Synthesis and Characterization of a Novel Nanofibrous Bioactive Prosthetic Sewing Cuff

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Statement of Purpose

Cardiac valve replacement using prosthetic valves is indicated when progression of degenerative disease or bacterial infection of the native valve results in valvular dysfunction. The number of prosthetic valve implants each year has increased with the aging population and a more aggressive approach to treat the mitral valve insufficiency, with the number estimated at greater than 50,000 each year. Bacterial infections, thrombosis and thromboembolism are major causes for the failure of these valves. The infections and thrombosis tend to localize at the sewing cuff/tissue interface.

The goal of our research is to develop a novel prosthetic cuff that will simultaneously provide localized infection-resistance and antithrombotic properties via release of drugs over an extended period of time. The electrospinning process incorporates a broad spectrum fluoroquinolone antibiotic moxifloxacin and a potent antithrombotic agent recombinant hirudin (rHir) into the sewing cuffs (bioactive sewing cuff). The synthesis process retains the biologic activity of these drugs, thereby enhancing the overall performance of the nanofibrous synthetic sewing cuff. The bioactive sewing cuffs were subjected to a rigorous drug release study and the wash solutions as well as the washed sewing cuffs were analyzed for residual drug content and activity. The results overwhelmingly suggest the improved performance of the electrospun sewing cuff as compared to the current standards.

Methods

Sewing Cuff Synthesis: Sewing cuffs (non-drug-loaded) were synthesized via electrospinning (espun) technology using polyester (control sewing cuffs). For the bioactive sewing cuffs, rHir and moxifloxacin were incorporated during the electrospinning process. For both cuffs, electrospinning was performed at room temperature, with the resulting sewing cuff that is 1.63mm thick. The electrospun material is after treated after electrospinning in order to remove the residual solvent.

Material Characterization: Random samples were selected from the electrospun sewing cuffs and examined via a JEOL JSM 5900LV electron microscope (15kV accelerating voltage, 500X and 2,500X magnification; gold sputter coated for 60 seconds) in order to qualitatively assess fiber size and distribution throughout the materials.

Physical Characterization: Control and drug loaded sewing cuffs were tested for tensile strength using a Q-Test Tensile Strength Apparatus (MTS Systems, Cary, NC). The samples were conditioned for 24 hrs at 25°C and 45% relative humidity. Segment stretching (crosshead speed= 50mm/min, gauge length= 2cm, load cell = 25 lbs) was then initiated and terminated on sample breakage. Suture retention was measured by placing a 24-gauge wire through one end of the sewing cuff. The samples are stretched at a similar rate as that for tensile testing and the force at which the wire tears the cuff is measured.

In vitro wash studies: Control and bioactive sewing cuffs were washed with PBS to study the duration and activity of the drug release. For each selected time period (1hr, 4hr, 24hr, 1 day, 2 days until day 30), two 5cm length segments of control and bioactive sewing cuffs were cut, weighed and placed into a 15ml Falcon tube along with 5ml PBS (37°C). The tubes were placed in a rugged rotator inversion mixer to carry out an intensive wash of the segments at 37°C. After each time period, the wash solution and respective segments were collected and analyzed for drug concentration and biologic activity. The antibiotic concentration were measured using spectrophotometry as well as spectrofluorometry while the antibiotic activity was measured using a zone of inhibition assay (standard moxifloxacin disc served as the positive control). rHir concentration in the wash solution was measured using a Lowry protein assay. Antithrombin activity of rHir released in the wash solution as well as remaining on the cuff surface was measured using a chromogenic assay.

Results

Figure 1A is a gross image of our novel nanofibrous bioactive sewing cuff as compared to a clinically-utilized cuff. Observation of the bioactive sewing cuff surface via SEM (Figure 1B) shows fiber distribution to range from 300nm to 3μm. Additionally, It appears that rHir and moxifloxacin are distributed throughout the electrospun material and not dispersed within the interstices of the material.

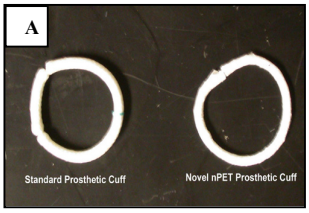


Figure 1A: Image of clinical vs. bioactive sewing cuff

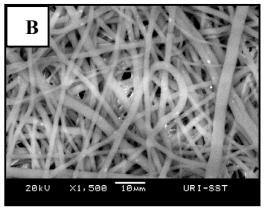
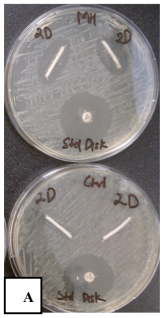


Figure 1B: SEM of bioactive sewing cuff

The wash studies reveal a drug release pattern which is initiated by a burst followed by a slow and low release. Even after 30 days of washing the sewing cuffs retained their antibiotic and antithrombotic activity (Figures 2A/2B). Both these properties are absent in the standard sewing cuffs pre or post wash.



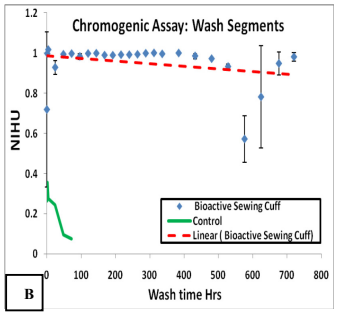


Figure 2A: Zone of Inhibition Assay Figure 2B: Thrombin Inhibition over Time

Conclusion:

We have been able to make a synthetic sewing cuff prototype which has antibiotic and antithrombotic properties that are retained even after a period of one month of rigorous washing. This advancement is a positive step towards next generation sewing cuffs. The next step is to assess overall healing and infection-resistance/thromboresistance *in vivo*.