Biocompatible and antibacterial gelatin-based polypyrrole cryogels

Biocompatible and antibacterial gelatin-based polypyrrole cryogels

Konstantin A. Milakin[a], Zdenka Capáková[b], Udit Acharya[a], Jan Vajdak[b], Zuzana Morávková[a], Jirí Hodan[a], Petr Humpolícek[b], Patrycja Bober[a'*]

[a] *Institute of Macromolecular Chemistry, Academy of Sciences of the Czech Republic, 162 06, Prague 6, Czech Republic*

[b] *Centre of Polymer Systems and Faculty of Technology, Tomas Bata University in Zlin, 760 01, Zlin, Czech Republic*

** Corresponding author. E-mail address: bober@imc.cas.cz (P. Bober).*

ABSTRACT

Polypyrrole-gelatin cryogels were synthesized by oxidative cryopolymerization of pyrrole in the presence of gelatin. Vibrational spectroscopy confirmed formation of doped polypyrrole. Mechanical and pore structure stability of the resulting macroporous materials (pore size 10-50 µm) was shown to increase with increasing of gelatin content in initial polymerization medium, reaching plateau at 6 wt%. Thermal stability of polypyrrole-gelatin cryogels was found to be enhanced in comparison to its individual components. Electrical conductivity of all prepared cryogels (2-5 S cm^{-1}) was similar to that of conventional polypyrrole. Cytotoxicity of polypyrrole-gelatin cryogels was found to decrease with increasing of gelatin concentration in the initial reaction mixture. The cryogel obtained using 8 wt% of gelatin showed the lowest cytotoxic effect reaching only mild cytotoxicity at 100% extract concentration. Polypyrrole-gelatin gels showed significant level of antibacterial activity without additional antibacterial agents.

Keywords: Polypyrrole, Gelatin, Cryogels, Conductivity, Cytotoxicity, Antibacterial properties

1. Introduction

Polypyrrole hydrogels is a promising class of materials due to the combination of conductivity and physicochemical properties of polypyrrole with mechanical properties of a polymer support which allows overcoming limited processibility of the conducting polymer **[1-9]**. They find their applications as tissue scaffolds [5], supercapacitors **[10]**, electrode materials **[11]**, drug release devices **[8]** and biosensors **[12]**.

Recently, a novel one-step approach for preparation of polypyrrole hydrogels was reported **[13]**. It involved oxidative polymerization of pyrrole in the frozen medium in the presence of poly(vinyl alcohol) which led to formation of soft and conducting cryogels with macroporous structure and uniform distribution of a conducting component across the gel volume. These cryogels were shown to have conductivity (18 S cm^{-1}) higher than conventional polypyrrole powder prepared without a polymer support **[14]**. Moreover, stability of conductivity values towards deprotonation at physiological pH and low cytotoxicity of the materials made them potentially attractive for biological applications.

Gelatin is a mixture of proteins which is obtained by hydrolysis of collagen **[15]**. Due to its biocompatibility, biodegradability and wide commercial availability at a relatively low cost, gelatin is widely used in biomedical and biochemical applications, especially for drug delivery and tissue engineering **[16-20]**. Gelatin and its derivatives are also attractive components for conducting hydrogels. Gelatin-based hydrogels containing polyaniline **[20,21]**, polypyrrole **[19,22]** and poly(3, 4-ethylenedioxythiophene) **[18,23,24]** are usually prepared in several steps: the gelatin gel is obtained after polymerization of a conducting component or the polymerization is performed after the gel formation. It complicates the procedure and introduces a factor of an uneven distribution of a conducting phase in the final material, which requires additional control. Therefore, we have decided to apply the above mentioned approach of cryopolymerization for a one-step synthesis of polypyrrole-gelatin cryogels which combine material uniformity provided by the preparation technique with intrinsic biocompatibility of gelatin.

In the present paper a single-step preparation of polypyrrole-gelatin cryogels have been performed by cryopolymerization. Influence of gelatin concentration in initial polymerization mixture on morphology, conductivity and mechanical properties of the materials has been studied. Cytotoxicity and antibacterial properties of the cryogels have been assessed.

2. Experimental section

2.1. Polypyrrole-gelatin cryogel preparation

For preparation of polypyrrole-gelatin cryogels, pyrrole (10 mmol, Sigma Aldrich) and iron (III) chloride hexahydrate (25 mmol, Sigma Aldrich) were dissolved separately in 25 ml of aqueous gelatin (from porcine skin, Fluka) solution each with concentration of gelatin varying from 2 wt% to 8 wt%. The monomer and oxidant solutions were mixed, quickly sucked into plastic syringes, frozen in dry ice/ethanol bath and left to polymerize in a freezer at — 24 °C for 7 days. After thawing at room temperature, the cryogels were removed from the syringes, washed with excess of water and freeze-dried. Some of the gels were washed with excess of 0.01 M hydrochloric acid, acetone and dried in air at room temperature for conductivity measurements.

2.2. Material characterization

Morphology of the freeze-dried materials was assessed using MAIA3 Tescan scanning electron microscope. Thermogravimetric analysis (TGA) was performed with a PerkinElmer Pyris 1 thermogravimetric analyzer in temperature range 30-900 °C at a rate of 10 °C min^{-1} in air. Static mechanical properties of the water-swollen cryogels (diameter 3 mm, length 60 mm) were evaluated on electromechanical testing machine Instron 6025/5800R equipped with a 10 N load cell at room temperature in deionized water and with a cross-head speed of 10 mm min^{-1}. Room temperature DC electrical conductivity was measured by van der Pauw method with four gold plated spring loaded electrodes on compressed pellets having 13 mm diameter and thickness 1 ± 0.3 mm at relative humidity 35 ± 5% using a Keithley 230 Programmable Voltage Source with a Keithley 196 System DMM in serial connection and a Keithley 181 Nanovoltmeter.

FTIR spectra of the samples were obtained using a Golden Gate Diamond ATR Top-Plate with a Thermo Nicolet NEXUS 870 FTIR spectrometer equipped with a DTGS detector in the wavenumber range from 600 to 4000 cm^{-1}.

Raman spectra were measured with a Renishaw InVia Reflex Raman microspectrometer. The spectra were excited with a diode 785 nm laser. The scattered light was registered with a Peltier-cooled CCD detector (576 x 384 pixels) and analyzed by the spectrograph with holographic grating 1200 lines mm^{-1} for the respective laser excitations.

2.3. Cytotoxicity analysis

Cytotoxicity was tested using mouse embryonic fibroblast cell line (ATCC CRL-1658 NIH/3T3) and performed in accordance with ISO 10 993 series. The ISO 10 993-5 was used for cytotoxicity testing and ISO 10 993-12 for preparation of extracts. The ATCC-formulated Dulbecco's Modified Eagle's Medium (Biosera) containing 10% of calf serum (BioSera) and 100 U ml^{-1} Penicillin/Streptomycin (GE Healthcare HyClone), was used as the culture medium in all experiments. The cytotoxicity testing was done using extracts of native material.

The extraction was done by pouring 0.1 g of tested material into 1 ml of culture medium and cultivation for 24 h at 37 °C with stirring. The parent extracts (100%) were filtered through 0.22 µm syringe filter (TPP) and then diluted with fresh culture medium to obtain a series of dilutions with concentrations of 75, 50, 25, 10, and 1% of extracts in culture medium. All extracts were used up to 24 h after preparation and all tests were performed in quadruplicates. Prior the testing, the cells were seeded at concentration 1 x 10^4 cells per well (96 well plates were used, TPP) and precultivated for 24 h. Subsequently, the culture medium was replaced with diluted extracts and cells were cultivated in the presence of tested extracts for 24 h. MTT cell proliferation assay kit (Duchefa Biochemie) was used to determine cell viability. The absorbance was measured at 570 nm and the reference wavelength was adjusted on 690 nm. The results are presented as reduction of cell viability in relative values when compared to reference (cells cultivated in medium without the extracts of tested materials).

2.4. Antibacterial testing

The antibacterial testing was conducted on Staphylococcus aureus (CCM 4516) and Escherichia coli (CCM 4517). The bacterial suspension (mixture of both strains) was diluted in nutrient broth to final concentration of 2.8 x 10^6 CFU/1 ml of E. Coli and 1.4 x 10^7 CFU/1 ml of S. Aureus and added to the samples (10 ml-340 mm^2). Samples with bacterial suspension were shaken for 24 h at 37 °C. As a reference only pure bacterial suspension without samples was used. The following day, bacterial suspensions were transferred from each sample into plastic tube and subsequently diluted following the decimal dilutions. The individual suspensions were homogenized and pipetted (1 ml) on Plate Count Agar. Bacterial suspensions were spread all over the agar surface. Plates were placed to the incubator in temperature 37 ° C for 24 h. The next day, the grown colonies were counted using a colony counter. Bacterial activity was determined by comparing the growth of colonies in samples against reference. The clean bacterial suspension was used as reference.

For each sample, the antibacterial activity was calculated using Equation **(1)**:

$$R = U_t - A_t \qquad (1)$$

R - the antibacterial activity;

U_t - the average of the common logarithm of the number of viable bacteria, in CFU/ml, recovered from the untreated test specimens after 24 h;

A_t - the average of the common logarithm of the number of viable bacteria, in CFU/ml, recovered from the treated test specimens after 24 h.

3. Results and discussion

Polypyrrole-gelatin cryogels were prepared by oxidative cry-opolymerization of pyrrole by iron (III) chloride in the presence of various concentrations of gelatin as a stabilizer. Syntheses in the presence of 2 and 3 wt% of gelatin led to formation of the gels that were mechanically unstable and were destroyed during washing. Therefore, only cryogels which were prepared at the concentrations of gelatin ranging from 4 to 8 wt% will be described further due to them having sufficient mechanical strength for being studied.

Influence of gelatin concentration on morphology of polypyrrole-gelatin cryogels was assessed by SEM (**Fig. 1**).

SEM images (**Fig. 1**) show that all polypyrrole-gelatin cryogels have macroporous structure with pore size in the range of 10-50 μm. The cryogel synthesized using the smallest amount of gelatin (4 wt%, **Fig. 1**a) has partially collapsed pores which might be explained by poor mechanical strength of the material due to a relatively low amount of a polymer support. In contrast, the materials prepared at higher amounts of gelatin (6 wt% and 8 wt%, **Fig. 1**b and c, respectively) have much better defined pores with approximate size of 10-25 μm. Therefore, it can be concluded that the increase of the gelatin amount in the reaction mixture leads to improvement of structural and mechanical stability of resulting polypyrrole-gelatin cryogels. To further reinforce this conclusion, tensile parameters of water swollen polypyrrole-gelatin hydrogels were investigated **(Table 1).**

As can be seen from **Table 1**, there is a notable improvement in mechanical characteristics of the cryogels such as tensile strain at break, tensile stress at break and tensile modulus with increasing gelatin content in the reaction mixture starting at 6 wt% of gelatin. Both tensile stress at break and tensile modulus increased $\sim$10 times for the cryogels prepared using $\geq$6 wt% of the polymer in comparison to the materials which contain less amount of the stabilizer. The fact that after increasing of the gelatin content up to 6 wt% mechanical parameters of the composite cryogels reach the plateau means that starting from this composition mechanical stability of the materials is determined primarily by gelatin. It should also be especially noted that polypyrrole-gelatin cryogels have much higher mechanical performance compared to polypyrrole-poly(vinyl alcohol) cryogels reported earlier **[13],** where the discussed tensile parameters were up to 10 times lower. Thus, substitution of poly(vinyl alcohol) with gelatin as the polymer stabilizer of the polypyrrole cryogels is beneficial for improvement of overall mechanical stability of the materials.

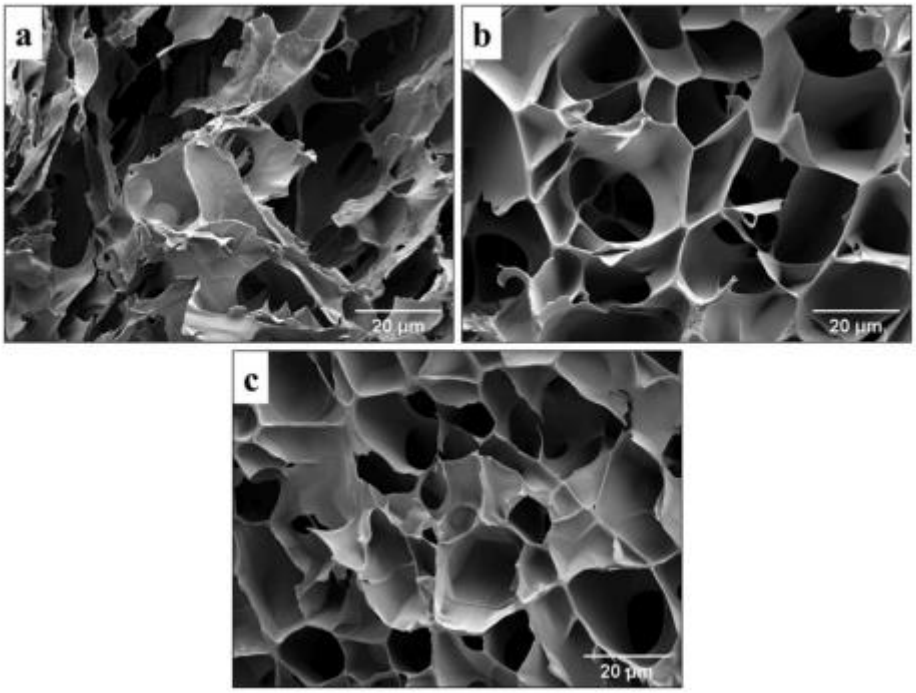

Fig. 1. SEM images of polypyrrole-gelatin cryogels synthesized using different gelatin concentrations: a) 4 wt%, b) 6 wt% and c) 8 wt%.

Table 1 Mechanical properties and conductivity of polypyrrole-gelatin cryogels prepared at various gelatin concentrations.

Gelatin concentration, wt %	Tensile strain at break, %	Tensile stress at break, kPa	Tensile modulus, kPa	Conductivity, S cm^{-1}
4	21 ± 2	2.6 ± 0.6	33 ± 9	3.6
5	16 ± 4	2.3 ± 0.3	24 ± 14	3.1
6	47 ± 9	18 ± 4	286 ± 24	1.9
7	32 ± 23	13 ± 7	357 ± 8	2.7
8	40 ± 7	10 ± 3	247 ± 91	4.7

Thermal stability of polypyrrole-gelatin cryogels was assessed by TGA **(Fig. 2).** As can be seen from **Fig. 2**, thermogravimetric curves of polypyrrole-gelatin cryogels look similar to the curve of pristine gelatin, which was used as a reference. They consist of 3 decomposition regions: up to about 150-200 °C corresponding to removal of water, ≈260-500 °C and ≈600-820 °C related to decomposition of different fragments of gelatin chains, containing proline and glycine, respectively **[25]**. It should be noted that a main mass loss of neat polypyrrole happens at about 300-580 °C **[26]** so in the case of polypyrrole-gelatin cryogels it can overlap with decomposition of gelatin. According to the published data **[26]**, pristine polypyrrole is fully decomposed at temperatures above 600 °C, while for the cryogels, full decomposition temperature is shifted to about 800 °C. It shows their enhanced thermal stability in the high temperature region compared to polypyrrole, which is likely attributed to the impact of gelatin having similarly high full decomposition temperature. Polypyrrole-gelatin cryogels have also much better thermal stability than pristine gelatin in the temperature region 300-800 °C. Increasing gelatin amount used for the cryogels preparation leads to higher cryogel mass loss in the mentioned temperature region shifting the material's behavior towards pristine gelatin.

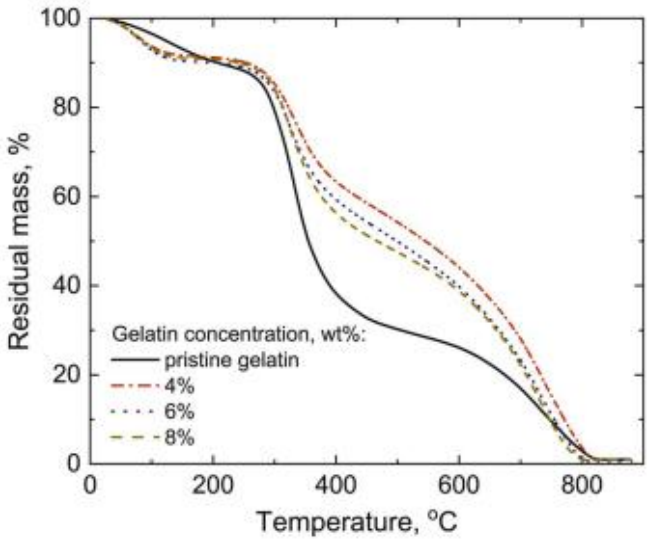

Fig. 2. Thermogravimetric curves of polypyrrole-gelatin cryogels prepared using different gelatin concentrations in the initial reaction mixture and pristine gelatin (recorded in air).

Therefore, we can conclude that addition of gelatin into the polypyrrole-based cryogels allows improvement of their thermal stability in comparison to individual components, however, it requires careful optimization in order to find a concentration of gelatin corresponding to a maximum of thermal stability.

According to our data **(Table 1)**, conductivity of polypyrrole-gelatin cryogels does not significantly depend on the gelatin content in initial polymerization mixture. For all the samples, conductivity was found to be in the range of 2-5 S cm^{-1} which is within the same order of magnitude as conventional polypyrrole powder prepared at room temperature without gelatin **[27]**. Therefore, it might be concluded that at the studied cryogel compositions content of polypyrrole is enough to form conducting pathways and negate the influence of non-conducting gelatin on the material conductivity.

Molecular structure of polypyrrole-gelatin cryogels was studied by vibrational spectroscopy. The FTIR spectra of the cryogels are compared with the FTIR spectra of pristine polypyrrole and gelatin **(Fig. 3)**. The bands of gelatin are located at 1088, 1235, 1440, 1524 and 1625 cm^{-1}, however, they overlap strongly with polypyrrole bands with the exception of the band at 1235 cm^{-1} that is not detected in the composites. The main FTIR bands of polypyrrole in the polypyrrole-gelatin cryogels are located at 1008 cm^{-1} (in-plane C-H and N-H deformation vibrations), 1128 cm^{-1} (ring breathing), 1272 cm^{-1} (C-N stretching), 1440 cm^{-1} (C-N stretching), 1524 cm^{-1} (C-C stretching in the pyrrole ring) and 1665 cm^{-1} (C-C stretching) **[28,29]**.

The Raman spectra of the cryogels **(Fig. 4)** correspond well to polypyrrole with its major bands at 922 cm^{-1} (ring deformation in neutral and polaron structures), 940 cm^{-1} (ring deformation in bipolaron structure), 1050 cm^{-1} (in-plane C-H deformation in neutral or polaron structures), 1082 cm^{-1} (in-plane C-H stretching in bipolaron structure), 1243 cm^{-1} (in-plane C-H deformation), 1328 cm^{-1} (ring stretching in neutral and polaron structures), 1380 cm^{-1} (ring stretching in bipolaron structure) and 1600 cm^{-1} (ring stretching in polaron and bipolaron structures) **[30-33]**. The Raman bands of gelatin should not overlap with those of polypyrrole as is seen from the Raman spectrum of pristine gelatin **(Fig. 4)**, but gelatin is not detected as its concentration is low and the polypyrrole signal is resonantly enhanced - specifically the bipo-laron structures. Therefore, according to the spectroscopy data, the prepared cryogels contain doped polypyrrole.

As can be seen from **Fig. 5**, cytotoxicity of polypyrrole-gelatin cryogels correlates with the amount of gelatin used for the preparation of the materials. With higher amount of gelatin, cytotoxicity decreases. The highest cytotoxicity was observed for the cryogels prepared at 4 wt% of gelatin, for which the cytotoxic effect disappeared only at the extract concentration 10%, while concentrations 50% and 75% reached moderate cytotoxicity and extract in concentration 100% even severe cytotoxicity. Anyway, all other materials did not reach higher than mild level of cytotoxicity at any extract concentration. In case of polypyrrole-gelatin cryogel prepared using 8 wt% of gelatin, even the extract concentration 75% had no harmful effect and 100% extract showed only mentioned mild cytotoxicity. Gelatin is a biocompatible material **[34]**, therefore it obviously does not cause any cytotoxic effect. Likewise, it was proved by Humpolícek et al. **[35]** that polypyrrole itself does not invoke cytotoxicity.

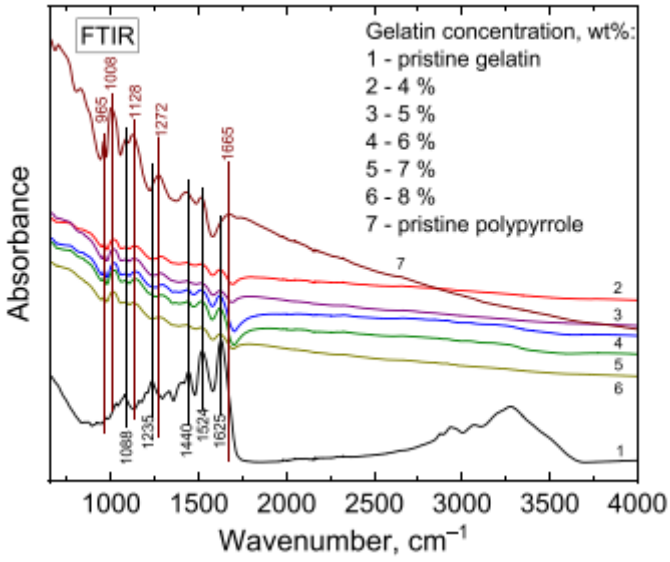

Fig. 3. ATR FTIR spectra of polypyrrole-gelatin cryogels in comparison with pristine polypyrrole and gelatin.

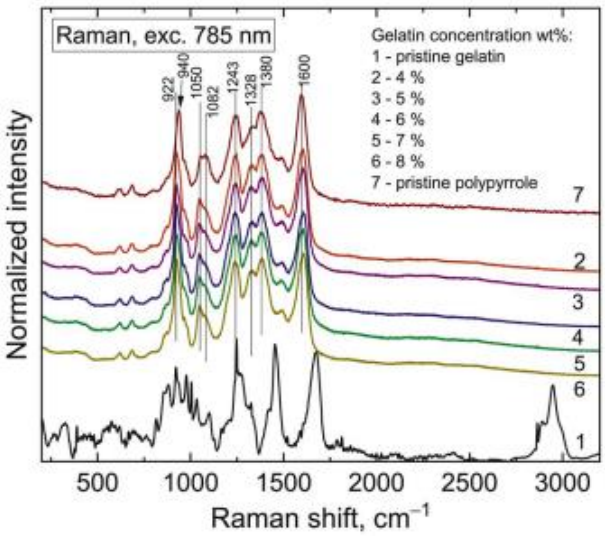

Fig. 4. Raman spectra of polypyrrole-gelatin cryogels, pristine polypyrrole and gelatin excited with the 785 nm laser line.

Hence, the cytotoxic effect is connected with potential byproducts generated during the polymerization. It should be especially noted that according to the cytotoxicity testing, gelatin supported materials achieved significantly better cytocompatibility compared to polypyrrole cryogels prepared in the presence of poly(vinyl alcohol) reported by Bober et al. **[13]** This effect might be potentially explained by better retention of toxic low-molecular weight products by the cryogels due to their interaction with gelatin, which decreases the overall cytotoxicity, or by lower amount of the produced byproducts when gelatin is used as a support instead of poly(vinyl alcohol).

According to **Table 2**, all studied polypyrrole-gelatin cryogels exhibit some level of antibacterial activity, which was considered either as weak or significant according the C SN EN ISO 20743: 2014. Cryogels prepared using 6 and 7 wt% of gelatin showed weak antibacterial activity whereas the other composites reached significant one. The highest antibacterial effectiveness was observed in case of the material synthesized in the presence of 8 wt% of gelatin. To the best of our knowledge, there is no study of antibacterial testing for polypyrrole cryogels or hydrogels. However, in many works the antibacterial effect of polypyrrole composites is supported by inorganic or metal particles, such as zinc oxide **[36]** or silver **[37]**. Silver nanoparticles are the most commonly used in this type of materials. For example, in the study of Wan and Li **[38]** cellulose aerogels functionalized with polypyrrole and silver nanoparticles inhibit growth of E. coli and S. aureus. In another study, membranes based on cellulose, polypyrrole and Ag-nanoparticles also showed antibacterial properties **[39].** However, in the mentioned studies the main antibacterial effect was caused by the use of Ag-nanoparticles. Nevertheless, application of silver nanoparticles for tissue engineering is still not well accepted **[40]**. There is a potential risk that they can lead to adverse effect such as inducing, for example, cytotoxicity or genotoxicity **[41].** In this study, the antibacterial effect was achieved with pure polypyrrole-gelatin cryogels without addition of any antibacterial agents.

4. Conclusions

Polypyrrole-gelatin cryogels were successfully prepared by efficient one-step oxidative cryopolymerization. The resulting composites, containing doped polypyrrole, were found to have higher thermal stability than the individual components. Increasing gelatin concentration in initial reaction mixture was shown to improve mechanical and structural stability as well as biocompatibility of the materials, while conductivity remained similar to the value of conventional polypyrrole powder. Polypyrrole-gelatin cryogels were found to have significant antibacterial effect without additional antibacterial agents. According to the described properties, we believe that polypyrrole-gelatin cryogels are novel, promising materials for potential biomedical applications.

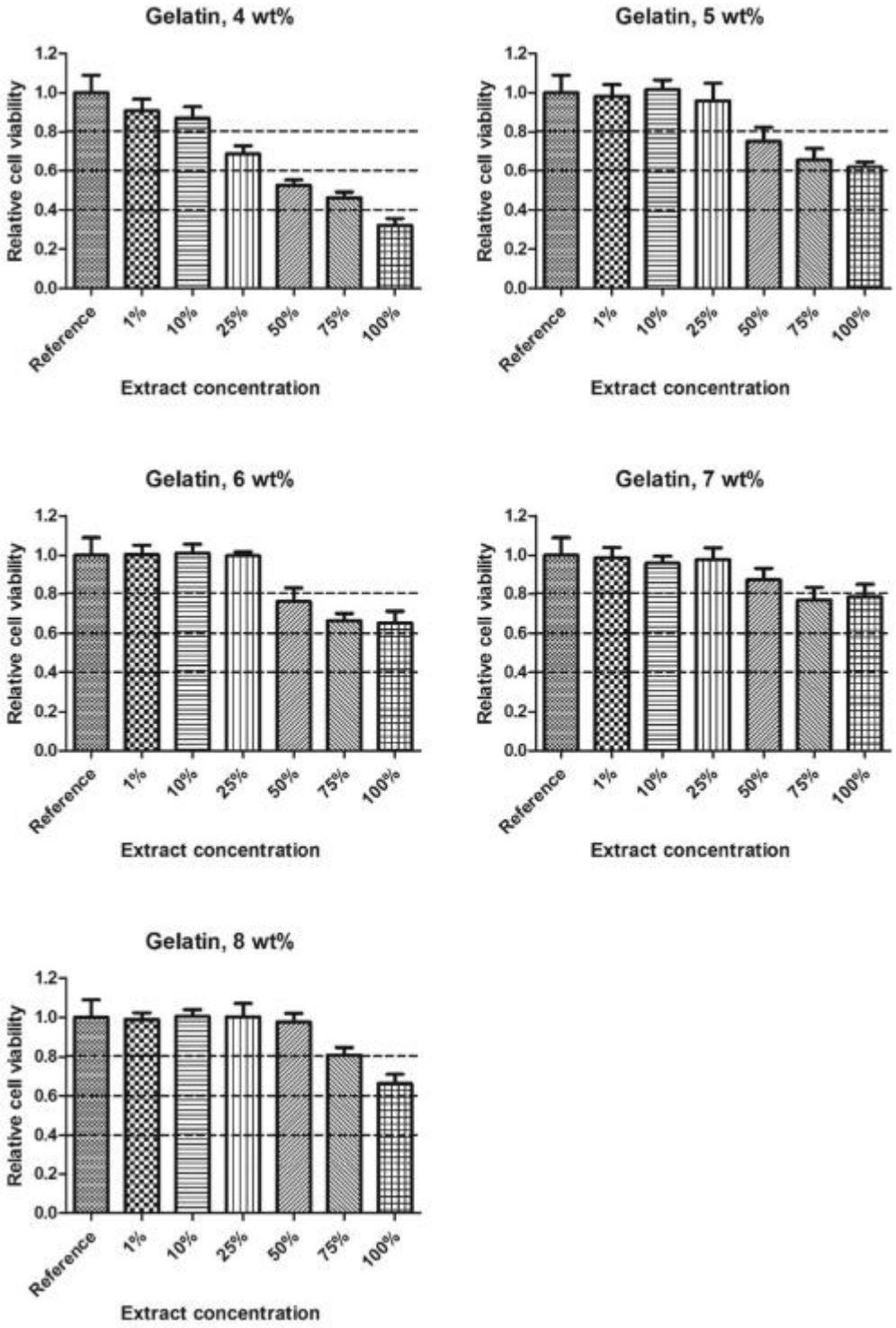

Fig. 5. Cytotoxicity of extracts from polypyrrole-gelatin cryogels prepared at different gelatin concentrations. The dashed lines highlight the limits of viability according to EN ISO 10993-5 where viability >0.8 means no cytotoxicity, 0.6-0.8 mild cytotoxicity, 0.4-0.6 moderate cytotoxicity, <0.4 severe cytotoxicity.

Table 2 Antibacterial properties of polypyrrole-gelatin cryogels synthesized in the presence of different gelatin concentrations.

Gelatin concentration, wt%	CFU/ml	R[a]
4	1.5×10^5	2.2
5	1.9×10^5	2.1
6	1.1×10^6	1.4
7	5.1×10^5	1.7
8	9.0×10^4	2.4
Reference	2.7×10^7	–

[a] *Note: According the CSN EN ISO 20743: 2014, the values of antibacterial activity 1 < R < 2 mean weak antibacterial effectiveness, 2 = R < 3 - significant antibacterial effectiveness, R > 3 - strong antibacterial effectiveness.*

References

[1] N. Sahiner, S. Demirci, N. Aktas, Superporous cryogel/conductive composite systems for potential sensor applications, J. Polym. Res. 24 (2017) 126, https:// doi.org/10.1007/s10965-017-1288-2.

[2] N. Sahiner, S. Demirci, In situ preparation of polyaniline within neutral, anionic, and cationic superporous cryogel networks as conductive, semi-interpenetrating polymer network cryogel composite systems, J. Appl. Polym. Sci. 133 (2016), https://doi.org/10.1002/app.44137.

[3] N. Sahiner, S. Demirci, The use of p(4-VP) cryogel as template for in situ preparation of p(An), p (Py), and p(Th) conductive polymer and their potential sensor applications, Synth. Met. 227 (2017) 11-20, https://doi.org/10.1016/j. synthmet.2017.03.003.

[4] B.W. Walker, R.P. Lara, E. Mogadam, C.H. Yu, W. Kimball, N. Annabi, Rational design of microfabricated electroconductive hydrogels for biomedical applications, Prog. Polym. Sci. 92 (2019) 135-157, https://doi.org/10.10Wj. progpolymsci.2019.02.007.

[5] J. Yang, G. Choe, S. Yang, H. Jo, J.Y. Lee, Polypyrrole-incorporated conductive hyaluronic acid hydrogels, Biomater. Res. 20 (2016) 31, https://doi.org/10.1186/ s40824-016-0078-y.

[6] H. Yuk, B. Lu, X. Zhao, Hydrogel bioelectronics, Chem. Soc. Rev. 48 (2019) 1642-1667, https://doi.org/10.1039/c8cs00595h.

[7] M. Tomczykowa, M.E. Plonska-Brzezinska, Conducting polymers, hydrogels and their composites: preparation, properties and bioapplications, Polymers 11 (2019) 350, https://doi.org/10.3390/polym11020350.

[8] R. Balint, N.J. Cassidy, S.H. Cartmell, Conductive polymers: towards a smart biomaterial for tissue engineering, Acta Biomater. 10 (2014) 2341-2353, https:// doi.org/10.1016/j.actbio.2014.02.015.

[9] A. Guiseppi-Elie, Electroconductive hydrogels: synthesis, characterization and biomedical applications, Biomaterials 31 (2010) 2701-2716, https://doi.org/ 10.1016/j.biomaterials.2009.12.052.

[10] C.R. Chen, H.L. Qin, H.P. Cong, S.H. Yu, A highly stretchable and real-time healable supercapacitor, Adv. Mater. 31 (2019), 1900573, https://doi.org/ 10.1002/adma.201900573.

[11] J. Hur, K. Im, S.W. Kim, J. Kim, D.Y. Chung, T.H. Kim, K.H. Jo, J.H. Hahn, Z.A. Bao, S. Hwang, N. Park, Polypyrrole/agarose-based electronically conductive and reversibly restorable hydrogel, ACS Nano 8 (2014) 10066-10076, https://doi.org/ 10.1021/nn502704g.

[12] S. Brahim, D. Narinesingh, A. Guiseppi-Elie, Polypyrrole-hydrogel composites for the construction of clinically important biosensors, Biosens. Bioelectron. 17 (2002) 53-59, https://doi.org/10.1016/S0956-5663(01)00262-7.

[13] P. Bober, Z. Capakova, U. Acharya, B.A. Zasonska, P. Humpolicek, J. Hodan, J. Hromadkova, J. Stejskal, Highly conducting and biocompatible polypyrrole/poly (vinyl alcohol) cryogels, Synth. Met. 252 (2019) 122-126, https://doi.org/ 10.1016/j.synthmet.2019.04.015.

[14] N.V. Blinova, J. Stejskal, M. Trchova, J. Prokes, M. Omastova, Polyaniline and polypyrrole: a comparative study of the preparation, Eur. Polym. J. 43 (2007) 2331-2341, https://doi.org/10.1016/j.eurpolymj.2007.03.045.

[15] M.C. Gomez-Guillen, B. Gimenez, M.E. Lopez-Caballero, M.P. Montero, Functional and bioactive properties of collagen and gelatin from alternative sources: a review, Food Hydrocolloids 25 (2011) 1813-1827, https://doi.org/10.1016/j. foodhyd.2011.02.007.

[16] S.S. Silva, J.F. Mano, R.L. Reis, Potential applications of natural origin polymer-based systems in soft tissue regeneration, Crit. Rev. Biotechnol. 30 (2010) 200-221, https://doi.org/10.3109/07388551.2010.505561.

[17] S. Huang, X.B. Fu, Naturally derived materials-based cell and drug delivery systems in skin regeneration, J. Contr. Release 142 (2010) 149-159, https://doi.org/ 10.1016/j.jconrel.2009.10.018.

[18] S. Oktay, N. Alemdar, Electrically controlled release of 5-fluorouracil from conductive gelatin methacryloyl-based hydrogels, J. Appl. Polym. Sci. 136 (2019) 46914, https://doi.org/10.1002/app.46914.

[19] M.K. Satapathy, B. Nyambat, C.W. Chiang, C.H. Chen, P.C. Wong, P.H. Ho, P. R. Jheng, T. Burnouf, C.L. Tseng, E.Y. Chuang, A gelatin hydrogel-containing nanoorganic PEI-Ppy with a photothermal responsive effect for tissue engineering applications, Molecules 23 (2018) 1256, https://doi.org/10.3390/ molecules23061256.

[20] S. Khorshidi, A. Karkhaneh, Hydrogel/fiber conductive scaffold for bone tissue engineering, J. Biomed. Mater. Res. 106 (2018) 718-724, https://doi.org/ 10.1002/jbm.a.36282.

[21] B. Bhowmick, M.M.R. Mollick, D. Mondal, D. Maity, M.K. Bain, N.K. Bera, D. Rana, S. Chattopadhyay, M. Chakraborty, D. Chattopadhyay, Poloxamer and gelatin gel guided polyaniline nanofibers: synthesis and characterization, Polym. Int. 63 (2014) 1505-1512, https://doi.org/10.1002/pi.4657.

[22] T. Tungkavet, N. Seetapan, D. Pattavarakorn, A. Sirivat, Improvements of electromechanical properties of gelatin hydrogels by blending with nanowire polypyrrole: effects of electric field and temperature, Polym. Int. 61 (2012) 825-833, https://doi.org/10.1002/pi.4149.

[23] M.M. Perez-Madrigal, F. Estrany, E. Armelin, D.D. Diaz, C. Aleman, Towards sustainable solid-state supercapacitors: electroactive conducting polymers combined with biohydrogels, J. Mater. Chem. 4 (2016) 1792-1805, https://doi. org/10.1039/c5ta08680a.

[24] A.R. Spencer, A. Primbetova, A.N. Koppes, R.A. Koppes, H. Fenniri, N. Annabi, Electroconductive gelatin methacryloyl-PEDOT:PSS composite hydrogels: design, synthesis, and properties, ACS Biomater. Sci. Eng. 4 (2018) 1558-1567, https:// doi.org/10.1021/acsbiomaterials.8b00135.

[25] A.S. de Menezes, C.M.R. Remedios, J.M. Sasaki, L.R.D. da Silva, J.C. Goes, P. M. Jardim, M.A.R. Miranda, Sintering of nanoparticles of alpha-Fe2O3 using gelatin, J. Non-Cryst. Solids 353 (2007) 1091-1094, https://doi.org/10.1016/j. jnoncrysol.2006.12.022.

[26] J. Kopecká, M. Mrlík, R. Olejnik, D. Kopecký, M. Vrnata, J. Prokes, P. Bober, Z. Morávková, M. Trchová, J. Stejskal, Polypyrrole nanotubes and their carbonized analogs: synthesis, characterization, gas sensing properties, Sensors 16 (2016) 1917, https://doi.org/10.3390/s16111917.

[27] Y. Li, P. Bober, M. Trchovaá, J. Stejskal, Polypyrrole prepared in the presence of methyl orange and ethyl orange: nanotubes versus globules in conductivity enhancement, J. Mater. Chem. C 5 (2017) 4236-4245, https://doi.org/10.1039/ c7tc00206h.

[28] M. Omastova, M. Trchová, J. Kovářova, J. Stejskal, Synthesis and structural study of polypyrroles prepared in the presence of surfactants, Synth. Met. 138 (2003) 447-455, https://doi.org/10.1016/S0379-6779(02)00498-8.

[29] A. Kausaite-Minkstimiene, V. Mazeiko, A. Ramanaviciene, A. Ramanavicius, Evaluation of chemical synthesis of polypyrrole particles, Colloids Surf. A 483 (2015) 224-231, https://doi.org/10.1016/j.colsurfa.2015.05.008.

[30] S. Gupta, Hydrogen bubble-assisted syntheses of polypyrrole micro/nanostructures using electrochemistry: structural and physical property characterization, J. Raman Spectrosc. 39 (2008) 1343-1355, https://doi.org/10.1002/jrs.2002.

[31] K. Crowley, J. Cassidy, In situ resonance Raman spectroelectrochemistry of polypyrrole doped with dodecylbenzenesulfonate, J. Electroanal. Chem. 547 (2003) 75-82, https://doi.org/10.1016/S0022-0728(03)00191-8.

[32] Y. Furukawa, S. Tazawa, Y. Fujii, I. Harada, Raman spectra of polypyrrole and its 2,5-13C-substituted and C-deuterated analogues in doped and undoped states, Synth. Met. 24 (1988) 329-341, https://doi.org/10.1016/0379-6779(88)90309-8.

[33] F. Chen, G. Shi, M. Fu, L. Qu, X. Hong, Raman spectroscopic evidence of thickness dependence of the doping level of electrochemically deposited polypyrrole film, Synth. Met. 132 (2003) 125-132, https://doi.org/10.1016/S0379-6779(02)00197-2.

[34] K. Ulubayram, E. Aksu, S.I. Gurhan, K. Serbetci, N. Hasirci, Cytotoxicity evaluation of gelatin sponges prepared with different cross-linking agents, J. Biomater. Sci. Polym. Ed. 13 (2002) 1203-1219, https://doi.org/10.1163/ 156856202320892966.

[35] P. Humpolicek, V. Kasparkova, J. Pachernik, J. Stejskal, P. Bober, Z. Capakova, K. A. Radaszkiewicz, I. Junkar, M. Lehocky, The biocompatibility of polyaniline and polypyrrole: a comparative study of their cytotoxicity, embryotoxicity and impurity profile, Mater. Sci. Eng. C 91 (2018) 303-310, https://doi.org/10.1016/). msec.2018.05.037.

[36] S. Pirsa, T. Shamusi, Intelligent and active packaging of chicken thigh meat by conducting nano structure cellulose-polypyrrole-ZnO film, Mater. Sci. Eng. C 102 (2019) 798-809, https://doi.org/10.1016/j.msec.2019.02.021.

[37] N. Marakova, P. Humpolicek, V. Kasparkova, Z. Capakova, L. Martinkova, P. Bober, M. Trchova, J. Stejskal, Antimicrobial activity and cytotoxicity of cotton fabric coated with conducting polymers, polyaniline or polypyrrole, and with deposited silver nanoparticles, Appl. Surf. Sci. 396 (2017) 169-176, https://doi.org/ 10.1016/j.apsusc.2016.11.024.

[38] C.C. Wan, J. Li, Cellulose aerogels functionalized with polypyrrole and silver nanoparticles: in-situ synthesis, characterization and antibacterial activity, Carbohydr. Polym. 146 (2016) 362-367, https://doi.org/10.1016/j. carbpol.2016.031.081.

[39] Z. Hanif, Z.A. Khan, M.F. Siddiqui, S. Park, S.J. Park, Polypyrrole-based conducting and antibacterial hybrid cellulose membranes: a study on the effect of UV exposure on the conductivity and formation of silver nanoparticles, Sensor. Mater. 31 (2019) 1927-1938, https://doi.org/10.18494/SAM.2019.2310.

[40] M. Akter, M.T. Sikder, M.M. Rahman, A.A. Ullah, K.F.B. Hossain, S. Banik, S. Hosokowa, T. Saito, M. Kurasaki, A systematic review on silver nanoparticles-induced cytotoxicity: physicochemical properties and perspectives, J. Adv. Res. 9 (2018) 1-16, https://doi.org/10.1016/jjare.2017.10.008.

[41] R. de Lima, A.B. Seabra, N. Duran, Silver nanoparticles: a brief review of cytotoxicity and genotoxicity of chemically and biogenically synthesized nanoparticles, J. Appl. Toxicol. 32 (2012) 867-879, https://doi.org/10.1002/ jat.2780.