NATIONAL UNIVERSITY OF SCIENCE AND TECHNOLOGY POLITEHNICA BUCHAREST

Doctoral School Chemical Engineering and Biotechnologies

PhD Domain: Chemical Engineering

PhD Thesis

NANOBIOMATERIALS

- Resume -

PhD Supervisor
Prof. PhD. Eng. Ecaterina Andronescu

PhD Student
Eng. Alexandra Elena Oprea (Stoica)

Bucharest

2023

Contents

Keywords: electrospinning; nanofibers; wound healing; wound dressings; tissue regeneration; antimicrobial agents; *in vitro*; *in vivo*; biocompatibility; fibrous silica, PVA; chitosan; usnic acid; recycled PET; magnetite.

List of figures

Figure 5. Zoom-in from the full spectrum (upside) to the monoisotopic peak of usnic acid, sample (red), reference (green), and simulated spectrum (black)

Figure 6. MALDI slide, sample (PET@Fe3O4@UA), reference compound, and scanning area.

Figure 7. Surface distribution of usnic acid M+H peak, C18H16O7

Figure 8. Surface distribution of usnic acid M+Na peak, C18H16O7Na

Figure 9. Graphical representation of the recorded absorbance values for *S. aureus* cultures, expressing the multiplication capacity of these cells after cultivation for 24 h in the presence of recycled PET polymer materials and control (planktonic microorganisms without materials). * $p < 0.05$; ** $p < 0.001$

Figure 10. Graphical representation of the absorbance values recorded for *P. aeruginosa* cultures expressing the multiplication capacity of bacteria cells after cultivation for 24 h in the presence of recycled PET polymer materials and control (planktonic microorganisms without materials). * $p < 0.05$; ** $p < 0.001$

Figure 11. Graphical representation of the absorbance values recorded for cultures of *C. albicans*, expressing the multiplication capacity of these cells after cultivation for 24 h in the presence of recycled PET polymer materials and control (planktonic microorganisms without materials). * $p < 0.05$; ** $p < 0.001$

Figure 12. Graphical representation of CFU/mL (colony forming units/mL) representing the number of *S. aureus* cells included in the monospecific biofilms developed on the surface of the materials obtained for 24 h, 48 h, and 72 h at 37 _C. (* $p < 0.05$; by comparing biofilm formation on PET control and corresponding UA containing PET, ns = not significant)

Figure 13. Graphical representation of CFU/mL (colony forming units/mL) representing the amount of *P. aeruginosa* cells included in the monospecific biofilms developed on the surface of the materials obtained for 24 h, 48 h, and 72 h at 37 _C. (* $p < 0.05$; by comparing biofilm formation on PET control and corresponding UA containing PET)

Figure 14. Graphic representation of CFU/mL (colony forming units/mL) representing the number of *C. albicans* cells included in the monospecific biofilms developed on the surface of the materials obtained for 24 h, 48 h, and 72 h at 37 _C. (* $p < 0.05$; ** $p < 0.001$ by comparing biofilm formation on PET control and corresponding UA containing PET)

Figure 15. The effects of PET@Fe3O4@UA subcutaneous implantation in mice on the C-reactive protein (CRP) levels at 24 h and 7 days post-surgery

Publication II – Internation Journal of Molecular Science

Publication III – Polymers

Figure 3. TEM images for nanostructured membranes of PET@Fe_3O_4@UA at a flow rate of 5 mL/h (PET@Fe_3O_4@UA_5)

Figure 4. The FT-IR spectra for PET@Fe_3O_4@UA membranes

Figure 5. Graphical representation of the recorded absorbance values for *S. aureus* cultures, expressing the multiplication capacity of these cells after cultivation, for 24h in the presence of recycled PET polymer materials and control (Planktonic microorganisms without materials)

Figure 6. Graphical representation of the absorbance values recorded for *P. aeruginosa* cultures expressing the multiplication capacity of bacteria cells after cultivation for 24 h in the presence of recycled PET polymer materials and control (Planktonic microorganisms without materials)

Figure 7. Graphical representation of the absorbance values recorded for cultures of *C. albicans*, expressing the multiplication capacity of these cells after cultivation for 24h in the presence of recycled PET polymer materials and control (Planktonic microorganisms without materials)

Figure 8. Graphical representation of CFU/mL (colony forming units/mL) representing the amount of *S. aureus* cells included in the monospecific biofilms developed on the surface of the materials obtained for 24h, 48h, and 72h at 37°C

Figure 9. Graphical representation of CFU/mL (colony forming units/mL) representing the amount of *P. aeruginosa* cells included in the monospecific biofilms developed on the surface of the materials obtained for 24h, 48h, and 72h at 37°C

Figure 10. Graphic representation of CFU/mL (colony forming units/mL) representing the amount of *C. albicans* cells included in the monospecific biofilms developed on the surface of the materials obtained for 24h, 48h, and 72h at 37°C

Figure 11. The effects of PET@Fe_3O_4@UA subcutaneous implantation in mice on the C-reactive protein (CRP) levels at 24 h and 7 days post-surgery

Figure 12. Biocompatibility analysis of PET_Fe_3O_4@UA_10 and PET@Fe3O4@UA_5 at 24 h and 7 days post-implantation. (a) H&E stain; (b) Masson-Goldner trichrome stain. Material (*); Barr 200 µm

Figure 13. TNF-α protein expression as revealed by confocal microscopy at 24 h and 7 days post-implantation. TNF-α is labeled green, and the nuclei are counterstained with DAPI.

List of tables

The **aim of the present study** was to obtain and characterize (structural and functional) of nanostructured membranes with fibrillar consistency obtained using electrospinning technique with applications in tissue engineering (TE).

The first section of this thesis provides a **literature background**, describing concepts referring to **nanomaterials** and **nanofibrous materials for TE (bone tissue engineering and wound dressing aplications)**, as well as electrospinning method, from basic concepts to challenges in electrospinning and nanofibrous scaffolds for tissue regeneration.

First, the thesis introduces the synthesis and evaluation of silica (SiO_2) nanofibrous mats produced through electrospinning and subsequent calcination with tetraethyl orthosilicate (TEOS) and polyvinyl alcohol (PVA). This is the first report on the development of fibrous silica architectures, using tetraethyl orthosilicate (TEOS) and polyvinyl alcohol (PVA) during the self-assembly electrospinning (ES) processing (a layer of flat fibers must first be created in self-assembly electrospinning before fiber stacks can develop on the fiber mat). The elemental and microstructural details of the produced scaffolds were assessed through a combination of infrared and thermal analyses, as well as advanced microscopic techniques. The study of the development of pure silica networks was made following a calcination process at a temperature of 500°C for a duration of 2 hours. These networks were found to be composed of nanofibers that were uniformly distributed and randomly orientated, exhibiting smooth surfaces. Subsequently, the samples underwent evaluation to determine their suitability for application in bone tissue engineering, employing a murine animal model. Calcined silica fibrous scaffolds (SiO2_0h aging and SiO2_1.5h aging) shown enhanced outcomes in mineralization and the generation of new bone. Additionally, the incorporation of vitronectin into calcined networks resulted in enhanced mineralization and facilitated the production of new bone through the promotion of cell adhesion to the fibers. The findings confirmed the suitability of electrospun silica nanofibers as effective options for scaffolding in bone tissue engineering applications.

Injuries and diseases of the skin require accurate treatment using nontoxic and noninvasive biomaterials, which aim to mimic the natural structures of the body. There is a strong need to develop biodevices capable of accommodating nutrients and bioactive molecules and generating the process of vascularization. Electrospinning is a robust technique, as it can form fibrous structures for tissue engineering and wound dressings. The best way of forming such meshes for wound healing is to choose two polymers that complement each other regarding their properties.

Second, the present doctoral thesis focuses on the manufacture and characterisation of an electrospun nanofiber mesh composed of polyvinyl alcohol, chitosan, and usnic acid. On the one hand, PVA is a water-soluble synthetic polymer widely used for the preparation of hydrogels in the field of biomedicine owing to its biocompatibility, water solubility, nontoxicity, and considerable mechanical properties. PVA is easy to subject to electrospinning and can offer strong mechanical stability of the mesh, but it is necessary to improve its biological properties. On the other hand, CS has good biological properties, including biodegradability, nontoxicity, biocompatibility, and antimicrobial properties. Still, it is harder to electrospin and does not possess as good mechanical properties as PVA. As these structures also allow the incorporation of bioactive agents due to their high surface area-to-volume ratio, the interesting point was to incorporate usnic acid into the structure as it is a natural and suitable alternative agent for burn wounds treatment which avoids an improper or overuse of antibiotics and other invasive biomolecules. The primary objective of this study is to investigate the potential applications of this nanofiber mesh in the field of wound healing. The physicochemical analysis demonstrated the presence of a fibrous morphology, with the fibers exhibiting diameters ranging from 14.86 nm to 75.06 nm. Notably, the majority of the fibers fell between the size range of 30 to 40 nm. The scanning electron microscopy (SEM) pictures revealed that the nonwoven assembly exhibited a linear morphology characterized by a random orientation. The SEM images revealed fibers with ramifications that exhibited a significantly porous structure, making them appropriate for utilization in wound healing due to their ability to mimic the natural extracellular matrix (ECM). The XTT assay demonstrated significant findings at both the 48-hour and 72-hour time points for the 5%PVA_2%CS_UA sample. Specifically, it exhibited a notable increase in cell viability, approximately 30% higher than that of the control group. The confirmation of this fact establishes the biocompatibility of the electrospun mesh with usnic acid, indicating its potential as a viable biodevice capable of supporting cellular proliferation and growth. The cell viability seen using fluorescence microscopy was found to be similar to that of the control group, indicating that the nanofiber meshes did not demonstrate any cytotoxic properties. The 5%PVA_2%CS_UA formulation exhibited enhanced anti-biofilm efficacy against the *S. aureus* strain, as observed from the perspective of biofilm growth. In brief, the results mentioned above significantly support the potential of applying electrospun nanofiber meshes composed of polyvinyl alcohol (PVA), chitosan, and usnic acid as a viable material for the purpose of wound healing.

The third objective of this study was to develop and characterize nanostructured membranes with fibrillar consistency based on recycled PET and magnetite nanoparticles functionalized with usnic acid by electrospinning technique. The obtained PET nanostructured membranes exhibited enhanced antibacterial and antibiofilm properties against both Gram-positive (*S. aureus*) and Gram-negative (*P. aeruginosa*) bacterial strains, as well as opportunistic yeast *C. albicans*. The samples obtained at higher feed rates exhibited superior antibacterial potential, which can be attributed to the development of denser meshes and a higher concentration of magnetite nanoparticles on their surface (as observed qualitatively). Furthermore, it was shown that the fibrillar mats nanoparticle-containing exhibited minimal toxicity when tested in both in vitro and in vivo conditions. The findings present novel possibilities for PET recycling, including its integration with diverse antimicrobial inorganic nanostructures to produce enhanced fibrillar materials with antimicrobial and antibiofilm characteristics. These developments have the potential for expanded utilization within the food industry, particularly in the context of food packaging applications. Additionally, their application in the biomedical field could be promising for the development of antimicrobial medical textiles.

A variety of materials has been developed by the utilization of the electrospinning process. The electrospinning techinque is characterized by a high level of complexity, with the final result being influenced by a wide range of parameters. This represents a challenge in determining the optimal parameters configuration for a certain application. Nevertheless, this technique also enables the control of fiber integrity and morphology by precisely adjusting these parameters. Frequently, it is advantageous to conduct spinning processes at ambient temperature due to the cost-effectiveness compared to operating at very high or low temperatures. All the materials obtained (in the form of nanofibrous mats) and studied for their properties demonstrated potential for **tissue engineering applications** and **wound healing** by bringing **novelty** in this **biomedical field**. Also, the **optimization of parameters** done in this work holds great value for the electrospinning deposition process of different types of materials.

ISI published papers included in the thesis

1. **Stoica, A.E.**; Bîrca, A.C.; Gherasim, O.; Ficai, A.; Grumezescu, A.M.; Oprea, O.-C.; Vasile, B.S.; Balta, C.; Andronescu, E.; Hermenean, A.O. Electrospun Fibrous Silica for Bone Tissue Engineering Applications. Pharmaceutics 2023, 15, 1728 **Q1 IF=5.4**

2. **Stoica, A.E.**; Albulet, , D.; Bîrca, A.C.; Iordache, F.; Ficai, A.; Grumezescu, A.M.; Vasile, B.S.; Andronescu, E.; Marinescu, F.; Holban, A.M. Electrospun Nanofibrous Mesh Based on PVA, Chitosan, and Usnic Acid for Applications inWound Healing. Int. J. Mol. Sci. 2023, 24, 11037. **Q1 IF=5.6**

3. **Stoica, A.E.**; Bîrca, A.C.; Mihaiescu, D.E.; Grumezescu, A.M.; Ficai, A.; Herman, H.; Cornel, B.; Rosu, M.; Gharbia, S.; Holban, A.M.; et al. Biocompatibility and Antimicrobial Profile of Acid Usnic-Loaded Electrospun Recycled Polyethylene Terephthalate (PET)—Magnetite Nanofibers. Polymers 2023, 15, 3282. **Q1 IF=5.0**

IF = 16

ISI published papers related to the thesis

4. **Stoica, A.E.**; Chircov, C.; Grumezescu, A.M. Nanomaterials for Wound Dressings: An Up-to-Date Overview. Molecules 2020, 25, 2699. **Q2 IF=4.6**

5. **Stoica, A.E.**; Chircov, C.; Grumezescu, A.M. Hydrogel Dressings for the Treatment of Burn Wounds: An Up-To-Date Overview. *Materials* 2020, *13*, 2853. **Q2 IF=3.4**

6. **Stoica, A.E.**; Grumezescu, A.M.; Hermenean, A.O.; Andronescu, E.; Vasile, B.S. Scar-Free Healing: Current Concepts and Future Perspectives. Nanomaterials 2020, 10, 2179. **Q1 IF=5.3**

7. **Stoica, A.E.**; Birca, A.C.; Pitigoi, M.L.; Grumezescu, A.M.; Vasile, B.S.; Holban, A.M.; Iordache, F.; Ficai, A.; Andronescu, E. Bioactive zinc oxide, egg albumin and cinnamon oil collagen, dressing, - accepted for publication in U.P.B. Sci. Bull. **Q4 IF=0.5**

ISI published papers during PhD studies

8. Vasile, B.S.; Nicoara, A.-I.; Surdu, V.-A.; Ene, V.L.; Neacsu, I.A.; **Stoica, A.E.**; Oprea, O.; Boerasu, I.; Trusca, R.; Vrabec, M.; et al. Fly-Ash Evaluation as Potential EOL Material Replacement of Cement in Pastes: Morpho-Structural and Physico-Chemical Properties Assessment. *Materials* 2022, *15*, 3092. **IF=3.4**

9. Nicoara, A.I.; **Stoica, A.E.**; Ene, D.-I.; Vasile, B.S.; Holban, A.M.; Neacsu, I.A. In Situ and Ex Situ Designed Hydroxyapatite: Bacterial Cellulose Materials with Biomedical Applications. *Materials* 2020, *13*, 4793. **IF=3.4**

10. Docea, A.O.; Calina, D.; Buga, A.M.; Zlatian, O.; Paoliello, M.M.B.; Mogosanu, G.D.; Streba, C.T.; Popescu, E.L.; **Stoica, A.E.**; Bîrcă, A.C.; et al. The Effect of Silver Nanoparticles on Antioxidant/Pro-Oxidant Balance in a Murine Model. *Int. J. Mol. Sci.* 2020, *21*, 1233. **IF=5.6**

11. Nicoara, A.I.; **Stoica, A.E.**; Vrabec, M.; Šmuc Rogan, N.; Sturm, S.; Ow-Yang, C.; Gulgun, M.A.; Bundur, Z.B.; Ciuca, I.; Vasile, B.S. End-of-Life Materials Used as Supplementary Cementitious Materials in the Concrete Industry. *Materials* 2020, *13*, 1954. **IF=3.4**

12. Grumezescu, A.M.; **Stoica, A.E.**; Dima-Bălcescu, M.-Ș.; Chircov, C.; Gharbia, S.; Baltă, C.; Roșu, M.; Herman, H.; Holban, A.M.; Ficai, A.; et al. Electrospun Polyethylene Terephthalate Nanofibers Loaded with Silver Nanoparticles: Novel Approach in Anti-Infective Therapy. *J. Clin. Med.* 2019, *8*, 1039. **IF=3.9**

13. Neacsu, I.A.; **Stoica, A.E.**; Vasile, B.S.; Andronescu, E. Luminescent Hydroxyapatite Doped with Rare Earth Elements for Biomedical Applications. *Nanomaterials* 2019, *9*, 239. **IF=5.3**

IF cumulative = 54.8

Selective Bibliography

1. Sajid, M. Nanomaterials: types, properties, recent advances, and toxicity concerns. *Current Opinion in Environmental Science & Health* 2022, *25*, 100319.

2. Bratovcic, A. Different applications of nanomaterials and their impact on the nvironment. *SSRG International Journal of Material Science and Engineering* 2019, *5*, 1-7.

3. Shahriar, S.M.S.; Mondal, J.; Hasan, M.N.; Revuri, V.; Lee, D.Y.; Lee, Y.-K. Electrospinning Nanofibers for Therapeutics Delivery. *Nanomaterials* 2019, *9*, 532.

4. Weng, T.; Zhang, W.; Xia, Y.; Wu, P.; Yang, M.; Jin, R.; Xia, S.; Wang, J.; You, C.; Han, C. 3D bioprinting for skin tissue engineering: Current status and perspectives. *Journal of tissue engineering* 2021, *12*, 20417314211028574.

5. Su, H.; Fujiwara, T.; Anderson, K.M.; Karydis, A.; Ghadri, M.N.; Bumgardner, J.D. A comparison of two types of electrospun chitosan membranes and a collagen membrane in vivo. *Dental Materials* 2021, *37*, 60-7.

6. Kalidas, S.; Sumathi, S. Mechanical, biocompatibility and antibacterial studies of gelatin/polyvinyl alcohol/silkfibre polymeric scaffold for bone tissue engineering. *Heliyon* 2023.

7. Gautam, S.; Sharma, C.; Purohit, S.D.; Singh, H.; Dinda, A.K.; Potdar, P.D.; Chou, C.-F.; Mishra, N.C. Gelatin-polycaprolactone-nanohydroxyapatite electrospun nanocomposite scaffold for bone tissue engineering. *Materials Science and Engineering: C* 2021, *119*, 111588.

8. Battafarano, G.; Rossi, M.; De Martino, V.; Marampon, F.; Borro, L.; Secinaro, A.; Del Fattore, A. Strategies for Bone Regeneration: From Graft to Tissue Engineering. *International Journal of Molecular Sciences* 2021, *22*, 1128.

9. Xue, N.; Ding, X.; Huang, R.; Jiang, R.; Huang, H.; Pan, X.; Min, W.; Chen, J.; Duan, J.-A.; Liu, P.; et al. Bone Tissue Engineering in the Treatment of Bone Defects. *Pharmaceuticals* 2022, *15*.

10. Xue, X.; Hu, Y.; Deng, Y.; Su, J. Recent Advances in Design of Functional Biocompatible Hydrogels for Bone Tissue Engineering. *Advanced Functional Materials* 2021, *31*, 2009432.