Nanoparticles modification for magnetic electrospun composites for tissue engineering

Anna Hlukhaniuk[1,2], Małgorzata Świętek[1], Vitalii Patsula[1], Beata Zasońska[2], Jiří Hodan[1], Antonín Brož[3], Marina Malić[3], Daniel Horák[1]

1. Department of Polymer Particles, Institute of Macromolecular Chemistry, Czech Academy of Sciences, CZECH REPUBLIC, Prague 162 06, Heyrovského nám. 2, E-mail: hlukhaniuk@imc.cas.cz

2. Faculty of Science, Charles University, CZECH REPUBLIC, Prague 128 00, Albertov 6

3. Institute of Physiology, Czech Academy of Sciences, CZECH REPUBLIC, Prague 142 20, Vídeňská 1083

Abstract – Polymer-based magnetic composites find multiple applications in the biomedical field. Good compatibility between polymer matrix and magnetic nanoparticles is a vital issue assuring their uniform distribution. Herein, a series of iron oxide nanoparticles grafted with poly(ε-caprolactone) (PCL) was produced and the effect of their composition on the properties of electrospun PCL-based composites was investigated.

Keywords – magnetic nanoparticles, polymer composites, tissue engineering, nanoparticles grafting.

Introduction

The recently observed rapid development of tissue engineering is possible thanks to the preceding progress in biology, chemistry, physics, and materials engineering. The ease of processing and wide selection of polymers, which translates into the possibility of matching them to specific tissues in terms of physiochemical properties, make polymers one of the most often used materials for tissue scaffolds [1-2]. Modification of polymeric matrix with various inorganic components allows enhancement of its mechanical properties and brings unique properties to scaffolds. For example, iron oxide-based nanoparticles (IONs), especially magnetite and maghemite, which are characterized by good magnetic properties and high biocompatibility, have been used to produce magnetic polymer-based scaffolds [3-4]. Noteworthy, a combination of magnetic polymer-based scaffolds and an external magnetic field has been reported to stimulate stem cells and bone cells to proliferate and differentiate, facilitate magnetic field-assisted drug delivery and fixation of a scaffold at the destination site, and accelerate the rate of polymer matrix degradation via magnetocaloric effect under alternating magnetic field [5-7]. Magnetic scaffolds have been also exploited in, e.g., cancer treatment via magnetic hyperthermia, targeted drug delivery systems, and diagnostics (magnetic resonance imaging and computed tomography) [4].

A crucial point in compiling nanoparticles and polymer matrix is their good chemical compatibility, which is necessary to avoid possible aggregation that may negatively affect the scaffold properties [8-9]. Herein, to provide good compatibility between IONs and PCL matrix, nanoparticles were grafted with PCL according to the novel Sipomer PAM 200 (SIPO)-mediated approach before their incorporation into the matrix. The scaffolds were prepared in the form of fibrous mats by the electrospinning technique, which is one of the most often exploited methods used to fabricate tissue scaffolds of controllable fiber size. Due to the high surface area-to-volume ratio, electrospun mats greatly mimic the extracellular matrix, facilitating cell attachment [10].

Experimental and Results

IONs denoted later as Fe_3O_4 were synthesized by the coprecipitation method according to the previously described procedure from aqueous solutions of iron chlorides in a Fe^{3+}/Fe^{2+} molar ratio of 2:1 [11]. The particles were magnetically separated and washed with water until a stable colloidal suspension was formed. The average diameter was 10.5 nm (SD 1.9) and PDI = 1.09.

A previously undescribed three-step procedure was used to prepare PCL-modified magnetic nanoparticles. First, the IONs surface was modified with SIPO followed by its functionalization with amino groups in the reaction with ethylenediamine (Fe_3O_4@SIPO-NH_2). Then, PCL was grafted from the nanoparticles' surface by ring-opening polymerization of ε-caprolactone (CL) (Fe_3O_4@PCL), which involved a reaction between the terminal hydroxyl group of CL and the amino groups exposed on the Fe_3O_4@SIPO-NH_2 surface. The particles were prepared with various CL to Fe_3O_4@SIPO-NH_2 ratios, Table 1.

Magnetic polymer composites were prepared by the electrospinning technique from the suspension of grafted nanoparticles in PCL solution in DCM/DMF (3:1 v/v). The content of Fe_3O_4@PCL was 2 wt.% in relation to the total weight of the polymer. The electrospun mat made of PCL was used as a control material. Composites containing nanoparticles were denoted as mPCL1-mPCL4, depending on the particles incorporated.

Results and discussion

Modified nanoparticles exhibited a lower propensity to aggregate compared to non-grafted particles, Fig. 1 a-b. The presence of PCL was confirmed by ATR-FTIR spectra, Fig. 1 c, by the presence of typical bands at 1,091 and 1,451 cm^{-1} of C-O stretching, two peaks at 1,735 and 1,652 cm^{-1} from stretching vibrations of C=O and C=C of SIPO, respectively. Asymmetric and symmetric stretching of CH_2 in methyl and methylene bonds appeared at 2,971, 2,906, and 2,870 cm^{-1}. The specific peak of the N-H bond at 1,652 cm^{-1} was registered for modified with SIPO and EDA nanoparticles. The amount of grafted polymer on the nanoparticles' surface was determined by TGA, Tab. 1. According to obtained results, the ratio of CL/Fe_3O_4 used during modification strongly influences the amount of grafted PCL.

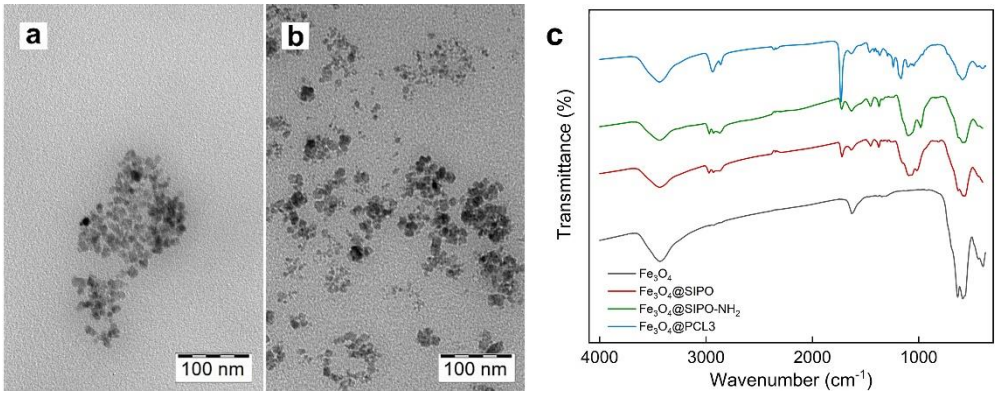

Fig. 1. TEM micrographs: (a) neat Fe_3O_4, (b) Fe_3O_4@PCL3, and (c) ATR-FTIR spectra of IONs.

Table 1.

Denotation	Amount of Fe_3O_4, mg	Ratio of CL/Fe_3O_4, wt/wt	Weight loss, %	Amount of grafted PCL, %
Fe_3O_4	---------	--------	4.5	---------
Fe_3O_4@PCL1	180	11.1/1	58.0	53.5
Fe_3O_4@PCL2	300	6.7/1	76.0	71.5
Fe_3O_4@PCL3	120	16.7/1	87.0	82.5
Fe_3O_4@PCL4	60	33.3/1	93.0	88.5

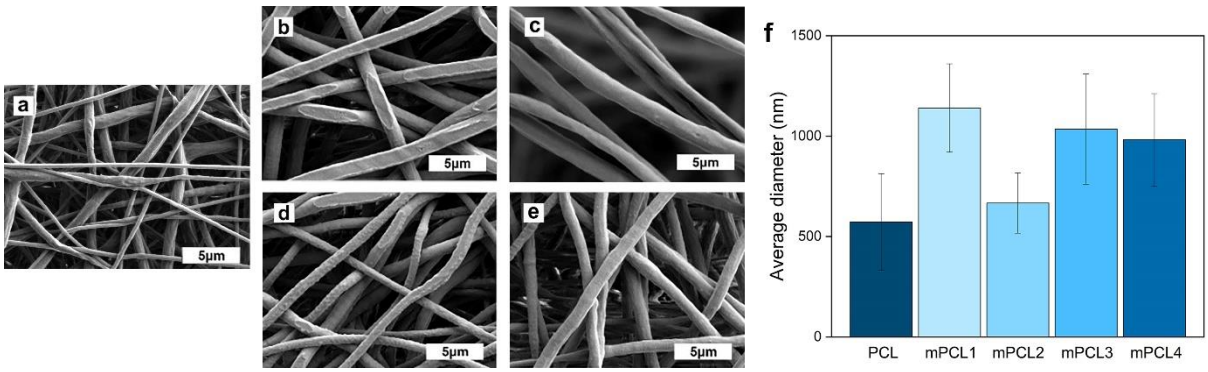

Fig. 2. SEM micrographs: (a) PCL, (b) mPCL1, (c) mPCL2, (d) mPCL3, and (e) mPCL4, and (f) average fiber diameters of PCL nanocomposites.

The morphology and fiber size of fabricated composite mats were analyzed based on obtained SEM photographs. All fibers showed smooth surfaces and untangled nets, Fig. 2 a-e. Change in fiber diameter revealed that the amount of grafted polymer on nanoparticles non-proportionally influenced the diameter changes in comparison to pure PCL samples, Fig. 2 f. However, the modification of polymer matrix with IONs leads to the increase in fiber size in all samples. Respectively, mechanical tests showed changes in tensile strength and Young's modulus for all composites in a different manner, Table 2. Composites with lower size PCL and mPCL2 are stiffer due to higher values of Young's modulus and smaller fibers. Also, the introduction of particles into the polymer matrix leads to a slight decrease in the water contact angle parameter, Table 2.

Table 2

Denotation	Tensile strength, MPa	Young's modulus, MPa	Water contact angle, º
PCL	7.9±0.8	25.9±1.9	139
mPCL1	5.7±0.4	16.6±1.4	136
mPCL2	11.9±0.8	28.5±1.8	127
mPCL3	10.3±0.6	22.4±2.2	135
mPCL4	3.6±0.3	16.9±1.6	136

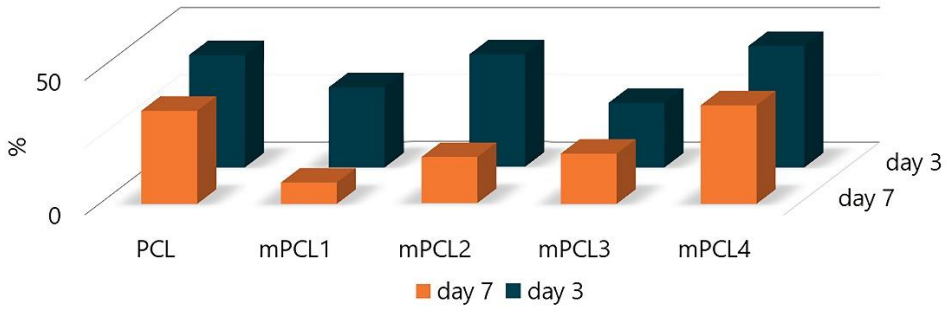

Fig. 3. Metabolic activity of SAOS-2 cells cultivated on PCL composites.

SAOS-2 osteosarcoma cells were cultivated on polymer mats to assess the biological properties of the materials. Cellular metabolic activity was measured on the 3. and 7. days of culturing, Fig. 3. The comparable metabolic activity of cells cultured on the PCL and mPCL4 mats and significantly different results for mPCL1 and mPCL4 indicated that both morphologies of composites and particles' composition influence the cellular response.

Conclusions

The proposed three-step surface modification of magnetic nanoparticles via grafting of PCL from the surface allows solving the popular problem of particles' low stability in polymer matrix while production of scaffolds for tissue engineering. The described procedure improved the stability and uniform distribution of nanoparticles along the matrix of a hydrophobic polymer, however influencing morphology and mechanical properties of fabricated electrospun nanofibers in a grafting-dependent manner. Moreover, the changes in metabolic activity of SAOS-2 osteosarcoma cells were registered. Composites with particles having 53.5 wt.% of grafted polymer induced the formation of thick fibers, however, with low mechanical properties but decreased toxicity. In contrast, particles containing 71.5 wt.% of IONs resulted in composites with the narrowest fibers and enhanced mechanical properties but high toxicity. Obtained results were not reported before and are valuable for further development of multifunctional scaffolds for biomedical applications.

Acknowledgments

The study was supported by the Czech Science Foundation (No. 20-07015S).

References

[1] Zhang, J., et al. (2014). 3D-printed magnetic Fe3O4/MBG/PCL composite scaffolds with multifunctionality of bone regeneration, local anticancer drug delivery and hyperthermia. J. Mater. Chem. B 2. DOI: 10.1039/C4TB01063A

[2] Farzin, A., et al. (2017) Multifunctional magnetic nanostructured hardystonite scaffold for hyperthermia, drug delivery and tissue engineering applications. Mater. Sci. Eng. C 70, 21–31. DOI: 10.1016/j.msec.2016.08.060

[3] Święt ek, M, et al. (2019). Mater Sci Eng C 104:109913. DOI: 10.1016/j.msec.2019.109913

[4] Liu, Q. et al. (2020). Ultrasmall Superparamagnetic Iron Oxide Labeled Silk Fibroin/Hydroxyapatite Multifunctional Scaffold Loaded With Bone Marrow-Derived Mesenchymal Stem Cells for Bone Regeneration. Front. Bioeng. Biotechnol. 8, 697. DOI: 10.3389/fbioe.2020.00697

[5] Eivazzadeh-Keihan, R. et al. (2020). Metal-based nanoparticles for bone tissue engineering. J. Tissue Eng. Regen. Med. 14, 1687–1714. DOI: 10.1002/term.3131

[6] Xia, Y. et al. (2018). Magnetic field and nano-scaffolds with stem cells to enhance bone regeneration. Biomaterials 183, 151–170. DOI: 10.1016/j.biomaterials.2018.08.040

[7] Yun, H. M. et al. (2016). Magnetic nanocomposite scaffolds combined with static magnetic field in the stimulation of osteoblastic differentiation and bone formation. Biomaterials 85, 88–98. DOI: 10.1016/j.biomaterials.2016.01.035

[8] Zare, Y., et al. (2016). Study of nanoparticles aggregation/agglomeration in polymer particulate nanocomposites by mechanical properties. Composites Part A: Applied Science and Manufacturing, 84, 158–164. DOI: 10.1016/j.compositesa.2016.01.020

[9] Liu, J., et al. (2011). Nanoparticle dispersion and aggregation in polymer nanocomposites: Insights from molecular dynamics simulation. Langmuir, 27(12), 7926–7933. DOI: 10.1021/la201073m

[10] Jun, I., et al. (2018). Electrospun fibrous scaffolds for tissue engineering: Viewpoints on architecture and Fabrication. International Journal of Molecular Sciences, 19(3), 745. DOI: 10.3390/ijms19030745