

FINAL PROGRAMME AND BOOK OF ABSTRACTS

4th International Conference on
Biomedical Polymers & Polymeric Biomaterials
15–18 July 2018, Kraków, POLAND



www.isbppb2018.org

The Comparison of Crosslinking Methods of Bicomponent PCL/gelatin Electrospun Nanofibres

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INTRODUCTION

In our laboratory, we optimized the method of obtaining bicomponent nanofibres made of polycaprolactone (PCL) with an addition of gelatin, through electrospinning from a green, cheap and safe for the operator solvent system – a mixture of acetic and formic acid (Fig. 1a)¹. Unfortunately, further in vitro biodegradation studies showed fast biopolymer leaching from the fibres. With loss of gelatin in the fibre structure and on its surface, the biofunctionality of a material decreases. It is reflected in its hydrophilicity, and as well as morphology and can be observed in scanning electron microscopy (SEM) images (Fig. 1b)². The solution to this predicament is crosslinking of gelatin within the fibre. We decided to investigate a set of different chemical crosslinking methods. Our main concerns were that the methods were as much low toxic as possible and had innovative potential.

EXPERIMENTAL METHODS

Four crosslinking agents were chosen: 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC), genipin, 1,4-butanediol diglycidyl ether (BDDGE) and transglutaminase. For each, a set of different concentrations and times were tested on a material with PCL to gelatin ratio 7:3 electrospun from acetic acid/formic acid (AA/FA) mixture solution. Crosslinked materials underwent then a 24 h biodegradation test in phosphate buffer saline in 37°C. To determine the crosslinking effectiveness and the changes of fibres' morphology, all samples, both right after crosslinking and after biodegradation test, were subjected to SEM imaging, as well as weight measurement to assess gelatin mass loss.

RESULTS AND DISCUSSION

Depending on the crosslinking agent used the results differed significantly. Our two main assessment criteria were: not less than 85% of gelatin mass left and maintained morphology after 24h in 37°C PBS. The main observations were:

- Both EDC/NHS and genipin were able to keep gelatin content within the satisfactory range (85% and up), with the use of relatively low crosslinking agent concentrations (1,25% /0,425% and 2% respectively).
- With the use of BDDGE, only high concentrations and long periods of time yield dependable good results of maintaining gelatin content.
- Transglutaminase, even in high dose, failed to adequately prevent gelatin loss or fibres morphology to deteriorate (fig. 1d).

- Only the materials crosslinked in EDC/NHS maintained satisfactory morphology after 24h biodegradation test. Fibres were smooth and not fused together (Fig. 1c).
- The most cost and time effective method was the use of EDC/NHS, as it required extremely small concentrations of compounds and a good result can be achieved in a couple of hours.

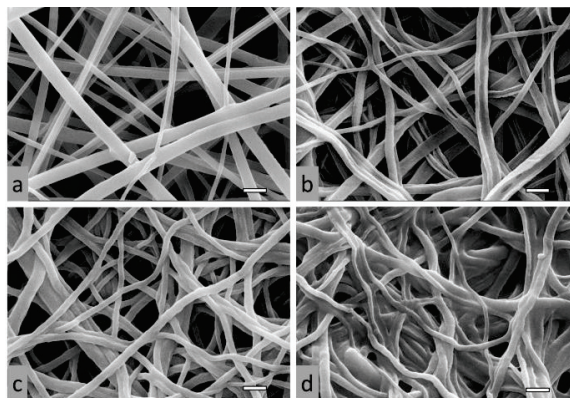


Fig. 1. SEM images of PCL/gelatin 7:3 nanofibres electrospun from AA/FA solvent mixture: a) untreated sample, b, c, d - samples after 24h in 37°C PBS, b) untreated sample with apparent linear groove-like sites remaining after gelatin leaching, c) sample crosslinked with EDC/NHS, d) sample crosslinked with transglutaminase; marker 1μm.

CONCLUSION

Based both on the effectiveness of preserving gelatin within the fibre, as well as maintaining as close morphology as possible to the untreated nonwoven material, EDC/NHS method of crosslinking PCL/gelatin nanofibres was chosen as the best from the tested group. Further, comprehensive research is planned to optimize the method including mechanical testing, prolonged biodegradation and cellular studies.

REFERENCES

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ACKNOWLEDGMENTS

This work was funded by the Polish National Science Center (NCN) under the Grant No.: 2015/17/N/ST8/02027.