Harnessing Hydrolized Silk Fibroin in Electrospun Nanofibers for Biomedical Applications

Marija B. Branković[1,*], Jana D. Baščarević[1], Marko N. Živanović[1], Nenad D. Filipović[2,3]

[1] Institute for Information Technologies Kragujevac, University of Kragujevac, Jovana Cvijića bb, 34000, Kragujevac, Serbia; e-mail: marija.brankovic@uni.kg.ac.rs, jana.bascarevic@uni.kg.ac.rs, marko.zivanovic@uni.kg.ac.rs

[2] Faculty of Engineering, University of Kragujevac, Sestre Janjić 6, 34000 Kragujevac, Serbia; e-mail: fica@kg.ac.rs

[3] Bioengineering Research and Development Center, Prvoslava Stojanovića 6, 34000 Kragujevac, Serbia; e-mail: fica@kg.ac.rs

* *Corresponding author*

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Abstract: This study focuses on the development of bioactive nanofibers by incorporating silk protein hydrolysate into polymer solutions composed of both synthetic and natural polymers. Electrospinning was used to fabricate fibers from solutions made of polyvinyl alcohol (PVA), polycaprolactone (PCL), and chitosan, each prepared with appropriate solvents to enhance process stability and fiber quality. The silk protein hydrolysate was incorporated to improve the biological properties of the resulting nanofibers, targeting potential biomedical applications such as wound healing and tissue engineering. Various challenges during electrospinning, including jet instability and phase separation, were addressed by adjusting solution compositions and processing parameters. The resulting nanofibers were uniform and free of bead defects, demonstrating the successful integration of silk protein with the polymers. However, the combination of PVA and PCL with silk protein hydrolysate presents both advantages and limitations, influencing the properties and performance of the electrospun nanofibers. Future work will involve comprehensive physicochemical and biological evaluations to fully explore the fibers' functionality and their potential for biomedical applications.

Keywords: hydrolized silk fibroin, polymers, electrospinning, nanofibers, bioengineering

1. Introduction

Electrospinning has emerged as continuous, cost-effective and relatively simple technique for producing ultrafine nanofibers [1]. These nanofibers exhibit a high surface-to-volume ratio, adjustable porosity with uniformly distributed pores, and physicochemical functionalization possibility, making them suitable for many applications [2]. Electrospinning utilizes a high-voltage electrohydrodynamic process to fabricate fibers from polymer-based liquids, such as solutions, melts, or emulsions, achieving diameters from nanometers up to several micrometers [3]. A basic

electrospinning setup consists of a high-voltage power supply, a syringe pump, a metallic nozzle and a collector [4].

Natural polymers offer superior biocompatibility and lower immunogenicity compared to synthetic materials, but their application in electrospinning can be hindered by issues such as partial denaturation and poor processability. The main advantage of synthetic polymers lies in their tunability, allowing precise control over properties like viscoelasticity, mechanical strength and degradation kinetics. However, some of them may exhibit limited biocompatibility and prolonged biodegradability [5].

Silk is a natural protein-based biopolymer, primarily derived from silkworms and spiders, and composed from two main proteins, fibroin and sericin [6]. Silk fibroin provides high tensile strength and mechanical stability to the silk fibers, while sericin contributes to their adhesive properties [7].

In this study, fibers were fabricated from both synthetic and natural polymers, incorporating silk protein hydrolysate.

2. Methodlogy

2.1 Materials

Hydrolyzed silk protein was obtained from Avena Lab, Farmadria d.o.o. The polymers used for the preparation of solutions included polyvinyl alcohol (PVA, Mw 89,000-98,000, ≥99% hydrolyzed), polycaprolactone (PCL, Mw 80,000), and chitosan (medium molecular weight), all purchased from Sigma-Aldrich. The following solvents were used: 80% acetic acid (Carl Roth), glacial acetic acid (Fisher Chemical), formic acid (≥98%, Honeywell), chloroform (VWR International), and ethanol (Fisher Bioreagents). Surfactant Tween 20 was also procured from Carl Roth.

2.2 Solution preparation

Four different polymer solutions were prepared for electrospinning.

In the first solution, 15% PVA (in dH_2O) and silk protein hydrolysate were mixed in an 80:20 volume ratio. PVA was dissolved at 80 °C for 2 hours, stirred for 1 hour, and then hydrolysate was added, stirring for another hour (the solution turned light yellow). To enhance electrospinnability, ~100 μL of Tween 20 was added dropwise with continuous stirring.

The second solution contained 15% PVA (in dH_2O) and 1% chitosan (in 80% acetic acid), prepared separately. PVA was heated at 80 °C for 2 hours and stirred for 1 hour; chitosan was stirred at room temperature for 2 hours. The solutions were mixed in an 80:20 ratio and stirred for 1 to 2 hours. Following this, 200 μL of silk hydrolysate was added and briefly stirred before electrospinning.

The third solution contained 10% PCL dissolved in a 70:30 chloroform-ethanol (96%) mixture, with a small amount of silk protein. The PCL was left overnight to dissolve, forming a whitish solution. After stirring for one hour to obtain a clear solution, 200 μL of hydrolysate was added dropwise, followed by an additional hour of stirring.

The final formulation consisted of 20% PCL dissolved in a 50:50 mixture of glacial and formic acids, supplemented with 200 μL of silk protein. The procedure mirrored the

previous one, with a shortened stirring period after silk addition to minimize protein denaturation.

2.3 Electrospinning process

Electrospinning was performed using a vertical setup in ambient conditions. Three milliliters of polymer solution were loaded into a plastic syringe attached to an injection pump. The high-voltage power supply connected its positive electrode to a 21-gauge metal needle and the negative electrode to a collector plate covered with aluminum foil. The distance between the needle tip and the collector was set to 11 cm, with flow rate and voltage adjusted individually for each solution.

3. Results and Discussion

The electrospinning process effectively produced continuous fibrous mats from all four prepared solutions. Optimized parameters were carefully determined and applied for each solution. Although morphological and chemical analyses were not conducted at this stage, visual results indicate a stable process and successful fiber formation.

3.1 Challenges in production of electrospun nanofibers

During parameter optimization, several challenges were encountered before achieving stable electrospinning and uniform, bead-free fibers. Water-based solutions (PVA-based) experienced jet instability and droplet formation due to low viscosity, which was resolved by increasing the polymer concentration. Also, the PVA/silk solution required a surfactant to improve conductivity. PCL-based solutions, particularly a chloroform-ethanol mixture, exhibited phase separation after the addition of silk hydrolysate, resulting in jet breakage. Prolonged stirring and adjusting solvent/additive ratios improved miscibility. Reducing the flow rate and increasing the voltage further stabilized the jet. A short tip-to-collector distance also caused incomplete fibers due to insufficient solvent evaporation. Increasing the distance resolved this, resulting in continuous, uniform fibers. Careful adjustment of all parameters was essential for reliable fiber formation.

4. Conclusions

The fabricated nanofibers exhibited consistent uniformity and absence of defects, indicating a successful integration of hydrolized silk protein within the polymer matrix. The combination of PVA and PCL with silk hydrolysate offers both advantages and limitations. PVA is hydrophilic, soluble in water, and compatible with aqueous silk solutions; however, it typically needs surfactants and post-processing to ensure stability. In contrast, PCL demonstrates excellent mechanical strength and long-term stability without crosslinking. However, it requires organic solvents, which may lead to phase separation with silk hydrolysate and present safety risks. Acid-based solvents improve conductivity but may denature proteins. Overall, PVA's rapid degradation makes it ideal for short-term applications such as wound dressings, whereas PCL's durability suits it better for long-term scaffold use. Further physicochemical and biological evaluation is necessary to fully understand and optimize the properties and performance of these composite fibers for biomedical applications.

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