Electronically-Controlled Drug Release System to Promote Wound Healing

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ABSTRACT

A controlled drug release system based on biocompatible and electroactive polymers (EAP) was built to promote wound healing. Negatively-charged as well as positively-charged drugs were loaded into EAP systems through electro-polymerization in the presence of prepolymer and supporting electrolytes. Release was precisely controlled by applying an electrical stimulus. For example, a negative potential applied to change the EAP from its oxidized state to its reduced state resulted in the release of the pre-loaded negatively-charged drugs from the polymer films into the surrounding solution. Additional work included miniaturization of the controlled release system into a two-electrode system with coin cell power source. Step-wise release of two negatively charged compounds (salicylate, a model compound, and ibuprofen sodium salt, an anti-inflammatory agent) and one positively charged anti-bacterial drug (lincomycin) has been demonstrated. The evidence supports the use of EAPs in a localized therapeutic delivery device for the enhancement of wound healing.

Keywords: controlled drug release system, electroactive polymer, wound healing, ibuprofen, lincomycin

1 INTRODUCTION

Acute and chronic wounds account for several billion dollars in treatment costs each year in the United States alone and the patient population is predicted to increase by several million over the next few years [1]. In order to enhance patient comfort and decrease medical costs, innovations in wound care are a necessary pursuit [1,2,3]. Certain cationic and anionic drugs (e.g. doxycycline and ibuprofen) released locally may show efficacy in promoting wound healing by decreasing local inflammation and fighting infection; two important factors in determining wound healing time [2,3]. Electroactive polymers (EAPs) respond to electrical stimuli by changing their chemical and/or physical states, which in turn may affect conductivity, pore sizes, and surface energy. Well-known examples of EAPs include polypyrrole (PPy), polythiophene, polyaniline and their derivatives. PAni has previously been shown to demonstrate conductivities on the order of 10^3 S/cm [4,5,6]. Drugs in this study were selected for their ability to aid in wound healing due to antibiotic, analgesic, or anti-inflammatory effects. We are currently developing several systems for drug delivery that are based on the use of applied potentials that can oxidize or reduce the polymer depending on the requirements of the system.

The electronically-controlled drug delivery system (eDDS) was prepared by the deposition of biocompatible EAP films *via* either chemical-polymerization or electropolymerization of pyrrole or thiophenes and their derivatives. Several derivatives of pyrrole and thiophenes have been polymerized and their role as potential drug delivery electrodes investigated. Cytotoxicity data for biocompatible polymers were acquired using mammalian cell lines. Loading of the drug molecules was achieved either by *in situ* polymerization of the monomers in the combined drug-monomer solution or by electrophoretic migration of the charged target drug from solution into the previously polymerized EAP films. Release was triggered by oxidation of the polymer for positively charged drugs or reduction of the polymer for negatively charged drugs.

This paper will present a few case studies among many of our successful eDDS in loading and release of negatively-charged and positively-charged drugs. The controlled release of negatively charged model drug compounds such as sodium salicylate, aztreonam, and ibuprofen, and positively charged drugs including lincomycin HCl, clindamycin HCl, and doxycycline, among others, was demonstrated using polymeric electrodes. Results of controlled release of salicylate, ibuprofen, and lincomycin will be discussed in this paper. In an effort to insure noncytoxicity in the polymer, drug, and polymer/drug systems, *in vitro* testing was developed and performed in concert with the Center for Biomedical and Life Sciences at Missouri State University. *In vivo* testing is also planned.

2 MATERIALS AND METHODS

2.1 Loading and Step-wise Release of Sodium Salicylate

Electro-polymerization of pyrrole was conducted in a three-electrode system at 0.7 V (vs. Ag/AgCl reference). Gold was used as the working electrode, and films were polymerized in an aqueous solution of 1 M KCl, 0.05 M sodium salicylate (Sigma-Aldrich, St. Louis, MO, USA), and 0.2 M distilled pyrrole (Sigma-Aldrich). The films were prepared using 0.1 C of charge. Films were rinsed with deionized (DI) water for 10 s.

The step-wise release of sodium salicylate was done in 0.1 M PBS (pH of 7.4). The release was performed using a three-electrode (Ag/AgCl as reference) configuration of the Gamry MultEchem potentiostat (Gamry Instruments, Warminster, PA, USA) with a -0.8 V (vs. Ag/AgCl) potential applied for 20 and 40 s pulse widths. A +0.5 V (vs. Ag/AgCl) was applied between pulses to minimize

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passive release. The release of sodium salicylate was monitored at 296 nm in real time using a UV probe (Ocean Optics USB2000+ in combination with a Mikropack DH-2000 UV-Vis light source and the Ocean Optics SpectraSuite software).

2.2 Loading and Step-wise Release of Ibuprofen Sodium

Films were generated using a CH Potentiostat in a three-electrode configuration at 0.9 V (vs. Ag/AgCl). With a stainless steel counter electrode and carbon fiber cloth as the working electrode, films were polymerized in an aqueous solution of 1 M KCl, 0.15 M ibuprofen sodium (Sigma-Aldrich, St. Louis, MO), and 0.3 M distilled pyrrole. The films were electrochemically polymerized using 2.8 C of charge and were subsequently rinsed twice in 2 L DI water with sonication for 10 and 20 minutes.
The buffer used for ibuprofen step release was 0.01 M PBS (pH of 7.4). The release was performed using a three-electrode (Ag/AgCl as reference) configuration of the Gamry MultEchem potentiostat with a voltage of +0.5 V applied for 400 s and then -0.5 V applied for 40 s to the working electrode versus Ag/AgCl for 5 complete cycles using carbon fiber cloth as a counter electrode. Release was monitored at 264 nm in real time using a UV probe.

2.3 Loading and Step-wise Release of Lincomycin HCl

Electro-polymerization of the conducting polymer film took place in an aqueous solution containing 0.1 M pyrrole monomer and 0.6 M polyvinylsulfate potassium salt (PVSK from Sigma-Aldrich, St. Louis, MO) to form PPy/PVSK. A constant potential of 0.9 V was applied to grow the PPy/PVSK film (0.1 C charge passed). Lincomycin was loaded by biasing the PVSK doped PPy at -0.5 V vs. Ag/AgCl for at least 1000 s in a 0.1 M lincomycin aqueous solution. Releasing lincomycin in 0.1 M PBS was achieved by applying +0.9 V (vs. Ag/AgCl). In both the loading and release of lincomycin, Pt button working electrodes and a Pt mesh counter electrode were used. Step-wise release of lincomycin from PPy/PVSK was controlled by alternately turning on and off the power source. The release profile was monitored using a UV probe at 210 nm.

2.4 Two-Electrode System Powered by Coin Cell

Designing a two-electrode system and powering it with a coin cell would simplify its operation for release and may be the format of the final device. A carbon cloth working electrode was loaded with salicylate through electro-polymerization of PPy at constant current of 50 mA for 600 s in 25 mL solution of 0.15 M salicylate, 0.3 M pyrrole monomer and 1 M KCl. The working electrode was rinsed with 2 L DI water with sonication for 20 min to remove physically adsorbed salicylate or soluble oligomers from its surface. The working electrode was then soaked into 25 mL 0.1 M PBS buffer. A carbon fiber cloth counter electrode was used. Two CR2032 Energizer 3 V coin cells were used in parallel to power the release system where the working electrode was connected to the negative side of the coin cell and the counter to the positive (Figure 1). Release was monitored at 296 nm using a UV probe. Twelve min were allowed for passive release and another 12 min for active release.

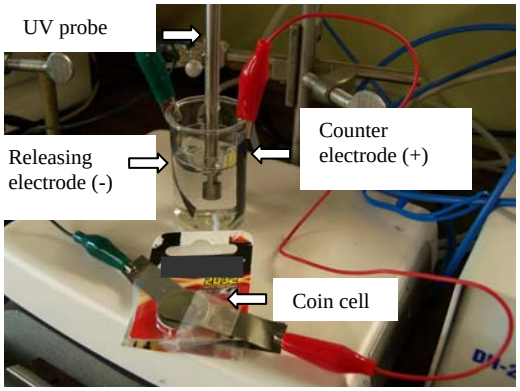

Figure 1. A two-electrode system powered by two 3 V coin cells to release salicylate from the working electrode with PPy.

2.5 Cytotoxicity Testing

RD muscle cells (ATCC: CCL-136) were selected to simulate tissue that would be in contact with any released drug in a full thickness wound. *In vitro* cytotoxicity was tested during active and passive release from drug loaded devices with the Cell Titer 96 viability assay. RD cells were seeded on glass coverslips in 24-well plates and allowed to grow for 24-48 h to confluency prior to cytotoxicity testing with polymer coated carbon fabric with drug for 2 h. The glass coverslips were then moved to 6-well plates for testing purposes. Devices were placed in each well with 5 mL of DMEM (Invitrogen) cell media containing 10% fetal bovine serum, 1% penicillin/streptomycin, and 0.5% Amphotericin B. The plates were then placed on a shaker. Using a constant voltage power supply developed by the Center for Applied Science and Engineering (CASE) at Missouri State University, a two-electrode system was biased to -1.5 V (working electrode loaded with ibuprofen sodium, blank carbon cloth counter electrode) for 20 min. After active release, all devices were removed from holders and placed in their respective wells. Plates were placed in a 37°C incubator with 5% CO_2 for 2 h. Cell Titer 96 reagent was added according to manufacturer's instructions and allowed to incubate at 37 °C for 1 h prior to absorbance measurements at 490 nm. Cell viability was determined by comparing the test absorbances to those of the control cells. A percent viability below 85% of untreated control used for

all toxicity limits. The final ibuprofen sodium concentration was later measured using HLPC-MS.

3 RESULTS AND DISCUSSION

3.1 Controlled Release from a Three-Electrode System

The real time release of negatively charged compounds from conducting polymer films was demonstrated. The release profiles for salicylate and ibuprofen are shown in Figure 2. These model drugs were released into phosphate buffered saline (PBS, pH 7.4) using step potentials applied as a negative bias for the saliciylate and ibuprofen. Although passive release was observed to both samples, electric bias of the salicylate- and ibuprofen-loaded polymer films showed significant increase in the release rate. For example, salicylate and ibuprofen were released at 0.44 µg/s and 23.8 µg/s actively (i.e upon the first negative bias) compared to 0.01 µg/s and 1.5 µg/s passively, respectively.

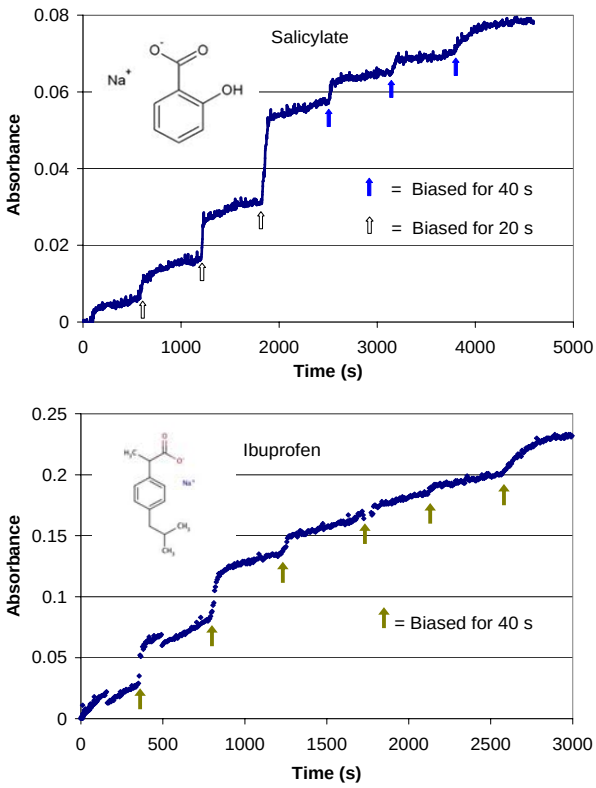

Figure 2. Stepwise release profiles of negatively charged drugs, salicylate (top) and ibuprofen (bottom), into PBS (pH 7.4) from the conductive polymer, PPy with negative bias (-0.5 ~ -0.8 V vs. Ag/AgCl).

To suppress passive release of negatively charged salicylate from PPy, positive potentials were applied to the working electrode. Three PPy films with sodium salicylate were prepared electrochemically under the samilar condition on carbon fiber cloth with 50 C charge. They were tested for 1 h passive release in 30 ml 0.1 M PBS, but subject to different potentials. They were applied 0, +0.5, +0.8 V vs. Ag/AgCl repectively for 1 h and passive release amount from each film was listed in Table 1. Results showed significant reduction of passive release by applying positive potentials.

Table 1. Suppression of passive release of salicylate in 0.1 M PBS by applying positive potentials.

Method	Passive Release after 1 hr (mg)
+0.0 V vs. Ref	15.0
+0.5 V vs. Ref	2.8
+0.8 V vs. Ref	1.7

Step-wise release of lincomycin HCl is shown in Figure 3. Upon applying a positive potential of +0.9 V (vs. Ag/AgCl), the release rate (0.173 µg/s) increased by nearly 10 times compared to passive release rate (0.028 µg/s), according to slopes of the two steps.

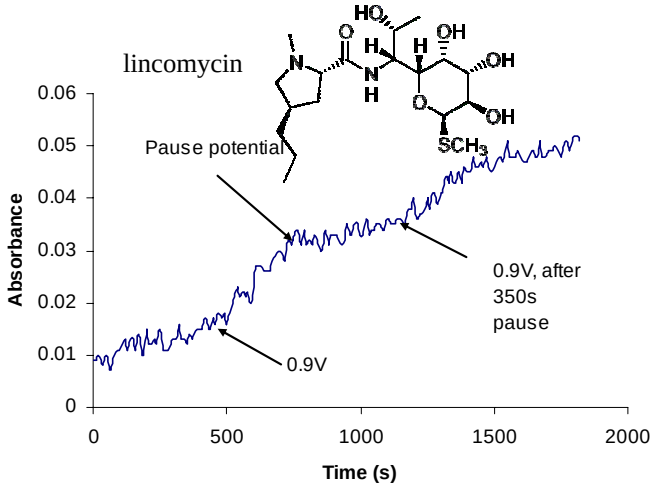

Figure 3. Step release of lincomycin(+) HCl from PPy/polystyrenesulfonate into PBS (pH 7.4) solution with positive bias (+0.9 V vs. Ag/AgCl).

3.2 Controlled Release from a Two-Electrode System

The two-electrode system powered by two coin cells showed controlled release of salicylate once connected (Figure 4). Passive release was observed before powering the device. Active release occurred immediately when the power was turned on (12 min). The UV probe was quickly saturated due to the large amount of release and absorbance plateaued in about 1 min. The successful demonstration of controlled release in a two-electrode system without a reference electrode has provided evidence that the overall design and operation of the system can be easily simplified.

This supports the notion of two-electrode EAP/drug systems in simple bandage constructs for use in wound healing.

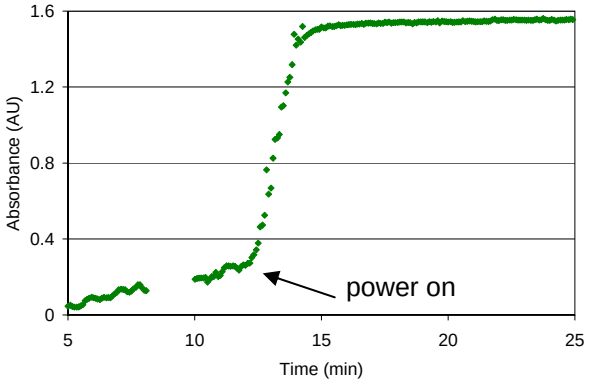

Figure 4. Release profile of sodium salicylate in PBS solution from a two-electrode system powered by two 3 V coin cells to release salicylate from the electrode with PPy.

3.3 Cytotoxicity

The cytotoxicity of the eDDS system (conducting polymer on carbon cloth loaded with ibuprofen sodium) was investigated. Carbon cloth was used as an electrode due to its flexibility and potential use in conjunction with a final bandage system. The RD cells were exposed to both active and passive release conditions using the ibuprofen-loaded PPy on carbon cloth. Controls (PPy alone) demonstrated no cytotoxicity in both the absence and presence of applied potentials (data not shown). Active release occurred for 20 min using a constant applied potential of -1.5 V in a two-electrode system. Samples were allowed to remain in the solution with the cells for 2 h to simulate bandaged wound conditions. Similar conditions were applied to controls. Passive release samples received no applied potential and were also incubated for 2 h. Cell viability measurements were evaluated using a modified MTT (3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay and all tested drugs and polymers were found to be noncytotoxic (>85% cell viability after a 2 h release). The early implications are that loaded ibuprofen sodium can be released *in vitro* in a controlled fashion without toxic effects from the drug, polymer, substrate, or electrical conditions. However, further testing is ongoing with this and other polymer/drug release systems.

4 CONCLUSION

An EAP-based controlled release system was successfully built to demonstrate release of two groups of drugs - negatively and positively charged - by the use of electrical stimulation. Step-wise release of these drugs was achieved when power was switched between on and off states. Transition from a three-electrode system into a simplified two-electrode system allowed for easy operation without compromising controlled release performance. The eDDS (polypyrrole with ibuprofen sodium) passed all cytotoxicity tests with the RD cells. The use of carbon fiber cloth as a working electrode allows for this system to be incorporated into a bandage in an effort to provide localized drug delivery. The delivery of therapeutic agents can be controlled to enhance wound healing by altering the dose and time of release using applied potential.

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