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UNIVERSITY OF CALIFORNIA
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Synthesis and Evaluation of Poly(3,4-ethylenedioxythiophene)
(PEDOT) Coated Magnesium for Nerve Regeneration

A Thesis submitted in partial satisfaction
of the requirements for the degree of

Master of Science

in

Bioengineering

by

Meriam Amel Sebaa

June 2012

Thesis Committee:

Dr. Huinan Liu

Dr. Valentine Vullev

Dr. Masaru Rao

Dr. Prudence Talbot

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The Thesis of Meriam Amel Sebaa is approved:

Committee Chairperson

University of California, Riverside

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1. Introduction: Conductive Biomaterials for Nerve Regeneration: a Review

1.1. Significance

Damage to a nerve is often labeled irreversible. Nerve cells are known to grow at early stages in human development and once fully developed (adult), most of them no longer regenerate. Nerve cell degeneration can occur at various levels causing all kinds of damage. Nerve damage can leave a patient terminally ill. Nervous system injuries affect over 90,000 people per year [1]. There are many diseases that can lead to central and peripheral nerve damage. Central nervous damage could be caused by traumatic brain injuries, strokes, ruptured aneurysms, ALS (Lou Gerhig's Disease), Alzheimer's, Parkinson's disease, multiple sclerosis, and spinal cord injuries. Some of the most common causes for peripheral nerve damage include diabetic neuropathy and accidents.

Amyotrophic Lateral Sclerosis (Lou Gerhig's Disease) is a motor neuron disease that affects 1 out of 100,000 people [2]. Damage to nerve cells in the brain and spinal cord lead to paralysis and inability to breathe because messages cannot be sent to the muscles. Around 62.3 out of 100,000 adults live with enduring functional impairments due to traumatic brain injuries [3]. An estimated 5.3 million Americans suffer from Alzheimer's disease. Approximately 50,000 Americans are diagnosed with Parkinson's disease yearly [4]. Around 250,000 to 350,000 people in the US have been diagnosed with multiple sclerosis. This disease destroys axon myelination in the brain and spinal cord.

Approximately 200 new cases are diagnosed each week [5]. There are about 250,000–400,000 people living with a spinal cord injury and around 13,000 people are subject to spinal cord injuries per year in the US [6]. According to National Health and Nutrition

Examination Surveys, 7.8% of the population in the US has diabetes (17.9 million people diagnosed). Of those, 60-70% have mild to severe forms of nervous system damage. Many lower-limb amputations occur as a result of nerve damage. Neural implants are becoming a necessity for treating patients with nerve damage. This is a very difficult task considering how complex the nervous system is. The central nervous system is far more complex than the peripheral nervous system in terms of repair due to the types of cells surrounding the axons. Damaged peripheral nerve cells can recover with proliferating Schwann cells promoting axonal growth. The central nervous system lacks Schwann cells making it an inhibitory environment in terms of neural regeneration [7].

1.2 Characteristics of Natural Nerves

Tissue regeneration in the CNS is deficient. This is due to the complexity of what occurs post injury. The results of these events lead to a non-conductive environment which is not favorable for tissue regeneration. This non-conductive environment is formed from glial scarring caused by astrocytes initiated by transforming growth factor β . The glial scarring consists of extracellular matrix proteins, myelin, astrocytes, and oligodendrocytes. The reactive astrocytes produce chondroitin sulfate proteoglycans which inhibit axonal growth. The scar tissue prevents neural tissue regeneration by acting as a barrier. This scarring exists and is necessary to repair the blood brain barrier. This is of concern when it comes to insertion of microelectrodes into the CNS for neural repair. Even a small electrode will initiate glial scarring which would reduce conductivity causing the electrode's effectiveness to decrease [8].

The peripheral nervous system has the ability to regenerate on their own under certain conditions with short distances [9]. Injury in the PNS leaves a gap between proximal and distal nerves. Schwann cells and macrophages remove myelin and debris from the site of injury and fill up a fibrin cable that forms around the injury gap [8]. PNS does not consist of astrocytes to cause glial scarring.

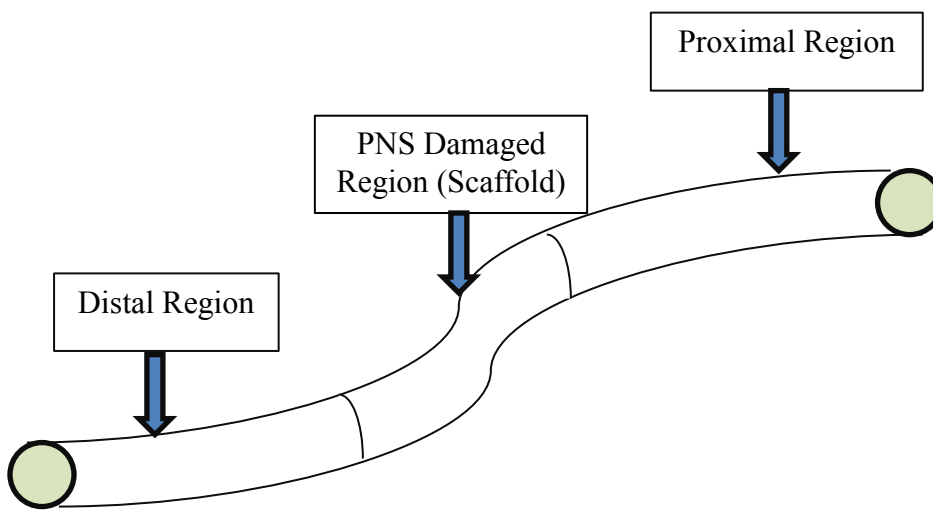


Figure 1: An example of a damaged nerve. Center region is where nerve guide channel is placed to help promote regeneration between damaged ends.

It is important to make sure the biomaterials used for nerve regeneration are similar to natural nerves. They must be able to connect with the nerve cells in order to attain positive results. When comparing biomaterials to the natural nerve, the natural nerve is conductive (the greater the diameter, the greater the conduction), with a resting membrane potential of -65 volts. The mechanical strength of a rat sciatic nerve has a Young's Modulus of 576 ± 160 kPa [10]. Another study on the mechanical properties of spinal nerve roots of Sprague Dawley rats by Singh, A., et al., reported maximum

strengths of 257.9 ± 111.3 kPa and 1.3 ± 0.8 MPa observed when stretched at a rate of 0.01mm/s and 624.9 ± 306.8 kPa and 2.9 ± 1.5 MPa observed when stretched at a rate of 15mm/s, respectively. These nerve roots were subject to tension and strain. [11]. Rabbit tibial peripheral nerve Young's Modulus was reported at 0.50 MPa [12]. The ultimate load of human ulnar nerves was reported at 65-155 and the medial nerves at 73-220 N [13].

In selecting the most compatible material for neural applications, one must consider that the nervous system is electrical. This requires the biomaterial to be conductive. Other important criteria include strength, and cyto-compatibility.

1.3. Biomaterials for Nerve Regeneration

Biomaterials play critical roles in nerve tissue regeneration since nerves cannot efficiently self-regenerate if at all. The biomaterials reviewed in this paper include conductive polymers polypyrrole and poly (3,4-ethylenedioxythiophene) (PEDOT), carbon nanotubes, piezoelectric zinc oxide (ZnO), and magnesium (Mg) metal. This paper will discuss these potential biomaterials by reviewing their mechanical properties, electrical properties, physical properties, cyto-compatibility, degradation properties, and in vitro/vivo performance for nerve regeneration.

1.3.1. Conductive Polymers

Conductive polymers are organic polymers that conduct electricity due to their backbones of contiguous sp^2 hybridized carbon centers. They can act like metals or semiconductors.

The following conductive polymers have exhibited great conductive properties for nerve regeneration.

1.3.1.1. Poly(3,4-ethylenedioxythiophene), (PEDOT)

Poly(3,4-ethylenedioxythiophene) (PEDOT), a sulfur containing conductive polymer, has been recognized to function similar to a wire; it has been observed to increase electrical signals within the nerve [14]. It has a tensile strength of 17.2 ± 5.1 MPa [15] with high electrochemical stability and a low band gap [16]. It is electrically conductive and more electrochemically stable than polypyrrole [17]. Conductivity can range from 780 to 2 775 S /cm [18]. It is light weight with a density of 1.5 g/cm^3 [19]. As far as biocompatibility, Lou, S.C. et al. [17] have reported that thin PEDOT films exhibit low intrinsic cytotoxicity and do not show inflammatory responses upon implantation. This makes them ideal for bio sensing and biomedical applications. It is a biocompatible and non-biodegradable polymer. PEDOT is not very soluble in water. In an acetonitrile and water mixture, EDOT concentration can be increased by a factor of 10, thus decreasing degradation. It degrades faster when exposed to air than liquid. In a physiological environment, little to no degradation occurs [17]. Their studies also showed that the cells selectively grew on the PEDOT coated areas of probes. In vitro studies by Richardson-Burns, S.M., et al., [20] showed that live cells embedded within the conductive polymer matrix remained viable for at least 120 hours following polymerization. PEDOT coatings on electrodes significantly enhanced the electrical properties compared to bare electrodes [20]. Toxicity of PEDOT was studied using overlay assays with L929 fibroblasts, elution, and direct contact tests on human neuroblastoma SH-SY5Y cells. They found no

cytotoxicity. They also tested PEDOT:heparin in vivo through heparin-coated implants in a rodent cortex. PEDOT:heparin surfaces were non-cytotoxic and showed no marked difference in immunological response in cortical tissue compared to pure platinum controls [21]. In vivo studies characterized the electrical functionality of PEDOT/peptide coated electrode in guinea pigs [17]. Results showed that high quality acute neural recordings were obtained via PEDOT/DCDPGYIGSR (peptide) coated electrodes [17].

1.3.1.2. Polypyrrole

Polypyrrole (Ppy) is a nitrogen-containing conductive polymer. Its breaking strength is 67 ± 6 MPa [22]. Its electrical conductivity can be as high as 2215 S/cm [16]. Under properly selective polymerizing conditions, 500 S/cm electrical conductivity was recorded [23]. It is very light weight, at 0.967 g/cm^3 [24]. Dissociated primary cerebral cortical cells cultured on Ppy samples found neural networks grew on all of the PPy surfaces in a study [25]. Ppy is biodegradable as well. The first order degradation rate constant varied between 2.8×10^{-5} and $3.4 \times 10^{-3} \text{ s}^{-1}$, covering corresponding half-period values of 6.8 h to 3.4 min [26]. Carbon nanotubes coated with Ppy were shown to support nerve growth [6]. Surgically implanted Ppy into the cerebral cortex of a rat showed that all versions of Ppy were at least as biocompatible as Teflon and even performed better in most cases.

Neurons and glial cells enveloped the implant [25]. Coated probes were implanted in a guinea pig's brain for 1, 2, and 3 week periods. They found significantly more neurofilament positive staining on the coated electrodes. This indicated that the coatings established strong connections with the neuronal structure in vivo. The first week tissue sections had no significant gliosis, the second week had a layer of non-neuronal tissue

consisting mostly of fibroblasts and extracellular membrane protein including fibronectin. Astrocytes started to form a loosely organized layer by the end of the third week [27]. In another study, specially shaped Ppy implants were fabricated to span the cerebral cortex of Sprague Dawley rats. Each side of the cerebral cortex was implanted. One of them was to determine if the solvent had any effect on the implant properties. The surrounding cortex enveloped the implants. Staining for macrophages was done and showed an increased presence of them around the implant site at the 3 week point, and a smaller amount around the 6 week point. The neural tissue would generally reform after the stab wound leaving a scar. The tissue bridged more completely the PPy lumen as opposed to the lumen of Teflon implants [28]. These conductive polymers show great potential to be used to coat implants to help with corrosion rates, increase mechanical integrity, and affect conductivity of biomaterials.

1.3.2. Carbon-based Nanomaterials

1.3.2.1. Carbon nanotubes

Carbon nanotubes are cylindrically shaped carbon molecules with a lot of potential use in nanotechnology. The tensile strength of a single walled carbon nanotube (SWNT) is 13–53GPa [29]. The electrical conductivity of a single walled carbon nanotube is 250-400 S/cm [30]. Its density is around 1.3 to 1.4 g·cm⁻³ [31]. Cytotoxicity of carbon nanotubes in terms of biological applications depend on the way the nanotube is synthesized, the size of the nanotube and the overall experimental setup. SWNTs can enter human cells and accumulate in the cytoplasm causing damage [32]. Generally, the harmful effects of the nanoparticles arise from the combination of high surface area, and the intrinsic

toxicity of the surface [33]. Studies have been done to show both toxic and non-toxic side effects of carbon nanotubes [33]. Carbon nanotubes are not biodegradable. In vitro studies have indicated that SWNTs functionalized by a covalent bonding method with phenyl-SO₃H or phenyl-(COOH)₂ groups generated less cytotoxic effects than aqueous dispersions of pristine SWNT stabilized with a surfactant. The cytotoxicity of covalently modified SWNT has been reported to be even further decreased with increasing side wall functionalization [33]. As mentioned earlier, CNTs have been shown to support nerve growth when coated with Ppy. Their conductive properties make them better suited as an interface with neurons to stimulate and record neural activity. Neurons cultured with CNTs showed enhanced neuronal electrical activity. They boost neuronal activity by providing a shortcut for electrical coupling between somatic and dendritic neuronal compartments [34]. It has been reported that SWNTs that are functionalized with phospholipids bearing polyethylene glycol (PEG) are very stable in vivo [35].

1.3.2.2. Graphene

Graphene is a flat carbon atom monolayer tightly packed in a honeycomb lattice. It has unique physical, chemical, and mechanical properties. It is mainly used as graphene oxide (GO) for biological applications. It's unique nanostructure and properties include high surface area and low cost. It has been reported to have a Young's Modulus of 0.5TPa [36] and 1TPa [37]. Graphene oxide nanoparticles have a bulk electric conductivity of 1000-2300 S/m [38] with a very low density of 0.77mg/m² [39]. The effects of graphene oxides (GO) on human fibroblast cells and mice was studied to report the biocompatibility of graphene. The human fibroblast cells were exposed to different

concentrations of GO for 5 days. The mice were divided into three groups of low dose, medium dose, and high dose GO plus a control group. The in vivo tests showed that GO below 20microg/mL exhibit low cytotoxicity with a cell survival rate of >80% while GO above 50microg/mL exhibited high cytotoxicity. Also, with increasing time of exposure to GO, the cell's survival decreased. Tests on the mice showed using pathology and light micrograph that GO was accumulating in the lungs, liver, and spleen [40]. This shows signs of dose and time dependent cyto-toxicity. A study on neural cell-cell interactions designed a flexible, transparent, and non-cytotoxic graphene/Polyethylene terephthalate (PET) film stimulator to examine its effects on cell-cell coupling. SHSY5Y human neuroblastoma cells were grown and two graphene electrodes separated by 2mm were connected to a power supply. They placed the cells on the PET film over the 2mm gap. When the electric field strength exceeded 450 mV/mm, the cells shrunk significantly. Weak electric fields on the other hand promoted new cell-cell coupling and strengthened existing cell-cell coupling [41].

1.3.3. Ceramic-based Nanomaterials

1.3.3.1. Piezoelectric Zinc Oxide

Piezoelectric materials generating electrical charges in response to mechanical strain may be used to stimulate axonal regeneration following nerve injury [42]. Theoretically, zinc oxide can provide a stimulatory cue for neural tissue regeneration when deformed mechanically through ultrasound [8]. Zinc Oxide has excellent mechanical strength, with a young's modulus of 150–240 GPa (thin films of ZnO)[43]. Its electrical resistivity is

about $3.3 \times 10^{-2} \Omega \cdot \text{cm}$ [44]. The density of ZnO is 5.606g/cm^3 , a bit more dense than the rest of the materials in this review. As far as cyto-compatibility, Song, W.H., et al. reported that ZnO particles had a dose-dependent toxic effect on Ana-1 cells without size-dependence. When the concentration of dissolved Zinc ions reached equilibrium, the zinc ion concentration lead to about 50% cell death [45]. Zinc Oxide is not water soluble but can be degraded by acids or bases. It also reacts with fatty acids in oils. Bases degrade the solid to give soluble zincates as follows: $\text{ZnO} + 2\text{NaOH} + \text{H}_2\text{O} \rightarrow \text{Na}_2(\text{Zn}(\text{OH})_4)$. ZnO with a polymer composite, have been shown to reduce astroglial cell activity which is critical in regeneration of functional neural tissue in the CNS [8]. The piezoelectric effect of the material can enhance both CNS and PNS regeneration [8]. Ultimately, this composite material, among other electrically active nano-materials, when fabricated into a NGC (nerve guidance channel) and implanted into the body, could reduce inhibitory cues which prevent healthy tissue regeneration and provide critical stimulatory cues which promote neural cell activity and axon growth [8].

1.3.4. Metals

1.3.4.1. Titanium

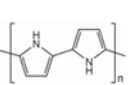
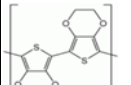
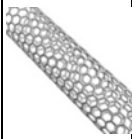
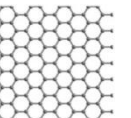
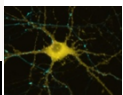
Titanium is a strong metal that is the current dominant material used for various biomedical applications. It has a young's modulus of 100-110GPa [46] and an ultimate strength of 3000MPa [46]. It's electrical resistivity is recorded at $420 \text{n}\Omega\text{m}$ [47]. It has a density of 4.5g/cm^3 [48]. Titanium dioxide nanoparticles (TiO_2 NPs) have been studied as well for biomedical applications. Particle size and exposure dose of TiO_2 NPs have an

important impact on pulmonary toxicity. Rats were intra-tracheally instilled with 0.5, 5, or 50 mg/kg of 5, 21, and 50 nm TiO₂ NPs. Pulmonary toxicity caused by 5nm particles caused more severe damage than 21 and 50nm particles [49]. A long term study on the toxicity of TiO₂ NPs was conducted on zebrafish in vivo for a 6 month period. The results showed obvious adverse effects on the fish including concentration and time dependent growth inhibition and a decrease in liver weight. The gills displayed thickening of edema. The TiO₂ NPs were distributed in the gills, liver, heart and brain [50]. The effects of TiO₂ NPs on neural stem cell proliferation rate and differentiation were studied. After incubating for the nanoparticles for 24 hours with the cells, the results showed inhibition on cell proliferation with concentrations larger than 100mg/mL. In studying the effects of TiO₂ NPs on differentiation of neural stem cells (NSCs), results showed obvious differentiation of NSCs into neurons with increasing neuronal concentration from 16.4% to 32.1% and glial cell decreasing concentration from 80% to 60% [51]. Titanium vascular closure staples (VCS) were applied to transected sciatic nerves of 36 Sprague Dawley rats. By day 5, regeneration was observed and advanced within four weeks. The use of VCS clips were shown to be faster and comparable to non-absorbable sutures in primary nerve repair[28]. The effects of TiO₂ NPs on hippocampal apoptosis was studied to investigate the molecular mechanism of it on mice. TiO₂ NPs (50mg/kg body weight) were intragastrically administered for 60 days and were found to accumulate in the hippocampus leading to apoptosis and impairment in spatial recognition memory [52].

1.3.4.2. Biodegradable Metal-Magnesium

Magnesium metal has shown promising biomimetic properties for bone regenerative medicine. The properties of magnesium will be explored as a potential biomaterial for nerve regeneration. Not much research has been done with magnesium studies as a biomaterial for nerve regeneration. It could have great potential use. As far as strength, Magnesium metal has excellent mechanical strength. Its tensile strength (pure Mg metal) is 180 – 220 MPa [53]. The electrical conductivity of Mg is 22 MS/m [54]. Its electrical resistivity is 43.9 nΩ·m. Magnesium metal is also very light weight. Its density is 1.738g/cm³[55]. Magnesium has a high corrosion rate. It degrades quickly. Polymer coatings help improve magnesium's biocompatibility and control the corrosion rate [56]. Magnesium is degradable in vivo. Its degradation rate according to Yunchang Xin and T.H., Paul K. Chu is 1.08mg/cm²h [57]. Not enough research (in vitro and in vivo) has been done on Magnesium involvement with neural cells for regenerative purposes

Table 1: Summary of biomaterial properties with potential use for nerve regeneration

Bio-material Property	Conductive Polymers		Carbon based materials		Piezo-electric Ceramic (ZnO)	Metals		Natural Nerve
	PP	PEDOT	CNT	Graphene, Graphene Oxide		Ti, TiO ₂ NPs	Mg	
Mechanic	Tensile strength 17 MPa [22, 58]	Tensile strength 17.2±5.1 MPa [15]	Tensile strength SWNT: 3.6 ± 0.4GPa [59] 13–53GPa [29]	Young's Modulus: 1 TPa [37] 0.5 TPa [36]	Young's Modulus of thin films of ZnO: 150–240 GPa [43]	Young's Modulus 100–110GPa Ultimate strength: 300MPa [46]	Tensile strength 180 - 220 MPa [53]	Young's modulus (rat): 576 ± 160 kPa [10] 0.50 MPa [12] Ultimate load (human): 65-155 and 73-220 N [13]
Electric	Electrical conductivity : 30-40 S/cm [22] 500 S/cm. [23] as high as 2215 S/cm. [16] 30-40 S cm ⁻¹ [22]	Electrical conductivity : 20- 50 S/cm [60] 132 S/cm [61]	Electrical conductivity (MWNT): 3000 S/m [62] Electrical conductivity (SWNT): 250-400 S/cm [30]	Electric conductivity (graphene): 0.96x10 ⁶ Ω ⁻¹ cm ⁻¹ [39] electric conductivity (GO): 1000–2300 S/m [38]	Electrical resistivity: about 3.3×10 ⁻² Ω·cm [44]	Electrical resistivity: 420 nΩ·m [47]	Electrical conductivity: 22 MS/m [54]	Electrical resistivity: 0.950 ± 0.002 Ω [63] Resting membrane potential: - 65mV [64]
Physical	 Density: 0.967 g/cm ³ [24]	 Density: 1.5g/cm ³ [19]	 Density: 1.3 - 1.4 g·cm ⁻³ [31]	 Density: 0.77 mg/m ² [39]	$Zn=O$ Density: 5.606g/cm ³ [65]	 Density: 4.5 g/cm ³ [48]	 Density: 1.738g/cm ³ [55]	 [67]
Cyto compatibility	Biocompatible: Neural networks grew on PPy surfaces [25]	Not cytotoxic [21] Excellent biocompatibility: low toxicity [19] Non-biodegradable yet biocompatible	Harmful effects based on high surface area, and intrinsic toxicity of surface [33] May or	Dose and time dependent cytotoxicity[40]	Dose-dependent toxicity [45]	Particle size and exposure determine toxicity [49]	Polymer Coatings help improve biocompatibility and control corrosion [56]	Bio-compatible

		le [20]	may not be cyto-toxic					
Degradation Property	Biodegradable first-order degradation rate constant between 2.8×10^{-5} and $3.4 \times 10^{-3} \text{ s}^{-1}$, [26]	Poor solubility in water. Used in organic media. [17] Little or no degradation in biological material.	Non-biodegradable	Non-biodegradable	Not water soluble [68]	Non-biodegradable	Degradable in vivo [56] Degradation rate: $1.08 \text{ mg/cm}^2 \text{ h}$ [57]	Bio-degradable
In vitro studies	Support nerve growth when coated CNT with ppy [69] Neural networks grew on all of the PPy surfaces [25]	Neuron-templated PEDOT coatings significantly enhanced the electrical properties compared to the bare electrode [20] Cells selectively grow on PEDOT [17]	provide a shortcut for electrical coupling between somatic and dendritic neuronal compartments [34]	Under weak electric field stimulation, stronger and new cell-cell coupling observed. [41]	Shown to reduce astroglial cell activity [8]	TiO ₂ NPs show advantages in differentiating NSCs into neurons [51]	Fast degradation rates of Mg-based biomedical implants impose severe limitations in vitro [70]	n/a
In vivo studies	Neurons and glial cells envelope implant [25] Guinea pig brain implanted coated probes show strong connections with neuronal structure [27] Neural tissue bridges PPy lumen [28].	Acute experiments in guinea pig show high quality acute neural recordings can be obtained through PEDOT/DC DPGYIGSR coated electrodes. [17]	SWNTs that are functionalized with phospholipids bearing polyethylene-glycol (PEG) are stable in vivo. [35]	GO resulted in pulmonary edema and granuloma formation at the dosage of 10 mg kg^{-1} body weight [71] Found GO accumulating in the lungs, liver, and spleen of mice [40]	Mechanically deformed ZnO provide electrical stimulus for neural tissue regeneration [8]	Titanium vascular closure staples faster nerve repair [28] TiO ₂ NPs lead to hippocampal apoptosis and impairment in spatial recognition and memory [52]	With controlled degradation (alloy or coating), Mg is biocompatible and degradable in vivo [72]	n/a

CHAPTER 1

Electrochemical Deposition and Evaluation of Electrically Conductive Polymer Coating on Biodegradable Magnesium Implants for Neural Applications

Abstract

In an attempt to develop conductive, biodegradable, mechanically strong, and biocompatible nerve conduits, pure magnesium (Mg) was used as the biodegradable substrate material to provide strength while the conductive polymer, poly(3,4ethylenedioxythiophene) (PEDOT) was used as a conductive coating material to control Mg degradation and improve cytocompatibility of Mg substrates. This study explored a series of electrochemical deposition conditions to produce a uniform, consistent PEDOT coating on Mg substrates. Both cyclic voltammetry (CV) and chronoamperometry coating methods produced adequate coverage and uniform coating. Low-cost stainless steel and copper electrodes can be used to deposit PEDOT coatings as effectively as platinum and silver-silver chloride electrodes. A concentration of 1M EDOT (monomer form of PEDOT) in ionic liquid was sufficient for coating Mg substrates with a surface area of 5mm x 5mm. Five cycles of CV with the potential ranging from -0.5V to 2.0V were used to produce consistent coatings for further evaluation. Scanning electron micrographs showed the micro-porous structure of PEDOT coatings. Energy Dispersive X-ray Spectroscopy (EDS) showed the peaks of sulfur, oxygen, and carbon, indicating sufficient PEDOT coating. Adhesion strength of the coating was measured using the tape test following the ASTM-D 3359 standard. The adhesion strength of PEDOT coating was within the classifications of 3B to 4B. Tafel

tests of the PEDOT coated Mg showed a corrosion current (I_{CORR}) of $6.14 \times 10^{-5} \text{A}$ and critical voltage of -1.10V , as compared with I_{CORR} of $9.08 \times 10^{-4} \text{A}$ with a critical voltage of -1.35V for non-coated Mg. The calculated corrosion rate for the PEDOT coated Mg was 8.6 mm/year , much slower than 126.9 mm/year for the non-coated Mg.

Keywords: poly(3,4ethylenedioxythiophene) (PEDOT), electrochemical deposition, electrodeposition, magnesium, neural, coatings.

1. Introduction

Treating patients with peripheral nerve damage is challenging due to the limited ability of nerves to self-regenerate. Current treatments for damaged nerves include nerve guide conduits, which are used to guide growth of injured nerves. It is crucial that foreign materials are non-toxic and can guide cell growth and differentiation into expected cell types when implanted into the body. Magnesium (Mg) was chosen as the metallic support due to its excellent mechanical strength, high electrical conductivity, neuro-protective capability, and biodegradability, all desirable properties. Mg has excellent mechanical strength. Specifically, the tensile strength of pure Mg is 180 – 220 MPa[53]. The electrical conductivity of Mg is 22 MS/m [54] and the electrical resistivity is 43.9 nΩ·m. Mg also has a light weight with a density of 1.738g/cm³. Moreover, Mg sulfate has been infused clinically as a neuro-protective agent [73-76]. Increasing Mg concentration in cerebrospinal fluid and brain has been shown to protect nerve system from further damage caused by acute stroke [75, 76] and cerebral ischemia [77]. Mg is biodegradable in physiological environment, with a fast degradation rate of 1.08mg/cm²h [57]. This rapid degradation leads to an unfavorable increase in surrounding pH. Polymer coatings help improve biocompatibility and control its corrosion rate[56]. These findings make Mg an excellent candidate for implantation as a nerve guide conduit if the release of Mg ions can be properly controlled with coatings.

The conductive polymer poly (3,4-ethylenedioxythiophene) (PEDOT) was chosen as the coating material due to its conductivity (electrical conductivity ranges found from 20 to 132 S/cm) and biocompatibility [19, 20]. PEDOT is a sulfur containing organic conductive polymer. It is also more electrochemically stable than its counterpart, polypyrrole[17], and has a low band gap [16]. Mg has a tensile strength of 17.2 ± 5.1 MPa [15] and a density of 1.5 g/cm^3 [19]. PEDOT has been observed to increase electrical signals within the nerve [14]. Previous studies showed nerves regenerated in PEDOT coated agarose based hydrogel tubes compared with polydimethylsiloxane (PDMS) and plain agarose tubes [14]. PEDOT films exhibited low intrinsic cytotoxicity and do not show inflammatory responses upon implantation [17]. This makes PEDOT ideal for neural applications. Moreover, the stability of PEDOT coatings was tested with regards to the peripheral nervous system [78]. Electrochemical impedance spectroscopies and pulse tests found that charge injection capacities did not decrease after 10 million pulses, which suggest PEDOT coating was stable [78]. PEDOT can serve as a viable alternative to polypyrrole for chronic, long-term neural implants [17, 79, 80]. The hypothesis is that PEDOT coatings on Mg will slow down the degradation rate of Mg in the body fluids, thus providing a good substrate for neuronal cell interactions. In this study, EDOT (monomer form of PEDOT) will be polymerized onto Mg substrates by electrochemical deposition. This study focuses on the coating development and characterization. The use of Mg as a structural support and PEDOT as a surface coating will provide a promising material for nerve regeneration.

Electrochemical deposition is a process by which ions in a solution are transferred via an electric field and deposited onto an electrode of interest. A voltage is applied to a working electrode, leading to reduction reaction. In turn, monomers in the electrolyte are oxidized. The oxidized monomers then polymerize. This is called electro-polymerization. A previous study has reported electrochemical deposition of PEDOT onto Mg substrates, which involved coating a 3.2mm diameter Mg surface area [56]. In order to apply coated Mg implants for nerve regeneration, a much larger surface area needs to be coated and examined. In this study, PEDOT will be electrochemically deposited to larger Mg substrates for future studies on cellular interactions. Briefly, voltages will be applied to Mg (working electrode) to oxidize the EDOT monomer. The oxidation of EDOT will produce radical ions. These radical ions would then polymerize. Since electron transfer is much faster than the diffusion of radical ions in solution, high local concentrations of the ions can be found near the working electrode. This allows for the polymerization to continue at the electrode interface. The objective of this study is to investigate the parameters of electrochemical deposition for PEDOT coating on Mg and to evaluate the coating properties.

2. Materials and Methods

2.1. Preparation of Magnesium Substrate

As-rolled Mg sheets of 99.9% purity (Goodfellow USA) were cut into 5mmx10mm in size, cleaned by sonication in ethanol (99.5% purity, Sigma-Aldrich) for 15 minutes at room temperature, and air-dried at room temperature. As-rolled Mg sheets have an oxidized surface induced during processing. To determine whether the oxidized versus

polished surface conditions of Mg affect the electrochemical deposition and coating properties, some samples were polished using 600, 800, and 1200 grit silicon carbide (SiC) paper (Ted Pella, Inc.) and cleaned with ethanol.

2.2. Electrochemical Deposition of PEDOT on Magnesium Substrate

A potentiostat (model 273A, EG&G Princeton Applied Research) operated by Power suite software and an electrochemical cell were set up and connected for electrochemical deposition (Figure 1). A 50mL glass beaker with a stir bar was used for the electrochemical cell setup. The electrochemical cell included three electrodes and a bath of electrolytes. The bath was prepared by mixing 3,4-ethylenedioxythiophene (EDOT, Sigma-Aldrich) and pristine 1-ethyl-3-methylimidazolium bis(trifluoromethylsulfonyl)imide (ionic liquid or IL, electrochemical grade, >99.5% purity, Covalent Associates, Inc.) to give a 1M EDOT concentration. Potentiostat leads were attached to their respective electrodes and the experimental parameters were inputted into the power suite software. A stir bar was utilized to help re-suspend the PEDOT settled on the bottom of the electrochemical cell and enhance polymerization of EDOT onto Mg substrates (Figure 2). Half of the Mg substrate (a 5mm x 5mm surface area) was immersed in the EDOT/IL bath for coating. The experiments began once the electrodes were secured and the parameters were set.

2.3. Key Parameters of Electrochemical Deposition

A variety of relevant deposition parameters were explored in this study, including the coating method (Cyclic Voltammetry versus Chronoamperometry), the purity of bath solution (pristine versus recycled EDOT/IL bath), type of electrodes, process duration at particular voltages, concentrations of EDOT/IL, stir bar usage during deposition, and post-coating treatment.

2.3.1. Cyclic Voltammetry and Chronoamperometry

One of two electrochemical deposition methods, that is, cyclic voltammetry (CV) or chronoamperometry, was utilized for PEDOT coating. The CV method involved the application of two different potentials for a number of cycles. One CV experiment involved cycling between -0.5V and 1.2V, and another involved cycling between -0.5V and 2.0V. Cycle duration varied between as short as 30 seconds per cycle to as high as 200 seconds per cycle. The scanning rate was set as a constant of 100mV/s.

Chronoamperometry involved the application of a constant potential for the duration of the coating process. For the chronoamperometry experiments, the potential was tested at 1.0V, 1.2V and 2.0V, and the duration of the electrochemical deposition ranged between 1000 and 2000 seconds.

2.3.2. Pristine versus Recycled EDOT/IL Bath

The effects of pristine versus recycled EDOT/IL bath on the coatings were investigated in this study as an effort to reduce the deposition cost. Initially, a freshly prepared 1M EDOT/IL bath solution (called pristine bath) was used for deposition. As the EDOT polymerized onto the Mg substrates, the solution changed color from clear to light

yellow, to opaque brown, and eventually to black. After deposition, the same bath was recycled to coat other Mg substrates. The color of the recycled baths turned darker with each re-use (Figure 3).

2.3.3. Electrodes

Reference electrodes for this experiment included silver-silver chloride (Ag/AgCl, CH Instruments), and copper (Cu, Miniscience). Counter electrodes included platinum (Pt, CH Instruments), and stainless steel (SS, Miniscience). Mg was utilized as the working electrode. A flat sheet of copper was cut into an approximate dimension of 10mm x 8mm x 0.1mm to produce the copper electrode. The stainless steel electrode was prepared similarly, with an approximate dimension of 14mm x 5mm x 0.2mm. Both electrodes were washed in ethanol ultrasonically for 15 minutes. The platinum electrode consisted of a wire that was coiled in a spring like shape to fit into the electrochemical cell. The silver/silver chloride electrode was enclosed in a glass sleeve with a permeable tip and was used directly as purchased. After establishing appropriate coating parameters using the Pt and Ag/AgCl electrodes, the Cu and SS electrodes were investigated as alternative electrodes. To compare the Pt and Ag/AgCl electrodes with the Cu and SS electrodes, Mg substrates were coated using CV ranging from -0.5 to +2.0V for 5 cycles at 200 seconds per cycle with a constant scanning rate of 100mV/s.

2.4. Post-coating Treatment

Three different procedures for post-coating treatment were investigated: (1) the samples were ultrasonically washed in ethanol for 5 minutes; (2) the samples were quickly rinsed with deionized (DI) water (Millipore) followed by ethanol; and (3) the samples were not

rinsed at all. Afterwards, all the PEDOT coated Mg samples were dried in a vacuum oven at room temperature for 24 hours.

2.5. Characterization of Surface Morphology and Composition of PEDOT Coating

After post-coating treatment, the surface morphology of PEDOT coated Mg samples was characterized using a field-emission scanning electron microscope (SEM, Philips XL-30) at a 15kV accelerating voltage. Energy dispersive X-ray spectroscopy (EDS) was utilized to analyze the surface composition and quantify elemental distribution.

2.6. Adhesion Strength of PEDOT Coating – ASTM D3359 Tape Test

The adhesion strength of PEDOT on the Mg substrate was measured by the tape test following ASTM D3359 standards. Briefly, the PEDOT coated surface was first marked with 5 x 5mm grids using a cutter. The 3M No. 3710 tape was attached and pressed onto the etched surface. The tape was then peeled off at a 180° angle. The remaining PEDOT coating was characterized for adhesion strength according to the ASTM classification system that ranges from 0B to 5B. A strong adhesion between the coating and substrate is classified as 5B in which no coating detaches from the substrate. A weak bond is classified as 0B in which 65% or more of the coating detaches.

2.7. Corrosion Potential of PEDOT Coated Mg versus Non-Coated Mg

The PEDOT coating was deposited using CV method for 5 cycles with the potential ranging from -0.5V to +2.0V for 200 seconds. The corrosion rate of the PEDOT coated Mg versus non-coated Mg was measured according to Tafel test. The test procedure was

carried out in a very similar manner as a CV experiment with the potential ranging from -2.0V to 0.5V for 30 seconds. The scanning rate was set as a constant of 100mV/s. All the samples were tested in a simulated body fluid (SBF) with a pH of 7.4. Using the potential versus current (reported in a log scale) polarization curves, the corrosion current was extrapolated and plugged into the following equation to determine the estimated corrosion rate:

$$CR = \frac{I_{CORR} \cdot K \cdot EW}{dA}$$

where CR= corrosion rate, I_{CORR} = corrosion current, K= constant , EW= equivalent weight, dA= area of substrate submerged.

3. Results

3.1. Morphology and Composition of PEDOT Coating

Scanning electron micrographs and EDS analyses showed that PEDOT coated Mg had different surface topography and composition as compared with non-coated, polished Mg (Figure 4). The PEDOT coating was prepared by CV method with the potential ranging from -0.5V to +2.0V for five cycles, 200 seconds per cycle. The PEDOT coating appeared to have a micro-porous morphology. The EDS spectrum of non-coated Mg showed a little amount of oxygen (<5 wt.%) due to Mg oxidation. The EDS analysis of PEDOT coated Mg showed the presence of S, C, O, N, F, and Mg.

3.2. The Effects of Coating Methods (CV versus Chronoamperometry) on Surface Microstructure and Composition

The PEDOT Coating was deposited using both CV and Chronoamperometry. During CV deposition, as the number of cycles or coating time increased, the coating thickness increased. Five cycles of CV with the potential ranging from -0.5V to +2.0V yielded a uniform coating. Figure 5a shows the SEM image of the PEDOT coated Mg using the CV method for 5 cycles in a pristine EDOT/IL bath. Figure 5c shows the EDS analysis of Figure 5a, indicating sufficient coating by the peaks of S, C, F, and O.

During chronoamperometric deposition, the greater the coating duration time, the thicker the coating. Higher voltages led to the appearance of coatings at a faster rate in the electrochemical cell. At the voltages below 1.2V, PEDOT coating did not form during the entire cycle of 2000 seconds. At the voltages of 2V or greater, the coatings formed within the first few hundred seconds of the cycle. Figure 5b shows the SEM image of the PEDOT coated Mg using the chronoamperometry method at 1.2 V for 2000 seconds in a pristine EDOT/IL bath. Figure 5d shows the EDS analysis of Figure 5b, indicating the presence of PEDOT coating.

3.3. Comparison of PEDOT Coatings Produced in Pristine versus Recycled EDOT/IL Bath

The PEDOT coatings were uniform with the use of a pristine EDOT/IL bath. A 1M concentration of EDOT/IL was sufficient for coating the Mg substrates with a 5mm x 5mm surface area. The pristine bath was easier to see through due to its clear color before

deposition. When the recycled baths were used, the coatings were still able to form; however, the larger particles of previously polymerized EDOT were deposited onto the Mg substrates, leading to a non-uniform surface morphology. Figure 6 shows SEM images of PEDOT coated Mg samples using CV method with the potential ranging from -0.5V to 2V for 200 seconds for 5 cycles. In Figure 6(a, c, and e), the PEDOT coating that was deposited in a pristine bath of EDOT/IL appeared uniform with well-defined particulate features it. In contrast, the PEDOT coating that was deposited in a recycled bath of EDOT/IL had non-uniform morphology, as shown in Figure 6(b, d, and f). As shown in Figure 6(e, f)), the elemental compositions of the PEDOT coatings were similar and both had high sulfur and low magnesium content, indicating the presence of PEDOT coating on Mg substrates. The PEDOT coating formed on the Mg substrates in both the pristine and recycled bath; however, the SEM images showed significant differences in coating morphology and uniformity.

3.4. The Effects of Different Electrodes on the PEDOT Coating

To investigate the use of potentially low-cost electrodes for PEDOT deposition, copper and stainless steel electrodes were used as reference and counter electrodes, respectively. Similar coating results were observed (Figure 7). Figure 7a shows PEDOT coated Mg using Cu and SS electrodes in a pristine EDOT/IL bath. EDS analysis (Figure 7b) shows that sufficient coating appeared on the Mg substrate and no copper ions were detected.

3.5. Other Parameters Affect PEDOT Deposition

The use of a polished surface versus as-rolled oxidized surface as substrates for electrochemical deposition seemed to have no effects on the coating morphologies and compositions based on SEM and EDS analyses. Ultrasonic wash in ethanol immediately after electrodeposition led to removal of the coating. Before setting the samples to dry, a quick rinse with DI water followed by ethanol was necessary to remove excess IL. Once the samples were vacuum dried for at least 24 hours, they were ready for further evaluations, such as SEM imaging, adhesion strength test, and corrosion test.

3.6. Results of PEDOT Adhesion Strength on Mg

Tape test can determine how well a coating is adhered to the substrate of interest. The tape test results showed that the coating adhesion strength was within the classifications of 3B to 4B. Figure 8a shows the surface of PEDOT coated sample before making a 5x5mm grid. Figure 8b shows the grids and Figure 8c shows the sample after tape test. Figure 8d shows the PEDOT remnants on the tape itself.

3.7. Improved Corrosion Potential

Figure 9 shows polarization curves of PEDOT coated Mg and non-coated Mg. After extrapolation, the corrosion current (I_{CORR}) for PEDOT coated Mg was 6.14×10^{-5} amps, yielding a corrosion rate of 8.6 mm/year; I_{CORR} for non-coated Mg was 9.08×10^{-4} amps, yielding a corrosion rate of 126.9 mm/year. The PEDOT coating did decrease the corrosion rate of Mg according to the Tafel test.

4. Discussion

4.1. Morphology and Composition of PEDOT Coating

Electrochemical deposition parameters affected the microstructure of the PEDOT coatings on Mg. When coated in a pristine bath, SEM images of the PEDOT coatings showed a uniform micro-porous structure, which matched with the results observed in previous studies[56, 81, 82]. The microstructure of PEDOT determines its conductivity[83]. The conductivity decreases with loosely packed polymers that have high inter-chain distances [84]. The electrochemical deposition method allows for precise control of polymer growth on the working electrode. The EDS spectra of the PEDOT coated Mg showed high sulfur (S), carbon (C), and oxygen (O) peaks and low Mg peaks, which indicated PEDOT coating on the substrate since PEDOT consists of S, C, and O. The Fluorine (F) peaks indicated that the anion F^- in the ionic liquid was transferred onto Mg surface during the electrochemical deposition [85].

4.2. Adhesion Strength of PEDOT Coating

ASTM standard tape tests are crucial for determining the adhesion strength of a coating, particularly for evaluating coating reliability under friction or wear [86]. The PEDOT coatings had high adhesion strength on the Mg substrates. High adhesion strengths have also been observed with PEDOT-PSS nano-composite films coated on titanium substrates [87]. Surface preparation has been shown to affect PEDOT adhesion strength on platinum, gold, indium tin oxide, and silver substrates [88]. The superior adhesion strength of coating was reported when the substrates were ultrasonically washed in acetone before deposition [88].

4.3. Corrosion Potential of PEDOT coated Mg versus non-coated Mg

The corrosion potential of PEDOT coated Mg increased by about 250 mV. This yielded a slower corrosion rate for the coated Mg. Similar results were reported in another study, showing the corrosion potential of PEDOT coated Mg increased by about 120 mV with a corrosion current decreased by about 50%. These findings indicated that PEDOT coatings indeed improved the corrosion resistance of Mg[56].

4.4. PEDOT Coated Mg for Potential Neural Applications

To further improve biological connection with neurons, co-deposition of the peptide DCDPGYIGSR onto the surface may be needed to enhance neuron binding abilities of the biodegradable Mg alloys. It has been reported that rat glial cells preferentially attached and grew on electrode sites containing the peptide after 24 hours of in vitro culture[17].

5. Conclusion

The PEDOT was successfully deposited on Mg substrates in a 1M EDOT/IL solution using the electrochemical method. The PEDOT deposition was achieved by either cyclic voltammetry (CV) or chronoamperometry method. With longer coating time or more CV cycles, more EDOT was polymerized onto the Mg substrate, leading to a thicker coating. Electrodes such as copper and stainless steel can be as effective as the more costly Pt and Ag/AgCl electrodes for this coating process. As the EDOT concentration increases in the pristine bath, the electrochemical deposition process can be used to coat larger Mg substrates for cell studies. Future studies are needed to determine the *in vitro* degradation

rate of PEDOT coated Mg implants and how cells would respond to the PEDOT coated Mg with various coating thickness.

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7. Figures

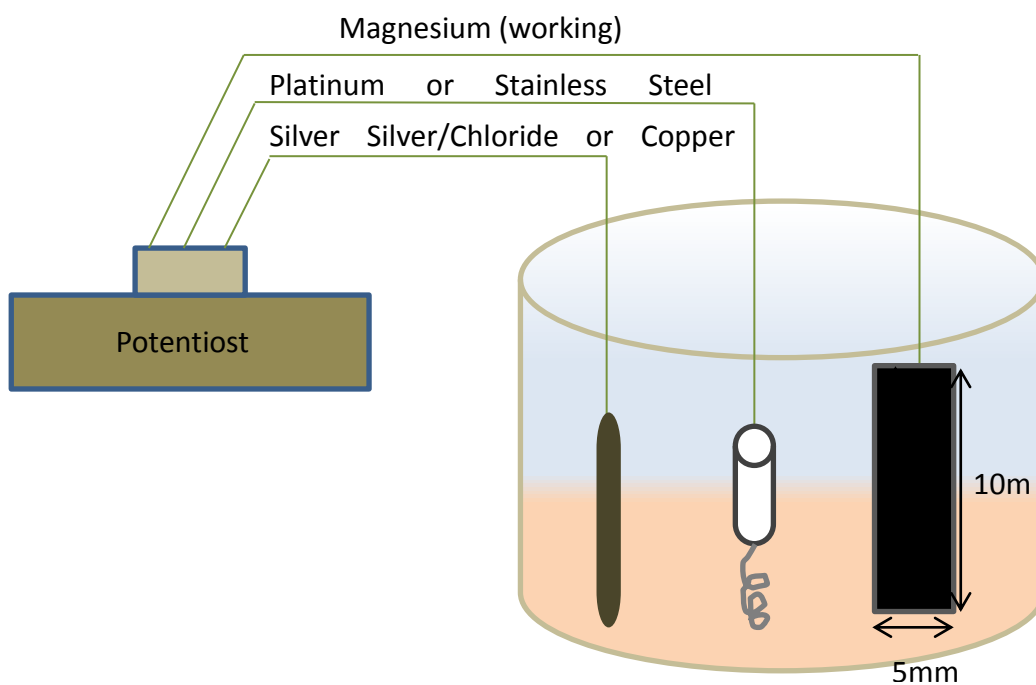


Figure 1: Schematic experimental set up for electrochemical deposition.

Reference electrodes were copper or silver/silver chloride, counter electrodes were stainless steel or platinum, and the working electrode was Mg. The optimal concentration of EDOT/IL was 1M for a 5mm x 5mm area of Mg substrates.

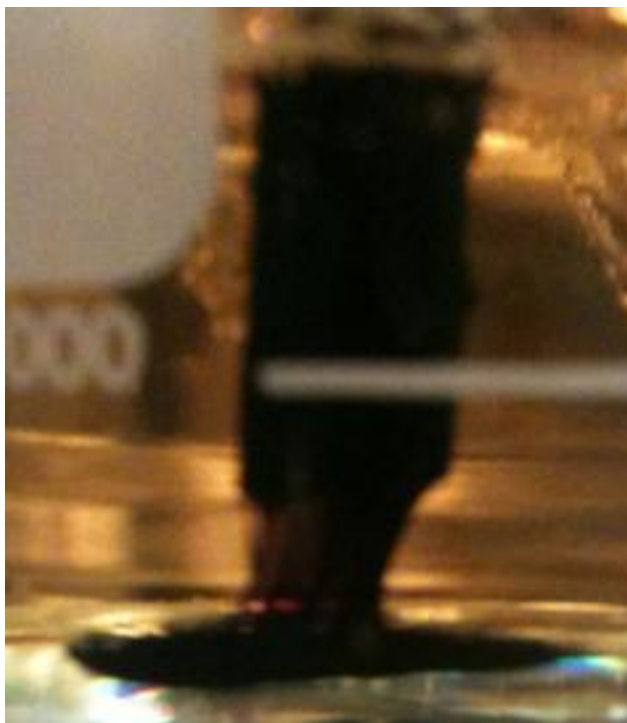


Figure 2: PEDOT formation (dark settling deposit) on Mg substrate in electrochemical cell. The use of magnetic stir bar helped redistribute settled PEDOT in solution.

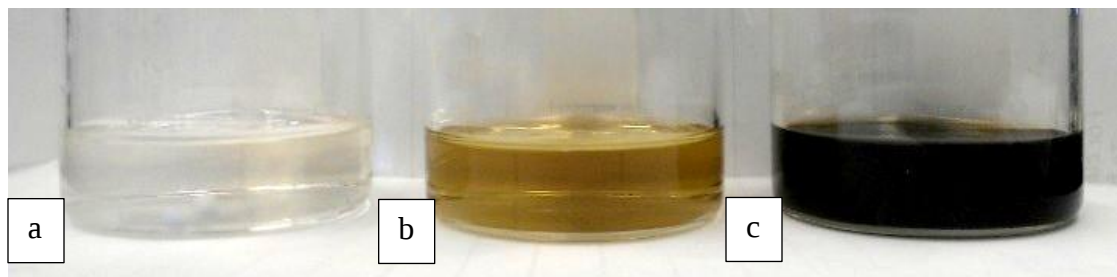


Figure 3: EDOT/IL baths after subsequent use for deposition. (a) Pristine EDOT/IL bath before deposition. (b) EDOT/IL bath after one use (CV for 5 cycles ranging from -0.5 to +1.2V). (c) EDOT/IL bath after more than 3 recycles.

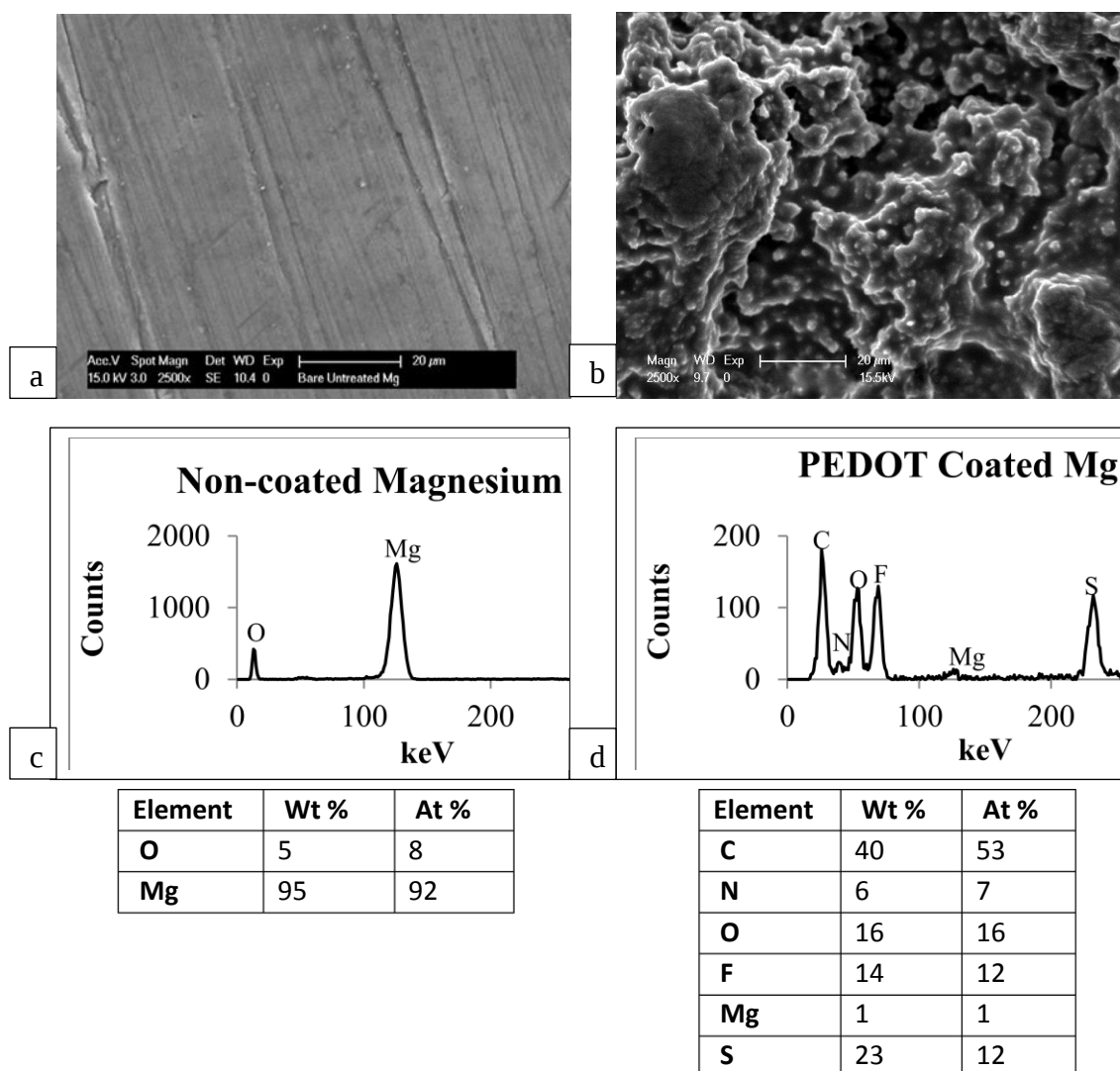
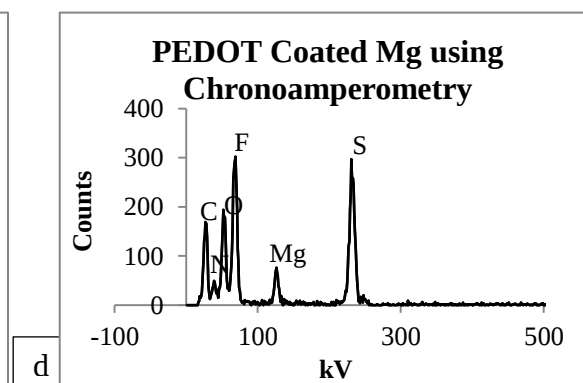
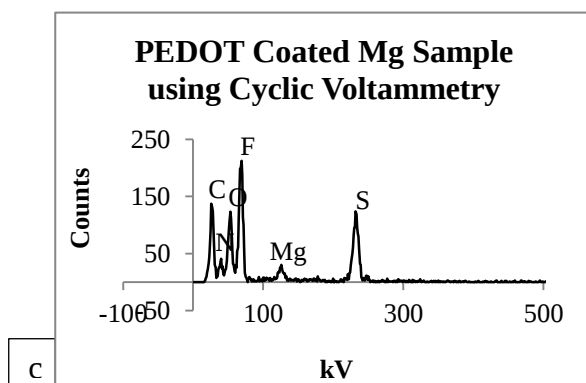
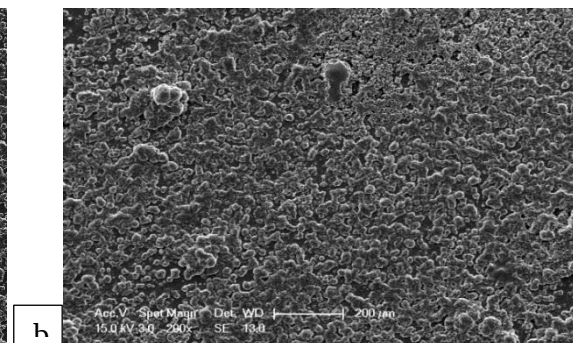
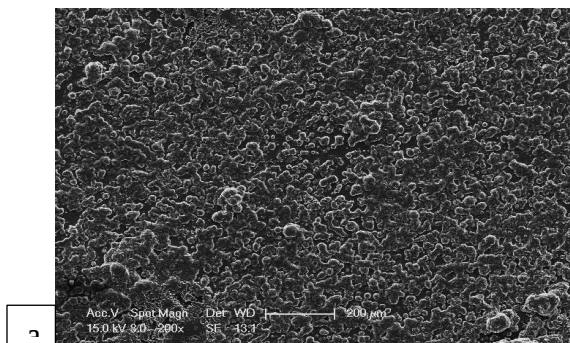


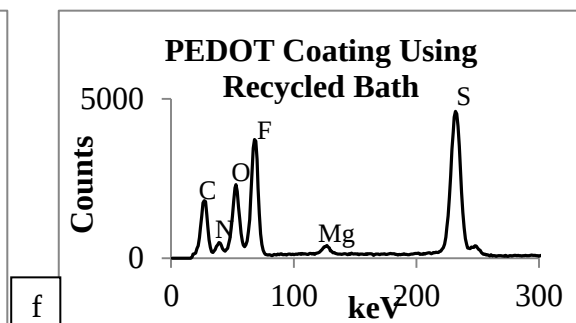
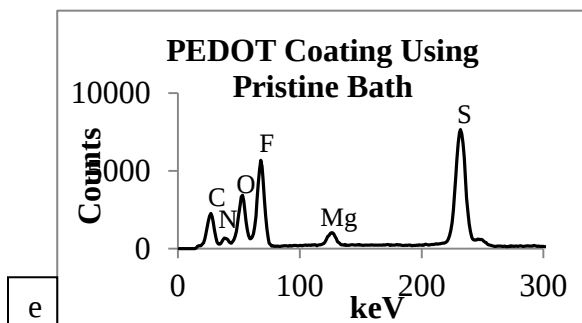
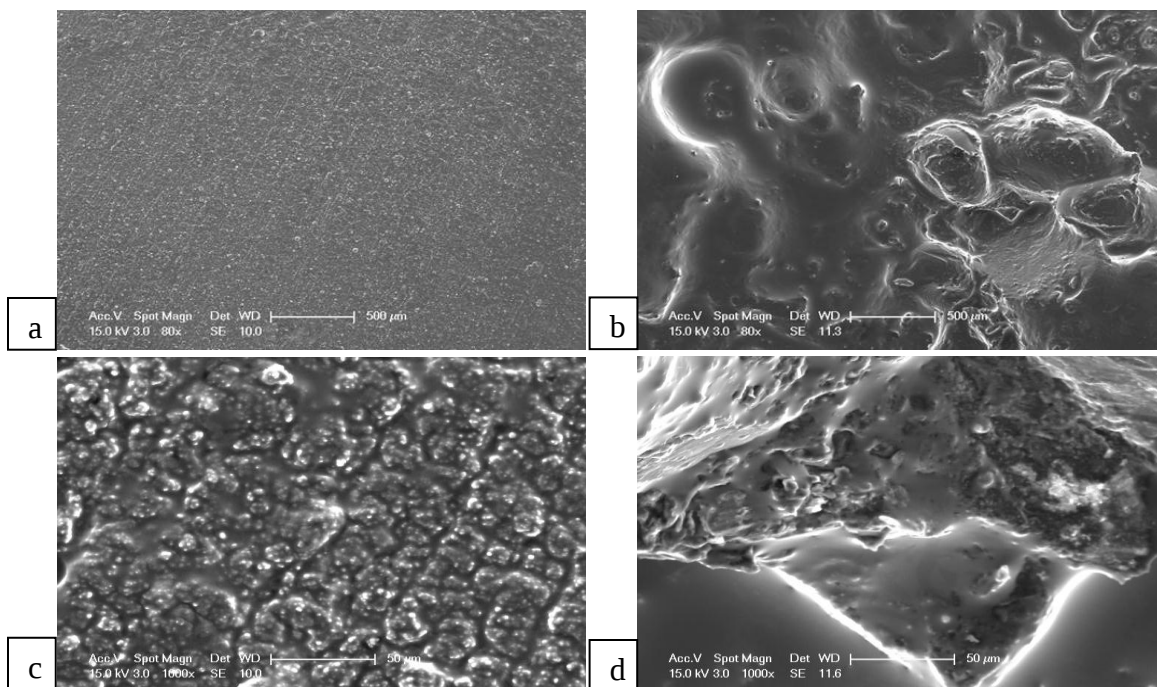
Figure 4: (a) SEM image of bare Mg substrate after polishing. (b) SEM image of PEDOT coated Mg after 5 cycles of CV deposition. Magnification bar = 20 µm at 2500x. (c) EDS analysis of bare Mg substrate. (d) EDS analysis of PEDOT coated Mg.



Element	Wt %	At %
C K	33	43
N K	10	11
O K	18	18
F K	23	19
MgK	3	2
S K	13	6

Element	Wt %	At %
C K	33	44
N K	10	12
O K	16	15
F K	23	19
MgK	2	1
S K	16	8

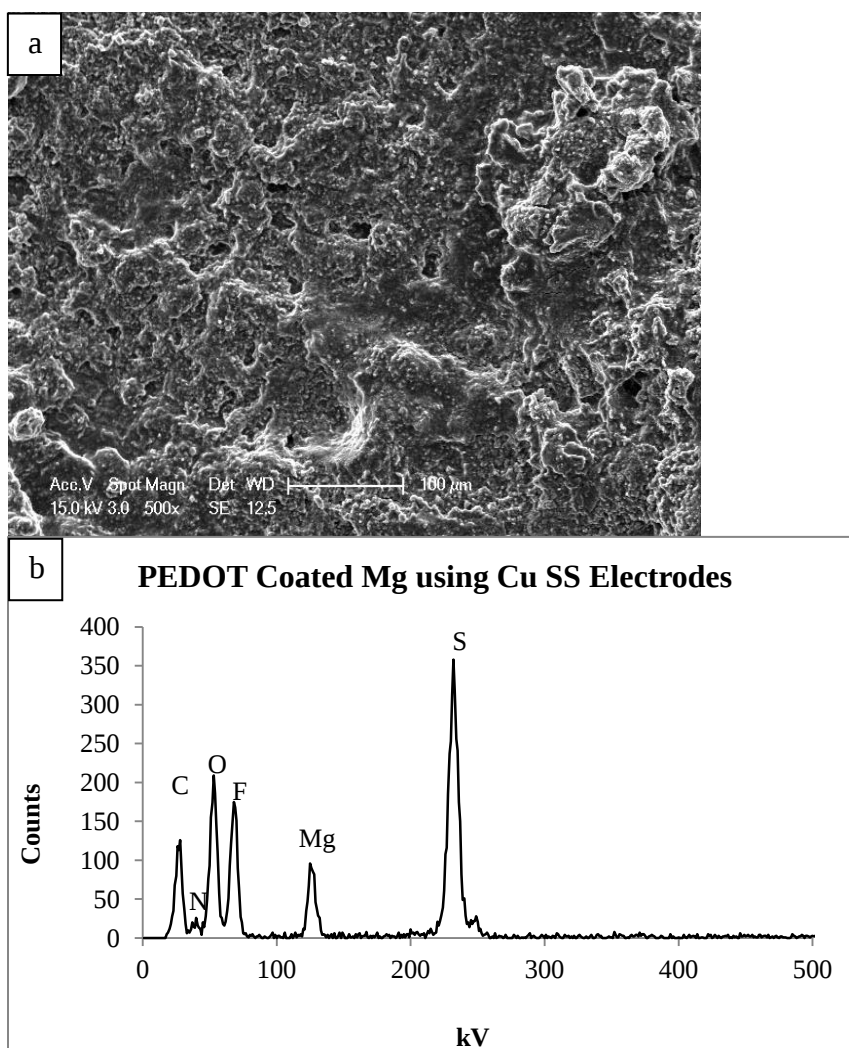
Figure 5: (a) SEM of PEDOT coated Mg using CV method for 5 cycles (200 seconds per cycle) in pristine EDOT/IL bath. (b) SEM of PEDOT coated Mg using chronoamperometry method for 2000 seconds in a recycled EDOT/IL bath. (c) EDS analysis conducted on image (a). (d) EDS analysis conducted on image (b). Scale bare reads 200 μ m at a magnification of 200x.



Element	Weight %	Atomic %
C	30	40
N	9	11
O	18	19
F	23	19
Mg	2	1
S	18	9

Element	Weight %	Atomic %
C	35	46
N	10	11
O	17	17
F	21	17
Mg	1	1
S	17	8

Figure 6: (a,c) SEM images of PEDOT coated Mg produced in a pristine bath at a magnification of (a) 80x and (c) 1000x. (b,d) SEM images of PEDOT coated magnesium produced in recycled bath at a magnification of (b) 80x (500 μ m scale bar) and (d) 1000x (50 μ m scale bar). The PEDOT was deposited by the CV method for five cycles. (e) EDS analysis conducted on image (a). (f) EDS analysis conducted on image (b). The EDS results showed that the elemental composition of PEDOT coating were similar; both had high sulfur and low magnesium content, indicating the presence of PEDOT coating on Mg substrates.



Element	Wt %	At %
C K	37	50
O K	23	23
F K	17	14
MgK	4	3
S K	19	10

Figure 7: (a) SEM image of the coated Mg substrate using copper and stainless steel electrodes. The PEDOT coating was deposited using CV method for 5 cycles ranging from - 0.5 to +2.0V at a scanning rate of 100mV/s. (b) EDS analysis conducted on image (a).

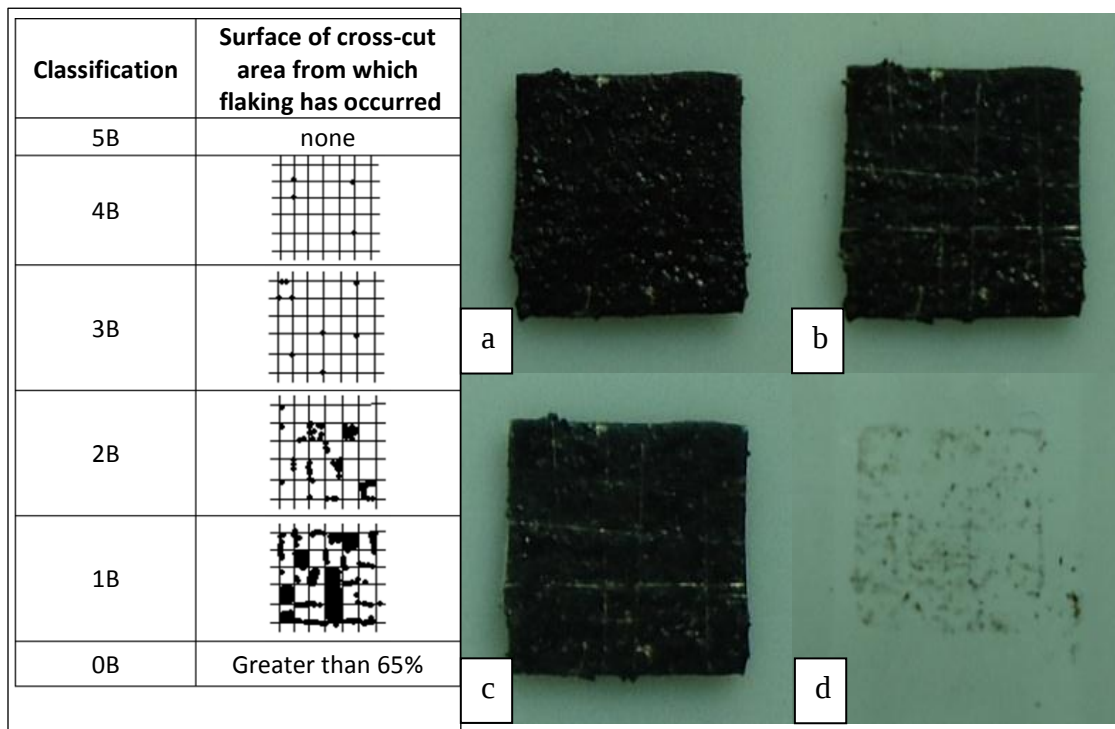


Figure 8: Adhesion strength of PEDOT coated Mg measured by tape test following ASTM D3359 standard. (a) PEDOT coating on Mg deposited by the CV method (5 cycles from -0.5V to 2V). (b) Grids of 5mmx5mm were created on PEDOT coating using a cutter. (c) Grid appearance after tape test. (d) PEDOT remnants on the tape. The classification of PEDOT coating adhesion strength was between 3B and 4B.

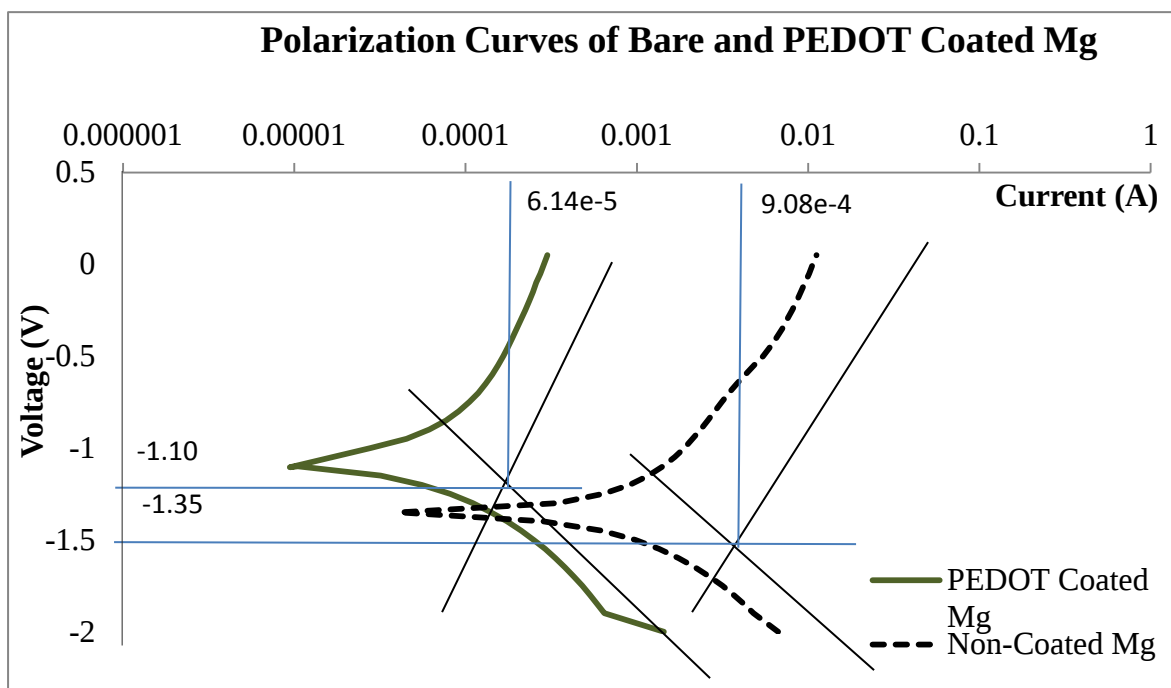


Figure 9: Polarization curves of PEDOT coated Mg and non-coated Mg in SBF (simulated body fluid) at pH 7.4. The coating was deposited using the CV method with the potential ranging from -0.5 to +2.0V for 5 cycles. Scanning parameters of Tafel test ranged from +0.5V to -2.0V at a scanning rate of 100mV/s. After extrapolation, the corrosion current (I_{CORR}) for PEDOT coated Mg was 6.14×10^{-5} amps, yielding a corrosion rate of 8.6 mm/year; I_{CORR} for non-coated Mg was 9.08×10^{-4} amps, yielding a corrosion rate of 126.9mm/year.

CHAPTER 2

Characterization of Poly 3, 4-(ethylenedioxythiophene) (PEDOT) Coated Magnesium and its Cytotoxicity Evaluated by Indirect Contact Human Embryonic Stem Cell Model

Abstract

H9 human embryonic stem cell (hESC) culture studies were conducted using magnesium (Mg) coated with a conductive polymer poly (3,4-ethylenedioxythiophene) (PEDOT) to study viability for potential neural applications. Previous studies involving bare Mg in contact with hESCs showed cytotoxic behavior due to Mg's high corrosion rate. In an attempt to slow the degradation rate of pure Mg, conductive polymer PEDOT coatings on Mg were explored using the sensitive human embryonic stem cell model. In this study, the Mg was coated with PEDOT for 2, 5, and 10 cycles ranging from -0.5 to 2.0V using cyclic voltammetry at a scanning rate of 100mV/s. Stem cells cultured with the Mg coated with PEDOT for 2 cycles were viable for a shorter amount of time when compared with the stem cells cultured with the 5 cycle PEDOT coated Mg.

Keywords: magnesium, nerve regeneration, poly (3,4-ethylenedioxythiophene), human embryonic stem cells, electrodeposition

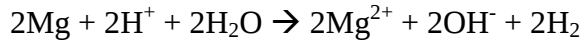
1. Introduction

Damaged nerves have limited ability to self-regenerate. Using materials to help guide damaged nerve regeneration must be carefully studied to avoid additional complications. It is crucial that foreign materials are non-toxic and are able to guide expected cell growth and differentiation when implanted into the body. Current treatments for damaged nerves include nerve guide conduits (NGCs), which are used to guide the connection of proximal and distal ends of severed nerves. NGCs can be constructed from material that is synthetic, natural, or a combination of both. The use of magnesium (Mg) as a structural support coated with the conductive polymer poly (3,4-ethylenedioxythiophene) (PEDOT) was investigated as a possible NGC material.

1.1. Advantages of Mg for Neural Applications

Mg metal has excellent mechanical strength. Its tensile strength (pure Mg metal) is 180 – 220 MPa [53]. The electrical conductivity of Mg is 22 MS/m [54]. It is also very light weight with a density of 1.738g/cm³ [55]. It is degradable *in vivo*, with a degradation rate of 1.08mg/cm²h [57]. Polymer coatings help improve biocompatibility and control its corrosion rate [56]. Magnesium sulfate is clinically used as a neuro-protective agent [73-76]. Mg infusion causes rises in cerebrospinal fluid and brain magnesium concentrations. It has been shown to protect against further damage with acute stroke [75, 76] and cerebral ischemia [77], and to prevent atrial fibrillation post coronary bypass surgery [89, 90]. These findings make Mg an excellent candidate for implantation as a nerve guide conduit once the release of Mg ions is properly controlled with coatings.

Mg's overall reaction with water is as follows:



Mg's reaction with water *in-vivo* results in the release of hydroxide ions, causing a rise in pH [91, 92]. With control of its degradation, such as coating, it can be a very useful material, particularly for neural applications.

1.2. Why PEDOT Coating is Necessary

In order to slow the rapid degradation of bare Mg, PEDOT was chosen as the coating material. PEDOT was used due to its conductivity (electrical conductivity ranges found from 20 to 132 S/cm) and biocompatibility [19, 20]. Also, it is more electrochemically stable than its counterpart, polypyrrole [17]. It is light weight, with a density of 1.5 g/cm³ [19]. PEDOT has been recognized to function similar to a wire; it has been observed to increase electrical signals within the nerve [14]. Construction of grafting material incorporated PEDOT as the lining of an agarose-based hydrogel tube. Nerve fiber regeneration and re-innervation of target skeletal muscles were tested. Results from this study showed high regenerating nerve fibers in PEDOT coated tubes compared with polydimethylsiloxane (PDMS) and plain agarose tubes[14]. PEDOT films have been shown to exhibit low intrinsic cytotoxicity and do not show inflammatory responses upon implantation. This makes them ideal for bio sensing and biomedical applications [17].

1.3. Why H9 hESCs is a Good Toxicity Model

H9 hESCs were used as the cell model due to their sensitivity. Stem cells are an important new tool for developing unique, *in vitro* model systems to test drugs and

chemicals, and potentially to predict or anticipate toxicity in humans [93]. Testing effects of PEDOT coated Mg degradation on hESCs is great place to start the investigation of this material.

1.4. Objectives

The objective of this study is to investigate the response of H9 hESCs to PEDOT coated Mg at varying coating cycles. The materials tested in this study include electrochemically deposited PEDOT onto Mg substrates for 2, 5, and 10 cyclic voltammetry cycles. This study was conducted using the novel human embryonic stem cell model.

2. Materials and Methods

2.1. Preparation of PEDOT Coated Magnesium Sample

As-rolled Mg samples (99-100% purity) were cut into 3.8 x 5mm squares with 10 x 2mm ends to be clamped to a potentiostat as the working the electrode (Figure 1). The samples polished using 600, 800, and 1200 grit silicon carbide abrasive papers (PACE Technologies) to remove any oxidation from the surface. After being polished, the samples were ultrasonically washed in ethanol for 15 minutes and air dried in a laminar flow hood for 10 minutes. The samples were then clamped to the working electrode of a potentiostat (model 273A, EG&G Princeton Applied Research) operated by Powersuite software. Platinum and Silver Silver/Chloride were used as the counter and reference electrodes, respectively. The samples were set in a 1M 3,4-ethylenedioxythiophene (EDOT, Sigma-Aldrich) in 1-ethyl-3-methylimidazolium bis(trifluoromethylsulfonyl)imide (ionic liquid, IL electrochemical grade, >99.5% purity, Covalent Associates, Inc.) bath for cyclic voltammetry coating. Three sample cycles were used for

this study. (1) The samples were set to coat for 2 cycles ranging voltages from -0.5V to +2.0V for 200 seconds at a scanning rate of 100mV/s. (2) The samples were set to coat for 5 cycles ranging voltages from -0.5V to +2.0V for 200 seconds at a scanning rate of 100mV/s. (3) The samples were set to coat for 10 cycles ranging voltages from -0.5V to +2.0V for 200 seconds at a scanning rate of 100mV/s. After coating, the samples were left to vacuum dry for 24 hours. Next, the samples were rinsed in ethanol and set under UV for 24 hours before cell culture.

2.2. Material Properties

2.2.1. *Characterization of Surface Morphology and Composition*

The PEDOT coated Mg were tested for coverage and composition via scanning electron microscopy and energy dispersive x-ray analysis. Characterization was performed using a Scanning Electron Microscope (Philips XL-30; Central Facility for Advanced Microscopy and Microanalysis at the University of California, Riverside) at a 15kV accelerating voltage. Energy dispersive X-ray spectroscopy (EDS) was utilized to analyze the surface composition and quantify elemental distribution. Ninety degree SEM mounts were used to determine the thickness of coated samples.

2.2.2. *Coating Adhesion Strength*

The samples were also tested for adhesion strength. ASTM D3359 standards were followed when performing tape tests and 3M no. 3710 tape was used to test the degree of attachment of PEDOT onto the Mg substrate. The tape test consisted of marking a 5 x 5mm grid into the PEDOT coated surface with subsequent attachment of 3M tape to the

etched surface. The tape was removed at a 180° angle. The remaining PEDOT on the coated Mg was characterized according to a classification system that ranges from 0B to 5B (refer to ASTM D3359). A strong adhesion between the coating and substrate is classified as 5B in which no coating detaches from the substrate. A weak bond is classified as 0B in which 65% or more of the coating detaches.

2.2.3. Corrosion Test

To determine an estimate of the corrosion rates of each sample, polarization curves were extrapolated using the potentiostat and Powersuite software. The samples were immersed in simulated body fluid for one CV cycle ranging from -2.0V to +2.0V. Using the potential vs log(current) graphs generated, the corrosion current and critical voltages were determined and plugged into the following equation to determine the estimated corrosion rate:

$$CR = \frac{I_{CORR} \cdot K \cdot EW}{dA}$$

(CR= corrosion rate, I_{CORR} = corrosion current, K= constant, EW= equivalent weight, dA= surface area immersed)

2.3. Human Embryonic Stem Cell Culture

H9 hESCs (WiCell) were stably transfected with an Oct4-eGFP reporter plasmid as previously described (Chatterjee et al., 2011). H9 OCT4 hESCs were maintained as feeder-free cultures on Geltrex (Invitrogen) in mTeSR[®]1 medium (Stem Cell Technologies). A 12-well tissue culture plate (BD Falcon) was used for the cell culture experiment. The 12-well plate was prepared by covering the wells with Geltrex matrix

(Invitrogen) for cell adhesion. A confluent T25 flask of H9 OCT4 was enzymatically detached with Accutase (Invitrogen) and centrifuged for 3 minutes at 800 rpm to form a pellet. The supernatant was removed and the pellet was re-suspended in 9 mL of mTeSR1. One mL of cells in mTeSR[®]1 medium was plated into each of the 12-well plates. The cells were incubated for 3 days under standard cell culture conditions (sterile, 37°C, 5% CO₂/95% air, and humidified environment). Every 24 hours, the mTeSR[®]1 media was replenished with fresh media. Then, the 2, 5, and 10 cycle PEDOT coated Mg samples were placed in trans-well inserts (Corning) in triplicate. Positive control wells consisted of mTeSR[®]1 media with H9 hESCs only. Finally, the 12-well plate was incubated in the Nikon BioStation CT, where phase and fluorescent images were obtained every 6 hours.

2.4. Stem Cell Imaging

Phase contrast and fluorescent images were recorded by the Nikon Biostation CT every 6 hours over the 72 hour incubation period. Three points were selected in each well to be imaged by the Nikon Biostation CT. The points were analyzed using ImageJ to quantify the colony growth (area) over time. The area of viable cells was determined using phase images for morphology with fluorescent images to show eGFP expression for viability and pluripotency.

2.5. pH of Media

The pH and Mg concentration of the medium collected every 24 hours during cell culture was obtained using a pre-calibrated pH meter.

2.6. ICP-AES Analysis of Mg Concentration in Media

inductively coupled plasma atomic emission spectroscopy (ICP-AES) (Perkin Elmer Optima 2000 DV). The ICP-AES was analyzed using a standard calibration curve with serial diluted $\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$ at 250, 125, 62.5, 31.25, and 15.63 ppm. The medium from Mg containing wells was diluted using DI water (MilliQ from a Millipore water purification system) in order to obtain values within the range of the standard curve.

3. Results

3.1. PEDOT coated Magnesium Samples Morphology

Figure 2 displays the morphology and chemical composition the 2, 5, and 10 cycle coated Mg. Higher coating cycles showed less Mg exposure according to the EDS spectrum. Figure 3 shows the coating thicknesses according to SEM. The Boxed regions show coating. The 2 cycle coating yielded a thickness of around 30 microns. The 5 cycle coating yielded a thickness of around 58 microns. The 10 cycle PEDOT coating yielded a thickness of around 72 microns.

3.2. Adhesion Strength

Figure 4 displays the ASTM D 3359 adhesion strength tape test results. Overall, the samples exhibited acceptable adhesion strength according to ASTM D3359 tape tests, with classifications between 3B and 4B.

3.3. Corrosion Rates

Corrosion tests showed a slowed degradation rate as follows $10 < 5 < 2$ cycle. Lower corrosion current values yield a slower corrosion rate. The more negative the critical

voltage, the faster it corrodes. The non-coated Mg had a critical voltage of -1.75 according to Tafel extrapolation test. The thickest 10 cycle coated Mg had a critical voltage of -0.75V according to Tafel extrapolation test. The 5 and 2 cycle coated Mg samples had critical voltages of -0.9V, and -1.1V, respectively. Figure 5 shows the polarization curves of non-coated, 2, 5, and 10 cycle coated Mg merged.

3.4. Cell Counts over Time

Figure 6 shows phase and fluorescent images of the cells growing at time points of 6, 24, and 54 hours for the 2 and 5 cycle PEDOT coated Mg. The cells cultured in wells with 2 cycle samples survived to about 24 hours. The cells cultured in wells with 5 cycle samples survived to about 54 hours. The cells plated in wells with no material grew to confluency. Figure 7 shows that the cells imaged by the BioStation and analyzed using ImageJ software exhibited a longer survival rate with the higher cycle coated Mg. The 2 cycle PEDOT coated Mg showed the cells were viable up to around 24 hours where most cells were no longer visible in phase contrast images nor were they expressing eGFP marker. The 5 cycle PEDOT coated Mg showed the cells were viable up to around 54 hours. The control wells grew to confluency under standard cell culture conditions. Figure 7 shows the growth curve of the cells over time after ImageJ analysis. For 2 and 5 cycle coated Mg, the curves appear to peak at 18 hours and decrease after 20 hours. This is observed from the cells stretching before cell death.

3.5. Media Showed Increase in pH for Mg with Lower Coverage

The media pH was measured for all samples every 24 hours. Figure 8 shows the medium from wells containing Mg coated for 2 cycles had a higher pH than that of the Mg coated for 5 cycles. The 2 cycle PEDOT coated Mg medium had a pH value of 8.3, the 5 cycle PEDOT coated Mg had a pH value of 8.0, and the wells with no sample maintained physiological pH of 7.3.

3.6. ICP-AES Analysis shows Mg Ion Concentration Increase in Lower Coverage Mg

Mg ion concentration in the media containing 2 cycle PEDOT coated Mg was higher than the 5 cycle coated Mg. Figure 9 shows the changes in Mg concentration over the 72 hours for the 2 cycle, 5 cycle, and blank wells. The control wells contain a small amount of Mg which is normal from the mTeSR[®]1 media.

4. Discussion

The microstructure of PEDOT is micro-porous [94]. This allows for some liquid interaction with the Mg underneath the coatings. As the Mg reacts with the media in vitro, the release of hydroxide ions causes a spike in pH. The Mg ion concentration was confirmed with ICP-AES analysis to be lower for samples coated under more cycles.

With thicker PEDOT coatings, the Mg surface is less exposed to the aqueous environment, leading to a less drastic change in pH. This makes the environment more favorable to the cells. Corrosion resistant coatings can significantly delay the initiation of

biodegradation [95]. Based on SEM/EDS analysis, it has been confirmed that the coatings significantly decreased the amount of Mg exposed.

With degradation of Mg in vitro, the H9 hESCs were unable to survive for long, especially for the Mg samples coated with fewer cycles. As the H9 hESCs begin to die, their morphology goes from tightly packed colonies, to stretched colonies, and eventually they become cellular debris.

5. Conclusion

Mg substrates were coated with the conductive polymer PEDOT using electrochemical deposition method. The coating of Mg with PEDOT was able to aid in decreasing the rate of cell death in vitro. With more coating cycles, less Mg ions were released into the media leading to longer viability. More studies are required to study the control of Mg ion release. Repeat experiments will be conducted with the 10 cycle PEDOT coated Mg as there were technical difficulties which slowed the acquiring of results. Also, testing the materials with more robust neuronal precursor cells needs to be investigated.

6. Acknowledgements

The authors would like to thank the NSF BRIGE award (CBET 1125801) and the University of California for financial support. We would also like to thank the Central Facility for Advanced Microscopy and Microanalysis (CFAMM) at the University of California, Riverside and the Stem Cell Core Facility. Also we would like to thank Chinh Nguyen, Lauren Wong, Lauren Richards for their help with ImageJ analysis.

7. Figures

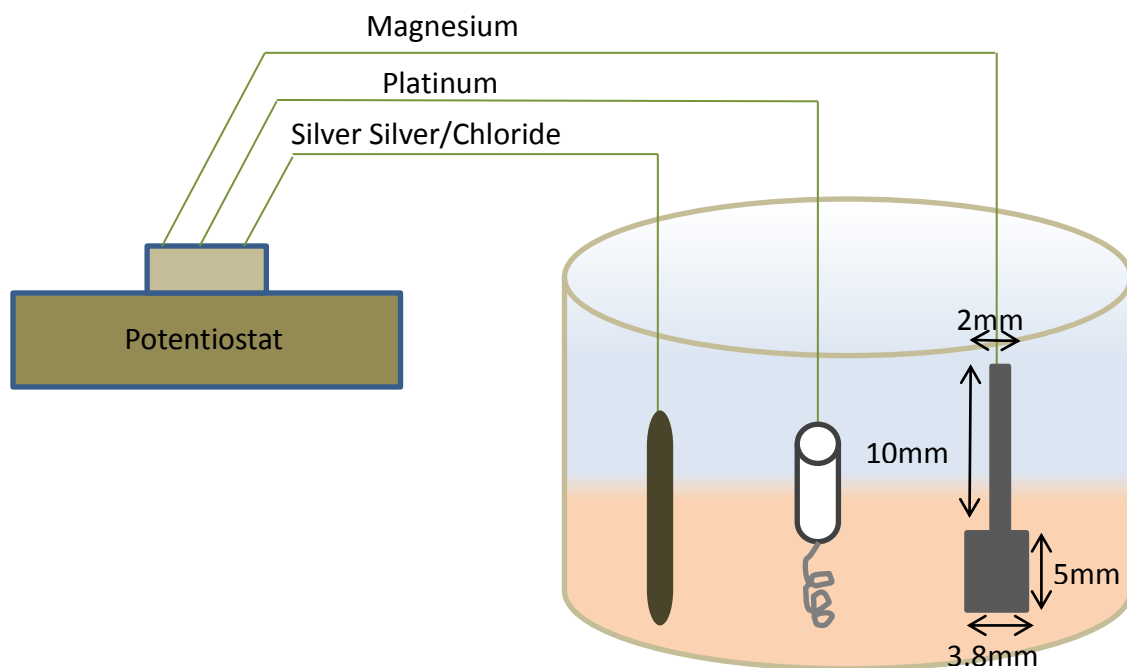


Figure 1: Schematic of electrochemical deposition of PEDOT onto Mg substrate. Deposition was conducted using cyclic voltammetry method ranging from -0.5 to 2V at a scanning rate of 100mV/s for 2, 5, and 10 cycles (200 seconds per cycle) in 1M EDOT solution.

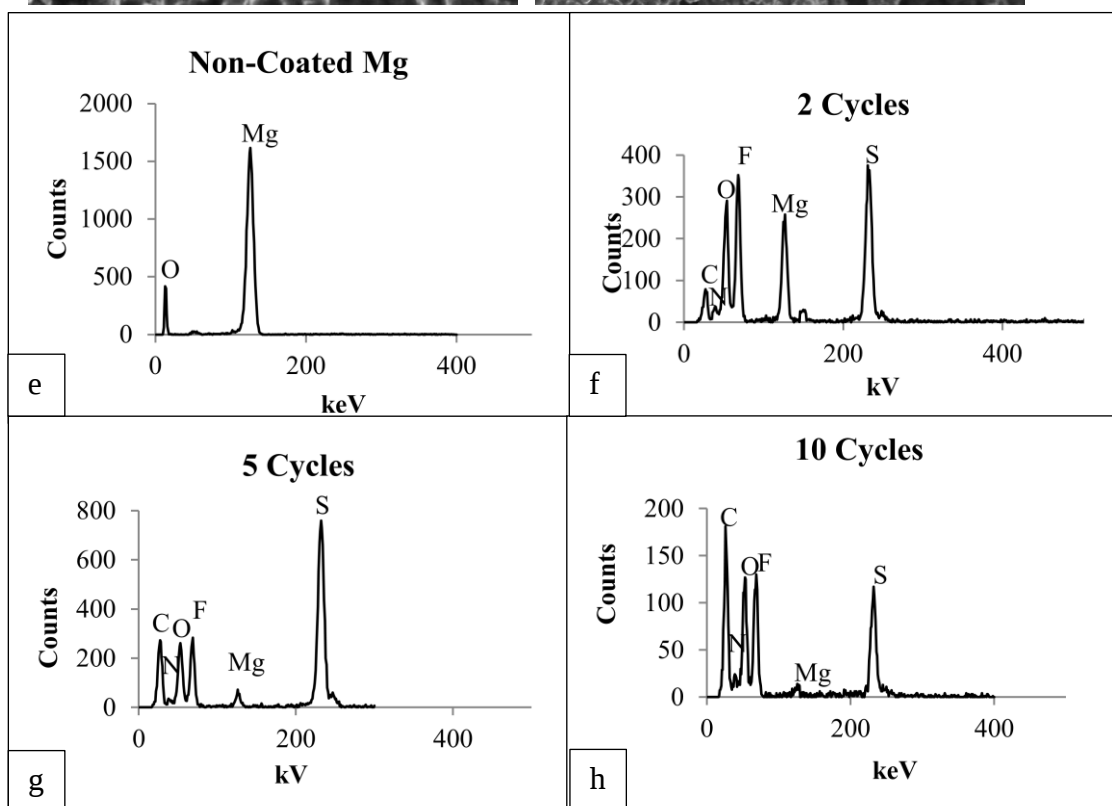
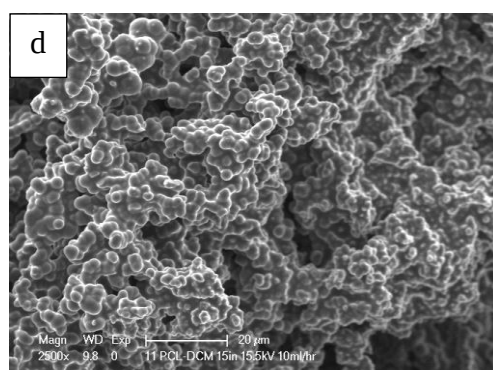
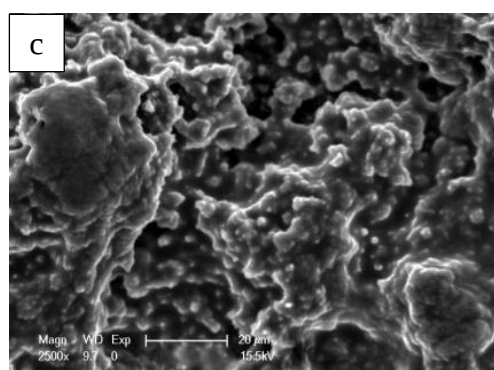
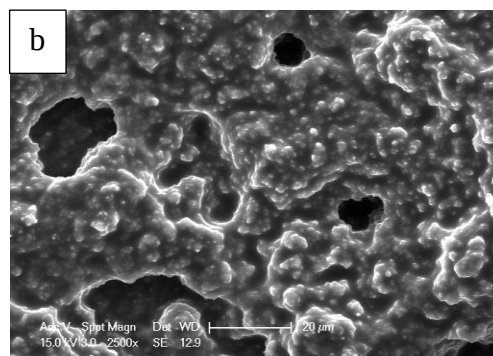
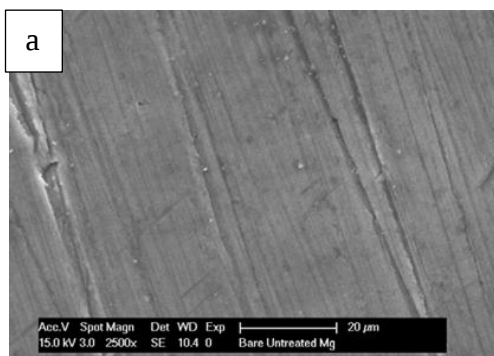


Figure 2: Scanning electron micrographs of (a) Non-coated Mg, (b) 2 cycle PEDOT coated Mg, (c) 5 cycle PEDOT coated MG, (d) 10 cycle PEDOT coated Mg, All samples were coated using CV method ranging from -0.5V to +2.0V. Energy Dispersive x-ray (EDS) analysis confirms chemical composition for: (e) non- coated Mg, (f) 2 cycle coated Mg, (g) 5 cycle coated Mg, and (h) 10 cycle coated Mg.

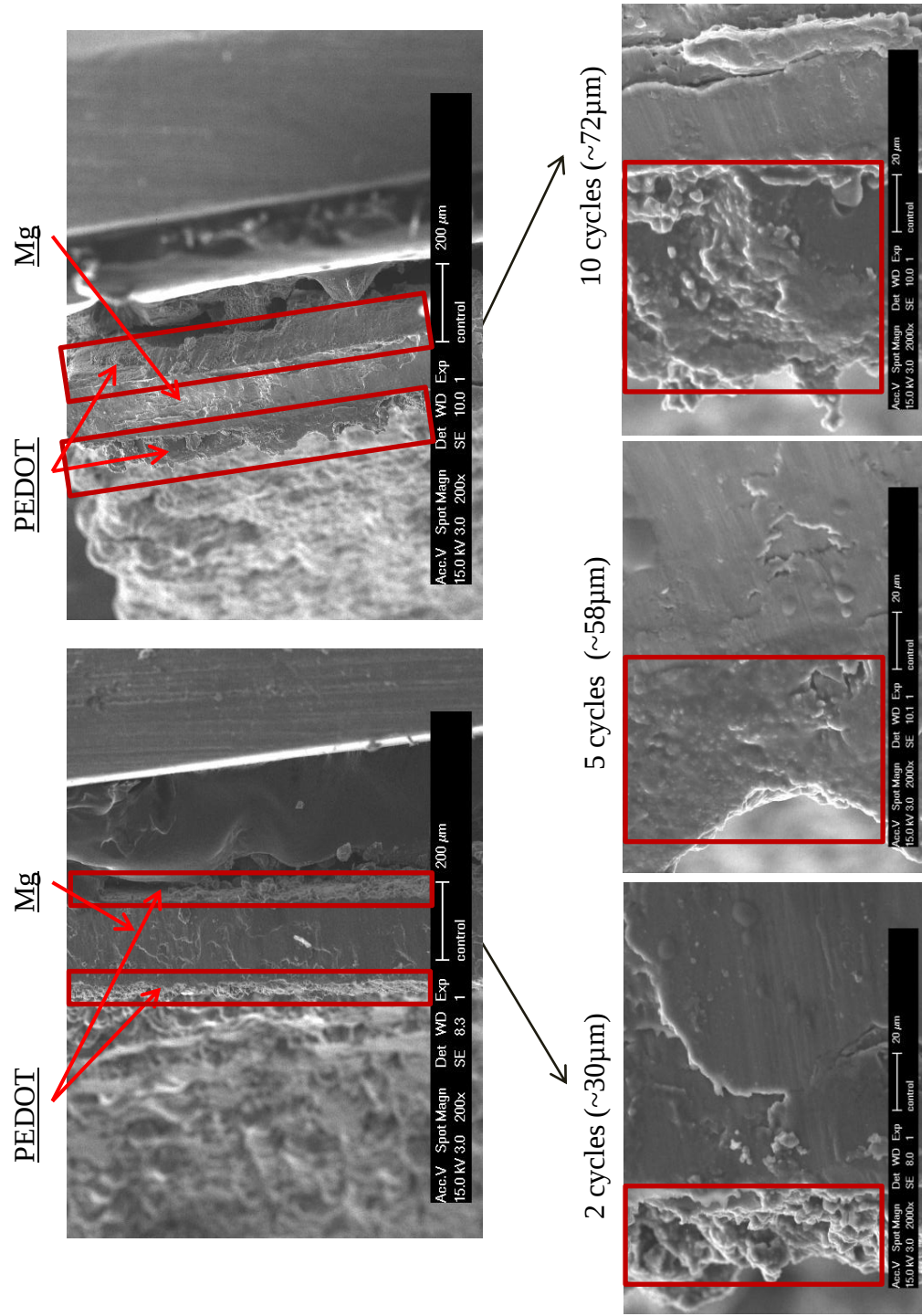


Figure 3: SEM micrographs of PEDOT coating placed on 90° mount. Boxed regions show coating. The 2 cycle coating yielded a thickness of around 30 microns. The 5 cycle coating yielded a thickness of around 58 microns. The 10 cycle PEDOT coating yielded a thickness of around 72 microns.

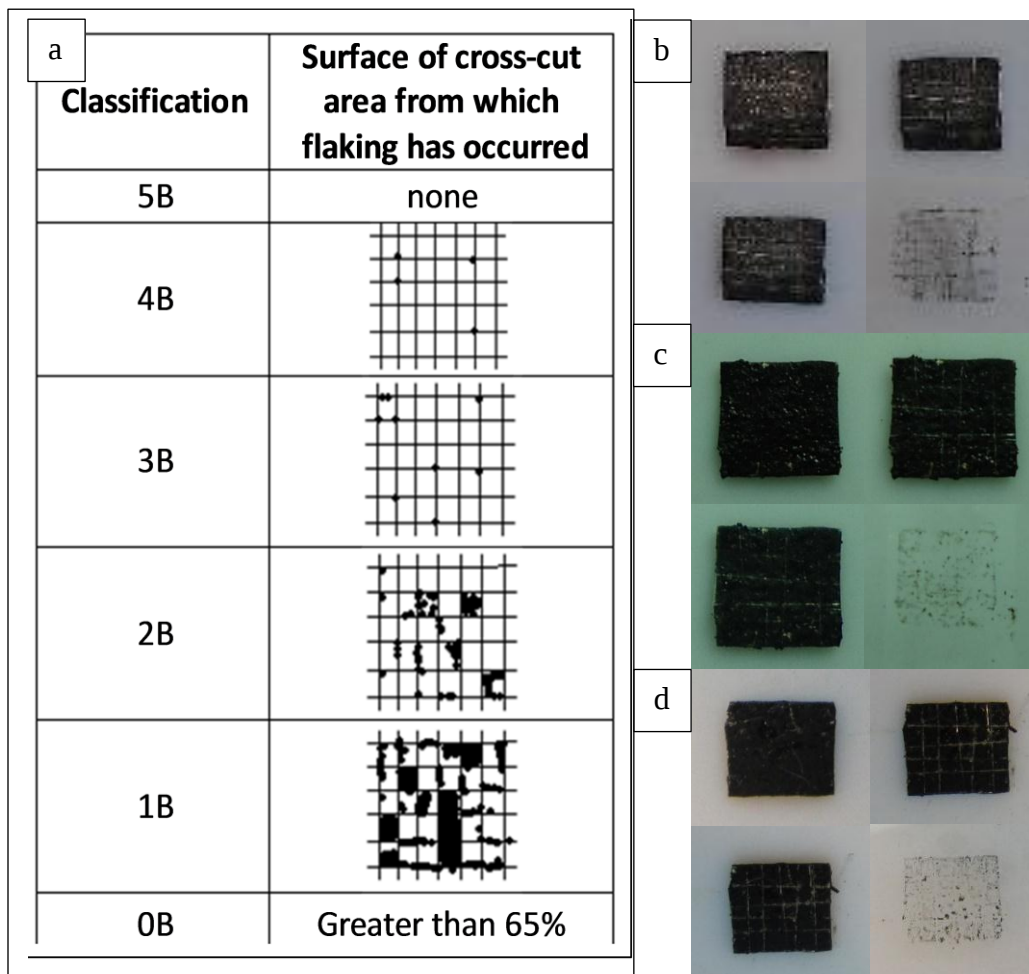


Figure 4: ASTM D3359 Adhesion strength tape test results. Top left shows PEDOT coated Mg before marking with blade. Top right shows PEDOT coated Mg after marking with blade. Bottom left shows PEDOT coated Mg after tape adhesion test. Bottom right shows remnants on tape. (a) Classification system according to ASTM D 3359, (b) 2 cycle PEDOT coated Mg (c) 5 cycle PEDOT coated Mg, (d) 10 cycle PEDOT coated Mg.

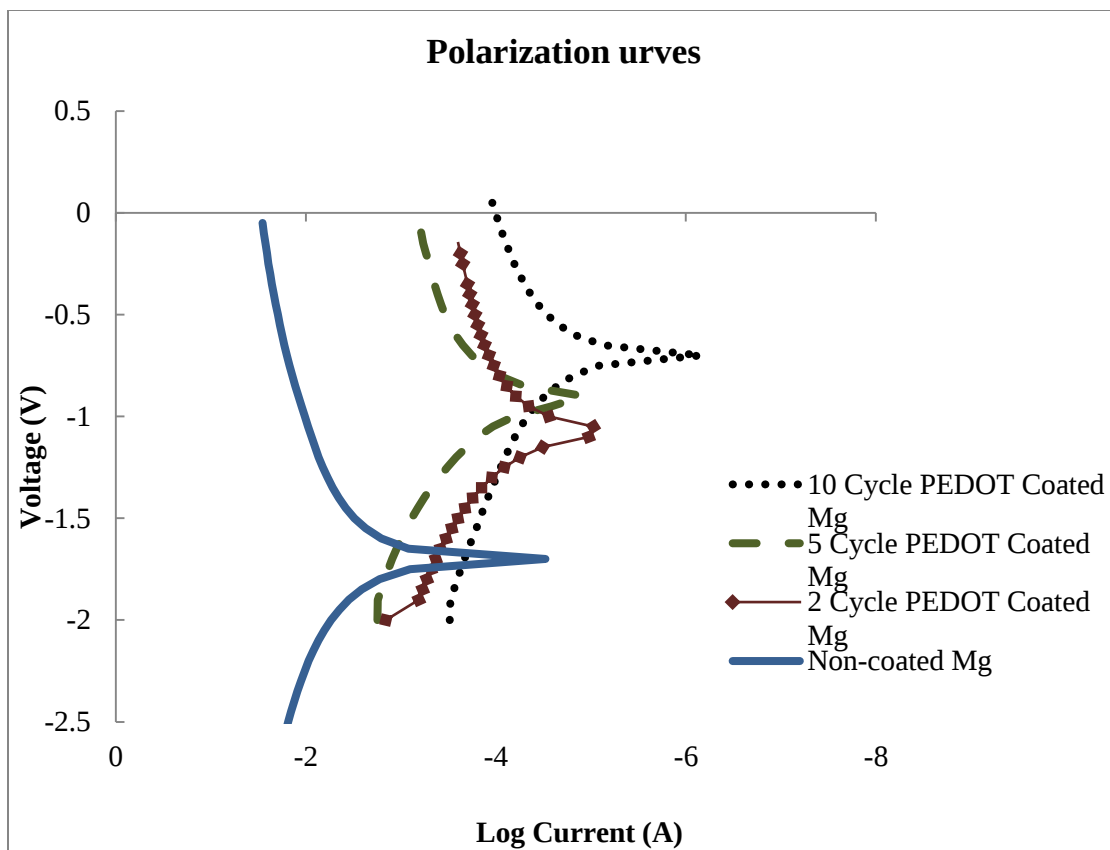
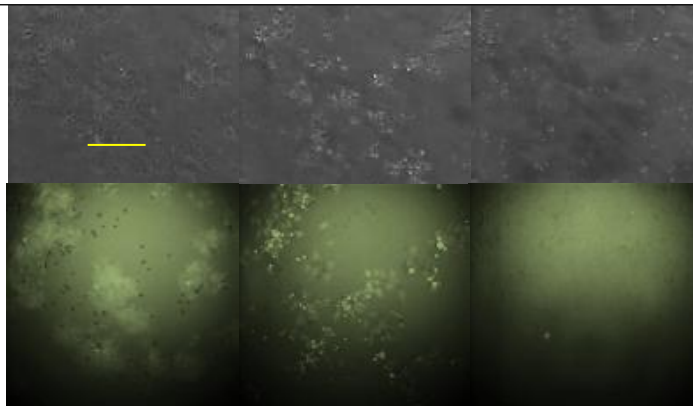
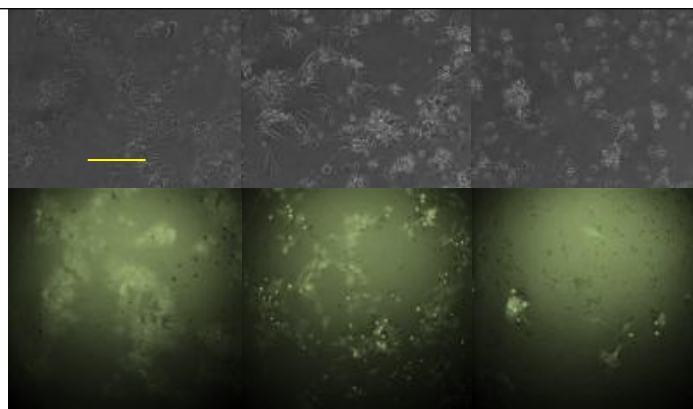


Figure 5: Polarization curves of different cycles. Lower critical voltages and higher corrosion currents yield higher corrosion rate. In this case, the higher cycle PEDOT coated Mg samples exhibit lower corrosion currents.

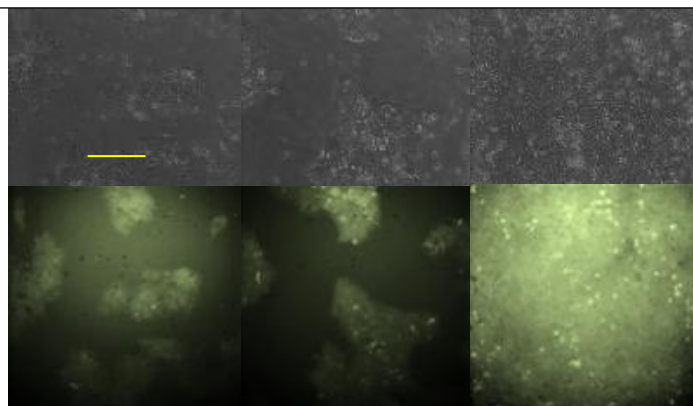
A. H9 hESC growth with CV PEDOT coated Mg for 2 cycles



B. H9 hESC growth with CV PEDOT coated Mg for 5 cycles



C. H9 hESC growth in positive control well (no material)



6 Hours

24 Hours

54 Hours

Figure 6: H9 hESCs growing on Geltrex in contact with PEDOT coated Mg by products via permeable trans-wells membrane over 72 hours. Scale bars = 0.2mm. Top rows contain phase images of cell growth to show morphology. Bottom rows contain fluorescent images expressing GFP via oct-4 promoter transfection expressing viability and pluripotency. All images were captured using Nikon Biostation CT. (A) Stem cell growth in contact with 2 cycle CV coated Mg. After 24 hours, cells were no longer viable. (B) Stem cell growth in contact with 5 cycle coated Mg. After 54 hours, cells were no longer viable. (C) Stem cell growth in blank reference wells with no material. Growth continued to confluence. (Scale bar = 0.2mm)

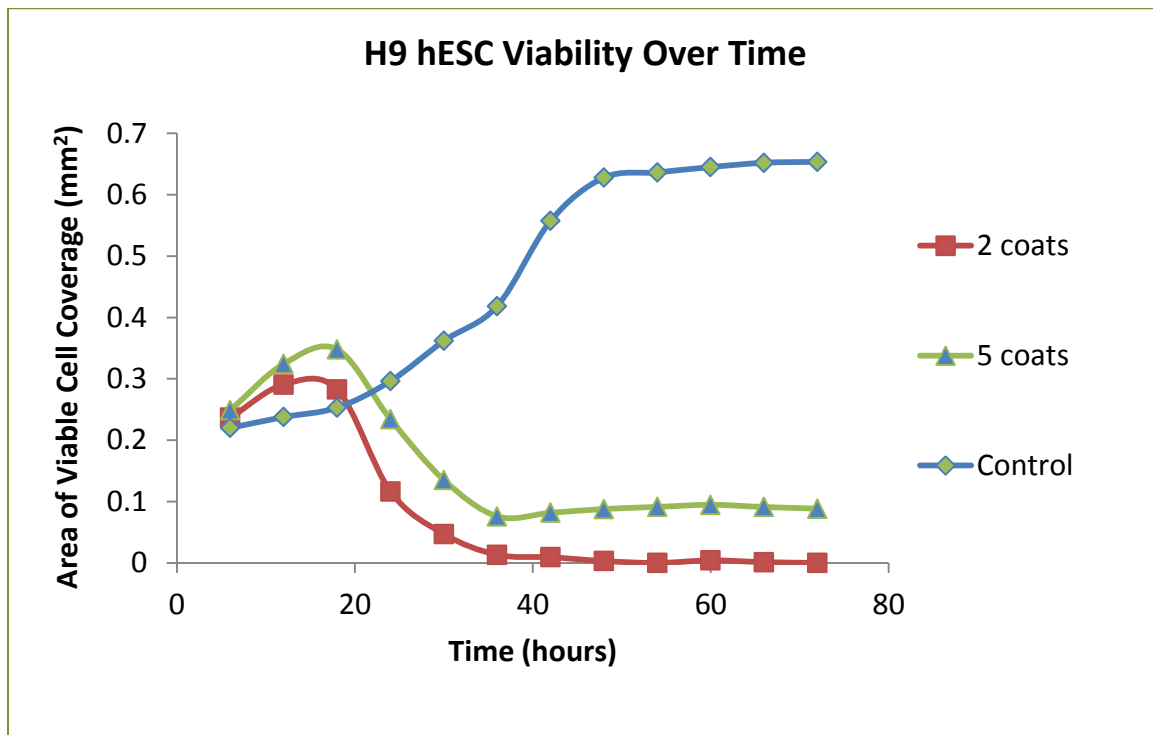


Figure 7: H9 hESC growth in area recorded every 6 hours for 72 hours. For 2 and 5 cycle coated Mg, the curves appear to peak at 18 hours and decrease after 20 hours. This is observed from the cells stretching before cell death. The control well cells continued to grow until they reached confluency.

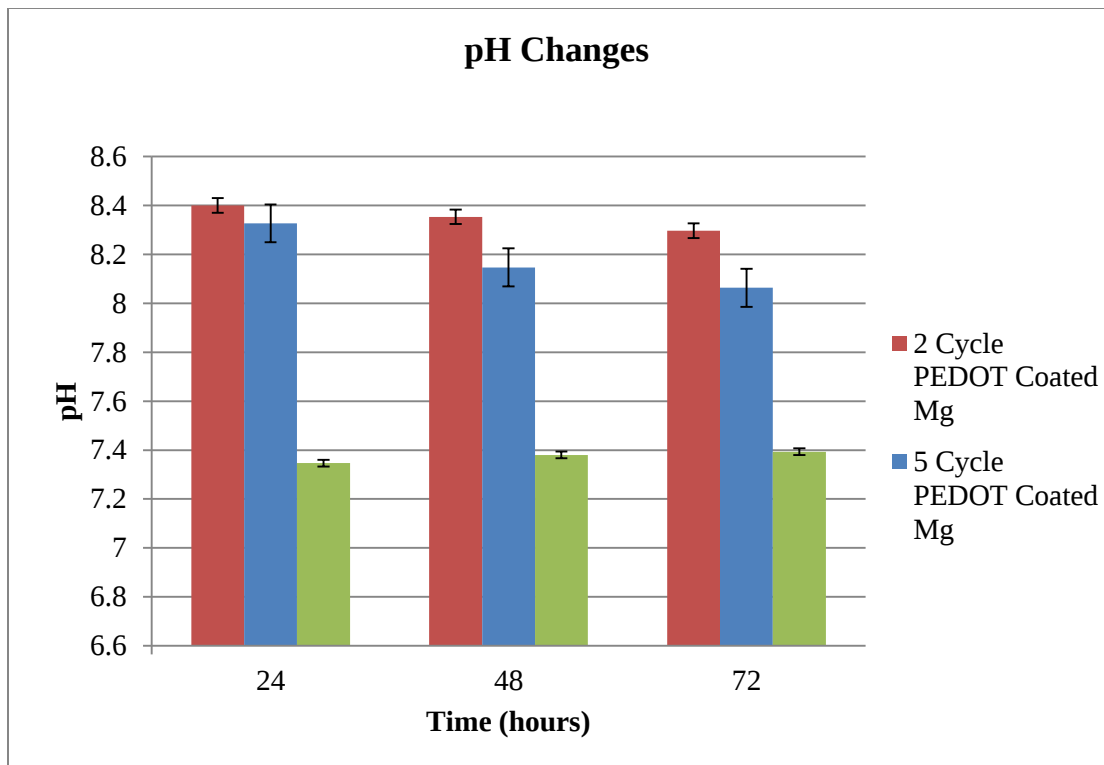


Figure 8: pH measurements of mTeSR[®]1 media was recorded every 24 hours by collecting media from the wells to determine how Mg affected the physiological environment of the H9 hESCs.

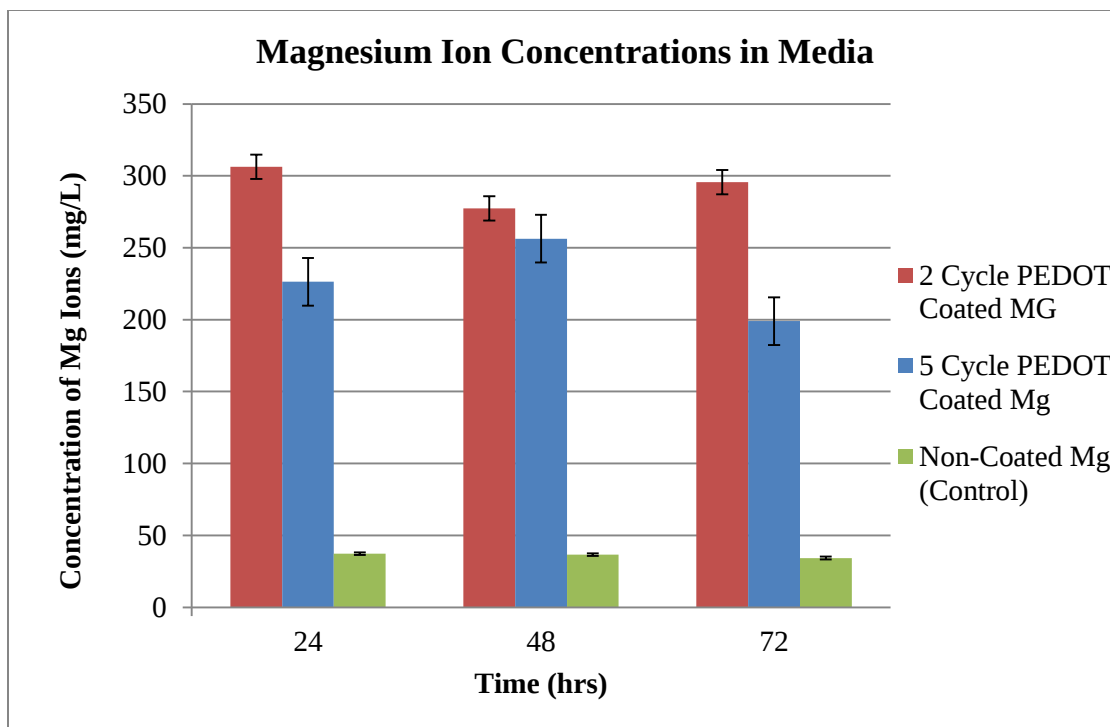


Figure 9: Magnesium concentrations recorded every 24 hours to determine the amount of Mg in media from degradation of Mg through the PEDOT pores during cell culture. The 2 cycle coated Mg allowed for a higher release of Mg ions than the 5 cycle coated Mg. The control wells contain basal amounts of Mg from the media used to culture these cells.

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