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Electrospun PLA/CSA-13 Antimicrobial Coatings for Spinal Instrumentation

The number of spinal fusion and instrumentation procedures is projected to drastically increase in the coming decades due to a rapidly aging population.[1] The invasiveness of these procedures **(Figure D)**, coupled with reduced immune function in elderly patients, can lead to an increase in bacterial colonization on the device and an increase in perioperative infection rates. In many cases, bacteria congregate and form a film that covers the implant surface. Microbes within this biofilm are capable of resisting treatment with systemic antibiotics, often requiring extensive revision surgery in order to remove the colonized device, debride infected tissue and repair structural damage at the surgical site.

The main goal of my research is to combat the formation of biofilms on spinal implants by modifying the interface between implant and tissue. To deliver targeted antimicrobial therapy in the spinal surgery environment and prevent these costly and dangerous infections, it is necessary to provide uniform coverage of the implant and selective protection against drug-resistant strains of bacteria. To do this, I propose using electrospinning to create an antimicrobial nanofiber construct for use in coatings and protective coverings in spinal surgical procedures. My previous research has demonstrated that selective antibiotics can be incorporated into electrospun fibers and that these fibers can be deposited conformally, as oriented fibers on titanium orthopedic device analogs. These electrospun coatings will resist the formation of biofilms, encourage the regrowth of native cells, improve integration of the device with native tissue, and provide a platform compatible with future cellular and nanoparticle-based therapies.

Electrospinning has become a preferred method for producing nanofibers for a wide variety of applications, such as precision filters, energy storage devices, engineered skin tissue, and drug delivery vehicles.[2] The phenomenon of electrospinning occurs when a fluid droplet is subjected to an electrical force, typically provided by a charged nozzle.[3] At a certain critical voltage, electrical force overcomes the surface tension of the liquid, initiating the formation of a fluid jet from a structure known as a Taylor cone **(Figure B)**. As the fiber jet travels towards a grounded collector, the solvent evaporates, leaving a solid nanofiber with a uniform, controllable diameter. The path of the nanofiber becomes increasingly chaotic as it moves farther from the nozzle due to a complex destabilization process. Upon collection, fibers are continuously deposited in a random array, forming a mat-like structure. Fiber mats can be firmly attached to an implant by electrospinning onto a surface that has been treated with a silane linking agent.

Through my design and optimization of the electrospinning device **(Figure A)**, I have been able to reproducibly synthesize poly-lactic acid (PLA) nanofibers with a uniform diameter of 254 ± 69 nm at a rate capable of uniformly coating a clinically relevant area of several square inches in 15-20 minutes. This uniform covering of biocompatible nanofibers, analogous to the fibrous extracellular matrix, provides a favorable substrate for cell growth and a vehicle for the delivery of selective antibiotics.

Additives in the initial electrospinning mixture provide functionality to the nanofiber. In order to create antimicrobial fibers, a cationic steroidal antibiotic, CSA-13, is incorporated into the biocompatible PLA electrospinning solution. Because the activity of CSA-13 mimics that of the body's antimicrobial peptides, it acts against a broad range of Gram-positive and Gram-negative bacteria, including drug resistant strains. CSA-13 is ideal for an antimicrobial coating because it maintains its functionality when immobilized on a surface and has been shown to reduce the development of bacterial resistance with repeated clinical use.[4] The incorporation of the CSA-13 molecule in a nanofibrous mat would provide a uniform barrier against biofilm formation on implant surfaces.

While a random array of fibers provides a nanostructured substrate capable of delivering the CSA-13 molecule, the ability to control the orientation of deposited fibers can further enhance an effective treatment system. Both nerve and bone cells have shown a preference for growth on oriented surfaces[5] and differentiating stem cells exhibit a well-documented substrate dependency. I have demonstrated two methods of achieving parallel arrays of nanofibers, one electrical and the other mechanical. Although fibers can be aligned electrically across an air gap between two grounded plates, the area that can be coated is limited to linear area of under three inches. To create alignment on a clinically relevant scale, I have designed and built a system wherein fibers can be drawn over a rapidly

rotating surface. This mechanical force is capable of producing even higher degrees of alignment **(Figure C)** than we were able to produce using parallel electrodes.

With the completion of the final electrospinning device, I will continue my work with single solution PLA/CSA-13 fibers, optimize the adhesion between coatings and implant analogs, and perform antimicrobial and cytotoxicity tests to determine the optimal composition of these coatings. Once efficacy of a nanofiber coating delivery platform is verified in the case of PLA/CSA-13, in depth physical and kinetic studies will be carried out with the goal of guiding the design of more complex nanofiber systems. These studies will be carried out using neutron imaging in collaboration with the Institut Laue-Langevin in Grenoble, France. Neutron scattering will provide vital information on the CSA-13 distribution within PLA fibers, the nature of the nanofiber-implant interface, and the release profile of CSA-13 from the fiber as it biodegrades.[6] These studies of the fundamental principles governing drug eluting electrospun nanofiber coatings will guide my future investigations into more complex nanofiber systems. The primary objectives for this phase of the project are creating core-sheath nanofibers and incorporating one or more bioactive compounds to provide additional antimicrobial action, inflammation control, or osteogenic activity.

To create these complex systems, we have designed a coaxial nozzle capable of producing core-sheath or hollow fibers, which can be used in the production of a hydroxyapatite ceramic laden fiber inside a biodegradable sheath filled with CSA-13. This combination material would be capable of reducing infection, promoting osteogenesis, and aiding in the incorporation of native bone. In addition, the release of silver, a natural oligodynamic antimicrobial, from the sheath can be tuned to match the infection and bone regeneration time scale for each operation. These types of electrospun nanofiber coatings will provide surgeons a new level of control over biofilm and deep tissue infections.

Works Cited

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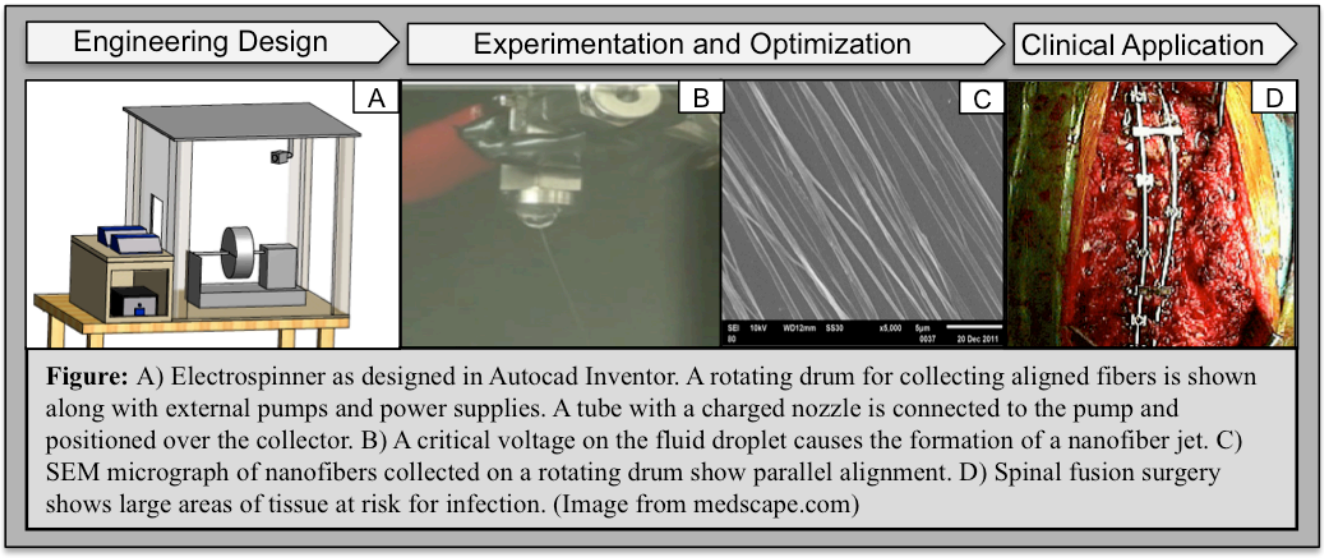

Figure: A) Electrospinner as designed in Autocad Inventor. A rotating drum for collecting aligned fibers is shown along with external pumps and power supplies. A tube with a charged nozzle is connected to the pump and positioned over the collector. B) A critical voltage on the fluid droplet causes the formation of a nanofiber jet. C) SEM micrograph of nanofibers collected on a rotating drum show parallel alignment. D) Spinal fusion surgery shows large areas of tissue at risk for infection. (Image from medscape.com)