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SHORT COMMUNICATION

Application of porogenes in production of porous polymers by supercritical foaming

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Biocomposite foam scaffolds of poly(ε-caprolactone) (PCL) with different porogenes were produced with batch foaming technique using supercritical carbon dioxide ($scCO_2$) as a blowing agent. In performed experiments composites were prepared from graphene-oxide (nGO), nano-hydroxyapatite (nHA) and nano-cellulose (nC), with various concentrations. The objective of the study was to explore the effects of porogen concentration and foaming process parameters on the morphology and mechanical properties of three-dimensional porous structures that can be used as temporary scaffolds in tissue engineering. The structures were manufactured using $scCO_2$ as a blowing agent, at two various foaming pressures (9 MPa and 18 MPa), at three different temperatures (323 K, 343 K and 373 K) for different saturation times (0.5 h, 1 h and 4 h). In order to examine the utility of porogenes, a number of tests, such as static compression tests, thermal analysis and scanning electron microscopy, have been performed. Analysis of experimental results showed that the investigated materials demonstrated high mechanical strength and a wide range of pore sizes. The obtained results suggest that PCL porous structures are useful as biodegradable and biocompatible scaffolds for tissue engineering.

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

1. INTRODUCTION

The development of chemical engineering is directly related to tissue engineering, an emerging interdisciplinary field aiming to produce biodegradable scaffolds that restore, maintain and improve damaged organs or tissues. A tissue scaffold is a three-dimensional structure that plays a crucial role as a temporary support for cell adhesion and it is used to facilitate transport of metabolites and nutrients (Chen et al., 2016). Over the last years, a variety of tissue scaffold fabrication techniques have been developed, including solvent-casting, particulate-leaching and freeze drying. However, these conventional methods suffer from various limitations. The main disadvantages are the use of large amounts of organic, not environment friendly solvents or high temperature required (Kramschuster et al., 2013). For this reason, novel methods are of interest and foaming process with the use of supercritical fluids has potential to overcome these drawbacks. The most commonly used supercritical fluid is carbon dioxide, due to its desirable attributes such as readily available and inexpensive, nontoxic, non-flammable, low critical parameters (T_c = 304.1 K and P_c = 73.8 bar) (White et al., 2012). Properties and characteristics of porous structures are dependent not only on the fabrication conditions but also on the type of materials. Synthetic polymers are promising scaffolding materials. Poly(ε-caprolactone) (PCL) is a semi-crystalline polymer of low melting tempera-

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ture of approximately 59–64 °C. It has always been a popular biomaterial due to its biocompatibility and slow degradation rate (up to 2 years) (Kramschuster et al., 2013). One of fundamental properties of scaffold is its sufficient mechanical strength that should closely match mechanical strength of neotissue. However, no single component polymer material can fulfil all functional requirements. Therefore, fabrication of composite materials seems to be an attractive strategy to develop multifunctional tissue scaffolds. In an attempt to improve mechanical properties and porosity of solid foams, bioactive calcium particle in the form of hydroxyapatite has attracted a lot of attention because of its similarity to the natural bone (Velasco et al., 2015). Nanocellulose, a natural polymer material extracted from native cellulose, possesses diverse characteristics different from traditional materials, including high Young's modulus (up to 167 GPa), high-surface area, possibility for chemical modifications and hydrophilicity. Materials based on this constituent are reported to be promising biomedical materials because of their excellent physical and biological properties, in particular their low cytotoxicity, biocompatibility and biodegradability (Dumrah, 2017). Also graphene-based materials have undergone rapid development in recent years due to their unique structure and excellent mechanical, optical and electrical properties. Graphene oxide (nGO) is a highly oxidised form of graphene whose structure consists of functional groups such as hydroxyls, carbonyls, carboxyls and epoxides. It is reported that addition of nGO to the polymer matrix enhances mechanical and thermal properties of commonly used biomaterials in tissue engineering (Ahadian et al., 2013).

The purpose of this study was to investigate the effects of supercritical foaming process parameters and porogen concentration on the properties of manufactured polymer-based scaffolds that can be applied as scaffolds in tissue regenerative engineering. The effects of temperature, pressure and saturation time on the final porous structure and mechanical, thermal properties of solid foams were particularly identified.

2. MATERIALS AND METHODS

In the performed experiments PCL pellets (D~3 mm) with number-average molecular weight $Mn = 80,000$ were obtained by Sigma Aldrich (Italy) and were used as a model polymeric material. Carbon dioxide (CO_2) with purity grade of 4.5, which was used as a blowing agent in foaming experiments, was purchased from Linde Gaz (Poland). Nano-hydroxyapatite powder and nano-cellulose powder were manufactured by Sigma Aldrich (Italy). Graphene oxide suspension in ethanol was supplied by Nanomaterials (Poland). Polymer-based nanocomposites were obtained by blending of biodegradable polymer – PCL with the reinforcement materials – porogenes. Composites containing 0–12 wt% of nano-hydroxyapatite (nHA), 0–12 wt% of nano-cellulose (nC) and 0–1.5 wt% of graphene-oxide (nGO) were prepared.

In the first part of experimental investigation, poly(ε-caprolactone) scaffolds incorporating hydroxyapatite, cellulose and graphene oxide nanoparticles were fabricated by supercritical foaming technique using high-pressure experimental system shown in Fig. 1.

Composite foaming experiments were conducted in a three-step batch process. First, a stainless steel high pressure vessel was filled with 3 grams of composite material that was melted and saturated with carbon dioxide under certain conditions. The structures were treated with $scCO_2$, at two various foaming pressures: 9 MPa and 18 MPa, at three different temperatures 323 K, 343 K and 373 K. Gas absorption led to formation of a homogeneous carbon dioxide-polymer mixture. This stage lasted 30 minutes, 1 hour or 4 hours. Thereafter, the mixture was cooled to 298 K and kept for 30 minutes under the constant pressure in order to enable nucleation of cells in the polymer matrix. The final stage of foaming process was rapid decompression to atmospheric pressure that led to the growth of foam bubbles. Finally, the composite porous structure was taken out of the stainless steel vessel and the residues of CO_2 were removed by evaporation.

In the second part of experiments mechanical properties and morphology of solid foams were analysed using specialised analytical methods.

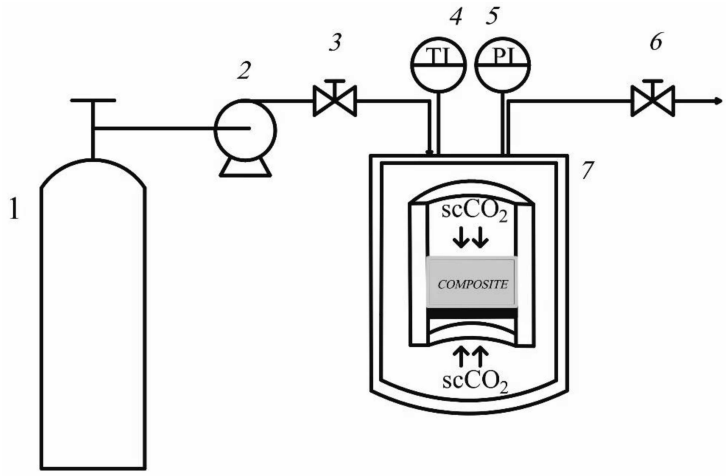

Fig. 1. Schematic diagram of the experimental system: (1) Carbon dioxide tank, (2) $scCO_2$ pump, (3) valve, (4) temperature indicator, (5) pressure indicator, (6) back pressure regulator, (7) high pressure vessel

2.1. Scanning Electron Microscopy

The morphology of cross-section fracture and side section of porous structures was analysed using Phenom scanning electron microscope (SEM). Sectioned foams were attached on SEM stubs using a double-sided tape. Then they were coated with gold for 2 min under an argon atmosphere with a K550× sputter coater. SEM microphotographs were taken at two different magnifications (600×, 2000×). Image software analysis (Axiovison, Carl Zeiss) was used to identify the average pore size D of scaffold materials, by applying the following equation

$$D = \frac{\sum_{i=1}^{n} \sqrt{\frac{4 \cdot P_i}{\pi}}}{n} \tag{1}$$

where P_i represents each pore area and n is a number of pores identified in the analysed sample.

2.2. Differential Scanning Calorimetry (DSC)

Thermal analysis was performed using a Mettler Toledo scanning calorimeter. A sample of about 8 mg was placed in a platinum crucible. Measurements were performed at a temperature range of 303–393 K with temperature increasing rate of 10 K/min under an argon flow rate of 30mL/min. The degree of crystallinity of polymer X_c was calculated from the following equation (Diaz-Gomez et al., 2016)

$$X_c = \frac{\Delta H}{x \cdot \Delta H^0_{PCL}} \tag{2}$$

where ΔH is a value of melting enthalpy obtained from DSC thermogram, ΔH^0_{PCL} is a value of melting enthalpy for 100% crystalline PCL (ΔH^0_{PCL} = 142 J/g), x the weight fraction of PCL in the sample.

2.3. Static Compression Test

Static compression tests were used to investigate the mechanical properties of obtained scaffolds. Three cuboid samples ($5 \times 5 \times 8$ mm) for each solid foam were tested. Measurements were carried out by Instron, the universal testing machine. Scaffolds were compressed to a total strain of 70% using a compression speed of 0.4 mm/min. The Young's modulus of solid foams was determined as the slope of the initial linear portion of stress-strain profile. Also compression strength was identified on the basis of that curve.

3. RESULTS AND DISCUSSION

The critical issue for scaffolds used for tissue engineering is the relation between mechanical properties and morphology sufficient to allow cells to adhere, proliferate and differentiate (Loh et al., 2013). Solid foams based on poly(ε-caprolactone) supplemented with 5% nHA, 5% nC, 0.2% nGO have fulfilled these requirements so they are favourable for biomedical applications.

The morphology and mechanical properties of porous materials prepared via $scCO_2$ foaming process are strongly dependent on the operating conditions such as pressure, saturation time and temperature.

The performed SEM analysis allowed to identify a microstructure of polymer-based composite materials obtained under various foaming process conditions. The effect of pressure on the scaffolds' morphology was studied at a constant saturation temperature of 343 K and saturation time of 1 h. SEM microscope images of cross-sections of the investigated composite porous structures evidencing the effect of process pressure on morphology of the samples are shown in Fig. 2.

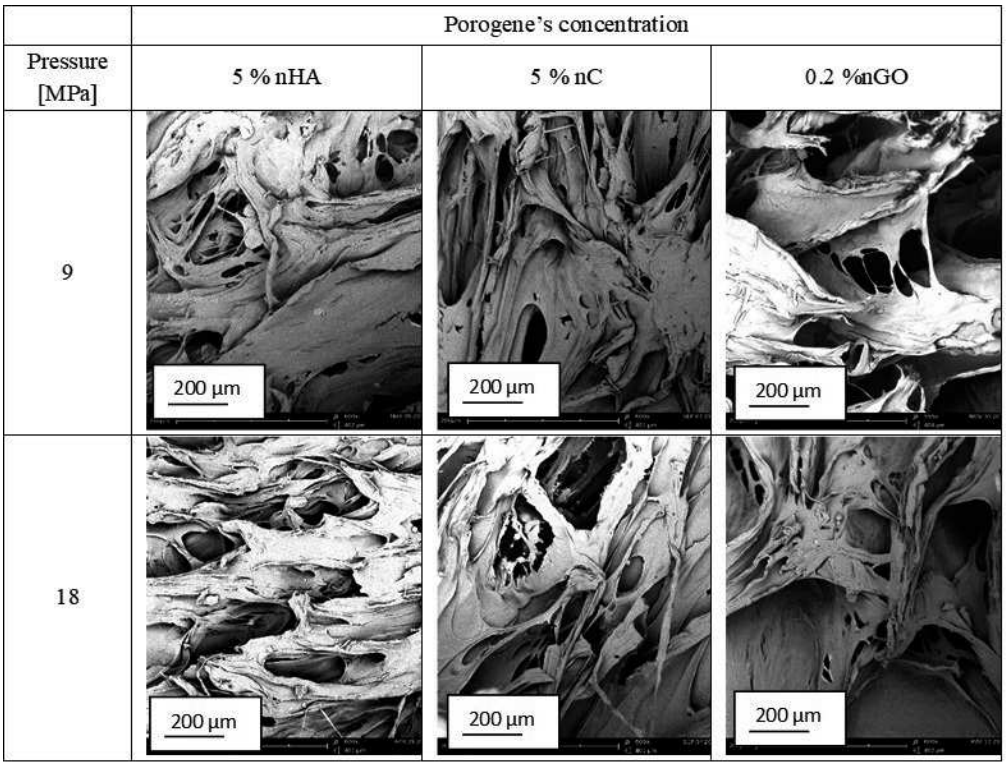

Fig. 2. Effect of process pressure on morphology of porous structures obtained at constant saturation temperature (343 K) and saturation time (1 h)

As presented in SEM microphotographs (Fig. 2), the pore sizes decreased with increase of pressure of polymer saturating. At a constant temperature, when pressure increases, more blowing agent is dissolved into the polymer matrix. This enhances the nucleation process and growth of pores. Moreover, a larger number of nuclei, which act as the centre of cell growth, are created. In general, much more pores of smaller size are formed when high pressure is applied.

The structures of all analysed porous composite foams are non-uniform, also different pore shapes are noticed. In principle, such a scaffold microstructure is most desirable for biomedical application.

The optimal pore size for porous structure is strictly dependent on a type of regenerated tissue. Values of an average pore size of composite structures obtained under constant pressure (18 MPa) and different saturation time and temperature are summarised in Table 1.

Table 1. Effect of foaming conditions on average pore size

Average pore size [μm]				
Pressure [MPa]		18		
Porogene's concentration		5% nHA	5% nC	0.2% nGO
T_{sat} [K]	323	11.17 (±0.84)	5.21 (±0.46)	23.19 (±2.54)
	343	22.43 (±1.61)	17.72 (±1.79)	23.97 (±2.96)
	373	15.27 (±1.86)	7.80 (±1.12)	15.54 (±1.76)
t_{sat} [h]	0.5	14.56 (±1.51)	16.06 (±1.48)	16.02 (±1.56)
	1	22.43 (±1.61)	17.72 (±1.79)	23.97 (±2.96)
	4	11.01 (±1.28)	15.67 (±2.53)	31.85 (±2.70)

Concluding, the application of supercritical carbon dioxide in the foaming process of PCL allowed to obtain composite porous structures with a wide range of average pore sizes. The effect of temperature on the average pore size of a final porous structure was identified. As presented in Tab. 1 average pore size increased with increase of foaming temperature from 323 to 343 K. At higher temperature, CO_2 solubility in composite matrix decreases which reduces the amount of dissolved blowing agent into this structure which is essential for the nucleation and growth of pores. Saturation of composite material at 343 K led to fabrication of scaffolds with the largest average pore size.

It was observed that an increase in saturation time led to creation of larger pores. The longer saturation time the more blowing agent is dissolved in polymer matrix. This results in obtaining pores of larger sizes in comparison to relatively short saturation times.

DSC assays were conducted to investigate the effects of nanoparticles on the crystallisation behaviour of the PCL matrix. As presented in Fig. 3, the melting temperature of composite was determined as the peak

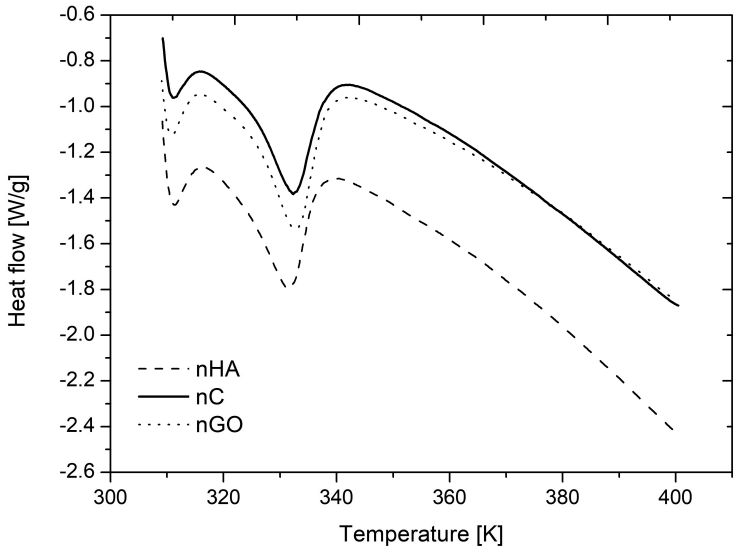

Fig. 3. DSC thermogram of composite porous structures obtained under the foaming conditions P_{sat} = 18 MPa, T_{sat} = 343 K, t_{sat} = 1 h

temperature of heating curve. Applying $scCO_2$ in polymer foaming process led to a decrease in melting point of the analysed materials. The melting temperature of obtained composite porous structures was lower than the melting point of 100% crystalline PCL. On the basis of DSC thermograph foams, degrees of crystallinity were defined. The largest value of crystallinity degree was observed for 5 wt% of nHA in a composite (Table 2).

Table 2. Compression Test and Differential Scanning Calorimetry results of composite foams

Porogene's concentration	Foaming process parameter		Static compression test		DSC	
			Young's modulus [MPa]	Compression strength C [MPa]	Degree of crystallinity [%]	Melting temperature [K]
Foaming process conditions: T_{sat} =343 K, t_{sat} =1 h						
5% nHA	Pressure [MPa]	9	1.446 (±0.21)	0.462 (±0.04)	22.10 (±2.19)	327.83 (±0.41)
		18	1.193 (±0.09)	0.507 (±0.05)	23.50 (±2.19)	326.83 (±0.41)
5% nC		9	1.303 (±0.20)	1.595 (±0.16)	18.55 (±1.85)	328.17 (±0.32)
		18	1.575 (±0.17)	1.331 (±0.47)	16.53 (±1.85)	328.17 (±0.32)
0.2% nGO		9	1.470 (±0.26)	14.872 (±4.81)	18.33 (±0.62)	327.50 (±0.22)
		18	1.698 (±0.09)	1.091 (±0.38)	17.38 (±0.62)	328.83 (±0.22)
Foaming process conditions: P_{sat} =18 MPa, t_{sat} =1 h						
5% nHA	Temperature [K]	323	1.702 (±0.19)	0.784 (±0.20)	23.16 (±2.19)	326.83 (±0.41)
		343	1.193 (±0.09)	0.507 (±0.05)	23.50 (±2.19)	326.83 (±0.41)
		373	1.098 (±0.23)	0.617 (±0.16)	23.56 (±2.19)	327.50 (±0.41)
5% nC		323	0.600 (±0.01)	0.676 (±0.05)	22.45 (±1.85)	327.50 (±0.32)
		343	1.575 (±0.17)	1.331 (±0.47)	16.53 (±1.85)	328.17 (±0.32)
		373	2.235 (±0.12)	2.382 (±0.21)	19.70 (±1.85)	328.83 (±0.32)
0.2% nGO		323	1.478 (±0.05)	1.036 (±0.03)	19.33 (±0.62)	328.83 (±0.32)
		343	1.698 (±0.09)	1.091 (±0.38)	17.38 (±0.62)	328.83 (±0.22)
		373	1.893 (±0.14)	5.189 (±4.23)	19.21 (±0.62)	328.83 (±0.22)
Foaming process conditions: P_{sat} =18 MPa, T_{sat} =343 K						
5 % nHA	Saturation time [h]	0.5	0.422 (±0.14)	0.816 (±0.26)	10.20 (±2.19)	329.50 (±0.41)
		1	1.193 (±0.09)	0.507 (±0.05)	23.50 (±2.19)	326.83 (±0.41)
		4	1.136 (±0.11)	0.448 (±0.12)	23.86 (±2.19)	328.17 (±0.41)
5 % nC		0.5	1.459 (±0.08)	4.127 (±2.17)	9.40 (±1.85)	328.83 (±0.32)
		1	1.575 (±0.17)	1.331 (±0.47)	16.53 (±1.85)	328.17 (±0.32)
		4	0.998 (±0.26)	0.736 (±0.35)	19.84 (±1.85)	326.83 (±0.32)
0.2 % nGO		0.5	3.281 (±0.40)	9.767 (±4.91)	21.80 (±0.62)	328.83 (±0.22)
		1	1.698 (±0.09)	1.091 (±0.38)	17.38 (±0.62)	328.83 (±0.22)
		4	0.633 (±0.10)	1.058 (±0.20)	19.99 (±0.62)	328.83 (±0.22)

A static compression test was performed to determine the mechanical properties of porous scaffolds that are strictly dependent on their porosity and density (Fröhlich et al., 2008). The results of experiments are summarised in Table 2. It was observed that for a relatively short saturation time of 0.5 h, the highest values of Young's modulus and compressive strength of composite scaffolds were achieved. The Young's modulus values increased with increase of pressure, whereas for a compressive strength values the opposite effect was observed. The increase of saturation temperature led to improvement of mechanical properties of solid foams.

4. CONCLUSIONS

The performed experimental investigations demonstrated that porogen concentration and foaming process parameters have a significant impact on the mechanical properties and morphology of obtained three-dimensional porous structures. The most appropriate foam for biomedical application was obtained by supplementing PCL with graphene-oxide (0.2 wt%) at the temperature of 343 K, under the pressure of 18 MPa and for the saturation time of 0.5 h. The structure was characterised by large average pore size and desirable mechanical and thermal properties. It has been proved that presence of the applied porogenes in a composite had no negative effect on foam structure and properties. The obtained results ensured that all investigated three-dimensional porous structures are suitable for potential application in tissue engineering.

SYMBOLS

D	an average pore size, μm
ΔH	value of melting enthalpy obtained from DSC thermogram, J/g
ΔH^0_{PCL}	value of melting enthalpy for 100% crystalline PCL, J/g
n	a number of pores identified in an analysed sample
P_i	each pore area in an analysed sample, μm^2
P_{sat}	saturation pressure, MPa
t_{sat}	saturation time, h
T_{sat}	saturation temperature, K
x	the weight fraction of PCL in the sample
X_c	degree of crystallinity of polymer, %

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