The process of magnesium ion modification of titanium surface and the sustained-release of magnesium ions from its surface

Hanako SAKATSUME1, Masatoshi TAKAHASHI1, Mary Wambui KANYI1,2, Yoshinaka SHIMIZU3 and Yukyo TAKADA1

1 Division of Dental Biomaterials, Tohoku University Graduate School of Dentistry, 4-1 Seiryo-machi, Aoba-ku, Sendai 980-8575, Japan
2 Moi Teaching & Referral Hospital, Box3 Nandi Road, Eldoret, Kenya
3 Division of Oral Pathology, Tohoku University Graduate School of Dentistry, 4-1 Seiryo-machi, Aoba-ku, Sendai 980-8575, Japan

Corresponding author, Yukyo TAKADA; E-mail: yukyo.takada.a1@tohoku.ac.jp

This study explored modification of an alkaline heat treated titanium surface, using magnesium ions, to improve bone compatibility through the sustained release of magnesium ions. Pure titanium surface was first subjected to alkaline treatment using 5 M NaOH then modified with magnesium through immersion in magnesium chloride solution before heating in a furnace at 600°C for 1 h. Use of at least 0.01 M magnesium chloride solution for at least 0.5 min, leads to introduction of 1.7 to 2.3 at% magnesium at a distribution close to saturation on the titanium surface. The modified titanium surface sustained long term release of magnesium ions in acidic solution for more than 168 h. It was further demonstrated that the process of sustained release of magnesium ions is influenced by pH and can be triggered by lowering it from neutral to 3.

Keywords: Surface modification, Magnesium, Titanium, Alkaline heat treatment, Bone compatibility

INTRODUCTION

The number of patients who recently wish to be treated using dental implants has increased by nearly five times more than that of around the year 2000. On the other hand, cases of peri-implantitis have led to increased unfavorable prognosis after treatment with dental implants1,2. Since tissues surrounding an implant are less resistant to plaque compared to periodontal tissues3, peri-implantitis is a big hurdle to the use of implants for treatment.

To counter this challenge, measures geared at suppressing peri-implantitis have been advanced from the material side as well. For example, it is reported that titanium alloyed with antimicrobial silver when subjected to acidic treatment exhibits excellent antibacterial effect compared to pure titanium4. Hanawa et al. reported that bone formation was improved when calcium-ion-implanted titanium inserted into rat tibia5. The authors of this study envisioned that use of sustained release metallic ions like calcium, at an appropriate concentration which does not exhibit harmful effects on cells, may promote bone formation. Magnesium which belongs to the alkaline earth metal as well as calcium is the fourth most abundant cations in the body6, and over 60% is accumulated in bone and teeth7. Moreover, a higher intake of magnesium has been proved to efficiently prevent reduction of bone mineral density (BMD) in patients with osteoporosis8. Additionally, some reports suggest that magnesium ions promote bone formation through the activation of osteoblasts9 and increase in bone morphogenetic protein (BMP-2)10. Another report indicates that magnesium implants show properties of less inflammatory response and fibrosis than conventional implant materials11. Safety against tissues when using magnesium implants has also been documented.

Although magnesium was focused on, and initial attempts to alloy titanium which was the principle material with magnesium proved difficult. This is because the melting point of titanium (1,668°C) being much higher than the boiling point of magnesium (1,091°C) under atmospheric pressure conditions, provides no shared temperature at which both metals can exist in liquid phases. Magnesium is however used to reduce titanium tetrachloride (TiCl4) in the process of refining titanium (Kroll method)12; a situation whereby its solid solubility limit to titanium is very low (less than 1% at, 600°C)13. Those technicalities made it impractical to introduce magnesium into titanium through the formation of a solid solution.

Another attempt at introducing magnesium onto the surface of titanium was done by first doing modifications on titanium surface to improve hard tissue compatibility. Surface modification methods can be roughly divided into two groups based on its mechanism of action. One category involves a modification of the surface morphology while the other involves a change in the surface composition or transformation of the existing phase14. A change in the surface morphology involves roughening of the titanium surface which contributes towards improved adhesion of bone by increasing the contact area and improving the mechanical fitting. Some of the surface treatment methods that modify the morphology include: blasting, acid etching, porous modification, titanium plasma spraying and anodic oxidation15. Methods that change the surface composition or transform the existing phase involve physicochemical process of forming apatite on the titanium surface. This process can be pre-initiated in the laboratory or induced in vivo. The former includes methods such as: thermal spray, vapor phase growth, simulated body fluid immersion, and electrochemical...
means. The latter involves calcium ion implantation, calcium titanate (CaTiO₃) coating, and alkaline heat treatment⁴⁰.

This study focused on alkaline heat treatment which is a simple surface-modification technique. Alkaline heat treatment is a surface modification method developed by Kokubo et al. in 1996⁴⁰ for improving bone compatibility of titanium⁴⁰. It involves heating titanium at 600°C for 1 h in the atmosphere after immersion of in a 5 M sodium hydroxide aqueous solution at 60°C for 24 h (alkaline treatment).

Alkaline heat treatment has already been used in the field of orthopedic surgery⁴⁰. It is also applicable in titanium alloys such as Ti-6Al-4V alloy¹⁷ and Ti-15Zr-4Nb-4Ta¹⁶ besides pure titanium. There are some studies that show sodium distributed on alkaline heat treated titanium surface was substituted with calcium¹⁹ or heavy metals (cobalt, chromium, copper)²⁰ and that incorporated silver ions imparted antibacterial properties²¹. However, there is insufficient information on substitution with magnesium.

On the other hand, research on biodegradable materials made of magnesium alloys is fairly common²²). Such magnesium alloys have been developed for controlling decomposition rate²³,²⁴ and for use in development of biodegradable orthopedic devices²⁵. On the contrary, non-degradable materials positively utilizing magnesium for the promotion of bone formation and imparting sustained release ability of magnesium ions have not been developed.

It is well known that the ionization tendency of magnesium is smaller than that of sodium and that solubility of the magnesium oxides and hydroxides in water is low²⁶). Therefore, there is a high possibility that sodium imparted by alkaline heat treatment in an aqueous solution can be substituted with magnesium and fixed as a compound with only a little solubility on titanium surface. In order to positively utilize such characteristics of magnesium; as we will mention our attempts into details later, we devised a method of modifying titanium surface with magnesium ions through alkaline heat treatment.

In this study, the purpose was to reveal a method of modifying titanium surface and its appropriate conditions using magnesium ions in a manner that allows sustained release of the ions from the modified surface.

MATERIALS AND METHODS

Production and preparation of titanium specimens
A pure titanium plate (JIS H 4600 (JIS 1), Kobe Steel, Tokyo, Japan) of 1 mm thickness was cut into rectangular 10×5 pieces. One side of the small titanium sheets was polished up to #1200 using SiC waterproof abrasive paper. After polishing, the specimens were subjected to ultrasonic cleaning with distilled water and ethanol. Specimens prepared as outlined above were those used for surface modification, observation of surface structure and analysis of element distribution. Out of the same titanium plate 10×10 mm square sheets were cut to generate specimens for elution test. All the surfaces and sides were polished up to #800 using SiC water resistant abrasive paper. After polishing, the specimens were ultrasonically cleaned with distilled water and ethanol.

Surface modification
Titanium specimens were immersed in 5 M sodium hydroxide solution maintained at 60°C for 24 h to carry out alkaline treatment¹⁵. The specimens after removal from the solution were gently rinsed off using distilled water, before further immersion in distilled water for 1 min.

Next, magnesium chloride solution of 15 graduated concentrations was prepared. The alkaline treated specimens were immersed in magnesium chloride of concentration within the range of 0 to 4 M and kept at 60°C, for a time period of between 6 s to 24 h (0.1 to 1,440 min). The specific concentrations and immersion times are shown in Table 1. The symbol “○” as it appears in the Table serves to positively identify conditions which were carried out in this study for the time and concentration parameters indicated. Three specimens were used for each set condition. After immersion, the specimen surface was rinsed gently with distilled water then further immersed in distilled water for 1 min. The specimen was then air dried before firing in in an electric furnace heated to 600°C for 1 h.

Observation of surface structure and elemental analysis
An image of each specimen surface after heat surface modification and firing process was observed using a scanning electron microscope (SEM, JSM-6060L, JEOL, Tokyo, Japan) equipped with an energy dispersive X-ray spectrometer (EDS, JED-2300, JEOL) set under acceleration voltage of 15 keV, working distance (WD) of 10 mm, and maximum magnification of 10,000. The surface of each specimen examined was enlarged 3,000 times. Qualitative and quantitative analysis using EDS was performed six times for each modified specimen that was examined. Concentrations of all elements distributed on the surface of the specimen as identified through qualitative analysis such as magnesium, titanium, and others were determined.

The surface structure of each specimen before and after the elution test was also observed. In addition, qualitative and quantitative analyses of elements distributed on these surfaces were carried out to determine if any changes or significant loss occurred after the release of magnesium ions during elution test.

Elution test
Specimen modified through immersion in 4 M magnesium chloride solution for 24 h and those that underwent alkaline heat treatment were subjected to this test. A total of six specimens were used for each surface modification (alkaline heat treated and magnesium ion modified) in each of the two types of solutions. Zero point nine percent sodium chloride solution and 0.001 M hydrochloric acid solutions were prepared to investigate release of ions in
neutral and low pH environments, respectively. Twenty milliliters of each of the two solutions was drawn and placed in plastic bottles kept at 37°C. Each bottle had its lid shut and vigorously shaken to saturate the solution with dissolved oxygen just before the specimen was ready for immersion.

Each specimen was retrieved and immersed into a bottle with a new solution after 1 h, 6 h, 12 h, 24 h (1 day), 48 h (2 days), 72 h (3 days), 96 h (4 days), 120 h (5 days) and 168 h (7 days). Magnesium and titanium ions found in the solutions from which the specimens were removed, over the different time periods, were quantitatively analyzed using an inductively coupled plasma emission spectrometer (ICP-AES, iCAP 6000 SERIES ICP Spectrometer, Thermo SCIENTIFIC, Tokyo, Japan). The obtained ion concentration was converted to dissolution mass (μg/cm²) per unit area of the specimen and was regarded as the amount of released ions.

**Statistical analysis**

Data obtained from the distribution of elements on each specimen surface and the elution tests was statistically analyzed using ANOVA and Tukey’s test at significance level of \( p<0.05 \).

**RESULTS**

**Surface structure**

Figure 1 shows the surface structure of both untreated titanium and alkaline heat treated titanium polished up to #1200 using SiC waterproof abrasive paper. Large polishing marks due to SiC abrasive grains were observed on the untreated titanium surface (Fig. 1a). Surfaces of alkali heat treated specimen exhibited a completely different structure; three-dimensional porous mesh structures in the range of several micro to nano microns in size were visible (Fig. 1b).

The process of surface modification with magnesium ions through immersion in magnesium chloride was studied as a function of both concentration and time, simultaneously. Different scenarios such as: high concentration per short time (4 M/0.5 min), high concentration per long time (4 M/24 h), low concentration per short time (0.005 M/0.5 min), and low concentration per long time (0.005 M/24 h) were tested and the structural appearances of the modified surfaces shown in Figs. 2a–d. Although there were slight differences in the size of pores depending on the observation site, a porous network structure was generally observed on the surfaces of specimen subjected to all the different conditions which was similar to the alkaline heat treated ones. The mesh structure was neither influenced by the concentration nor the immersion time, as no significant difference was observed in the surface structure of the specimen subjected to the different conditions.

**Magnesium concentration on ion-modified surface**

Figure 3 shows the qualitative analysis of the different elements distributed on the magnesium modified and
alkaline heat treated titanium surfaces. Alkaline heat treated titanium surface (Fig. 3a), shows on EDS a peak corresponding to sodium in addition to those of titanium and oxygen. However, the peak corresponding to sodium disappeared as one that matches that of magnesium appeared in addition to those of titanium and oxygen on the magnesium modified titanium surface (Fig. 3b).

Next, the concentration of magnesium chloride used for ion modification was expressed on the ordinate axis, whereas immersion time was done on the abscissa axis. The different ion modification conditions were represented on a scatter diagram. The concentration of magnesium ions measured/identified on the modified surface under the different immersion time and magnesium chloride concentration conditions was categorized into 4 levels: (Mg<the detection limit, the detection limit≤Mg<1.4 at%, 1.4 at%≤Mg<1.7 at%, 1.7 at%≤Mg≤2.3 at%). These levels were expressed using different shapes on the plot as shown in Fig. 4.

The four color-coded areas represent the concentration of magnesium ions distributed on the surface under the different conditions. The average concentration of magnesium ions incorporated onto the modified titanium surface was within the range of 0 (detection limit) to 2.3 at%.

The dark gray region represents the conditions (time and magnesium chloride concentration) that resulted in the highest concentration of magnesium being fixed on the modified titanium surface. For a specimen immersed in more than 0.01 M concentration of magnesium chloride for 0.5 min or more; the concentration of magnesium ions detected was 1.7 to 2.3 at% and a state of near saturation was attainable regardless of the specific condition.

The gray region represents conditions which produced a concentration of magnesium being fixed on the modified surface in the range of 1.4 to 1.7 at%. Notably, higher concentrations of magnesium chloride solution and longer immersion times resulted in higher concentrations of magnesium ions absorbed and distributed on the surface.

The light gray region represents conditions that resulted in adsorbed magnesium concentration of 1.4 at% or less. Within this region, magnesium concentration did not increase any further even with prolonged immersion. Use of less than 0.005 M concentration of magnesium chloride for less than 10 min of immersion time, resulted in surfaces where magnesium could not be detected (below the detection limit) and is represented by the white region.

Beyond an immersion time of 60 min, there was no dependence relationship between the concentration of fixed magnesium and the immersion time. When the concentration of the magnesium chloride used was 0.01 M or more, the absorbed magnesium measured reached near saturation levels regardless of the immersion time.

**Amounts of released ions from modified and unmodified titanium surfaces**

The amounts of ions (μg/cm²) released from the
magnesium ion modified and unmodified titanium (alkaline heat treated) surfaces are shown in Fig. 5. It shows the total amounts of ions released into either sodium chloride solution (0.9% NaCl) or the acidic solution (0.001 M HCl+0.9% NaCl) for 168 h (7 days).

Alkaline heat treated titanium surfaces released titanium below detection limit into sodium chloride and 0.24 μg/cm² into acidic solution. Magnesium ion modified titanium specimen, released titanium ions of slightly below determination limit into both solutions. The amount of titanium ions into the acidic solution from magnesium modified surface was drastically low compared to that from of alkaline heat treated surfaces. The amount of magnesium ions released into sodium chloride and the acidic solution was 0.28 and 0.34 μg/cm², respectively. The ions released into sodium chloride were significantly less than those released into the acidic solution.

**Time course of the released magnesium ions**

The amount of released magnesium ions against the immersion times is shown in Fig. 6. The ions released into sodium chloride solution within 1 h of immersion was 0.23 μg/cm², which accounts for about 82% of the total ions released in 168 h. The amount decreased sharply to 0.012 μg/cm² after 6 h, and thereafter gradually until it reached the detection limit after 96 h. In the acidic solution; the released amount of ions within 1 h of immersion was 0.24 μg/cm² which greatly reduced to 0.015 μg/cm² after 6 h. However, release of magnesium ions continued even after 168 h of immersion.

**Surface structures and residual magnesium concentration**

A comparison of the structure of titanium surfaces before and after elution test is shown in Fig. 7. In both cases, a three-dimensional porous structure of less than

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**Fig. 5** Amount of ions released from unmodified (alkaline heat treated) and magnesium ion modified titanium into sodium chloride and acidic solutions.

**Fig. 6** Amounts of magnesium ions released from the magnesium ion modified titanium into the sodium chloride solution and the acidic solutions every 1–24 h.

**Fig. 7** Surface structure of magnesium ion modified titanium before and after the elution test.

**Fig. 8** Concentration of introduced magnesium distributed on the magnesium ion modified titanium surfaces before and after the elution test into sodium chloride and acidic solutions for 168 h.
several microns was observed. In addition, there was no significant difference between the concentrations of magnesium fixed on the titanium surface before and after the elution test in any of the solutions as shown in Fig. 8. Therefore, the amount of magnesium absorbed and retained on the surface through ion modification was much more than that of ions released within 168 h.

**DISCUSSION**

**Introduction of magnesium onto titanium surface**

As mentioned earlier, magnesium is the fourth most abundant cations in the body, equal to about 1 mol (24 g) in an adult human body and over 60% is accumulated in bone and teeth. Moreover, a higher intake of magnesium has been proved to efficiently prevent reduction of BMD in patients with osteoporosis. Therefore, an attempt to develop a bioactive titanium surface with ability to sustain release of magnesium ions is useful. In this study, developing a method of introducing and fixing magnesium onto titanium was the greatest hurdle, in the quest to develop a modified titanium implant surface that supports bone formation and remodeling. As mentioned in the introduction section, magnesium can only form a solid-solution state with titanium at 600°C of less than 1 at% and the boiling point of magnesium is about 600°C lower than the melting point of titanium. Therefore, it is not possible to alloy magnesium to titanium through the usual melting method.

As a method of introducing magnesium to titanium, attempts were also made to form an alloy by gas-solid reaction between magnesium vapor and titanium solid. However, it was not possible to form a solid solution involving magnesium on the titanium surface in our pre-experiment. Since alloying in bulk was difficult, a consideration was made to introduce magnesium through alkaline heat treatment leading to formation of a compound containing magnesium on the surface of titanium. In order to introduce magnesium on the titanium surface, untreated titanium, alkaline heat treated titanium (after sintering), alkaline treated titanium (before sintering) were prepared and were immersed in solutions containing only magnesium chloride and both of magnesium chloride and sodium hydroxide. The pre-experiment revealed a suitable combination of the conditions for introducing magnesium on the titanium surface. The best combination resulted in the manner roughly similar to the method of calcium-modified titanium reported by Kizuki et al.

As shown in Fig. 9, alkaline treated titanium was immersed in magnesium chloride solution to modify the surface with magnesium ions before firing. It was found that the structure of magnesium ion modified titanium surface, through this method of alkaline heat treatment, maintains a network structure that is not dependent on either magnesium chloride concentration or immersion time as shown in Fig. 2. The titanium passive film formed changes to titanate ion (HTiO$_3^-$) which is a peroxide ion formed electrochemically at passivating potential as pH increases to 12 or higher. Therefore, when titanium is immersed in sodium hydroxide solution; the passive film formed dissolves to generate sodium titanate (Na$_x$H$_{2-x}$Ti$_2$O$_{4y}$; 0<x<2, y=2, 3, 4) and a network structure develops. Subsequent immersion in magnesium chloride solution of which pH is 4.2 to 4.3 provides stability to the network structure already formed after alkaline treatment.

During standard alkaline heat treatment, a layer of sodium hydrogen titanate is initially formed on the surface after alkaline treatment which upon firing in the atmosphere, heating, turns into sodium titanate. Sodium is incorporated into the titanium surface in addition to titanium and oxygen as shown in Fig. 3. Interestingly, magnesium instead of sodium was detected in magnesium ion-modified titanium surfaces. In the process of modifying alkaline treated titanium through immersion in magnesium chloride sodium was substituted with magnesium. Subsequent firing resulted in formation of magnesium titanate structure, proof that magnesium was successfully incorporated and fixed on the titanium surface.

Sun and Li reported that heavy metal ions such as copper, nickel and cobalt substituted sodium ions in its network structure and remained on the titanium surface even after firing. Magnesium has a smaller ionization tendency than sodium, thus electrochemical substitution by oxidation-reduction reaction is possible. In addition, the solubility of magnesium hydroxides and oxides in water is low due to formation of sparingly soluble salts. Once substitution occurs to form a salt, it is difficult for dissolution of its ion to occur in solution. Based on the above facts, it was thought possible to introduce and immobilize magnesium onto the titanium surface by modifying with magnesium ions after alkaline treatment then subjecting the surface to heat treatment.

**Conditions for magnesium ion modification**

The conditions for magnesium ion modification (combination of magnesium chloride concentration and immersion time) can be roughly mapped into four zones.
depending on the concentration of magnesium ions incorporated detected on the titanium surface as shown in Fig. 4.

The dark gray region shows that magnesium in the range of 1.7 to 2.3 at% exists on the titanium surface. It was found that the concentration of magnesium distributed within the area reached near saturation levels regardless of the specific condition. This study proved that magnesium can be fixed onto a titanium surface to a nearly saturated state by immersion in magnesium chloride of 0.05 M or more for only 0.1 min (6 s).

The zone corresponding to the represents conditions of short immersion (less than 10 min) and low concentration magnesium chloride (0.005 M and less) that resulted in magnesium concentration of below detection limit. The area sandwiched between the dark gray and white regions, depicted a relationship whereby the higher the magnesium chloride concentration or the longer the immersion time; the higher the magnesium concentration distributed on the titanium surface. However, saturation could not be reached even after 24 h of immersion.

Magnesium can be fixed onto the titanium surface to a nearly saturated state within range of conditions that fall under the dark gray region. However, the specimens used for ion modification testing in this study were 10×5×1 mm in size (surface area: 130 mm²). The volume of magnesium chloride solution used for each specimen was 10 mL. Therefore, a decrease in the volume of solution used or an increase in the surface area of the specimen would lead to a reduction in the absolute amount of magnesium chloride per unit area. The reduction would translate to inability to attain saturation of magnesium on the surface even under the optimum conditions of saturation identified and mentioned above.

Even when ion modification is carried out under the optimum conditions (surface area of 130 mm² and solution volume of 10 mL); it is necessary to use magnesium chloride solution of at least 0.01 M concentration in order to fix magnesium to levels close to saturation. Although the difference in immersion times that favour saturation depends on the concentration of magnesium chloride solution, the amount of magnesium ions in magnesium chloride required to saturate the titanium surface with magnesium is calculated to be at least 7.7×10⁻⁷ mol/mm² of the specimen surface area. Therefore in order to incorporate magnesium to the titanium surface at a concentration close to saturation, concentration of more than 0.01 M magnesium chloride containing magnesium ions of 7.7×10⁻⁷ moles for every mm² of specimen surface is required.

**Sustained release of magnesium ions**

A small amount (0.24 μg/cm²) of titanium ions was released from the unmodified (alkaline heat treated) titanium specimen in acidic solution. On the other hand, the magnesium ion modified titanium specimen released 0.28 to 0.34 μg/cm² magnesium ions. Contrastingly, the amount of titanium ions released from modified specimen in both sodium chloride and acidic solutions was lower than the determination limit. The release of magnesium ions could have suppressed the release titanium ions even in the acidic solution. Since it is assumed that the oxidation number of magnesium in magnesium titanate is in the range of 0 to 2 valence and it is smaller than bivalent, release of divalent ions from the surface will cause it is surrounding to become negatively charged. The negative charge in turn suppresses oxidation of titanium and prevents release of titanium.

The amount of magnesium ions released from the modified titanium reached the maximum within the first 1 h of immersion in each solution. The peak value was 70 to 85% of the amount released in 168 h (7 days). Throughout magnesium ions were continuously released. The sustained release into sodium chloride solution stopped after 120 h, but continued beyond 168 h in the acidic solution even though it was in minute quantities. Therefore, this study demonstrates that a decrease in pH can trigger or control sustained release of magnesium ions.

The average amount of magnesium ions released into acidic solution per 24 h (per day) calculated between 24 to 168 h, is 0.013 μg/cm². The amount released when the sustained slow release continues for 6 months is approximately 10 times that of the first 1 h, which is still a small amount. Since there was no structural difference observed on the surface, no significant difference in the magnesium concentration distributed on the modified surface before and after the elution test the weight loss after long term sustained release of ions was negligible.

Furthermore, if the pH around magnesium ion-modified titanium was decreased by inflammation in vivo, the sustained release of magnesium ions occurs and can be expected to lead to activation of osteoblasts leading to a reduction in bone resorption.

**CONCLUSIONS**

It was possible to modify the titanium by incorporating magnesium ions onto the surface in this study and the following conclusions can be drawn.

1. The titanium surface was modified with magnesium ions through this process: Pure titanium was immersed in 5 M sodium hydroxide solution at 60°C for 24 h then immersed in magnesium chloride solution at the same temperature before firing in the atmosphere at 600°C for 1 h. It was noted that immersion in magnesium chloride solution of at least 0.01 M for 0.5 min or more; magnesium of 1.7 to 2.3 at% which is close to saturation could be introduced on the titanium surface.

2. Immersion beyond a time of 60 min did not have any dependence relationship between the concentration of fixed magnesium ions and the immersion time. When the concentration of the magnesium chloride used was 0.01 M or more, the magnesium ions fixed onto the titanium surface reached near saturation levels regardless of the
immersion time.
3. Specimen of titanium modified with magnesium, sustained release of magnesium ions into both 0.9% sodium chloride and 0.001 M hydrochloric acid (acidic solution) for 120 h and more than 168 h, respectively. The concentration of magnesium ions distributed on the modified titanium surface after the elution tests did not show a significant decrease. Furthermore, it was demonstrated that the sustained release can be controlled by pH changes with low pH acting as a trigger.

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