Failure of Fiber Formation in Electrospun Polyurethane/Chitosan/Elastin Composite Membranes: The Effect of a Temperature-driven Synthesis Method of Electrospinning Solution

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Abstract

The fabrication of composite synthetic vascular grafts mimicking the native extracellular matrix is highly desirable in tissue engineering. In this study, we attempted to fabricate a small-diameter vascular graft membrane composed of Polyurethane (PU), Chitosan (Ch), and Elastin (Es) using an electrospinning technique with varying rotating collector speeds. However, scanning electron microscopy (SEM) analysis revealed a complete failure of fiber formation across all rotational speeds (0, 50, 100, and 150 rpm). We hypothesize that the elevated temperature (35°C) used during the preparation of fabric solution components increased solvent evaporation, thereby raising the viscosity of the final composite gel to a level that caused fiber breakage during the room-temperature electrospinning process.

Description

Cardiovascular diseases frequently require surgical interventions utilizing synthetic vascular grafts; however, widely used materials such as expanded polytetrafluoroethylene (ePTFE) and Polyethylene terephthalate (Dacron) often face limitations in small-diameter applications (< 6 mm) [1-3] due to complications like thrombosis, intimal hyperplasia, and compliance mismatches [4-6]. To overcome these limitations, composite grafts utilizing highly biocompatible materials—specifically Polyurethane (PU), Chitosan (Ch), and Elastin (Es) are being explored. The use of these biocompatible biomaterials provides a significant advantage in tissue engineering, as they accelerate functional tissue formation [7] and offer a three-dimensional cell-supporting scaffold that encourages cellular attachment, proliferation, and differentiation in static culture [8-10]. Additionally, they significantly enhance tissue fidelity and preserve favorable marker gene expression profiles that are essential for robust cell replication

and functional maturation under a dynamic cell culture environment [11]. Furthermore, Elastin is a particularly crucial extracellular matrix protein, comprising approximately 50% of the dehydrated weight of human arteries [12], providing necessary tissue elasticity, and heavily reducing platelet adhesion.

In our previous work, we successfully fabricated a highly porous, small-diameter PU/Ch composite electrospun membrane that demonstrated excellent morphological characteristics suitable for vascular graft applications [13]. Building upon this success, this experiment aimed to incorporate Elastin by fabricating a PU/Ch/Es membrane using a rotating collector at various speeds to promote aligned, porous, small-diameter fibers. The electrospinning solution consisted of PU, Ch, and Es at a volumetric ratio of 9.6:0.30:0.10.

Despite successful continuous fiber formation in the PU/Ch control groups, the PU/Ch/Es composite failed to produce a fibrous membrane. As shown in **Figure 1,** Scanning Electron Microscopy (SEM) imaging revealed a complete absence of elongated fibers across all tested rotational speeds (0, 50, 100, and 150 rpm). The resulting morphology appears as a dense, film-like surface characterized by irregular, fused globular formations and a total lack of interconnected porous structures.

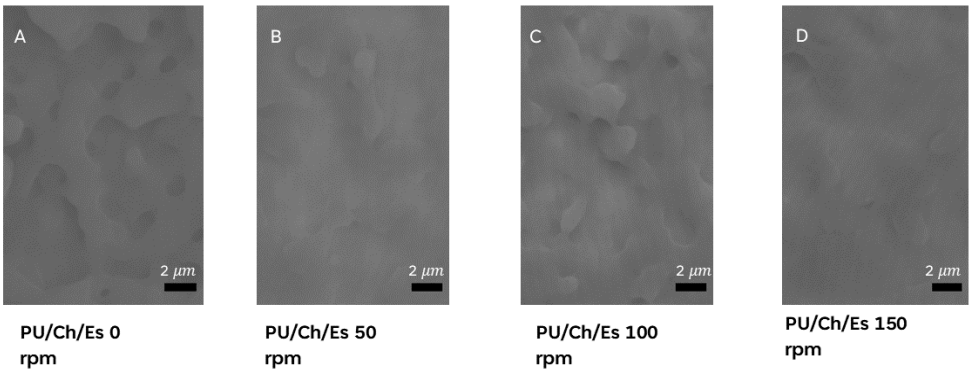

Figure 1: SEM images of PU/Ch/Es membranes at (A) 0 rpm, (B) 50 rpm, (C) 100 rpm, and (D) 150 rpm speeds.

Crucially, this structural failure was physical rather than chemical. Attenuated total reflectance-Fourier transform infrared spectroscopy (ATR-FTIR) was performed on the deposited membranes (**Figure 2**). The spectra confirmed the successful chemical incorporation of Elastin into the PU/Ch matrix, highlighted by the distinct presence of the Amide II (N–H deformation) peak at 1520 cm^{-1} in the PU/Ch/Es samples, a peak absent in the pure PU and PU/Ch controls. Furthermore, the overall lower peak intensities in the PU/Ch/Es spectra indicate a significantly thinner membrane deposition compared to the pure PU control.

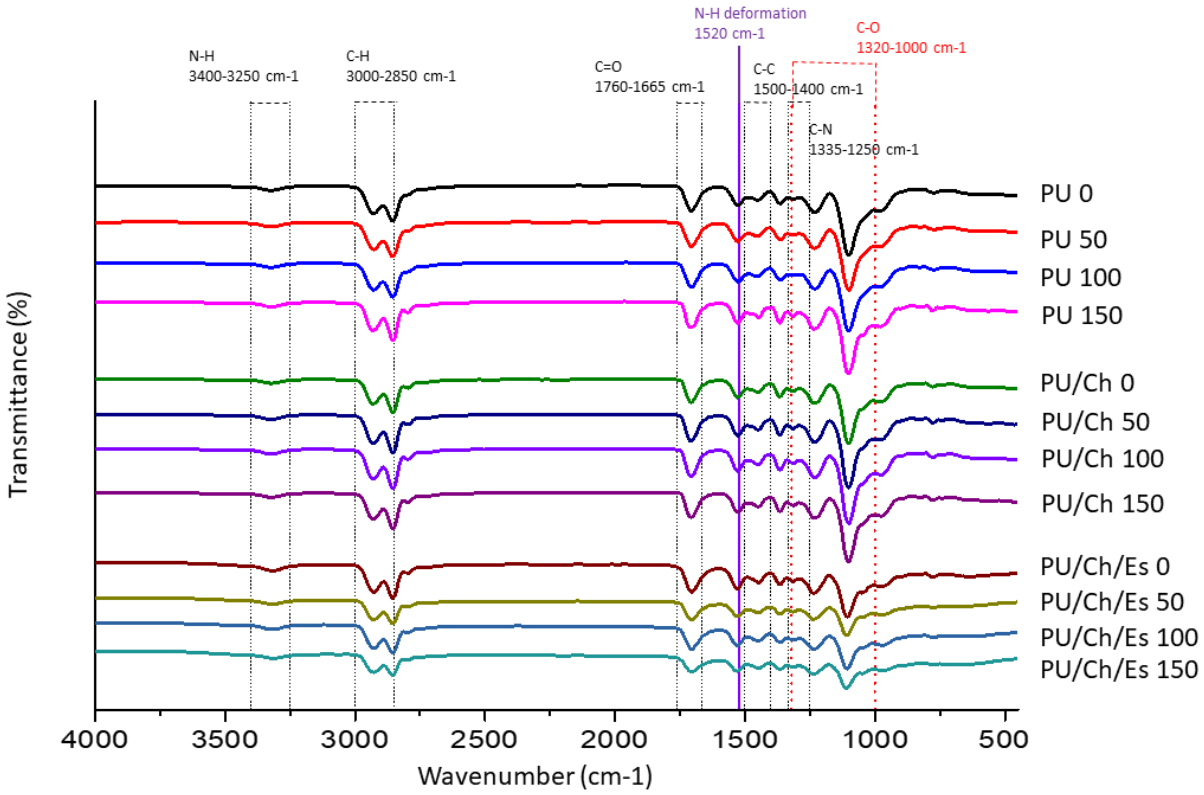

Figure 2: ATR-FTIR of PU, PU/Ch, and PU/Ch/Es membranes.

Aa FTIR analysis confirmed presence of Es and Ch in PU matrix, the cause of this morphological failure is thought to be attributed to the physical solution preparation parameters. The Es solution was stirred at 35°C to achieve a uniform 1% (w/v) concentration prior to mixing with the PU and Ch solutions. Elevated temperatures in electrospinning solutions can significantly enhance the rate of solvent evaporation. This rapid evaporation likely lowered the

solvent volume, consequently increasing the viscosity of the polymer gel prior to the electrospinning process. High viscosity can drastically restrict the drawing of the fiber, causing the jet to break and wet-deposit onto the collector, resulting in the fused, non-porous film observed in the SEM images.

Because the resulting membrane failed to form the requisite porous, fibrous nanoscale structure required to mimic the extracellular matrix and support cell proliferation, this specific PU/Ch/Es fabrication method at 35°C temperature is deemed inapt for vascular graft applications.

Materials and Methods

• **Materials:** Chitosan powders, Elastin from bovine neck ligament, N,N-dimethylformamide (DMF), acetic acid, Trizma base, and sodium tripolyphosphate were supplied by Sigma-Aldrich, Germany. Tecoflex EG-80A medical-grade thermoplastic (PU) was purchased from Lubrizol, USA.

• **Solution Preparation:** PU was dissolved in DMF at an 8% (w/v) concentration. Chitosan was dissolved in DMF at a 3% (w/v) concentration. Elastin was dissolved in a 0.2 M Triz buffer (prepared with deionized water) and stirred at 35°C for 24 hours to achieve a 1% (w/v) solution.

• **Electrospinning Mixture:** The final electrospinning solution was mixed at a ratio of 9.6 mL PU, 0.3 mL Chitosan, and 0.1 mL Elastin.

• **Electrospinning Parameters:** The process utilized an applied voltage of 12 kV, a flow rate of 0.5 mL/hour, and a tip-to-collector distance of 15 cm. A 10 mL syringe connected to a 21-G stainless steel needle was mounted to a syringe pump (SP20, NFiber, USA).

• **Collection:** A rotating mandrel wrapped in aluminum foil was used as the collector, with rotational speeds set to 0, 50, 100, and 150 rpm.

• **Characterization:** The morphology of the resulting deposition was observed using a Scanning Electron Microscope (Hitachi, TM300, Japan) after being sputter-coated with platinum for 1 minute. Chemical composition was analyzed using an ATR-FTIR spectrometer (Frontier spectrometer, PerkinElmer, UK), and image processing was conducted using ImageJ software (1.35a, Rasban.W, USA).

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