Optimization of genipin crosslinking in electrospun blood protein scaffolds for autologous vascular implantation

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1 Motivation

Electrospun vascular grafts derived from patient blood proteins enable personalized, immunocompatible implants.

Crosslinking necessary due to solubility in water. Optimization of ethanol–water solvent composition to balance structural stability and protein preservation (avoid full denaturation).

Objective: develop a genipin-based crosslinking strategy for electrospun plasma protein scaffolds (porcine model for preclinical autologous processing).

2 Materials and Methods

Porcine plasma purified via diafiltration, concentrated using rotary evaporation, blended with polyethylene oxide (PEO) to enable electrospinning without cytotoxic solvents.

Nonwoven fibers fabricated using a vertical electrospinning setup. Crosslinking performed with 5% w/w genipin in ethanol–water mixtures (30–96 vol%).

Morphology analyzed by scanning electron microscopy (SEM).Protein stability and secondary structure assessed using differential scanning calorimetry (DSC) and Fourier-transform infrared spectroscopy (FTIR).

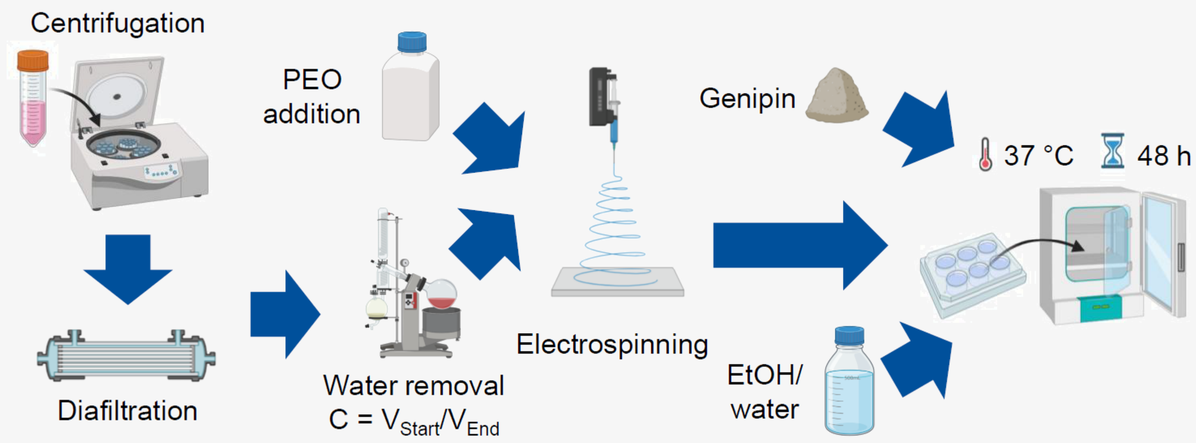
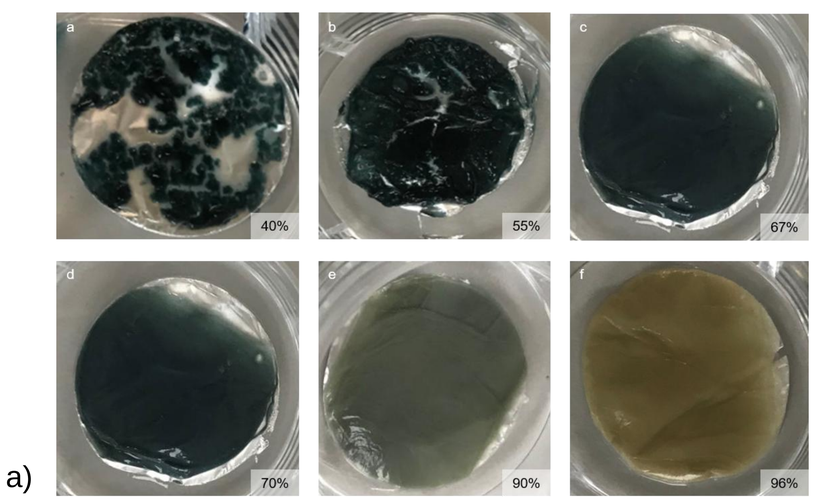
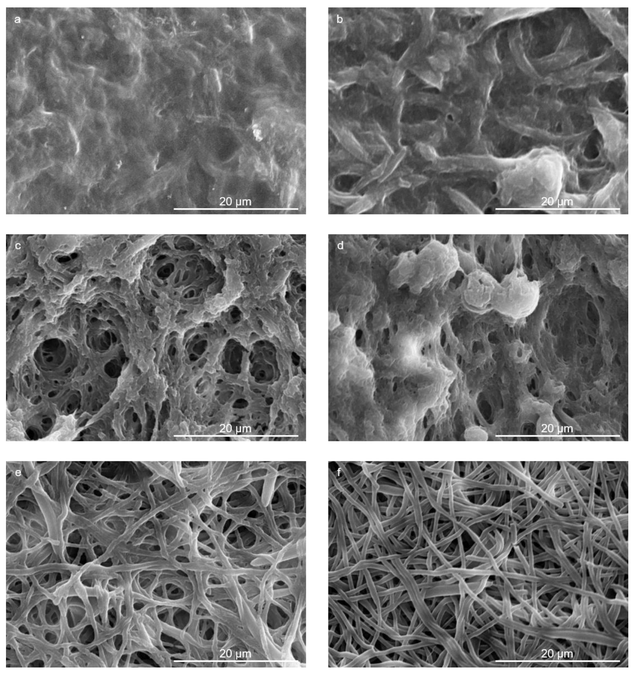
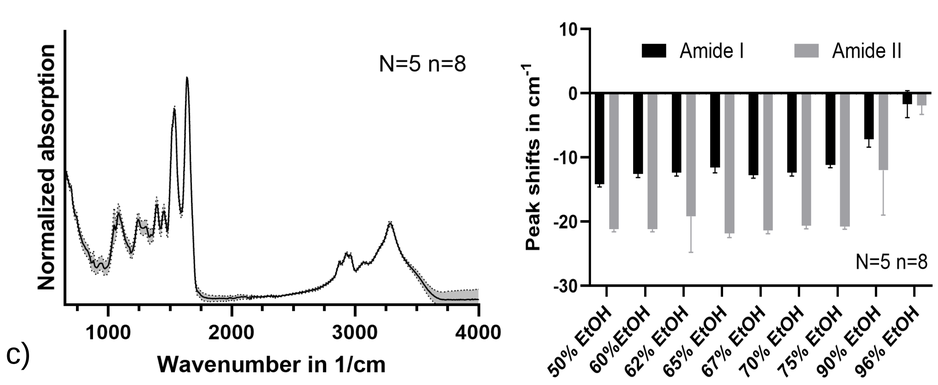


Fig. 1: Work flow to create electrospun fiber mats from porcine blood plasma proteins including separation of blood components, diafiltration of plasma and water removal followed by electrospinning and crosslinking with genipin.

3 Results







a)

b)

c)

Fig. 2: (a) Scaffolds after the crosslinking process in different crosslinking solutions. The scaffolds a-c were crosslinked with genipin in a 40 %, 55 % and 67 % (v/v) ethanol solution. Blue discoloration was more pronounced at lower ethanol concentrations; the two samples 90 % and 96 % (v/v) showed a yellow tinge. All samples appeared homogeneous and intact with no signs of dissolution. (b) SEM-images of crosslinked scaffolds for volumetric ethanol concentrations of 40 % (a), 50 % (b), 55 % (c), 62 % (d), 70 % (e) and 96 % (f). The magnification was 2500. (c) Left: Normalized FTIR absorption spectra for non-crosslinked fiber nonwovens. Mean values with standard deviations are shown. Right: Peak shifts of the Amide I (1600-1650 cm−1) and Amide II (1500-1550 cm−1) bands for crosslinked fiber nonwovens compared to the non-crosslinked control group.

Optimal crosslinking window identified at 70–90 vol% ethanol; stable fibrous morphology retained:

Low ethanol (<55 vol%): scaffold dissolution prior to effective crosslinking.

High ethanol (>90 vol%): protein aggregation and reduced genipin reactivity due to limited amino group accessibility.

FTIR: reduced amide band shifts indicate minimal denaturation and preserved secondary protein structure.

DSC: thermal stability in 70–90 vol% range comparable to native plasma fibers, confirming balanced crosslinking and structural preservation.

4 Conclusions / Outlook

Reproducible fabrication of protein-based vascular scaffolds from whole blood plasma demonstrated; preservation of native biochemical properties and structural porosity.

Platform enables future autologous graft production using human plasma for patient-specific applications.

Next steps: evaluation of mechanical performance, endothelialization under physiological conditions and immunoresponse.

Goal: advance toward clinically translatable, personalized vascular implants.

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