Antibiotic Release Using Nanostructured Polypyrrole-coated Titanium to Decrease Staphylococcus Epidermis Colonization

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Statement of Purpose:

Implant infection is difficult to treat and can result in revision surgery [1]. The biofilm formed by bacteria and the relatively poor delivery of antibiotics from the blood stream to the infected site contribute to problems with infected implant treatments [2]. In this study, the local administration of antibiotics was achieved by coating polypyrrole (PPy) doped with penicillin/streptomycin (P/S) on titanium. P/S release was triggered by applying a voltage. The PPy doped P/S allowed for the delivery of antibiotics directly to the infected site on a titanium orthopedic implant upon demand. In this manner, this study supports the continued investigation of titanium coated with PPy for in situ nano-biosensor applications which can determine events on the implant surface and release drugs on demand to reverse potential problematic events (such as infection).

Methods:

I. Electrodeposition of polypyrrole doped with P/S

Pyrrole monomers (0.1M; Sigma) were oxidized with penicillin-streptomycin (P/S; $C_{37}H_{57}N_9O_{16}S$; Hyclone) onto 1 cm^2 of gold-palladium coated titanium (Ti) in 1 M phosphate buffer saline (PBS; pH=7.2; Gibco). Electropolymerization was carried out by cyclic voltammetry (Epsilon EC; Bioanalytical) using a three-electrode system. The working electrode was gold-palladium coated Ti (AuPd-Ti), the counter electrode was platinum gauze, and the reference electrode was silver/silver chloride. A sweep voltage was applied to the electrodeposited PPy doped P/S on AuPd-Ti from 0 V to 1.1 V with a scan rate of 100 mV/s and 10 cycles.

II. Bacteria adhesion

A bacteria cell line (Staphylococcus epidermis; ATCC 35984) was obtained in freeze-dried form. The pellet was rehydrated in Luria broth. Cells were used at the 2nd passage and then frozen in glycerol and Luria broth (1:1). A sterile 10 µl loop was used to withdraw bacteria from the 2nd bacteria passage and were inoculated a polystyrene centrifuge tube with 3 ml of Luria broth. The tube of bacteria was agitated with a shaker (250 rpm) at 37°C for 16 hours before bacteria seeding. Bacteria concentration was assessed at an optical density of 562 nm at 30% transmittance (McFarlan Scale estimated 900 million bacteria/ml). This bacteria concentration was dilute to 10 million/ml with Dulbecco's modified eagle medium (DMEM; Gibco) supplemented with 10% fetal bovine serum (Hyclone), 50 nM β-glycerophosphate (Sigma) and 50µg/ml ascorbate (Sigma). Bacteria adhesion was investigated on PPy[P/S]-Ti, PPy, and conventional Ti. All samples were washed twice with Tris-buffered saline before culture. Bacteria were seeded at 10 million/sample and were allowed to adhere in a standard incubator (at 37°C and 5% CO_2 in humidified air). After 1 hr of a bacterial incubation, voltages were applied to PPy[P/S]-Ti in order to release P/S with 3, 5, 10, 15, 20 cycles of cyclic voltammetry at room temperature. Then, all samples were stained with the Live/Dead BacLight bacteria viability kit (Molecular Probes), and images were captured by fluorescence microscope (Leica Microsystem) at 20X.

Results:

Results showed that after 1 hour, PPy[P/S]-Ti drastically decreased bacteria colonies after electrical stimulation to release P/S compared to PPy[P/S]-Ti, Ti and PPy (Fig. 1). This suggests that P/S released from PPy can significantly reduce S. epidermis colonization.

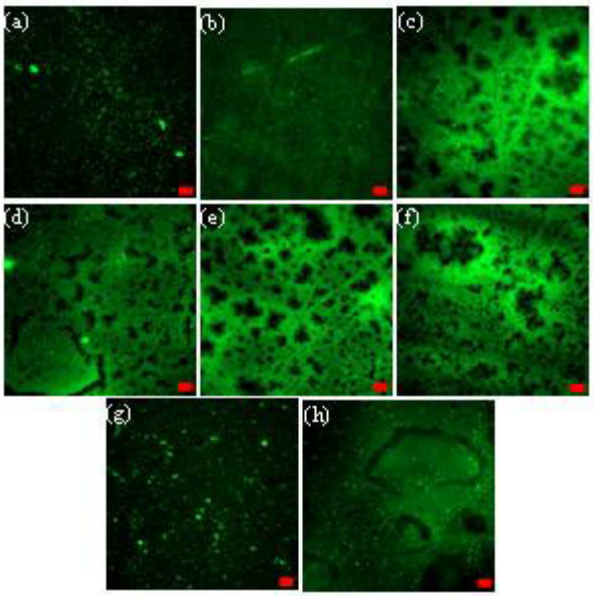

Figure 1. (a) Green fluorescent images of S. epidermis colonization on: (a) PPy[P/S] (no P/S release), (b) PPy[P/S] (release triggered after 3 cycles), (c) PPy[P/S] (for 5 cycles), (d) PPy[P/S] (for 10 cycles), (e) PPy[P/S] (for 15 cycles), (f) PPy[P/S] (for 20 cycles), (g) conventional Ti, and (h) PPy without P/S. The P/S releases were performed through cyclic voltammetry after 1hr of bacterial incubation in vitro. (Scale bar = 50 µm).

Conclusions:

This work demonstrated that P/S can be released to lyse bacteria by applying voltages to the PPy doped P/S. Previously, the cumulative P/S concentration after electrical stimulation to PPy[P/S]-Ti was determined. The concentration of P/S released increased with respect to the number of applied voltage cycles [3]. Coupled with previous studies [4], this study continues to support the use of Ti with PPy-coatings to serve as in situ nano-biosensors that can release drugs on demand to increase implant efficacy.

Acknowledgements:

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References:

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