Utilization of Nanofibrous Polyester Materials as Drug/Protein Release Vehicles

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Background

Over 13 million medical devices are utilized annually in the United States, ranging from such simple devices as hernia repair mesh, wound dressings and catheter cuffs to more complex implantable devices such as the total implantable heart, left ventricular assist devices and prosthetic arterial grafts. All medical devices are prone to complications of infection, unregulated cellular growth, and undesirable blood clotting behavior.

Currently available biomaterials do not emulate the dynamic biologic and reparative processes that occur in normal tissue to overcome these complications. Thus, a novel biomaterial for use in a wide range of medical devices to direct or enhance the normal healing processes of native tissue would improve patient morbidity and mortality.

The goal of this study was to synthesize and characterize *in vitro* novel nanofibrous materials that contained biologically-active agents, ranging from small organic molecules to proteins. Our hypothesis was that the nanofibrous materials would serve as a "reservoir", slowly releasing the active agents over an extended period of time. We employed electrospinning technology in order to synthesize the nanofibrous polyester materials. A major benefit of this process is that the polyester nanofibers are formed at low temperatures, unlike standard polyester fibers which are extruded as a melt at high temperatures. The low temperature permits the structure of the active compounds to remain intact, thus retaining their biological activity. Additionally, no exogenous binder agents or polymers are required to incorporate the respective agents.

Methods

Electrospinning Methodology: A polyester solution (20%) was prepared in a solvent system. Additionally, two other polymer solutions containing either the broad-spectrum antibiotic Ciprofloxacin (Cipro) or the potent antithrombin agent recombinant hirudin (rHir) were prepared. For each polymer, a 5ml syringe with stainless steel 18-gauge blunt spinnerets (0.5mm internal diameter) was filled with the polymer solution and placed onto a Harvard Apparatus syringe pump. The perfusion rate was set at 3ml/hour at 25ºC. A PTFE-coated stainless steel mandrel (diameter = 4mm) was set at a jet gap distance of 15cm from the tip of the needle. The mandrel was then grounded to the power source, with the positive high potential source connected to the needle. Perfusion of the polymer was then started upon application of the current to the tip of the needle (15kV). After electrospinning onto the mandrel, the tubular constructs (nPET, nPET-Cipro and nPET-rHir) were air-dried at 60°C overnight.

Determination of Tensile Strength: Knitted and nPET materials (7mm width, 3cm length; n=3/test condition) were measured and cut. A Q-Test Tensile Strength Apparatus (MTS Systems, Cary, NC) was calibrated according to manufacturer's specifications in a climate-controlled environment. Segment stretching (crosshead speed = 50mm/min, gauge length = 2cm, load cell = 25 lb) was then initiated and terminated upon segment breakage.

In Vitro Wash Studies: nPET, nPET-Cipro and nPET-rHir segments (0.5cm segment length, n = 3 segments/ time interval/ segment treatment) were placed into 5ml of phosphate buffered saline (PBS) followed by continuous agitation using Rugged Rotator inversion mixer (33 r.p.m.) at 37°C. Wash solutions were sampled at acute (0, 1, 4 and 24 hours) and chronic (24 – 480 hours) time periods, with replacement of the wash solution with a fresh 5ml of PBS after sampling.

Cipro/rHir Release: For Cipro release, the absorbance of wash solutions were read at 322nm (PBS blank) using a Beckman DU640 UV/VIS spectrophotometer. A standard curve using known Cipro concentrations ranging from 0 - 100μg/ml was prepared. This Cipro standard curve was then used to extrapolate the antibiotic concentration within the wash solutions. For rHir, a Lowry protein assay was performed on the nPET and nPET-rHir wash solutions in order to determine protein concentration released at each time period.

Antimicrobial Activity via Zone of Inhibition: Washed nPET (n = 3 segments/time interval) and nPET-Cipro (n = 9 segments/time interval) segments were then evaluated for antimicrobial activity. A stock solution of *S. aureus* was thawed at 37°C for 1 hour. Upon thawing, 1μl of this stock was added to 5ml of Trypticase Soy Broth (TSB) and incubated overnight at 37°C. From this solution, 10μl was streaked onto Trypticase Soy Agar (TSA) plates. nPET and nPET-Cipro segments were then embedded into the streaked TSA plates and placed into a 37°C incubator overnight. Standard 5μg Cipro Sensi-Discs (n = 3) were also embedded at each time interval as a positive control. The zone of inhibition each piece was determined, taking the average of 3 individual diameter measurements. Zone size (mm) over time was determined for each parameter evaluate

Antithrombin Activity by Immobilized rHir: nPET-rHir segments with non-specifically and covalently bound ^{125}I-rHir were examined for antithrombin activity using a chromogenic assay for thrombin S-2238. Thrombin concentrations of 1, 2.5, 5 and 10 NIHU were evaluated. The assay was started by the addition of 1ml of 100μM S-2238 with the change in absorbance per minute monitored at 15-second intervals for 3 minutes at 410nm.

Results/Discussion

Using electrospinning technology, novel nanofibrous materials comprised of nPET, nPET-Cipro and nPET-rHir were synthesized. By varying such parameters as polymer solution concentration, spinning voltage, spinning time and spin gap distance, we controlled the mean fiber diameter and bulk material porosity and thickness.

There was a marked difference between the break load of the knitted Dacron (42 ± 9 pounds force) and nPET (3.7 ± 0.9 pounds force) segments. This difference in breaking load was expected due to the significantly greater wall thickness of the knitted Dacron material. The other physical properties such as the percent strain at maximum load and percent strain at break were comparable.

Cipro was spun into the nPET material as shown by fluorescence. nPET-Cipro segments subjected to the extensive washing regime showed antibiotic release over the 20 day study period. Antimicrobial activity was also demonstrated over the same time period. In contrast, nPET control segments had no antimicrobial activity t any of the time periods evaluated.

rHir, similar to the Cipro, was also incorporated into the nPET material. rHir release into the wash solution was evident throughout all of the time periods examined as determined using a Lowry Protein assay. Antithrombin activity of nPET-rHir surfaces, examined using a chromogenic assay for thrombin, showed significant thrombin inhibition at the various thrombin concentrations evaluated.

Conclusions

This study demonstrates that Cipro as well as rHir can be individually incorporated into a nanofibrous polyester material. Additionally, these compounds are slowly released over an extended washing period. Lastly, these nanofibrous surfaces can maintain localized biological activity due to the drug elution from the nanofibrous material.