

The ExcellMater project for advancements in biomaterials and 3D *in vitro* culture systems for applications in pharmacy and biomedicine

Bojana Obradović, Jasmina Stojkowska, Ivana Banićević, Anđela Radisavljević, Marija Jovanović, Miloš Petrović, Mirjana Rajilić-Stojanović, Petar Uskoković, Dušica Stojanović, Vesna Radojević

University of Belgrade, Faculty of Technology and Metallurgy, Karnegijeva 4, 11000 Belgrade, Serbia

Introduction

The Twinning project ExcellMater aims to achieve significant advancements in biomaterials engineering towards novel medical devices by particularly reinforcing the S&T capacity of the Faculty of Technology and Metallurgy, University of Belgrade (FTM). One of the topics being pursued is development of novel biomaterials aimed for controlled drug delivery produced by electrospinning and 3D printing. The obtained biomaterials were characterized in detail by various physical and chemical methods and the next step in translation is biological characterization. Biomimetic bioreactors in conjunction with appropriate biomaterials can support 3D cultures of cells, so to address weaknesses of the traditional 2D cell cultures and animal tests, and provide physiologically relevant biomaterial evaluation, tissue and tumor engineering, antitumor drug testing and development of personalized therapies.

Here we present advancements at FTM towards practical applications: 1) use of the previously developed antibacterial electrospun nanofiber mats for coatings of urinary catheters; 2) gelatin and polyvinylpyrrolidone (PVP) printed scaffolds with alendronate sodium trihydrate (ALN); 3) a 3D osteosarcoma model.

Materials and methods

1) Cefazolin or yarrow loaded polycaprolactone (PCL) nanofiber mats (PCL/CEF and PCL/YAR, respectively) were fabricated from PCL solutions

containing CEF (20 wt%) or YAR (*Achillea millefolium*) plant extract powder (10 wt%) by using the blend electrospinning method (Radisavljevic et al., 2018, 2023). Commercially available silicone and rubber urinary catheters were coated with the mats by using adhesive n-butyl-2-cyanoacrylate. Adhesion strength was determined by a peel test and antibacterial activity was investigated against standard strains *S. aureus* and *E. coli*.

2) Scaffolds with the 1:1 mass ratio of gelatin (porcine skin, type A) and PVP K30 with 5% ALN on a dry polymer basis, were prepared by the semi-solid extrusion 3D printing method. The scaffolds were characterized by the FTIR analysis and regarding the tensile properties and *in vitro* drug release kinetics.

3) Macroporous composite Ca-alginate scaffolds (discs: ~9.5 mm diameter, ~4.5 mm thick) were produced by controlled gelation of Na-alginate solution containing hydroxyapatite (HAP) microparticles followed by lyophilization (Stojkowska et al., 2020). The scaffolds were seeded with murine K7M2-wt osteosarcoma cells (15×10^6 cells/ml scaffold volume) and cultured for 7 days in perfusion bioreactors ("3D Perfuse", Innovation Center FTM, Belgrade, Serbia) at the continuous medium flow (40 $\mu\text{m/s}$ superficial velocity), while static cultures served as a control. The scaffolds were assessed by the MTT test and histological and immunocytochemical analyses.

Results and discussion

PCL/CEF and PCL/YAR nanofiber mats were successfully produced and coated onto commercial

catheters with retained biological activity. Good adhesion was achieved in all samples (Fig. 1) with slightly higher adhesion strengths obtained for silicone catheters. All catheters containing bioactive substances have shown antibacterial activity with CEF being more pronounced. Still, PCL/YAR nanofiber mats could be used as a safe and effective source of natural antioxidants and antibacterial agents suitable for urinary catheter coatings.

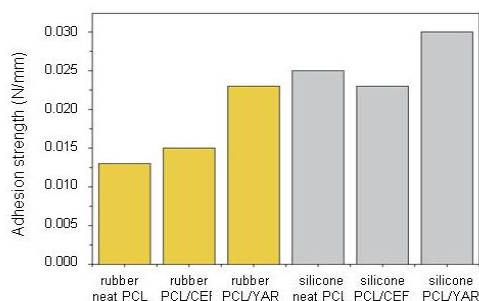


Fig. 1. Adhesion strengths of nanofiber mats coated onto a rubber (yellow) or a silicone (grey) catheter: neat PCL, PCL/CEF and PCL/YAR

3D printed gelatin/PVP scaffolds loaded with ALN (confirmed by the FTIR analysis) as a drug for osteoporosis treatments, have shown the total drug release into phosphate buffered saline over 150 min (Fig. 2). The release profile in the initial period (up to 60% ALN released) followed the first order kinetics with the rate constant of 0.8 h^{-1} . These results indicate potentials of the scaffolds to be used for local and sustained ALN delivery, thus solving the problem of low absorption from the gastrointestinal tract when administered orally.

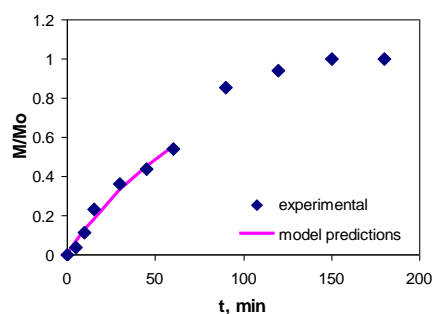


Fig. 2. Fractional release of ALN (released per the total amount, M/Mo) over time: experimental data (symbols) and the first order kinetics model in the initial stage (line)

Osteosarcoma cells dispersed throughout the macroporous Ca-alginate/HAP scaffolds initially forming loosely bound aggregates, which further densified into spheroid-like structures expressing pluripotency-associated genes after the 7-day culture (Fig. 3). The spheroids in the perfusion culture were more abundant

with higher amounts of extracellular matrix, especially reticular fibers, and with better oriented α -tubulin as compared to static controls. The obtained results show potentials of the developed 3D osteosarcoma model for utilization in studies of *in vitro* tumor development.

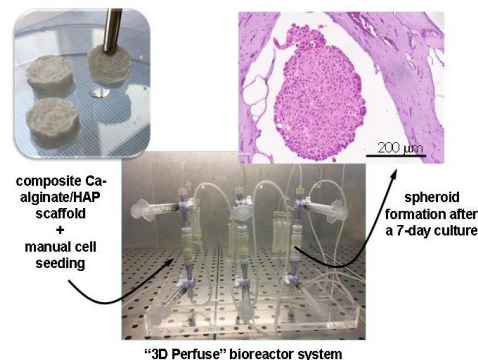


Fig. 3. 3D osteosarcoma model: Ca-alginate/HAP scaffolds were seeded with osteosarcoma cells and cultured for 7 days in “3D Perfuse” bioreactors resulting in spheroid formation (H&E stain; scale bar = 200 μm)

Conclusion

The novel biomaterials as well as *in vitro* 3D culture systems developed at FTM show high potentials for further practical utilization in pharmacy and biomedicine, which is supported by the ExcellMater project.

Acknowledgements: European Union’s Horizon 2020 research and innovation program (GA 952033).

References

- Radisavljevic, A., Stojanovic, D.B., Perisic, S., Djokic, V., Radojevic, V., Rajilic-Stojanovic, M., Uskokovic, P.S., 2018. Cefazolin-loaded polycaprolactone fibers produced via different electrospinning methods: characterization, drug release and antibacterial effect, *Eur. J. Pharm. Sci.* 124, 26-36. doi: 10.1016/j.ejps.2018.08.023
- Radisavljevic, A., Stojanovic, D.B., Petrovic, M., Radojević, V., Uskokovic, P., Rajilic-Stojanovic, M., 2023. Electrospun polycaprolactone nanofibers functionalized with *Achillea millefolium* extract yield biomaterial with antibacterial, antioxidant and improved mechanical properties. *J. Biomed. Mater. Res. A* 111, 962-974. doi:10.1002/jbm.a.37481.
- Stojkowska J., Zvicer J., Andrejevic M., Janackovic Đ., Obradovic B., Veljovic Đ., 2021. Novel composite scaffolds based on alginate and Mg-doped calcium phosphate fillers: enhanced hydroxyapatite formation under biomimetic conditions, *J. Biomed. Mater. Res. B*, 109, 2079-2090. doi:10.1002/jbm.b.3485