

Preparation of Three-Dimensional Scaffolds from Degradable Poly(ether)esterurethane by Thermally-Induced Phase Separation

Karola Luetzow,¹ Thomas Weigel,¹ Michael Schossig,¹ Karl Kratz,^{1,2} Andreas Lendlein^{*1,2}

Summary: Highly porous scaffolds with an overall volume up to 30 cm³ and porosities in the range from 80% to 93% were prepared from degradable poly(ether)esterurethane by thermally-induced phase separation (TIPS) and subsequent solvent sublimation. The variation of the polymer concentration when applying a fixed TIPS cooling protocol led to an adjustment in pore size as well as in pore size distribution. While polymer concentrations of 5, 7.5 and 10 wt% resulted in averaged pore sizes ranging from 70 to 90 μ m with a broad pore size distribution (according to μ -CT analysis) for polymer concentrations of 15 wt% a significant lower averaged pore size of 50 μ m with a narrow pore size distribution was obtained.

Keywords: scaffold formation; thermally-induced phase separation; tissue engineering; urethane

Introduction

The demand for biomaterial-based regenerative therapies is high and is expected to increase because of increasing life expectancy and changes in activity behavior as well as nutrition. Biomaterial induced endogeneous regeneration, in which biomaterials are used to support and guide tissue growth, is considered a very promising approach to meet this demand.^[1–5] This approach demands degradable scaffolds, which are able to guide tissue growth and degrade as the extracellular matrix of the regenerated tissue has been developed. In this context the mechanical properties and the morphological properties of the applied scaffolds play a key role and should mimic the extracellular matrix of the tissue to be regenerated. Consequently, polymer based scaffolds with tailored elastic properties,

adjustable degradation behavior^[6,7] and the appropriate morphology^[3] are required. In tissue regeneration approaches usually scaffolds exhibiting a highly porous, interconnected morphology with pore sizes ranging from a few μ m to several hundred μ m were applied. The optimum pore size of a scaffold has to be adapted to the specific cell type e.g. a minimum pore size of 5 μ m was reported to support neovascularization, while pore sizes in the range of 75–400 μ m are suitable for osteoid and bone regeneration.^[8,9] The desired scaffold morphology can be controlled by selecting the appropriate processing techniques e.g. CO₂ foaming, leaching approaches or thermally-induced phase separation (TIPS).^[10,11] TIPS is a technique for scaffold formation prepared from polymer solution, in which a solvent is used which shows poor solubility at low temperature and good solubility at elevated temperatures. By cooling the polymer solution to a temperature below the binodal, a phase separation is induced thermally and the polymer solution separates into a polymer-rich and a polymer-lean phase. The demixing behavior is described in a phase diagram of a binary solution. Lowering the temperature slowly

¹ Centre for Biomaterial Development, Institute of Polymer Research, Helmholtz Zentrum Geestacht, Kantstr. 55, D-14513, Teltow, Germany
E-mail: andreas.lendlein@hzg.de

² Berlin-Brandenburg Centre for Regenerative Therapies, Berlin, Germany

leads to a binodal separation into a polymer-rich and a polymer-lean phase. A rapid cooling leads to a spinodal separation of the two phases. The phase separation process can be followed by a coarsening, in which the polymer-solvent system is kept at a defined temperature between the phase separation temperature and freezing point to achieve pore enlargement.^[12,13] Removal of the solvent, e.g. by sublimation or washing processes,^[14] renders the final scaffold. Scaffolds prepared by TIPS are normally open-porous and show pores in the range of 50–200 μm . Many scaffolds have been prepared by quenching the polymer solution directly at -20°C , -80°C or even in liquid nitrogen.^[15] However, larger pore sizes of up to 400 μm have been reported by applying a slower cooling procedure and by focusing on coarsening of the polymer solution.^[13]

In this work we explored whether highly open porous scaffolds can be achieved from degradable poly(ether)esterurethanes by using TIPS technique consisting of a specific cooling protocol and in how far the scaffold morphology can be controlled by parameters like the polymer concentration. For this study we chose a semi-crystalline poly(ether)esterurethane named PDC,^[16] which was synthesized by the prepolymer method via co-condensation of poly-(p-dioxanone)diol (PPDO) and poly(ϵ -caprolactone)diol (PCL) using an aliphatic diisocyanate according to a method described in.^[17] Poly(p-dioxanone)diol as telechelic precursor was prepared by ring-opening polymerization of p-dioxanone with ethylene glycol as initiator.^[16] The thermoplastic multiblock copolymer PDC was selected as multifunctional biomaterial combining degradability and excellent elastic properties enabling a shape-memory capability.^[17–19] For PDC a linear mass loss was reported in hydrolytic and enzymatic degradation experiments, whereby the degradation behavior could be controlled by the composition e.g. the ratio of the PCL and PPDO precursors.^[6,7,17,20] Furthermore, the in vivo degradation of PDC is characterized by a fragmentation and a pro-angiogenic effect was observed.^[18,21]

Experimental Part

Polymer Synthesis

The multiblock copolymer PDC was synthesized via co-condensation of identical weight contents of poly(ϵ -caprolactone)diol (trade name CAPA 2205, Solvay Caprolactones, Warrington, U.K.) with a number average molecular weight of $M_n = 2000 \text{ g} \cdot \text{mol}^{-1}$ and poly(p-dioxanone)diol ($M_n = 5900 \text{ g} \cdot \text{mol}^{-1}$) in the presence of 2,2(4),4-trimethyl-hexanediisocyanate (TMDI). PDC had a molecular weight of $M_n = 60500 \text{ g} \cdot \text{mol}^{-1}$ (polydispersity: 3.02) and exhibited two distinct melting temperatures in differential scanning calorimetry (DSC) analysis (determined from the second heating run) at $T_m = 36 \pm 1^\circ\text{C}$, attributed to the crystalline PCL domains and at $T_m = 88 \pm 1^\circ\text{C}$ related to the crystalline PPDO domains. Furthermore, two well separated glass transitions could be observed in DSC analysis at $T_g = -59 \pm 1^\circ\text{C}$ related to the amorphous PCL domains and at $T_g = -21 \pm 1^\circ\text{C}$ for the amorphous PPDO domains, which demonstrate the completely phase separated morphology of the poly(ether)-esterurethane.

The bulk PDC polymer sample exhibited an overall degree of crystallinity index of $\chi_c = 25.3\%$ as calculated from the ΔH_m values obtained in DSC analysis. The overall crystallinity index χ_c, PDC was calculated according to the equation (1) using the following enthalpy values for 100% crystallinity of PCL and PPDO: $\Delta H_m^0, \text{PCL} = 135 \text{ J} \cdot \text{g}^{-1}$ [22] and $\Delta H_m^0, \text{PPDO} = 102 \text{ J} \cdot \text{g}^{-1}$ [23] and $\Delta H_m, \text{PCL} = 18.3 \text{ J} \cdot \text{g}^{-1}$ and $\Delta H_m, \text{PPDO} = 10.1 \text{ J} \cdot \text{g}^{-1}$ determined by DSC.

$$\chi_c, \text{PDC} = \left[\left(\frac{\Delta H_m, \text{PCL}}{\Delta H_m^0, \text{PCL}} \right) + \left(\frac{\Delta H_m, \text{PPDO}}{\Delta H_m^0, \text{PPDO}} \right) \right] \cdot 100\% \quad (1)$$

Scaffold Formation

PDC polymer was dissolved in 1,4-dioxane (Merck, Germany) in different concentrations (2.5, 5, 7.5, 10, 15 wt%) at elevated

temperature of 80 °C. The solution was cooled down to room temperature and filled into plastic vials with a inner diameter of 3.1 cm and a total volume of 40 cm³ (25 g or 10 g solution per vessel, depending on aimed scaffold height). In the next step, the sample was cooled down to 12 °C, where it was kept for 16 h, afterwards to +6 °C, where it was held for 1.5 h, and finally to +3 °C, a temperature below the freezing point of 1,4-dioxane, where it was held for 2.5 h. A cooling rate of 1 K/min was applied during the aforementioned cooling procedures. The solvent was removed by sublimation in a freeze dryer (ALPHA 2-4 LSC, Martin Christ Gefriertrocknungsanlagen GmbH, Germany) at +5 °C and 1 mbar for 21 h, and then another 3 h at 0.003 mbar.

Solvent Residue

The solvent residue content of the foam prepared from 5 wt% PDC solution was checked by dissolving 0.5 g of a foam sample in 1.5 g N-Methyl-2-pyrrolidone (NMP, Merck, Germany) at 50 °C. The sample is injected into the headspace (Hewlett Packard, Headspace Sampler HP7694, USA) and is equilibrated for 30 min at 90 °C and is finally transferred into the GC (Gaschromatograph 5890 Series II, Hewlett Packard, USA) where it is heated from 100 °C to 200 °C. For the detection a column (DB 624, J&W Scientific, USA) having a flame ionization detector is used.

X-ray Microtomography (μ CT)

The morphological characterization of the scaffolds was carried out with an x-ray micro computer tomography system (Pro-Con X-ray GmbH, Germany). The sample is irradiated by an x-ray beam and the transmitted intensity is detected with help of a CCD detector. The absorption of the sample is proportional regarding density. In this way, the material with the higher x-ray absorption generates a shadow picture on CCD detector. The sample rotates in steps of 0.45° until a nearly complete rotation of 360° is reached. Transmission light pictures are generated by this procedure. A three-dimensional picture can be reconstructed

with the modular constructed software “MAVI” (Fraunhofer Institut Techno- und Wirtschaftsmathematik, Kaiserslautern, Germany). The three-dimensional shadow pictures are used for quantitative determination of pore size distributions and the average pore size including its standard deviation.

Scanning Electron Microscopy (SEM)

The foams were cut in liquid nitrogen, fixed on holders with a conductive adhesive, and sputtered with 1.5 nm Pt/Pd (Polaron SC7640, Great Britain). The prepared samples were investigated using a LEO 1550 VP electron microscope with a Schottky-Emitter (Zeiss, Germany) at an acceleration voltage of 3 kV with a maximal resolution of 2.5 nm. Pore size measurements were carried out with the software Scandium (Olympus Soft Imaging Solutions GmbH, Germany). For each sample 19 to 39 pores were evaluated.

Determination of the Porosity, Accessibility of the Pores by Nitrogen and Foam Density

The relative content of pores, which are accessible to nitrogen gas (“open pores”), was determined by pycnometer measurements and had a maximum standard deviation of 1%. The measurements were carried out according to the instructions of the manufacturer in a 60 cm³ test cell at 20 °C in a Ultrafoam Pycnometer 1000 (Quantachrome Instruments, USA) using nitrogen as a displacement fluid (6 psi) and 10-fold repetition of each measurement. The foam density was calculated from the geometric volume and the mass of the foam sample. The total porosity corresponds to the ratio of the foam volume and the volume of the corresponding compact, non-foamed PDC. The density of PDC (1.16 g · cm⁻³) was determined by pycnometry.

Results and Discussion

For the TIPS process an appropriate solvent had to be chosen which can be

sublimated and has suitable solvation properties, i.e. good solvent at elevated temperatures, poor solvent at lower temperature. 1,4-dioxane and dimethylcarbonate were selected as possible solvents. The cloud point of a 5 wt% PDC in the corresponding solvent (1,4-dioxane or dimethylcarbonate) was determined. The 5 wt% solution was prepared at 80 °C and slowly cooled down to determine the temperature at which a significant change in turbidity (the cloud point) can be noticed. A cloud point for 1,4-dioxane of 30 °C and for dimethylcarbonate of 47 °C was determined. Further cooling down of the solutions to room temperature led to a slight deposit for dioxane and for a strong deposit for dimethylcarbonate. Since dimethylcarbonate showed a high deposit already at room temperature, foam formation from this solvent can not be considered reasonable and thus dioxane was selected for further investigations. For the cooling regime of the TIPS technique, a temperature sequence was chosen, in which the polymer

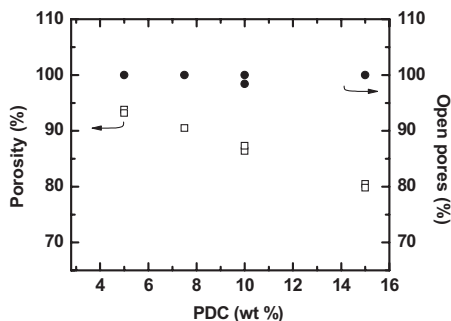


Figure 1.

Scaffold porosity (□) and percentage of open pores (●) in dependence of polymer concentration.

solution was cooled from room temperature, which is below the cloud point of the polymer solution, down to 12 °C, which is slightly above the freezing point of pure 1,4-dioxane, and then to 3 °C, which is the freezing point of the solution. The temperature of 12 °C was held for 16 h to allow coarsening of the solution and to decrease funnel formation in the center of the scaffold.

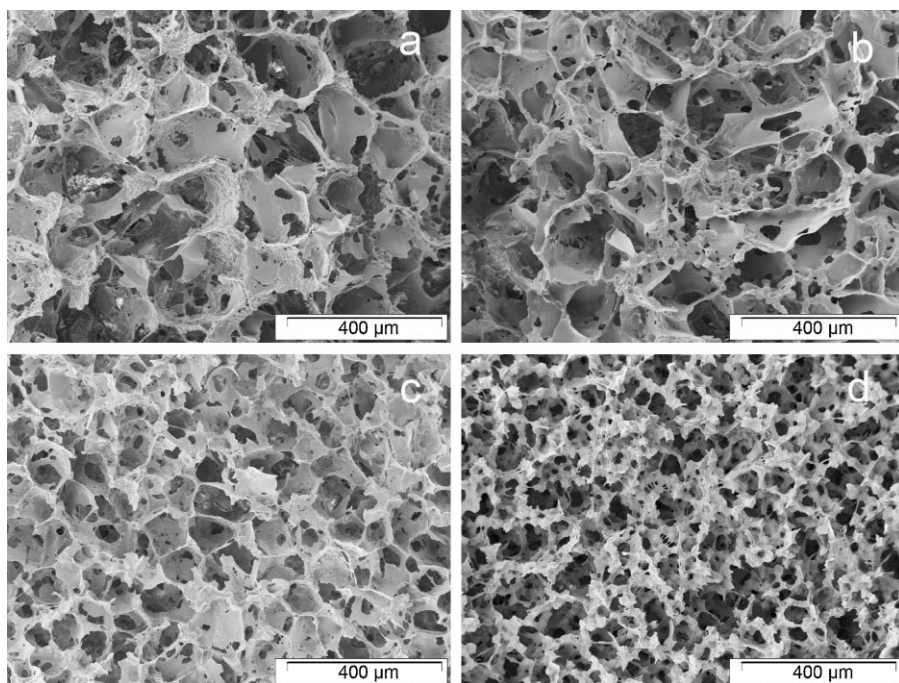


Figure 2.

SEM pictures of the foams' cross-sections at the foam center; PDC-5 (a), PDC-7.5 (b), PDC-10 (c), PDC-15 (d).

Highly porous PDC foams having a diameter of 2.8 cm and an overall volume up to 30 cm³ were fabricated with the TIPS technique and sequential sublimation of the solvent when applying the cooling program. Foams prepared from 5 wt% (PDC-5), 7.5 wt% (PDC-7.5) and 10 wt% (PDC-10), and 15 wt% (PDC-15) solution of PDC in 1,4-dioxane showed most favorable morphological properties, lower concentrations led to fragile foam structures. The PDC-5 foam was investigated for possible solvent residue. However, no residual solvent above the detection limit of 5 ppm could be found.

Pycnometry measurements showed porosities of the scaffolds between 80%

for PDC-15 and 93% (PDC-5) and revealed the aimed open porous morphology of the scaffolds. A complete accessibility of the pores by nitrogen (Figure 1) was found for all scaffolds. This also corresponds with the fact that no residual solvent was found for the PDC-5 foam after freeze-drying. The open porous morphology of the scaffolds was also verified by SEM investigations in which the interconnectivity can be clearly seen in the pore walls (Figure 2).

The average pore size estimated by SEM gradually decreased from $186 \pm 56 \mu\text{m}$ for the PDC-5, to $170 \pm 50 \mu\text{m}$ for the PDC-7.5, to $113 \pm 26 \mu\text{m}$ for the PDC-10 and $101 \pm 31 \mu\text{m}$ for the PDC-15. The pore size of the SEM sample was measured on the

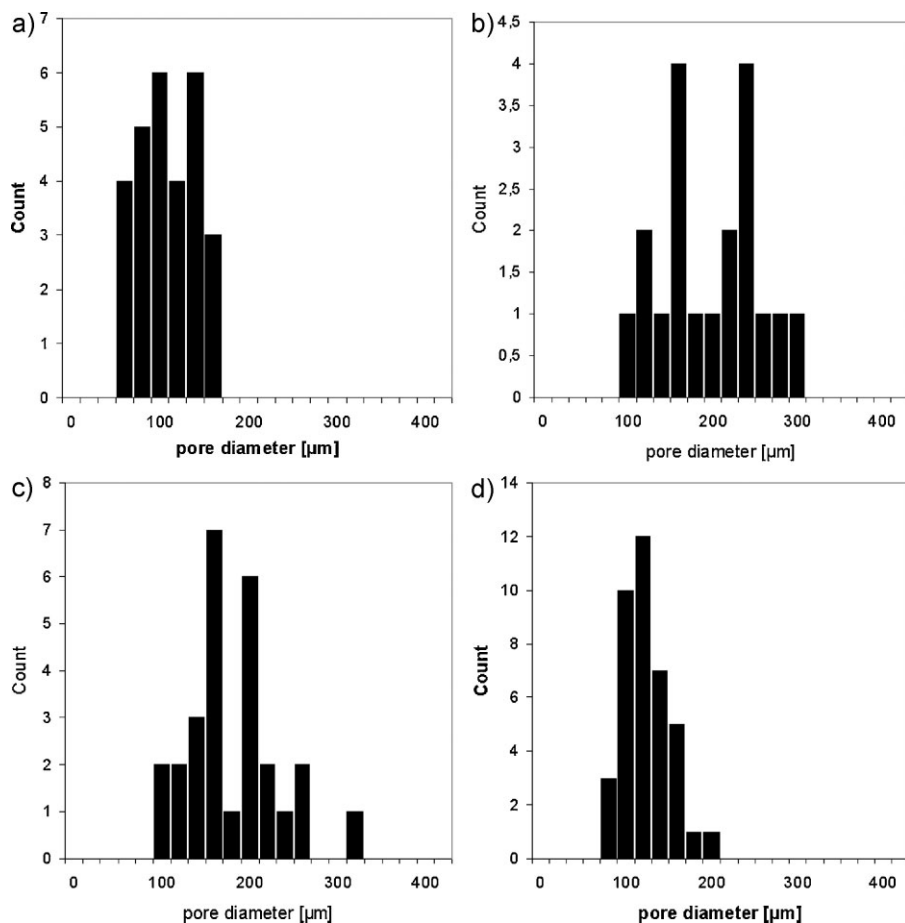


Figure 3. SEM evaluation of the foams PDC-5 (a), PDC-7.5 (b), PDC-10 (c), PDC-15 (d) showing the pore diameter distribution.

cross-cut of each sample. The largest diameter of the recognizable pores was determined. The pore diameter distribution is shown in Figure 3. PDC-15 shows a narrow pore diameter distribution, while PDC-7.5 and PDC-10 show the broadest size distributions. PDC-15 also shows a different morphological appearance than the other three scaffolds presumably because of different phase separation process.

Another method to determine the pore size is μ CT evaluation in which several ten thousand pores are evaluated on computer basis, however with a lower resolution than the SEM. The μ CT evaluation uses a three-dimensional evaluation, whereby a pore is defined as an accumulation of a defined number of voxel of a defined grey shade. This method evaluates more of the smaller pore sizes, which are more likely to be omitted in the manual SEM evaluation. Furthermore it is likely that some of the small “ μ CT-pores” are counted as inter-connectivities within the SEM investigation. In case of SEM determination of the pore diameter, one should take into account that the fracture takes place at the weakest points of the foam, and it is a 2D evaluation of the pores, in which pores

are defined by pore walls visible to the eye. Thus pores, which are not clearly visible to the eye, tend to be neglected. Taking the above mentioned facts in account, a difference in the determined average pore size of both methods is not surprising. The average pore size, which was calculated from the relative pore number, changed from $69 \pm 35 \mu\text{m}$ for the PDC-5, to $88 \pm 45 \mu\text{m}$ for the PDC-7.5, to $76 \pm 38 \mu\text{m}$ for the PDC-10 and $47 \pm 15 \mu\text{m}$ for the foam prepared from the PDC-15 polymer solution (Figure 4 and 5). The foams PDC-5, PDC-7.5 and PDC-10 showed a broad pore diameter distribution, in case of PDC-5 the pore diameter had a higher amount of smaller pore diameters. PDC-15 showed a strikingly narrow size distribution.

Conclusion

We demonstrated that open-porous foams from PDC could be successfully prepared by TIPS technique. We found that the pore diameter and its distribution can be tailored if the concentration of the polymer solution is varied while all other parameters, e.g. cooling procedure, are kept constant.

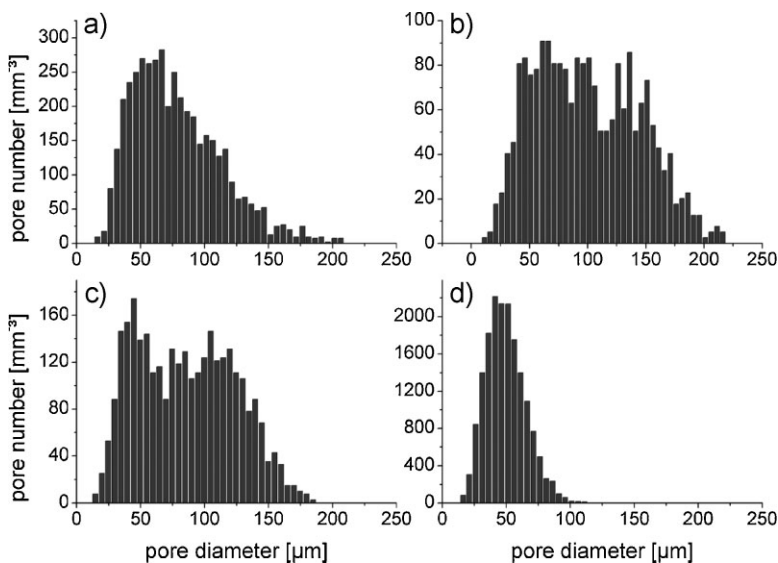


Figure 4. μ CT evaluation of the foams PDC-5 (a), PDC-7.5 (b), PDC-10 (c), PDC-15 (d) showing the pore diameter distribution.

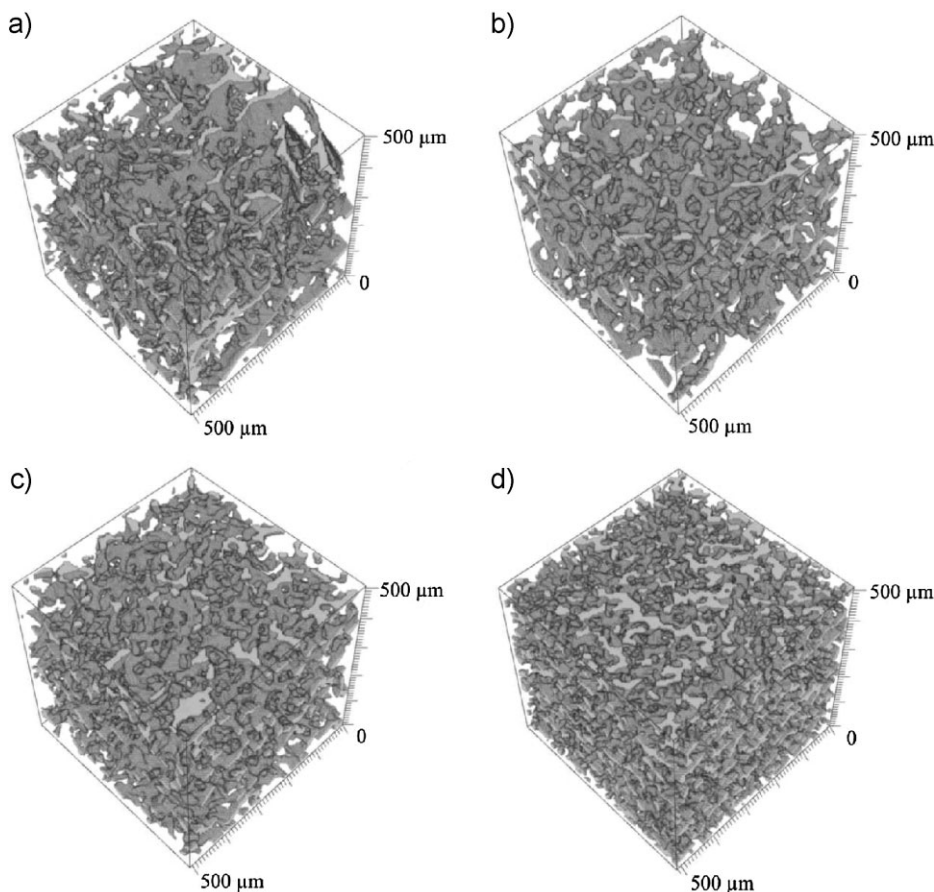


Figure 5.

μ CT evaluation showing a 3D reconstructing of the foams PDC-5 (a), PDC-7.5 (b), PDC-10 (c), PDC-15 (d).

Preparation of scaffolds from a 5 wt% polymer solution led to an average pore size of $69 \pm 35 \mu\text{m}$ according to μ -CT analysis, while a 15 wt% polymer solution had an average pore size of $47 \pm 15 \mu\text{m}$. The porosities can be tailored in dependence of the polymer concentration between 93% for the lowest polymer concentration (PDC-5) and 80% for the highest polymer concentration (PDC-15). Thus it is expected that PDC scaffolds are well-suited for applications in regenerative medicine because they show favorable morphological properties, e.g. open-porosity with pores in the micrometer range, and have the polymer's intrinsic properties of biodegradability and pro-angiogenic effect.

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