A Layer-by-layer Approach to Incorporate Bioactive Factors into Electrospun Meshes while Preserving Protein Secondary Structure

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Introduction & Motivation

Electrospun meshes – popular in several tissue engineering applications – are often functionalized with therapeutic proteins to enhance their bioactivity and thus improve their ability to elicit specific cell responses. However, traditional approaches to incorporate sensitive bioactive factors into electrospun meshes expose the proteins to harsh processing conditions (e.g., hydrophobic polymers, organic solvents, high voltage) which can negatively impact protein structure and function. Alternative strategies that help retain protein structure/function post-incorporation can improve the biological/therapeutic potential of functionalized meshes.

Objectives

This work presents an approach to create sandwich composites using a layer-by-layer fabrication technique that permits the incorporation of proteins into electrospun composites while retaining protein secondary structure. Specifically, we use an air-sprayer to sandwich a protein-loaded layer between two electrospun fibrous layers. To demonstrate the benefits of this approach, we incorporate two model proteins bovine serum albumin (BSA) and lysozyme into the air-sprayed layer and subsequently evaluate composite mechanics, protein secondary structure and release kinetics (**Figure 1**).

Experimental

- **Electrospinning**: Poly(lactic-co-glycolic acid)
- **Air-spraying**: Alginate and calcium chloride
- **Proteins**: Bovine serum albumin (BSA) or lysozyme

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- Layer-by-layer fabrication of composites by electrospinning and air-spraying.
- Alginate cross-linked *in situ* with calcium chloride.

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- Composite-90 and Composite-180 samples (based on the relative positions of the air-sprayers)
- Control sample prepared without alginate layer

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Microscopy | Spectroscopy | Tensile testing | Circular dichroism | Protein release

Figure 1: Study framework

Morphology and composition

- Bright-field, fluorescence and scanning electron microscopy of samples collected during various stages of composite preparation revealed three layers in the sandwich: a bottom fiber layer, a middle air-sprayed alginate layer and a top fiber layer (**Figure 2**). As proof-of-concept, rhodamine was pre-loaded into the air-sprayed alginate during fabrication in order to visualize under fluorescence microscopy.
- FTIR spectroscopy (**Figure 3**) revealed peaks for the functional groups present in PLGA (indicated by X) and the incorporated BSA or lysozyme (indicated by # & * respectively) for the composites. Alginate peaks were observed in both samples in the 3200-3400 cm^{-1} range. Control samples prepared without alginate showed peaks only for PLGA.

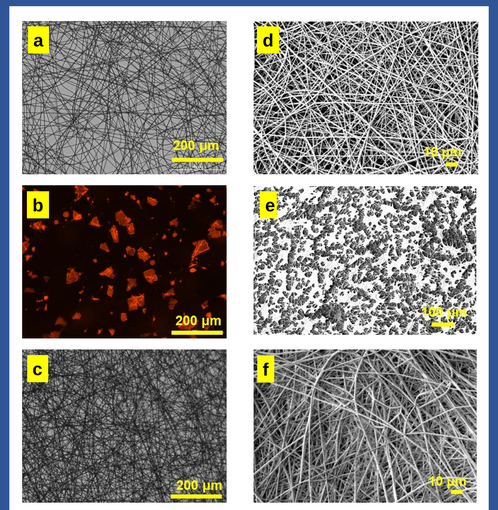

Figure 2: Microscopy images of (a) bottom fibrous layer, (b) middle alginate layer with rhodamine, (c) top fibrous layer; (d,e,f) corresponding scanning electron micrographs

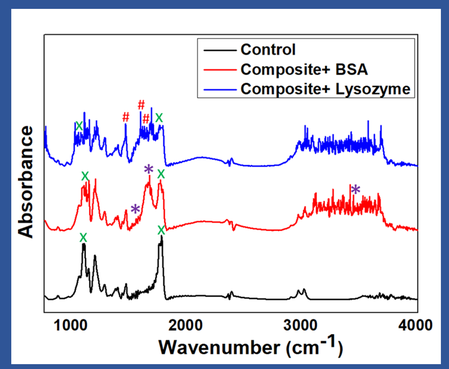

Figure 3: FTIR spectra of composites & control

Mechanical properties

- Uniaxial tensile testing was conducted to analyze the mechanical stability of the composites (**Figure 4**).
- Composite 90 and composite 180 samples had moduli of 14.7 ± 3.3 and 16.7 ± 4.2 MPa respectively compared to 9.9 ± 0.56 MPa for the control. Strain at failure values for the composites were 19.2 ± 0.54 and 25.2 ± 0.33 compared to 15.2 ± 0.70 for the control.

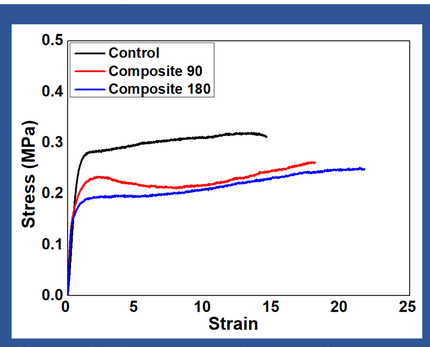

Figure 4: Representative tensile stress-strain curves for composites and control

Protein secondary structure

- Circular dichroism spectra for BSA extracted from both composites was comparable to the native spectrum (**Figure 5**) and the secondary structure was pre-dominantly α-helical (~55%). When incorporated in a similar manner, lysozyme secondary structure was also comparable to the control at ~40% α-helix and 20% β-sheets (**Figure 5**).

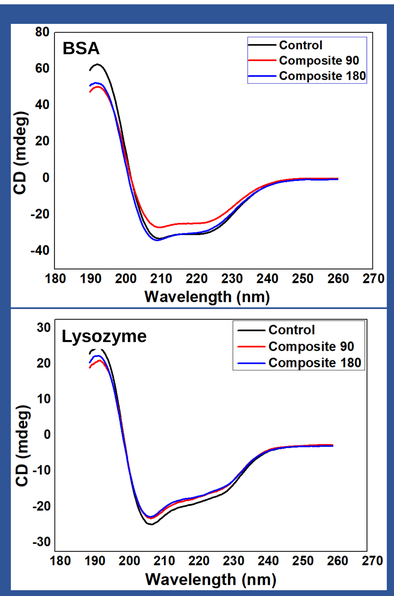

Figure 5: Representative CD spectra of proteins extracted from composites

Protein release kinetics

- Incorporated proteins were released from the composites in a controlled manner *in vitro* **(Figure 6).**
- The differences in release profiles may be attributed to differential alginate cross-linking between the composite-90 and composite-180 samples, differential isoelectric points of the two proteins and their inherently different sizes.

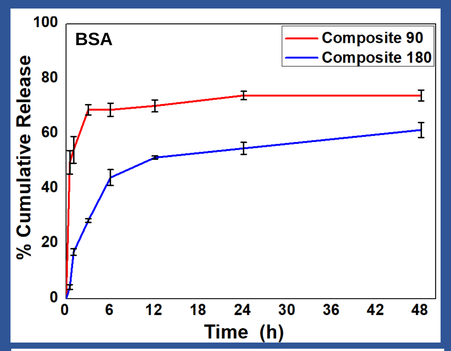

Figure 6: *In vitro* cumulative release of BSA and lysozyme from the composites

Conclusions & future work

- An approach to safely incorporate proteins into electrospun composites was developed.
- The approach aids retention of protein structure and offers control over the release of two model proteins.
- Future work will include the incorporation of cytokines and evaluation of *in vitro* biological responses.

References

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