

Melatonin-Loaded Crosslinked Chitosan Nanofibers via Microfluidic Blow Spinning for Wound Healing

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Introduction

Chronic wounds remain a major healthcare burden due to delayed healing and high risk of infection. Crosslinked chitosan nanofibrous patches mimicking the extracellular matrix promote wound healing and controlled drug delivery due to their high porosity and surface area (1). Melatonin further enhances healing through its antioxidant and anti-inflammatory properties (2).

Conventional nanofiber production methods often require multiple processing steps, limiting scalability. To overcome this, our group previously developed a microfluidic blow-spinning (MBS) technique capable of producing crosslinked chitosan nanofibers in a single step.

In this study, we investigate if MBS can be used to produce melatonin-loaded crosslinked chitosan nanofibers and achieve sustained drug release for wound healing applications (3).

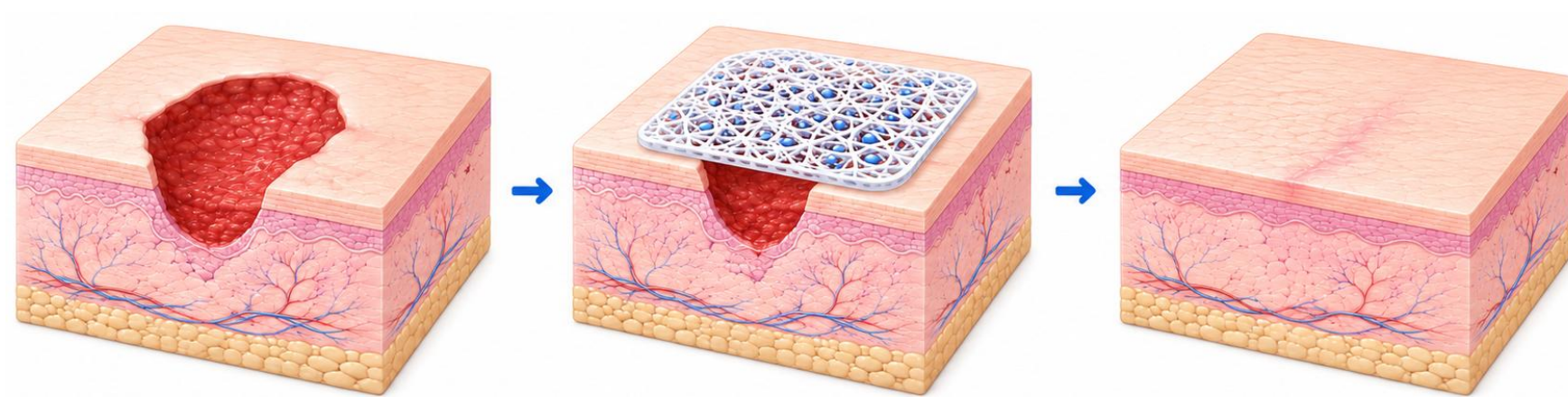


Fig. 1 - Wound healing promoted by nanofibrous patch loaded with melatonin.*

Methods

Melatonin-loaded nanofibers were produced via MBS (Fig. 2). Morphology was assessed using scanning electron microscopy (SEM). Nanofibers were also characterized regarding water stability, swelling and drug release. *In vitro* biocompatibility and ROS production were evaluated in HUVEC cells.

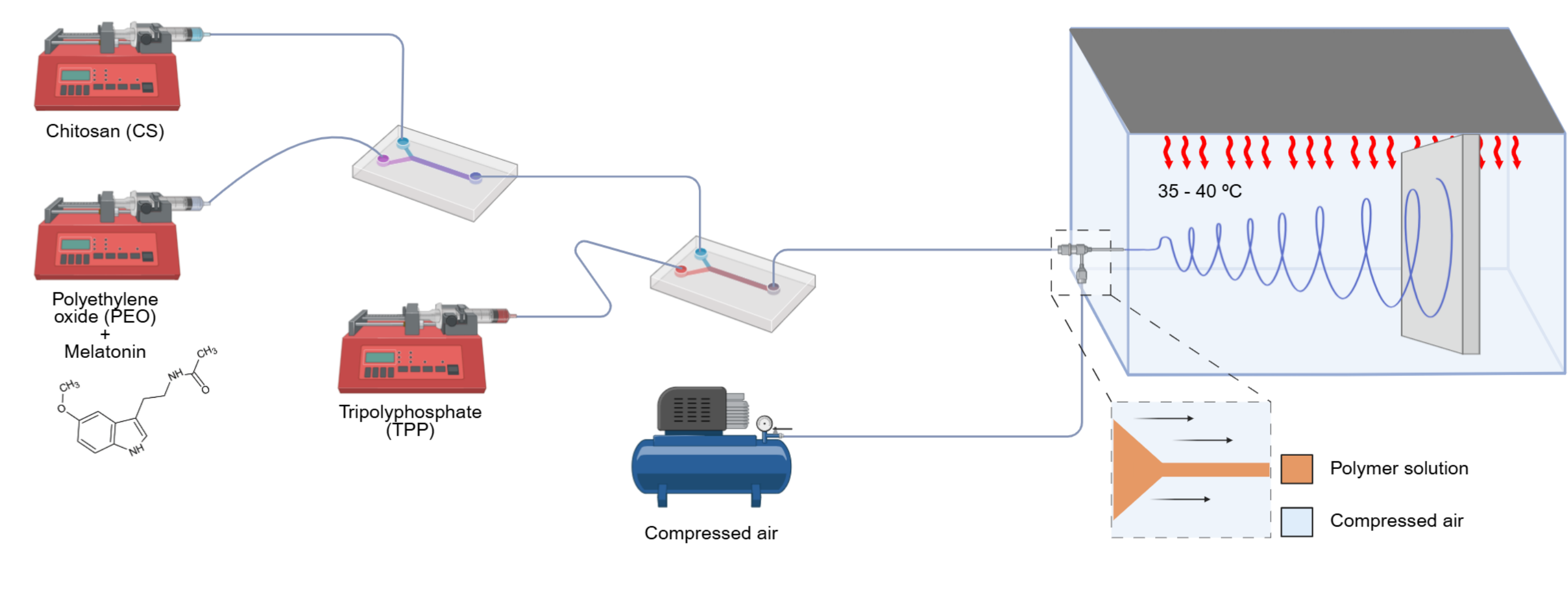


Fig. 2 - Set-up of the MBS apparatus used to produce melatonin-loaded crosslinked chitosan nanofibers.

Results

Nanofibers morphology and diameter evaluation

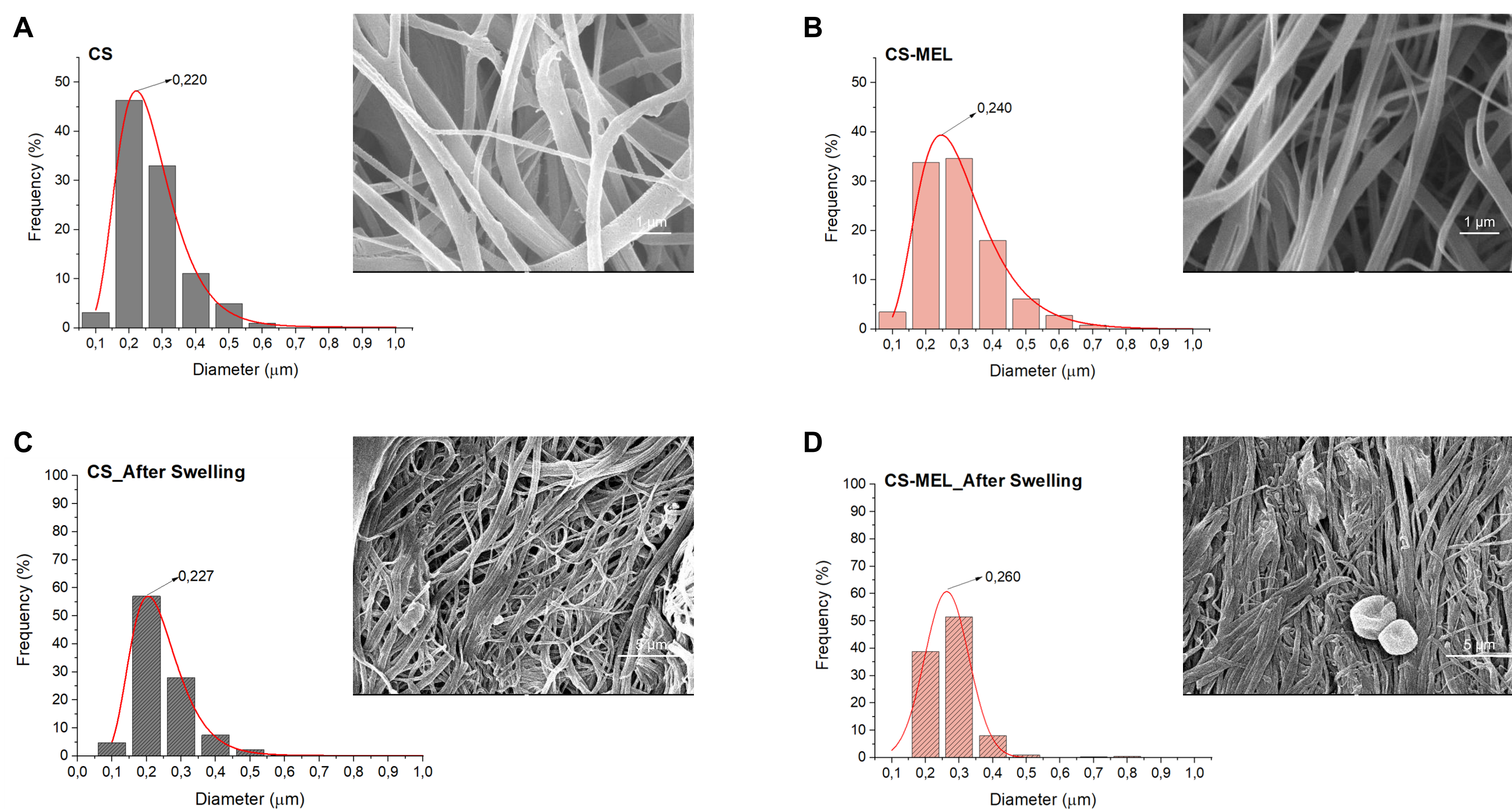


Fig. 3 - SEM images confirmed the successful spinning of melatonin-loaded chitosan nanofibers (CS-MEL) and preserved structure after swelling. No statistically significant differences in fiber diameter were observed between unloaded and melatonin-loaded formulations. (Abbreviations: CS, chitosan; MEL, melatonin)

In Vitro Biocompatibility and ROS Production

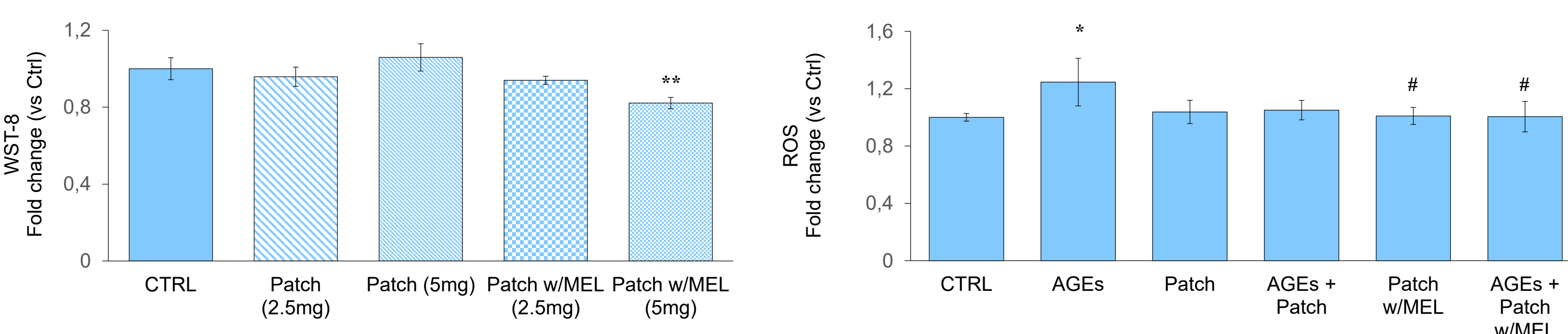


Fig. 4 - All samples are biocompatible (>70% cell viability), despite significant viability decrease induced by the patch w/MEL (5mg). Patches w/MEL (2,5 mg) significantly reduced AGEs-induced intracellular ROS production. $p < 0,01^{**}$, $p < 0,05^{*}$ vs Ctrl, $p < 0,05^{#}$ vs AGEs. (Abbreviations: AGEs, advanced glycation end products; MEL, melatonin).

Swelling ratio and water stability

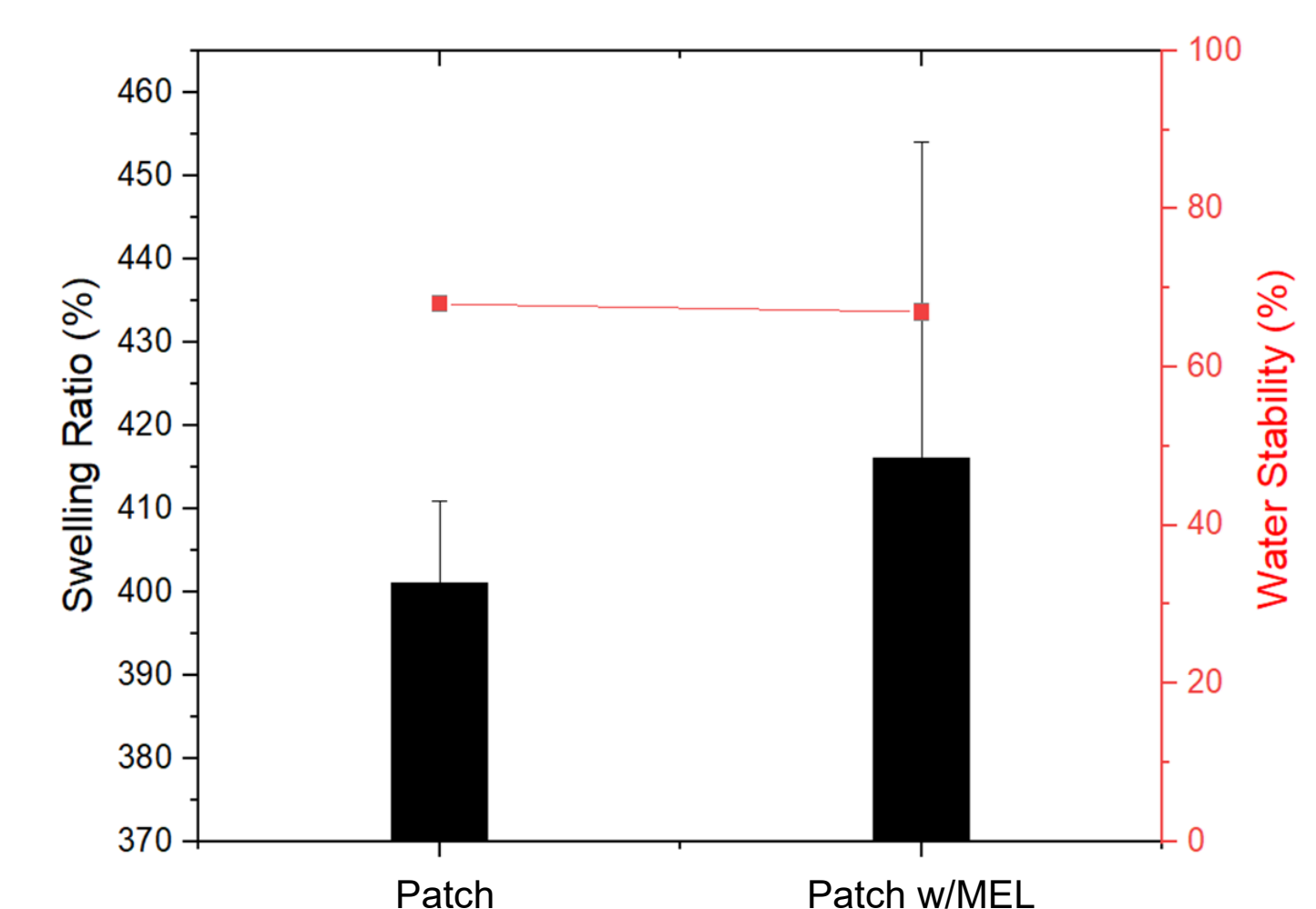


Fig. 5 - Melatonin-loaded nanofibers showed higher swelling capacity while water stability was preserved with minor mass loss, suitable for wound exudate absorption while retaining adequate moisture. (Abbreviations: MEL, melatonin)

Drug release profile

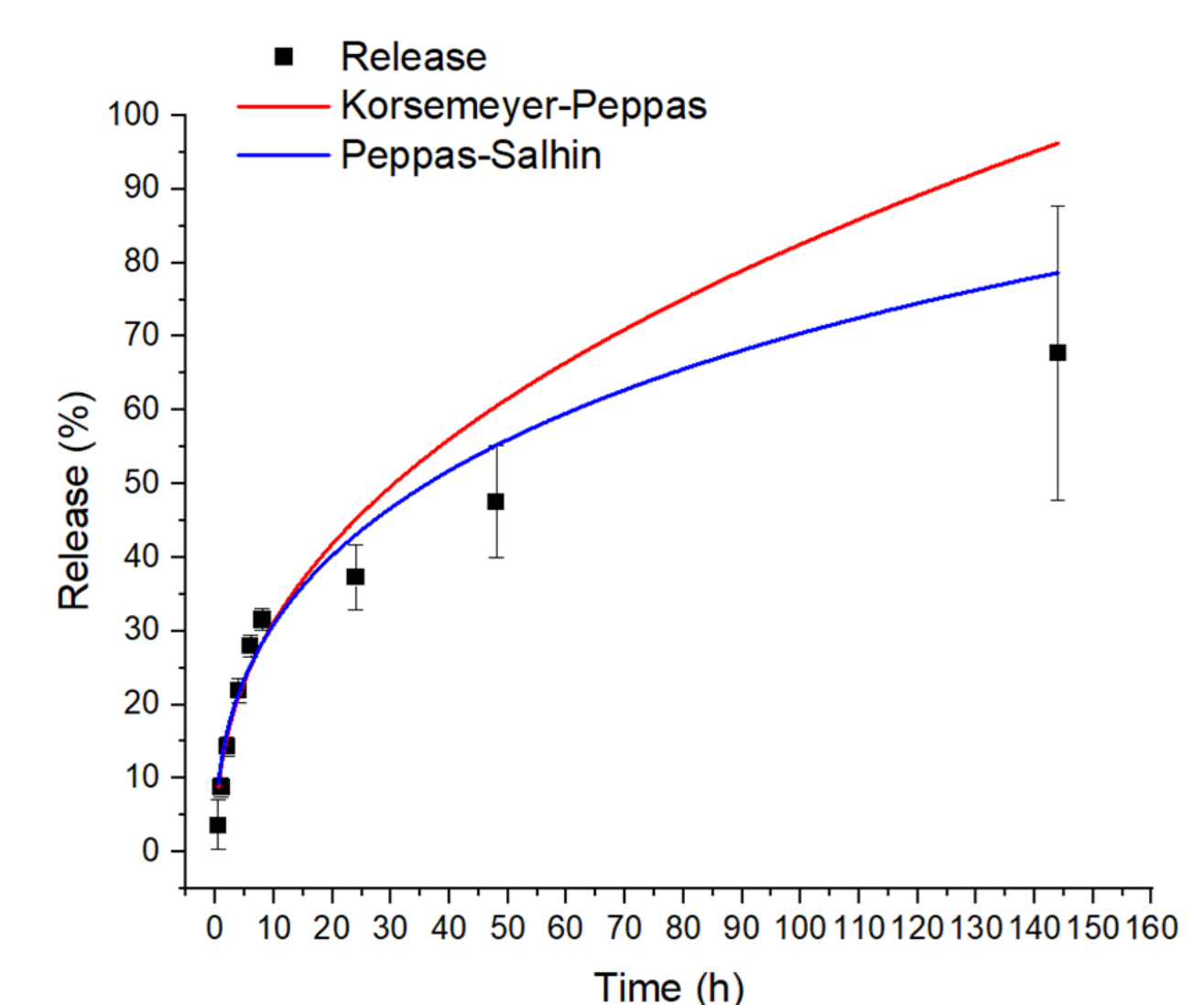


Fig. 6 - Drug-release study revealed sustained melatonin release and release profile was best described by Peppas-Sahlin model ($R^2 = 0,901$), suggesting diffusion-controlled release without influence of erosion or swelling ($m = 0,42$).

Conclusion

Melatonin-loaded crosslinked chitosan nanofibrous patches were successfully produced via MBS, exhibiting adequate fiber diameter, water stability and swelling capacity. The observed controlled and sustained melatonin release, biocompatibility and reduction in ROS production highlight the potential to promote wound healing. Future studies will investigate angiogenesis and inflammatory response modulation.

Acknowledgments

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References

- (1) S. N. Kalva, et al., "Active agents loaded extracellular matrix mimetic electrospun membranes for wound healing applications," *J Drug Deliv Sci Technol*, 2021, vol. 63, p. 102500.
- (2) E.H. Sohn, et al., "Melatonin's Impact on Wound Healing," *Antioxidants*, 2024, vol. 13, no. 10, p. 1197.
- (3) A. F. Matos, et al., "Optimized microfluidic blow spinning process for single-step manufacturing of crosslinked chitosan nanofibers using a quality-by-design approach," *Int J Biol Macromol*, 2025, vol. 321, p. 146279. (*) Image generated with AI.