J Mater Sci: Mater Med (2009) 20:719–724
DOI 10.1007/s10856-008-3622-z

Development of polycaprolactone/chitosan blend porous scaffolds

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Received: 24 June 2008 / Accepted: 15 October 2008 / Published online: 6 November 2008

Abstract Polycaprolactone (PCL) and chitosan were blended to fabricate porous scaffolds for tissue-engineering applications by employing a concentrated acetic acid solution as solvent and salt particles as porogen. These scaffolds showed well-controlled and interconnected porous structures. The pore size and porosity of the scaffolds could be effectively modulated by selecting appropriate amounts and sizes of porogen. The results obtained from compressive mechanical measurements indicated that PCL/chitosan could basically retain their strength in their dry state compared to individual components. In a hydrated state, their compressive stress and modulus could be still well maintained even though the weight ratio of chitosan reached around 50 wt%.

1 Introduction

Synthetic biopolymers have attracted a great deal of attention in the biomedical applications since last two decades. Of different types of synthetic biopolymers, biodegradable polyesters, mainly including poly(glycolic acid), poly(lactic acid), poly(hydroxyl butyrate), and poly(ε-caprolactone) (PCL) as well as their copolymers, have been widely used for the different biomedical purposes [1–5]. Among these polyesters, PCL is of very interest due to its soft- and hard-tissue compatibility and easy processing (melting point, ca. 60°C). Its non-toxic degradation products can be either metabolised via the tricarboxylic acid cycle or eliminated by direct renal secretion [6]. Nevertheless, like other kinds of biodegradable polyesters, applications of PCL scaffolds could be limited by several its drawbacks [7]: (1) absence of cell recognition sites on the surface of the PCL-based materials; (2) hydrophobicity; (3) neutral charge contribution; and (4) possible acidic degradation products hydrolyzed from the ester linkages. In addition, PCL has a slow degradation rate compared to other aliphatic polyesters due to its high crystalline character. Numerous efforts, therefore, have been directed to overcome these drawbacks. The most common strategy is to blend PCL with other synthetic or natural polymers [8–11].

Chitosan is a linear naturally occurred polymer and usually obtained by alkaline deacetylation of chitin. It has been very frequently used in different biomedical areas because of its unique advantages [12], including biocompatibility, biodegradability, hydrophilicity, non-antigenicity, non-toxicity, and anti-microbial activity as well as bioadherence and cell affinity. Moreover, the amino groups in its backbone render chitosan a weakly basic characteristic. However, the deficiency of chitosan is its less flexibility in regulating the mechanical properties and especially, the mechanically weak features in the wet state. On the basis of above-described respective characteristics associated with PCL and chitosan, it can be figured out that chitosan and PCL have some mutually complementary properties. It is therefore reasonable to expect that their individual deficiencies would be overcome if PCL and chitosan could be well blended

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together. Although it is difficult to blend PCL with chitosan at a fully miscible level, several efforts have been dedicated to blending these two components together [13, 14] by using hexafluoro-2-propanol (HFIP) or a 77% acetic acid solution as solvents, respectively. However, in the first case, HFIP is a very expensive solvent; and in the second case, the reported technique for fabricating PCL/chitosan scaffolds is very complicated and laborious, frequently resulting in products with unstable pore structures. Therefore, developing a new technique for fabricating porous PCL/chitosan scaffolds becomes quite necessary.

In the present work, a new and effective processing technique for building porous PCL/chitosan scaffolds was presented. The preparation and characterization of these scaffolds with related compressive mechanical behaviors in both dry and wet states were reported.

2 Experimental

2.1 Materials

Chitosan and PCL (CAPA 6800) were supplied by Fluka and Solvay Chemicals, respectively.

The viscosity-average molecular weight of chitosan was determined as $8.94(\pm0.26) \times 10^6$ using 0.25 M CH_3CO OH/0.25 M CH_3COONa as a solvent system based on our previous method [15], and the corresponding degree of deacetylation was measured as 78.3 (±1.4)% using UV spectrometry, following a known technique [16]. Phosphate buffered saline (PBS) packets were purchased from Sigma, and all other chemicals were obtained from Aldrich and used without further purification.

2.2 Fabrication of scaffolds

Fine NaCl particles (density: 2.165 g/cm^3) were prepared by using a large analytical mill and the milled salt particles were sieved into two groups of mesh sizes: 60–70 (210–250 μm) and 70–100 (150–210 μm). PCL was dissolved in glacial acetic acid with various concentrations changing from 2.0 to 15.0 wt%. Chitosan was dissolved in 70% acetic acid solutions to prepare 0.5–1.0 wt% solutions. Two kinds of solutions were mixed together at different weight ratios but the final concentration of the solvent in the mixture should be adjusted to around 80%. To each mixture, a prescribed amount of salt particles was introduced and the resultant mixture was homogenized while being concentrated by heating at 50°C with stirring. The so produced gel-like mixture was degassed using a centrifuge and then cast into membrane onto a Teflon dish, followed by drying at 50°C in an oven. These samples were then leached in a 5.0 wt% NaOH solution to remove salt particles, and repeatedly washed with distilled water until neutral pH was reached. After that, they were dehydrated by using 90% CH_3OH for 12 h and 99.7% CH_3OH for additional 12 h, and totally dried in vacuum at ambient temperature.

To determine the volume ratio of salt particles to the PCL/chitosan blends, the density of some solid PCL/chitosan membranes with various weight ratios of components was measured via a floating method and using carbon tetrachloride (density: 1.586 g/ml) and *n*-heptane (density: 0.683 g/ml) as mixed solvents. The density of the solid membranes was used to calculate the feed volume percent of salt particles.

A series of PCL/chitosan scaffolds was fabricated and they were named as PCL/ch25, PCL/ch50, and PCL/ch75, respectively. The number following ch denoted the weight ratio of chitosan to PCL in the scaffolds. Some pure PCL or chitosan scaffolds were also manufactured with the same technique and used as controls.

2.3 Characterization

The porosity of scaffolds was measured using a specific gravity bottle based on Archimedes' Principle according to a known method [17], and the porosity of scaffolds was calculated as follows:

$$\text{Porosity}(\%) = [(W_2 - W_3 - W_S)/(W_1 - W_3)] \times 100 \quad (1)$$

where W_1 is the weight of specific gravity bottle filled with ethanol; W_2, the weight of specific gravity bottle including ethanol and scaffold (ethanol above the mark was removed); W_3, the weight of specific gravity bottle taken out the ethanol-saturated scaffold from W_2; W_S, the weight of scaffold.

A mercury intrusion porosimeter (AutoPore IV 9500, Micromeritics) was employed to determine the pore size distribution of the porous scaffolds. Mercury was filled progressively changing from a value of filling pressure of 3.4 kPa to that of 414 MPa. The relationship between applied pressure and the minimum size pore into which mercury will be forced to enter is [18]

$$d = -4\gamma \cos\theta/P \quad (2)$$

where γ is the surface tension, θ, the contact angle between mercury and the sample, and P, the pressure required to force mercury into a pore having a diameter of d.

The scaffolds were cut into strips, coated with gold-palladium and examined using SEM (Philips, XL-30) for their morphologies. The average pore size was determined using a computed image analyzer (Malvern Instruments) by measuring the approximate diameters of pores at 100 different points in a 712 × 484 SEM image for each specimen.

2.4 Compressive mechanical test

The compressive mechanical parameters were measured in both dry and wet state using an INSTRON universal testing machine (model 4206). The dry scaffolds were cut into circular pieces with a dimension of 10-mm in diameter and around 3-mm in thickness. The crosshead speed was set at 1 mm/min. Stress-strain data were collected from load and displacement measurements. In the case of the hydrated samples, after being immersed in a PBS solution (pH 7.4), the samples were transferred into several tubes with a sintered glass bottom, and the excess of absorbed water was removed using a centrifuge (IEC 21000) at 2,000 rpm for 1 min. The hydrated samples were also cut into round pieces with the same diameter indicated above but with a thickness of around 4-5 mm, and tested under the same conditions. All specimens were compressed at ambient temperature. In the both cases, the compressive modulus (E) was defined as the initial linear modulus and the stress at 10% strain was recorded as σ_{10}.

Analysis of variance (ANOVA) was conducted using commercially available statistical software (SPSS 15.0 for Windows) to examine whether significant differences existed between the measured data.

3 Results and discussion

3.1 Morphologies of scaffolds

In the present investigation, it was found that PCL and chitosan solutions could be well blended into clear and almost colorless mixtures by employing a 80% acetic acid solution as the solvent without any visible phase separation such as delamination of component solutions or the direct precipitation of components although it was unknown that whether these two components were miscible at a microcosmic scale. In order to homogenously disperse salt particles inside the PCL/chitosan solutions, the mixtures containing PCL, chitosan and salt particles were further stirred in open beakers at 50°C for required durations until they formed into concentrated gel-like fluids. At the end of this stage, the color of the mixture could turn into uniform light yellow depending on the weight percent of chitosan. However, there was no any visible phase separation observed during the concentration procedure since the colors of PCL and chitosan are white and light brown in the present case, respectively, and any visible precipitation is easy to be checked. Based on many trial experiments, it was observed that by controlling the volume ratio of salt particles in a mixture higher than 75 vol%, the particles loaded inside the scaffolds could be almost completely leached out without remnants. In addition, the pore size and porosity of the resultant scaffolds could be effectively modulated by varying the amount of the salt particles. Figure 1 presents a few representative SEM images of scaffolds with interconnected porous structures. Many square-shaped pores or some pores with right-angled sides were easily observed inside the PCL/ch25 or PCL/ch50 scaffolds (see Fig. 1a, b), indicating the evidence of the

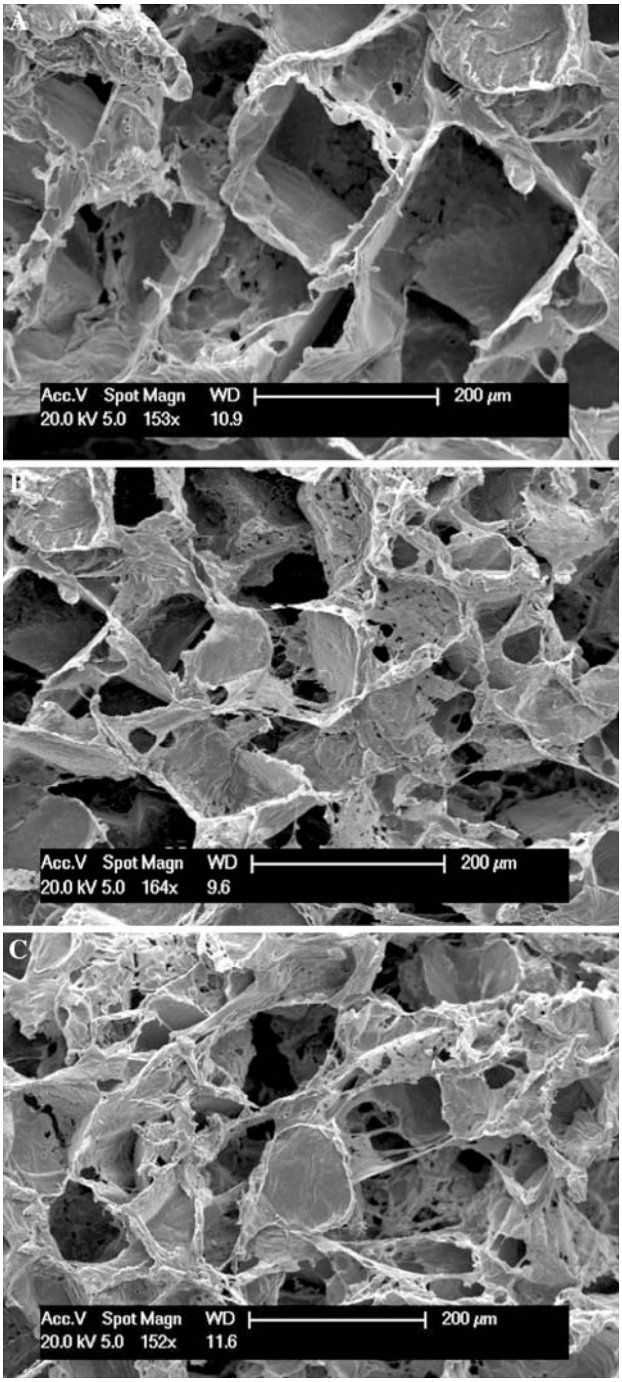

Fig. 1 SEM micrographs of scaffold sections (salt particles volume ratio: 80 vol%, particle size: 150–210 µm). **a** PCL/ch25, **b** PCL/ch50, **c** PCL/ch75

trace left by salt particles. However, the pores in PCL/ch75 scaffolds were displayed with quite irregular shapes. Since these scaffolds were fabricated with the same volume amount of salt particles the differences in the morphologies should be mainly ascribed to the proportions and properties of the components.

3.2 Parameters of scaffolds

To figure out effect of the components on pore sizes the distribution of pore sizes was measured and the collected curves were displayed in Fig. 2. It can be seen that (1) all depicted pore-size distribution curves basically display an approximate Gaussian distribution but in different modes; and (2) as the weight ratio of chitosan increases the patterns are integrally moved forwards to the lower pore size interval. As described in the experimental section, the salt particles inside the scaffolds had been leached out by using a 5.0 wt% NaOH solution and the resultant scaffolds were extensively washed with distilled water, inevitably, chitosan component inside would swell to a certain extent during these procedures. After being dehydrated, compared to the particle-size of the salt particles, more pores with a smaller pore size inside the scaffolds would be retained, and this could become more significant as the content of chitosan was increased, thus leading to more pores with a smaller pore size and irregular pore shapes.

Expect for the effect of the components on the pore-size distribution, it is worth further examining the impact of salt particle size on the pore-size distribution. In this case, the composition proportion of the components needs to be maintained constant to keep the same baseline. PCL/ch50 scaffold was selected as an example because of its equivalent components. Figure 3 shows the variations in the pore-size distribution curves of PCL/ch50 scaffolds. It was observed that when salt particles with smaller sizes (150–210 μm) were employed, the corresponding scaffolds also exhibited smaller pore sizes, illustrating by the pore-size distribution at a lower interval; in contrast, scaffolds would show larger pore sizes while some salt particles with greater sizes (210–250 μm) were used, evidencing by the curve distributed over a higher interval. Based on the these curves and the marked peak values (see P(I) and P(II) denoted in Fig. 3, $P < 0.01$), it could be concluded that the salt particle size could dominantly modulate the pore-size of the scaffolds.

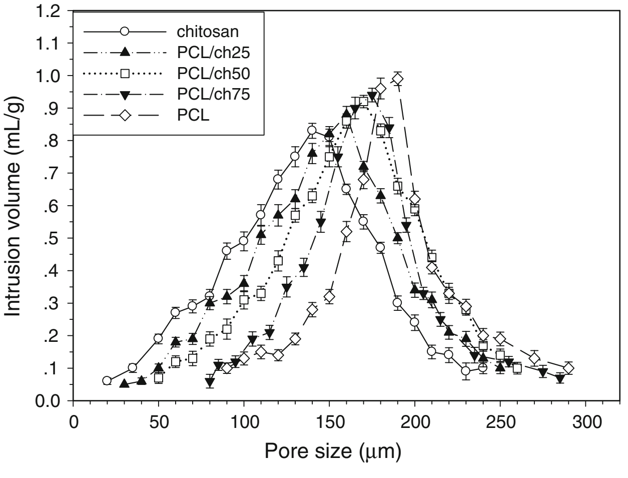

Fig. 2 Variations in pore-size distribution of scaffolds with various composition proportions of the components (salt particles volume ratio: 80 vol%, particle size: 150–210 μm)

Figure 4 presents the changes in the porosity of scaffolds with both proportions of components and the volume ratio of salt particles. The data registered in Fig. 4 revealed that by keeping the volume ratio of salt particles constant

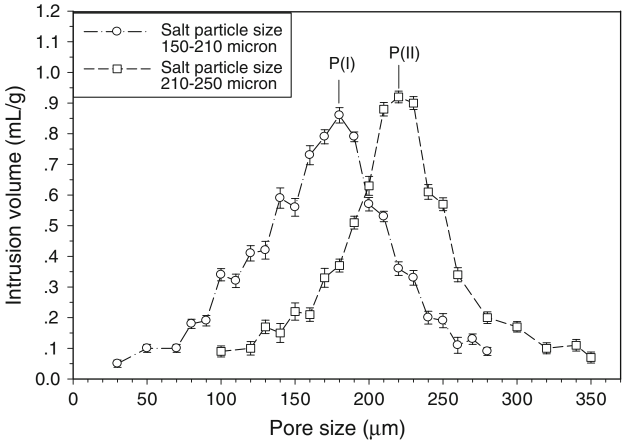

Fig. 3 Variations in pore-size distribution of PCL/ch50 scaffolds with the salt particle size (salt particle volume ratio: 85 vol%)

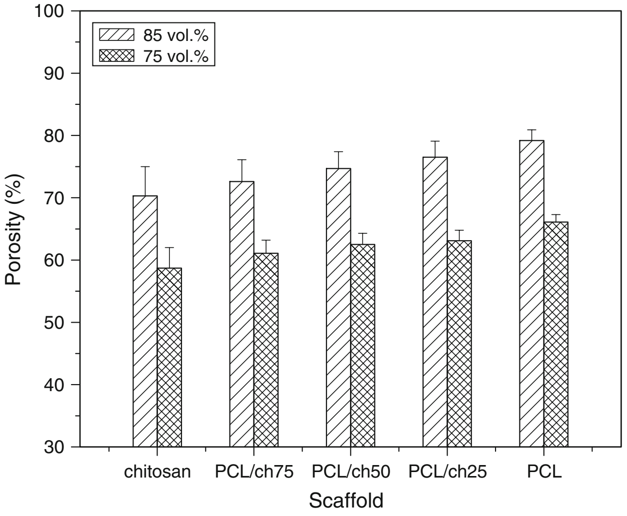

Fig. 4 Changes in porosity of scaffolds with the composition proportions of the components and the volume ratios of the salt particles

the resultant PCL/chitosan scaffolds did not notably alter ($P > 0.05$) their porosity although the proportions of components varied considerably; and on the other hand, provided that the proportions of components were fixed the porosity of scaffolds could significantly vary with the volume ratio of salt particles. In the present study, the density of PCL and chitosan was measured as 1.12 and 1.21 g/cm^3, respectively, meaning that only a small difference in the density would exist among the PCL/chitosan blends with various weight ratios of PCL to chitosan. As mentioned in the experimental section, salt particles were added into PCL/chitosan blend solutions based on the volume ratios. Therefore, when a prescribed volume ratio was selected, only a very small difference in the real amount of salt particles would exist in different PCL/chitosan scaffolds, leading to insignificant changes in their porosity as long as these salt particles could be completely leached out. The curves in Fig. 4 revealed that the porosity of the scaffolds could be well controlled by changing the volume ratio of salt particles.

3.3 Compressive mechanical properties of scaffolds

The compressive mechanical properties of porous scaffolds are of particular importance in tissue engineering since they are very closely linked to the dimension-maintaining ability and durability in practical operations and applications. To eliminate the effect of pore parameters on the properties of scaffolds and mainly focus on the compressive mechanical properties of scaffolds, by carefully optimizing processing conditions, a great number of scaffolds having proximately similar pore parameters were prepared and the relevant data were summarized in Table 1.

Figure 5 shows compressive stress–strain curves of several dry scaffolds, and the data of compressive modulus, E, and compressive stress at 10% strain, σ_{10}, were collected and listed in Table 2. In general, the compressive stress-strain curves for porous foams composed of non-brittle polymer materials exhibit a few distinct characteristics: linear elastic deformation at small strain, flexure deformation for the elastic materials or yielding deformation for the elasticplastic materials at somewhat large strain, followed by a plateau region at apparently large strain and a solidifying region in which the stress sharply increases at very large strain [19]. All curves in Fig. 5 display these four characteristics and have not shown any yielding deformation, indicating that these porous scaffolds possess well-defined compressive mechanical properties. The data in Table 2 indicate that, compared to pure porous PCL or chitosan scaffolds, both E and σ_{10} of blend scaffolds in their dry state showed relatively low values. It is well known that both PCL and chitosan are semi-crystalline polymers. The crystallinity of each component inside the scaffolds would be certainly decreased because the individual components could suppress the recrystallized ability each other [13], which would certainly result in decreased E and σ_{10}. However, there were no significant differences ($P > 0.05$) recorded among the PCL/chitosan scaffolds, revealing that these PCL/chitosan scaffolds could basically maintain their compressive strength in their dry state compared to individual components.

Table 1 Pore parameters of scaffolds

Scaffold samples	Pore-size interval (μm)	Average pore-size (μm)	Porosity (%)
PCL	90–280	181.6 ± 7.5	72.1 ± 3.1
PCL/ch25	80–280	173.2 ± 8.3	73.6 ± 4.2
PCL/ch50	50–260	171.9 ± 9.1	70.9 ± 3.9
PCL/ch75	30–250	174.3 ± 7.8	69.4 ± 4.5
Chitosan	20–240	169.6 ± 9.4	68.2 ± 5.2

The values in table were the average data from five specimens for each sample. The pore size of scaffolds was estimated from their SEM images in the cross-section area

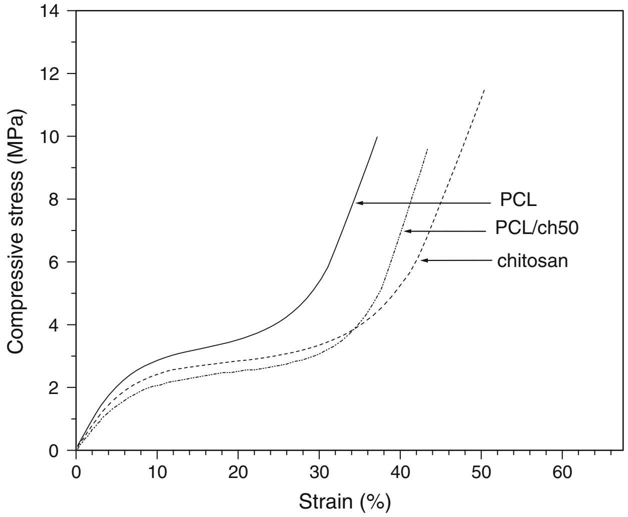

Fig. 5 Compression stress–strain curves of dry scaffolds

Several plots for hydrated porous scaffolds are represented in Fig. 6. It is observed from Fig. 6 and Table 2 that hydrated pure porous chitosan scaffolds have a low strength and a very poor dimensional stability, indicating by a large deformation and greatly decreased compressive E and σ_{10} (see Table 2). Chitosan is a semicrystalline polymer and is also highly hydrophilic because of its polar groups. In a hydrated state, some microcrystalline domains of chitosan, which are previously formed in a dry state through hydrogen bonding formed between inter- and intra-molecules [20], will no long exist, as a result, both E and σ_{10} of pure porous chitosan scaffolds could be extremely

Table 2 Compressive modulus (E) and stress at 10% strain (σ_{10}) of scaffolds

Scaffold samples	Dry state		Hydrated state[a]	
	E (MPa)	σ_{10} (MPa)	E(MPa)	σ_{10} (MPa)
PCL	37.1(±3.09)	2.57(±0.17)	34.1(±1.09)	2.01(±0.07)
PCL/ch25	31.5(±2.21)	2.12(±0.13)	27.6(±2.12)	1.59(±0.11)
PCL/ch50	30.2(±2.13)	2.05(±0.09)	14.4(±2.03)*	0.94(±0.12)*
PCL/ch75	29.7(±2.29)	1.88(±0.12)	6.7(±1.49)	0.31(±0.09)
Chitosan	33.6(±2.76)	2.21(±0.08)	0.32(±0.13)	0.023(±0.007)

Values in table were recorded as the average from six specimens

[a] Samples were hydrated in a PBS solution under ambient atmosphere for 2 h

* $P < 0.01$, the differences were evaluated as significant ones

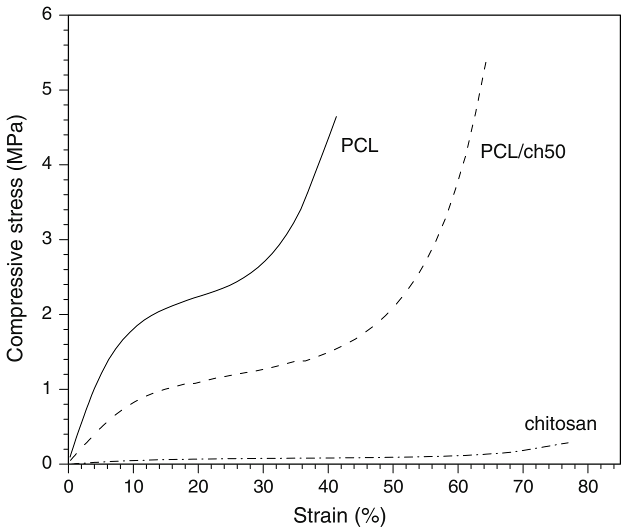

Fig. 6 Compression stress–strain curves of hydrated scaffolds

low in their wet state. By blending with PCL, the present PCL/chitosan scaffolds could obtain pronouncedly improved compressive strength. The data listed in Table 2 proved that the compressive stress and modulus of the PCL/chitosan scaffolds could be still well maintained in their wet state even though the weight ratio of chitosan reaches around 50 wt%.

4 Conclusions

The newly developed technique for fabricating polycaprolactone/chitosan blend porous scaffolds was proved to be successful. The obtained scaffolds could show interconnected porous structures with various pore sizes and porosities. The pore parameters of the scaffolds could be effectively modulated via varying the size and amount of employed porogen. The resultant scaffolds could basically retain their compressive strength in their dry state compared to individual components. In addition, their compressive stress and modulus could be still well maintained in a hydrated state even though the weight ratio of chitosan reached around 50 wt%.

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