Responsive Drug Eluting Nanotechnology Biosensor Systems for Bone Implants

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Statement of Purpose: In a previous study, it was shown that multiwalled carbon nanotubes (MWCNTs) grown out of anodized nanotubular titanium (Ti) (MWCNT-Ti) can serve as an effective sensor to determine if osteoblasts (bone-forming cells), bacteria, or inflammatory cells are present next to an implant surface [1]. The objective of this present study was to further the development of such sensors by incorporating a drug eluting system controlled by electrical stimulation to decrease infection and an inflammatory response.

Methods:

1. Electrochemical Polymerization (Electrodeposition)

Pyrrole monomers (0.1M) were either oxidized with 6.82 mM penicillin/streptomycin (P/S) or 0.125 mM dexamethasone (Dex) onto 1 cm^2 of AuPd on Ti in a 1 M phosphate buffer saline at a pH of 7.2. Electrochemical polymerization was carried out by cyclic voltammetry using a three electrode system. The working electrode was gold palladium coated Ti, the counter electrode was platinum gauze, and the reference electrode was silver/silver chloride. Potentials of 0 V to 1.1 V (a scan rate of 100 mV/s and 10 repeat scans) were used for electrodepositing.

2. Electrically-Controlled Drug Release

P/S and Dex were released from polypyrrole (PPy) into PBS buffer (pH 7.2) by applying sweeping voltages from 1 V to 1 V for up to 25 cycles with a scan rate of 100 mV/s. The cumulative release of P/S and Dex were determined in PBS buffer (pH 7.2) during electrical stimulation. The P/S release into PBS was determined quantitatively by using a BCA protein assay kit (Thermo Scientific). Dex was measured at an absorbance of 245 nm under a Lambda 35 UV vis spectrophotometer (PerkinElmer). The absorbance of PPy was subtracted from the light absorbance at 562 nm and 245 nm. Five samples of each drug were analyzed and then used to determine the average cumulative release profile with a standard curve for each drug.

3. Macrophage Adhesion

Mouse macrophages (TIB-186; ATCC) were seeded onto PPy-Ti and PPy[Dex]-Ti in RPMI 1640 media (Hyclone) supplemented with 10% FBS (Hyclone) and 1% P/S (Hyclone) in a standard cell cultured environment (at 37°C and 5% CO_2 in humidified air) at 10 million cells/cm^2. Macrophages were cultured onto PPy-Ti and PPy[Dex]-Ti for 4 hours before the application of voltage using cyclic voltammetry (scan rate of 100 mV/s from -1 V to 1V for 5 cycles). After cyclic voltammetry was applied, macrophages on PPy-Ti and PPy[Dex]-Ti were allowed to grow for either 4 or 9 hours. The experiments were repeated 3 different times.

4. Bacteria Culture

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 bacteria were inoculated in a polystyrene centrifuge tube with 3 ml of Luria broth. The tube of bacteria was then 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). Bacteria were seeded at 10 million cells/sample and were allowed to adhere in a standard incubator (at 37°C and 5% CO_2 in humidified air).

Results: The increase in the amount of P/S or Dex released after electrical excitation was significant until five cycles of sweeping voltages. The cumulative P/S and Dex released approached 80% of the drug release (Figure 1) and became relatively stable at that time. Importantly, absorbance curve stability also corresponded to the disappearance of the reduction peaks in cyclic voltammetry.

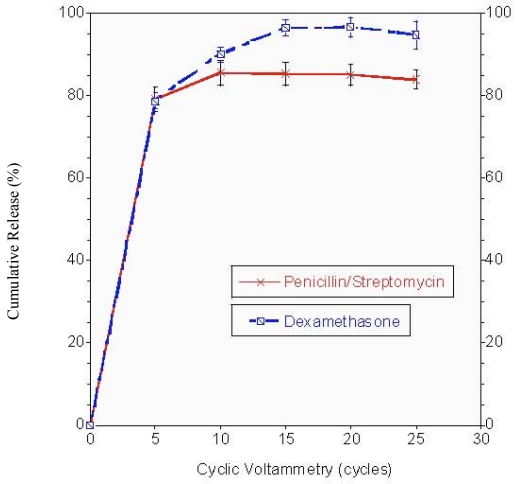

Figure 1. The cumulative concentration of P/S and Dex released after electrical stimulation (scan rate = 100 mV/s from -1 V to 1 V).

Conclusions: The results from a previous study showed that the PPy-drug films enhanced osteoblast adhesion to form bone, *in vitro*, whereas they inhibited fibroblast adhesion and scar tissue formation, when compared to Ti [2]. This present study showed that P/S can be released from such sensors and can decrease the amount of bacteria and macrophage density on Ti implants. The results also revealed a controllable, reliable, typical biphasic release profile for both P/S and Dex upon voltage application. Therefore, drug-embedded polypyrrole films, which were electrodeposited on MWCNT-Ti, may be able deploy drugs to reduce the prevalence of both septic and aseptic complications.

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References:

[1] Sirivisoot S et al. Nanotechnology. 2008;19; 29510.
[2] Sirivisoot S et al. in:E Kny (Eds.). Nanocomposite Materials, Solid State Phenomena. 2009;151; 197-202.