Combining Breath Figures and Supercritical Fluids To Obtain Porous Polymer Scaffolds

Marta Castaño,† Enrique Martínez-Campos,‡,§ Mercedes Pintado-Sierra, § Carolina García, † Helmut Reinecke,† Alberto Gallardo,†,‡ Juan Rodríguez-Hernandez, † and Carlos Elvira*†‡

†Department of Applied Macromolecular Chemistry, Institute of Polymer Science and Technology (ICTP-CSIC), Juan de la Cierva 3, 28006 Madrid, Spain
‡Institute of Biofunctional Studies (IEB), Tissue Engineering Group, (UCM), Associated Unit to the Institute of Polymer Science and Technology (ICTP-CSIC), Paseo de Juan XXIII 1, 28040 Madrid, Spain
§Institute of Organic Chemistry (IQOG-CSIC), Juan de la Cierva 3, 28006 Madrid, Spain

ABSTRACT: Supercritical fluids technology is a clean methodology to foam polymeric materials. However, this technique provides only the formation of inner porosity, whereas the so-called skin layer is commonly observed at the polymer surface. This article describes a new method for the preparation of outer and inner porous poly(ε-caprolactone) (PCL) scaffolds by combination of supercritical CO2 (SCCO2) foaming and the breath figures technique. In the first step, experiments with a SCCO2 reactor were performed at 35–45 °C, 100–250 bar, and 1–20 min depressurization time. The effect of these parameters in the formation of inner porosity was investigated for an adequate optimization. In a late stage, to provide also surface porosity to the polymeric samples and remove the skin layer, the breath figures technique was employed. The evaluation of porosity was determined by scanning electronic microscopy, mercury porosimetry, and micro X-ray computerized tomography scanning processing the images obtained with the ImageJ software. The results of this study using these two complementary techniques showed the existence of interconnectivity between inner and outer porosity of the samples. Furthermore, thermal transitions and crystallinity of the PCL samples have been analyzed by differential scanning calorimetry. Finally, a preliminary biological evaluation of the resulting scaffolds with mouse endothelial cells (C166-GFP) was performed to assess their biocompatibility and cellular viability.

1. INTRODUCTION

Porous biodegradable polymer scaffolds are being applied in tissue regeneration processes1 as the number of people who receive life-saving organs and tissue transplantation is limited by a shortage of donor tissue. A great variety of biomaterials as synthetic polymers, ceramics, and naturally derived proteins are being used for the preparation of these scaffolds.2 Different techniques have been developed to fabricate these polymers into porous scaffolds for tissue engineering applications.3–5 In this sense, supercritical CO2 (SCCO2) is a well-known technology to obtain porous structures of polymeric materials. Its advantages with respect to other techniques rely on its green character (CO2 is recyclable and elude the undesirable use of organic solvents), low economical costs, easy achievable critical points, nontoxicity, nonflammability, and easy control of the porosity and pore size by the depressurization process when using the appropriate temperature and pressure conditions.6,7 However, the main disadvantage that this technique presents when foaming polymer samples is the appearance of the so-called “skin layer” which avoid the formation of external porosity. Regarding the skin layer, the rapid diffusion of the embedded fluid out of the sample edges results in the formation of this dense nonporous skin layer that can be decreased, to a certain extent, by an increase in pressure.8

Different approaches have been carried out to prevent the formation of a skin layer and form an entirely porous structure. In that sense, Mooney et al.9 incorporated NaCl particles to the polymer solution of poly(3,4-lactic-co-glycolic acid) before gas foaming. The leaching of this porogen after fabrication of the polymer foam created an interconnected open pore network. Barry et al.10 in 2004 simply removed the skin layer from the scaffolds before cell culture when they used the gas-foaming method to create poly(ethyl methacrylate)/tetrahydrofurfuryl methacrylate foams. Hori et al.11 prepared skinless polymer foams by treating poly(methyl methacrylate) (PMMA) with SCCO2 that is, by immersing the PMMA in SCCO2 with ethanol to nucleate, and then incubated in a hot water bath to induce nuclear growth. The obtained polymer

Received: August 14, 2018
Accepted: September 11, 2018
Published: October 4, 2018
foams had a low density, and cells were connected to each other to make channels through the skin layer.

Our group described recently the preparation of poly(ε-caprolactone) (PCL) biodegradable porous membranes with outer and inner porosity just by SCCO$_2$ treatment, displaying surface hierarchical macroporosity which could be tailored by careful control of the pressure, in the range of 150−250 bar, and depressurization processes in several steps, showing also pore interconnectivity between both membrane faces. In this case, the thickness of the employed membranes played an important role to obtain outer and inner porosity, as those with thickness higher than 90 μm showed the appearance of the so-called skin layer.

In the present work are combined the SCCO$_2$ technology, which provides the internal porosity, with the breath figures (BF) technique which affords the external porosity in PCL samples independently of the sample thickness. This technique is based on the short immersion of the porous samples in an organic solvent in a moist atmosphere. During the evaporation of the thin solvent layer deposited at the polymer surface, the interfacial temperature decreases thus leading to water vapor condensation. The condensation process finally produces water droplets at the sample surface which, after evaporation of both solvent and condensed water, provide the external porosity. SCCO$_2$ pressure, temperature, and depressurization are crucial parameters that were evaluated to obtain the internal porosity, as well as different immersion times for the BF technique were employed to obtain connection between the external and internal porosity. The thermal properties of the foamed PCL are also described in terms of changes in glass transition temperature, crystallinity, and melting point. Finally, cell culture studies were carried out on the prepared porous PCL samples using mouse endothelial cells analyzing the metabolic activity with a view to possible applications in tissue engineering.

2. RESULTS AND DISCUSSION

2.1. Control of the Internal Porosity of the PCL Scaffolds. Although all polymeric samples treated exclusively with SCCO$_2$ showed a nonporous outer skin layer with variable thickness, the pore distribution as well as the average pore size depends on the experimental conditions. Therefore, the optimization of the inner porosity was carried out by varying three parameters: temperature, pressure, and depressurization time.

The first parameter explored was the temperature. Figure 1 shows the scanning electronic microscopy (SEM) micrographs for samples prepared at different temperatures, between 35 and 45 °C, keeping the pressure constant at 200 bar and the depressurization time at 5 min. On the one hand, at 35 and 37 °C, no porosity can be observed in the center of the cylindrical samples. At 35 °C, the thickness of the porous layer is about 400 μm, and at 37 °C, the thickness of the porous layer is about 800 μm. The absence of porosity in the center of the polymer is associated with the low temperature used. At this temperature, the viscosity of the polymeric matrix (largely below the melting point of PCL) is too high and limits the diffusion of CO$_2$ within the saturation time employed. This fact can also be due to the noncompleted solubility of CO$_2$ in the polymer sample.

On the other hand, as depicted in Figure 1, at temperatures between 40 and 45 °C, the polymer samples showed inner porosity in the whole fractured area. Table 1 shows the average pore size of the samples for the different conditions employed. At 40 °C a more homogeneous distribution with an average pore size of 77±17 μm can be seen, while at 45 °C this value is 106±29 μm and the size distribution is more heterogeneous. This observation can be related to a partial collapse of the pores due to a process temperature close to the melting temperature of the polymer after the absorption of CO$_2$. The larger pore sizes observed at 45 °C are due to the fact that the viscosity of the polymer is lower and the gas diffusivity increases allowing the pores to grow.

As a result of the experiments depicted above, the temperature employed for the rest of experiments was 40 °C, as at this value pores are obtained in the whole sample while remaining below the melting temperature. Interestingly, this temperature has also been selected and used by other authors that employed PCL as polymeric material.

The second parameter explored to control the internal structure of the PCL scaffolds was the pressure employed. In Figure 2, the SEM images of the porous samples obtained varying the pressure employed are represented. At 100 bar (Figure 2a) (the lowest pressure tested), a mixture of areas with rather large pores (up to 500 μm in diameter) and solid areas can be distinguished. Using these conditions, the SCCO$_2$ has not been dissolved enough to generate the appropriate pore size.
number of nuclei that can grow afterward. A rather homogeneous distribution can be observed in the fractured area for the other three experimental pressures (Figure 2b–d). Moreover, the average pore size decreases when the pressure increases (Table 1). This effect is associated with an increase in dissolved CO2 in the polymer matrix, which generates more available nuclei for the formation and growth of the pores. As a consequence, more pores showing smaller size are generated.15

Finally, the third experimental condition analyzed was the depressurization time. In Figure 3, the different SEM images obtained by varying the depressurization time are depicted. When the depressurization time increases, that is, depressurization takes place at slower rate, the average pore size also increases (see also Table 1). The reason for this observation is that the nucleation process competes with the growth of the pores, as a result of the gas diffusion through them. On the one hand, when the depressurization rate is high, nucleation takes place faster and a great amount of pores are generated. The development of each pore will occur fast, so that the effects of diffusion will be negligible, and the resulting structure will have an uniform pore size distribution. On the other hand, when the nucleation process is slow, the resulting pores will be bigger than the rest because of a higher diffusion of the gas, and the final structure will have a larger pore size distribution.19

2.2. Analysis of the Pore Interconnection. A major requirement for the application of porous materials for cell growth is the formation of interconnected pores that enable the cells to migrate and simultaneously permit the flow of nutrients inside the scaffold. As depicted in Figure 4, both closed pores as well as a majority of interconnected pores have been observed in the cross section SEM micrographs of the samples. Besides, the high porosity values obtained by mercury porosimetry (Table 2) revealed the existence of connection.
between the pores. It is important to note that non-interconnected pores would not have permitted mercury to fill all the free space in the sample. Interestingly, it is worth mentioning that porosity increases when pressure increases at a given depressurization time and can be, therefore, modulated.

Although, by micro-computerized tomography, it has also been possible to show the good interconnection between the inner pores, we will discuss these analyses in the next section.

2.3. Effect of SCCO2 on the Thermal Transitions and Crystallinity of the Polymer.

Differential scanning calorimetry (DSC) analyses were made to determine the influence of the SCCO2 on the thermal transitions of the processed porous PCL. For comparative purposes, as a control sample, a nontreated PCL was employed and the measured \( T_g \) was \(-60 \) °C and the \( T_m \) was \( 73 \) °C, while the crystallinity was \( 50.6\% \).

Table 3 shows the values obtained for a selection of samples, and it can be observed that the \( T_g \) in all cases is very similar to the one of the control sample, with variations between \(-62 \) and \(-59 \) °C. Nevertheless, the changes observed in the \( T_m \) are greater, going from \( 6 \) to \( 14 \) °C less than the untreated PCL independently of the pressures employed or the depressurization times used. The difference between the thermal transitions after the BF technique in comparison to the ones just treated with SCCO2 under same conditions is also negligible. Regarding the crystallinity, it was observed that, in general, these were higher in comparison to the control PCL. This behavior, also reported by other authors,\(^\text{17,20,21}\) can be explained due to the fact that the experimental temperature is close to the \( T_m \) resulting in a higher mobility of the chains, thus enabling the polymer chain reorganization and improving the crystal formation. It is also worth noting that the \( T_m \) decrease can be attributed to the formation of smaller crystals, as they do not have enough time to crystallize as bigger ones.

2.4. Surface Porosity and Interconnection with Inner Porosity.

After the optimization of the parameters of the SCCO2 technique, tests were conducted to study the formation process of the pores on the surface of the scaffolds by the BF technique. This study was focused on the samples obtained at 250 bar saturation pressure and 40 °C temperature which were the most homogeneous in terms of pore size. The interconnection of the pores was studied by SEM (Figures 5 and 6) and by microcomputerized X-ray tomography (Figure 7).

### Table 2. Porosity Measured by Mercury Porosimetry for Samples Treated with SCCO2 at Different Saturation Pressures

| Sample  | Pressure [bar] | Depressurization Time [min] | Porosity [%] |
|---------|----------------|-----------------------------|--------------|
| E4-P100 | 100            | 5                           | 71.9         |
| E4-P150 | 150            | 5                           | 76.8         |
| E4-P200 | 200            | 5                           | 79.1         |

### Table 3. DSC Results for Untreated PCL, PCL Treated under Different Pressure, and Depressurization Time Conditions with the SCCO2 Foaming Technique, and PCL Samples after the BF Technique

| Sample  | Pressure [bar] | Depressurization Time [min] | Immersion in CHCl₃ [s] | \( T_g \) [°C] | \( T_m \) [°C] | Crystallinity [%] |
|---------|----------------|-----------------------------|------------------------|--------------|--------------|------------------|
| PCL control |                |                             |                        | \(-60\)       | 73           | 50.6             |
| E4-P100B | 100            | 5                           | 3                      | \(-60\)       | 65           | 56.0             |
| E4-P150C | 150            | 5                           | 7                      | \(-60\)       | 67           | 54.3             |
| E1-T40  | 200            | 5                           | 5                      | \(-62\)       | 59           | 45.3             |
| E1-T40B | 200            | 5                           | 7                      | \(-61\)       | 67           | 57.2             |
| E4-P250I | 250            | 5                           | 10                     | \(-61\)       | 64           | 51.4             |
| E4-P250C | 250            | 5                           | 5                      | \(-61\)       | 65           | 55.3             |
| E4-P250E | 250            | 10                          | 5                      | \(-59\)       | 67           | 51.2             |
| E4-P250F | 250            | 10                          | 5                      | \(-59\)       | 67           | 67.7             |
| E4-P250G | 250            | 20                          | 5                      | \(-62\)       | 65           | 52.3             |
| E4-P250H | 250            | 1                           | 5                      | \(-61\)       | 65           | 51.1             |
From the SEM analysis of the samples shown in Figure 5, it can be observed that for immersion times between 5 and 7 s in CHCl₃ in areas where the dense skin layer thickness is in the range of 10–30 μm, the pores formed at the surface are clearly interconnected with those inside the sample (Figures 6 and 7). This means, for samples treated with SCCO₂ at high pressures (250 bar) and long immersion times for BF, connection between inner porosity (SCCO₂) and outer porosity (BF) is obtained. For 1 and 3 s immersion time, that is, shorter immersion times, the solvent did not penetrate the skin layer and the interconnection did not occur. Additional experiments were carried out at longer immersion times (from 8 s until 30 s) to study whether the interconnection can be still improved (see Figure 6). For samples treated with SCCO₂ under the same conditions (250 bar, 40 °C and 5 min depressurization time), the connection between the inner and the outer pores is appreciable with no significant differences. From 10 s onward, the effect is not further improved because a wide (ca. 300 μm) nonporous outer layer is formed. This effect of the immersion time may be due to two different reasons. On the one hand, the outer skin of the polymer has been, at least to some extent, dissolved in CHCl₃, reducing the thickness of the dense skin. On the other hand, the swelling of the PCL surface by the CHCl₃ employed and its evaporation leads to deeper pores.

Another interesting aspect to be observed is the differences in pore size obtained by the SCCO₂ technique and BF. Although BF generally form pores with sizes ranging from 5 to 20 μm, foaming with SCCO₂ produced larger pores (50–150 μm for the conditions used in this work). This aspect can be advantageous in tissue engineering due to the fact that small pore sizes improve cell response on the surface, while inner bigger pores allow that, after cell seeding, the tissue penetrates in the scaffold and improves the integration of the material until its degradation.²²

2.5. Evaluation of the Cell Adhesion and Biocompatibility of the PCL Porous Scaffolds. To test suitability of the PCL scaffolds for tissue engineering purposes, a cytocompatibility assay has been performed. Endothelial C166-GFP cell line has been used, because of their autofluorescence and its optimal behavior in terms of cell growing over biomaterials.

After 7 days of culture, all samples allowed endothelial cell adhesion and proliferation. As it can be observed in Figure 8, nontreated PCL scaffolds allowed only small cell clumps proliferation, supporting a reduced number of cells with a poorly attached morphology over their surface. Nevertheless, this situation changed for the three treated PCL samples. It was possible to detect bigger cell colonies adhered in the PCL surface and inside the pores, at several depth levels (Figure 8b–d). Especially, cell proliferation was increased over E4-P250-5 and E4-P250-10, where endothelial cells located and grew into interconnected scaffold cavities.

In addition, a metabolic activity study of these samples was also performed to quantify cell proliferation. As a result, all scaffolds showed similar values of cell activity (Figure 8e), demonstrating that treatment did not decrease biocompatibility. On the contrary, a slight upward trend was detected over specific scaffolds, such as E4-P250-5.

Regarding these preliminary biological results, double SCCO₂ and BF techniques over PCL scaffolds improved cell behavior in comparison with nontreated PCL. This finding suggests an interesting application in bone and cartilage tissue engineering purposes. On the one hand, PCL characteristics include high mechanical strength, bioresorbability, and a proper degradation rate compatible with bone tissue proliferation.²³ On the other hand, the introduction of inner and surface porosity in bone implants has shown to increase cell culture activity.²⁴ Moreover, after bone matrix synthesis, sample–tissue interface is enhanced, improving implant fixation and promoting final success. Although endothelial cell results are interesting by themselves, mainly because vascular growth is also needed in these bone regenerative applications, a deeper study including osteoblasts and/or osteoclasts and specific differentiation markers would ensure this clinical use.
3. CONCLUSIONS

The combination of BF and supercritical fluids (SCCO₂) techniques allows the preparation of PCL porous scaffolds with interconnected outer and inner porosity. Their inner porosity was obtained by SCCO₂ treatments by keeping the temperature constant at 40 °C, decreasing the pore size when increasing the pressure, and increasing when slowing the depressurization rate, showing all samples an outer skin layer. SCCO₂ porous scaffolds were found to be interconnected. The SCCO₂ treatment effect on the PCL thermal transitions showed constant Tm and crystallinity slightly increased. By BF, samples previously treated by SCCO₂ at 40 °C and 250 bar were submitted to different CHCl₃ immersion times (up to 10 s), providing surface porosity that was found by SEM and micro X-ray computerized tomography scanning analysis, to be connected to the inner one, showing pores from 5 to 20 μm by BF and from 50 to 150 μm by SCCO₂. Finally, preliminary cell adhesion and biocompatibility of the porous scaffolds exhibited good cell adhesion and proliferation into the interconnected scaffolds, maintaining the biocompatibility and improving cell behavior with respect to nontreated PCL.

4. EXPERIMENTAL SECTION

4.1. Materials. PCL (50 000 g mol⁻¹) was supplied by Perstorp. Liquid carbon dioxide was purchased from Carburos Metálicos with a 99.99% purity. Chloroform (CHCl₃) was supplied by Sigma-Aldrich.

4.2. Preparation of the Samples. The samples were prepared by extrusion of PCL pellets. The process conditions were 60 °C and velocity, with a nozzle diameter of 3 mm. In each experiment, three samples were tested at the same time and placed in 4 mm diameter tubes to keep the cylindrical shape.

4.3. Preparation of the Porous Structures. 4.3.1. Inner Porosity. PCL cylinders were placed in a SCCO₂ reactor (Thar R100W, 104 mL) which operated under different conditions: 35−45 °C temperature, 100−250 bar pressure, and 1−20 min depressurization time. The SCCO₂ system consists of a CO₂ tank, which delivers CO₂ to a high pressure pump precooled using a cryostat at 4 °C, reaching the reactor at the desired pressure and temperature. The system remained at these conditions for a processing time of 90 min using a CO₂ flow of 5 g/min for all the cases and then depressurization takes place until atmospheric pressure.

4.3.2. Superficial Porosity. Samples with different inner pore sizes (between 50 and 150 μm) were chosen to obtain superficial porosity by the BF technique. For this purpose, PCL cylinders pretreated with SCCO₂ were immersed during different times (1−30 s) in chloroform under saturated relative humidity (over 90%) conditions at room temperature in a closed chamber. The samples were left to dry inside the chamber for 24 h.

4.4. Characterization. 4.4.1. Determination of Pore Size. The porous scaffolds were frozen in liquid nitrogen and fractured. Their cross sections were analyzed by SEM (XL30ESEM Philips) at an accelerating voltage of 25 kV. The morphology of the pores, the thickness of the skin layer (in the case of samples treated only by SCCO₂) and the average pore size were determined by this technique. SEM micrographs were analyzed with the ImageJ software. Average pore sizes were determined by measuring the diameter of 30 pores of the image and obtaining their average and standard deviation.

4.4.2. Mercury Porosimetry. The determination of the percentage of porosity was performed using the unit PoreMaster-33, Quantachrome Instruments.

4.4.3. Computerized Tomography. The interconnectivity of the pores was studied by computerized tomography with the unit CT-SCAN-XT-H-160 (Nikon). This technique allows to take two-dimensional images while the sample rotates 360° and to obtain a volumetric three-dimensional reconstruction with the proper software.

4.4.4. Differential Scanning Calorimetry. Thermal transitions and crystallinity of the samples were analyzed by DSC (Mettler Toledo DSC 822e). The polymer was heated from −90 to 100 °C at a rate of 10 °C/min. The melting temperature, Tm, was determined using the value of the enthalpetic transition peak, and the glass transition temperature, Tg, was taken as the middle point of the transition. The percentage of crystallinity was calculated through the ratio between ΔH and ΔHf.

4.5. Biological Studies. 4.5.1. Cell Culture and Cell Seeding. The cell studies were carried out using C166-GFP, a mouse endothelial cell line (ATCC CRL-2583). For culturing cells on the scaffolds, the endothelial cells were seeded singly over the samples in supplemented Dulbecco’s modified Eagle’s medium, and the polymers were placed in a 24-well plate in maintenance medium, incubated at 37 °C with 5% CO₂ in a humidified incubator. For experiments on PCL scaffolds, cells were seeded at a density of 5000 cells/scaffold, and three scaffolds of each sample type were used for each experiment. Samples were observed and fluorescent images were captured at 168 h using an Olympus BX51 microscope.

4.5.2. Metabolic Activity Study. Metabolic activity of cells was measured by alamarBlue assay at 72 and 168 h. AlamarBlue dye (10% of the culture volume) was added to each well, containing living cells seeded over samples, and incubated for 90 min. The fluorescence (λex/λem 535/590 nm) of each well was measured using a plate-reader (Synergy HT, BioTek).

AUTHOR INFORMATION

Corresponding Author
*E-mail: celvira@ictp.csic.es (C.E.).

ORCID

Enrique Martinez-Campos: 0000-0002-7110-3651
Alberto Gallardo: 0000-0003-4614-4299
Carlos Elvira: 0000-0001-6007-5774

Notes

The authors declare no competing financial interest.

ACKNOWLEDGMENTS

Authors would like to acknowledge to MAT2013-42957-R and MAT2016-78437-R (FEDER—EU) for the financial support.

REFERENCES

(1) Jafari, M.; Paknejad, Z.; Rad, M. R.; Motamedian, S. R.; Eghbal, M. J.; Nadjmi, N.; Khoshtaei, A. Polymeric scaffolds in tissue engineering: a literature review. J. Biomed. Mater. Res., Part B 2017, 105, 431−459.
(2) Stratton, S.; Shelke, N. B.; Hoshino, K.; Rudraiah, S.; Kumbar, S. G. Bioactive Polymeric Scaffolds for Tissue Engineering. Bioact. Mater. 2016, 1, 93−108.
(3) Wu, D.; Xu, F.; Sun, B.; Fu, R.; He, H.; Matyjaszewski, K. Design and Preparation of Porous Polymers. *Chem. Rev.* 2012, 112, 3959–4015.

(4) Das, S.; Heasman, P.; Ben, T.; Qiu, S. Porous Organic Materials: Strategic Design and Structure-Function Correlation. *Chem. Rev.* 2017, 117, 1515–1563.

(5) Tan, L.; Tan, B. Hypercrosslinked Porous Polymer Materials: Design, Synthesis, and Applications. *Chem. Soc. Rev.* 2017, 46, 3322–3356.

(6) Beckman, E. J. Supercritical and near-critical CO2 in green chemical synthesis and processing. *J. Supercrit. Fluids* 2004, 28, 121–191.

(7) Kazarian, S. G. Polymer Processing with Supercritical Fluids. *Polym. Sci., Ser. C* 2000, 42, 78–101.

(8) Goel, S. K.; Beckman, E. J. Generation of microcellular polymeric foams using supercritical carbon dioxide. I: Effect of pressure and temperature on nucleation. *Polym. Eng. Sci.* 1994, 34, 1137–1147.

(9) Harris, L. D.; Kim, B.-S.; Mooney, D. J. Open Pore Biodegradable Matrices Formed with Gas Foaming. *J. Biomed. Mater. Res.* 1998, 42, 396–402.

(10) Barry, J. J. A.; Gidda, H. S.; Scotchford, C. A.; Howdle, S. M. Porous Methacrylate Scaffolds: Supercritical Fluid Fabrication and in Vitro Chondrocyte Responses. *Biomaterials* 2004, 25, 3559–3568.

(11) Morisaki, M.; Ito, T.; Hayvali, M.; Tabata, I.; Hisada, K.; Hori, T. Preparation of Skinless Polymer Foam with Supercritical Carbon Dioxide and its Application to a Photoinduced Hydrogen Evolution System. *Polymer* 2008, 49, 1611–1619.

(12) Pintado-Sierra, M.; Delgado, L.; Aranaa, I.; Marcos-Fernández, A.; Reinecke, H.; Gallardo, A.; Zeugolis, D.; Elvira, C. Surface hierarchical porosity in poly(ε-caprolactone) membranes with potential applications in tissue engineering prepared by foaming in supercritical carbon dioxide. *J. Supercrit. Fluids* 2014, 95, 273–284.

(13) Muñoz-Bonilla, A.; Fernández-García, M.; Rodríguez-Hernández, J. Towards hierarchically ordered functional porous polymeric surfaces prepared by the breath figures approach. *Prog. Polym. Sci.* 2014, 39, 510–554.

(14) Zhang, A.; Bai, H.; Li, L. Breath figure: a nature-inspired preparation method for ordered porous films. *Chem. Rev.* 2015, 115, 9801–9868.

(15) Markočič, E.; Skerget, M.; Knez, Ž. Effect of Temperature and Pressure on the Behavior of Poly(ε-caprolactone) in the Presence of Supercritical Carbon Dioxide. *Ind. Eng. Chem. Res.* 2013, 52, 15594–15601.

(16) Tisvintzelis, I.; Pavlidou, E.; Panayiotou, C. Biodegradable polymer foams prepared with supercritical CO2-ethanol mixtures as blowing agents. *J. Supercrit. Fluids* 2007, 42, 265–272.

(17) Xu, Q.; Ren, X.; Chang, Y.; Wang, J.; Yu, L.; Dean, K. Generation of Microcellular Biodegradable Polycaprolactone Foams in Supercritical Carbon Dioxide. *J. Appl. Polym. Sci.* 2004, 94, 593–597.

(18) Léonard, A.; Calberg, C.; Kerckhofs, G.; Wevers, M.; Jérome, R.; Pirard, J.-P.; Germain, A.; Blacher, S. Characterization of the porous structure of biodegradable scaffolds obtained with supercritical CO2 as foaming agent. *J. Porous Mater.* 2008, 15, 397–403.

(19) Barry, J. J. A.; Silva, M. M. C. G.; Popow, V. K.; Shakesheff, K. M.; Howdle, S. M. Supercritical Carbon Dioxide: Putting the Fizz into Biomaterials. *Philos. Trans. R. Soc., A* 2006, 364, 249–261.

(20) Jenkins, M. J.; Harrison, K. L.; Silva, M. M. C. G.; Whitaker, M. J.; Shakesheff, K. M.; Howdle, S. M. Characterisation of microcellular foams produced from semi-crystalline PCL using supercritical carbon dioxide. *Eur. Polym. J.* 2006, 42, 3145–3151.

(21) Kiran, E.; Liu, K.; Ramsdell, K. Morphological changes in poly(ε-caprolactone) in dense carbon dioxide. *Polymer* 2008, 49, 1853–1859.

(22) Civantos, A.; Martínez-Campos, E.; Ramos, V.; Elvira, C.; Gallardo, A.; Abarrategi, A. Titanium Coatings and Surface Modifications: Toward Clinically Useful Bioactive Implants. *ACS Biomater. Sci. Eng.* 2017, 3, 1245–1261.