Corrosion and Biocompatibility Improvement of HA-Coated Magnesium-Based Alloys as Bone Implant Materials

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***Abstract* — Magnesium alloys attract great attention as prospective bone implants due to their biocompatibilities, physical properties and an ability to degrade completely under physiological conditions, what eliminates the need for surgical reintervention. The main problem of developing advanced Mg alloys for medical application is matching degradation with tissue healing rate. Orthopaedic metallic implants should maintain its mechanical property for at least 3 month to avoid the second fracture occurrence resulting from their fast degradation.**

The purpose of this research was assessment of in-vitro corrosion and surface morphology after short term in-vivo implantation of Mg based implant covered by HA.Mg alloys with the addition of Zr(0,65%), Al(1,85%) and Nd(1,25%) were used.

Ca–P-based coatings were used to improve the corrosion resistance of magnesium and its alloys as well as their surface bioactivity. Hydroxyapatite (HA) coatings were obtained on Mg alloy substrates by dipping method.

Simulated body fluid (SBF; pH 7,4) with ion concentrations approximately equal to those of human blood plasma resembling physiological conditions and citrate buffer with pH 5 - simulating inflammation were selected as modeling environments for in-vitro degradation test. The rod samples were implanted into the tibia bone of rats and after 1 and 5 days of implantation were taken out to observe cells adhesion on surface samples. SEM was used to assess surface morphology after *in-vitro* and *in-vivo* tests.

We determined different mechanisms of HA layer corrosion - SBF solution causes the partial dissolution, while citrate solution caused complete disappearance of the coating. HA coated layer coused lower degradaion without significant pH change during the static immersion test in SBF and citrate buffer. The HA coating favored cell adhesion and rapid fibrous tissue formation.

Keywords — Mg alloy; corrosion; HA-coating; implantation

I. INTRODUCTION

Nowadays trauma and orthopaedic surgery cannot be imagined without biomaterials. Bone substitutes, such as biodegradable polymers, metals, ceramics, and bioactive glasses are widely used to facilitate bone fracture healing or to compensate for a lack or loss of bone tissue [1, 2]. Among mentioned kinds of materials, magnesium alloys attract great attention as prospective bone implants due to their biocompatibilities, physical properties and an ability to degrade completely under physiological conditions, what eliminates the need for surgical reintervention. First attempts of applying biodegradable Mg alloys as a material for osteosynthesis were made in 1906 by Lambotte and resulted in clinical failure [3, 4]. Despite the fact that recently considerable researches were performed for creation an ideal orthopaedic metallic implant, there remains a lot of critical factors to solve in this field.

Mg alloys have a number of advantages before other bone substitutes. Mg is an essential element inside human body: it performs a function of cofactor to many enzymes and stabilization of DNA and RNA structure [2]. There are no evidence for toxic effect of Mg in human body and therefore, it could be safety used as a main compound of orthopedic implant [5]. Moreover, some researches show that Mg could have stimulatory effects on new bone formation [6]. Mg alloys have superior mechanical properties: density and Young`s modulus (shows an ability to resist deformation) close to those of natural bone compare with other commercial bone implants [7].

Mg and its alloys have high electronegative potentials and can, therefore, degrade in aqueous solutions via an electrochemical reaction, which produces magnesium hydroxide and hydrogen gas. In physiological conditions where a high chloride concentration exists, Mg alloy present a faster degradation rate, because Mg hydroxide can rapidly convert into highly soluble Mg chloride, which accelerates corrosion of an alloy [7]. Fast and uncontrolled corrosion associated with hydrogen ion release remains a serious problem of Mg alloys

degradation [2,3]. Hydrogen gas, which is formed and accumulated within the surrounding tissues during degradation, lead to inflammatory reactions. Severe pH and osmolarity changes have negative influence on organism in general. In addition, fast resorption of metal implant can lead to mechanical instability before bone healing is completed [2, 3, 8, 9].

Hence, the main problem of developing advanced Mg alloys for medical application is matching degradation with tissue healing rate. Orthopaedic metallic implants should maintain its mechanical property for at least 3 month to avoid the second fracture occurrence resulting from their fast degradation [5, 8].

Many optimization procedures were developed lately for improvement of Mg alloys corrosion resistance. They include alloy composition design [5, 10, 11] and surface modifications [2, 7, 12]. The alloying elements in self-resorbable Mg alloys should be selected not only on the improvement of mechanical properties, but also on the consideration of degradation and biocompatibility [1]. It means that elements with potential toxicological problems should be ideally avoided or only used in minima and in acceptable amounts if they cannot be excluded from the design [5].

Surface modification represents one of the most effective ways not only to reduce and control the degradation behavior but also to improve the surface biocompatibility of Mg-based implants [13]. Currently, a wide range of mechanical, chemical and physical methods are used for surface modification of Mg alloys, including coating creation techniques. Hydroxyapatite (Ca(PO4)6(OH)2, HA) resembles the structure of mineralized bone and therefore, has been widely used as the coating of bone implant materials. It has such properties as excellent biocompatibility, nontoxicity, bioactivity, bone inductivity, and stability [14].

The purpose of this research was assessment in-vitro corrosion and surface morphology after short term in-vivo implantation of Mg based implant covered by HA.

II. MATERIALS AND METHODS

2.1 Materials

Mg alloys with the addition of Zr(0,65%), Al(1,85%) and Nd(1,25%) were used.

Ca–P-based coatings were used to improve the corrosion resistance of magnesium and its alloys as well as their surface bioactivity. Hydroxyapatite (HA) coatings were obtained on Mg alloy substrates by dipping method. Mg-alloy substrates were sequentially washed in acetone and 96% ethanol solutions and triple washed in distilled water. Initial solutions for coating deposition contained 0.1 M $CaCl_2$ and 0.06M Na_2HPO_4. Basic pH is obtained due to partial corrosion of Mg-alloy substrates during immersion in water solution as well as rough surface which is necessary for better coating adhesion. Experimental conditions are described in the table 1

Coating of sample 4 was not uniform and not enough to cover all surface of the sample. The coating obtained at room temperature (22 ºC) need enough time for crystallization so it is more uniform after 24-48 hours of crystallization (samples 1-3). At 50ºC fast coating formation was observed, besides the corrosion rate was also increased. At 30ºC the obtained coatings were the most uniform (samples 5,8,9).

Simulated body fluid (SBF; pH 7,4) with ion concentrations approximately equal to those of human blood plasma resembling physiological conditions and citrate buffer with pH 5 - simulating inflammation were selected as modeling environments.

2.2 In-vitro degradation

4 types of samples were used for the hydrogen evolution test such as Mg-alloy and 5,8,9 with HA coating. The samples were immersed separately in 50 mL of SBF and citrate buffer with an initial pH value of 7.4 ± 0.02 and 5 ± 0.02 in a water bath at 37 ◦C. The immersed samples were placed in fresh solution every day. The pH value of the SBF was monitored every 24 h during the immersion period. Static experiment determines the long-time-dependent changes in the pH level of model solution.

TABLE I. *EXPERIMENTAL CONDITIONS OF HA COATINGS DEPOSITION ON MG-ALLOY SUBSTRATES BY DIPPING METHOD.*

Sample number	Experimental conditions	Δm, g
1	24 h, t=22ºC	0.003
2	24 h, t=22ºC	0.004
3	48 h, t=22ºC	0.01
4	2 h, t=22ºC	0
5	1h, t=30ºC	0.005
6	1h, t=50ºC, polished	0.004
7	1h, t=50ºC	0
8	0.5 h, t=30ºC	0.003
9	1.5 h, t=30ºC	0.001

2.3 In-vivo tes

All animal experiments were conducted according to the ISO 10993-2:1992 animal welfare requirements. The rod samples were implanted into the tibia bone of rats. Implants after 1 and 5 days of implantation were taken out to observe cells adhesion on surface samples.

2.4 Material characterization

To access the surface morphology of Mg implants and for determination of any cell adhesion on it we performed scanning electronic microscopy using REMMA102 (SELMI, Ukraine). To avoid surface charge accumulation, samples were covered with thin (30-50 nm) layer of silver in the vacuum set-up VUP-5M (SELMI, Ukraine).

Samples after in-vivo test were fixed in 2,5% glutaraldehyde solution for 24 hours, then washed with distilled water and dehydrated through ethanol series with increasing concentrations (35%, 50%, 70%, 80%, 95%x2 times, 100%). After samples were dried in desiccators at room temperature conditions, they were mounted on to a SEM sample stub with a double-sided sticky tape.

2.4 Statistic

Data were expressed as means ± standard deviation. Student's t-test on unpaired data was used to assess the statistical significance of the difference. Statistical significance was assumed at a confidence level of 95% ($p < 0.05$).

III. Results and Discussion

3.1 In-vitro degradation test.

Mg-alloy are being destroyed under physiological conditions. The minimal changes of pH values were identified among implants *HA Mg-alloy 9* - 7,49± 0,20 and 5,24± 0,1

	pH₀7,4	***pH₀5***
Mg-alloy	8,47 ± 0,25	5,77 ± 0,58
HA Mg-alloy 5	8,01± 0,15	5,29± 0,11
HA Mg-alloy 8	7,74± 0,11	5,27± 0,13
HA Mg-alloy 9	7,49± 0,20	5,24± 0,1

Table 1 – Hydrogen output levels from different types of Mg-alloy in the static study in 24 hours

Two samples were selected for further study: HA Mg-alloy 9 -through less changes in pH values and Mg-alloy for negative control.

3.2 Material characterization

Fig.1.(a-g) showed the SEM surface images of Mg-alloys and HA-coated implants after one and five days of implantation. After test with SBF Mg-alloys sample surface was coarse and the surface presented a crackled appearance supposed due to fast degradation.

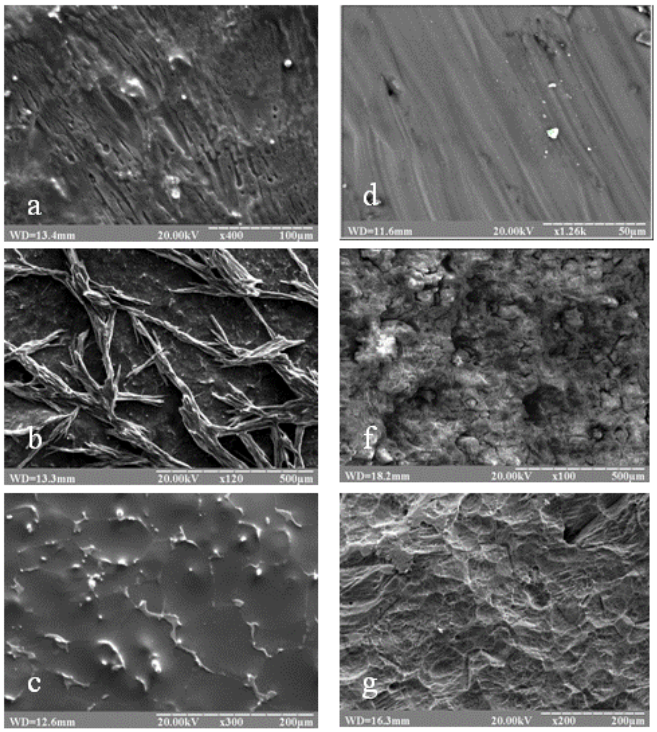

Fig. 1 – SEM images surface of HA Mg-alloys (a,b,c) and Mg-alloys (d,f,g) surface before and after degradation test in 24 hours: before immersion (a,d); after immersion in SBF (b,f), citrate solution (c,g)

For the HA coated sample, the surface was covered by the typical needle-like HA (Fig.1.b). After test with citrate buffer magnesium alloy samples had cellular-like surface. HA-coated sample had a relatively smooth surface (Fig.1.c).

On first day after implantation the surface structure of Mg-alloy samples have changed. The surface of implants was covered with small crack network structure. Adhesion of cells and collagen fibers is visualized on surface. After 5 days of implantation (Fig.2.b), the surface of uncoated sample was destroyed due to severe corrosion. Besides the formation of serious crack, we detected cell migration and tissue development.

Coated samples demonstrate higher amount of cells and collagen fibers. In five days after implantation samples were coated by several layers of connective tissue (Fig.2.d).

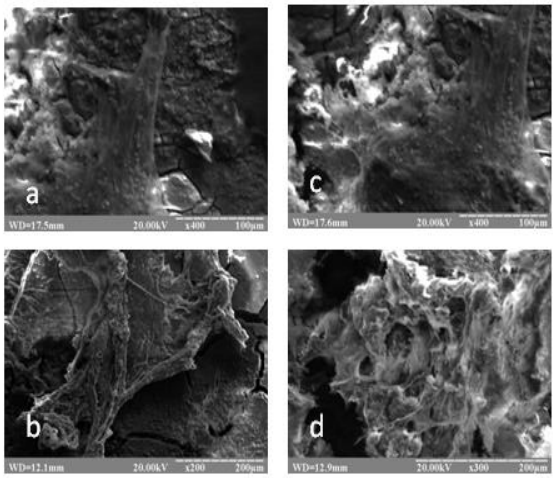

Fig. 2 – SEM images surface of Mg-alloys (a,b,) and HA Mg-alloys (c,d) after one (a,c) and five (b,d) days implantation.

Low or fast corrosion rate might pose problems during in-vitro experiments because gas formation and increase of pH might prevent cell adhesion and proliferation. HA could provide protective layer over metallic surface.

The analysis of the corrosion exhibits the increase of cell attachment after five days of implantation. The SEM pictures revealed the differences in the surface appearances and shapes of attached cells between non-coating and HA-coated Mg-alloy. It is predicted that the HA coating of Mg implants will improve the attachment of soft tissue. It is known, that HA is an attractive agent for osteoblast cell line fibroblast like cells. The adhesion of fibroblasts on implants is a presents favorable conditions for soft tissue integration. The number of adherent cells on HA-coated Mg-alloy was significantly higher than on non-coated samples. Two mechanisms can play significant role in cell adhesion: 1) HA can prevent cell toxicity of pure Mg alloy and 2) corrosion resistance can increase call viability on implant surface.

HA coating layer may maintain the corrosion resistance of Mg-based alloys. Consequently local accumulation of hydrogen and fast increase of local pH value are reduced as evidenced by insignificant changes of pH in model solutions.

V. Conclusion

We determined different mechanisms of HA layer corrosion - SBF solution causes the partial dissolution, while citrate solution caused complete disappearance of the coating. HA coated layer couse lower degradaion without significant pH change during the static immersion test in SBF and citrate buffer. The HA coating favored cell adhesion and rapid fibrous tissue formation.

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