Precipitation of Magnesium Apatite on Pure Magnesium Surface during Immersing in Hank's Solution

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A new artificial bone concept by magnesium alloys is proposed to think much importance on its homogenization with a surrounding natural hard and soft tissue. Magnesium is an essential element for human body, so that magnesium bone implants can be expected to be toxicity free even though magnesium dissolved into human soft tissue. In addition, magnesium base artificial bone has vivo-adaptively to growing bone cells once vivo-coating is formed on the surface of magnesium in the inside of soft tissue. In the present paper, its chemical behavior in Hank's solution (HBSS (+)) is described to simulate biochemical reactions of magnesium in the human body. An effect of heat treatment of magnesium on its chemical behavior is also investigated. Specimens of $10 \times 20 \times 2 \text{ mm}^3$ were used for examining chemical behaviors of commercial grade pure magnesium (3N-Mg) in a HBSS (+) for various holding time (25–700 h). Specific mass gain of each specimen was measured, the surface microstructure was observed by a scanning electron microscope, identification of reaction products were examined by x-ray diffraction measurements. Chemical compositions of reaction products were also analyzed by an energy dispersion x-ray spectrometry. Mass change of heat-treated 3N-Mg, which was heat-treated at 803 K for 90 ks increased with immersing time in HBSS (+) though that of other heat-treated 3N-Mg unstably decreased in HBSS (+). Magnesium reacted with HBSS (+) and then a magnesium apatite was precipitated on the heat-treated 3N-Mg specimen surface. The magnesium apatite should be described as $(\text{Ca}_{0.86}\text{Mg}_{0.14})_{10}(\text{PO}_4)_6(\text{OH})_2$.

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1. Introduction

Medical implantation has become an essential solution to deal with osteoporosis, bone fracture or tooth decay. From the medical-engineering point of view, there are many requirements for optimum design for artificial bone, dentures or implants. Bio-compatibility is the first barrier to be overcome. High specific strength and high fracture toughness are needed for life time extension of these man-made parts. Adaptive weight and geometric size must be also taken into account to reduce the burden on patients. Among various material selections, the first candidate might be titanium and its alloys, glass-ceramics or polymers. In fact, various kinds of biomaterials are in commercial: titanium alloy for SS Hip system,¹⁾ alumina ceramic or zirconia ceramic modular heads, titanium, cobalt-chromium alloys with/without apatite coating for stem,²⁾ ultra high molecular weight poly ethylene for socket,²⁾ or apatite, alumina, or zirconia for artificial bones and ball heads in hip joint.³⁾

Owing to the recent reports,^{4,5)} even pure titanium, which has been believed as a corrosion-less material in human body, solved in vivo, leading to a trigger to induce disease or metallic allergy. Glassy-ceramics like A-W glass⁶⁾ are insufficient both in strength and toughness.⁷⁾ Hence, the above material selection is still open to be discussed from new bioengineering sense.

In the present paper, the advanced artificial bone design is first introduced to have key items understood for material selection of hard tissues. Three items are indicated as key technologies: bio-compatibility, bio-adaptability and mechanical properties. As before mentioned, artificial bone material must

be not only toxicity-free but also compatible to the soft tissue through a buffer coating or film formed in vivo. In order to allow the bone soft tissue to grow and form a vivo-system, a hard tissue material has to accommodate adaptive porosities or open cell structure to be contact with growing soft tissues and to house the vivo-formed tissue system. With consideration of daily life of patients, sufficient strength, ductility and toughness must be equipped with hard tissue material.

Magnesium base graded cellular structure is proposed as a new concept of artificial bone to satisfy these three key items. In general, magnesium has been believed only as an easy-corrosive material in water; initial mass must be reduced in corrosion. The present paper first states that mass gain occurs through reaction of magnesium with constituents of human body. Hanks' solution is utilized to simulate the circumstance of human body. This mass gain comes from formation of magnesium base apatite coating on the surface of magnesium sample. This new finding proves that magnesium and its alloys should have bio-compatibility in vivo.

2. Artificial Bone Design

Natural bone is an ideal limit for any artificial bone structure to be targeting. Owing to its perfect compatibility to soft tissue, broken head-bone can be replaced with hipbone by operation and original bone can be reproduced in vivo. The first step to reach the above perfect biocompatibility is homogenization of interface between natural and artificial bones. In order to be free from bio-negativity, it should have a surface property that enhances to grow bone due to stimulate osteoblast and then apatite from the natural bone forms on

Fig. 1 A structure on the cross section of an ideal artificial bone based on a new concept of biomaterials. (a) Image on inserting the magnesium cell structural implant. Arrow shows that materials is selected to stimulated bone cell, to be possible to insert a medicine capsule for promoting bone growth (activation of bone cell), (b) Image of penetrating natural bone into a cell structural magnesium artificial bone implant, (c) image of homogenization with natural bone.

the surface. There are many investigations to make such a situation, for example, apatite coating method, which various techniques have been applied to coat apatite on an artificial bone.

Kokubo⁸⁾ has suggested that hydroxide-alkaline aqueous solution treatment drives to form natural apatite precipitates on an artificial bone material and results in direct growth of apatite on the treated artificial bone material. This apatite precipitation mechanism certainly provides importance of interfacial control between natural bone system and artificial bone. Since the artificial bone material remains its original state and little fibrous tissue is formed in the interface, however, homogenization between two bone systems through interface is never sufficient in the human body. Further advancement is required to improve the bioadaptivity of this kind interface.

New type of artificial bone concept is necessary to aim for homogenization with the natural bone by exhausting xenobiotic material and allowing bone growth through interface.

Recently, Kawamura *et al.*⁹⁻¹¹⁾ have made binary and ternary alloy search and successfully developed new type of magnesium alloys by rapid quenching. This alloy has become free from the conventional trade-off-balancing curve between strength and ductility and jumped into a lower boundary limit of design-goal region: (550 MPa, 4%) to (450 MPa, 10%). This strength - ductility balance is comparable to most of wrought titanium alloys; it becomes superior to most of titanium alloys with respect to the specific strength. Then, magnesium alloy has a potential to be working instead of the titanium alloys.

Figure 1 summarizes a new artificial bone concept on the basis of a graded magnesium alloy cell structure. Pore size and its graded distribution are designed to simulate porous structure of natural bone. At the initial stage, magnesium cellular structure¹²⁾ is covered with magnesium-base apatite coating in vivo. Different from conventional ceramics and titanium alloys, solid part of magnesium cells is gradually re-

acted in vivo to hard tissue by homogenization to surrounding soft tissues. Since significant inner stresses are absent in the inside of cellular materials, formation mechanism of hard and tissue growth to reproduce the lost bone is not prevented in the daily life of patients.

3. Experimental Procedures

From an extruded sheet, the magnesium specimen with the purity of 99.9% (abbreviated as 3N-Mg as the beneath) was prepared: 10 mm in width, 20 mm in length, and 2 mm in thickness. A hole of 1 mm in diameter was drilled at a corner of each specimen for immersing it in HBSS (+). Then all specimens were rinsed in acetone using an ultrasonic cleaner for 0.6 ks.

A chemical composition of HBSS (+) in 1 liter H₂O is listed in Table 1.

HBSS (+) is well known as an artificial nutritious solution using for cultivating animal cell.

Low corrosion resistance of magnesium is surely caused on reactivity with chlorine ion and hydro-oxy ion in the present study. There are much chlorine ions in HBSS (+) as well as human body. In order to recrystallize deformed grains of 3N-Mg, heat treatment of 3N-Mg specimens were carried out under the conditions as shown in Table 2.

Table 1 Chemical contents of HBSS (+) in H₂O of 1 liter

NaCl	KCl	Na ₂ HPO ₄ ·2H ₂ O	KH ₂ PO ₄	MgSO ₄ ·7H ₂ O	CaCl ₂	NaHCO ₃
8 g	0.4 g	0.06 g	0.06 g	0.2 g	0.14 g	0.35 g

Table 2 Conditions of heat treatment of 3N-Mg

Temperature	Time	Ambient
803 K	3.6, 14.4, 32.4, and 90 ks	Atmosphere

These specimens were polished by aluminum particle of 0.1 μm in mean diameter with buff followed rinsing in acetone using ultra-sonic cleaner for 0.6 ks.

Change of mass of each specimen was also measured by a digital microbalance before and after immersing in HBSS (+). In order to normalize the change of mass of each specimen, specific mass gain should be defined by the equation as the follow:

$$\text{Specific mass gain} = \frac{M_{\text{after}} - M_{\text{before}}}{M_{\text{before}}}, \quad (1)$$

where M_{before} denotes mass of specimen before dipping into HBSS (+) and M_{after} that after dipping in HBSS (+).

The surface microstructure was observed by a scanning electron microscope (SEM, Hitachi S800 equipped with energy dispersion x-ray analyzer by Philips), identification of reaction product was examined by an x-ray diffraction (XRD, Philips X'Pert MPD) using irradiating Cu-K α (40 kV–55 mA).

4. Results and Discussion

4.1 Effect of heat treatment on reaction product during immersing in HBSS (+)

In general, the specific mass change in biochemical reaction of 3N–Mg specimen depends on the immersing time. The polished 3N–Mg specimens were immersed in HBSS (+) from 90 ks (25 h) to 2.25 Ms (625 h). Figure 2 shows the relationship between the specific mass change and the immersing time in the case of polished 3N–Mg without heat treatment. HBSS (+) solved around 98% to 99% of original mass during duration of 810 ks (225 h). This fast solution rate is similar to that of AZ31.¹³⁾ This might be partially because a heterogeneous structure of 3N–Mg prepared by extruding process.¹⁴⁾

Then annealing treatment of 3N–Mg was carried out under atmosphere at 803 K for various holding time to recrystallize its microstructure. Figure 3 shows a typical microstructure on the cross section of 3N–Mg, which was rinsed in acetone using a ultra-sonic cleaner for twice of 0.6 ks and heat-treated at 803 K for 32.4 ks (9 h). The modified layer of 20 μm in thickness could be clearly observed even after ultra-sonic rinsing.

Figure 4 shows XRD profile on the surface of heat-treated

Fig. 2 Ratio of change of mass during immerse in HBSS (+) as functioned with holding time.

3N–Mg after rinsing in acetone using a ultra-sonic cleaner. All peaks are identified as Mg ($\circ$) and MgO (Δ); the modified layer in Fig. 2 would be consisted of MgO and Mg. However, microstructure of the modified layer can not be clearly observed as dual phase of MgO and Mg. The volume fraction of MgO is smaller than that of Mg, because intensity of XRD for MgO in Fig. 4 is smaller than that of Mg. Anyway, heat-treated 3N–Mg can be expected to improve its chemical behavior rather than non-treated 3N–Mg, since MgO is well known to be extreme stable substance even at elevated temperature and to be its high corrosion resistance.

Figure 5 shows specific mass gain of 3N–Mg specimen during immersing in HBSS (+) for 90 ks (25 h) as functioned with heat treatment time at 743, 773, and 803 K. The magnesia powder, which was resulted in oxidation due to heat treatment of magnesium under atmosphere was almost completely removed from the 3N–Mg specimen surface by rinsing it in acetone using a ultra-sonic cleaner. However, the modified layer is surely remained on the specimen surface as shown in Fig. 3, since the specific mass gain of 3N–Mg in-

Fig. 3 A typical microstructure on the cross section of 3N–Mg heat-treated at 803 K for 32.4 ks (9 h). S^{ex} represents an external surface, S^{in} an interface between the modified layer and matrix of 3N–Mg, and E thickness of the modified layer, respectively.

Fig. 4 XRD profile on the surface of 3N–Mg of twice super-sonic rinsing followed heat-treated at 803 K for 32.4 ks (9 h). $\circ$: Mg [JCPDS, 04-0829], Δ : MgO [JCPDS, 35-0821].

Fig. 5 Ration of change of mass during immerse in HBSS (+) for 90 ks as functioned with various heat treatment times at 743, 773, and 803 K. Δ : Heat treated at 743 K, ∇ : at 773 K, $\circ$: 803 K.

creases clearly by the oxidation due to the heat treatment at 803 K as the process time. Kuwahara *et al.* already reported that the specimen of 3N-Mg, which was surface modified at 743 K for 3.6 ks, was almost melted by immersing in HBSS (+) for 90 ks (25 h) (the specific mass gain = -98.35%).¹³⁾

On the contrary, the specific mass gain is converted to plus stably by the heat treatment at 803 K longer than for 32.4 ks (9 h), that is, the heat treatment of 803 K results in increasing mass (the specific mass gain = -1.79% for 3.6 ks, -0.87% for 14.4 ks, $+0.63\%$ for 32.4 ks, $+0.15\%$ for 57.6 ks, and $+1.46\%$ for 90 ks). Such facts indicate that the heat treatment at 803 K can surely suppress the solution of 3N-Mg in HBSS (+), besides, the longer heat treatment increases the larger specific mass gain. It can be expected to suppress the solution of 3N-Mg to HBSS (+) rather than that at lower temperature of 743 or 773 K.¹³⁾

These results of the heat treatment should be required to discuss about the effect of interstitial atoms such as oxygen or nitrogen on suppression of solution of 3N-Mg in HBSS (+), since magnesium reacts easily and simultaneously with oxygen then MgO should be formed on its surface.

4.2 Precipitations of magnesium apatite on the specimen surface during immersing in HBSS (+)

Figure 6 shows a comparison of the specific mass gain of 3N-Mg heat-treated at 803 for various time as functioned with cumulative immersing time and immersing mode, that is, in the first mode and second mode type the specimens were heat-treated at 803 K for 32.4 ks (9 h) and 90 ks (25 h), respectively, and then immersed continuously in HBSS (+), while in the third and forth type the specimens were heat treated at 803 K for 32.4 ks (9 h) and 90 ks (25 h), respectively, and then immersed in HBSS (+) with exchanging the solution every 360 ks (100 h).

It was clearly shown that the mass gain increased in the specimen heat treated for 90 ks during immersing without exchanging solution though all specimens shown in Fig. 6 were also rinsed for twice 0.6 ks in acetone using an ultrasonic cleaner.

Figure 7 shows XRD profile of the marked specimen in Fig. 6, which was immersed in HBSS (+) for 7 times of 360 ks (100 h). There are some peaks after apatite and metallic magnesium. However, metallic magnesium peaks, for ex-

Fig. 6 Comparison of ratio of change of mass of 3N-Mg heat-treated at 803 for various time as functioned with cumulative immersing time and immerse mode.

Mark	Heating time, t/ks	Immerse mode
Δ	32.4	Exchanging HBSS (+) for every 3.6 Ms
$\blacktriangle$	32.4	Continuous immersion
$\bullet$	90	Exchanging HBSS (+) for every 3.6 Ms
$\circ$	90	Continuous immersion

Fig. 7 XRD profile on a precipitation on the 3N-Mg heat-treated at 803 K for 90 ks (25 h). $\odot$: Apatite [JCPDS, 74-0565], $\circ$: Mg [JCPDS, 04-0829], Δ : MgO [JCPDS, 35-0821], $\times$: MgCl_2 [JCPDS, 37-0774].

Table 3 Chemical composition on the precipitation from HBSS (+) on 3N-Mg by EDX (mass%)

Specimen conditions of annealing and immerse		Mg	Ca	P	Cl
803 K-25 h	700 h Cumulative immerse for 100 h	6.69	64.35	28.96	None

ample around $2\theta = 34.4$ deg. can be detected due to x-ray penetration. No peaks after magnesium chloride (MgCl_2) could be identified as the reason as the result of chemical analysis by EDX as shown in Table 3, which shows chemical composition of the precipitated apatite.

Standing on these facts, it can be qualitatively thought about a mechanism of the increase of mass as the follows:¹⁵⁾

- (1) The heat treatment of 3N-Mg in atmosphere forms a "reacted layer" or "effected layer" on all the surfaces. Besides, the layer has enough strength for fracture induced by ultrasonic vibration energy though almost MgO powder should be removed from the specimen surface by ultrasonic cleaner.

Table 4 Kinds of ions in apatite as $M_{10}(PO_4)_6X_2$

Position	Occupied atoms
M	Ca^{2+} , Na^+ , H^+ , Ba^{2+} , Pb^{2+} , Zn^{2+} , Mg^{2+} , Fe^{2+} , Mn^{2+} , Al^{3+} , and so on
X	OH^- , F^- , Cl^- , Br^- , O^{2-} , CO_3^{2-} , and H_2O

- (2) Chemical reaction rate of the layer for solving out into HBSS (+) is small.
- (3) Magnesium apatite is precipitated on the specimen surface due to absorbed Ca^{2+} and PO_4^{3-} from HBSS (+).
- (4) Precipitated apatite, which contents magnesium ion suppresses solution of 3N-Mg substrate.

In general, chemical formula of apatite can be written as $M_{10}(PO_4)_6X_2$ and many ions can occupy the positions of M and X as shown in Table 4.¹⁶⁾

Chlorine ion in HBSS (+) tends to occupy X site of apatite as dissolve ion rather than formation of $MgCl_2$ as shown in Fig. 7, though chlorine is rarely detected on the precipitation. Then, a chemical formula can be written as $(Ca_{0.86}, Mg_{0.14})_{10}(PO_4)_6(OH)_2$ from calculation based on the results as shown in Table 3 and Fig. 7, under some assumptions in which mole ratio of Ca/P was kept constant at 1.67 and both hydrogen and oxygen are always supplied sufficiently to the precipitation from HBSS (+). However, the result of $(Ca_{0.86}, Mg_{0.14})_{10}(PO_4)_6(OH)_2$ does not agree with an analysed chemical composition as shown in Table 3. A ratio of $(Ca + Mg)/P$ is equal to 2.01 bigger than 1.67, since the electron beam at 25 keV would penetrate to the 3N-Mg substrate throughout the precipitation of magnesium apatite.

Such chemical behavior of 3N-Mg seems to be beneficial to prepare an artificial bone or other implants made of magnesium. An artificial bone made of magnesium is possible to be safely used, since magnesium apatite is introduced instead of original metallic magnesium, which fades out. Furthermore, the precipitation of magnesium apatite can't distinguish from natural bone, since magnesium bone would be harmonized with natural bone. Accordingly, magnesium is possible to apply to a biomaterial in the near future, especially to enable to homogenize with natural bone.

5. Conclusions

To examine a possibility of magnesium as a biomaterial, chemical behavior of commercial grade of pure magnesium (3N-Mg) is examined by immersing in Hank's solutions with Ca and Mg HBSS (+). Moreover, heat treatment process is also examined to improve the chemical behavior of 3N-Mg.

The main results are shown as the followings:

- (1) Magnesium apatite precipitated on the heat-treated 3N-Mg specimens during immersing 3N-Mg in HBSS (+).
- (2) A chemical formula of a magnesium apatite can be written as $(Ca_{0.86}, Mg_{0.14})_{10}(PO_4)_6(OH)_2$.
- (3) Heat treatment of 3N-Mg is considered to homogenize it and to enhance precipitation of magnesium apatite. And its condition is recommended in the range of the present study as follows:
Temperature: 803 K. Heat treatment time: 90 ks.
- (4) The modified layer didn't destroy by ultra-sonic rinsing in acetone for twice 0.6 ks.
- (5) A possibility is suggested to prepare an artificial bone or other implants, besides a homogenized artificial bone material.

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