Transactions on Additive Manufacturing Meets Medicine
Trans. AMMM, 2024, Vol. 6, No 1, Article ID 1848, 4 Pages

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Original Research Article

Investigation of cellulose acetate nanofibers using a DMSO based solvent system

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Electrospinning is a highly adaptable process for producing nanofibers with a wide variety of applications. The resulting fibers are recognized for their biocompatibility, exceptional surface-to-volume ratios, porosity, and adjustable composition properties, making them promising scaffolds for tissue engineering. Previous research already demonstrated successful electrospinning of cellulose acetate. However, these studies used N,N-dimethylformamide (DMF) as a solvent, a substance linked to carcinogenic and mutagenic effects. Therefore, a less toxic solvent system consisting of acetone and dimethylsulfoxide (DMSO) was used in this study. The investigation focuses on the electrospinning process using cellulose acetate (CA). Initially, the appropriate solvent system substituting acetone/DMF was identified by comparing rheological measurements of different acetone/DMSO compositions with a 3:1 acetone/DMF solvent system described in the literature at constant polymer concentration. With the appropriate acetone/DMSO composition found, various CA solutions with polymer concentration ranging from 12% to 16% (w/v) were investigated. The surface tension was measured via a tensiometer and the morphology and diameter distribution of the electrospun fibers were analyzed using scanning electron microscopy (SEM). Furthermore, the wettability of the electrospun meshes were determined through contact angle measurements. Defect-free fibers are achieved at all polymer concentrations exhibiting uniform morphology and diameters in the nanometer range, indicating the successful fabrication of CA nanofibers. The contact angle measurements showed hydrophilic behavior on all investigated meshes. This study provides valuable insights into the fabrication of CA electrospun fibers which hold promise for various applications, including biomedical scaffolds, scaffolds for cultured meat, filtration membranes and food packaging materials.

I. Introduction

Tissue engineering is a multidisciplinary field that focuses on restoring, maintaining, and enhancing tissue functionality through the development of biological substitutes. Central to this field is the use of porous 3D scaffolds, which act as physical substrates facilitating cell attachment, proliferation, and differentiation. These scaffolds emulate the extracellular matrix (ECM)—the body's natural framework that cells attach to and communicate with—providing the necessary mechanical support for cellular structure and growth while permitting nutrient and waste exchange [1].

Cellulose acetate is an affordable, renewable material noted for its biocompatibility, biodegradability, and non-toxicity, thereby rendering it highly applicable to tissue engineering [2]. Studies have explored its use in various applications, including wound dressings, dermal substitutes, and engineered tissues [3].

One technique for fabricating cellulose acetate scaffold structures in tissue engineering is electrospinning. This process involves the electrostatic extrusion of polymer solutions through a fine nozzle leading to ultrafine fiber meshes with interconnected pores. Their high surface-to-volume ratios, significant porosity, and tunable composition have garnered considerable interest for use as biocompatible scaffolds [4]. Previously, cellulose acetate was successfully electrospun using an acetone/dimethyl-formamide (DMF) solvent system [5]. However, DMF is subject to restrictions by the European Chemicals Agency (ECHA), being listed in the "Candidate List of Substances of Very High Concern for Authorization" due to its potential hazards [6].

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In this study, the iterative adjustment of the solvent system for electrospinning cellulose acetate fibers through varying concentrations of dimethylsulfoxide (DMSO) was explored. The resultant nanofiber properties were characterized using morphological analysis and wettability assessment.

II. Material and methods

II.I. Polymer solution

Cellulose acetate (CA) powder (Mn ~30.000 g/mol) was obtained from Sigma-Aldrich (St. Louis, USA). Dimethylsulfoxide (DMSO ≥ 99.5%) and Acetone (C_3H_6O ≥ 99.7%) were obtained from Carl Roth (Karlsruhe, Germany) as solvents for the polymer solution.

II.II. Preparation of nanofibers

Cellulose acetate was dissolved in acetone/DMSO (77.5/22.5%) (v/v) to obtain a polymer solution with 12% (w/v), 14% (w/v) and 16% (w/v). The polymer solution was fed into a 5 ml syringe (B. Braun SE, Melsungen, Germany) attached to a 0.8 mm steel nozzle. The electrospinner device used was a NS1 NanoSpinner (Inovenso Ltd., Istanbul, Turkey) using a single-nozzle electrospinning setup with a static collector. The electrospinning process utilized an electric voltage of 16 kV, 10 cm working distance and a flow rate of 0.9 ml/h at room temperature.

II.III. Characterization of spinning solution and nanofibers

The rheological properties of the polymer solutions were characterized using an MCR 302e rheometer (Anton Paar GmbH, Graz, Austria) equipped with a plate-plate geometry of 50 mm. Measurements were obtained in the software Anton Paar RheoCompass (Anton Paar GmbH, Graz, Austria) with a shear rate from 0.1 to 1000 s-1 and with three measurements per polymer solution. The acquired measurements were subsequently averaged.

Surface tension measurements were conducted using a Lauda TD 1C tensiometer (Lauda Dr. R. Wobser GmbH & Co. KG, Lauda-Königshofen, Germany) via the plate method with three measurements per solution.

The nanofiber morphology was examined using a SUPRA 25 scanning electron microscope (SEM) (Carl Zeiss AG, Oberkochen, Germany) equipped with an SE2 detector (Everhart-Thornley) and operating at an electron high tension (EHT) of 3.00 kV. To ensure conductivity, the electrospun meshes were coated with a thin layer of gold. Diameter measurements were performed using the ImageJ plugin DiameterJ [7].

The wettability of the electrospun meshes was assessed through contact angle (CA) measurements using a video-based optical contact angle measuring system, OCA 40 Micro (DataPhysics Instruments GmbH, Filderstadt, Germany). A 5 µl drop of distilled water was placed on each mesh, and the contact angle was subsequently measured. The experiments were conducted at room temperature, repeated six times, and the results were averaged.

III. Results and discussion

III.I. Solvent systems and solution properties

To achieve the objective of substituting DMF with DMSO, the viscosity of the solvent was first examined to minimize impact on the spinning process. Given that DMSO possesses a significantly higher viscosity (2.14 mPa·s) compared to DMF (0.85 mPa·s), the DMSO content was reduced compared to the DMF content described in literature [5]. Figure 1 illustrates the average viscosity of polymer solutions with various solvent compositions, exluding standard deviations for better visibility, which ranged from 0.53 to 26.75 mPa·s. The DMSO content in the acetone/DMSO mixture was gradually increased from 15% to 30% (v/v). Additionally, the viscosity of an acetone/DMF (3:1) mixture, as successfully utilized by dos Santos et al. [5], was examined. 12% (w/v) cellulose acetate (CA) was added to all solvent systems.

The recorded flow curves indicate a significant influence of DMSO content on the viscosity. The solution with the lowest DMSO content of 15% (v/v) exhibited the lowest viscosity. With rising DMSO content a steady increase in viscosity is observed, up to a DMSO content of 25% (v/v). The flow curve for a DMSO content of 30% (v/v) shows an unexpectedly higher profile. While the viscosities of the solutions with 15% (v/v) and 25% (v/v) DMSO only show an increase of around 20-30 mPa·s, the increase from 25% to 30% (v/v) DMSO is in the range of 80-90 mPa·s. Furthermore, the investigation demonstrates that the flow curve for 22.5% (v/v) DMSO shows the greatest congruence with the acetone/DMF (3:1) system.

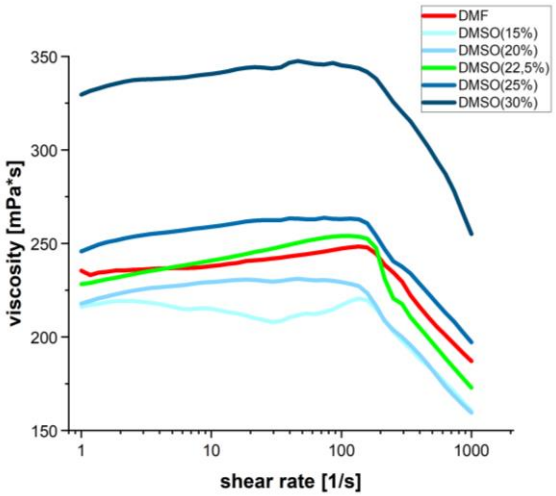

Figure 1: Viscosity measurements of different solvent compositions with 12% (w/v) of cellulose acetate.

Preliminary experiments investigated the influence of solvent composition on process and Taylor cone stability. At very low percentage with 5-10% (v/v) of DMSO a rapid

drying of the Taylor cone occurred after a few minutes, leading to clogging at the tip of the nozzle. Extrusion of material halted initially until the dried surface was ruptured by the continuing flow of material which allowed a temporary resumption of the electrospinning process.

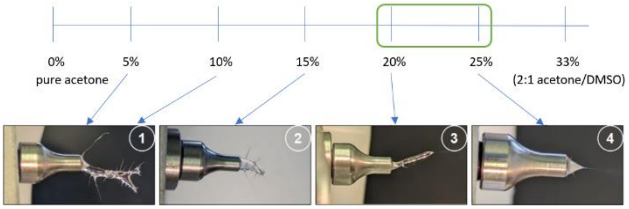

Figure 2: Taylor cone behavior for selected acetone/DMSO compositions.

However, the newly formed Taylor cone rapidly dried out as well. This cycle of drying and breakthrough resulted in the formation of a large, heavily branched polymer structure at the tip of the nozzle, as illustrated in Figure 2-1. This issue is most likely caused by the fact that acetone is fast evaporating with an evaporation rate of 2 relative to diethylether. With a DMSO content of 15% (v/v), polymer formation at the tip of the nozzle still occurred, but this process was significantly delayed and the resulting polymer structure was noticeably smaller (Figure 2-2). At a DMSO concentration of 20% (v/v), the disruption was reduced to such an extent that the process was stable for a duration of 20 minutes. During this period, only a small amount of polymer formed on the nozzle, which did not visibly impair the extrusion of the material (Figure 2-3). With a DMSO content of 25% (v/v) in the spinning solution, disruptions such as drying of the Taylor cone were not observed (Figure 2-4). Instead, a stable Taylor cone with continuous material extrusion was seen throughout the experiment. However, the low acetone content prevented the full evaporation of the solvent resulting in a moist deposit on the collector.

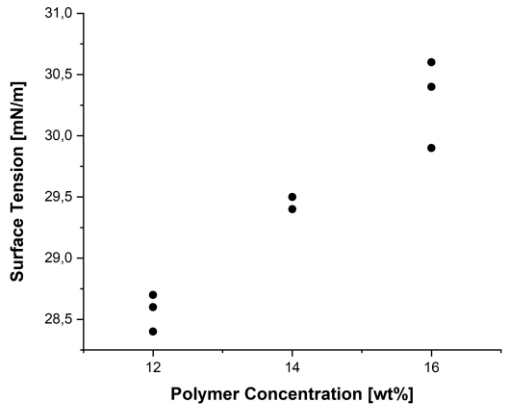

Figure 4: surface tension of CA increases with polymer concentration.

The optimization of the DMSO content led to a concentration of 22.5% (v/v), at which a good balance between the formation of a stable Taylor cone and the drying properties of the solution was established. With this specific composition, continuous spinning without clogging or moist fiber depositions could be observed.

Surface tension measurements were also conducted for different polymer concentrations. Figure 4 illustrates that with higher polymer concentration the surface tension also increases. This is also observed in other research with regard to electrospinning cellulose acetate [8].

III.II. Characterization of electrospun scaffolds

Nanofiber scaffolds were successfully obtained by electrospinning. The SEM images of CA meshes show bead-free, smooth and flexible interconnected porous mats. At a concentration of 12% (w/v), the average fiber diameter is 272 nm ± 94 nm. By increasing the polymer concentration to 14% (w/v), an increase in the fiber

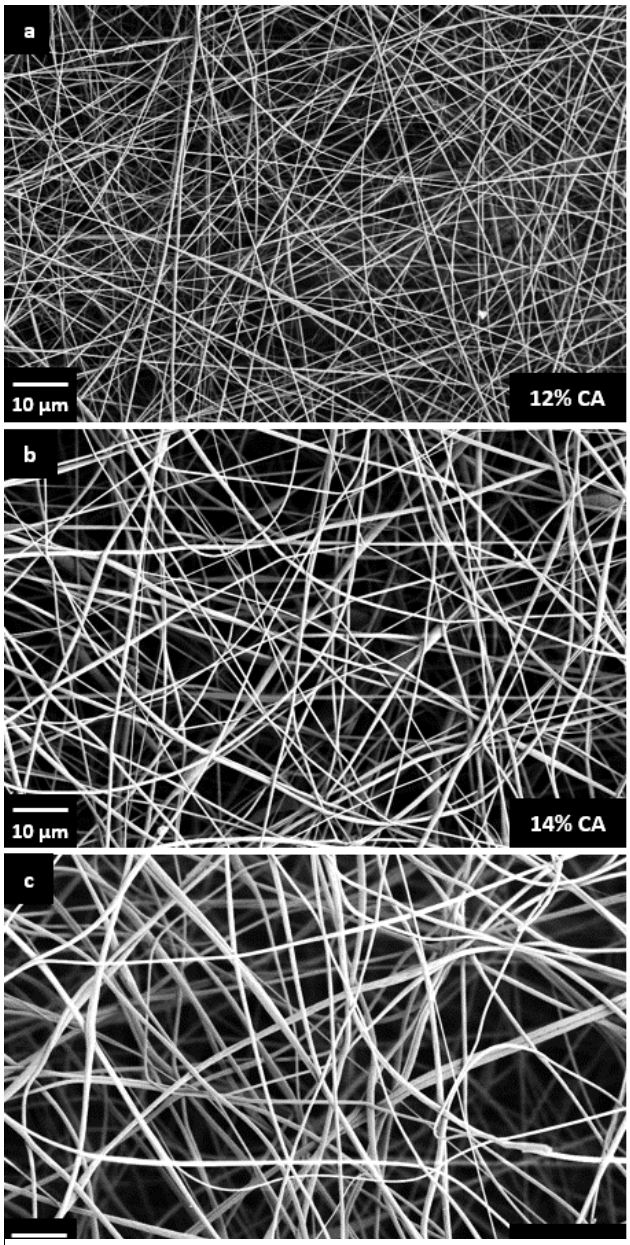

Figure 3: SEM images of cellulose acetate mesh with a polymer content of 12% (a), 14% (b) and 16% (c) (w/v).

diameter to an average of 317 nm ± 123 nm was observed. Finally, at 16% (w/v) CA content the diameter was 398 ± 158 nm (Figure 3).

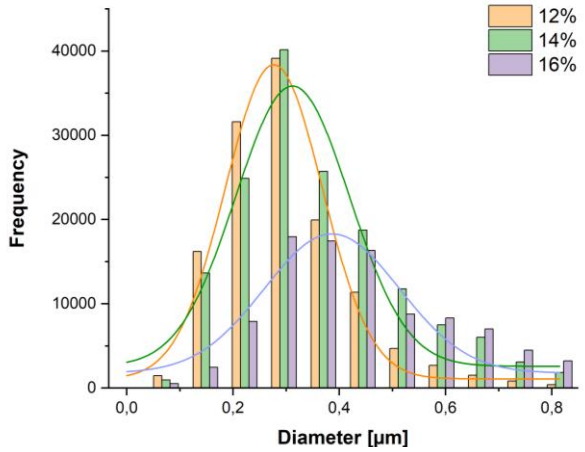

Figure 5: Histogram of CA fiber diameter for 12, 14 and 16% (w/v) polymer concentration.

Furthermore, the distribution of fiber diameters gets wider with higher polymer concentration. As seen in Figure 5, the standard deviation increases from 94 nm for 12% (w/v), to 123 nm for 14% (w/v) and finally 158 nm for 16% (w/v). This can be explained by the increase in surface tension which had already been observed. An increase in surface tension can prevent the charged jet from splitting since the repulsive forces among the charges must be stronger than the surface tension for the jet to split. As a result, as the concentration of the solution rises, the average diameter of the fibers significantly increases and the diameter distribution widens [9, 10].

The results of the contact angle measurements can be seen in Figure 6. The electrospun meshes with a polymer concentration of 12% (w/v) exhibit the highest average contact angle with 63.07±14.39°. Increasing the concentration to 14% (w/v) results in a smaller average contact angle of 59.04°±10.52°. Finally, increasing the polymer concentration to 16% (w/v) reduces the contact angle to 52.67°±10°.

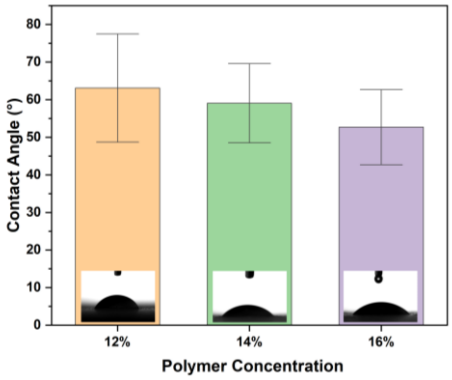

Figure 6: Contact angle measurements of 12, 14 and 16% (w/v) CA.

IV. Conclusions

In this study, DMF was successfully replaced by DMSO as part of a solvent system for electrospinning cellulose acetate. Different polymer concentrations and solvent systems were investigated by rheological and morphological examinations. Successful electrospinning was achievable across all examined concentrations. Bead-free, uniform fibers could be produced at all concentrations. Contact angle measurements were successfully conducted on the electrospun meshes and showed hydrophilic behavior on all investigated polymer concentrations.

This study demonstrated that N,N-dimethylformamide (DMF) can be successfully substituted with dimethylsulfoxide (DMSO) while achieving a continuous electrospinning process without clogging and producing bead-free fibers. The fabrication of electrospun meshes from biopolymers such as cellulose acetate exhibits significant potential for biomedical applications.

Acknowledgments

Research funding: We would like to thank the Federal Ministry of Education and Research (BMBF) for the funding, reference: 031B1295B. Conflict of interest: Authors state no conflict of interest.

Author's statement

Conflict of interest: Authors state no conflict of interest. Informed consent: Informed consent has been obtained from all individuals included in this study. Ethical approval: The conducted research is not related to either human or animals use.

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