In vitro effect of magnesium inclusion in sol–gel derived apatite

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Abstract

Magnesium-containing apatite coatings were prepared on Ti6Al4V substrates by sol–gel dip coating method. Standard simulated body fluid (SBF) was used to evaluate the bioactivity of the coatings. A series of the coatings according to the composition (Ca_{10-x}Mg_x(PO_4)_6(OH)_2, where x=0 to 2, is synthesized and immersed in the standard SBF for periods of 7 to 35 days for direct deposition of apatite layer from the SBF solution. Scanning electron microscopy (SEM) was used to examine the morphology changes of the SBF apatite layer that occurred during in vitro immersion. X-ray diffractometry, Fourier Transformation Infra-Red Spectroscopy and X-ray Photoelectron Spectroscopy were used to analyse the phases, chemical groups and composition of the sol–gel coating. Results show that as the sol–gel coating contains magnesium, this promotes deposition of apatite layer from SBF. As x ≤ 1, SBF immersion gives rise to a dense apatite layer. However, as x ∝ 1, selected dissolution of the deposited layer takes place, which results in serious pitting on the surface. Also, Mg ions from the dissolution of the sol–gel coating during immersion in the SBF apparently played a role in the subsequent deposition of apatite on the coating, evidence of Mg was found in the apatite layer.

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1. Introduction

The application of apatite coatings on titanium alloy substrates has been widely studied for clinical applications due to their superb osteointegration properties[1–3]. Hydroxyapatite (Ca_{10}(PO_4)_6(OH)_2, HA) and β-tricalcium phosphate (β-Ca_3(PO_4)_2, β-TCP) are most popular because they can form chemical bonds directly with bone tissues without the intervention of soft tissues[4,5].

However, coatings like pure HA can dissolve while β-TCP has high dissolution rate in body fluid which affects the long-term stability. Addition of fluorine has been found effective in retardation of the coating dissolution in SBF[6,7]. Substitution of cations like K⁺, Na⁺, Zn^{2+}, Mn^{2+} and Mg^{2+} in the apatite structure to mimic natural bone has received much attention[8–11] to take advantage of their roles in bone metabolism, promoting catalytic reaction and controlling biological functions. Undoubtedly, magnesium is one of the most important bivalent ions associated with biological apatites[12]. Okazaki et al. [13] studied the crystallographic behavior of fluoridated hydroxyapatite (FHA) containing Mg^{2+} and CO_3^{2−} ions and found that both ions caused decrease in crystallinity of the FHA. Fanovich et al.[14] sintered hydroxyapatite with low concentrations of Mg^{2+} at 1200 °C and established that addition of 1% magnesium improves the microstructure and the microhardness of the samples. Too much Mg addition causes MgO agglomeration, resulting in a diminution in microhardness. Kannan et al. [15] prepared magnesium whitlockite (β-TCP) via aqueous precipitation and found that the β-TCP powders had higher density and contracted lattice constants and unit cell volumes with respect to that of pure β-TCP powders. Suchanek et al. [16] synthesized magnesium-substituted hydroxyapatite powders by the mechanochemical–hydrothermal method with Mg substitution levels as high as 28.4 wt%.

The essential requirement for a bioactive material to bond to living bone is the formation of bonelike apatite on the material surface in the living body[4]. The in vivo apatite formation can be
reproduced in a simulated body fluid (SBF) [17,18]. The bio-
activity of sol–gel derived magnesium apatite coatings on
Ti6Al4V substrate in simulated body fluid has not been sys-
tematically assessed. Apatite coatings of different magnesium
contents were soaked in SBF solution in this study to elucidate the
precipitation of the bone-like apatite on the coatings.

2. Experimental procedures

2.1. Coating preparation

Calcium nitrate tetrahydrate (Ca(NO\textsubscript{3})\textsubscript{2}·4H\textsubscript{2}O, Sigma-
Aldrich, AR), phosphorous pentoxide (P\textsubscript{2}O\textsubscript{5}, Merck, GR) and
magnesium nitrate hexahydrate (Mg(NO\textsubscript{3})\textsubscript{2}·6H\textsubscript{2}O, Sigma-
Aldrich, AR) were selected to prepare Ca-precursor, P-precursor
and Mg-precursor. A designed amount of Mg-precursor and Ca-
precursor was mixed to form the Ca\textsubscript{10−}\textit{x}Mg\textsubscript{x}(PO\textsubscript{4})\textsubscript{6}(OH)\textsubscript{2}, where
\textit{x}=0/2, 1/2, 2/2, 3/2, 4/2. The subsequent coatings were labeled as M0, M1,
M2, M3 and M4 respectively.

Titanium alloy (Ti6Al4V) substrate (20×30×1.2 mm\textsuperscript{3}) was
dipped vertically into the sol and withdrawn at a speed of 3 cm/
min at the first dip to allow formation of a thin but good bond
layer. The sol-coated substrate was then immediately transferred
to an oven at 150 °C to dry for 15 min followed by firing at
600 °C for 15 min. To reach a coating thickness of about 2 μm,
dipping–drawing–drying–firing process was repeated 4
times at a new drawing speed of 4.5 cm/min to allow thicker
coating at each dipping.

2.2. In vitro test in standard SBF solutions

The standard SBF solution was prepared according to
Kokubo’s protocol [19] by dissolving appropriate quantities of
the relevant reagent-grade chemicals in deionized water: NaCl,
NaHCO\textsubscript{3}, KCl, K\textsubscript{2}HPO\textsubscript{4}·3H\textsubscript{2}O, MgCl\textsubscript{2}·6H\textsubscript{2}O, HCl (1 M),
CaCl\textsubscript{2}, Na\textsubscript{2}SO\textsubscript{4} and NH\textsubscript{2}C(CH\textsubscript{2}OH)\textsubscript{3}. Reagents were added,
one by one after each reagent was completely dissolved in the
deonized water according to the order and amount given in
Table 1. After all the reagents were added and dissolved, the
solution was then raised to 37 °C and maintained at this tem-
perature while titrating the solution to a pH of 7.4 with 1 M HCl
solution. The inorganic ion concentrations in the standard SBF
solution are close to human blood plasma.

The coatings were placed in sterilized bottle containing
solution with a liquid/area ratio of 50 ml/cm\textsuperscript{2}. Before soaking in
the solution, the samples were washed ultrasonically in acetone
for 10 min and then sterilized in ethanol. The soaking bottles
were kept for various periods of 7, 10, 15, 20, 25, 30, and
35 days at 37±0.1 °C. After immersion, the samples were taken
out, gently washed with deionized water and dried at room
temperature before characterization.

2.3. Coating Characterization

The surface morphology of the coating was observed using
Scanning Electron Microscopy (SEM, JEOL JSM-5600LV). The
phase characterization was conducted by X-ray diffraction anal-
ysis (XRD, Philips, PW1830) using monochromatic CuKα
radiation at a step size of 0.02°. Analysis of chemical groups was
conducted using Fourier Transformation Infra-Red Spectrosco-
py (FTIR) at a Bio-Rad FTS-3000 spectrometer in the range of

Table 1
Reagents for preparing SBF (pH 7.40, 1 L)
<table>
<thead>
<tr>
<th>Reagent</th>
<th>Amount</th>
<th>Order</th>
</tr>
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<tbody>
<tr>
<td>NaCl</td>
<td>7.996 g</td>
<td>1</td>
</tr>
<tr>
<td>NaHCO\textsubscript{3}</td>
<td>0.350 g</td>
<td>2</td>
</tr>
<tr>
<td>KCl</td>
<td>0.224 g</td>
<td>3</td>
</tr>
<tr>
<td>K\textsubscript{2}HPO\textsubscript{4}·3H\textsubscript{2}O</td>
<td>0.228 g</td>
<td>4</td>
</tr>
<tr>
<td>MgCl\textsubscript{2}·6H\textsubscript{2}O</td>
<td>0.305 g</td>
<td>5</td>
</tr>
<tr>
<td>1 M–HCl</td>
<td>35–40 ml</td>
<td>6</td>
</tr>
<tr>
<td>CaCl\textsubscript{2}</td>
<td>0.278 g</td>
<td>7</td>
</tr>
<tr>
<td>Na\textsubscript{2}SO\textsubscript{4}</td>
<td>0.071 g</td>
<td>8</td>
</tr>
<tr>
<td>NH\textsubscript{2}C(CH\textsubscript{2}OH)\textsubscript{3}</td>
<td>6.057 g</td>
<td>9</td>
</tr>
</tbody>
</table>

Fig. 1. SEM micrographs of the sol–gel derived pure HA coating soaked in SBF for a) 10 days b) 35 days.
4000 – 400 cm^{-1} in transmission mode with a resolution of 4 cm^{-1}. The composition of the apatite precipitated from the SBF was determined by X-ray Photoelectron Spectroscopy (XPS, Kratos-Axis Ultra System) using monochromatic Al K\textsubscript{\alpha} X-ray source (1486.7 eV).

3. Results and discussion

3.1. The morphologies of the apatite layer precipitated from the SBF

Fig. 1 shows the characteristics of HA surface after soaking in SBF for 10 and 35 days (Fig. 1a and b respectively). After 10 days, a new apatite layer (the “secondary coating”) is formed on top of the HA coating surface. However the apatite layer does not covered the original HA surface completely. After 35 days, the apatite layer densify (Fig. 1b). Morphologies of magnesium apatite coatings after 10 days in SBF are shown in Fig. 2. The amount of magnesium incorporation was found to have an impact on the secondary coating formation. For samples M1 and M2, the apatite layer is dense and compact, and with many nuclear boundaries. On M3, small holes formed on the surface and on M4, the holes are obvious and much larger than that on M3.

Compared with the pure HA primary coating (c.f., Fig. 1) or with increasing level of Mg incorporation, increase in “nuclear” sites is observed. In atomic structure, hydroxyapatite builds

| Table 2 | Molar concentration of Ca, P and Mg in the sol–gel derived coatings |
|--------------------------|--------------------------|--------------------------|--------------------------|--------------------------|--------------------------|
| x in sol                 | M0 | M1 | M2 | M3 | M4 |
| x in coating             | 0  | 0.5| 1.0| 1.5| 2.0|
| Ca/P                     | 1.67| 1.57| 1.50| 1.46| 1.60|
| (Ca+Mg)/P                | 1.67| 1.66| 1.67| 2.41| 3.20|

Fig. 3. Cross-section morphology of M2 in SBF after 35 days.
up with a central Ca(OH)$_2$ and three surrounding Ca$_3$(PO$_4$)$_2$ groups. When magnesium is incorporated, the central calcium atom is substitute by magnesium [11]. Since the ionic radius of Mg$^{2+}$(0.69 Å) is considerably shorter than that of Ca$^{2+}$(0.99 Å) [20], substitution of Mg$^{2+}$ into Ca$^{2+}$ position distorts the HA structure and the structure more resembles $\beta$-TCP. To denote the existence of Mg, it is often written as $\beta$-TCMP (where M stands for Mg), which is also a kind of whitlockite [20–22].

As $x=0.5$ (sample M1), both HA and $\beta$-TCMP phases co-exist in the primary coating. The secondary coating on M1 exhibits characteristics of both M0 and M2. The secondary coating is smooth, dense and compact. As $x \gg 1$, however, on the surfaces of M3 as shown in Fig. 2, serious “pitting” indicates severe dissolution. This becomes even more severe at $x=2.0$ (sample M4). The pores are distributed uniformly over the whole surface. The increase of dissolution rate on M3 and M4 is consistent with the higher magnesium content (Table 2) (as compared with that designed in the sols).

Prolonged soaking of the $\beta$-TCMP in SBF promotes the deposition of apatite layer. The typical cross-section morphology of M2 in SBF for 35 days (M2) is shown in Fig. 3. It shows that about 2 μm thick secondary coating is formed on the sol–gel derived primary apatite layer. The secondary coating is dense and uniform, no pores and voids are observed. Fig. 4 shows the surface morphology of the secondary coating formed on M1 and M2 after 35 days in SBF. The nucleation of the new apatite on the former formed surface can be clearly seen. The phenomenon of the growth of secondary coating indicates that the incorporation of magnesium in the apatite structure to form $\beta$-TCMP has good biological response in SBF.

3.2. Phase, group and composition of the apatite coatings after SBF soaking

Typical XRD patterns of the apatite coatings before and after soaked in SBF are shown in Fig. 5. There are three phases observed in the patterns: the substrate (open circles), the HA (solid circles) and the $\beta$-TCMP (solid star). Before SBF immersion, the HA peaks on M0 and $\beta$-TCMP peaks on M2 are prominent. After 35 days immersion, the similar crystal apatite peaks can be observed on the samples. These results indicate
that the newly formed “secondary coating” keeps the same crystal phase as the “primary coating”.

Fig. 6 shows typical FTIR profile of apatite coatings after 35 days soaking in SBF. The coatings have similar chemical groups: PO$_4^{3-}$, CO$_3^{2-}$ and OH$^-$, typical chemical groups of carbonate apatite structure. The FTIR profile of M0 has a large H$_2$O peak that can not be observed on other patterns. The depletion of H$_2$O in the magnesium apatite samples indicates that the magnesium in the primary apatite coatings can affect the growing process of new apatite in the SBF solution. It follows that the secondary coating is denser (as is observed in Fig. 3).

The compositions of the apatite deposition after 35 days soaking in SBF are analyzed with XPS and the typical survey scan spectra on M1 are shown in Fig. 7. Since XPS affects only about 2 μm thick Fig. 3, therefore the XPS results give the compositions of the secondary coatings: Ca, P, O and C together with a small peak for Mg. (This does not happen in the gel derived apatite) contains Mg, the secondary coating (i.e., the apatite deposition directly from SBF) will then contain Mg. Therefore, incorporation of magnesium in the sol–gel derived apatite promotes inclusion of magnesium in the SBF precipitated apatite.

4. Conclusion

The incorporation of magnesium in the sol–gel derived apatite (Ca$_{10-x}$Mg$_x$(PO$_4$)$_6$(OH)$_2$) promotes deposition of apatite layer directly from simulated body fluid (SBF). As $x \leq 1$, deposition from SBF gives rise to dense apatite layer. However, as $x \gg 1$, selected dissolution of the deposited layer takes place, which results in serious pitting on the surface.

The incorporation of magnesium in the sol–gel derived apatite promotes inclusion of magnesium in the SBF precipitated apatite.

Acknowledgements

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References


Fig. 7. Typical XPS survey scan spectra of the SBF-derived apatite layer on M1 (soaked in SBF for 35 days). (Inset: narrow scan spectra of magnesium).