Electrospun poly(ε-caprolactone) nanofiber mats with gallic acid and glucosamine sulfate for cartilage repair

Anđela Radisavljević[1,*], Dušica Stojanović[2], Huan Meng[3], Zhen Li[3], Sibylle Grad[3] and Petar Uskoković[2]

[1]University of Belgrade, Innovation Centre of the Faculty of Technology and Metallurgy, Karnegijeva 4, 11120 Belgrade, Serbia
[2]University of Belgrade, Faculty of Technology and Metallurgy, Karnegijeva 4, 11120 Belgrade, Serbia
[3]AO Research Institute Davos, Clavadelerstrasse 8, 7270 Davos, Switzerland

Keywords: Electrospinning; nanofibers; anti-inflammatory drugs; bovine chondrocytes

Hem. Ind. **78(1S)** 26 (2024)

Available on-line at the Journal web address: http://www.ache.org.rs/HI/

INTRODUCTION: Cartilage defects are a common clinical problem, and tissue engineering has become a promising approach for cartilage regeneration because of its high efficiency [1]. The goal of this research was the development of poly(ε-caprolactone) (PCL) nanofiber mats with incorporated active substances gallic acid (GA) and glucosamine sulfate (GAS) as an unexplored alternative for cartilage repair.

EXPERIMENTAL: PCL granules, GA powder, dimethylformamide (anhydrous, 99.8 %, DMF), and dichloromethane (DCM) were obtained from Sigma-Aldrich. The GAS was received from Goodwill Pharma, Serbia. PCL was dissolved in the mixture of DMF/DCM (1:4) solvents to make 10 wt.% PCL solution followed by the addition of surfactant Span 80 (1 % v/v). The aqueous solution of GA and GAS was slowly dripped into PCL solution (1 % of GA and GAS relative to the weight of polymer), to form w/o emulsions. For comparison, neat PCL nanofibers without incorporated drugs were also produced. The emulsion electrospinning method (vertical electrospinning setup CH-01, Linari Engineering, Italy) was applied to produce nanofiber mats where the process parameters were as follows: the flow rate of 3 cm^3h^{-1} and the distance of 10 cm from the collector for both neat PCL and PCL/GA/GAS solutions. At the same time, the voltage was adjusted to 18 kV and 14.5 kV for the neat PCL and PCL/GA/GAS solutions, respectively. Bovine chondrocytes were seeded onto PCL-based nanofiber mats and were cultured in a chondropermissive medium for 7 days, with an added inflammatory factor (Interleukin IL-1β) to induce an inflammatory process in the system. In the pure drug groups GA and GAS, the chondrocytes were seeded in well plates, with the addition of the drug in the medium. After that, the DNA content within the scaffold-cell constructs was measured by Hoechst 33258 dye assay using calf thymus DNA (Sigma-Aldrich) as a standard. Glycosaminoglycan (GAG) content within the scaffold-cell constructs was measured with dimethylmethylene blue dye binding assay using bovine chondroitin sulfate (both Sigma-Aldrich) as the standard. Nitric oxide (NO) production was measured with a Griess kit. All tests were performed in triplicates from one donor.

RESULTS AND DISCUSSION: After seven days of cell culture, pure GA and GAS drugs retained their biological activity, and their incorporation into PCL nanofibers decreased the inflammatory process, as shown by a reduction of NO release, compared to neat PCL. The neat PCL group had the greatest extracellular matrix (ECM) production. The neat PCL can promote chondrocytes proliferation, as reported in several previous studies, and chondrocytes seeded into PCL scaffolds showed the synthesis of cartilage-specific ECM proteins [2].

CONCLUSIONS: Based on the obtained results, PCL nanofiber mats with GA and GAS could be used as a relevant drug scaffold with pronounced anti-inflammatory effects which may be a treatment method for inflammatory cartilage defects.

Acknowledgements: This work was supported by the ExcellMater project (European Union's Horizon 2020 No. 952033).

REFERENCES

[1] Jovanović M, Petrović M, Cvijić S, Tomić N, Stojanović D, Ibrić S, Uskoković P. 3D Printed Buccal Films for Prolonged-Release of Propranolol Hydrochloride: Development, Characterization and Bioavailability Prediction. Pharmaceutics 2021; 13: 2143. https://doi.org/10.3390/pharmaceutics13122143

[2] Rao Z, Dong Y, Liu J, Zheng X, Pei Y., Tang K. Genipin-crosslinked gelatin-based composite hydrogels reinforced with amino-functionalized microfibrillated cellulose. *Int. J. Biol. Macromol* 2022; 222: 3155. https://doi.org/10.1016/j.ijbiomac.2022.10.088

*Corresponding author E-mail: aradisavljevic@tmf.bg.ac.rs