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INVESTIGATION OF HIGHLY POROUS POLY(E-CAPROLACTONE) SCAFFOLDS

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Abstract

Porous polycaprolactone based scaffolds were prepared by compression molding and particulate leaching technique. As pore-forming agent, three fractions (125–250 μm, 250–500 μm and 500–1000 μm) of common table salt-grains were utilized during the processing. The porosity was varied from 50 up to 90 percent, and the compressive characteristics were investigated as a function of porosity. The power-law was found valid in the case of compressive characteristics; however due to the manufacturing not only the strain of densification but also the strain of elasticity was highly dependent on the porosity and the salt-grain size.

Keywords: scaffold; compressive properties; polycaprolactone; cellular solid; foam; tissue engineering

Introduction

Tissue engineering is a rapidly emerging field of biomaterial science, which aims to overcome the drawbacks of current tissue, organ replacements and the lack of donors[1,2]. Several methods, processes, materials have been developed; however they are still not widely utilized. The tissue regeneration can be achieved by several methods (e.g. guided tissue regeneration, or creating human tissues in laboratory), but for each trial we have to provide porous templates[3,4]. Materials and manufacturing methods of such templates have been studied by chemists and chemical engineers, but mechanical aspects have not been fully understood yet. To create a porous template called scaffold biodegradable, bioabsorbable materials are the most attractive, as they can be eliminated after the fulfillment of the implant function without any inflammatorial reactions by standard metabolic pathways. Poly(ε-caprolactone) (PCL) is an aliphatic biodegradable polyester, which has a long degradation time in the range of two-three years[5]. PCL is Food and Drug Administration (FDA) approved biocompatible material which is often used as suture. Due to its slow degradation it is attractive not only for suturing but also for long term applications such as drug delivery systems or bone tissue engineering[6,7].

Feijen et al. prepared studies[8,9] on the manufacturing of porous PCL based scaffolds. E.g. in 2002, they prepared scaffolds with different salt fractions by coagulation process and with different temperatures by freeze drying process[8]. They investigated the surface morphology by scanning electron microscope (SEM) and porosity by estimating the volume and weight. They found that both methods are capable of obtaining highly porous scaffolds with highly interconnective structure. The porosity of salt-leached samples showed linear correlation; however at 80w% initial porogen content the porosity was ca. 70%, till at 97w% salt content the porosity was about 92%. In 2003, they published another paper[9] on PCL and poly(d-, l-lactide) scaffolds manufactured by the same methods. They performed compression tests to calculate the compressive Young's modulus, which was then plotted as a function of porosity. They found exponential decrease of the modulus, and the obtained scaffolds had less than 2 MPa modulus in each case. They presented their results on power-law relationship (equation 1):

$$Ec = k_{PCL}\,(1-p_0)^{2.59} \qquad (1)$$

where E_c – compressive Young's modulus of the cellular solid, k_{PCL} – a material constant, p_0 – porosity; and 2.59 shape factor the porogen.

An early recommendation of power law was done by Gibson and Ashby in the late 80ies when they published a book[10] on cellular solids which has been one of the basic books concerning the mechanical properties of porous materials. They overviewed the cellular solids with both open and closed cells. For scaffolding the properties of open cell foams are more relevant.

As they suggested the compressive curve can be divided into three independent regimes *(Figure 1). "The linear elasticity is controlled by cell wall bending and if the cells are closed cell faces stretching. During compressive loading the plateau is associated with collapse of the cells by elastic buckling in elastomeric foams. When the cells have almost completely collapsed opposing cell walls touch and further compresses the solid itself, giving the final region of rapidly increasing stress"*[10]. To describe the stress-strain behavior of cellular solids different equations were defined which can give a good approximation of the mechanical properties.

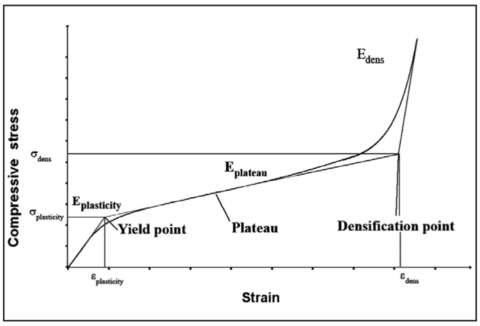

Figure 1. Typical regimes of compressive stress-strain curves of polymeric foams

The aim of the present study was to prepare scaffolds based on polycaprolactone, furthermore to investigate the power law in the case of obtained porous templates. For the experiments different grain-sizes of pore-forming agent were utilized, and the compressive characteristics were investigated as a function of porosity.

Experiments

Polycaprolactone (with an 80 kDa number average molecular weight, and 130 kDa weight average molecular weight) supplied by Sigma-Aldrich was used as matrix material, and sodium chloride (NaCl) was utilized as pore-forming agent (porogen). Both the chemicals were vacuum-dried before used. The NaCl was fractionized; and the 125–250 μm, 250–500 μm and 500–1000 μm fractions of grains were utilized for the experiments.

The scaffold was obtained by compression molding and particulate leaching technique. As a very first step the polymer and sodium chloride crystals were mixed at different weight ratios in a Brabender type internal mixer at 120 °C till torque equilibrium. Later the mixing process the obtained mixtures were chopped up and compression molded in a Collin type hot press at 120 °C and 100 bar pressure for 5 minutes; then cooled by cold water. The obtained samples were immersed into distilled water for porogen extraction. The final stage of the sample manufacturing was the vacuum drying at 40 °C till mass change was not measurable.

During the study we have concentrated on the compressive characteristics of porous templates as the most probable load in the human bone is the compression. For this purposes unconstrained compression tests were performed at room temperature with 1 mm/min velocity of the cross-head on a Zwick Z020 universal testing machine, and minimum 150 specimens were measured per series.

To characterize the cross-cut morphology of the porous PCL scaffolds JEOL 6380LA scanning electron microscope was used. Prior the test samples were gold-coated.

Results and discussion

The advantage of particulate leaching techniques is that the porosity and the pore-size can be easily controlled by the fraction and the amount of pore-forming agent. The pore-shape correlates well with the crystal geometry as during the processing the molten polymer flows around the salt crystals and cools down; the shape is fixed, and later the salt is leached out. During this method the pore geometry is not influenced by the processing caused shrinkage and warpage of the samples. *Figure 2* represents the cross-cut morphology of scaffolds which contain pores in three different ranges such as 125–250 μm *(Figure 2A),* 250–500 μm *(Figure 2B),* 500–1000 μm *(Figure 2C).* It was found that the pore-forming agent undergoes some fragmentation during manufacturing resulted in smaller pore-size which could be expected. The fragmentation was depending on both porosity and pore-size. If the porogen content was high, the cracking effect was more aggressive than at low porogen content; and the fragmentation was less pronounced in case of small grain sizes than in case of big ones. The mentioned fragmentation changed the microstructure of the scaffolds which predicts the difference of theoretical and experimental results as the pore-size is a function of the porosity, and pore-size as well.

The most relevant is the compressive characteristic of the scaffold to fulfill its function. In order to identify the relationship between the porosity and the compressive characteristics, compression tests were performed on specimens with 2 mm height and 12 mm diameter at different porosities (in the range of 50-92%). Based on the stress-strain curve the yield and densification strength, the young and plateau modulus, and finally the strain of elasticity and densification were determined. First, the compressive modulus of the scaffolds

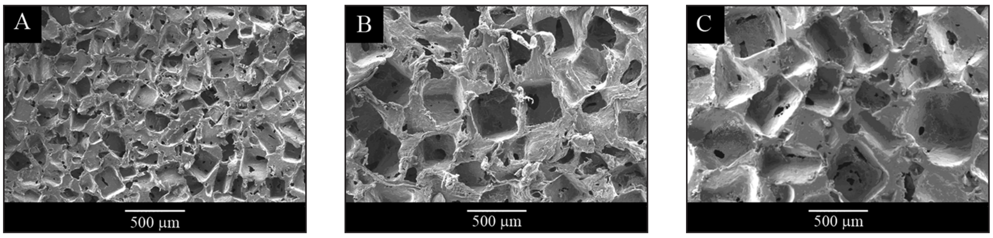

Figure 2. SEM picture of compression molded salt-leached scaffold prepared by different fraction of sodium chloride having 75% porosity. Prepared by A: 125–250 μm, B: 250–500 μm, C: 500–1000 μm grain-size of NaCl

was investigated as a function of porosity which showed good correlation to the power-law, which means that it is a power function of porosity.

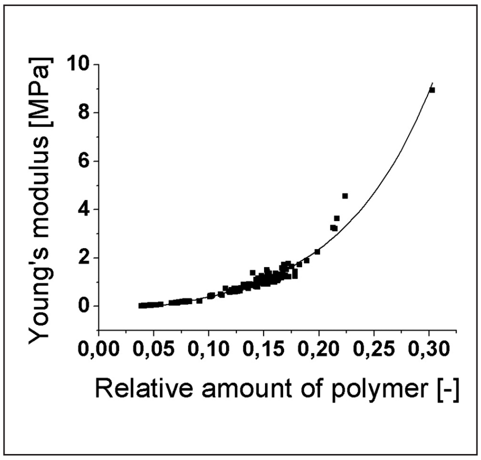

Figure 3. The compressive modulus as a function of relative polymer amount

It is shown on *Figure 3,* and by the equation it can be written

$$E_c = 10^A \cdot (1-p)^B \qquad (2)$$
$$\lg(E_c) = A + B \cdot \lg(1-p) \qquad (3)$$

where E_c – compressive modulus [MPa], p – porosity [-], A, B – material constants relating to the pore geometry and the compressive modulus of the bulk polymer. The exact parameters of equations are shown in the *Table 1.*

salt grain size [μm]	Compressive characteristics	A	B	R^2
125-250	Young's modulus	2.556 ± 0.072	2.777 ± 0.129	0.997
	Yield strength	1.584 ± 0.054	2.819 ± 0.096	0.999
	Modulus of plateau	1.882 ± 0.073	2.455 ± 0.106	0.998
	Densification strength	1.739 ± 0.050	2.286 ± 0.081	0.998
250-500	Young's modulus	2.344 ± 0.063	2.427 ± 0.107	0.999
	Yield strength	1.437 ± 0.063	2.512 ± 0.115	0.992
	Modulus of plateau	1.853 ± 0.033	2.402 ± 0.056	0.997
	Densification strength	1.736 ± 0.037	2.232 ± 0.064	0.997
500-1000	Young's modulus	2.109 ± 0.071	2.083 ± 0.117	0.998
	Yield strength	1.318 ± 0.048	2.246 ± 0.087	0.997
	Modulus of plateau	1.552 ± 0.049	1.949 ± 0.092	0.985
	Densification strength	1.515 ± 0.013	1.951 ± 0.029	0.997

Table 1. Parameters of the equations between the logarithmic value of the porosity and logarithmic values of the compressive characteristics

The further compressive characteristics also correlate well with the power-law. In *Figure 4* all the investigated characteristics are shown in case of scaffold having 250–500 μm pore-size. The experimental results showed that all the compressive characteristics increases with decreasing porosities; however by theory the plateau modulus supposed to be approximately 0 if the pores are open. This increasing plateau modulus indicates that if the porosity decreases the pore-interconnectivity decreases as well; some pores are closed where the air pressure starts to increase resulted in the increasing modulus of plateau during the compression test.

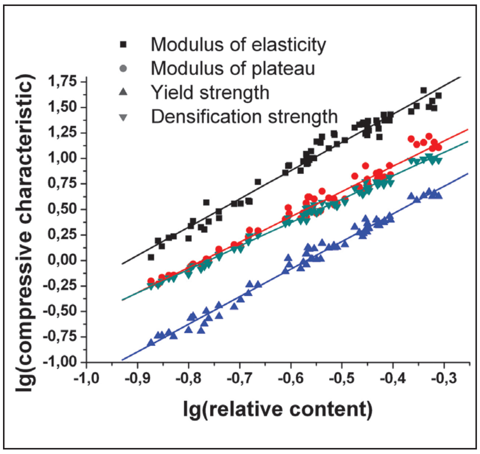

Figure 4. Typical relationship equations between the logarithmic value of the porosity and logarithmic values of the compressive characteristics made of the 250–500 μm salt grain size

The *Table 1* comprises all A, B parameters obtained at different pore sizes. By Gibson's theory the strain of the elasticity is independent on the porosity. It was found that the slope of the modulus of plateau and densification strength were only slightly dependent on the pore-size while the Young's modulus and the yield strength were highly dependent. It was found at relatively low pore-size (125–250 μm) that the slope of yield strength and modulus of elasticity has similar values which predicts that the board of elasticity is not a function of the porosity, while this dependency was more pronounced with increasing pore-size.

To characterize the stress-strain behavior of PCL porous templates it is also necessary to understand the relationship between the porosity and the strain of elasticity and densification. *Figure 5* below shows the experimental data related to the strain of elasticity and densification in case of scaffolds which have pores in the range of 250–500 μm. Linear equation was fitted to both of the data series, expressed by the equation 4:

$$S = C + D \cdot p_0 \qquad (4)$$

where S – investigated strain value [%], C, D – constants depending on the pore-size, the pore-geometry, p_0 – porosity [%].

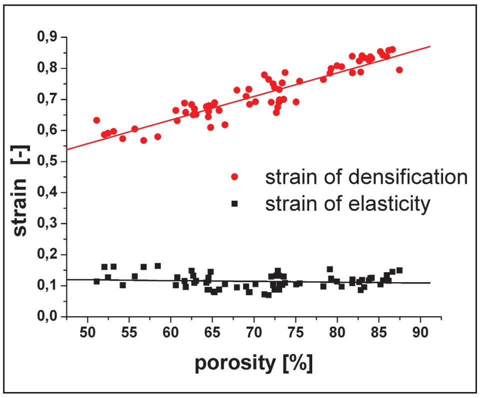

Figure 5. Typical relationship between the porosity and the strain values in case of scaffolds made of the 250–500 μm salt grain size

Both strain values are linear functions of the porosity; however the slope of the linear function fitted on the strain of densification and porosity is higher than in case of strain of elasticity. In case of small pores (125–250 μm) the parameter of the strain of elasticity *(Table 2)* shows that the strain of elasticity follows Gibson's theory (it is constant); however significantly (two times) higher value than the suggested value (0.5). The strain of elasticity is independent on the porosity, and its value is about 0.105.

salt grain size [μm]	Strain characteristics	C	D	R^2
125 - 250	strain of elasticity	8.833 ± 2.366	0.021 ± 0.033	0.488
	strain of densification	-9.818 ± 4.290	1.096 ± 0.053	0.987
250 - 500	strain of elasticity	15.718 ± 4.034	-0.066 ± 0.056	-0.863
	strain of densification	6.280 ± 3.784	0.913 ± 0.052	0.990
500 - 1000	strain of elasticity	24.801 ± 2.044	-0.163 ± 0.030	-0.942
	strain of densification	20.392 ± 4.037	0.745 ± 0.054	0.992

Table 2. Parameters of the equations between the porosity and strain values

At the same time a slight dependency on porosity was found in case of 250–500 μm, while it is significant in case of large pore-size (500–1000 μm). This basic difference, which was found between the theory and our experimental results, may be a consequence of the grain breakage during the processing. If the amount of porogen increases, the probability of rubbing increases resulting in smaller pore-size. This phenomenon is more pronounced in case of large grain-sizes. The explanation is partially confirmed by the fact that the board of elasticity is converging to one value in case of high porosity, and the difference is more significant in case of low porosities.

The slope of the strain of densification was also dependent on the pore-size; if the pore-size increases; its slope decreases. If the slope is high it means that the pores can close easily so the entrapped gas increase the modulus fast in the high indicating the material is supposed to be dense. If the slope is low, it means that the pore system can be closed not as easily due to the high interconnectivity of the pores.

To show the results of the comparison of the experimental and the calculated results the stress-strain map of the scaffolds *(Figure 6)* which were prepared by compression molding combined with particulate leaching technique made of the 250–500 μm grain-size fraction of sodium chloride was prepared; however this kind of stress-strain maps could be prepared based on the equations *(Table 1* and *Table 2)* in each case.

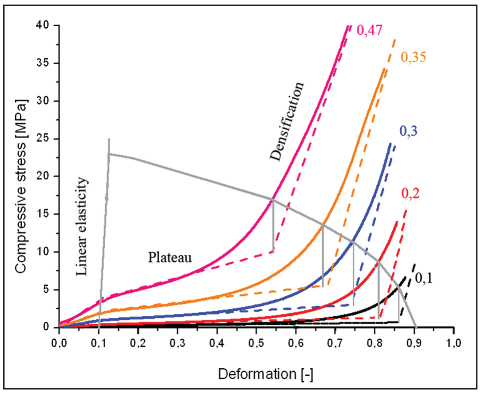

Figure 6. Stress-strain map of scaffolds having pores in the range of 250–500 μm

Based on the map, the same observations can be done as it was written above, just as the strain of elasticity is slightly increasing as the porosity is decreasing. The solid line shows the exact result of the measurement, while the dashed line is the calculated value which shows very good accuracy especially in the linear elastic regime which is the most probable range of the implant applications.

Conclusions

Different fractions of common table salt were utilized to obtain porous scaffolds made of polycaprolactone by compression molding and particulate leaching. The specimens had porosity in the range of 50–92 percent, and the structural and compressive characteristics were investigated. It was found that during the manufacturing of specimens the large grains undergoing cracking resulted in slightly different pore-sizes than the expected value, which phenomenon was more pronounced in case of high initial porogen contents.

Based on the compressive tests, the yield and densification strength, the Young's and plateau modulus, furthermore the strain of elasticity and densification were determined. Based on the experimental results linear curves were fitted on log-log plots, which showed that the power-law is valid for the obtained scaffolds; however the strain of elasticity differed from the theory as it was dependent on both the pore-size and porosity.

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