Corrosion of Bioresorbable Magnesium Alloy (AZ91D) in pH-Buffered, Osteogenic Culture Medium

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Introduction: Magnesium-9%aluminum-1%zinc alloy (AZ91D) is a bioresorbable metal with mechanical properties similar to bone and whose degradation (corrosion) products are thought to stimulate osteogenic differentiation of mesenchymal stem cells (MSCs). These materials hold promise for developing novel orthopedic devices and bone tissue engineering scaffolds with biomimetic mechanical properties, tailored degradation, and stimulation of osteogenesis. The objective of this study is to evaluate the corrosion properties of AZ91D immersed in a biologically relevant electrolyte (pH-buffered osteogenic culture media) over the same time period (1-21day) as forthcoming experiments that will evaluate MSC osteogenic differentiation on AZ91D.

Methods: Discs of AZ91D were wet sanded to 600 grit, sonicated in 70% EtOH, exposed to UV light for 30 mins, and mounted in a corrosion chamber that exposed $3.9cm^2$ of the metal. This chamber was filled with 8mL of osteogenic media (α modified eagle's media with 10% fetal bovine serum, 10nM dexamethasone, 0.05mM L-ascorbic acid-2-phosphate, and 10mM beta-glycerophosphate) and placed in 37°C, 5%CO_2 incubator. The media was exchanged every 3 days. At 3, 6, 15, and 21 days incubation a carbon counter electrode and a silver/silver chloride reference electrode (via agar bridge) were inserted in the chamber and electrochemical impedance spectroscopy (EIS) was conducted (100kHz-5mHz, ±10mV) at the open circuit potential of the AZ91D (working electrode). The impedance spectra were fit to the circuit model shown in Fig 1e which accounts for the inner barrier layer and the outer corrosion product layer of the interface. The Rs is the solution resistance and the charge transfer resistance of the inner and outer layer are modeled by the Ri and Ro respectively. The Warburg component (W) models diffusion processes at the interface. The constant phase element (CPE) magnitudes (Qo and Qi) and the exponents (m and n) are used to calculate the capacitances (Co and Ci) through the following equations:

$$C_o = Q_o^{1/m}(R_s^{-1} + R_o^{-1})^{(m-1)/m} \qquad C_i = Q_i^{1/n}(R_s^{-1} + R_i^{-1})^{(n-1)/n}$$

After EIS, the samples were extracted from the chambers, rinsed in purified water, and analyzed with a scanning electron microscope (Hitachi SU8000) in backscattered mode (BSE) and with energy dispersive spectroscopy (EDS). Five areas were examined per sample. A minimum of 3 samples were analyzed at each timepoint. One way ANOVA (α=0.05) was conducted to compare outcomes at different times followed by Tukeys post-hoc.

Results: The impedance results (Fig 1a) reveal that the inner barrier layer has significantly greater resistance, capacitance, and homogeneity (higher CPE exponent) as compared to the outer corrosion layer at all times. Examining the individual impedance components over time indicates there are significant electrochemical changes occurring at the interface out to 15 days, but that after 15 days there are no impedance changes. The Ri
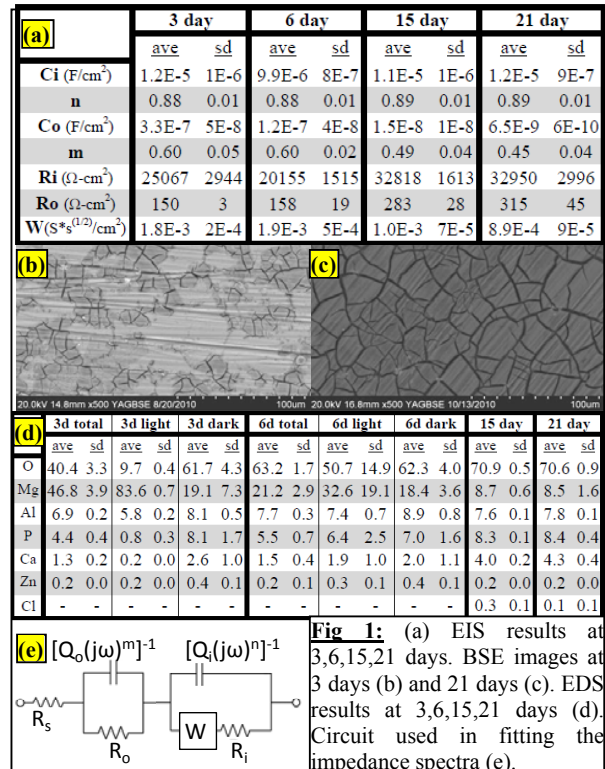
(a)	3 day ave	3 day sd	6 day ave	6 day sd	15 day ave	15 day sd	21 day ave	21 day sd
Ci (F/cm²)	1.2E-5	1E-6	9.9E-6	8E-7	1.1E-5	1E-6	1.2E-5	9E-7
n	0.88	0.01	0.88	0.01	0.89	0.01	0.89	0.01
Co (F/cm²)	3.3E-7	5E-8	1.2E-7	4E-8	1.5E-8	1E-8	6.5E-9	6E-10
m	0.60	0.05	0.60	0.02	0.49	0.04	0.45	0.04
Ri (Ω-cm²)	25067	2944	20155	1515	32818	1613	32950	2996
Ro (Ω-cm²)	150	3	158	19	283	28	315	45
W (S*s^(1/2)/cm²)	1.8E-3	2E-4	1.9E-3	5E-4	1.0E-3	7E-5	8.9E-4	9E-5

(d)	3d total ave	sd	3d light ave	sd	3d dark ave	sd	6d total ave	sd	6d light ave	sd	6d dark ave	sd	15 day ave	sd	21 day ave	sd
O	40.4	3.3	9.7	0.4	61.7	4.3	63.2	1.7	50.7	14.9	62.3	4.0	70.9	0.5	70.6	0.9
Mg	46.8	3.9	83.6	0.7	19.1	7.3	21.2	2.9	32.6	19.1	18.4	3.6	8.7	0.6	8.5	1.6
Al	6.9	0.2	5.8	0.2	8.1	0.5	7.7	0.3	7.4	0.7	8.9	0.8	7.6	0.1	7.8	0.1
P	4.4	0.4	0.8	0.3	8.1	1.7	5.5	0.7	6.4	2.5	7.0	1.6	8.3	0.1	8.4	0.4
Ca	1.3	0.2	0.2	0.0	2.6	1.0	1.5	0.4	1.9	1.0	2.0	1.1	4.0	0.2	4.3	0.4
Zn	0.2	0.0	0.2	0.0	0.4	0.1	0.2	0.1	0.3	0.1	0.4	0.1	0.2	0.0	0.2	0.0
Cl	-	-	-	-	-	-	-	-	-	-	-	-	0.3	0.1	0.1	0.1

(e) $[Q_o(j\omega)^m]^{-1}$ $[Q_i(j\omega)^n]^{-1}$ R_s R_o W R_i

Fig 1: (a) EIS results at 3,6,15,21 days. BSE images at 3 days (b) and 21 days (c). EDS results at 3,6,15,21 days (d). Circuit used in fitting the impedance spectra (e).

significantly increases from 3 to 15 days, but the Ci and n do not change over time. The Ro significantly increases while Co significantly decreases over 3 to 15 days and indicates a thickening of the outer corrosion layer. The low values of m (~0.5) at 15 and 21 days indicate the outer layer growth is heterogeneous and introduces a diffusion barrier. The Warburg parameter indicates diffusional impedance is significantly greater at 15 and 21 days as compared to 3 and 6 days. BSE images of the AZ91D surface at 3 days (Fig 1b) shows a patchwork of light colored regions separated by darker/cracked regions. EDS shows (Fig 1d) the dark regions have significantly higher atomic % of oxygen (O) and significantly lower atomic % magnesium (Mg) as compared to the light areas and indicates that the cracked areas are where corrosion (oxidation) products formed. Fig 1c is a typical BSE image of the AZ91D at 15 and 21 days and shows that the entire surface is composed of the dark/cracked region. The EDS results at 15 and 21 days indicate that corrosion products have formed over the entire surface as evidenced by uniform (cracked) surface morphology and the elemental composition of the surface being dominated by O. At 6 days the surface morphology (images not shown) and EDS data show the AZ91D is at an intermediate transition between the 3 and 15 day results.

Conclusions: The impedance, morphology, and the chemistry showed mutually supportive results and provide a basis for exploring how the time course of AZ91D corrosion influences MSC osteogenic differentiation.