Scientific paper

Protein Release from Biodegradable Poly(ε-Caprolactone)-Chitosan Scaffolds Prepared in $scCO_2$

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Abstract

To study the release patterns of protein bovine serum albumin (BSA), porous poly(ε-caprolactone)-chitosan scaffolds with entrapped BSA were fabricated by using supercritical CO_2 for its potential use in tissue engineering applications. An emulsion, consisting of a polymer-solvent solution and buffer protein solution was saturated with $scCO_2$ at 12 MPa and 37 °C and then rapidly depressurized through a release valve causing bubble nucleation and precipitation of the composite material. The controlled total protein release from biodegradable poly(ε-caprolactone) with 5% chitosan (w/w) scaffolds was assessed by Bradford protein assay. After 16 to 20 days of protein release testing, 58.8% of the protein was released from composite with PCL (M_w = 10,000 g/mol) and 43.9% from composite with PCL (M_w = 60,000 g/mol). Preliminary studies for characterization of the prepared composite biomaterials using FTIR spectra, ESEM photo analysis and DSC analysis have been carried out.

Keywords: Protein release; PCL polymer; chitosan; scaffold; supercritical CO_2

1. Introduction

Biodegradable porous polymer scaffolds are often required for reconstruction or regeneration of organ function in tissue engineering applications, alone or in combination with a bioactive agent. They mimic the extracellular matrix (ECM), so they must provide a sustainable template for cell attachment, proliferation, and differentiation. Biocompatibility, absorbability, appropriate mechanical strength, and porous structure are some of the basic requirements of scaffolds. Biodegradability is a very convenient feature of polyesters, and that is why they are so promising and extensively utilized, since ceramic and metal implants require a second surgical operation, to remove them from the body.[1–3] Furthermore, in bone tissue engineering, the material should form an osteoconductive structural support for the newly formed bone and consist of interconnected pores, which have an important role in supporting cell penetration, new tissue ingrowth, nutrient diffusion, and neovascularization.[4–6]

Poly(glycolic acid), poly(lactic acid), poly(hydroxyl butyrate), poly(ε-caprolactone) (PCL) and their copolymers are the most frequently used synthetic biodegradable polymers in tissue engineering. PCL is highly appealing because of its physical-chemical and mechanical characteristics. It has been broadly investigated on account of its soft- and hard-tissue compatibility. Its low melting point (ca. 60 °C) allows easy processing. Its non-toxic degradation products are easily metabolized or secreted from the body. PCL is approved by the Food and Drug Administration (FDA) for use in biomedical applications. However, as with other biodegradable polyesters, its use could be limited by some drawbacks. The absence of cell recognition sites on the surface of scaffolds can lead to poor cell affinity and adhesion. Its hydrophobicity can prevent the cells from penetrating into the porous structure. Neutral charge distribution can cause a lack of interaction with the ECM. Acidic degradation products are formed during hydrolysis of PCL and could possibly cause side effects. It has a very

slow degradation rate, due to high crystallinity. Therefore, PCL is often mixed with natural polymers to enhance its bioactivity.[1,5,7] Chitosan (CS) is a linear polysaccharide. Its numerous good features, such as biocompatibility, biodegradability, non-antigenicity, non-toxicity, antibacterial properties, and bio-adherence have induced its use in biomedical areas. When combined with PCL, hydrophilic chitosan can modulate the hydrophobicity of PCL, which increases the biocompatibility, cellular affinity, wettability and permeability of matrices.[6,7]

Tissue engineering applications require scaffolds with high surface area to volume ratios. This is crucial for effective cell seeding onto the biomaterial support and for efficient nutrient acquisition, so that rapid proliferation and sufficient physiological activity can be achieved. Porous polyester scaffolds can be formed by many techniques, each of which results in different biomaterial characteristics, therefore, the most convenient method may depend on the application.[8] In the last two decades, there has been significant progress in using $scCO_2$ for polymer foaming. This is a non-toxic, non-flammable, chemically inert and environmentally safe gas.[9] Furthermore, it is inexpensive and offers a useful alternative to organic solvents that can be potentially harmful to cells. CO_2, dissolved in the polymer, decreases the viscosity, acts as a plasticizer and allows processing at lower temperatures. This foaming technique enables control of the size and distribution of pores by selecting suitable processing conditions, like temperature, depressurization rate and solubilisation pressure (gas concentration in the polymer). Good solubility of CO_2 in polymers can be additionally influenced by temperature, pressure and weak interactions with functional groups of polymers.[5,10]

With the ability to control the morphology of the composite, supercritical fluid technology has also overcome the problem of incorporating biologically active species into polymeric composites without a change in activity. The combination of gas-like viscosity and liquid-like density makes $scCO_2$ an ideal medium for making polymer-based materials containing bioactive species. Interaction of $scCO_2$ with amorphous polymers leads to depression of the glass transition temperature (T_g); under these conditions, the polymer is plasticized, which significantly lowers the viscosity and allows incorporation of insoluble bioactive particles into the polymer. Additionally, no solvent residues remain in the material after processing.[11,12]

To create new composite material scaffolds with specific physical-chemical properties PCL (M_w = 10,000 and 60,000 g/mol) was mixed with 5% chitosan (w/w) and further, bovine serum albumin (BSA) was incorporated. We have used BSA as a model protein for preliminary studies of controlled release from prepared porous scaffolds. Measurement of total protein release was performed by Bradford protein assay. As a prior characterization of the prepared material, FTIR, ESEM and DSC analysis on clean PCL and CS and their composites were performed.

2. Experimental

2. 1. Materials

Bovine serum albumin (BSA) (CAS: 9048-46-8) was provided by Sigma-Aldrich. Dimethyl chloride (CAS: 75-09-2), was purchased from Fluka Analytical. Poly(ε-caprolactone) (PCL) of molar weight 10,000 g/mol (CAS: 24980-41-4) and of 60,000 g/mol (CAS: 24980-41-4) and chitosan (CAS: 9012-76-4) were purchased from Sigma-Aldrich. The polymers were delivered as powders and were used without further purification. Moisture content in the polymers, determined gravimetrically by means of an HB43-S Compact Halogen Moisture Analyzer, was lower than 0.10 wt. % for each polymer. CO_2 (99.998%) was supplied by Messer (Slovenia).

2. 2. Scaffold Preparation in $scCO_2$

Before processing with $scCO_2$, an emulsion consisting of a water (aqueous) phase and an organic composite solution phase was prepared by sonicating the immiscible phases.

The water phase consisted of 20 mg BSA dissolved in 200 μL of phosphate buffer at pH 7.4. The organic phase consisted of 500 mg PCL + CS mixture dissolved in 1 mL of dimethyl chloride. Prior to supercritical processing, the emulsion was pipetted into the small cylindrical mold and then immediately placed into a high-pressure view equilibrium cell made of stainless steel (Sitec AG, Zurich, CH) (Figure 1).[13] The high-pressure cell was pressurized to 12 MPa with CO_2 by a high-pressure pump (NWA PM-101). The pressure inside the cell was measured by an electronic pressure gauge (WIKA Alexander Wiegand GmbH & Co. KG, Alexander-Wiegand-Straße, Klingenberg, Germany). The temperature of the cell was kept constant using a heating jacket and was observed using calibrated thermocouple immersed in the cell. The pressure deviation was ± 0.01 MPa and the total temperature deviation was 0.1 °C. When the CO_2 reached the desired working pressure, a stable 20 mL/h flow-rate of CO_2 was produced through the pressure cell by a syringe pump manufactured by ISCO (Lincoln, NE) to fully extract the dimethyl chloride. The emulsion consisting of BSA and a biodegradable polymer was saturated with $scCO_2$ for 18 h to easily incorporate insoluble bioactive particles into the polymer by lowering the glass transition temperature (T_g) and eliminating all solvent residues from the material. In the last step, the high pressure cell was rapidly depressurized at a controlled depressurization rate of 0.1 MPa/s by opening the release valve in order to create a microporous composite scaffold. After the depressurization step, the composite scaffold was removed from the mold and sectioned into three parts for further protein release studies. Prior to material characterization, additional scaffolds were prepared without incorporating BSA protein.

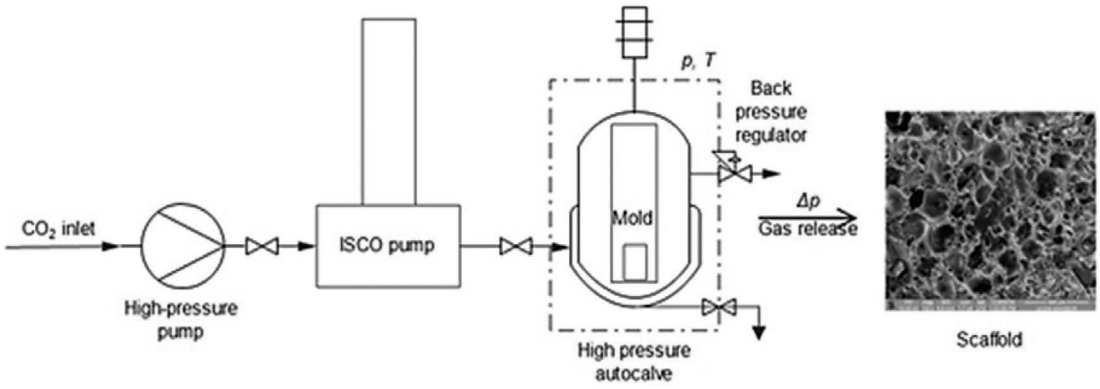

Figure 1. Diagram of the system used to generate biodegradable scaffolds with $scCO_2$.

2. 3. Characterization of Prepared Scaffolds

The porosity of the prepared scaffolds was measured with a graduated cylinder.[1,14] It was calculated as given in the equation (1):

$$\text{Porosity}\ (\%) = \frac{(w_2 - w_3 - w_s)/\rho_e}{(w_1 - w_3)/\rho_e} \times 100 \qquad (1)$$

where w_1 is the weight of graduated cylinder filled with ethanol, w_2 the weight of the graduated cylinder, ethanol and scaffold, w_3 the weight of the graduated cylinder and ethanol after removing the scaffold, w_s the weight of the scaffold and p_e the density of absolute ethanol used in the analysis.

2. 3. 1. DSC Analysis

Differential scanning calorimetry (HP DSC 1, Mettler Toledo) was performed on pure PCL (M_w = 10 000, 60 000 g/mol) and on the prepared porous scaffolds to confirm the composition of PCL and CS and to define the percentage of crystallinity. Samples were placed in sealed aluminium pans. Measurements were held at ambient pressure at a temperature range from 25 °C to 600 °C with a temperature rate of 10 °C/min.

Based on the DSC curves, the melting temperature (T_m), heat of fusion (ΔH_m) and crystallinity of the samples were determined. The crystallinity of the PCL fracture (X_c) in the samples was calculated with equation (2) [15]:

$$X_c = \frac{\Delta H_m}{\Delta H_m^\circ \cdot w} \times 100 \qquad (2)$$

where ΔH_m is the specific melting enthalpy of the sample, ΔH_m° is the melting enthalpy of 100% crystalline poly (ε-caprolactone) (76.9 J/g), and w is the weight fraction of PCL in the blend.[16]

2. 3. 2. FTIR Spectra and ESEM Analysis

The FTIR spectra were recorded on a Fourier Transform infrared instrument (Bruker Platinum-ATR) equipped with OPUS Optik GmBH software in the range from 400 to 4400 cm^{-1} of wavelength.

Pores size for the resulting composite scaffolds generated in $scCO_2$ were determined using environmental scanning electron microscopy (ESEM) Quanta 200 3D (FEI Company, Hillsboro, OR), which allows observation of translation and non-sample without prior preparation (polymer materials, biological and medical samples).

2. 4. Protein Release Studies

Each of the porous PCL-CS composites incorporated with BSA was divided into three smaller units of 50 mg, to verify the uniformity of protein release. Each section was placed into 2 mL microcentrifuge tubes, containing 1 mL of phosphate buffer (PBS) with pH 7.4. In order to simulate the conditions in the human body, the tubes were incubated at 37 °C. The amount of the released BSA was determined by the Bradford method.[17] First measurement was performed after 3 hours and second after 24 hours from the start of the study. Then, Bradford assay was repeated every few days. After each measurement, fresh PBS was added to the microcentrifuges containing parts of the composites.

3. Results and Discussion

Results for the characterization of prepared composite material using FTIR spectra, ESEM photo analysis, DSC analysis and finally protein release studies are presented in the following subsections.

3. 1. DSC Analysis

DSC analysis was performed on two pure PCLs with different molar masses (M_w = 10,000 and 60,000 g/mol) and on porous scaffolds after processing with CO_2 to confirm the composition formation of the mixture between PCL and CS. As seen in Table 1, the melting point of both composites was lower than the melting point of pure PCL.

Table 1. Melting temperatures and specific melting enthalpies of the samples.

Material	T_m (°C)	ΔH_m (J/g)
PCL 10,000	67.58	71.31
PCL 10,000 + 5% CS	62.88	65.17
PCL 60,000	73.04	66.62
PCL 60,000 + 5% CS	66.23	55.04

That coincides with the previously published results by She et al.[18], where the melting point of the composites was decreasing with the increase of the amount of CS in the mixture. PCL 10,000 based composite material has a higher crystallinity (89.2%) than the PCL 60,000 based composite (75.3%). Degradation of materials was observed at a temperature of approximately 420 °C, and a slight decrease in decomposition temperature of the composites can be obtained, compared to pure PCL.

3. 2. FTIR Analysis

The FTIR analysis for raw materials, pure PCL, CS, and other obtained composites was made. Figure 2 shows the spectra of raw materials, PCL (M_w = 10,000 g/mol), CS and the composite material. Figure 3 shows the spectra of pure PCL (M_w = 60,000 g/mol), CS and their composite. The characteristic peak of pure PCL that corresponds C=O stretching vibration of the carbonyl group in ester is at 1732 cm^{-1} for PCL (M_w = 10,000 g/mol) and at 1734 cm^{-1} for PCL (M_w = 60,000 g/mol). The spectra of pure CS shows a broad peak for the O-H stretch at 3408 cm^{-1}. The peak at 1656 cm^{-1} represents N-H stretching. Another peak for 1° amine in CS at 1076 cm^{-1} represents the C-N stretch. C=O stretching vibrations were detected in both composites, at 1735 cm^{-1} for the composite with PCL (M_w = 10,000 g/mol) and at 1734 cm^{-1} for the composite with PCL (M_w = 60,000 g/mol). In the blend of PCL and CS, interactions between the carbonyl groups of PCL and the hydroxyl and amine groups of CS can occur, which leads to the formation of ester and amide bonds. However, there are no major absorptions shifts of the characteristic functional groups to be seen when the spectra of pure PCL is compared to the spectra of the composites. This could indicate that no molecular interactions occurred between functional groups, a finding which corresponds to results from the previous studies.[19] Peaks in the range from 2850 cm^{-1} to 3000 cm^{-1} represent alkyl (sp^3 hybridization of C atoms) stretching vibrations in pure materials and in the composites. This could indicate that no molecular interactions occurred between functional groups, a finding that corresponds to results of Neves et al.[7] who have constructed composite materials by blending CS and PCL to make 3D fiber-mesh scaffolds for articular cartilage tissue repair. On the contrary, spectra in Wu et al.[19], where composites were made with layer-by-layer assembly technique, show

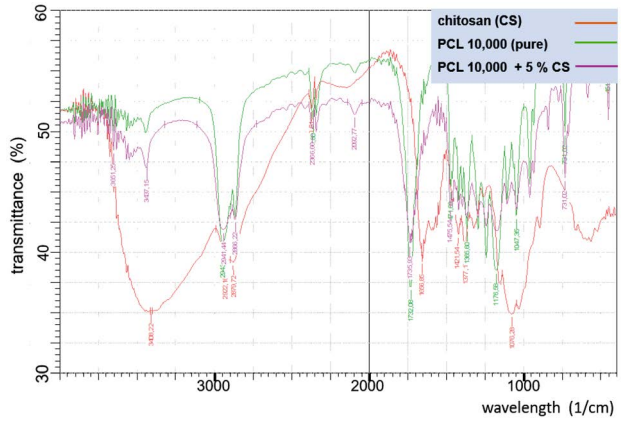

Figure 2. The FTIR spectra of raw materials (PCL (M_w = 10,000 g/mol) and CS) and the composite material.

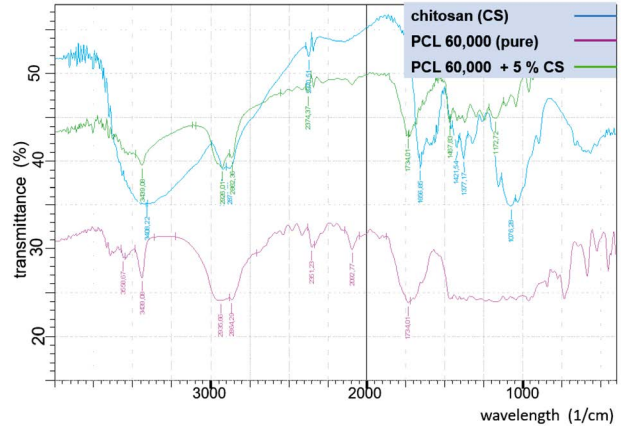

Figure 3. The FTIR spectra of raw materials (PCL (Mw = 60,000 g/mol) and CS) and the composite material.

some new strong peaks when spectrum of pure CS is compared to blend (PCL+CS). FTIR of PCL and CS composites, prepared by She et al.[18], have confirmed that the intensity of characteristic peaks increases with increase of CS content.

3. 3. ESEM Analysis and Porosity

Figure 4 (a and b) shows an ESEM micrograph of PCL-CS scaffolds foamed with $scCO_2$ at 12 MPa and 37 °C. ESEM analysis showed that resulting porous scaffolds have a closed cell structure, which enables better isolating properties because of its greater stiffness and toughness and lower permeability. The diameters of the pores vary from 50 to 130 μm when PCL 10,000 + 5% CS is used; and from 40 to 140 μm when PCL 60,000 + 5% CS is used, so they are considered to be macropores (Table 2).

For the pore growth period, a constant moderate depressurisation rate of 0.1 MPa/s was selected. At quicker depressurization rates more pores could be generated but with undesired smaller pore sizes.[20] Similarly, the lowest possible operating pressure of 12 MPa was used in order to

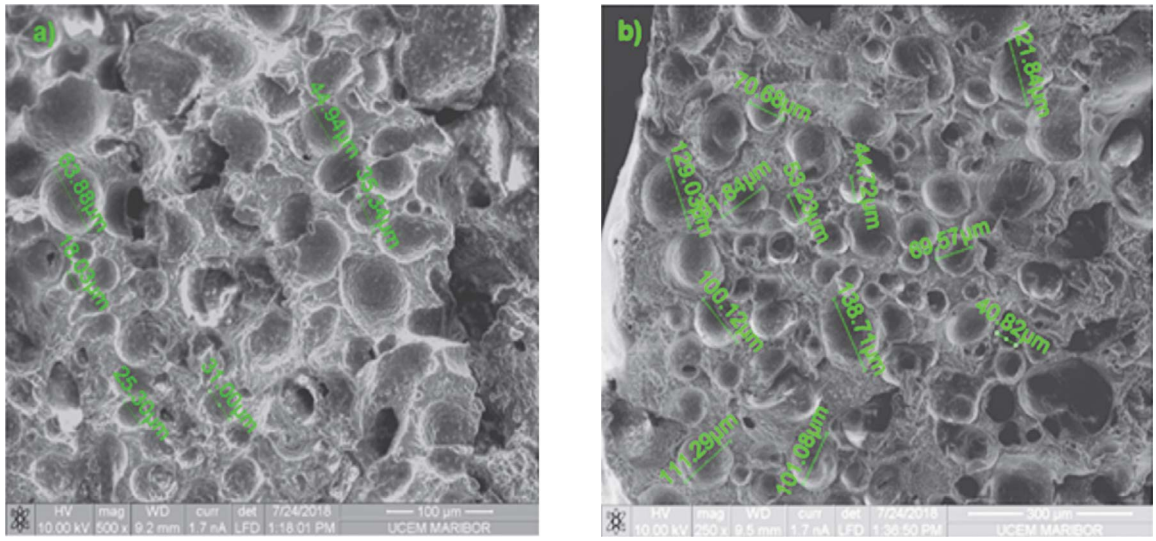

Figure 4. Environmental scanning electronic microscope image of a) PCL 10,000 + 5% CS and b) PCL 60,000 + 5% CS scaffolds.

Table 2. Preparation of biodegradable composite material at an applied pressure of 15 MPa and a temperature of 37 °C.

Composite	Molar mass (g/mol)	Depressurization rate (MPa/s)	Pore size interval (μm)	Porosity (%)
PCL 10,000 + 5% CS	10,000	0.1	50–130	80.1
PCL 60,000 + 5% CS	60,000	0.1	40–140	75.1

achieve the melting behavior of a polymer PCL in contact with $scCO_2$ at 37 °C. At selected supercritical conditions, PCL undergoes to partial or complete melting at 37 °C which is well below its melting point at ambient pressure.[21] Increasing pressure above the selected one results in higher rate of dissolved gas in biomaterial matrix that creates more nuclei and decreases the number of suitable pore sizes for tissue engineering.[22]

Porosity was calculated from. and was in both composites relatively high, above 75%. Biomaterials with a porosity greater than 70% and a size of pores around 100 μm are required to allow vascularization and tissue ingrowth.[23] Additionally, used supercritical foaming method generated high porous biomaterial scaffolds without toxic organic residuals and a waste of the materials.[24] Supercritical foaming method can be easily combined with other scaffold fabrication techniques like salt leaching, breath figure method and thermal induced phase method to obtain better pore interconnectivity and higher porosity.[25]

3. 4. Protein Release Monitoring

Total protein release monitoring was assessed for the PCL 10,000 + 5% CS and PCL 60,000 + 5% CS scaffolds by dividing each of them into three equal parts. The influence of molecular mass of PCL and time of protein release from composite polymers on total concentration of released BSA was studied. As presented in Figure 5, BSA loaded scaffolds showed two-stage release profiles. Larger concentrations of protein were released in the first few days from both investigated scaffolds, and then release of protein was constant, since the total protein concentration linearly increased with increase in release time. The main share of the protein from PCL–based scaffolds was released within the first 16 days. After that time, concentrations of protein in PBS solutions were zero or negligibly small, regardless of the tested composite. Higher total protein release was observed for the scaffold PCL 10,000 + 5% CS compared to the scaffold PCL 60,000 + 5% CS. This can be related to scaffold porosity, which is approximately 5% higher in the

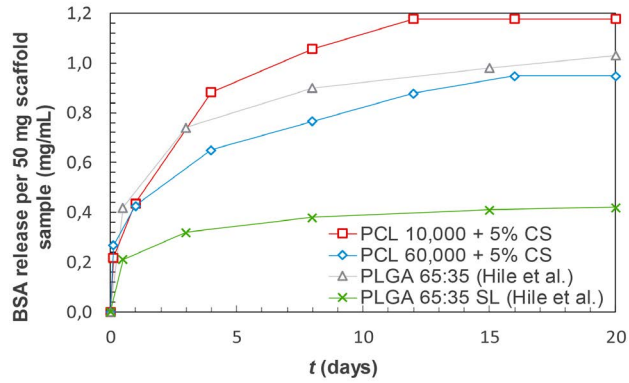

Figure 5. Total concentration of released BSA from PCL 10,000 + 5% CS and PCL 60,000 + 5% CS scaffolds vs. time of release, compared to the literature data.[21]

case of PCL 10,000 + 5% CS. With high porosity, the lightly trapped BSA in the micropores can be easily released.[26] Similar controlled release protein patterns were observed when BSA and basic fibroblast growth factor (bFGF) was encapsulated in poly(lactic-co-glycolic) acid (PLGA 65:35) generated in $scCO_2$.[27] The overall release rates from the scaffolds foamed with CO_2 were up to twice as high compared to release patterns of PLGA 65:35 by the salt leaching method. This can be related to protein loss due to the distilled water required for salt leaching.[28]

The BSA release was normalized to the initial protein entrapped in the scaffold, as shown in Figure 6. At the beginning, the release of proteins follows the same curve for both tested scaffolds and after 4 days of protein release, the PCL 10,000 + 5% CS shows better release profile, which

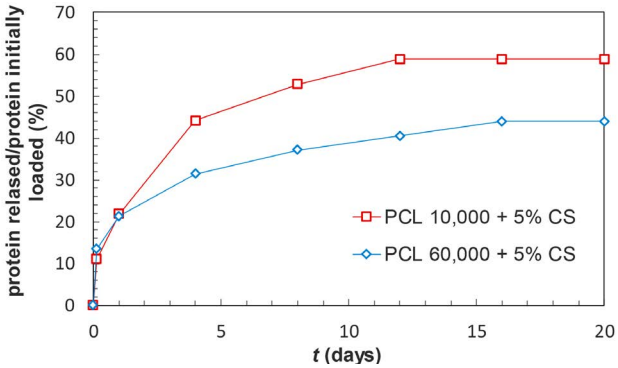

Figure 6. Total protein release normalized to the initial protein entrapped in the scaffold (for PCL 10,000 + 5% CS and PCL 60,000 + 5% CS) vs. time of release.

can be attributed to better porosity of the material. 58.8% of the protein was released from the composite with PCL (M_w = 10,000 g/mol) and 43.9% from composite with PCL (M_w = 60,000 g/mol) after 16 or 20 days of protein release testing.

4. Conclusion

Porous PCL-CS composite scaffolds with entrapped BSA were generated in $scCO_2$ as constructs for tissue engineering. Before the protein release studies, scaffolds were prepared for characterization of the composite material without incorporated BSA. DSC analysis confirmed the miscibility of the blend with a decrease in the melting points of the composites, compared to pure PCL. ESEM analysis showed that the resulting porous scaffolds have a closed cell structure that yields better isolating properties because of higher toughness and lower permeability.

When used as a model protein loaded into scaffolds, BSA showed two-stage release profiles. Larger concentrations of protein were released in the first few days in both investigated scaffolds. After 16 to 20 days of protein release testing, 58.8% of the protein was released from composite with PCL (M_w = 10,000 g/mol) and 43.9% from composite with PCL (M_w = 60,000 g/mol). The ability to simply incorporate and release thermolabile proteins at a controlled rate demonstrates the potential for using $scCO_2$ as a blowing agent for creating scaffolds and opens up the frontier for investigating encapsulation of healing agents and therapeutic enzymes for tissue engineering applications.

Table 3. Concentrations of protein, released from three equal parts of scaffolds (PCL 10,000 + 5% CS and PCL 60,000 + 5% CS).

Time (days)	Protein concentration in scaffold part 1 (mg/mL)	Protein concentration in scaffold part 2 (mg/mL)	Protein concentration in scaffold part 3 (mg/mL	Average concentration (mg/mL)	Total roteinp release (mg/mL)
PCL 10,000 + 5% CS					
0	0	0	0	0	0
0.125	0.249	0.204	0.205	0.219	0.219
1	0.169	0.162	0.320	0.217	0.436
4	0.113	0.680	0.550	0.448	0.883
8	0.106	0.291	0.121	0.173	1.056
12	0.122	0.144	0.096	0.121	1.177
16	0	0	0	0	1.177
20	0	0	0	0	1.177
PCL 60,000 + 5% CS					
0	0	0	0	0	0
0.125	0.385	0.118	0.302	0.268	0.268
1	0.090	0.103	0.281	0.158	0.426
4	0.123	0.398	0.152	0.225	0.651
8	0.108	0.115	0.121	0.115	0.765
12	0.018	0.079	0.080	0.113	0.878
16	0.060	0.068	0.083	0.070	0.949
20	0	0	0	0	0.949

5. Acknowledgement

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6. References

1. Y. Wan, H. Wu, X.Cao, S. Dalai, *Polym. Degrad. Stab.* **2008**, *93*, 1736–1741. **DOI:**10.1016/j.polymdegradstab.2008.08.001
2. H.-Y. Cheung, K-T. Lau, T.-P. Lu, D. Hui, *Composites, Part B.* **2007**, *38*, 291–300. **DOI:**10.1016/j.compositesb.2006.06.014
3. M. Leitgeb, K. Heržič, G. Hojnik Podrepšek, A. Hojski, A. Crnjac, Ž. Knez, *Acta Chim. Slov*. **2014**, *61*, 145–152.
4. S. Kovačič, N. B. Matsko, G. Ferk, C. Slugovc, *Acta Chim. Slov*. 2014, *61*, 208–214.
5. B. Dariš, P. Ferk, E. Markočič, Ž. Knez, *Acta Medi. Bio.* **2016**, *9*, 42–48.
6. R. M. Jin, N. Sultana, S. Baba, S. Hamdan, A. F. Ismail, *J. Nanomater*. **2015**, *2015*. **DOI:**10.1155/2015/357372
7. S. C. Neves, L. S. Teixeira Moreira, L. Moroni, R. L. Reis, C. A. Van Blitterswijk, N. M. Alves, M. Karperien, J. F. Mano, *Biomaterials*. **2011**, *32*, 1068–1079. **DOI:**10.1016/j.biomaterials.2010.09.073
8. M. Whitaker, R. Quirk, S. Howdle, K. Shakesheff, *J. Pharm. Pharmacol*. **2001**, *53*, 1427–1437. **DOI:**10.1211/0022357011777963
9. M. Škerget, Ž. Knez, *Acta Chim. Slov*. **2007**, *54*, 688–692.
10. E. Markočič, M. Škerget, Ž. Knez, *Ind. Eng. Chem. Res*. **2013**, *52*, 15594–15601. **DOI:**10.1021/ie402256a
11. S. M. Howdle, M. S. Watson, M. J. Whitaker, V. K. Popov, M. C. Davies, F. S. Mandel, J. D. Wang, K. M. Shakesheff, *Chem. Commun*. **2001**, 109–110. **DOI:**10.1039/b008188o
12. A. Salerno, J. Saurina, C. Domingo, *Int. J. Pharm*. **2015**, *496*, 654–663. **DOI:**10.1016/j.ijpharm.2015.11.012
13. G. Kravanja, M. K. Hrnčič, M. Škerget, Ž. Knez, *J. Supercrit. Fluids*. **2016**, *108*, 45–55. **DOI:**10.1016/j.supflu.2015.10.013
14. J. Yang, G. Shi, J. Bei, S. Wang, Y. Cao, Q. Shang, G. Yang, W. Wang, *J. Biomed. Mater. Res*. **2002**, *62*, 438–446. **DOI:**10.1002/jbm.10318
15. A. R. C. Duarte, J. F. Mano, R. L. Reis, *Polym. Degrad. Stab*. **2010**, *95*, 2110–2117. **DOI:**10.1016/j.polymdegradstab.2010.06.020
16. E. de Paz, A. n. Martín, S. Rodríguez-Rojo, J.Herreras, M. a. J.Cocero, *J. Chem. Eng. Data*. **2010**, *55*, 2781–2785. **DOI:**10.1021/je900997t
17. Kruger, N. J. in The protein protocols handbook, Springer, 2009, pp. 17–24. **DOI:**10.1007/978-1-59745-198-7_4
18. H. She, X. Xiao, R.Liu, *J. Mater. Sci*. **2007**, *42*, 8113–8119. **DOI:**10.1007/s10853-007-1706-7
19. H. Wu, Y. Wan, X. Cao, S. Dalai, S. Wang, S. Zhang, *Matter. Lett*. **2008**, 62, 2733–2736. **DOI:**10.1016/j.matlet.2008.01.029
20. V. N. Malheiro, S. G. Caridade, N. M. Alves, J. F. Mano, *Acta Biomater*. **2010**, *6*, 418–428. **DOI:**10.1016/j.actbio.2009.07.012
21. L. J. M. Jacobs, M. F. Kemmere, J. T. F. Keurentjes, *Green Chem*. **2008,** 10.7, 731–738. **DOI:**10.1039/b801895b
22. L. J. White, V. Hutter, H. Tai, S. M. Howdle, K. M. Shakesheff, *Acta Biomater*. **2012**, 8.1, 61–71. **DOI:**10.1016/j.actbio.2011.07.032
23. A. Salerno, S. Zeppetelli, E. Di Maio, S. Iannace, P. A. Netti, *Macromol. Rapid Commun*. **2011**, *32*(15), 1150–1156. **DOI:**10.1002/marc.201100119
24. M. A. Fanovich, J. Ivanovic, D. Misic, M. V. Alvarez, P. Jaeger, I. Zizovic, R. Eggers, *The J. Supercrit. Fluids.* **2013,** 78, 42–53. **DOI:**10.1016/j.supflu.2013.03.017
25. L. D. Gomez, A. Concheiro. C. A. Lorenzo, C. A. G. González, *Carbohydr. Polym*. **2016,** 142, 282–292. **DOI:**10.1016/j.ijpharm.2017.05.038
26. K. Zhang, Y. Wang, J. Jiang, X. Wang, J. Hou, S. Sun, Q. Li. *J. Mater. Sci*. **2019,** 54, pp 5112–5126. **DOI**: 1https://doi.org/10.1007/s10853-018-3166-7
27. H. Nie, S. T. Khew, L. Y. Lee, K. L. Poh, Y. W. Tong, C.-H. Wang, *J. Controlled Release*. **2009**, 138, 64–70. **DOI:**10.1016/j.jconrel.2009.04.027
28. D. D. Hile, M. L. Amirpour, A. Akgerman, M. V. Pishko, *J. Controlled Release*. **2000**, 66, 177–185. **DOI:**10.1016/S0168-3659(99)00268-0
29. S. B. Lee, Y. H. Kim, M. S. Chong, S. H. Hong, Y. M.Lee, *Biomaterials*. **2005**, 26, 1961–1968. **DOI:**10.1016/j.biomaterials.2004.06.032

Povzetek

Z namenom študija sproščanja proteina BSA iz kompozitnega materiala, smo pripravili s superkritičnim CO_2 porozne pene iz polikaprolaktona in hitozana, ki bi bile potencialno uporabne v tkivnem inženirstvu. Emulzijo, ki sestoji iz polimera, topila in puferne raztopine s proteinom smo nasitili s superkritičnim CO_2 pri 12 MPa in 37 °C in nato z odprtjem izhodnega ventila hitro zmanjšali tlak, ki je povzročil nukleacijo in nastanek poroznega kompozitnega materiala. Kontrolirano sproščanje proteina iz biorazgradljivih pen polikaprolaktona in 5 % hitozana (w/w) smo merili z Bradfordovo metodo za določevanje proteinov. Po 16 do 20 dneh sproščanja se je skupno izločilo iz kompozita s PCL (M_w=10,000 g/mol) 58.8 % proteina in iz kompozita s PCL (M_w = 60,000 g/mol) 43 %. Preliminarno smo izvedli študije za karakterizacijo pripravljenih kompozitnih biomaterialov z uporabo FTIR spektrov, ESEM foto analize in DSC analize.