2nd Conference on Green Chemistry and Sustainable Coatings

greenchemco

Pisa, Italy, 28th-30th June 2023

Using electrospinning to incorporate probiotics for middle ear bacteriotherapy

Beatrice Cecchini[1,#], Roberta Rovelli[1,#], Bahareh Azimi[2], Semih Esin[2], Giovanna Batoni[2], Serena Danti[1]

[1] *Dept. of Civil and Industrial Engineering, University of Pisa, Pisa, Italy.*
[2] *Dept. of Translational Research and New technologies in Medicine and Surgery, University of Pisa, Pisa, Italy.*
[#] *These authors contributed equally to this work.*

Telephone: +390502217874, serena.danti@unipi.it

Keywords: alginate, microparticles, fibers.

Introduction

Due to the alarming phenomenon of antimicrobial resistance, the use of antibiotics to treat upper respiratory tract-related diseases, such as otitis media (OM), is not resolutive. Bacteriotherapy, which relies on the administration of probiotics to compete with the infectious pathogens, is emerging as an attractive option [1]. However, adequate systems for the delivery of probiotics to the target site are not yet established. In recent years, electrospinning and electrospray have emerged as suitable biofabrication techniques to produce micro/submicrometric fibers and particles, which can be loaded with a wide range of cells [2]. Nevertheless, supporting data, describing the bacteria encapsulation via electrospinning, are still scarce.
The present work aims at fabricating multi-dimensional probiotic delivery systems to treat middle ear infections via bacteriotherapy. In particular, the electrospinning technique is exploited to produce microparticles (0D) and fiber (1D) to fiber meshes (2D) for the topical application in the tympanic membrane.

Experimental

Alginate (MW = 427 kDa, M/G ratio = 0.7) microparticles were obtained through electrospray. A 2 w/v% alginate in water solution was electrosprayed (V = 22 kV, flow rate = 2.0 mL/h, distance = 9 cm) in a 2 w/v% calcium chloride bath and crosslinked overnight. Particles were re-suspended in water and observed (t = 0) with an inverted optical microscope to characterize the shape and dimensions. Degradation tests in PBS (T = 37°C) were performed to assess the stability of the system in simulated biological conditions. The same solution containing model commercial probiotics was electrosprayed using the same parameters and observed (t = 0) with the optical microscope for comparison.
Alginate microfibers were electrospun (V = 28 kV, flow rate = 2.0 mL/h, distance = 5.5 cm) by employing a solution of 2 w/v% high molecular weight alginate (Mw = 495 kDa, M/G ratio = 1.9) and 2 w/v% low molecular weight alginate (Mw = 51 kDa, M/G ratio = 0.3) dissolved in a mixture of water and glycerol (1:1 glycerol to water volume ratio). The fibers were collected on a rotating

collector (rotational speed = 15 rpm) partially submerged in a 2 w/v% calcium chloride solution and subsequently observed with scanning electron microscopy (SEM). Alginate/poly(ethylene oxide) (PEO) nanofibers were electrospun (V = 10-16 kV, flow rate = 0.5 mL/h, distance = 9 cm) using a solution of 3 w/w% PEO (Mw = 1000 kDa) and 70:30 (w/w) alginate (Mw = 51 kDa, M/G ratio = 0.3):PEO, with the addition of 1 w/w% of Triton-X as a surfactant. The fibers were collected on a rotating collector (rotational speed = 15 rpm).

Results and Discussion

Probiotic-free microparticles showed an initial diameter of 346.5 ± 10.0 μm and the degradation tests revealed a good stability (for up to two weeks) in simulated biological conditions. Probiotic-laden microparticles exhibited a diameter of 395.5 ± 23.5 μm and a slightly more elongated shape with respect to probiotic-free particles.

Electrospinning alginate is challenging due to its rigid molecular structure, lack of entanglement formation capacity and polyelectrolytic nature. Therefore, the use of an alginate with high molecular weight and M/G ratio, as well as the presence of glycerol as a co-solvent, was necessary to achieve the successful formation of pure alginate fibers. However, this method could not yield long, continuous fibers. Therefore, an alternative strategy, i.e., the use of PEO as a support polymer, was exploited to achieve the desired characteristics for the fibrous meshes.

Conclusions

The method is promising to produce 0D, 1D and 2D delivery systems for the treatment of middle ear otitis. However, further studies are necessary to improve the electrospinnability of alginate and evaluate the viability of probiotics after the procedure, as well as the efficacy of bacteriotherapy in *in vitro* and *in vivo* models.

Acknowledgments:

This project has not received external funding.

References

[1] Van den Broek, M. F., De Boeck, I., Kiekens, F., Boudewyns, A., Vanderveken, O. M., & Lebeer, S. (2019). Translating recent microbiome insights in otitis media into probiotic strategies. Clinical microbiology reviews, 32(4), e00010-18.

[2] Diep, E., & Schiffman, J. D. (2021). Encapsulating bacteria in alginate-based electrospun nanofibers. Biomaterials Science, 9(12), 4364-4373.

Biography

Beatrice Cecchini is currently enrolled in the Materials and Nanotechnology Master’s Degree at University of Pisa, offered in cooperation with Scuola Normale Superiore. She has a Bachelor’s Degree in Chemical Engineering (University of Pisa). She is interested in polymers, especially hydrogels, and she is currently working on her thesis project on bacteriotherapy.