

National Textile Center

FY 2004 New Project Proposal

Project No.

F04-NS26

Competency: **Fabrication**

Single-step protein surface-attachment to electrospun fibers

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Objective: The objective of this research is to develop a single-step processing route for the production of synthetic fibers possessing specific bioactive surface functionalities. The approach proposed here to achieve this objective utilizes electrospinning of conventional synthetic polymers in conjunction with novel synthetic-bioorganic hybrid copolymers as the carriers for the introduction of prescribed bioactive functionalities. This strategy will incorporate covalently-linked oligopeptides on the electrospun fibers as surface functionalities that can interact actively with biological systems. A variety of applications can be realized depending on the primary structure of the surface-bound oligopeptide. While the focus in the present project will be to design antimicrobial or antifungicidal surfaces, this methodology can be equally useful in the design of other biocompatible, biorepulsive or bioattractive fiber surfaces.

Relevance to NTC Mission: The development of bioactive textiles for use in biomedical applications, military uniforms and civilian clothing represents a key growth area for advanced textiles. More specifically, the development of bioactive medical textiles is timely as the occurrence of hospital-related infection continues to increase. The design of bioactive military uniforms is urgently needed to protect soldiers from the repercussions of biowarfare. Similar design can be used on a daily basis to reduce odor in everyday clothing. This work could lead to the development of new bioactive materials with controllable/targeted functionality that could be incorporated directly into electrospinning operations. It will likewise improve the current level of understanding in polymer and textile science by elucidating the chemical factors needed to synthesize the hybrid copolymers required and the material and process factors responsible for enrichment of the oligopeptides on the surface of synthetic polymer fibers.

State of the Art: Fibers produced from polymeric materials are critical in a variety of fields ranging from surgical sewing material to components in ultralight, high-strength composite materials. The mechanical properties of fibers are dominated by the bulk material comprising the fiber. However, fibers "communicate" with their environment through their interfaces, in which case there is a great demand to control surface properties and functionality. Modification of fiber surfaces proceeds along pathways comparable to those employed to modify planar surfaces. The methods of surface treatment depend strongly on the nature of the material used to produce the fiber and include covalent grafting of polymers¹, physical treatment (e.g., plasma treatment²⁻⁴), physisorption^{5,6}, chemisorption⁷⁻⁹ and chemical derivatizing^{4,10}. The covalent attachment of functional compounds is the approach of choice to introduce complex or fragile functionalities permanently. Unfortunately, this approach usually requires multiple steps, since anchoring functionalities must be established on the fiber surface prior to introduction of linkers and, finally, the molecule carrying the desired functionality¹¹. In the case of multifunctional molecules (e.g., proteins), attachment is often non-regioselective, leading to ill-defined activity and function of the resultant biomolecules.

Designer fiber surfaces exhibiting specific bioactive functionalities are important since they are capable of directing and controlling a desired biological response^{4,12-14}. For example, surfaces that are bioadhesive¹⁴⁻¹⁷ or bio-repulsive^{18,19} can attract or repel specific proteins. This could lead to selective docking of desired cell surfaces or prevent the tagging of surfaces as foreign, which would result in an immune response. Surface functionalities that can actively interact with biological systems are widely applicable in, for instance, the field of antimicrobial or antifungicidal packaging to extend the shelf life of food²⁰. Fibers with bioactive surfaces can be applied to surgical

sewing materials where it can prevent an inflammatory response or stimulate wound healing^{21,22}. By processing the fibers into fabrics, antimicrobial filters, bandages or clothing can be realized. The design and development of such fabrics can be readily extended to smart military uniforms capable of multifunctional service.

Approach: The focus of this project is to develop a new, single-step approach towards fibers with bioactive surface functionalities (Figure 1). Established electrospinning techniques will be applied to co-spin a homogeneous blend of a synthetic polymer (forming the bulk fiber) and a synthetic-bioorganic hybrid copolymer as the bioactive functionality carrier. For the hybrid polymer, an anchor polymer that is compatible with the bulk fiber polymer is covalently linked to an oligopeptide with desired biological activity to produce a block copolymer. During the electrospinning process, demixing of the nonpolar fiber material and the polar oligopeptide is anticipated to occur due to differences in polarizability (Figure 2). Since the anchor block of the copolymer remains entangled with the bulk fiber polymer, a covalent tethering of the peptide functionality to the fiber is thereby achieved.

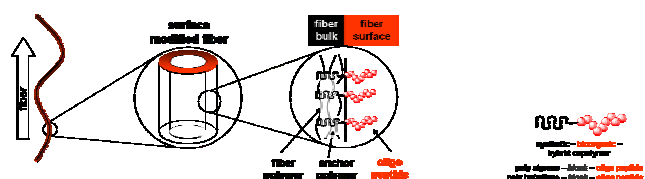


Figure 1. Microstructure of the surface-functionalized fiber (left) and the copolymer used for this purpose (right).

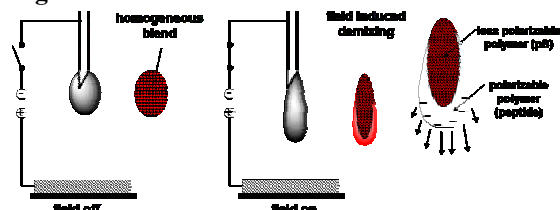


Figure 2. Proposed mechanism of electrospinning a fiber containing a synthetic-bioorganic hybrid copolymer.

Synthesis of the hybrid copolymer

The hybrid block copolymer consists of a synthetic polymer and an oligopeptide and is synthesized via solid-phase supported peptide synthesis followed by on-support coupling of the synthetic block²³, as illustrated in Figure 3.

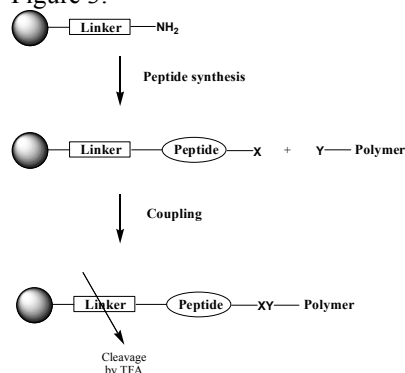


Figure 3. Synthesis scheme of hybrid copolymers.

The solid-phase supported peptide synthesis yields oligopeptides exhibiting a defined primary structure with up to ~30 amino acids in length^{24,25}. To avoid difficulties resulting from a complex peptide structure, a simple peptide sequence such as (glutamic acid-*alt*-serine)₁₀ (ES)₁₀ will be used at the start of this project. Even if the (ES)₁₀ is not specifically chosen for its biological activity, basic antimicrobial activity studies can be performed in the future, since anionic peptides sometimes exhibit antimicrobial potential²⁶. Use of this simple peptide will permit evaluation of the process and analysis conditions. As the project develops, more active oligopeptides will be selected on the basis of their activity. Cationic peptides are suitable candidates in this vein since they combine a high antimicrobial potential with high selectivity for bacteria and non-toxicity for mammalian cells²⁷. This is due to their specific binding, permeation and disintegration of the negatively-charged outer bacterial membrane, whereas the predominantly zwitterionic membranes of mammalian cells are not attacked. Hybrid copolymers containing cationic peptides fit within the issue of incompatibility between the bulk fiber material and strong differences in

polarizability, which are necessary requirements for the proposed mechanism of field-enhanced phase separation and surface enrichment during electrospinning. The synthetic block of the hybrid copolymer is prepared via living anionic or controlled radical polymerization. These provide access to a variety of polymers including, but not limited to, polystyrene, polybutadiene, polyacrylates, polyester or polyamides with well-defined molecular weights, relatively low polydispersities and a high level of end functionalities. Due to the ease of synthesis, the anchor polymer used in the first phase of this project will most likely be polystyrene. As the project develops, the synthesis of the copolymer will be adapted towards the more commonly used fiber-forming polymers listed above.

Choice of fiber polymer

At the start of the project, the synthetic polymer forming the bulk fiber and dominating the mechanical properties of the resultant fiber will be chosen based on the ease of availability, processability, compatibility with the anchor polymer and incompatibility with the oligopeptide. For this reason, we shall explore the electrospinning conditions required to generate fibers of polystyrene or a commercial copolymer thereof. Surprisingly, very little has been reported on the electrospinning of styrenic polymers, and so we shall develop a baseline upon which to build our project, as well as a new database of electrospun fibers. Upon proof of concept using this model system, fiber polymers will be selected on the basis of specific applications identified by us with the assistance of industrial and government advisors. Suitable candidates for the fiber polymer include nylon, polyethylene, polypropylene, polyurethane, polylactides and recombinant spider silk. A subset of these polymers suitable for detailed investigation within the time frame of the project will be chosen and subsequently investigated.

Process and characterization steps

Co-spinning of the bulk fiber and hybrid polymers will be performed from solution. The conditions will be varied sufficiently to achieve a wide range of fibers in order to ascertain the efficacy of peptide surface enrichment. Due to strong differences in polarizability of the bulk polymer and the peptide block of the copolymer, the applied electric field is expected to induce phase separation during the spinning process. The extent to which such field-induced demixing (and peptide surface enrichment) occurs will depend on factors such as the applied electric field and the residence time of the fiber within the field. The key parameters governing phase demixing and surface enrichment will be identified through a statistical design, followed by a more systematic variation, of the process parameters. Evidence of surface enrichment will be achieved through chemical and morphological analysis conducted with transmission electron microscopy (TEM), energy-dispersive x-ray spectroscopy (EDS), x-ray photoelectron spectroscopy (XPS) and, if necessary, scanning transmission x-ray microscopy (STXM). In TEM, the peptide can be selectively stained, and its spatial distribution on the surface and in the interior of the fibers can be determined. The EDS and XPS analyses can provide a more quantitative description of composition, whereas STXM (conducted at the National Synchrotron Light Source at Brookhaven National Laboratory) can be used to identify the peptide on the basis of its functional constitution. While select antimicrobial assays may be conducted here, no extensive microbiology testing is planned during the course of this process/material-related project.

This Year's Goal:

The first year accomplishment.

A series of hybrid copolymers will be synthesized, and polystyrene (and its copolymers) will be electrospun into fibers to identify critical process parameters. Peptide-enriched fibers will be produced under different sets of process conditions, and the resultant peptide-enriched fibers will be characterized. Information acquired during this phase will guide subsequent steps with more conventional fiber-forming polymers. With these data, we shall endeavor to attract additional industrial and federal support. At least one manuscript will be prepared for publication in a high-profile peer-reviewed journal, and our results will be presented at no fewer than two international conferences.

Outreach to Industry:

We shall work closely with several companies (e.g., Gentex and Foster-Miller), as well as the U.S. Department of Defense, to explore commercial potential in bioactive textile applications. If our results are successful, industrial and/or government partnerships will be initiated for scale-up.

New Resources Required:

A cryoultramicrotome used to prepare fiber sections for TEM is requested for use in conjunction with this project.