Surface Construction Of Zn Doped Hydroxyapatite/mgf2 Multilayer Coatings For The
Biomedical Mg Materials By A Novel Conversion/sol-gel Method

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Introduction:
Mg alloy is now an attractive biodegradable material especially in orthopedic and cardiovascular
applications. It also has similar physical and mechanical properties with natural bone. However, it has
high corrosion rate in physiological fluid and might easily lost its function as a restoration material in the
human body.
In general, alloying and coating are two common methods to improve its poor corrosion resistance. Sol-
gel method is the most commonly used coating method because it can be easily handled, and the most
important thing is that it is easy to incorporate with other elements during the solution process.
Hydroxyapatite (HA) is believed to enhance the interaction between the bone tissue and the implant. In
addition, some research also said that Zn increases the biocompatibility of HA, which then increase the
activity of osteoblast in bone growth.
Sol-gel coatings are well studied for titanium-based orthopedic implants or scaffolds and generally result
in porous, nano-crystalline, and thin to thick coatings with controlled microstructure at relatively low
processing temperatures suitable for Mg substrates (m.p.=650oC). This article presents the preliminary
results on a novel procedure to create a functional multilayer, and also evaluates the in-vitro corrosion
behavior and biocompatibility.

Methods:
A. Mg material and pretreatment process
Pure Mg (99.98%) was used as starting material, the specimens were immersed in HF for 12 hours at
room temperature. On account of this process, the specimens are called as fluoride pre-treated
specimens (FP).
B. HA and ZnHA coatings manufacture
All the calcium phosphate samples were prepared by a sol-gel method which was performed in the four
basic steps including stirring, gelation, drying and sintering. In this experiment, all solutions used were
controlled at 0.5 M. Two precursors, P2O5 and Ca (NO3)2 • 4H2O were produced by dissolving each
source in C2H5OH. The precursors were mixed by titration and fixed Ca/P atom ratio at 1.67. The pH
value of mixed Ca/P solution was fixed at pH 8 by adding NH4OH, and this sol-gel was called as HA
solution. Calcium phosphate sol-gel solutions were coated on Mg substrate using a spin coating
machine, and then calcination step was done using the vacuum furnace at 500 oC for 2 hours.
C. Material characterizations
Calcium phosphate sols and HA coated Mg had been analyzed using XRD, SEM and EDS to get
information about phase compound, and also influenced zinc addition in the calcium phosphate sol-gel.
D. in-vitro degradation test
The electrochemical test was performed in a corrosion cell that contained 350mL of r-SBF at 37 °C. The measurements were performed using a conventional three-electrode set up where 1 cm² exposed surface area was placed in r-SBF solution as working electrode, the reference electrode was an Saturated Calomel Electrode, and the counter electrode was the platinum plate. Tafel’s extrapolation method was applied to determine the corrosion current density (Icorr) and polarization resistance (RP) with scan rate 1 mV/sec.

In-vitro degradation behavior was evaluated by immersion test. All the specimens had been immersed in r-SBF solution for 1, 3, 7 days at 37 °C and the weight loss were measured. The r-SBF solution was changed every 2 days to keep the pH value stable.

E.Cell culture and in-vitro cytotoxicity test

The cytotoxicity tests were carried out by indirect method using MG-63 osteoblast-like cell following ISO 10993-5. After incubating the cells for 1, 3 and 6 days, The MTS solution was then added to each 96-well and incubated for 4 hours. The absorbance of the MTS-treated solution was measured by ELISA reader at 490 nm. After the experiment, the cell relative growth rate (RGR) was calculated according to the following formula: RGR=[(ODtest-ODblank)/(ODnegative-ODblank)]x100%

Results:
A. The effect of Zn content on crystallinity of CaP sols
Zinc doped to calcium phosphate cause the crystalline phase changes, some of hydroxyapatite peak change to apatite, forming chemical composition [Ca10Znx(PO4)6(O2x(OH)2-2x)]. And it can also change to other calcium zinc phosphate compounds at 27.94o, 29.34o, and 31.16o. Moreover, some new peaks appear by increasing zinc contents to 5% and 10% of calcium phosphate sol-gel that are apatite compounds at two theta 49.9o and calcium zinc phosphate compound at 34.48o and 36.22o. Interestingly, when the percentage of zinc were 0.5% and 1% the intensity of tricalcium phosphate (2θ ~ 29.34o) was increased, and by adding 5% and 10% of zinc which the crystalline phase of TCP disappeared being changed with calcium zinc phosphate. Details can be seen on Fig.1A

B.Interfacial properties
The continuing line of passive layer (MgF2) is obvious on the substrate of FP. The percentage retention on the substrate after the peeling-off test has been measured that FP-ZnHA is about 83.3% and 18% for FP-HA. High residual deposited on the substrate confirmed good adhesion zinc-doped calcium phosphate on Mg phosphate as shown in Fig.1B.

C.in-vitro corrosion assessment
Fig.2A shows that Zn doped coating can improved corrosion resistant. Indeed, 5% zinc doped HA has slightly higher corrosion resistant than HA. Immersion test of the treated and untreated Mg specimens in SBF solution (Fig.2B) shows different corrosive effect of the specimen by time immersion (1, 3, and 7 days). HA coated specimens has adverse effect in r-SBF solution and the worst group was FP-HA which shows lots of weight loss compared to the other groups at 7th immersion day. ZnHA coated surface, according to electrochemical test and corrosion rate shows better achievement than HA coated surface.

D.in-vitro cytotoxicity test
Fig.3 shows the viability of MG63 cells expressed as a percentage of the viability of cells cultured after cultured in extracting of Mg, FP, FP-HA and FP-ZnHA specimens for 1, 3, and 7 days culture. It can be seen that Mg substrate without treating extracting medium shows lower Relative Growth Rate (RGR), and the FP-ZnHA shows high percentage of RGR value.
**Discussion:**
Zinc-doped calcium phosphate increased the casting ability on the substrate (refer to Figure 3.) and also produced a denser layer than undoped calcium phosphate layer. Precipitation of calcium phosphate on Mg phosphate tended to have a granular deposition. The zinc-doped calcium phosphate prefer smaller grain size compare to the undoped layer. The refined particles, therefore, produced a more compact layer than the coarse grain particles. As a result, the compact layer will give higher protection against the corrosive medium. Moreover, zinc ion is more important than calcium in bone formation because zinc regulates the level of circulating IGF-1 to stimulate osteoblast. This experiment confirmed that zinc-doped calcium phosphate enhanced the viability of MG63 cells.

**Significance:**
As a biodegradable implant, the corrosion rate of Mg has to be controlled to match the wound healing rate at implant site. In the present study, surface conversion using HF and coated Mg surface with zinc-doped calcium phosphate can reduce the corrosion rate of pure Mg and also increase the cell viability of MG63.
Figure 2A. Potential Dynamic curves
Figure 2B. Corrosion rate of magnesium in r-SBF
Figure 1A. XRD spectra of Zn-doped Calcium Phosphate calcinated at 500°C
Figure 1B. Cross-section and Peeling-off Test
Figure 3. Cytotoxicity test

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