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Water Resistance and Morphology of Electrospun Gelatine Blended with Citric Acid and Coconut Oil

Vodoodpornost in morfologija elektropredene želatine, mešane s citronsko kislino in kokosovim oljem

Original Scientific Article/*Izvirni znanstveni članek*
Received/*Prispelo* 04-2018 • Accepted/*Sprejeto* 06-2018

Abstract

Bio-polymer gelatine can be found in a broad variety of applications, mostly in the food industry. Moreover, it is used in the encapsulation of active pharmaceutical ingredients. In electrospinning, it is used for drug release, but can also strongly influence the morphologies of nanofiber mats when blended with other polymers. In a recent project, we studied the influence of adding citric acid and coconut oil to gelatine electrospinning solutions. While the former can be used to modify gelatine nanofiber diameters and create diverse morphologies between the fibres and sprayed droplets of different shapes, the latter results in an electrospraying process and additionally increases water resistance, suggesting a possible use of the combination for tailored drug release.
Keywords: nanofiber mat, water resistance, electrospraying

Izvleček

Biopolimerno želatino uporabljajo na številnih področjih, še posebno v živilski industriji, pa tudi pri kapsuliranju aktivnih farmakoloških sestavin. Pri elektropredenju jo uporabljajo za sproščanje zdravil. Ko jo mešajo z drugimi polimeri, pa lahko pomembno vpliva na morfologijo nanovlaknatih kopren. V nedavnem projektu sta bila preučevana vpliva dodatka citronske kisline in kokosovega olja k raztopljeni želatini za potrebe elektropredenja. Medtem ko se citronska kislina lahko uporabi za spreminjanje premera nanovlaken iz želatine in ustvarjanje različne morfologije med vlakni in razpršenimi kapljicami različnih oblik, pa povzroči kokosovo olje elektrorazprševanje in dodatno poveča vodoodpornost, kar kaže na možno uporabo kombinacije za načrtovano sproščanje zdravil.
Ključne besede: nanovlaknata koprena, vodoodpornost, elektrorazprševanje

1 Introduction

Gelatine belongs to bio-polymers which are often electrospun to create nanofiber mats for diverse purposes. Gelatine nanofibers can be used to mimic an extra-cellular matrix [1, 2] and are therefore expected to provide good growth and proliferation conditions for cells. Gelatine is known to bind cell surfaces via fibronectin binding better than native collagen [3]. However, gelatine has a disadvantage since it is water-soluble, which makes crosslinking after the electrospinning necessary, e.g. with a heat-treatment in the presence of different other chemicals [4, 5], addition of diverse acids [6] or toxic materials, such as glutaraldehyde [8].

Another possibility is using gelatine blended with other polymers. Blending silk fibroin with gelatine, for example, resulted in an increased nanofiber diameter and hydrophobicity, while the mechanical properties decreased [8]. Testing diverse PAN/bio-polymer blends, gelatine was found to be the only bio-polymer which significantly increased the PAN fibre diameters, an effect which survived wetting the samples although no cross-linking step

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*Tekstilec, 2018, **61**(2), 129-135*
DOI: 10.14502/Tekstilec2018.61.129-135

was included [9]. Similarly, large fibre diameters could not be gained by modifying spinning and solution parameters of pure PAN [10]. Electrospinning PVA/gelatine blends was used to create scaffolds with modified hydrophobicity and morphology [11]. Similar results were found in ethyl cellulose/gelatine blends, which could be tailored between hydrophobic and hydrophilic properties, showing tuneable water stability [12]. Zein/gelatine blends were found to show high crystallinity, resulting in the preservation of a porous 3D structure gained by electrospinning, which was not possible in pure gelatine or pure zein nanofibers [13].

Blended with cellulose acetate, gelatine loaded with gabapentin was found to significantly increase injury regeneration in rats [14]. Similarly, gelatine-coated poly(butylene succinate) nanofiber mats could be used to immobilize thrombin, a haemostat, resulting in shorter haemostasis times and less blood loss than commercial gelatine sponges when tested in a rat liver model [15].

In the research, apart from some pre-tests with respect to pure gelatine, we examined the influence of citric acid on water resistance and morphology of pure gelatine fibres. This acid was shown to increase water stability of collagen/PEO nanofibers [16], of gelatine scaffolds which were not produced by electrospinning [17], and of electrospun native collagen fibres [18]. It must be mentioned that according to the literature, the bio-polymer which should be crosslinked and the citric acid need to be incubated for several hours and should afterwards be electrospun from the solution containing a high ratio of ethanol or similar solvents [19]. The latter was not possible in the electrospinning machine used in this project. Thus, our experiments aim at investigating whether the electrospinning process itself can replace the incubation process due to the large forces and high dynamics working during nanofiber formation. Electrospinning both materials together without former incubation has, to the best of our knowledge, not been reported before in the scientific literature and therefore represents a new approach to create water resistant gelatine nanofiber mats. If working, it would allow creating waterproof gelatine nanofiber mats without the necessity of using toxic cross-linkers afterwards, which could lead to the use of gelatine nanofiber mats for medical or biotechnological applications.

Additionally, the influence of coconut oil – which is known to work as a plasticizer [20] – was tested. Coconut oil also shows good antibacterial, anti-inflammatory and anti-viral properties [21, 22]. Electrospinning blends of gelatine and coconut oil were not found in the literature either, and might not only increase the possible application of such electrospun nanofiber due to combining the intrinsic medical properties of both materials, but may also stabilize gelatine to increase its water resistance.

2 Materials and methods

For electrospinning, a needleless electrospinning machine "Nanospider Lab" from Elmarco (Czech Republic) was used. The spinning parameters were as follows: temperature in the spinning chamber (21 ± 1) °C, relative humidity 33%, carriage speed 75–150 mm/s, substrate speed 0 mm/min, electrode distance 175–220 mm, electrode-substrate distance 50 mm, high voltage 70–75 kV between the lower electrode and the grounded upper electrode, and nozzle diameter 0.6–0.9 mm, depending on the viscosity of the spinning solutions. A polypropylene (PP) substrate (from Elmarco) was used as the substrate during electrospinning. A sketch of the electrospinning machine is shown in Figure 1.

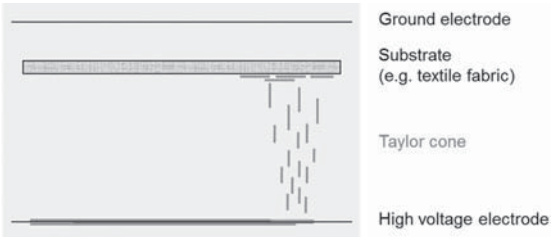

Figure 1: Electrospinning setup in "Nanospider Lab"

Gelatine powder was purchased from Abtei (Germany), coconut oil from BioWise (Germany). The following spinning solutions were prepared from these materials, using aq. dest. as a solvent (all fractions are given as weight percentage, all spinning solutions besides the one with coconut oil had room temperature during electrospinning):

- G1: 35% gelatine,
- G1b: 35 wt.-% gelatine, boiling the solution and cooling down before spinning,
- G2b: 40 wt.-% gelatine, boiling the solution and cooling down before spinning,
- GCA1: 33 wt.-% gelatine + 3 wt.-% citric acid (solid),

– GCA2: 31 wt.-% gelatine + 14 wt.-% citric acid (solid),
– GCA3: 27 wt.-% gelatine + 24 wt.-% citric acid (solid),
– GCA4: 24 wt.-% gelatine + 33 wt.-% citric acid (solid),
– GCO: 26 wt.-% gelatine + 26 wt.-% coconut oil (heated to ~ 40 °C before spinning to reduce viscosity).

An optical examination of nanofiber mats was performed using a confocal laser scanning microscope (CLSM) VK-9000 (Keyence, Germany) with a nominal magnification of 2000×. All scale bars have the dimension of 10 µm.
Water resistance tests were performed by immersing the nanofiber mats completely into water for 1 min and letting them dry at room temperature afterwards.
Fibre diameters were measured on 10 fibres per image using ImageJ 1.51j8 (National Institutes of Health, USA).

3 Results and discussion

The first experiments aimed at investigating the influence of boiling the gelatine solution on the nanofiber mat morphology, as compared to its working at room temperature. Figure 2 depicts nanofiber mats, prepared from the solutions G1 and G1b, spun at the voltage of 70 kV. The images show that, on the one hand, the fibres became thinner after boiling the solution, and on the other hand, spherical agglomerates were formed. Thinner fibres can be explained by the reduction of gel-building abilities of gelatine when it is boiled. Both changes in the nanofiber mat morphology are not desired; the relatively thick gelatine nanofibers are often preferable for the application in cell growth, compared to the thinner ones.
Consequently, in the next test, a slightly higher concentration of gelatine was used to prepare a solution which had to be boiled again before the spinning to slightly reduce the viscosity and thus support the spinning process. Higher concentrations often result in thicker fibres, as investigations of other polymers have shown [10,23]. Figure 3 depicts the results of this experiment. Unfortunately, in this way only relatively few, chaotically arranged nanofibers were produced, which after the spinning duration of 35 min merely covered the thick substrate fibres, without creating a visible nanofiber mat.

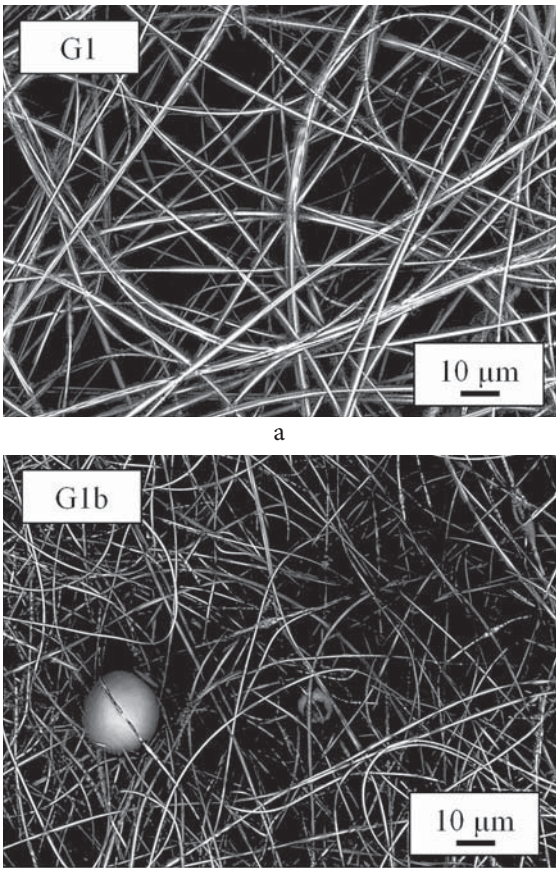

Figure 2: Nanofiber mat, created from solutions with 35% gelatine, prepared at room temperature (left panel, diameters (770 ± 210) nm) and after boiling, respectively (right panel, diameters (440 ± 130) nm)

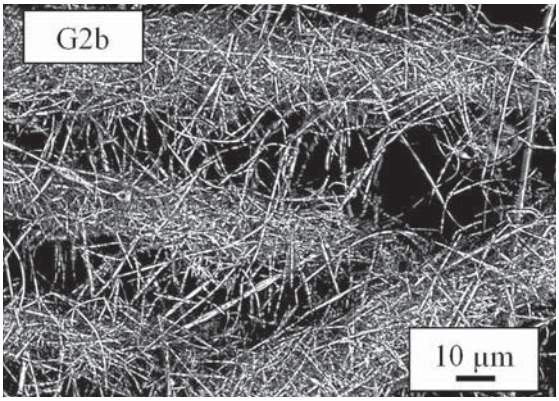

Figure 3: Nanofiber mat, created from solution with 40% gelatine, prepared after boiling (diameters (580 ± 140) nm)

Since former experiments in our group revealed a similar trend to significantly higher spinnability for slightly lower gelatine concentrations, the experiments with gelatine/citric acid blends were performed with reduced gelatine concentrations and without boiling.

The results are depicted in Figure 4. While sample GCA1, containing the highest gelatine and the lowest citric acid concentration, resulted in a mat of nanofibers with reduced diameters, increasing the citric acid concentration and at the same time decreasing the amount of gelatine resulted in electrospraying instead of electrospinning, with strongly changed droplet morphologies between spiky arrangements of very short nanofibers (GCA2) and smooth drops in a broad variety of diameters (GCA4). This finding can be explained by the well-known suppression of the gelling properties of gelatine in the presence of acids. The most interesting structure, however, is visible in sample GCA3. Here, a relatively thick coating on substrate fibres can be recognized, on top of which a labyrinth-like structure is revealed, looking like a strongly cross-linked net of thick gelatine fibres. It should be mentioned that the width of these features is approx. 1.5–2 μm, i.e. thicker than the nanofibers gained for sample GCA1 with the diameters in the range of 600–900 nm.

Unfortunately, the tests of water resistance of these nanofiber mats showed that all electrosprayed or electrospun structures were completely washed off of the substrate. The same occurred at all tests to increase water resistance by spraying citric acid onto pure gelatine fibre mats or vapour coating the electrospun gelatine mats with citric acid. It does not seem to be possible to increase water resistance of gelatine nanofiber mats in this way. Apparently, the incubation step mentioned in the literature cannot be replaced by the electrospinning process itself.

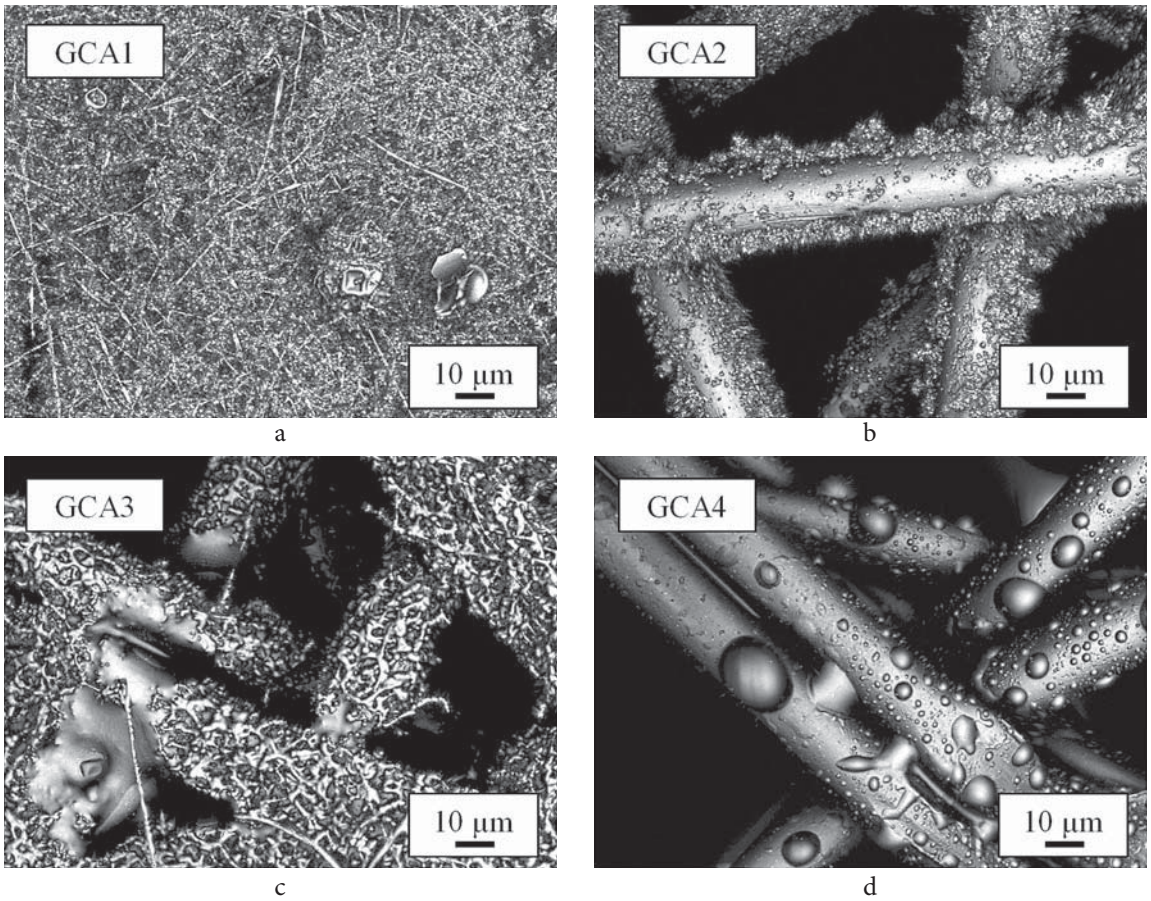

Figure 4: Nanofiber mats and electrosprayed samples GS1–GS4, prepared combining gelatine and citric acid, using carriage speed of 75 mm/s, electrode distance of 200 mm and high voltage of 75 kV

Finally, an experiment was performed combining gelatine with coconut oil. The solution could only be spun in a relatively warm state, i.e. for a limited time of approx. 30 min after heating the solution and putting it into the spinning equipment. The results of the electrospinning process and of wetting the sample afterwards are depicted in Figure 5.

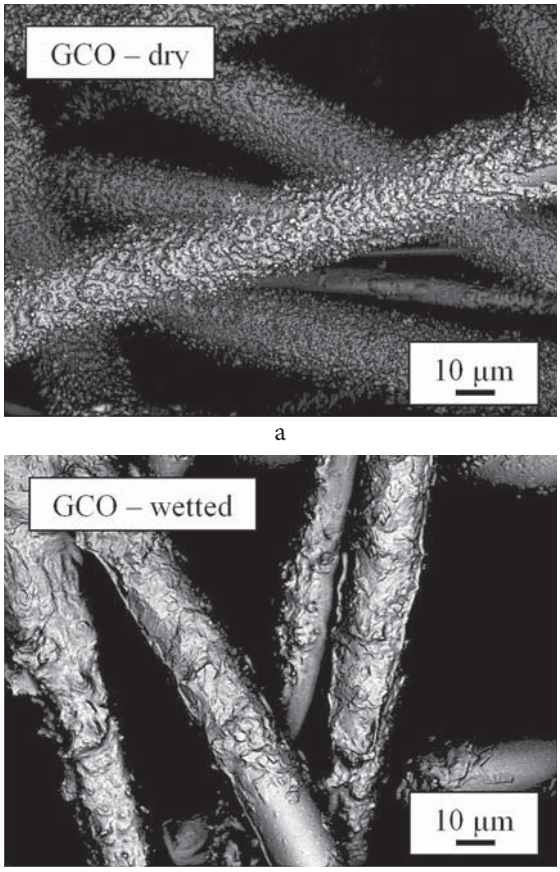

Figure 5: Electrosprayed samples GCO, prepared from warm gelatine/coconut oil solution, using carriage speed of 75 mm/s, electrode distance of 200 mm and high voltage of 75 kV

Figure 5 again shows an electrosprayed coating of the support nonwoven with another morphology than the other samples. Typically, electrospraying results in single, round dots, such as for sample GCA4. A similar structure, however, can be created by electrospraying poloxamer/dextran blends [24].
Opposite to the tests with gelatine/citric acid, which aimed at increasing water resistance compared to pure gelatine, adding coconut oil could indeed reduce the water solubility of the gelatine spray-coating. As depicted in Figure 5 (right panel), the surface morphology clearly changed after wetting the sample with a drop of water which dried on the fabric at room temperature, not washing off the coating. This finding is consistent with former experiments on casein, for which water stability could be significantly increased by adding wax or paraffin oil to the spinning solution [25]. We assume that this reduction of water solubility can be attributed to closing small pores in the coating, thus reducing the contact area between gelatine and water [26]. This suggests further experiments with different gelatine : coconut oil ratios to create nanofibers which may also show increased water resistance, making them suitable for a slow release of medical drugs etc.

4 Conclusion and outlook

To conclude, this study shows that citric acid can be used to modify the nanofiber mat morphology and create not only thinner fibres than with pure gelatine, but also different sprayed structures, including a labyrinth-like surface. Coconut oil, on the other hand, has indeed increased the water resistance of the resulting electrosprayed coatings.
Future research will concentrate on combining both features, i.e. creation of nanofiber mats or labyrinth-like coatings with increased water resistance, possibly also using the combination of coconut oil and citric acid to enable the use of such materials for drug delivery and similar applications where a large surface : volume ratio of nanofibers should be combined with slow dissolving in aqueous environments.

References

1. SROUJI, Samer, KIZHNER, Tali, SUSS-TOBI, E., LIVNE, E., ZUSSMAN, Eyal. 3-D Nanofibrous electrospun multilayered construct is an alternative ECM mimicking scaffold. *Journal of Materials Science: Materials in Medicine*, 2008, **19**(3), 1249–1255, doi: 10.1007/s10856-007-3218-z.
2. NAGHIBZADEH, Majid, ADABI, Mahdi. Evaluation of effective electrospinning parameters controlling gelatin nanofibers diameter via modelling artificial neural networks. *Fibers and Polymers*, 2014, **15**(4), 767–777.

3. PANKOV, Roumen, YAMADA, Kenneth M. Fibronectin at a glance. *Journal of Cell Science*, 2002, **115**, 3861–3863, doi: 10.1242/jcs.00059.
4. MINCHEVA, Rosica, MANOLOVA, Nevena, PANEVA, Dilyana, RASHKOV, Iliya. Preparation of polyelectrolyte-containing nanofibers by electrospinning in the presence of a non-ionogenic water-soluble polymer. *Journal of Bioactive and Compatible Polymers*, 2005, **20**(5), 419–435, doi: 10.1177/0883911505057447.
5. NAGHIBZADEH, Majid, FIROOZI, Saman, NODOUSHAN, Fatemeh Sadeghian, ADABI, Mohsen, KHORADMEHR, Arezoo, FESAHAT, Farzaneh, ESNAASHARI, Seyedeh Sara, KHOSRAVANI, Masood, ADABI, Mandi, TAVAKOL, Shima, PAZOKI-ROROUDI, Hamidreza, ADEL, Moein, ZAHMATKESHAN, Masoumeh. Application of electrospun gelatin nanofibers in tissue engineering. *Biointerface Research in Applied Chemistry*, 2018, **8**(1), 3048–3052.
6. CHOMACHAYI, Masoud Dadras, SOLOUK, Atefeh, AKBARI, Somaye, SADEGHI, Davoud, MIRAHMADI, Fereshteh, MIRZADEH, Hamid. Electrospun nanofibers comprising of silk fibroin/gelatin for drug delivery applications: Thyme essential oil and doxycycline monohydrate release study. *Journal of Biomedical Materials Research Part A*, 2018, **106**(4), 1092–1103, doi: 10.1002/jbm.a.36303.
7. TAVASSOLI-KAFRANI, Elham, GOLI, Sayed Amir Hossein, FATHI, Milad. Fabrication and characterization of electrospun gelatin nanofibers crosslinked with oxidized phenolic compounds. *International Journal of Biological Macromolecules*, 2017, **103**, 1062–1068, doi: 10.1016/j.ijbiomac.2017.05.152.
8. LAHA, Anindita, YADAV, Shital, MAJUMDAR, Saptarshi, SHARMA, Chandra S. In-vitro release study of hydrophobic drug using electrospun cross-linked gelatin nanofibers. *Biochemical Engineering Journal*, 2016, **105**, 481–488, doi: 10.1016/j.bej.2015.11.001.
9. WEHLAGE, Daria, BÖTTJER, Robin, GROTHE, Timo, EHRMANN, Andrea. Electrospinning water-soluble/insoluble polymer blends. *AIMS Materials Science*, 2018, **5**(2), 190–200, doi: 10.3934/matersci.2018.2.190.
10. GROTHE, Timo, WEHLAGE, Daria, BÖHM, Tobias, REMCHE, Alexander, EHRMANN, Andrea. Needleless Electrospinning of PAN Nanofiber Mats. *Tekstilec*, 2017, **60**(4), 290–295, doi: 10.14502/Tekstilec2017.60.290-295.
11. PEREZ-PUYANA, Victor, JIMENEZ-ROSADO, Mercedes, ROMERO, A., GUERRERO, Antonio. Development of PVA/gelatin nanofibrous scaffolds for Tissue Engineering via electrospinning. *Materials Research Express*, 2018, **5**(3), 035401, doi: 10.1088/2053-1591/aab164.
12. LIU, Yuyu, DENG, Lingli, ZHANG, Cen, FENG, Fengqin, ZHANG, Hui. Tunable physical properties of ethylcellulose/gelatin composite nanofibers by electrospinning. *Journal of Agricultural and Food Chemistry*, 2018, **66**(8), 1907–1915, doi: 10.1021/acs.jafc.7b06038.
13. DENG, Lingli, ZHANG, Xi, LI, Yang, QUE, Fei, KANG, Xuefan, LIU, Yuyu, FENG, Fengqin, ZHANG, Hui. Characterization of gelatin/zein nanofibers by hybrid electrospinning. *Food Hydrocolloids*, 2018, **75**, 72–80, doi: 10.1016/j.foodhyd.2017.09.011.
14. FARZAMFAR, Saeed, NASERI-NOSAR, Mahdi, VAEZ, Ahmad, ESMAEILPOUR, Farshid, EHTERAMI, Arian, SAHRAPEYMA, Hamed, SAMADIAN, Hadi, HAMIDIEH, Amir-Ali, GHORBANI, Sadegh, GOODARZI, Arash, AZIMI, Arian, SALEHI, Majid. Neural tissue regeneration by a gabapentin-loaded cellulose acetate/gelatin wet-electrospun scaffold. *Cellulose*, 2018, **25**(2), 1229–1238, doi: 10.1007/s10570-017-1632-z.
15. CHENG, Hui-Hui, XIONG, Jiang, XIE, Zhi-Ning, ZHU, Ya-Ting, LIU, Yu-Man, WU, Zhong-Yin, YU, Jian, GUO, Zhao-Xia. Thrombin-loaded Poly(butylene succinate)-based electrospun membranes for rapid hemostatic application. *Macromolecular Materials and Engineering*, 2018, **303**(2), 1700395, doi: 10.1002/mame.201700395.
16. GAO, Jing, GUO, Huiwen, ZHAO, Linshuang, ZHAO, Xinzhe, WANG, Lu. Water-stability and biological behavior of electrospun collagen/PEO fibers by environmental friendly crosslinking. *Fibers and Polymers*, 2017, **18**(8), 1496–1503, doi: 10.1007/s12221-017-7319-0.
17. JIANG, Qiuran, XU, Helan, CAI, Shaobo, YANG, Yiqi. Ultrafine fibrous gelatin scaffolds with deep cell infiltration mimicking 3D ECMs for soft tissue repair. *Journal of Materials Science: Materials in Medicine*, 2014, **25**(7), 1789–1800, doi: 10.1007/s10856-014-5208-2.

18. JIANG, Qiuran, REDDY, Narendra, ZHANG, Simeng, ROSCIOLI, Nicholas, YANG, Yiqi. Water-stable electrospun collagen fibers from a non-toxic solvent and crosslinking system. *Journal of Biomedical Materials Research Part A*, 2013, **101**(5), 1237–1247, doi: 10.1002/jbm.a.34422.
19. JIANG, Qiuran, REDDY, Narendra, YANG, Yiqi. Cytocompatible cross-linking of electrospun zein fibers for the development of water-stable tissue engineering scaffolds. *Acta Biomaterialia*, 2010, **6**(10), 4042–4051, doi: 10.1016/J.ACTBIO.2010.04.024.
20. CHAVEZ GUTIERREZ, Miguel, DEL CARMEN NUNEZ-SANTIAGO, Maria, ROMERO-BASTIDA, Claudia Andrea, MARTINEZ-BUSTOS, Fernando. Effects of coconut oil concentration as a plasticizer and Yucca schidigera extract as a surfactant in the preparation of extruded corn starch films. *Starch – Stärke*, 2014, **66**(11–12), 1079–1088, doi: 10.1002/star.201400062.
21. EL-NAGGAR, Mehrez E., SHAFIE, Shaarawy A. El., HEBEISH, A. Development of antimicrobial medical cotton fabrics using synthesized nanoemulsion of reactive cyclodextrin hosted coconut oil inclusion complex. *Fibers and Polymers*, 2017, **18**(8), 1486–1495, doi: 10.1007/s12221-017-7390-6.
22. MUKTAR, Muhammad Zulhemi, ROSE, Laili Che, AMIN, Khairul Anuar Mat. Formulation and optimization of virgin coconut oil with Tween-80 incorporated in gellan gum hydrogel. In *3rd Electronic and Green Materials International Conference 2017 : AIP Conference Proceedings*. Krabi, Thailand, 2017, **1885**, 020044-1, doi: 10.1063/1.5002238.
23. SABANTINA, Lilia, MIRASOL, José Rodríguez, CORDERO, Tomás, FINSTERBUSCH, Karin, EHRMANN, Andrea. Investigation of needleless electrospun pan nanofiber mats. *AIP Conference Proceedings*, 2018, **1952**, 020085, doi: 10.1063/1.5032047.
24. BÖTTJER, Robin, GROTHE, Timo, WEHLAGE, Daria, EHRMANN, Andrea. Electrospraying poloxamer/(bio-)polymer blends using a needleless electrospinning machine. *Journal of Textiles and Fibrous Materials*, 2018, **1**, 1–7, doi: 10.1177/2515221117743079.
25. BIER, Marie Carolin, KOHN, Sophia, STIERAND, Antonia, GRIMMELSMANN, Nils, HOMBURG, Sarah Vanessa, RATTENHOLL, Anke, EHRMANN, Andrea. Investigation of eco-friendly casein fibre production methods. *IOP Conf. Series: Materials Science and Engineering*, 2017, **254**, 192004, doi: 10.1088/1757-899X/254/19/192004.
26. LAI, Huey-Min PADUA, Graciela W. Properties and microstructure of plasticized zein films. *Cereal Chemistry*, 1997, **74**, 771–775, doi: 10.1094/CCHEM.1997.74.6.771.