Research article

Agrimonia Eupatoria L.-Incorporated Electrospun Nanofibers and Cotton Composite for Antibacterial Wound Dressing Applications

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Abstract.
Composite wound dressings which combine the suitable properties of distinct materials into one dressing are currently being explored, in combination with different types of bioactive compounds, to enhance the healing process and avoid skin infections. In the present work, poly(vinyl alcohol) and chitosan nanofibers containing *Agrimonia eupatoria* L. were fabricated using a needleless electrospinning method (through Nanospider technology) and deposited on top of a 2,2,6,6-tetramethylpiperidine-1-oxyl (TEMPO)-oxidized cotton textile dressing. The negatively charged carboxyl groups on the cotton fibers interacted with the positively charged amino groups of chitosan, which was previously blended with poly(vinyl alcohol) and *Agrimonia eupatoria* L. to produce the nanofiber layer. The properties of the produced composite materials were analyzed to determine the dressing's potential for antimicrobial wound dressing applications.

Keywords: cotton, textile dressing, electrospun nanofibers, *Agrimonia eupatoria* L., antibacterial composite wound dressings

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1. Introduction

Nowadays, a wide a range of wound dressings has been fabricated using different materials and physical forms from several techniques in order to cover the wound site, provide protection against external agents, and efficiently support the healing process [1,2]. However, wound dressings from natural textile materials, like cotton, remain one of the most used due to their low cost, limited side effects, easy handling and fabrication, as well as a suitable mechanical support and robust protection against external threats [3]. Furthermore, cotton textile materials are non-toxic, haemostatic, non-allergic, biocompatible, biodegradable, and easily tailored to the wound [3]. Nevertheless, cotton wound dressings can lead to maceration of the wound and cause trauma and pain when

removed, delaying the healing process and making skin more susceptible to infection [3,4]. To overcome such limitations and improve their application as wound dressings, different surface modifications have been reported. Among the various techniques, the chemical modification of cotton using 2,2,6,6-tetramethylpiperidine-1-oxyl (TEMPO) has received considerable attention due to its ability to increase the negative charge density onto the cotton surface [5,6]. This enables the cotton fibers to be functionalized with other materials, namely with electrospun nanofibers which exhibit structural features similar to those presented by the native skin's extracellular matrix (ECM). Moreover, electrospun nanofibers present a high surface area to volume ratio and high porosity which help to maintain an appropriate wound environment for healing, and allow the delivery of the bioactive compounds that are effective in wound healing, improving their therapeutic performance [4,7]. As such, composite wound dressings that combine the benefits of different materials have been highlighted as a promising alternative [1,3].

Concerning that, in the present work, Poly(vinyl alcohol) (PVA) and Chitosan (CS) nanofibers containing *Agrimonia eupatoria* L. (AG), a medicinal plant widely known for its traditional bioactive properties, non-toxicity, limited side effects, and low price acquisition, were electrospun using a needleless electrospinning method onto a cotton dressing to both protect the wound from infections and enhance the healing process.

2. Material and methods

Preparation of Crude AG Extract. The AG extraction was carried out by maceration using ethanol solvent in a ratio of 1:20 (w/v).

Production of the TEMPO-oxidized cotton-electrospun nanofibers composite materials. Firstly, the surface of cotton dressing was oxidized using the 0.0125% (w/v) TEMPO/0.125% (w/v) NaBr/ 3.2% (v/v) NaClO system at pH 10.5, one of the most common surface chemical modification methods of cotton fibers, which directly oxidizes the primary alcohol of cellulose to negatively-charged carboxyl groups, increasing the cotton negative surface charge density [5,6].

In turn, PVA and CS solutions were prepared separately by dissolving 10% (w/v) PVA powder in distilled water and 2% (w/v) CS in 0.1 M glacial acetic acid, respectively. Subsequently, the pH of CS solution was adjusted to 5 using 0.1 M HCl, producing a positive charge, and then added to the PVA solution in a ratio of 30:70. Lastly, AG (5.0 wt.%) was added to the PVA_CS blend before the electrospinning process. The PVA_CS and PVA_AG_CS blends were electrospun on top of the previously TEMPO-activated cotton using the Nanospider needleless system (Nanospider laboratory machine NS

LAB 500S from Elmarco S.R.O., Liberec, Czech Republic) by applying a voltage of 75 kV, an electrode rotation of 55 Hz, and a working distance of 13 cm, at room temperature.

Evaluation of the properties of the produced TEMPO-oxidized cotton-electrospun nanofibers composite materials.

Characterization of the composite materials' morphology. The produced TEMPO-oxidized cotton-electrospun nanofibers composite materials and the electrospun PVA_CS and PVA_AG_CS nanofibers were analyzed by Scanning electron microscopy (SEM) (SEM, S-3400N, Hitachi, Tokyo, Japan) at an accelerating voltage of 20 kV to characterize their morphology and the electrospun fibers' diameter were examined using an image analysis software (Image J, National Institutes of Health, Bethesda, MD, USA).

Determination of the composite materials' porosity. The porosity of each material of the produced TEMPO-oxidized cotton-electrospun nanofibers composites was measured by the liquid displacement method using ethanol as displacement liquid, according to the method described in [7].

Evaluation of the composite materials' wettability. The wettability of each material of the produced TEMPO-oxidized cotton-electrospun nanofibers composites was evaluated by determining the water contact angle (WCA) at their surfaces using the Dataphysics Contact Angle System OCAH-200, operating in static mode, at room temperature.

Evaluation of the composite materials' swelling capacity. The sweeling capacity of the dried TEMPO-oxidized cotton-electrospun nanofibers composite materials (W_0) was determined by immersing them in PBS (pH = 5.5) at 37°C and removed from the media at predetermined time points. Excess water on the surface was removed with a filter paper, and the swollen samples weighed (W_t) immediately. The swelling ratio was calculated according to the equation described in [7].

Evaluation of the composite materials' antibacterial activity. The TEMPO-oxidized cotton-electrospun nanofibers composite materials' antibacterial performance was studied through the Standard Test Method for Determining the Activity of Incorporated Antimicrobial Agent(s) in Polymeric or Hydrophobic Materials (ASTM E2180-07 standard) by using *Staphylococcus aureus* (*S. aureus*) (gram-positive bacterium) and *Pseudomonas aeruginosa* (*P. aeruginosa*) (gram-negative bacterium) as models. A filter paper control (pore size of 0.22 µm) was used as control and the log (CFU/mL) was calculated for each material.

Evaluation of the composite materials' cytotoxicity. The cytotoxicity of the produced TEMPO-oxidized cotton-electrospun nanofibers composite materials was analyzed

through an MTT assay, according to the ISO 10993-5:2009 guidelines (Biological evaluation of medical devices—Part 5: Tests for in vitro cytotoxicity). To accomplish that, the produced materials were sterilized by UV irradiation (254 nm, ≈ 7 mW cm^{-2}) for 1 hour, and then placed into 24-well plates in direct contact with normal human dermal fibroblasts (NHDF) cells during 1, 3, and 7 days.

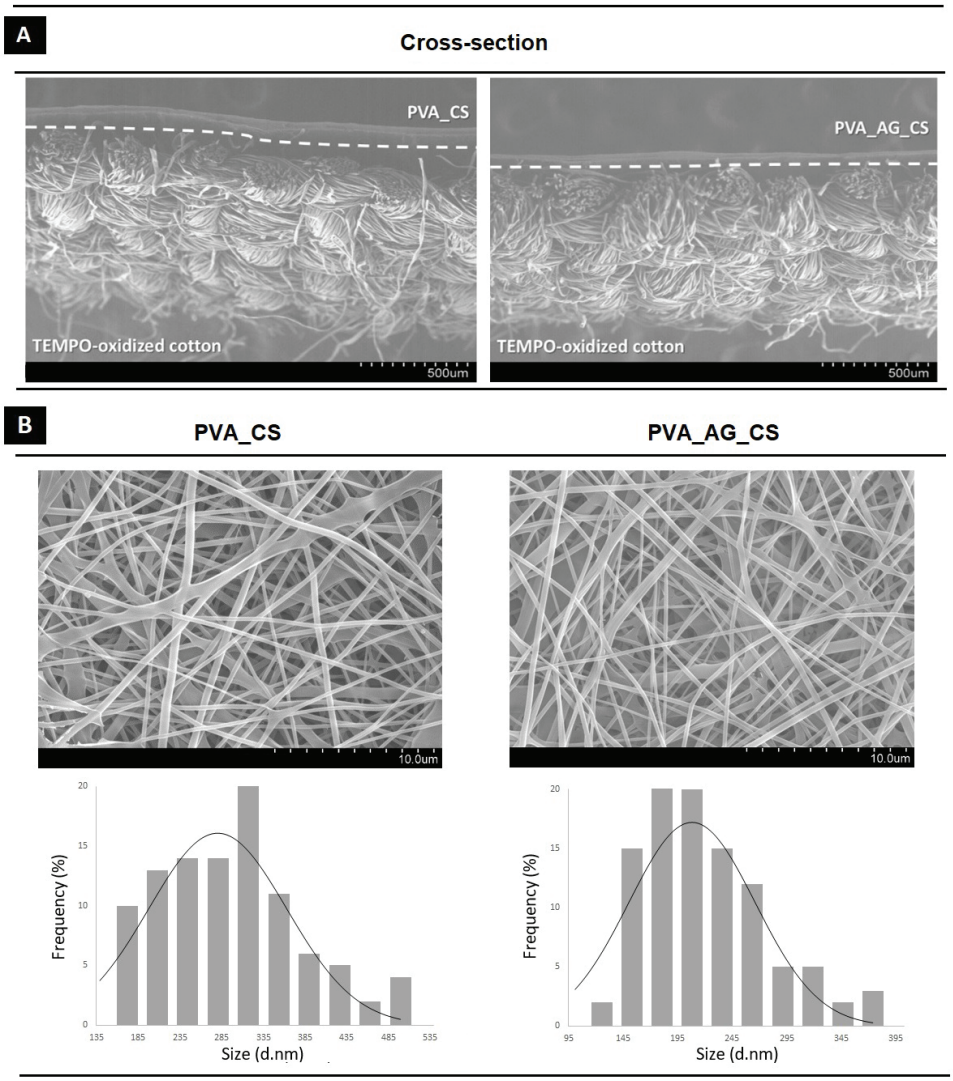

Figure 1: Morphological characterization of the produced 2,2,6,6-tetramethylpiperidine-1-oxyl radical (TEMPO)-oxidized cotton/ Poly(vinyl alcohol) (PVA)_Chitosan (CS) materials with and without crude *Agrimonia eupatoria L.* (AG) extract. Cross-sections of the produced composite materials (**A**); surface morphologies and respective diameter distribution of the electrospun nanofibers' layers (PVA_CS and PVA_AG_CS) (**B**).

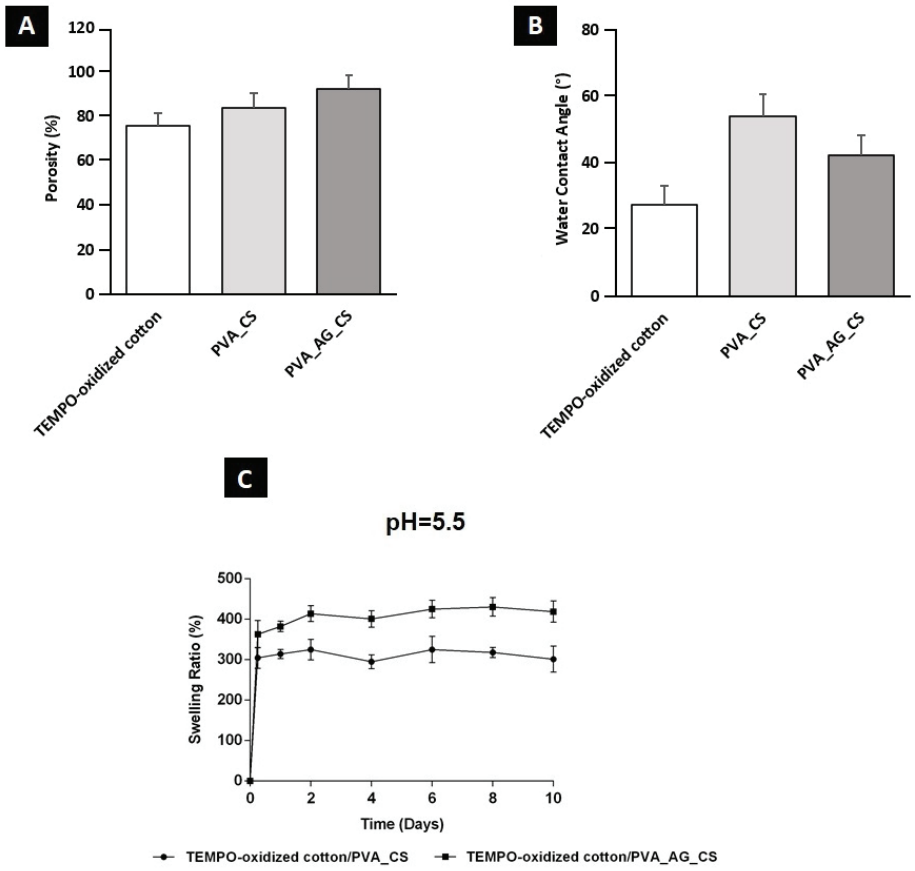

Figure 2: Characterization of the porosity (**A**), wettability features by the water contact angle (WCA) (**B**), and the swelling profile at pH=5.5 (**C**) of the produced composite materials.

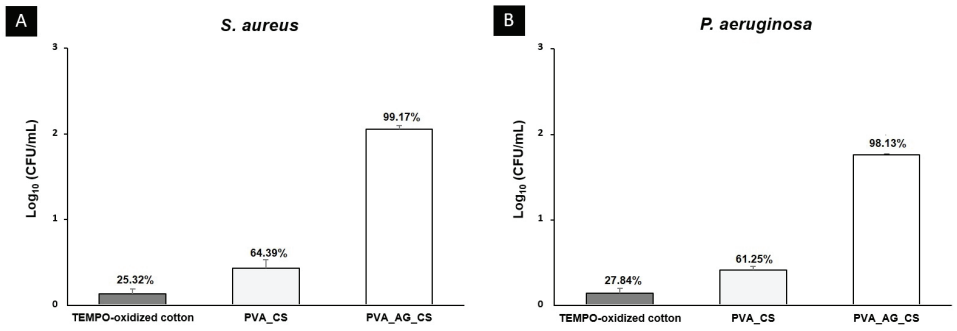

Figure 3: Evaluation of the TEMPO-oxidized cotton, PVA_CS and PVA_AG_CS' antibacterial properties against *S. aureus* (**A**) and *P. aeruginosa* (**B**).

3. Results and discussion

The obtained results demonstrated that from cross-section of produced TEMPO-oxidized cotton-electrospun nanofibers composite materials (TEMPO-oxidized cotton/PVA_CS and TEMPO-oxidized cotton/PVA_AG_CS) it is possible to identify the

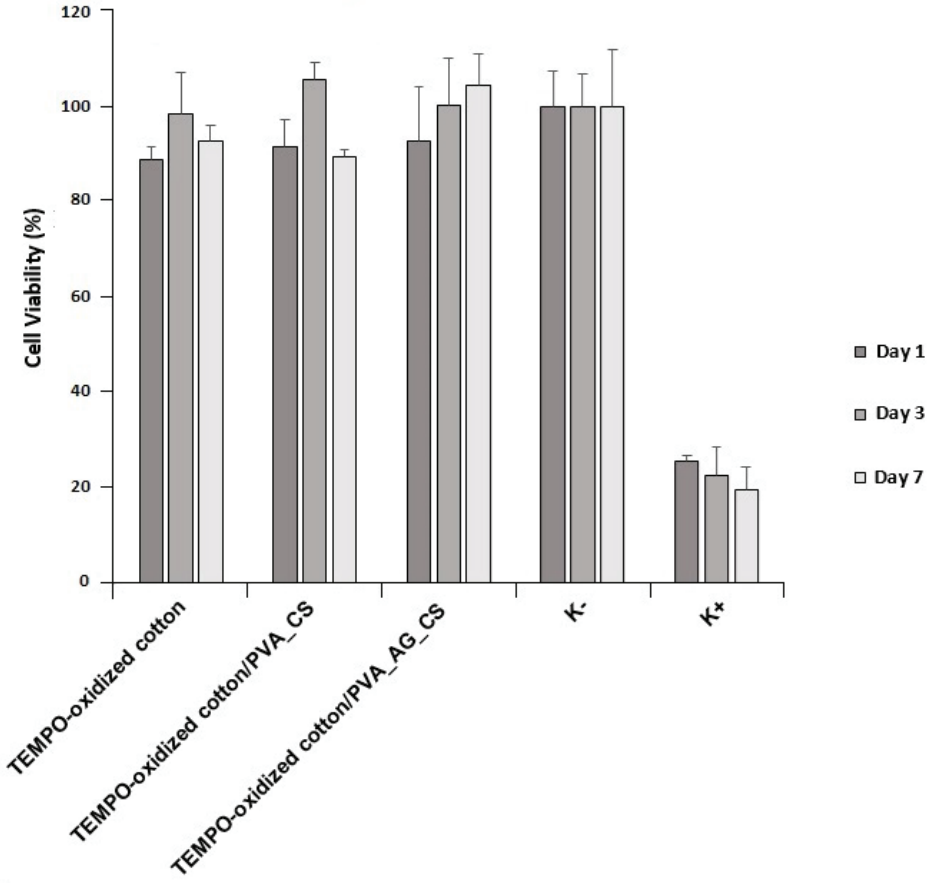

Figure 4: Evaluation of the normal human dermal fibroblast (NHDF) cell viability after 1, 3, and 7 days in contact with the TEMPO-oxidized cotton and the produced composite materials.

two different materials' layers, Fig. 1A. In addition, the electrospun nanofibers' layers (PVA_CS and PVA_AG_CS) presented uniform and homogeneous fibers with average diameters of 280.20 ± 82.65 nm and 208.11 ± 57.92 nm, respectively, similar to those found at the collagen fibers in the native skin's extracellular matrix (ECM) (50–500 nm) [8], increasing its structural similarity with the native skin tissue, Fig. 1B.

Moreover, the electrospun PVA_AG_CS nanofibers' layer of the produced composite materials displayed a greater porosity (92.77 ± 6.01%), a moderate hydrophilic character (42.37 ± 7.52º), and a desired swelling capacity (~400%), confirming the nanofibers ability to ensure a moist wound environment and exudate absorption, as well as to improve cell adhesion and proliferation, Fig. 2.

Besides, AG-incorporated into PVA_CS's nanofibers layer showed an inhibitory effect against *S. aureus* (99.17 ± 4.05%) and *P. aeruginosa* growth (98.13 ± 0.88%), Fig. 3, while the *in vitro* cytotoxicity assay on normal human fibroblast (NHDF) cells demonstrated more than 80% cell viability, even after 7 days, confirming its biocompatibility, Fig. 4.

4. Conclusions

These findings highlight that the TEMPO-oxidized cotton/PVA_CS composite dressing material containing AG extract represents a promising approach to be used as an antibacterial wound dressing due to the potential benefits of AG, particularly its excellent antibacterial activity against pathogenic bacteria and low tendency to develop bacterial resistance, as well as the enhanced properties regarding the combination of two distinct materials, like the mechanical protection, wettability, porosity, and swelling ratio.

Acknowledgments

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