Poly (3,4-ethylenedioxythiophene) Graphene Oxide Composite Coatings for Controlling Magnesium Implant Corrosion

Kasey Catt^a, Huaxiu Li^a, X. Tracy Cui^{a,b,c,*}

^aDepartment of Bioengineering, University of Pittsburgh, Pittsburgh, PA, USA

^bCenter for the Neural Basis of Cognition, University of Pittsburgh, Pittsburgh, PA, USA

^cMcGowan Institute for Regenerative Medicine, University of Pittsburgh, Pittsburgh, PA 15260, USA

**Corresponding author, 5057 Biomedical Science Tower 3, 3501 Fifth Avenue,*

Pittsburgh, PA, USA. E-mail: xic11@pitt.edu; Fax: +1-412-648-9076; Tel: +1-412-383-6672

Abstract:

Magnesium (Mg) is a promising biodegradable implant material because of its appropriate mechanical properties and safe degradation products. However, *in vivo* corrosion speed and hydrogen gas production need to be controlled for uses in biomedical applications. Here we report the development of a conducting polymer 3,4-ethylenedioxythiophene (PEDOT) and graphene oxide (GO) composite coating as a corrosion control layer. PEDOT/GO was electropolymerized on Mg samples in ethanol media. The coated Mg samples were subjected to various corrosion tests. The PEDOT/GO coating significantly reduced the rate of corrosion as evidenced by lower Mg ion concentration and pH of the corrosion media. In addition, the coating decreased the evolved hydrogen. Electrochemical analysis of the corroding samples showed more positive corrosion potential, a decreased corrosion current, and an increase in the polarization resistance. PEDOT/GO corrosion protection is attributed to three factors; an initial passive layer preventing solution ingress, buildup of negative charges in the film, and formation of corrosion protective Mg phosphate layer through redox coupling with Mg corrosion. To explore the biocompatibility of the coated implants *in vitro*, corrosion media from PEDOT/GO coated or uncoated Mg samples were exposed to cultured neurons where PEDOT/GO coated samples showed decreased toxicity. These results suggest that PEDOT/GO coating will be an effective treatment for controlling corrosion of Mg based medical implants.

Statement of Significance:

Coating Mg substrates with a PEDOT/GO composite coating showed a significant decrease in corrosion rate. While conducting polymer coatings have been used to prevent

corrosion on various metals, there has been little work on the use of these coatings for Mg. Additionally, to our knowledge, there has not been a report of the combined use of conducting polymer and GO as a corrosion control layer. Corrosion control is attributed to an initial barrier layer followed by electrochemical coupling of the PEDOT/GO coating with the substrate to facilitate the formation of a protective phosphate layer. This coupling also resulted in a decrease in hydrogen produced during corrosion, which could further improve the host tissue integration of Mg implants. This work elaborates on the potential for electroactive polymers to serve as corrosion control methods.

Key words: conducting polymer; graphene oxide; magnesium; corrosion control

1. Introduction:

High strength to weight ratio, excellent mechanical properties, and biodegradability makes magnesium attractive for a variety of biomedical implant applications[1-3]. The ability of Mg implants to completely degrade in the body prevents the need for implant removal surgery and minimizes side effects due to the chronic presence[4-8]. In vivo corrosion of Mg implants result in harmless byproducts, granted that the corrosion rate is appropriately controlled[3, 4, 9, 10]. In addition, Mg is essential in numerous biological processes and increased Mg levels have been shown to be beneficial to osseointegration as well as neural protection and regeneration[7, 11-13]. Mg's mechanical properties are also superior to current biodegradable polymers[14]. Despite the promise of Mg as a biodegradable implant material; there have been multiple reports on rapid in vivo corrosion of Mg [1, 6, 15-21]. Rapid degradation results in premature mechanical failure and can lead to harmful buildup of corrosion products, such

as hydroxide ions, which increases the local pH, and hydrogen gas, which results in gas bubble formation around the implant. In order to prevent such uncontrolled and rapid in vivo degradation, many approaches have been investigated to control corrosion, including alloying, coatings, and surface treatments.

Conducting polymer (CP) coatings have shown promise in their ability to increase corrosion resistance of metals. CPs can be electropolymerized directly on metal surface along with appropriate dopant molecules resulting in a conductive and electroactive film that can have unique redox reactions with the underlying metal[22]. With multiple different types of CPs and a greater number of dopants, many mechanisms have been proposed to explain CP corrosion control. These mechanisms have been thoroughly reviewed elsewhere [22-24] and include: a barrier layer, ennobling, anodic protection, formation of a modified CP metal interface, facilitation of a protective oxide, shifting of the electrochemical interface, and use of dopant released from the CP to facilitate a more corrosion resistant surface layer. Research also suggests that CPs can electrochemically protect exposed areas of the substrate and act as a self-healing coating[25, 26].

In addition to CPs, graphene and graphene oxide (GO) have been shown to provide effective corrosion control. Layers of graphene sheets alone can act as a barrier to prevent corrosion[27-29]. GO sheets, due to negatively charged functional groups, can repel anions that normally facilitate corrosion[30]. We hypothesize, that when coupled with the CP coating as immobile anionic dopant, additional corrosion protection benefits may be obtained. As the positive backbone of the CP is reduced via the electrons generated by metal corrosion, large dopants will remain physically trapped in the film

resulting in a net negatively charged film. Such effect has been previously reported with polypyrrole CP doped with dodecylsulfate [22].

The aforementioned corrosion studies involving conducting polymers have been mostly focused on metals such as aluminum and stainless steel, while few have investigated the use of CPs to control magnesium corrosion[31, 32]. In our previous work we show that PEDOT film electrodeposited on Mg surfaces in ionic liquid reduced the corrosion current[31]. In this work we developed a method to electrochemically polymerize poly 3,4-ethylenedioxythiophene (PEDOT) doped with GO on Mg to utilize the synergistic properties of both CP and GO. The effect of the PEDOT/GO coating on Mg corrosion was evaluated and the mechanism of corrosion protection investigated. Additionally, the effect of the coating on cellular toxicity of Mg was assessed with primary neuron cultures.

2. Materials and Methods:

2.1 Materials:

Single layer Graphene Oxide sheets were purchased from Cheap Tubes (Cambridgeport, VT). 200proof ethanol was purchased from Decon Labs Inc. (King of Prussia, PA). Magnesium ribbon $\geq 99.5\%$ (3 mm wide x 0.2 mm thick), 3,4-Ethylenedioxythiophene (EDOT) monomer, methanol, laminin (from Engelbreth-holmswurm murine sarcoma basement membrane), and nitrocellulose were purchased from Sigma-Aldrich. PBS 10x concentrate (136mM NaCl, 2.7mM Potassium chloride, 10mM Phosphate Buffer) was purchased from EMD Millipore (Billerica, MA) and diluted using de-ionized water from a Millipore Milli-Q system. StanBio Liquicolor Magnesium Test and Live Dead stain were purchased from Fisher Scientific. Conductive silver paint

was purchased from Electron Microscopy Sciences (Hatfield, PA). Two-part epoxy was purchased from Leco (St. Joseph, MI). Glutamax 100x, B27 supplement, and Neurobasal media for neuron culture were purchased from Gibco (Grand Island, NY). Primary neurons were isolated from e18 rat cortices from Brain Bits (Springfield, IL).

2.2 Polymerization of the PEDOT/GO film:

GO was added to ethanol at a concentration of 10 mg/ml and the resulting solution was sonicated for 30 minutes in a bath sonicator FS110H (Fisher Scientific). Following sonication, 20 μ l/ml EDOT and 35 μ l/ml deionized water were added and dissolved into the GO ethanol solution to create the polymerization solution. Magnesium ribbon was cut into 2 cm long strips, rinsed with ethanol, and blotted dry.

Polymerizations, and all electrochemical measurements, were conducted on a Gamry Potentiostat FAS2/Femtostat using Gamry Framework Software (Warminster, PA).

Polymerization consisted of a two-electrode system with Mg ribbon working electrode and a gold counter/reference electrode. Polymerization was conducted in 300 μ l of fresh polymerization solution and a potential of 0.6 V vs. counter was applied until a charge of 10 mC was reached. Coated length was 9 mm. Following polymerization, electrodes were gently dipped in ethanol and then dried at -20°C for two days, followed by drying at 3°C for one day. Following this drying process, the PEDOT/GO forms a thin adherent black film on the Mg ribbon. Samples were stored at ambient temperature post drying.

2.3 Corrosion characterization

Mg corrosion in aqueous solution, with or without the PEDOT/GO coating, involves anodic and cathodic reactions as shown in equation 1-4 below. The assessment of

corrosion often targets different reaction products. Multiple methods to characterize corrosion have been reported in literature [1, 2, 9, 33, 34]. To obtain a comprehensive understanding, we utilized multiple metrics to analyze the corrosion of PEDOT/GO coated and uncoated samples.

2.3 Tafel scan

A three-electrode system was utilized with Ag/AgCl reference, platinum counter, and Mg working electrode. Coated or uncoated samples were immersed 5 mm into PBS for the scan ensuring only the coated portion of the PEDOT/GO sample was exposed to the PBS (supplemental Fig. 1). Open circuit potential (OCP) was recorded for 600s, or until it reached a stability of 0.05 mV/s. Following OCP determination, the sample was scanned from -0.25V to +0.25V vs. OCP. This scan provides a current vs. voltage graph

displaying the anodic and cathodic half reactions. The low current valley in the graph is the position where the anodic and cathodic half reactions are in equilibrium. The potential where this occurs is the corrosion potential (E_{corr}). Additionally, the current where the half reactions are in equilibrium can be extrapolated from the linear portions of the cathodic and anodic arms of the tafel scan. The point of intersection of these linear extrapolations is the corrosion current (i_{corr}).

2.4 Long term corrosion test (pH, Mg ion, OCP, and Electrochemical Impedance Spectroscopy):

The tafel scan is a one-time analysis that damages the sample affecting further readings. For long-term quantification of corrosion, analysis of corrosion byproducts and non-damaging electrochemical techniques were utilized. PEDOT/GO coated and uncoated Mg samples were electrically connected using conductive silver paste and copper wire to the back of the Mg sample. Samples were then mounted in epoxy only leaving 5 mm length exposed. This resulted in a fixed area of the sample being exposed to the corrosion solution, with only coated portions being exposed for PEDOT/GO samples (supplemental Fig. 2). Corrosion was conducted in PBS using 20 ml/cm² media volume to surface area ratio. Each day samples were transferred to fresh PBS and OCP and Electrochemical Impedance Spectroscopy (EIS) measurements were conducted. Mg ion concentration and pH measurements were taken from the corrosion media of the previous day.

2.4.1 OCP and EIS:

Electrochemical tests, OCP and EIS, have a negligible effect on the corrosion reaction and can be repeated daily without affecting sample behavior. Immediately after transferring samples into fresh PBS, a three-electrode system was used to measure the OCP and EIS with a Ag/AgCl reference, platinum counter, and Mg working electrode. OCP is a real time representation of E_{corr} displayed in the Tafel scans. OCP was recorded for 600 s, or until it reached a stability of 0.05 mV/s. Following OCP, EIS was taken from 100 kHz to 0.1 Hz with an applied potential of 0 V vs. OCP and a voltage amplitude of 10 mV. EIS data and equivalent circuit modeling were conducted using Gamry EchemAnalyst software to provide a better understanding of the sample/electrolyte interface and the effect of the coating on corrosion.

The equivalent circuit used to describe coated and uncoated samples can be seen in Fig 6. This is a common circuit used to describe corroding samples with coatings [24, 35-39], and has particularly been applied to magnesium with either an inherent oxide/hydroxide layer or other topically applied coatings[40-43]. This circuit consists of solution resistance (R_s), coating capacitance (CPE_c), pore resistance (R_{po}), double layer capacitance (CPE_{dl}), and charge transfer resistance (R_{ct}). Circuit components R_{po} and CPE_c represent properties of the coatings on the surface of the Mg where R_{po} indicates the resistance to ion transfer from the solution to the underlying Mg through the coating and in areas where the coating begins to fail, and CPE_c represents the capacitance of the coating. Values R_{ct} and CPE_{dl} describe the electrochemical reactions occurring at the Mg solution interface where the corrosion media infiltrated through the coatings. Here R_{ct} is the faradaic charge transfer accomplished through chemical changes, in this case oxidation of the Mg. R_{ct} therefore describes the sample's corrosion. CPE_{dl} and CPE_c are

constant phase elements representing a double layer capacitance that is not a perfect capacitor defined by equation 6 below[44, 45].

$$Z_{CPE} = \frac{1}{(j\omega)^a Q} \quad (6)$$

Here ω is the angular frequency in rad/sec, a indicates how close behavior is to an ideal capacitor, and Q is the constant phase element coefficient with units $s^a/\Omega\text{cm}^2$. For a , a value of 1 describes a capacitor with a phase angle of 90° (an ideal capacitor) while a value of 0.5 represents a phase angle of 45° indicating a resistive characteristic making up 50% of Z_{CPE} .

2.4.2 pH measurement

The reaction of untreated Mg in aqueous media can be seen in equation 3. Oxidation of Mg (equation 2) resulting in is balanced by the reduction of water and formation of OH^- and H_2 (equation 1)[1, 34]. To quantify the OH^- corrosion byproduct, pH of the corrosion media was measured daily using Fisher Scientific Accumet Ab15 pH meter.

2.4.3 Mg ion concentration

A StanBio Magnesium Liquicolor (Xylidyl Blue) assay was used to quantify the Mg produced during oxidation (equation 2). 200 μl /well of the liquicolor assay solution was added to the 96 well plate. Corrosion solution was diluted in half with PBS. Three 10 μl samples were taken from each dilute corrosion solution and placed into individual wells containing the liquicolor solution and mixed. Samples reacted at room temperature for 10 minutes and Absorbance at 520 nm was tested using a Spectromax M5 spectrophotometer.

2.5 Hydrogen evolution measurement:

Similar to Mg ion concentration and pH, hydrogen evolution measurements monitor a Mg corrosion byproduct. Hydrogen is a product of the reduction of water in the cathodic half reaction (equation 1). PEDOT/GO coated Mg and uncoated Mg samples were mounted in epoxy on glass microscope slides leaving 5 mm of the sample exposed (supplemental Fig. 3). Samples were then placed in a 2 L crystallization dish filled with PBS. 2 ml plastic pipettes were modified so that the tip was closed and the opposite end was flared out. These pipettes were filled with PBS and placed over the corroding Mg samples. Hydrogen evolved from the samples was quantified as it collected at the top of the pipette.

2.6 SEM and EDX analysis:

SEM analysis was conducted using a Jeol JSM 6330F SEM. Accelerating voltage was 3 kV. Samples were analyzed before corrosion and after 22 days of corrosion in PBS, refreshing PBS every day. Following corrosion, samples were dipped in DI water followed by ethanol, air dried, and stored in a desiccator until imaging. Coating integrity and surface morphology of coated and uncoated samples were analyzed. Due to lack of EDX analysis capability on JEOL JSM 6330F, chemical analysis was conducted on a Jeol JSM6510 SEM using an Oxford Instruments X-ray analyzer and Inca Software. Working distance was 10 mm and accelerating voltage was 20 kV. Elemental identification was conducted automatically through the Inca EDX software with the three dominant elements identified being magnesium, oxygen, and phosphorus.

2.7 Neuron Culture

Coated and uncoated Mg samples were placed in culture media (Neurobasal media, 10% Horse serum, 2mM L-alanyl-L-glutamine, 4ml b27 50x/ml media, and 1% PennStrep) at a surface area to volume ratio of 1.25 ml/cm². Corrosion was conducted in a cell culture incubator at 37°C and 5% CO₂. Following corrosion for 24 or 72 hours, different concentrations of corrosion media were added to neurons cultures.

96 well plates were pre-coated with nitrocellulose, cast from 1 cm²/ml methanol solution, followed by laminin (40 µl/ml) adsorption then rinsed with PBS. Primary neurons from e18 rat cortices were dissociated via trituration and were plated at 30,000 cells/well. Neurons were allowed to grow in unaltered media for 1 day. After one day, the culture media was replaced with 100% corrosion media as well as corrosion media diluted with fresh culture media to 50% and 10%.

2.7.1 LDH Assay:

Cytotoxicity was tested utilizing an LDH assay where the amount of LDH released by damaged cells into the media was quantified after exposure to 24-hour corrosion media. 50 µl cell supernatant was removed and combined with 50 µl assay reaction solution. Reaction proceeded for 30 minutes at room temperature followed by addition of 50 µl of the assay stop solution. Absorbance was measured at 490 nm (LDH) and 680 nm (background) using a Molecular Device M5 plate reader.

2.7.2 Live dead staining:

To further assess longer-term corrosion media toxicity, analysis was also conducted where samples were allowed to corrode for 72 hours. Following exposure to

corrosion solution, the media was removed and replaced with staining solution 2 μM CalceinAM and 2 μM Ethidium Homodimer-1 in PBS. After 10 min of incubation, images were taken on a Leica DMI 4000B inverted scope. Counting was conducted with ImageJ manually using 'Cell Counter' add-on by Kurt De Vos.

2.8 Statistics

Statistical analysis was conducted using a two-tailed Student's T-Test in Microsoft Excel. Significance is considered $p < 0.05$. Data is presented as mean $\pm$ standard error of the mean.

3. Results

3.1 Film formation

Electropolymerization of PEDOT requires an oxidizing potential that can drive Mg corrosion. To minimize this corrosion and increase PEDOT/GO film adhesion, polymerization was conducted in pure ethanol as it does not facilitate Mg corrosion[46]. However, visible film growth was not achieved. Water was added at increasing concentrations (0.5 to 50 μl water/ml ethanol GO polymerization solution) to the polymerization solution and the film growth was inspected visually. Only when water concentration reached 35 $\mu\text{l}/\text{ml}$, the electropolymerization yielded visibly uniform brown film fully covering the substrate, while greater concentrations resulted in films with visible bubbles exposing the underlying Mg corrosion during the polymerization.

Following polymerization, the drying protocol was also optimized. PEDOT/GO films after polymerization were in a solvated form and showed a visible volume decrease during drying. Drying at ambient temperatures resulted in quick drying films that displayed non-uniform thicknesses, visually observed as darker patches, as well as

bubbles and cracks in the coating. To slow the drying, samples were placed at -20°C for two days followed by storage at 4°C for one day. Following this process, films presented uniform color and no appearance of cracks or bubbles. Polymerization at optimized conditions resulted in uniform films with a surface texture (Fig. 1A and 2 A&C) indicating that the GO sheets are protruding from the surface of the polymer film[47].

3.2 SEM and EDX analysis

Images of un-corroded samples as well as samples corroded in PBS for 22 days were analyzed under SEM (Figs. 1 and 2). Following corrosion, PEDOT/GO coated samples display cracks in the coating as well as areas where the coating delaminated. PEDOT/GO samples showed coating delamination of varying degrees with one sample having a large portion of the coating removed and two remaining largely intact throughout the 22 day corrosion. It is likely that the cracking of the film visualized under SEM was exacerbated by the SEM sample drying preparation. Of interest is the morphology of the underlying Mg after corrosion. Images revealed cracked plate like morphology of exposed Mg around the PEDOT/GO coated areas (Fig. 2D). There is also evidence that the cracked plate like morphology was present underneath the PEDOT/GO coating as seen through the cracks in the coating (Fig. 2F). Uncoated Mg samples, however, predominantly displayed an amorphous morphology with only some areas of cracked plate like morphology on the edge (Fig. 2E). EDX analysis was conducted on both the cracked and amorphous morphologies and it was found that the scale morphology displayed higher percentage of oxygen and phosphorus than the amorphous areas (Fig. 3). This suggests that the uncoated samples were predominantly covered with

a $\text{Mg}(\text{OH})_2$ while the PEDOT/GO coated samples displayed a $\text{Mg}_3(\text{PO}_4)_2$ in the areas where the PEDOT/GO coating began to fail.

3.3 Corrosion Characterization

3.3.1 Tafel scan

Representative tafel scans, seen in Fig. 4, display a positive shift in the corrosion potential (E_{corr}) for the PEDOT/GO coated Mg. The PEDOT/GO coating shifted the E_{corr} of uncoated Mg (-1.705 V) in the noble direction to -1.55 V following coating.

Additionally, the PEDOT/GO coating decreased the corrosion current, extrapolated from the linear portions of the cathodic (red line Fig. 4) and anodic (blue line Fig. 4) arms of the tafel scan. Corrosion current was decreased 75% to 12.3 μA for PEDOT/GO coated samples from 49.6 μA for uncoated samples.

3.3.2 Magnesium ion analysis

Mg ion concentration values, summed over days, and the accompanying linear regression analysis can be seen in Fig. 5A. The Mg ions were released at a relatively constant rate over days for the uncoated samples while the PEDOT/GO coating showed deviation from the linear trend at initial days. PEDOT/GO coated Mg released significantly less Mg ions in the corrosion media than untreated Mg every day for the period examined, with a 41.1% reduction in average daily release. The increase in variation between the PEDOT/GO sample coatings at later time points is a result of the coating delamination occurring at slightly different rates.

3.3.3 pH analysis

Summed OH^- present in the corrosion solution and the accompanying linear regression analysis can be seen in Fig. 5B. Similar to the Mg ion release profile, the concentration of OH^- increased linearly over days for the uncoated Mg and the trend is not as linear for the PEDOT/GO coated samples. Overall, uncoated samples displayed a significantly greater amount of OH^- in the corrosion solution than the PEDOT/GO coated samples with PEDOT/GO samples displaying a 43% decrease in the average daily OH^- production. Again, the increased variability at later time points for PEDOT/GO coated samples is attributed to polymer delamination.

3.3.4 Electrochemical tests

3.3.4.1 OCP

It can be seen in Fig. 5C that the OCP values for the PEDOT/GO coated samples were more positive than the uncoated samples at all time points, which is indicative of lower corrosion rates. There was a sharp negative shift in corrosion potential in the first

24 hrs. for the PEDOT/GO coated samples to a voltage of -1.66 V from initial -1.58 V.

This drop is likely due to film cracking during the first 24 hours leading to a compromised barrier function. However, despite the drop, the OCP of the coated sample is still more positive than the uncoated at -1.72 V. Coated samples showed an average 0.035 ± 0.002 V greater OCP than uncoated samples throughout the duration of the experiment.

3.3.4.2 EIS

The radius of the Nyquist plot arc in Fig. 5D from EIS represents the sample's corrosion resistance. The imaginary and real components of the AC impedance are displayed with higher frequency stimuli nearer to position (0,0) and frequency values decreasing as the data proceeds clockwise. For a simple RC circuit composed of a resistor serially linked to a capacitor and charge transfer resistance in parallel, the Nyquist plot intersects the x-axis twice. The first intersect at very high frequency indicates solution resistance of the media, while the second intersect at very low frequencies is the sum of the solution resistance and charge transfer resistance since the capacitor is an open circuit at such low frequencies. Therefore the diameter of the arc is approximated as the charge transfer resistance. In the case of a corroding metal, charge transfer is associated with the corrosion reaction of the sample. Therefore, larger radii indicating increasing resistance to charge transfer and consequently higher corrosion resistance[48, 49]. Here the uncoated sample shows an initial increase in the radius of the Nyquist arc followed by a decrease towards the end of the experiment. The initial increase is due to the formation of a $\text{Mg}(\text{OH})_2$ (equation 4) layer after immersion in the corrosion media. This hydroxide

layer is known to be unstable in chloride solutions [1] and the decrease in protection at day 22 is likely due degradation of the coating by Cl^- . Conversely, the PEDOT/GO coatings showed an increase in the radius of the Nyquist arc over initial days and the protection was maintained for the duration of the experiment. This is due to the barrier layer properties as well as the facilitation and continued maintenance of the $\text{Mg}_3(\text{PO}_4)_2$ corrosion protection layer.

3.3.4.3 Equivalent Circuit Analysis

Equivalent circuit analysis employed constant solution resistance due to constant PBS concentration and low resistance of the solution. For PEDOT/GO, R_{po} and CPE_c represent properties of both the PEDOT/GO coating and the passive phosphate layer. For the uncoated samples, R_{po} and CPE_c represent the oxide and hydroxide layers formed on the surface. There is very little difference between the coated and uncoated samples for the pore resistance (R_{po}) fluctuating between ~ 100 and $\sim 200 \Omega$ indicating that the $\text{Mg}(\text{OH})_2$ coating on the uncoated samples had similar ability to prevent the solution from reaching the underlying Mg as did the passive layer in combination with the PEDOT/GO coating. This is likely due to the amorphous morphology of the $\text{Mg}(\text{OH})_2$, the visible damage to the conducting polymer during corrosion, and the cracks of the phosphate layer visualized under SEM. Additionally, while the magnesium hydroxide may have prevented corrosion media infiltration, it is continually being eroded away by the chloride ions in the corrosion media. Coating capacitance (CPE_c) was greater for coated samples during the first 13 days which is attributed to the increase in surface area facilitated by the conducting polymer and its nanotopography [50]. Excessive water infiltration of the oxide/hydroxide film on the uncoated samples likely caused the

increase in capacitance after day 15. As can be seen in Fig. 6, PEDOT/GO coated samples had a significantly higher R_{ct} than uncoated samples with an average $7.0 \pm 1.4 \text{ k}\Omega$ greater R_{ct} indicating increased resistance to faradic corrosion. Double layer capacitance (CPE_{dl}) is another component representing the metal/solution interface. Uncoated samples exhibited increased CPE_{dl} indicating a greater surface area of metal in contact with the corrosion solution. This is likely due to the increased pitting corrosion and the amorphous morphology of the corrosion products on the surface of the uncoated samples. This data, especially the continually significant increase in R_{ct} of the coated samples, serves as further evidence that the PEDOT/GO coating improves Mg corrosion resistance.

3.4 Hydrogen evolution

Cumulative H_2 evolution from coated and uncoated samples can be seen in Fig. 7. Every day the production of H_2 is significantly less for the PEDOT/GO coated samples than the uncoated. Overall, PEDOT/GO samples resulted in a 67% decrease in average daily hydrogen production, indicating that the coating is preventing corrosion or bypassing the water reduction reaction by PEDOT/GO reduction. PEDOT/GO coated samples did display small isolated bubbles after about 24 hours of immersion, however those bubbles were stagnant and increased in volume much more slowly than the bubbles being released from the uncoated Mg samples.

3.5 Cytotoxicity

3.5.1 LDH assay

Results in Fig. 8A show a significant decrease in sample toxicity after coating. 100% corrosion media exposure showed a 40% decrease in toxicity as a result of PEDOT/GO coating. Cytotoxicity of cells exposed to 50% corrosion media showed an 86% decrease in toxicity for PEDOT/GO coated Mg. At 10% corrosion solutions, coated samples trended towards being less toxic, though not significant. Presumably, at such high dilution, the differences between the coated and uncoated samples are negated.

3.5.2 Live/Dead assay

As can be seen in Fig. 8B, corrosion media of coated samples showed a significant 25% reduction in cytotoxicity at a dilution of 50% over the corrosion media of uncoated Mg. With 10% corrosion media, there is a slight trend towards coated samples being less toxic, though not statistically significant. Large amounts of death were observed for both coated and uncoated samples at 100% media concentration to the point where quantification could not be completed.

4. Discussion

This study investigates the ability of the PEDOT/GO coating to control Mg corrosion. PEDOT/GO corrosion protection was observed and assessed with multiple techniques including monitoring corrosion byproducts and electrochemical analysis. This work reports on the successful electropolymerization of PEDOT/GO on Mg and displays the coating's ability to reduce corrosion rates and hydrogen evolution as well as improve neuronal biocompatibility.

Previous reports of electropolymerization in non-aqueous media noted that small amounts of water improved polymer growth[51-53]. This improvement was attributed to increasing the permittivity of the polymerization solution. Addition of water to the ethanol based polymerization solution decreased the electrostatic repulsion of the radical mer units during polymerization resulting in the polymerization of the PEDOT/GO film on the Mg surface.

Assessment of corrosion byproducts showed a significant decrease in corrosion with the application of the PEDOT/GO coating. Comparisons of the electrochemical data similarly showed that the PEDOT/GO coating improved corrosion resistance. This corrosion protection can be the result of barrier properties of the PEDOT/GO coating as well as the potential electrochemical coupling of the redox properties of the PEDOT/GO with the underlying Mg. Following long-term corrosion, SEM and EDX analysis revealed distinctly different surface morphologies and chemical makeups. Uncoated samples displayed an amorphous $\text{Mg}(\text{OH})_2$ while the exposed areas of Mg substrate beneath the PEDOT/GO displayed a scale like $\text{Mg}_3(\text{PO}_4)_2$. These data suggest that the PEDOT/GO coating facilitated the formation of a magnesium phosphate corrosion layer in the areas where the corrosion solution penetrated the film.

Early values for Mg^{2+} , OH^- , and H_2 show low corrosion rates for PEDOT/GO samples compared to the uncoated Mg largely due to barrier layer corrosion protection provided by the PEDOT/GO. This PEDOT/GO barrier protection may have been further improved through reactions between oxidizing Mg and PEDOT/GO. Following reduction of the polymer backbone, large GO sheets will remain trapped in the polymer film

resulting in a net negative charge in the film [54]. This can improve resistance to penetrating corrosion promoting negative ions like Cl^- [22, 55].

During corrosion of uncoated samples, Mg^{2+} from the anodic half reaction and OH^- from the cathodic half reaction react with one another to form $\text{Mg}(\text{OH})_2$ on the surface (equation 4) illustrated in Fig. 9A[1, 34]. This $\text{Mg}(\text{OH})_2$ film is porous, non-uniform, and chemically unstable making it a poor corrosion protection layer[56, 57]. The increase in EIS nyquist arc is likely due to the formation of this semi protective $\text{Mg}(\text{OH})_2$ while the lack of maintenance of the increased arc radii suggest this layer's instability. Similarly, the increase in between sample variability for Mg^{2+} , OH^- , and H_2 measurements of uncoated samples is likely due to the instability as well as the continual formation and erosion of the $\text{Mg}(\text{OH})_2$ layer.

Between days 5 and 10 in the corrosion solution, the between sample variability for corrosion byproduct quantification (Mg^{2+} , OH^- , and H_2) begins to increase for PEDOT/GO coated samples. This suggests that the PEDOT/GO coatings are starting to fail at different rates. In these localized regions where the coating fails, there is Mg corrosion. At these loci, the water reduction cathodic water reduction (equation 1) is replaced by reduction of the PEDOT backbone and the generation of OH^- diminished[58]. As a result, in areas where the PEDOT/GO fails, there is an increased probability that Mg^{2+} will react with the phosphate from the corrosion solution. This mechanism is displayed in Fig. 9B. Similarly, other groups have noted that conducting polymers facilitate the formation of a passive oxide layer on other metals[24, 59-63] in a manner similar to that proposed here. Additionally, reactions displayed in Fig. 9 are similar to those proposed for phosphate based anodized coatings[64].

The distinctly different surface morphologies and chemical makeups between uncoated and PEDOT/GO samples post corrosion support the reaction scheme in Fig. 9. Uncoated samples displayed an amorphous $\text{Mg}(\text{OH})_2$. Exposed areas of Mg substrate beneath the PEDOT/GO displayed a scale like $\text{Mg}_3(\text{PO}_4)_2$. These data suggest that the PEDOT/GO coating facilitated the formation of a magnesium phosphate corrosion layer in the areas where the corrosion solution penetrated the film.

Magnesium phosphate coatings like the one observed on PEDOT/GO samples provide increased corrosion resistance in that they are a denser and insoluble protection layer[65-70]. Phosphate conversion coatings on other metals and phosphate-based coatings on Mg have shown promise as corrosion prevention coatings [65, 66, 70-72]. Corrosion of Mg alloys in solutions containing phosphate at concentrations mimicking biologic fluids have shown a precipitation of a semi corrosion-protective phosphate layer[73, 74]. Phosphate containing layers have also been deposited on steel and Mg alloys by immersion into magnesium phosphate baths[64, 67, 68]. Phosphate conversion coatings and phosphate coatings applied through plasma electrolytic oxidation (PEO) on Mg and Mg alloys have shown improved corrosion protection as well as a self-healing deposition of a phosphate protective coating in areas where the original coating was damaged[64, 75]. These ‘self-healed’ phosphate locations displayed a surface with cracked morphology, similar to that observed beneath PEDOT/GO.

Because of the allure of a degradable magnesium nerve guide implants[76, 77], *in vitro* primary neuronal culture was used as test bed to begin assessing the effect of the PEDOT/GO coating on cytotoxicity of Mg implants. Our lab has previously shown that PEDOT/GO film itself is exceptionally biocompatible and promotes the attachment and

neurite extension of primary neurons[47, 54, 78]. The lowered toxicity afforded by the PEDOT/GO coating on Mg is likely due to two factors. Firstly a decrease in Mg ion concentration, which at elevated levels, has been shown to be toxic to neurons[79, 80]. Secondly a more neutral pH due to the lack of OH⁻ production as the reduction of the polymer takes the place of water reduction[22]. Additionally, a potential benefit not demonstrated by corrosion media exposure tests is the prevention of hydrogen production. Hydrogen bubbles formed during corrosion cause tissue dislocation of the implant and result in implant failure [18, 21, 81]. The reduction of H₂ formation by the PEDOT/GO coating may serve to further improve in vivo implant biocompatibility in future experiments. While these results suggest the PEDOT/GO coating improve neuronal biocompatibility of Mg implants, additional *in vivo* performance needs to be assessed. Similarly, for other potential applications of Mg (ex. orthopedic and vascular), tissue specific *in vitro* and *in vivo* biocompatibility will need to be investigated.

This PEDOT/GO coating has shown the ability to slow magnesium corrosion, improve biocompatibility, and electrochemically decrease the production of hydrogen gas, all of which are important properties for improving the clinical potential of Mg implants. In addition to these benefits, future exploration will involve the release of anti-inflammatory drugs from the coating to reduce the host tissue response [54].

Additionally, the GO sheets exposed at the surface of the film provide loci for surface functionalization which can allow for improved tissue interaction with the implant[47]. The combination of improved corrosion resistance, the ability to release drug at the site of corrosion, as well as improve tissue interaction through surface cues make this

composite material a promising versatile coating for numerous potential applications of degradable Mg implants.

5. Conclusion

Here PEDOT/GO coatings were electropolymerized on Mg ribbon as uniform and adherent coatings. Coated Mg samples showed decreased corrosion rates when evaluated with Mg^{2+} concentration and pH of corrosion media, hydrogen evolution, and electrochemical techniques. Initially, PEDOT/GO coatings acted as passive barrier layers. Following diffusion of media through the film, the coating facilitated continued corrosion resistance through an increase in the negative charge on the film and the formation of a magnesium phosphate layer. *In vitro* neuron cytotoxicity experiments show that the PEDOT/GO coating improves the neuronal biocompatibility of the Mg samples. This research provides new insight into the potential for conducting polymers to act as effective corrosion control coatings on magnesium biomedical implants through multiple mechanisms. Such coatings will be highly desired for various Mg implant applications from cardiovascular stents to nerve guides.

Acknowledgement: The project described was supported by the National Science Foundation Grants 0748001 and ERC-0812348, and National Institute of Health R01NS062019.

References:

- [1] G. Song, A. Atrens, Understanding magnesium corrosion: a framework for improved alloy performance, *Advanced Engineering Materials*. 5(12) (2003) 837-858.
- [2] N.T. Kirkland, N. Birbilis, M.P. Staiger, Assessing the corrosion of biodegradable magnesium implants: A critical review of current methodologies and their limitations, *Acta Biomaterialia*. 8 (2012) 925-936.
- [3] J. Wang, Y. He, M. Maitz, B. Collins, K. Xiong, L. Guo, Y. Yun, G. Wan, N. Huang, A surface-eroding poly(1,3-trimethylene carbonate) coating for fully biodegradable magnesium-based stent applications: Toward better biofunction, biodegradation and biocompatibility, *Acta Biomaterialia*. 9(10) (2013) 8678-8689.
- [4] B. Shaw, E. Sikora, S. Virtanen, Fix, Heal and Disappear: A new approach to using metals in the human body, *The Electrochemical Society Interface*. (45-49) (2008).
- [5] J. Walker, S. Shadanbaz, T. Woodfield, M. Staiger, G. Dias, Magnesium biomaterials for orthopedic application: A review from a biological perspective, *J Biomed Mater Res Part B*. (2014).
- [6] F. Witte, N. Hort, C. Vogt, S. Cohen, K. Kainer, R. Willumeit, F. Feyerabend, Degradable biomaterials based on magnesium corrosion, *Current Opinion in Solid State and Materials Science*. 12(5-6) (2008) 63-72.
- [7] F. Witte, V. Kase, H. Haferkamp, E. Switzer, A. Myer-Lindenberg, C.J. Wirth, H. Windhage, In vivo corrosion of four magnesium alloys and the associated bone response, *Biomaterials*. 26 (2005) 3557-3563.
- [8] R. Erbel, C.D. Mario, J. Bartunek, J. Bonnier, B.d. Bruyne, F. Erne, M. Haude, B. Heublein, M. Horrigan, C. Ilesley, D. Bose, J. Koolen, T. Luscher, N. Weissman, R. Waksman, Temporary scaffolding of coronary arteries with bioabsorbable magnesium stents: a prospective, non-randomized multicenter trial, *The Lancet*. 369 (2007) 1869-1875.
- [9] A. Doepke, D. Xue, Y. Yun, W. Vanooij, B. Halsall, W. Heineman, Corrosion of organosilane coated Mg4Y alloy in sodium chloride solution evaluated by impedance spectroscopy and pH changes, *Electrochimica Acta*. 70 (2012) 165-170.
- [10] H. Hornberger, S. Virtanen, A. Boccaccini, Biomedical coatings on magnesium alloys- A review, *Acta Biomaterialia*. 8 (2012) 2442-2455.
- [11] K. Muir, Magnesium for neuroprotection in ischaemic stroke, *CNS Drugs*. 15(12) (2001) 921-930.
- [12] H. Pan, M. Sheu, H. Su, Y. Chen, C. Chen, D. Yang, W. Chiu, F. Cheng, Magnesium supplement promotes sciatic nerve regeneration and down-regulates inflammatory response, *Magnesium Research*. 24(2) (2011) 54-70.
- [13] R. Elin, Assessment of magnesium status for diagnosis and therapy, *Magnesium Research*. 23(4) (2010).
- [14] V.d.V. K, P. Kiekens, Biopolymers: overview of several properties and consequences on their applications, *Polymer Testing*. 21 (2002) 433-442.
- [15] F. Witte, The history of biodegradable magnesium implants: A review, *Acta Biomaterialia*. 6 (2010) 1680-1692.
- [16] J. Reifenrath, D. Bormann, A. Meyer-Lindenberg, Magnesium alloys - corrosion and surface treatments, in: F. Czerwinski (Ed.) *INTECH*, 2011.

- [17] G. Song, S. Song, A possible biodegradable magnesium implant material, *Advanced Engineering Materials*. 9 (2007) 298-302.
- [18] G. Song, Control of biodegradation of biocompatible magnesium alloys, *Corrosion Science*. 49 (2007) 1696-1701.
- [19] B. Zberg, P. Uggowitzer, J. Löffler, MgZnCa glasses without clinically observable hydrogen evolution for biodegradable implants, *Nature Materials*. 8 (2009) 887-891.
- [20] T. Kraus, S. Fischerauer, A. Hanzi, P. Uggowitzer, J. Löffler, A. Weinberg, Magnesium alloys for temporary implants in osteosynthesis: In vivo studies of their degradation and interaction with bone, *Acta Biomaterialia*. 8 (2012) 1230-1238.
- [21] J. Kuhlmann, I. Bartsch, E. Willbold, S. Schuchardt, O. Holz, N. Hort, D. Hoche, W. Heineman, F. Witte, Fast escape of hydrogen from gas cavities around corroding magnesium implants, *Acta Biomaterialia*. 9 (2013) 8714-8721
- .
- [22] T. Ohtsuka, Corrosion protection of steels by conducting polymer coating, *International Journal of Corrosion*. 2012 (2012).
- [23] D. Tallman, G. Spinks, A. Dominis, G. Wallace, Electroactive conducting polymers for corrosion control Part 1: General introduction, *Journal of Solid State Electrochemistry*. 6 (2002) 73-84
- .
- [24] D. Tallman, G. Spinks, A. Dominis, G. Wallace, Electroactive conducting polymers for corrosion control Part 2. Ferrous metals, *Journal of Solid State Electrochemistry*. 6 (2002) 85-100.
- [25] G. Paliwoda-Porebska, M. Stratmann, M. Rhowerder, K. Potje-Kamloth, Y. Lu, A. Pich, H. Adler, On the development of polypyrrole coatings with self-healing properties for iron corrosion protection, *Corrosion Sciences*. 47 (2005) 3216-3233.
- [26] D. Kowalski, M. Ueda, T. Ohtsuka, Self-healing ion-permselective conducting polymer coating, *Journal of Materials Chemistry*. 20 (2010) 7630-7633.
- [27] A. Krishnamurthy, V. Gadhamshetty, R. Mukherjee, Z. Chen, W. Ren, H.-M. Cheng, N. Koratkar, Passivation of microbial corrosion using graphene coating, *Carbon*. 56 (2013) 45-49.
- [28] E.T.Y. Lih, R.b.M. Zaid, T.L. Ling, K.F. Chong, Facile corrosion protection coating from graphene, *International Journal of Chemical Engineering and Applications*. 3(6) (2012) 453-455.
- [29] D. Prasai, J.C. Tuberquia, R.R. Harl, G.K. Jennings, K.I. Bolotin, Graphene: corrosion-inhibiting coating, *ACS Nano*. 6(2) (2012) 1102-1108.
- [30] R. Prabakar, Y.-H. Hwang, E.G. Bae, D.K. Lee, M. Pyo, Graphene oxide as a corrosion inhibitor for the aluminum current collector in lithium ion batteries, *Carbon*. 52 (2013) 128-136.
- [31] X. Luo, X.T. Cui, Electrochemical deposition of conducting polymer coatings on magnesium surfaces in ionic liquid, *Acta Biomaterials*. 7(1) (2011) 441-446.
- [32] V. Truong, P. lai, B. Moore, R. Muscat, M. Russo, Corrosion protection of magnesium by electroactive polypyrrole/paint coatings, *Synthetic Materials*. 110 (2000) 7-15.
- [33] A. Atrens, G.L. Song, F. Cao, Z. Shi, P. Bowen, Advances in Mg Corrosion and Research Suggestions, *Journal of Magnesium and Alloys*. 1 (2013) 177-200.

- [34] G. Baril, G. Galica, C. Deslouis, N. Pebere, B. Tribollet, V. Vivier, An impedance investigation on the mechanism of pure magnesium corrosion in sodium sulfate solutions *Journal of the Electrochemical Society*. 154(2) (2007) C108-C113.
- [35] X. Li, B. Faber, B. Minch, H. Castaneda, Analysis of soft coating corrosion performance on carbon steel using electrochemical impedance spectroscopy, *Corrosion Engineering, Science and Technology*. 70(6) (2014) 615-626.
- [36] P. Bonora, F. Deflorian, L. Fedrizzi, Electrochemical impedance spectroscopy as a tool for investigating underpaint corrosion, *Electrochimica Acta*. 41(7/8) (1996) 1073-1082.
- [37] S. Ahn, Y. Choi, J. Kim, J. Han, A study on corrosion resistance characteristics of PVD Cr-N coated steels by electrochemical method, *Surface and Coatings Technology*. 150(2-3) (2002) 319-326.
- [38] J. Pan, D. Thierry, C. Leygraf, Electrochemical impedance spectroscopy study of the passive oxide film on titanium for implant application, *Electrochimica Acta*. 41(7-8) (1996) 1143-1153.
- [39] A.M.A. Alsamuraee, H. Jaafer, H. Ameen, A. Abdullah, Electrochemical impedance spectroscopic evaluation of corrosion protection properties of polyurethane/polyvinyl chloride blend coatings on steel, *American Journal of Scientific and Industrial Research*. 2(5) (2011) 761-768.
- [40] E.-S. Sherif, Corrosion behavior of magnesium in naturally aerated stagnant seawater and 3.5% sodium chloride solutions, *International Journal of Electrochemical Science*. 7 (2012) 4235-4249.
- [41] K. Brunelli, M. Dabala, I. Calliari, M. Magrini, Effect of HCl pre-treatment on corrosion resistance of cerium based conversion coatings on magnesium and magnesium alloys, *Corrosion Science*. 47(4) (2005) 989-1000.
- [42] A. Rudd, C. Breslin, F. Mansfeld, The corrosion protection afforded by rare earth conversion coatings applied to magnesium, *Corrosion Science*. 42(2) (2000) 275-288.
- [43] S. Lamaka, M. Montemor, A. Galio, M. Zheludkevich, C. Trindade, L. Dick, M. Ferreira, Novel hybrid sol-gel coatings for corrosion protection of AZ31B magnesium alloy, *Electrochimica Acta*. 53(14) (2008) 4773-4783.
- [44] J.-B. Jorcin, M.E. Orazem, N. Pébère, B. Tribollet, CPE analysis by local electrochemical impedance spectroscopy, *Electrochimica Acta*. 51(8-9) (2006) 1473-1479.
- [45] J. Bisquert, G. Garcia-Belmonte, P. Bueno, E. Longo, L.O.S. Bulhões, Impedance of constant phase element (CPE)-blocked diffusion in film electrodes, *Journal of Electroanalytical Chemistry*. 452 (1998) 229-234.
- [46] P. Kruze, Corrosion and surface protections, in: F. Horst, B. Mordike (Eds.), *Magnesium Technology*, Springer, Berlin Heidelberg, 2006.
- [47] X. Luo, C.L. Weaver, S. Tan, X.T. Cui, Pure graphene oxide doped conducting polymer nanocomposite for bio-interfacing, *J. Mater. Chem. B*. 1 (2013) 1340-1348.
- [48] U. Rammelt, P. Nguyen, W. Plieth, Protection of mild steel by modification with thin films of polymethylthiophene, *Electrochimica Acta*. 46 (2001) 4251-4257.
- [49] A. Ganash, Effect of current density on the corrosion protection of poly(o-toluidine)-coated stainless steel, *Int. J Electrochem. Sci*. 9 (2014) 4000-4013.

- [50] C. Weaver, H. Li, X. Luo, T. Cui, A graphene oxide/conducting polymer nanocomposite for electrochemical dopamine detection: origin of improved sensitivity and specificity, *Journal of Materials Chemistry B*. 2 (2014) 5209-5219.
- [51] F. Beck, M. Oberst, Electrocatalytic deposition and transformation of polypyrrole layers, *Synthetic Materials*. 28(1-2) (1989) 43-45.
- [52] A. Diaz, J. Bargon, Electrochemical synthesis of conducting polymers, in: T. Skotheim (Ed.), *Handbook of conducting polymers*, Marcel Dekker Inc, New York, 1986, pp. 87-90.
- [53] A. Diaz, B. Hall, Mechanical properties of electrochemically prepared polypyrrole films, *IBM Journal of Research and Development*. 27(4) (1983) 342-347.
- [54] C. Weaver, J. LaRosa, X. Luo, X.T. Cui, Electrically controlled drug delivery from graphene oxide nanocomposite films, *ACS Nano*. 8(2) (2014) 1834-1843.
- [55] G. Song, A. Atrens, D.S. John, X. Wu, J. Nairin, The anodic dissolution of magnesium in chloride and sulphate solutions, *Corrosion Science*. 39(10-11) (1997) 1981-2004.
- [56] B. Shaw, Corrosion resistance of magnesium alloys, *ASM Handbook*. 13A (2003) 692-696.
- [57] S. Salman, M. Okido, Anodization of magnesium (Mg) alloys to improve corrosion resistance, in: G.-L. Song (Ed.), *Corrosion Prevention of Magnesium Alloys*, Woodhead Publishing Limited 2013, p. 199.
- [58] P. Kinlen, V. Menon, Y. Ding, A mechanistic investigation of polyaniline corrosion protection using the scanning reference electrode technique, *Journal of the Electrochemical Society*. 146(10) (1999) 3690-3695.
- [59] B. Wessling, Corrosion prevention with an organic metal (polyaniline): surface ennobling, passivation, corrosion test results, *Materials and Corrosion*. 47 (1996) 439-445.
- [60] B. Wessling, Passivation of metals by coating with polyaniline: Corrosion potential shift and morphological changes, *Advanced Materials*. 6(3) (1994) 226-228.
- [61] A. Talo, P. Passiniemi, O. Forsen, S. Ylassaari, Polyaniline/epoxy coatings with good anti-corrosion properties, *Synthetic Metals*. 85 (1997) 1333-1334.
- [62] W. Lu, R. Elsenbaumer, B. Wessling, Corrosion protection of mild steel by coatings containing polyaniline, *Synthetic Materials* 71 (1995) 2163-2166.
- [63] P. Li, T. Tan, J. Lee, Corrosion protection of mild steel by electroactive polyaniline coatings, *Synthetic Metals*. 88 (1997) 237-242.
- [64] K. Murakami, M. Hino, K. Nakai, S. Kobayashi, A. Saijo, T. Kanadani, Mechanism of corrosion protection of anodized magnesium alloys, *Materials Transactions*. 49(5) (2008) 1057-1064.
- [65] K. Chong, T. Shih, Conversion-coating treatment for magnesium alloys by a permanganate-phosphate solution, *Materials Chemistry and Physics*. 80(1) (2003) 191-200.
- [66] T. Ishizaki, R. Kudo, T. Omi, K. Teshima, T. Sonoda, I. Shigematsu, M. Sakamoto, Magnesium hydroxide/magnesium phosphate compounds composite coating for corrosion protection of magnesium alloy by a combination process of chemical conversion and steam curing, *Materials Letters*. 68(1) (2012) 122-125.

- [67] C. Lin, C. Lee, W. Li, Y. Chen, G. Fang, Formation of phosphate/permanganate conversion coating on AZ31 magnesium alloy, *Journal of the Electrochemical Society*. 153(3) (2006) B90-B96.
- [68] M. Morks, Magnesium phosphate treatment for steel, *Materials Letters*. 58(26) (2004) 3316-3319.
- [69] H. Zang, G. Yao, S. Wang, Y. Liu, H. Luo, A chrome-free conversion coating for magnesium-lithium alloy by a phosphate-permanganate solution, *Surface and Coatings Technology*. 202(9) (2008) 1825-1830.
- [70] W. Zhou, D. Shan, E.-H. Han, W. Ke, Structure and formation mechanism of phosphate conversion coating on die-cast AZ91D magnesium alloy, *Corrosion Science*. 50(2) (2008) 329-337.
- [71] R. Arrabl, E. Matykina, F. Viejo, P. Skeldon, G. Thompson, Corrosion resistance of WE43 and AZ91D magnesium alloys with phosphate PEO coatings, *Corrosion Science*. 50(6) (2008) 1744-1752.
- [72] H. Zhang, G. Yao, S. Wang, Y. Liu, H. Luo, A chrome-free conversion coating for magnesium-lithium alloy by a phosphate-permanganate solution, *Surface and Coatings Technology*. 202(9) (2008) 1825-1830.
- [73] L. Xu, E. Zhang, D. Yin, S. Zeng, K. Yang, In vitro corrosion behavior of Mg alloys in a phosphate solution for bone implant application, *Journal of Material Science: Materials in Medicine*. 19 (2008) 1017-1025.
- [74] Y. Xin, V. Kaese, Z.-T. Hou, H. Tao, G. Tang, P. Chu, Influence of aggressive ions on the degradation behavior of biomedical magnesium alloy in physiological environment, *Acta Biomaterialia*. 4 (2008) 2008-2015.
- [75] S. Yagi, K. Kuwabara, Y. Fukuta, K. Kubota, E. Matsubara, Formation of self-repairing anodized film on ACM522 magnesium alloy by plasma electrolytic oxidation, *Corrosion Science*. 73 (2013) 188-195.
- [76] J. Vennemeyer, T. Hopkins, J. Kuhlmann, W. Heineman, S. Pixley, Effects of elevated magnesium and substrate on neuronal numbers and neurite outgrowth of neural stem/progenitor cells in vitro, *Neuroscience Research*. 84 (2014) 72-78.
- [77] J. Vennemeyer, T. Hopkins, M. Herschcovitch, K. Little, M. Hagen, D. Minteer, D. Hom, K. Marra, S. Pixley, Initial observations on using magnesium metal in peripheral nerve repair, *J Biomater Appl*. 29(8) (2015) 1145-1154.
- [78] C. Weaver, X.T. Cui, Directed neural stem cell differentiation with a functionalized graphene oxide nanocomposite, *Advanced Healthcare Materials*. Accepted (2015).
- [79] W. Dribben, L. Eisenman, S. Mennerick, Magnesium induces neuronal apoptosis by suppressing excitability, *Cell Death and Disease*. 1(63) (2011).
- [80] T. Huang, Z. Wang, L. Wei, M. Kindy, Y. Zheng, T. Xi, B. Gao, Microelectrode array-evaluation of neurotoxic effects of magnesium as an implantable biomaterial, *Journal of Materials Science & Technology*. 32(1) (2016) 89-96.
- [81] M. Staiger, A. Pietak, J. Huadmai, G. Dias, Magnesium and its alloys as orthopedic biomaterials: A review, *Biomaterials*. 27(9) (2006) 1728-1734.

Figure 1

Figure 2

Figure 3

Figure 4

Figure 5

Figure 6

Figure 7

Figure 8

Figure 9

A

B