Study on the in vitro degradation behavior of pure Mg and WE43 in human bile for 60 days for future usage in biliary

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Abstract
Mg and its alloys have been tested in both bone and blood environment, but the degradation process in human bile have not been reported yet. In this case, commercial pure Mg and WE43 alloy were investigated in human bile to see the corrosion performance as potential biliary stent materials. The results revealed the weight loss of WE43 after 60 days immersion was only 1.87%. Moreover, WE43 performed a superior corrosion resistance to pure Mg, owing to more formation of the secondary reaction product Mg(H2PO4)2. The formation of H2PO4/CO3 instead of PO4/CO3 may be due to the fact that the compositions in the bile can prevent the formation of PO4/CO3 as it is the main composition of gallstone. The much slower corrosion rate of the WE43 alloy in human bile indicated that it may be good candidates as biliary stent materials.

1. Introduction
Benign biliary strictures are common results from cholecystectomy. In general, two therapies, including surgical and endoscopic, were adopted to cure the strictures. Endoscopic stenting provides options for those who cannot tolerate surgery procedure. So far, polymeric stents, either permanent or degradable, were reported to perform displacement and lack of strength [1,2]. Biodegradable Mg and its alloys have attracted increasing attention both as orthopedic implants and coronary stents [3,4]. Mg-based DREAMS 2G coronary stents have been reported a comparable clinical performance to biodegradable polymer stents [5]. Mg and its alloys may be the new kind of biodegradable materials for bile duct stent. Despite it was reported that only 9% residual weight of Mg-6Zn after 3 weeks implantation in the common bile duct (CBD) of rabbits [6], previous researches have demonstrated a quick degradation of the material in bone environment and also deviation of in vitro and in vivo tests [7]. Moreover, the degradation process of Mg and its alloys in human bile is still uncertain.

In this study, as-extruded WE43 alloy with pure Mg as control were immersed in fresh human bile for 60 days to investigate the corrosion performance. The in vitro degradation behavior were characterized, which may provide guidance for further application of Mg and its alloys alloy as bile duct stent.

2. Materials and methods
Commercial pure Mg (99.95%) and as-extruded WE43 samples (Ø10 mm × 2 mm) were machined, mechanically polished up to 2000 grit, ultrasonically cleaned in acetone, absolute ethanol and distilled water, and then sterilized by ultraviolet. Human bile from T-tube drainage were donated by patients undergoing cholecystectomy with exploration in Peking University People's Hospital.

The samples were weighed prior to immersion tests. After that, the sterilized samples were immersed at 37 °C according to ASTM-G31-72. The pH value of the human bile and the hydrogen evolution were monitored during the first 20 days of immersion test. At 3, 10 and 60 days, the samples were collected from bile, rinsed with distilled water, dried in open air. The corrosion products were characterized by XRD (DMAX 2400, RIGAKU). Changes on surface morphologies were observed with ESEM coupled with EDS (Quanta 200FEG, FEI). The samples were weighed and weight loss was calculated before and after totally eliminating the corrosion products by chromic acid (200 g/L). Mg2+ concentration were investigated with ICP-AES (Inductively Coupled Plasma-Atomic Emission Spectrometer, LEEMAN). Five immersion duplicates were used.

3. Results and discussion
Fig. 1(a) shows the hydrogen evolution from the human bile
incubating pure Mg and WE43. WE43 exhibited a faster hydrogen evolution rate during the first 2 days, which might result from the multi-phase structure. With time prolonging, less hydrogen were generated from WE43 during the determined process. Moreover, pH value monitoring confirmed that the pH value of human bile without samples started at 7.69 and remained relatively stable with slight fluctuation around 7.6 during the first 20 days. Pure Mg and WE43 performed a similar trend where the pH value decreased during the first 10 days and then increased during the following 10 days. The decrease of pH value may be due to bacteria activity in the human bile since some patients may have bile reflux due to the abnormal of pyloric sphincter. The lower pH increment of WE43 also indicated a slower degradation rate compared to pure Mg.

Fig. 1(c) and (d) exhibit the weight loss and Mg$^{2+}$ concentration after 60 days immersion tests. Less weight loss was gathered from WE43 than pure Mg immersed in human bile after 3, 10 and 60 days immersion. After 60 days immersion, the weight loss

![Fig. 1.](image1.png)

![Fig. 2.](image2.png)
percentage of WE43 was just 1.87 ± 0.04%, which is significantly lower than pure Mg. Moreover, the calculated corrosion rate through weight loss after 60 days immersion were only 0.074 ± 0.002 mm/y and 0.1296 ± 0.0002 mm/y for WE43 and pure Mg, respectively, which are significantly lower than immersed in Hank's solution or SBF [8,9]. The Mg2+ concentration of WE43 after 60 days immersion was significantly lower than pure Mg.

Fig. 2 shows the surface morphologies and EDS results of pure Mg and WE43. Both pure Mg and WE43 were covered by relatively uniform corrosion product after 60 days immersion. Moreover, the corrosive effects caused by human bile seemed to be less damaged than SBF [10], which indicated that Mg and its alloys may corrode slower in human bile environment compared with other kinds of body fluids. EDS results demonstrated high concentration of C and O, which may be the organics adsorbed by the surface. Thus there might be compositions adhered and aggregated on the surface, which form a rather solid layer to protect the materials from further corrosion.

Fig. 3 illustrates the XRD patterns of both pure Mg and WE43 alloy before and after 60 days immersion in human bile. WE43 alloy was composed of α-Mg, Mg41Nd5 and Mg24Y5 before tests. After 60 days immersion, Mg(H2PO4)2 instead of common Ca/P corrosion products was detected on the surface of both pure Mg and WE43 alloy, with higher intensity detected on WE43 alloy.

Fig. 4 illustrates the different corrosion mechanisms between Hank’s solution and human bile for pure Mg and WE43. Former literature has demonstrated H2PO4− reduced the corrosion rate of WE43 alloy in SBF [11]. Common human liver bile consists of 97.5 g/dl water, 1.1 g/dl bile salts, 0.04 g/dl bilirubin, 0.1 g/dl cholesterol, 0.12 g/dl fatty acid, 0.04 g/dl lecithin, 145 mEq/L Na+, 5 mEq/L K+, 5 mEq/L Ca2+, 100 mEq/L Cl− and 28 mEq/L HCO3− [12]. Meanwhile, Ca3(PO4)2 was thought to be one of the main composition of gallstone. However, the relatively stable composition especially bile acid and lecithin help prevent the formation of gallstone [13,14]. That may be the reason why Mg(H2PO4)2 instead of Ca3(PO4)2 was formed. Moreover, Mg(H2PO4)2 may be a secondary reaction product. First, Mg dissolved in human bile and generated hydrogen, either with H+ in the weak acid environment attributed to bacteria activities or H2O in the alkaline environment. Then the pH of the bile increased and the bacteria activity was inhibited. The phosphates in human bile then reacted with Mg2+ under the inhibition of gallstone and lead to the formation of Mg(H2PO4)2. In addition, the H2PO4− may also result from complex enzymatic reaction of lecithin, which need to be further investigated.

Previous researches have demonstrated a minimum of 60 days of duration was needed to provide mechanical integrity as well as avoid early postoperative strictures. Our in vitro results indicated good corrosion resistance of WE43 after 60 days immersion in static human bile, which is eligible for biliary application. Further work will concentrate on the in vivo evaluation of Mg alloy as biliary stents.

4. Conclusions

In this study, a 60 days immersion test in human bile under the consideration of minimum duration to provide mechanical support was adopted for pure Mg and WE43 was conducted. Hydrogen evolution test revealed less evolved hydrogen from WE43. After 60 days immersion, the weight loss percentage of WE43 was only 1.87%. The reduced corrosion rate may be attributed to Mg(H2PO4)2. The Mg(H2PO4)2 instead of Ca/P was thought to be the second reaction product, due to gallstone prevention caused by the bile acid and lecithin in human bile. Based on the above facts, WE43 showed good in vitro corrosion resistance in human bile.

Fig. 3. X-Ray diffraction patterns of pure Mg and WE43 alloy before and after 60 days immersion.

Fig. 4. Schematic illustration on corrosion mechanisms of Mg alloys in Hank's solution based on our previous study [8] (a) and human bile in this study (b).
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