Polishing marks were observed on all of the Mg substrates
15 since the coating layers were semi-transparent and only a few micrometers thick. A
16 large number of bright or dark spots were clearly observed on the whole surface of the
17 uncoated sample. These are corrosion products or pits. Corrosion of the PLLA- and
18 PVC₃₀-coated samples was observed locally. Coatings with PVC₃₀ or PLLA appear
19 effective in retarding corrosion of the Mg substrate. However, on the PVC₆₀-coated
20 sample surface, corrosion products and pits were observed, similar to those observed on
21 the uncoated sample. This suggests that the PVC₆₀ coating was ineffective in
22 suppressing the initial degradation of the Mg substrate.

23 Figure 3 shows SEM images of the surfaces of the coated samples after immersion
24 in α -MEM+FBS for 7 days. There were a few pits of approximately 0.5–1.0 μ m in

1 diameter on the PLLA- and PVC₃₀-coated surfaces. However, 1.0–2.0- μm diameter
2 pores were observed on the PVC₆₀-coated surface., The formation of the larger pores on
3 the PVC₆₀-coated surface probably relates to the lower molecular weight of the PLLA
4 component and a higher concentration of vaterite particles than in the other coating
5 materials (Fig. 3 (c)). The PLLA component with a low molecular weight degrades at an
6 earlier stage of immersion, decreasing the pH of the surrounding solution and causing
7 detachment of the vaterite particles from the coating layer. The higher vaterite
8 concentration also encourages pore formation. This early degradation of the PLLA
9 component and pore formation may accelerate the corrosion of the Mg substrate.

10 An image of the Mg^{2+} ions released into α -MEM+FBS during the immersion is
11 shown in Fig. 4 (a). α -MEM+FBS initially contained 20 $\mu\text{g}/\text{mL}$ of Mg^{2+} ions. The
12 amount of Mg^{2+} ions released increased with immersion time. The Mg^{2+} ions released
13 from the uncoated Mg sample reached more than 10 $\mu\text{g}/\text{mm}^2$ after 7 days of immersion.
14 The sample with the PVC₆₀-coating released almost the same amount of Mg^{2+} ions as
15 the uncoated Mg sample after a period of 3 days. After 7 days, higher amounts of Mg^{2+}
16 ions were released from the sample with the PVC₆₀-coating than the uncoated Mg
17 sample. This agrees with the observations made using optical and electron microscopy,
18 suggesting the lower molecular weight component of the PLLA and a higher vaterite
19 concentration accelerated the substrate degradation.

20 However, the PLLA- and PVC₃₀-coating caused the Mg substrate to degrade slowly
21 over a period of 7 days. After 7 days, the amount of Mg^{2+} ions released was 2–3 $\mu\text{g}/\text{mm}^2$.
22 The PLLA- and PVC₃₀-coating are suitable for the suppression of the Mg degradation
23 under these experimental conditions. The coatings did not delaminate during immersion
24 in α -MEM+FBS. This suggests that the coating layers are strongly bonded as a bonding

1 strength of 0.8–1.0 MPa. These bonding strengths were enough for avoiding the
2 detachment of the coatings in these experimental conditions. However, it is necessary to
3 evaluate the stability of the coating layers for a longer period of time with a longer
4 immersion test.

5 Figure 4 (b) and (c) show the concentration of Ca^{2+} and P^{5+} ions in α -MEM+FBS
6 over an immersion period of 7 days. α -MEM+FBS initially contained about 78.1 $\mu\text{g/mL}$
7 of Ca^{2+} and 34.3 $\mu\text{g/mL}$ of P^{5+} . There was no change in the Ca^{2+} and P^{5+} ion
8 concentrations for the uncoated and PLLA-coated samples during immersion. The Ca^{2+}
9 and P^{5+} ion concentrations in the PVC-coated samples decreased slightly in
10 α -MEM+FBS with increasing immersion time. The decrease in the concentration may
11 be because of the adsorption of these ions on the sample surface or the precipitation of
12 insoluble salts such as calcium phosphate even though the precipitates were not
13 observed on the surface of the sample or glass container with the naked eye.

14 The top surfaces of the samples were analyzed by EDX before and after immersion
15 in α -MEM+FBS. The concentrations of Ca and P after immersion in α -MEM+FBS are
16 shown in Figure 5. On the PVC_{30} -coated surface, the initial concentration of Ca and P
17 atoms was 20.1 and 1.5 atom %, respectively. The PVC_{30} -coated surface had an increase
18 in the Ca and P content with an increase in immersion time. The Ca/P ratio on the
19 PVC_{30} -coating surface was 6.87 after immersion for 7 days. On the PVC_{60} -coated
20 surface, the concentration of Ca and P atoms was initially 61.3 and 3.8 % respectively.
21 The PVC_{60} -coated surface had a higher Ca content than the PVC_{30} -coated surface
22 because of the higher vaterite content in the coating. There was no significant difference
23 in the Ca content during immersion. However, the P content on the PVC_{60} -coated
24 surface increased with the time. The Ca/P ratio on the PVC_{60} -coating surface was 6.71

1 after immersion for 7 days. Our previous work [13], demonstrated the usefulness of
2 PVC for apatite formation in the simulated body fluid. The Ca/P ratios on the PVC₃₀-
3 and PVC₆₀-coated surfaces were far from the stoichiometric Ca/P ratio of
4 hydroxyapatite (1.67) since the Ca in the PVC₃₀ and PVC₆₀ films was also counted. It is
5 possible that calcium phosphate compounds are formed on the PVC-coated surface by
6 adsorbing P⁵⁺. However, further investigation is needed to determine the details of the
7 precipitates on the sample surfaces.

8

9 **3.3. pH values near the sample surfaces**

10 Figure 6 shows the pH of α -MEM+FBS near the sample surface after immersion of
11 a period of up to 4 days. The initial pH of α -MEM+FBS in air was approximately 8.3.
12 After 6 h in the CO₂ incubator, it decreased to 7.55 and was stable. This was because the
13 CO₂ gas dissolved into the medium and dissociated in the incubator. The pH of the
14 medium near the uncoated sample surface decreased to 7.7 after a few hours. It then
15 slowly reached 7.8 after 4 days. Degradation of the Mg increased the pH of the medium.
16 The pH of the medium near the PLLA-coated sample surface decreased to around 7.0.
17 Near the surface of the PVC₃₀- and PVC₆₀-coated samples, the pH decreased to between
18 7.4 and 7.5. The low molecular weight PLLA component in these coatings may degrade
19 at an early stage of immersion, inducing local acidification. However, the pH of the
20 media near the PVC₃₀- or PVC₆₀-coated surface was higher than that of the
21 PLLA-coated surface. This is probably because of the partial dissolution of the vaterite
22 particles, which produces carbonate ions and buffers the medium. In the case of
23 PVC-coated samples, the degradation of the PLLA component in the PVC-coated
24 samples overcomes the effect of the OH⁻ generated by the Mg substrate degradation.

1 However, the carbonate ions derived from the vaterite buffered the medium, resulting in
2 a pH of 7.4. This is closer to the pH of blood plasma, suggesting that PVC can maintain
3 the pH balance of the medium with the degradation of PLLA and vaterite.

5 **3.4. Cell proliferation**

6 Figure 7 shows the cell proliferation of the MC3T3-E1 cells on the uncoated and
7 coated samples. Using the WST-8 assay, it was confirmed that the absorbance of the
8 formazan in the WST-8 reagent correlates with the number of viable cells [19, 20].
9 There were no significant differences in cell viability among the samples that were
10 cultured for 1 day. After culturing the cells for 3 days, the number of viable cells on the
11 coated samples was significantly higher than on the uncoated sample. The difference in
12 cell viability between the samples was more obvious after 7 days of culture. The
13 number of viable cells on the PVC-coated samples was significantly higher than on the
14 PLLA-coated and uncoated samples. The PVC₆₀-coated samples had the greatest
15 number of viable cells.

16 Surface roughness is a key factor for cell attachment and proliferation; however the
17 surfaces employed in this study had a similar surface roughness of 0.08–0.20 μm . This
18 could not explain the difference in the cell viability of the samples. Surface wettability
19 is another important factor influencing cell attachment [21]. The average static contact
20 angles of the uncoated, PLLA-, PVC₃₀- and PVC₆₀-coated surfaces were 61 ± 2 , 95 ± 5 ,
21 75 ± 3 and 72 ± 4 degrees, respectively. The error is given by the standard deviation. The
22 relatively high hydrophobicity of the PLLA-coated surface may correlate with the lower
23 cell growth after 3 and 7 days of culture. The slightly lower contact angles of the

1 PVC-coated samples may relate to the amount of vaterite particles in the composites.
2 This may contribute to higher cell growth after 3 and 7 days of culture.

3 It is possible that the ions released from the samples influenced cell proliferation.
4 It was reported that Ca^{2+} ions released from the samples enhanced cell proliferation of
5 osteoblasts [22, 23]. This implies that PVC coatings have an influence on
6 cytocompatibility. However, the Ca^{2+} ion concentration in the α -MEM+FBS after the
7 PVC_{30} -coated samples were immersed for 7 days was $137 \mu\text{g/mL}$. The Ca^{2+} ion
8 concentration when the PVC_{60} -coated samples were immersed in α -MEM+FBS was
9 $134 \mu\text{g/mL}$. There was almost no difference in the Ca^{2+} ion concentration in the
10 α -MEM+FBS with different PVC-coated samples. Although the optimum concentration
11 was not determined, Mg^{2+} ions were reported to stimulate cell attachment and
12 proliferation of osteoblasts [24, 25]. The PVC_{60} -coated samples had the highest
13 concentration of released Mg^{2+} ions (Fig. 4). This suggests that Mg^{2+} ions promote cell
14 proliferation. However, the cells could not proliferate sufficiently on the uncoated
15 sample, which released a similar concentration of Mg^{2+} ions to the PVC_{60} -coated
16 samples. The stability of the surface structures under biological conditions is a key
17 consideration for cytocompatibility [26]. The surface of the uncoated sample had some
18 instability because of the high reactivity of Mg (Fig. 3 (a)). The effect of the pH of the
19 medium on cell behavior was previously reported [27, 28]. The higher pH of
20 α -MEM+FBS near the uncoated sample surface may inhibit cell growth on the sample
21 surface. The lower pH near the PLLA-coated sample surface is lower than that of
22 human blood plasma (7.4), and may also suppress the cell growth on the sample surface.

23

24 4. Conclusion

1

2 To control the rapid degradation of Mg and improve its cytocompatibility, two types
3 of poly(L-lactic acid)/vaterite composite materials (PVCs) were coated on the Mg
4 substrate. The surface of the PVC₃₀-coated sample had no pores and the coating was
5 maintained after immersion in α -MEM+FBS for 7 days, suppressing the degradation of
6 the Mg substrate. A large number of 1.0–2.0- μ m diameter pores were observed on the
7 PVC₆₀-coated surface. This coating had no effect on the suppression of the Mg substrate
8 degradation, probably because of the pores in the coating. The pH of α -MEM+FBS near
9 the coatings remained close to that of human body fluid. This might be because of the
10 degradation of the PLLA matrix, the amount of dissolution of vaterite from the PVC and
11 the degradation of the Mg substrate. The number of cells of MC3T3-E1 on the
12 PVC₆₀-coated sample was higher than on the PLLA- and PVC₃₀-coated sample. The
13 Ca²⁺ and Mg²⁺ ions that originated from the samples and the stability of the surface
14 structures are believed to have influenced the cell proliferation. The degradation rate
15 and the cell viability of the PVC-coated surface on the Mg substrate were controlled by
16 the vaterite content of the PVC. Based on the results, the PVC-coating containing 30
17 wt % vaterite can suppress the degradation of the Mg substrate and enhance the
18 proliferation of osteoblast-like cells. PVC coatings on Mg and its alloys may be
19 effective for preparing new types of biodegradable metallic bone plates or screws.

20

21

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Figure Captions

Fig. 1 SEM images of (a) the PLLA-coated sample, (b) the PVC₃₀-coated sample and (c) the PVC₆₀-coated sample.

Fig. 2 Optical images of the surface of the samples after immersion in α -MEM+FBS for 7 days: (a) uncoated sample; (b) PLLA-coated sample; (c) PVC₃₀-coated sample; (d) PVC₆₀-coated sample.

Fig. 3 SEM images of the coated surfaces after immersion in α -MEM+FBS for 7 days: (a) PLLA-coated sample; (b) PVC₃₀-coated sample; (c) PVC₆₀-coated sample.

Fig. 4 (a) The amount of Mg²⁺ ions released from the sample into α -MEM+FBS (b) the amount of Ca²⁺ ions and (c) the amount of P⁵⁺ ions in α -MEM+FBS after immersion of (○) the uncoated sample, (◇) PLLA-coated sample, (■) PVC₃₀-coated sample and (▲) the PVC₆₀-coated sample.

Fig. 5 Percentage of Ca and P atoms on the (a) PVC₃₀-coated surface and (b) PVC₆₀-coated surface after immersion in α -MEM+FBS.

Fig. 6 Change in the pH of α -MEM+FBS during incubation in 5 % v/v CO₂ at 37 °C after soaking: (a) α -MEM+FBS only; (b) uncoated sample; (c) PLLA-coated sample; (d) PVC₃₀-coated sample (e) PVC₆₀-coated sample.

Fig. 7 Absorbance as a function of the number of cells on each sample after culturing the MC3T3-E1 cells (* $p < 0.05$ as compared using the student's t -test).

Figures

Fig. 1

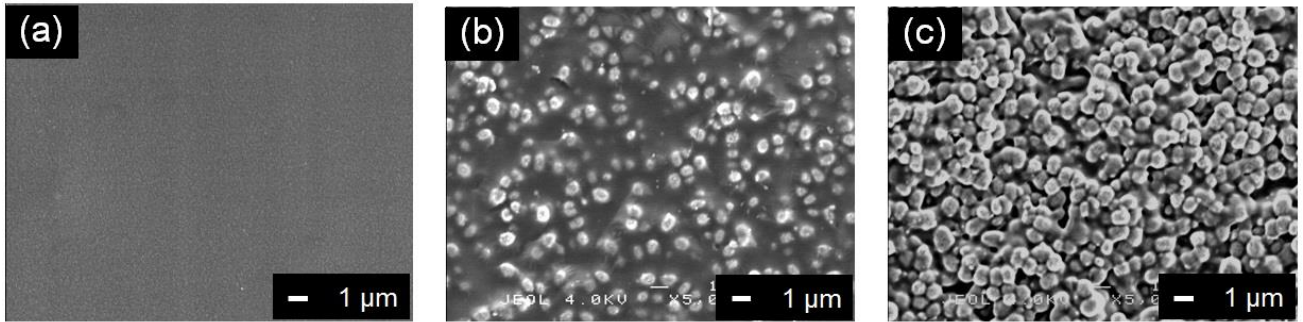


Fig. 2

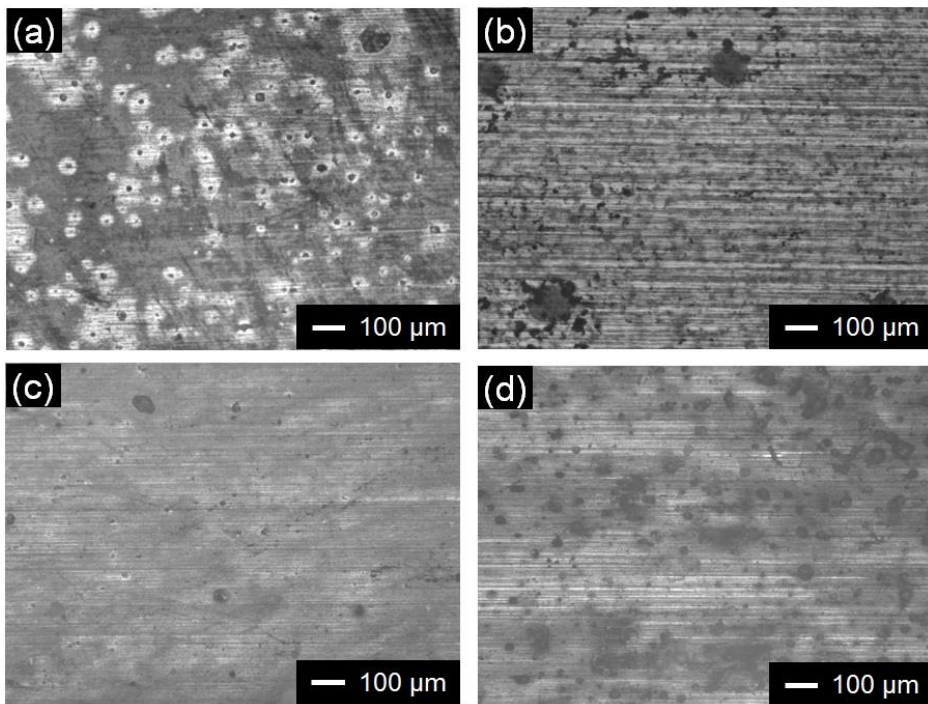


Fig. 3

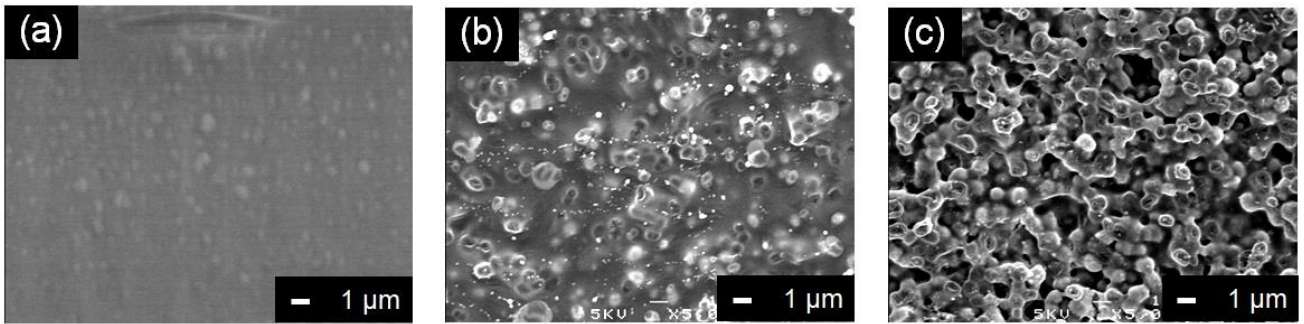


Fig. 4

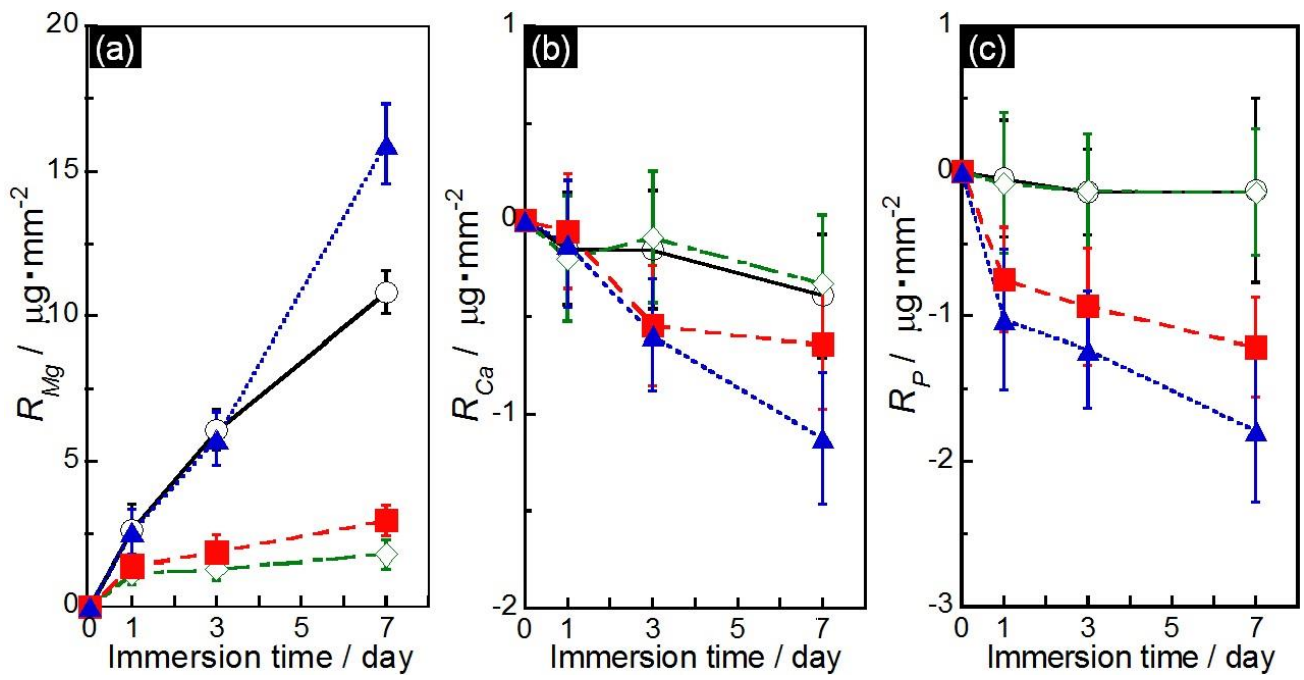


Fig. 5

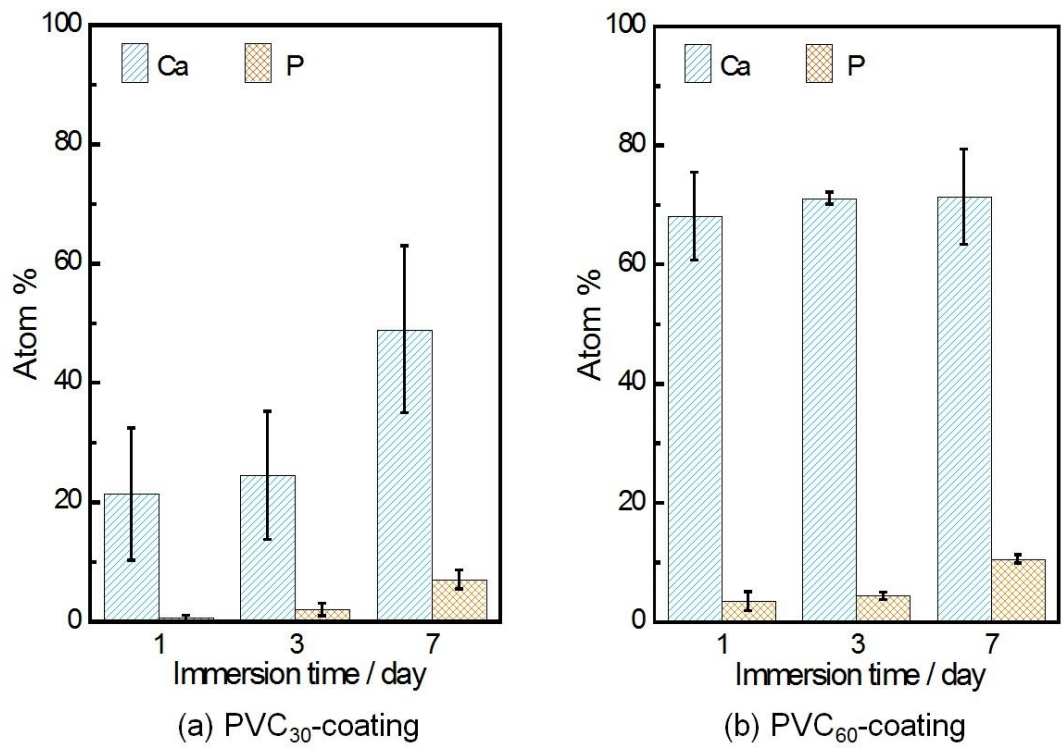


Fig. 6

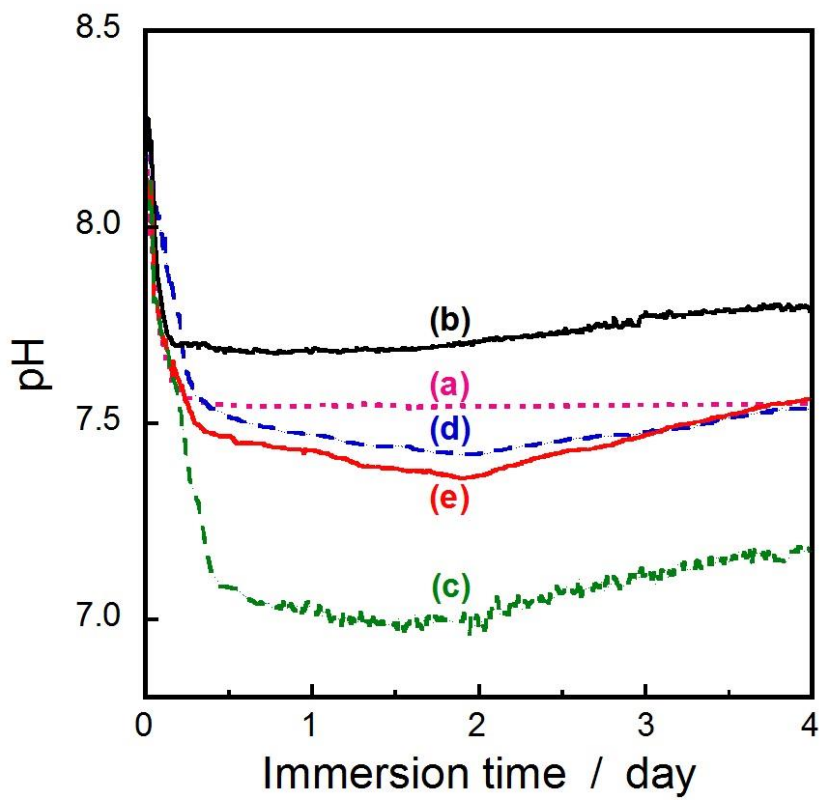


Fig. 7

