THE INFLUENCE OF SUPERCRITICAL FOAMING CONDITIONS ON PROPERTIES OF POLYMER SCAFFOLDS FOR TISSUE ENGINEERING

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The results of experimental investigations into foaming process of poly(ε-caprolactone) using supercritical CO_2 are presented. The objective of the study was to explore the aspects of fabrication of biodegradable and biocompatible scaffolds that can be applied as a temporary three-dimensional extracellular matrix analog for cells to grow into a new tissue. The influence of foaming process parameters, which have been proven previously to affect significantly scaffold bioactivity, such as pressure (8-18 MPa), temperature (323-373 K) and time of saturation (1-6 h) on microstructure and mechanical properties of produced polymer porous structures is presented. The morphology and mechanical properties of considered materials were analyzed using a scanning electron microscope (SEM), x-ray microtomography (μ-CT) and a static compression test. A precise control over porosity and morphology of obtained polymer porous structures by adjusting the foaming process parameters has been proved. The obtained poly(ε-caprolactone) solid foams prepared using $scCO_2$ have demonstrated sufficient mechanical strength to be applied as scaffolds in tissue engineering.

Keywords: supercritical fluids, supercritical foaming, poly(ε-caprolactone), tissue engineering, scaffold

1. INTRODUCTION

Bone defects being the result of injuries or diseases are a serious problem posing enormous challenge for modern regenerative medicine. The development of methods aiming at regeneration of damaged tissue, as opposed to their replacement, is an alternative to surgical therapy (Gloria et al., 2012). Tissue engineering is an interdisciplinary, rapidly evolving area of research which draws upon materials engineering, chemistry, biology and medicine (Tarabasz and Henczka, 2016). In recent years the development of modern technologies has been mainly focused on the advancement in research of innovative, efficient, cost-effective and environmentally safe methods of the production of functional porous structures. New products may be useful for a wide range of applications, for example as biodegradable scaffolds for the culture of bone cells (Curia et al., 2015; Gualandi et al., 2010; Karimi et al., 2012; Kweon et al., 2003). There are numerous production methods of polymer porous materials applicable to biomedical practices, such as solvent casting-particle leaching, thermally induced phase separation, injection molding, extrusion, foaming and electrospinning (Guan et al., 2005). Some of these techniques require processing of the polymer material at relatively high temperature, which prevents embodiment of thermosensitive compounds, such as medicaments or growth factors into foam structure during the production process (Liao et al., 2012; Markočič et al., 2013; Tsivintzelis et al., 2006; Tsivintzelis et al., 2007).

The polymer foaming process with the use of supercritical fluids is an alternative to traditional methods of production of functional porous structures, carried out with the application of environmentally

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harmful, volatile organic solvents, which may also lead to a reduction in the activity of processed biodegradable polymers (Gualandi et al., 2010; Tayton et al., 2012). The exercitation of a safe medium such as supercritical carbon dioxide in the process allows to eliminate the above mentioned disadvantages. Supercritical carbon dioxide (scCO$_2$) is the most commonly used supercritical fluid, possessing such attributes as moderate values of critical parameters, chemical passivity, non-flammability, non-toxicity, wide availability and low cost (Nalawade et al., 2006). Its unique characteristics, such as gas like low kinematic viscosity, liquid like density, high compressibility and pressure-dependent solubility of various substances, are beneficial for applications of scCO$_2$ in industrial technologies (Curia et al., 2015; Guan et al., 2005; Karimi et al., 2012). Carbon dioxide applied as a supercritical process medium can be easily removed from porous products simply by depressurization. However, it should be mentioned that in some cases the contact of polymer material with supercritical carbon dioxide may lead to unfavorable changes in its structure and properties (Tarabasz et al., 2016). Poly(ε-caprolactone) (PCL) is a polymer commonly used in tissue regenerative engineering. It is a biodegradable aliphatic semi-crystalline polyester of a low melting temperature of about 333 K (Curia et al., 2015; Guan et al., 2005; Karimi et al., 2012; Kweon et al., 2003). In the foaming process of PCL using scCO$_2$ one can distinguish the following stages: melting and saturating the polymeric material with scCO$_2$ at appropriate parameters, cooling and depressurization of saturated polymer matrix, and finally nucleation and growth of gas cells which leads to formation of the final porous structure (Gualandi et al., 2010; Jacobs et al., 2007; Karimi et al., 2012; Reverchon et al., 2008). The course of these stages strongly affects the final properties of the obtained porous structures, which must meet certain specific characteristics when applied as scaffolds. In particular, appropriate pore size, sufficient porosity and defined mechanical properties are considered to be fundamental parameters in the field of tissue engineering and they should be accustomed to the type of regenerated tissue (Gloria et al., 2012; Gualandi et al., 2010; Guan et al., 2005; Karimi et al., 2012; Markočič et al., 2013). Pertinent control of the properties of produced scaffolds is executed with the appropriate definition of the foaming process parameters and with the use of adequate composite materials.

The purpose of this work was to identify the effect of supercritical foaming process parameters on the properties of obtained poly(ε-caprolactone) porous structures and to establish the key features affecting their usability for bone tissue engineering. In particular, the effects of pressure, temperature and time of scCO$_2$ polymer saturation on 3D microstructure and mechanical properties of the polymer porous product are presented.

2. METHODS

Commercially available poly(ε-caprolactone) in form of pellets (D˜ 3 mm) with Mn = 80,000 manufactured by Sigma Aldrich (Italy) was used as a model polymeric material. The melting temperature and density of the material was equal to 333 K and 1.19 g/cm3, respectively. Carbon dioxide (purity of 4.5) was purchased from Linde Gaz (Poland) and used in experiments as the foaming agent. In the first part of experimental investigations, scCO$_2$ - based foaming polymer process was carried out. A scheme of experiment setups including a scCO$_2$ pump, a high pressure cell and a process parameter control unit was reported previously (Tarabasz and Henczka, 2016). The foaming experiments were conducted using the procedure presented subsequently. The high pressure cell was filled with 1.5 g of polymer, which was melted, contacted and saturated with scCO$_2$ in batch mode. This stage of the process was carried out in the following conditions: temperature in the range of 323 - 373 K, pressure 8 - 18 MPa, and saturation time of 1 - 6 h. Consecutively, the carbon dioxide-polymer mixture was cooled to 298 K, kept over 30 min under a constant pressure and finally rapidly decompressed to atmospheric pressure. In the second part of experiments the morphology and mechanical properties of obtained porous structures were identified. Scanning electron microscope (SEM) examination and x-ray microtomography (μ-CT) were applied in the analysis of the

microstructure of the produced polymer porous materials. The morphology of side surface and cross-section fracture of the porous structure at different magnifications was analyzed with the use of Phenom scanning electron microscope SEM (FEI COMPANY, Netherlands). The samples were first sectioned using a stainless steel scalpel and then a coat of gold was applied for 2 min in argon atmosphere with the use of EMITECH K550X sputter coater (Quorum Technologies, Warsaw). The porosity and the interconnectivity of the pores were evaluated with the use of microtomography X-ray scanning (μ-CT, SkyScan 1172, Bruker). Static compression tests to evaluate the mechanical properties of the polymer foams were conducted using the universal testing machine Instron 5566 (Instron, USA). Prior to the compression testing, the obtained structures were cut into cuboids of identical dimensions: 5.0 mm×5.0 mm×10.0 mm. The Young's modulus and compression strength of solid foams were determined on the basis of stress–strain profiles.

3. RESULTS AND DISCUSSION

Scanning electron microscope examination allowed the identification of the microstructure of polymer porous materials obtained under different foaming process conditions. In Fig. 1a and Fig. 1b the effect of experimental conditions on morphology of solid - gas structures is presented. The microstructure of foam strongly depends on the process parameter.
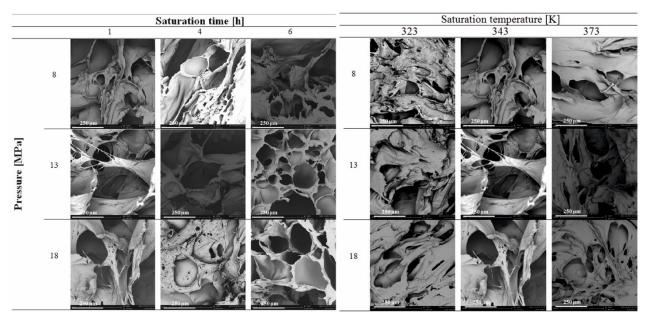

Fig. 1a. Effect of saturation time on morphology of porous structures

Fig. 1b. Effect of saturation temperature on morphology of porous structures

On the basis of SEM microphotographs, the pore sizes and pore size distribution of solid foams were defined, with the use of image analysing software. The performed analysis showed that the foaming method applied in this procedure resulted in generating a porous structure with partially closed pores. The effect of pressure and scCO$_2$ polymer saturation time on the pore size distribution of porous structures acquired by polymer foaming is shown in Fig. 2. Frequency distribution indicates the index in percentage of the number of pores occurring in respective pore size intervals after the range of target pore sizes is divided into separate intervals.

For a relatively short saturation time of 1 hour the increase of pressure results in obtaining a more uniform porous structure which forms small pores of size of 10 μm order, whereas for a long saturation time the opposite effect is observed. An increase in pressure of scCO$_2$ polymer saturation performed for

6 hours leads to creation of larger pores and significant homogeneity reduction of obtained porous structure. The effects of pressure and temperature of $scCO_2$ polymer saturation on the pore size distribution is shown in Fig. 3.
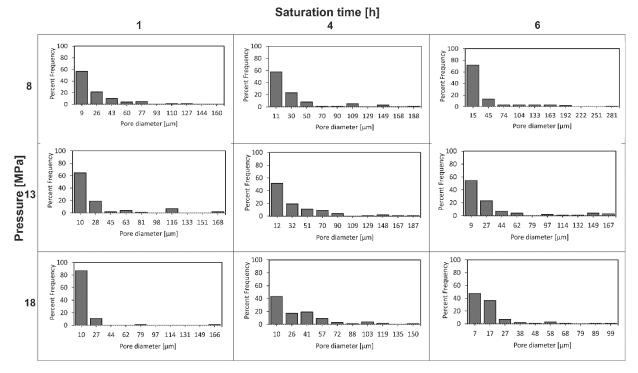

Fig. 2. The effect of pressure and time of saturation on pore size distribution of porous polymer in saturation temperature of 343 K
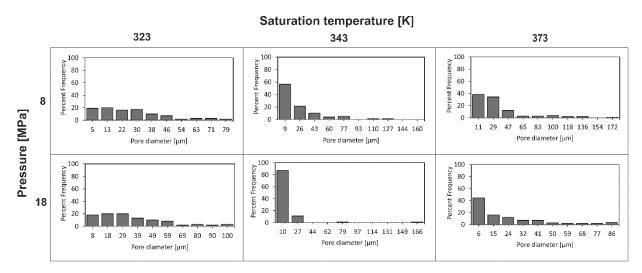

Fig. 3. The effect of pressure and temperature of saturation on pore size distribution of porous polymer for saturation time of 1 hour

In general, execution of the foaming process with the application of high pressure and short saturation time and with moderate pressure and long saturation time promotes the formation of uniform microstructure in polymer material, being the result of the melting qualities of polymer under moderate and high pressure. On the other hand, performing saturation of polymer at the temperature of 343 K leads to production of highly non-uniform porous structures. Increasing solubility of CO_2 in polymer matrix leads to the decrease of melting temperature, whereas the hydrostatic pressure causes this temperature to increase. Reportedly, CO_2 rapidly dissolves into polymers at moderate pressures, and the solubility of CO_2 increases linearly with rising pressure. The solubility effect is dominant in the

moderate pressure range, while the hydrostatic pressure effect becomes significant under higher pressures when the polymer becomes saturated and the solubility of CO_2 reaches a constant value (Takahashi et al., 2012).

The microstructure of a porous scaffold should be defined in accordance with the kind of tissue or organ being regenerated. The values of an average pore size and surface pore density characterizing the porous polymer obtained under different conditions and identified with the use of the AxioVision software are presented in Table 1. The application of supercritical fluids in the foaming process allowed to obtain polymer structures with the average pore size in the range from 15 to 45 μm and density oscillating between 2×10^4 and 12×10^4 pores/cm^2 of various uniformity.

Table 1. The effect of foaming conditions on average pore size and surface pore density of porous polymer

Pressure [MPa]		Average pores size [μm]			Average surface density of pores [10^4 pores/cm^2]		
		8	13	18	8	13	18
t sat [h]	1	25.38 (± 2.83)	26.97 (± 3.62)	11.75(± 1.99)	3.62 (± 1.21)	3.49 (± 0.69)	10.42 (± 2.40)
	4	31.33 (± 3.77)	37.84 (± 3.72)	35.70 (± 3.06)	4.04 (± 1.41)	2.83 (± 0.51)	2.19 (± 0.71)
	6	35.16 (± 5.22)	33.13 (± 4.29)	18.83 (± 1.74)	4.61 (± 1.24)	5.63 (± 1.89)	8.31 (± 2.55)
T sat [K]	323	27.28 (± 1.86)	29.38 (± 1.97)	36.91 (± 2.43)	5.41 (± 1.03)	4.21 (± 0.94)	2.95 (± 0.36)
	343	25.38 (± 2.83)	26.97 (±3.62)	11.75 (±1.99)	3.62 (±1.21)	3.49 (±0.69)	10.42 (±2.40)
	373	36.73 (± 3.34)	46.52 (± 3.07)	23.30 (± 2.16)	1.75 (± 0.18)	2.35 (± 0.41)	5.07 (± 0.67)

Table 2. Compression test results of the polymer foams

T_{sat} [K]	P_{sat}/P_{foam} [MPa]	T_{sat} [h]	Young's modulus [MPa]	Compressive strength [MPa]	Maximum strain [%]
323	8		20.20 (±6.62)	5.001 (±0.95)	70.022 (±0.003)
	13		4.307 (±0.38)	1.460 (±0.21)	70.007 (±0.01)
	18	1	19.480(±7.71)	4.686 (±1.51)	70.011 (±0.01)
343	8		2.671 (±0.72)	0.776 (±0.13)	70.014 (±0.005)
	13		1.794 (±0.47)	0.380 (±0.15)	57.742 (±12.33)
	18		1.626 (±0.54)	0.499 (±0.17)	70.007 (±0.01)
	8		1.908 (±0.54)	0.367 (±0.09)	61.317 (±8.67)
	13	4	1.302 (±0.41)	0.374 (±0.11)	58.925 (±8.07)
	18		1.763 (±0.66)	0.597 (±0.20)	57.890 (±12.12)
	8		1.033 (±0.62)	0.330 (±0.08)	69.997 (±0.01)
	13	6	7.337 (±1.82)	1.517 (±0.47)	65.184 (±4.84)
	18		2.974 (±0.31)	0.856 (±0.09)	70.019 (±0.01)
373	8		3.931 (±0.86)	1.032 (±0.12)	68.168 (±1.84)
	13	1	5.241 (±1.48)	1.346 (±0.38)	70.003 (±0.01)
	18		1.403 (±0.30)	0.309 (±0.08)	48.565 (±15.77)

The microstructure of scaffolds was defined with non-destructive x-ray microtomography. The performed μ-CT studies resulted in identification of the key properties of the obtained porous polymer, such as the interconnectivity of the pores, the porosity and foam microstructure. It was

observed that pores inside of foams were interconnected with irregular shape and porosity of structure ranging from 57 to 84 % which is optimal for bone growth (Chen Chuan-Xin et al., 2016; White et al., 2012). Moreover, the mechanical properties of scaffolds were analyzed with the static compression tests. The results of the compressive mechanical test of polymer foams obtained under different foaming conditions are summarized in Table 2.

The values of Young's modulus and compressive strength of these scaffolds depend on density and porosity of foams. The obtained porous structures were compressed to total strain of 70 % using a compression speed of 0.4 mm·min^{-1}. Significant differences in measured mechanical parameters of the porous structures obtained at different foaming conditions could be observed. This phenomenon makes it possible to prepare scaffolds with properties correlated with specific biomedical applications. In particular, both Young's modulus and compressive strength decreased with increasing porosity and pore size. Moreover, the most rigid structure was obtained in the lowest temperature of polymer saturation. The values of these parameters in the lower range seem to be analogous to natural bone. The compression test results ensure that obtained porous structures are suitable for potential applications in bone tissue engineering (Mathieu et al., 2016).

4. CONCLUSIONS

The study findings demonstrate that scCO$_2$ application with moderate pressures and temperatures during the foaming process results in attainment of poly(ε-caprolactone) scaffolds of three-dimensional homogeneous morphologies with porosities in the order of almost 85% and pore sizes ranging from 10 to 45 μm. The influence of process parameters of supercritical carbon dioxide polymer saturation on the final attributes of obtained polymer porous structures has been identified. The most uniform porous structures with the smallest pores of average size of 11.75 μm were obtained in the foaming process carried out with polymer saturation performed at the temperature of 343 K and under the pressure of 18 MPa for the period of 1 hour. The applied method allows production of solid foams with well-controllable internal microstructure and mechanical properties favorable for cell proliferation and differentiation, and consequently tissue regeneration.

REFERENCES

Chen Chuan-Xin, Liu Qian–Qian, Xin Xin, Guan Yi-Xin, Yao Shan-Jing, 2016. Pore formation of poly(ε-caprolactone) scaffolds with melting point reduction in supercritical CO$_2$ foaming. *J. Supercrit. Fluids*, 117, 279-288. DOI: 10.1016/j.supflu.2016.07.006.

Curia S., De Focatiis D.S.A., Howdle S.M., 2015. High-pressure rheological analysis of CO$_2$-induced melting point depression and viscosity reduction of poly (ε-caprolactone). *Polymer*, 69, 17–24. DOI: 10.1016/j.polymer.2015.05.026.

Gloria A., Causa F., Russo T., Battista E., Della Moglie R., Zeppetelli S., De Santis R., Netti P.A., Ambrosio L., 2012. Three-dimensional poly (ε-caprolactone) bioactive scaffolds with controlled structural and surface properties. *Biomacromolecules*, 13, 11, 3510–3521. DOI: 10.1021/bm300818y.

Gualandi C., White L.J., Chen L., Gross R.A., Shakesheff K.M., Howdle S.M., Scandola M., 2010. Scaffold for tissue engineering fabricated by non-isothermal supercritical carbon dioxide foaming of a highly crystalline polyester. *Acta Biomater.*, 6, 1, 130–136. DOI: 10.1016/j.actbio.2009.07.020.

Guan J., Fujimoto K. L., Sacks M. S., Wagner W. R., 2005. Preparation and characterization of highly porous, biodegradable polyurethane scaffolds for soft tissue applications. *Biomaterials*, 26, 3961-397. DOI: 10.1016/j.biomaterials.2004.10.018.

Jacobs L.J.M., Danen K.C.H., Kemmere M.F., Keurentjes J.T.F., 2007. A parametric study of polystyrene-co-methylmethacrylate foams using supercritical carbon dioxide as a blowing agent. *Polymer*, 48, 3771-3780. DOI: 10.1016/j.polymer.2007.05.002.

Karimi M., Heuchel M., Weigel T., Schossig M., Hofmann D., Lendlein A., 2012. Formation and size distribution of pores in poly(ε-caprolactone) foams prepared by pressure quenching using supercritical CO_2. *J. Supercrit. Fluids*, 61, 175-190. DOI: 10.1016/j.supflu.2011.09.022.

Kweon H.Y., Yoo M.K., Park I.K., Kim T.H., Lee H.C., Lee H.-S., Oh J.-S., Akaike T., Cho C.-S., 2003. A novel degradable polycaprolactone networks for tissue engineering. *Biomaterials*, 24, 5, 801–808. DOI: 10.1016/S0142-9612(02)00370-8.

Liao X., Zhang H., He T., 2012. Preparation of porous biodegradable polymer and its nanocomposites by supercritical CO_2 foaming for tissue engineering. *J. Nanomaterials*, 2012, Article ID 836394. DOI: 10.1155/2012/836394.

Markočič E., Škerget M., Knez Ž., 2013. Effect of temperature and pressure on the behavior of poly (ε-caprolactone) in the presence of supercritical carbon dioxide. *Ind. Eng. Chem. Res.*, 52, 15594-15601. DOI: 10.1021/ie402256a.

Mathieu L.M., Mueller T.L., Bourban P.E., Pioletti D.P., Muller R., Manson J.A.E., 2006. Architecture and properties of anisotropic polymer composite scaffold for bone tissue engineering. *Biomaterials*, 27, 905-916. DOI: 10.1016/j.biomaterials.2005.07.015.

Nalawade S.P., Picchioni F., Janssen L.P.B.M., 2006. Supercritical carbon dioxide as a green solvent for processing polymer melts: Processing aspects and applications. *Prog. Polym. Sci.*, 31, 19-43. DOI: 10.1016/j.progpolymsci.2005.08.002.

Reverchon E., Cardea S., Rapuano C., 2008. A new supercritical fluid-based process to produce scaffolds of tissue replacement. *J. Supercrit. Fluids*, 45, 365-373. DOI: 10.1016/j.supflu.2008.01.005.

Takahashi S., Hessler J.C., Kiran E., 2012. Melting behaviour of biodegradable polyesters in carbon dioxide at high pressures. *J. Supercrit. Fluids*, 72, 278-287. DOI: 10.1016/j.supflu.2012.09.009.

Tarabasz K., Henczka M., 2016. Experimental investigations into foaming of biodegradable polymers using scCO2. *Inż. Ap. Chem.*, 55, 40-41.

Tarabasz, K., Krzysztoforski, J., Szwast, M., Henczka, M., 2016. Investigation of the effect of treatment with supercritical carbon dioxide on structure and properties of polypropylene microfiltration membranes. *Matt. Letters*, 163, 54-57. DOI: 10.1016/j.matlet.2015.10.010.

Tayton E., Purcell M., Arvold A., Smith J.O., Kalra S., Briscoe A., Shakesheff K., Howdle S.M., Dunlop D.G., Oreffo R.O.C., 2012. Supercritical CO_2 fluid-foaming of polymers to increase porosity: a method to improve the mechanical and biocompatibility characteristics for use as a potential alternative to allografts in impaction bone grafting? *Acta Biomater.*, 8, 1918-1927. DOI: 10.1016/j.actbio.2012.01.024.

Tsivintzelis I., Angelopoulou A.G., Panayiotou C., 2007. Foaming of polymers with supercritical CO_2: An experimental and theoretical study. *Polymer*, 48, 5928-5929. DOI: 10.1016/j.polymer.2007.08.004.

Tsivintzelis I., Pavlidou E., Panayiotou C., 2006. Biodegradable polymer foams prepared with supercritical CO_2-ethanol mixtures as blowing agent. *J. Supercrit. Fluids*, 42, 265-272. DOI: 10.1016/j.supflu.2007.02.009.

White L.J., Hutter V., Tai H., Howdle S.M., Shakesheff K.M., 2012. The effect of processing variables on morphological and mechanical properties of supercritical CO_2 foamed scaffolds for tissue engineering. *Acta Biomater.*, 8, 61-71. DOI: 10.1016/j.actbio.2011.07.032.