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Characterization of chitosan and polycaprolactone membranes designed for wound repair application

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Abstract Polycaprolactone (PCL) and chitosan (Ch) are nontoxic, biocompatible, and biodegradable polymers of vast interest for wound repair. The aim of this work was to prepare Ch/PCL membranes in different proportions (90:10 and 80:20 w/w) in the presence and absence of the surfactant Pluronic F68 (PF68). The membranes were evaluated regarding morphology, thermal behavior, and viscoelastic properties. Sample swelling and degradation in phosphate-buffered saline (PBS), simulated body fluid (SBF), and fetal bovine serum (FBS) were determined by differential scanning calorimetry (DSC) and dynamical mechanical analysis (DMA), while cell toxicity to L929 and Vero fibroblasts was evaluated using the MTT reduction assay and cell proliferation, by DNA quantification and confocal laser microscopy. After 60 days in SBF, marked Ch matrix loss and advanced degradation of PCL particles were noticed by scanning electron microscopy (SEM). No significant differences in melting temperature (T_m) and enthalpy (ΔH_m) were detected by DSC. However, the surfactant increased the ΔH_m. After 30 days, the membranes obtained in the presence of PF68 had absorbed more blood serum and were more degraded after exposure to simulated blood fluid for 30 days. All membranes had low cytotoxicity, and higher cell proliferation was noticed for samples obtained in the presence of the surfactant. In conclusion, the Ch/PCL membranes showed satisfactory degradability and biocompatibility, which enhances their potential for application in wound repair.

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Introduction

Extensive studies in the field of regenerative medicine are focused on the development of cells, tissues, and organs for the purpose of restoring body function through implants. It is assumed that function repair and restoration as well as tissue regeneration are the best treatments for the loss or failure of an organ [1]. Different polymeric membranes have been produced for the recovery of skin lesions such as burns and wounds caused by chronic illnesses or resulting from accidents. In particular, skin dressings based on polysaccharides such as chitosan (Ch) have frequently been studied for this purpose [2–9].

Chitosan is a polysaccharide produced by chitin deacetylation and may be chemically modified to expand

its properties [10]. Chitin is commonly found in the exoskeleton of crustaceans, insects, and some fungi and its application in the clinical field, as well as that of many of its derivatives, is being widely studied [11–14]. Ch has several advantages such as low cost and high commercial availability. Since Ch consists of molecules with multiple positive charges, this polysaccharide can interact with molecules that have negative charges, such as glycosaminoglycans present in the extracellular matrix. Ch has antimicrobial activity [15] is soluble in weak acids (at pH values lower than 6.3) and is easily processed in the form of films and porous scaffolds [16, 17]. Its degradation is caused by the action of the enzyme lysozyme, and the degradation rate varies depending on the degree of deacetylation of the material, being higher for highly deacetylated Ch [18].

Polycaprolactone (PCL), on the other hand, is a linear aliphatic polyester [19], and like some other synthetic polymers belonging to the polyesters group, it has been used clinically for many years in the production of reabsorbable surgical sutures and controlled drug release devices. PCL degradation occurs in three phases. In the first stage the material goes through a nonenzymatic degradation process resulting in the susceptibility of the ester bonds to hydrolysis [20]. In the second stage, short-chain oligomers are formed, reducing the PCL molecular weight. In the last stage, the low molecular weight PCL is phagocytosed by macrophages and rapidly degraded in ε-hydroxycaproic acid, which is metabolized by entering the tricarboxylic acid (TCA) cycle, finally being eliminated by renal excretion [21].

Previous work [7, 8, 20] focusing on the use of Ch mixed with PCL showed very promising results regarding the development of new polymer hybrids. Sarasam and Madihally [21] report good results with regard to biomechanical aspects of the blend. However, there is still room for improvement of the material's biological performance. Thus, the different solubilities of Ch and PCL may be a constraint.

The miscibility problems of Ch and PCL mixtures may be at least partially circumvented through with the use of surfactants such as Pluronic F68 (PF68). This particular noncytotoxic compound, a triblock copolymer of hydrophilic polyethylene oxide and hydrophobic polypropylene oxide, is widely used to protect animal cells from aeration and agitation-related injuries in stirred culture [22, 23].

Thus, the main objective of this work was to produce Ch and PCL membranes with different mass ratios in the presence and absence of the compatibilizing agent PF68. The membranes were characterized regarding their mass gain and stability in different fluids; their morphological, mechanical, and thermal properties before and after exposure to simulated body fluid (SBF); and their effect on fibroblasts cultured in vitro.

Materials and methods

Production of the membranes

The Ch/PCL blend membranes were prepared with Ch (with at least 85% of deacetylation from crab shells, Sigma-Aldrich) solution at 1% in acetic acid (Synth) at 2% and PCL (800,000 g/mol, Sigma-Aldrich) solution (0.1 and 0.5%) in glacial acetic acid. The Ch solution was mixed with the PCL solution under mechanical agitation (800 rpm) at a flow rate of 100 mL/h and a controlled temperature (40 °C). The Ch/PCL membranes were produced using different polymer mass proportions (90:10 and 80:20 Ch:PCL) in the presence and absence of the surfactant PF68 at 0.05 and 0.1%. The materials were dried at room temperature (solvent casting), neutralized with 0.1 M NaOH (Synth) solution for 30 min and washed with 2 L of distilled water. The samples were sterilized with ethylene oxide at Acecil Central de Esterilização Comércio, Indústria Ltda (Campinas, SP, Brazil), washed three times with air to remove residual ethylene oxide and stored at room temperature for at least 1 week before testing.

Characterization of the membranes

The effect of sample exposure to SBF, prepared according to Kokubo et al. [24], for 15, 30, and 60 days (changing the solution every 5 days), was evaluated by optical microscopy (GX51, Olympus), scanning electron microscopy (SEM) (Jeol JXA 840A, 40 mA and 10 kV), differential scanning calorimetry analysis (DSC-STA 409C, Netzsch) performed at temperatures from room temperature to 250 °C with a heating rate of 10 °C/min and dynamical mechanical analysis (DMA, Triton) at 0.1 N tension with a scan frequency of 0.01–16 Hz in a phosphate buffered saline solution (PBS) at 37 °C (average and SD of three samples of each material).

The swelling capacity of each membrane type (1 g per sample) in SBF, PBS, and fetal bovine serum (FBS) was assessed by measuring the samples' mass variation (in triplicate) after immersion in 10 mL of each solution (pH 7.4). The results were calculated using Eq. 1, where M_S was the swollen sample mass, and M_D was the dry sample mass. The mass loss was calculated with Eq. 2, where M_{Df} was the final mass of the dried membrane after exposure to different solutions and M_D was the initial mass of dried samples before the degradation test.

$$C_i = \frac{(M_S - M_D)}{M_D} \quad (1)$$

$$V_m = \frac{(M_D - M_{Df})}{M_D} \times 100 \quad (2)$$

Tensile properties were also studied according to the ASTM D882-02 standard [25] in Tinius Olsen H5H-S

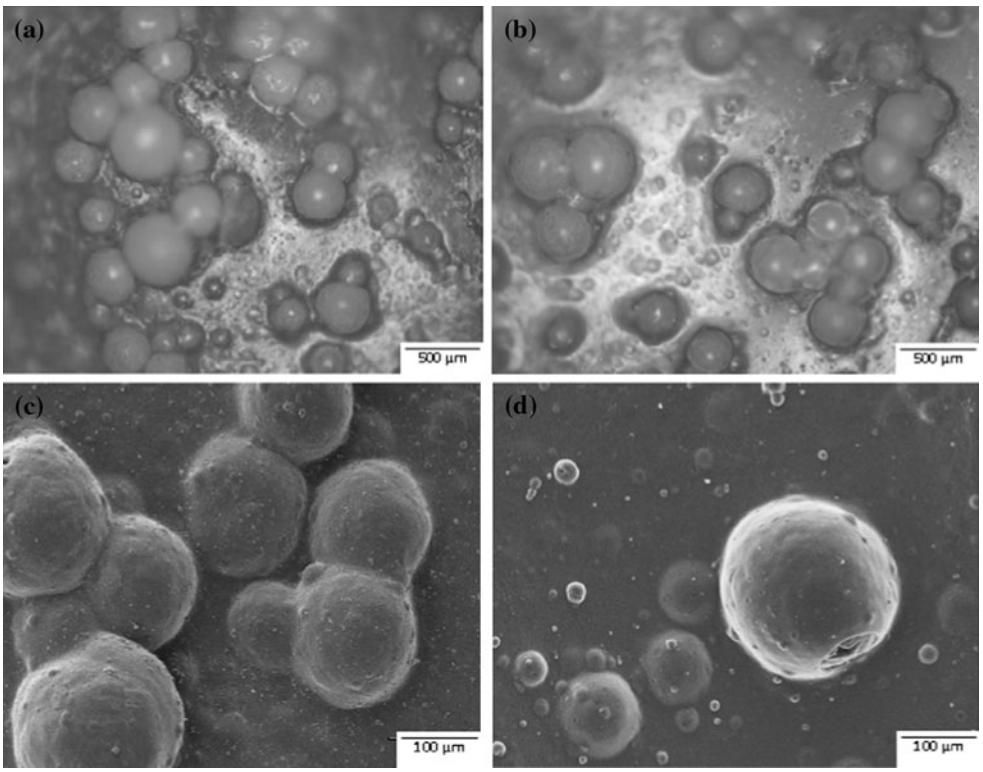

Fig. 1 Membrane morphology without PF68 observed by optical microscopy: **a** Ch/PCL 80:20 (%w/w) and **b** Ch/PCL 90:10 (%w/w). SEM images of membranes in the absence of Pluronic: **c** Ch/PCL 80:20 (%w/w) and **d** Ch/PCL 90:10 (%w/w)

equipment at 23 ± 2 °C and 50% relative humidity, using a test speed of 10 mm/min. Ten dry samples were tested per condition. The results obtained were stress at break and elongation.

The biocompatibility of the materials was analyzed by direct [26, 27] and indirect exposure [28, 29] of the samples (in triplicate) to L929 and Vero fibroblasts (ATCC, USA). The cells were cultured in DMEM medium supplemented with 10% FBS and 1% antibiotic (streptomycin at 3×10^{-4} mol/L and penicillin at 5×10^{-4} mol/L, Cultilab). After 90% cell confluence, the cells were removed with trypsin (0.05%, Cultilab) and seeded in 24- and 96-well plates (Corning) at 1×10^4 cells or 2×10^3 cells/well concentration for the direct and indirect exposure tests, respectively. The membrane samples were submersed in the culture medium and placed on the cell monolayer. After 24 h, cell viability was analyzed using methylene blue at 0.5% (Sigma) and cell morphology was evaluated through optical microscopy (Olympus SZ 40).

In the case of the indirect exposure test, the material extracts were obtained by incubation of the samples in complete culture medium at 37 °C in a concentration of 0.1 g/mL for 24 h. Latex samples were used as a cytotoxic positive control and the culture plate itself was considered the negative control. The MTT (3-[4,5-dimethyl-2-thiazolyl]-2,5-diphenyl-2*H*-tetrazolium bromide) reduction method (Sigma) was used to measure cell viability in this case. The optical densities were measured at 590 nm with a plate reader (BioTek). DNA extraction (Quant-iT, Invitrogen) was performed for samples (in triplicate) and cell morphology was analyzed through confocal laser microscopy after fixing with a 4% formaldehyde solution and dying with DAPI (4′-6-diamidine-2-phenylindole at 0.2%) to visualize the nuclei (in blue) and with phalloidin (at 1%), which binds to the cell cytoplasm F-actin (in red).

Groups were compared by using the independent paired *t* test with $p < 0.05$ indicating statistical significance of the control (cell culture plate).

Results and discussion

Morphology of membranes exposed and not exposed to SBF

When used in tissue engineering, polymeric membranes and other devices are in direct contact with different types of tissues. For this reason, these materials must have properties that favor their application, such as homogeneity, controlled rate of degradation, and appropriate cellular responses.

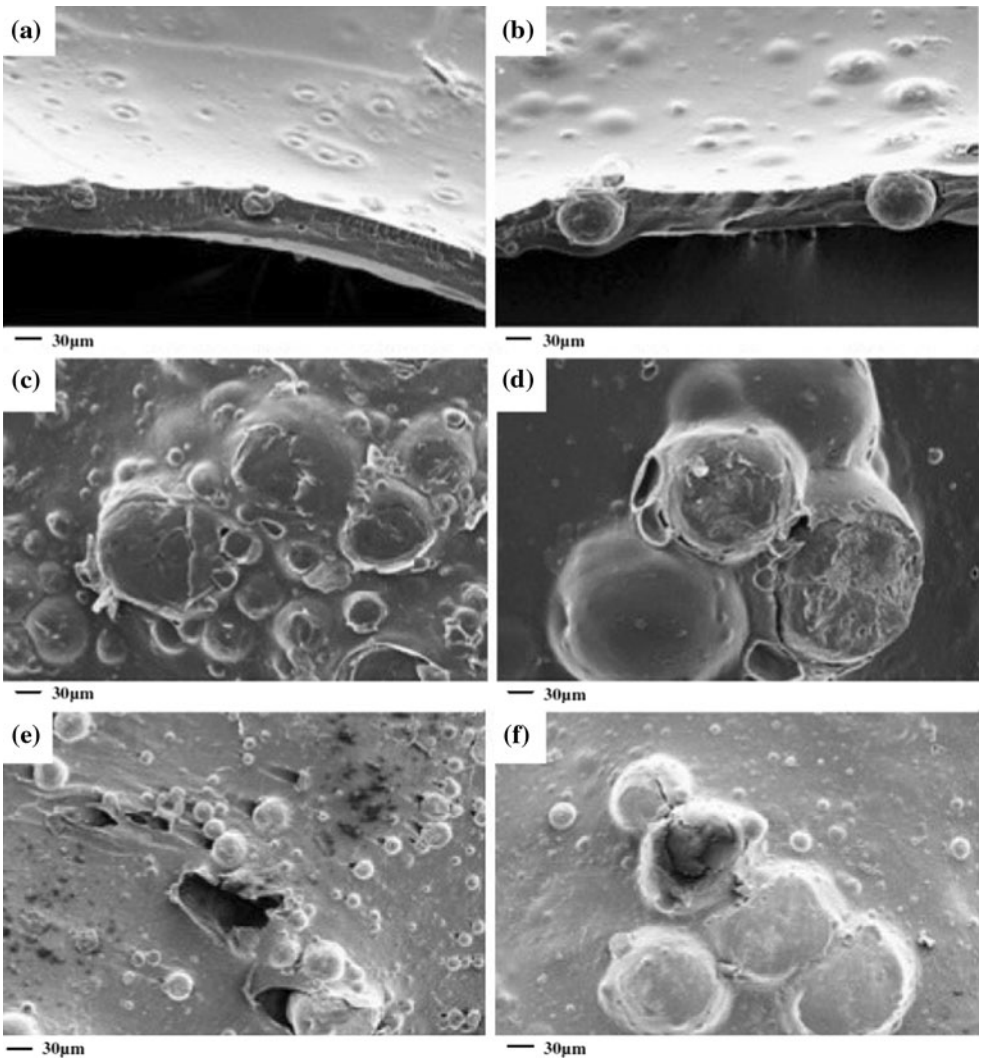

Fig. 2 Morphology of membrane surfaces evaluated by scanning electron microscopy. Cross-sections of Ch/PCL membranes: **a** 80:20 (%w/w) and **b** 90:10 (%w/w). Morphology of membrane surfaces prepared with Ch/PCL at 80:20 and 90:10 (%w/w) ratios after degradation in SBF for 30 days (**c** and **d**, respectively) and 60 days (**e** and **f**, respectively). All membranes prepared in the presence of 0.1% Pluronic F68

Table 1 Swelling capacity of Ch/PCL membranes prepared with different proportions in different solutions (PBS, SBF, and FBS) and mass loss after incubation in SBF

Material	PCL (%)	PF68 (%)	Swelling capacity (g/g)			Mass loss in SBF (%)	
			PBS	SBF	FBS	15 days	30 days
Ch	–	–	2.0	1.5	3.5	26.7	41.8
Ch/PCL	10	–	1.4	0.8	3.6	0.0	1.0
Ch/PCL	10	0.05	1.7	1.7	4.6	1.5	2.5
Ch/PCL	10	0.1	3.5	1.7	2.5	13.8	14.1
Ch/PCL	20	–	1.5	1.8	–	3.4	3.9
Ch/PCL	20	0.05	1.6	1.7	4.3	3.8	4.2
Ch/PCL	20	0.1	1.3	1.2	9.3	6.8	8.9

The morphology of the membranes prepared with different proportions of Ch and PCL was observed before and after incubation in SBF. The surface of membranes in the absence of PF68 was analyzed by optical microscopy (Fig. 1a, b) and by SEM (Fig. 1c, d). PCL spherical particles (with average diameters of around 100–150 μm) could be observed distributed in the Ch matrix (Fig. 1a, b). The materials with PF68 showed smaller particles

Table 2 Differential scanning calorimetry analysis of Ch/PCL membranes prepared with different polymer proportions in the presence and absence of PF68

Material	PCL (%)	PF68 (%)	T_c (°C)	T_m (°C)	ΔH_m (J/g)
Ch/PCL	20	–	31.5 ± 0.2	58.0 ± 0.8	8.0 ± 0.3
Ch/PCL	20	0.05	21.7 ± 1.2	59.4 ± 1	13.0 ± 0.7
Ch/PCL	20	0.1	20.9 ± 0.4	57.1 ± 0.7	13.5 ± 0.5
Ch/PCL	10	–	22.0 ± 0.6	58.1 ± 0.2	7.2 ± 0.4
Ch/PCL	10	0.05	23.1 ± 0.7	59.5 ± 1.4	7.1 ± 0.8
Ch/PCL	10	0.1	22.1 ± 1.1	58.6 ± 0.9	9.9 ± 0.7
PCL	100	–	23.0 ± 0.5	66.3 ± 0.2	136.0 ± 0.3

Table 3 Differential scanning calorimetry analysis of membranes prepared with different Ch/PCL proportions before and after degradation in SBF for 15, 30, and 60 days

Material	PCL (%)	PF68 (%)	Period (days)	T_c (°C)	T_m (°C)	ΔH_m (J/g)
Ch/PCL	20	–	15	29.7 ± 0.5	57.3 ± 0.6	17.0 ± 0.4
Ch/PCL	20	–	30	21.8 ± 1.2	56.5 ± 0.9	11.0 ± 0.5
Ch/PCL	20	–	60	19.2 ± 0.9	56.6 ± 0.3	15.3 ± 0.7
Ch/PCL	20	0.1	30	21.6 ± 0.7	59.2 ± 0.2	11.4 ± 0.6
Ch/PCL	20	0.1	60	18.9 ± 0.4	57.1 ± 0.7	8.5 ± 0.4
Ch/PCL	10	–	15	21.8 ± 0.3	57.6 ± 0.2	13.1 ± 0.5
Ch/PCL	10	–	30	21.7 ± 0.1	58.9 ± 0.8	8.8 ± 0.8
Ch/PCL	10	–	60	19.5 ± 1.4	54.5 ± 0.2	0.4 ± 0.6
Ch/PCL	10	0.1	30	21.6 ± 0.8	58.7 ± 0.5	7.8 ± 0.3
Ch/PCL	10	0.1	60	19.3 ± 1.3	56.9 ± 0.7	15.8 ± 0.7

Table 4 Analysis of mechanical resistance (tension) of Ch/PCL membranes prepared with different proportions in the presence and absence of PF68

Material	PF68 (%)	Stress at break (MPa)	Elongation (%)
Ch	–	3.1 ± 0.5	5.0 ± 2.4
PCL	–	14.2 ± 1.4	154.9 ± 1.2
Ch/PCL 80:20	–	5.5 ± 1.9	7.2 ± 1.5
Ch/PCL 90:10	–	9.8 ± 2.5	5.7 ± 0.71
Ch/PCL 80:20	0.1	3.3 ± 1.0	4.0 ± 1.0
Ch/PCL 90:10	0.1	8.4 ± 2.6	3.7 ± 1.1

(30–60 μm) and more homogeneous distribution than the membranes without the surfactant (Fig. 2a, b). Surfactant addition improves the dispersion of PCL in the Ch solution in such a way that phase separation was not clearly observed during membrane preparation. However, afterwards it was noticed that PCL was not fully dispersed in the Ch matrix and the presence of PF68 caused a reduction in the material's mechanical properties. Mi et al. [7] showed that irregularities on Ch membrane surface should enhance fibroblast adhesion and proliferation. Therefore, the presence of the PCL particles dispersed on the surface could improve cell performance when they are cultured on these materials.

Sample degradation was not observed after incubation in SBF for up to 15 days. Nevertheless, extensive degradation of the Ch matrix could be observed at days 30 and 60 of incubation in SBF (Fig. 2c–f). PCL particles in advanced degrees of degradation were also noticed (Fig. 2c, d), and after incubation for 60 days, many of these particles had been almost completely removed from the membranes (Fig. 2e, f). Even for the membrane incubated in SBF for 60 days no calcification was detected, indicating that the system does not promote deposition of apatite. In this case the membranes were developed for wound repair, but different strategies to promote apatite deposition are available for promoting osteoconductivity and bone repair. The inclusion of bioactive inorganic particles in the material should be a strategy for bone tissue engineering [13].

Membrane behavior regarding stability after exposure to physiological solutions

The swelling capacity and stability regarding mass loss of the Ch/PCL membranes prepared in the presence of the surfactant showed significant differences from the samples without PF68 (Table 1). The performance of the samples in

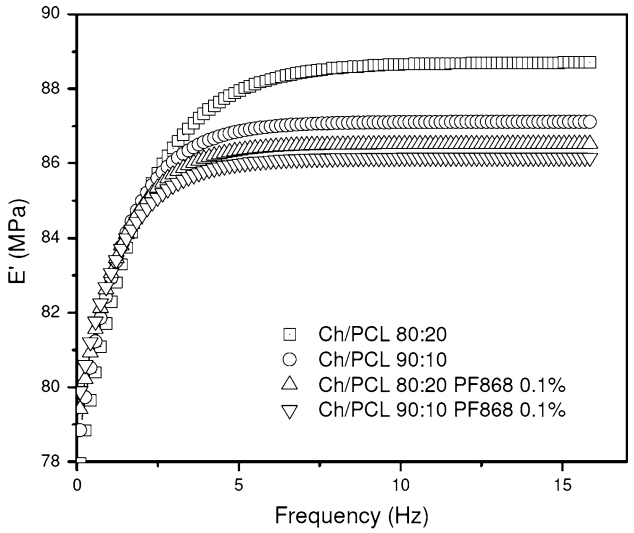

Fig. 3 Storage modulus (E') curves of chitosan and polycaprolactone membranes in the presence and absence of PF68

relation to absorption of PBS and SBF was similar, but an increase in absorption capacity values was observed when the samples were in FBS. In fact, the membranes composed of 20% PCL without the surfactant had been completely dissolved in FBS after 24 h. As for the stability of the membranes in SBF, the addition of PF68 resulted in a higher weight loss; however, the blends did not achieve the same degree of degradation as membranes prepared with only Ch. Similarly, Ch membranes prepared in the presence of other polymers, such as polyglycol (PEG), showed increased swelling rate in water as the Ch content of the materials increased on the structure [30].

Thermal properties characterization

The membranes' thermal properties obtained by DSC analysis are shown in Table 2. The use of different Ch/PCL proportions and the addition of PF68 resulted in only slight differences in membrane crystallization temperatures (T_c), which were similar to that of samples prepared with only PCL, except for the membrane obtained with the 80:20 Ch/PCL ratio. Their melting temperatures (T_m) were lower than for pure PCL; however, these results were much closer to those of isolated PCL. Thus, the melting enthalpy of the blends (ΔH_m) was significantly lower than that of pure PCL. In previous studies, materials containing only PCL showed similar modifications in phase transition temperature in hydrolytic degradation when water was used as the solvent [20, 31]. The same authors also observed decreases in T_c and T_m as the PCL crystalline phase was degraded.

Regarding stability in SBF, the Ch/PCL membranes incubated in SBF for 15, 30, and 60 days showed few changes in the phase transition (crystallization and melting) temperatures according to the DSC analysis (Table 3). During the first 15 days of incubation in SBF, the membranes prepared in the absence of surfactant showed increased melting enthalpy (ΔH_m), which may be attributed to a higher degree of crystallinity resulting from loss of the amorphous PCL phase. Samples degraded for 30 days showed lower ΔH_m, though, and this phenomenon could be a result of the hydrolysis of the PCL crystalline phase. Materials exposed to the longest degradation period (60 days) showed a decrease in crystallization temperature. The peak was not well defined, possibly as a consequence of the lower crystalline percentage of PCL. The melting temperature also decreased, probably because of the high degradation state of the material.

Characterization of mechanical performance

Tensile tests were performed with the purpose of comparing the mechanical properties of the blends obtained to those of dry membranes composed solely of PCL or Ch. A greater enhancement of the maximum stress and of the

Table 5 Behavior of Vero and L929 cells directly exposed to different Ch/PCL membranes

Material	PCL (%)	PF86 (%)	Vero cells		L929 cells	
			Zone index	Lysis index	Zone index	Lysis index
Ch	–	–	0	1	1	1
Ch/PCL	20	–	0	1	0	0
Ch/PCL	20	0.1	1	1	1	1
Ch/PCL	20	0.05	1	1	1	1
Ch/PCL	10	–	1	1	1	1
Ch/PCL	10	0.1	1	1	1	1
Ch/PCL	10	0.05	1	2	1	1
PCL	100	–	0	1	1	1
Latex	–	–	4	4	4	4
Negative control	–	–	0	0	0	0

A zone index (IZ) of zero means no cytotoxicity, while an IZ of four refers to severe cytotoxicity. Equivalent analysis applies to the lysis index

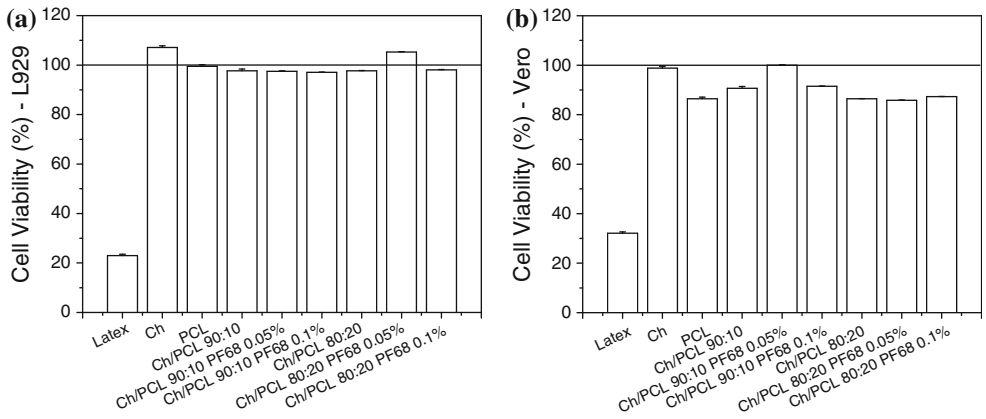

Fig. 4 **a** L929 cell and **b** Vero cell viability after indirect exposure to Ch/PCL membranes prepared with different polymer proportions in the presence and absence of PF68

elongation was noticed for the PCL/Ch membranes than for the isolated Ch membrane (Table 4); however, in all cases the results were closer to those obtained for Ch alone, probably because the proportion of this component in the mixtures was substantially higher. As the experiments were performed in the dry state, the amorphous component of Ch was in its glass state [32], explaining why the Ch was more brittle than the PCL. The materials with PF68 showed a small reduction in mechanical resistance.

Regarding the data from the DMA, the membranes showed lower storage modulus (E') when prepared in the presence of PF68, particularly the 90:10 Ch/PCL formulations (Fig. 3). This result can be associated with the fact that these preparations have a high swelling capacity in PBS (Table 3). The behavior observed corroborates data reported by Caridade et al. [33], who analyzed rates of absorption of water/ethanol solutions by Ch. The Ch storage modulus decreases with the increase in water content. As the surfactant increases the swelling capacity of the 90:10 Ch/PCL membrane in PBS solution, E' is reduced more than that of membranes prepared without the addition of PF68. The membrane richer in PCL has a higher storage modulus, which can be explained by the elastomeric nature of the Ch upon immersion at 37 °C [32].

Behavior of Vero and L929 cells cultured in the presence of the membranes

Analysis of cytotoxicity by direct exposure of Vero cells and L929 to the samples showed that the membranes had mild or no toxicity (Table 5). Cell proliferation occurred in the presence of all formulations with low lysis levels. A slight loss of cell monolayers (less than 20%) was noticed, characterizing only a mild toxicity of most of the Ch/PCL membranes. However, this result can be partially attributed to compression of the cell monolayer by the tested material, decreasing oxygen availability to the cells.

As for the indirect cytotoxicity assay, the samples were incubated in culture medium in the absence of cells and then the effects of their extract on cell growth were analyzed after 3 days. Cell viabilities above 90% were observed for the L929 cell line (Fig. 4a), while Vero cells were shown to be a little more sensitive to the Ch/PCL membrane extracts, given that viabilities mostly around 80% were detected (Fig. 4b). Overall, the blends of Ch and PCL may be considered as cytocompatible, corroborating previously published data [21].

Cell proliferation and morphology were also analyzed by confocal microscopy using the L929 cell line. After 3 days of incubation, some cells were found on top of the membranes without the surfactant (Fig. 5a, b). Many attached fibroblasts were noticed during the analyses of the membranes with Pluronic PF68 and satisfactory proliferation was detected in both formulations (Fig. 5c, d). The highest cell proliferation was observed on the Ch/PCL 80:20 membranes prepared in the presence of PF68. This result was also assessed through DNA extraction analysis, which showed higher concentrations on the membranes prepared with the larger proportion of PCL (Fig. 6). The results for these membranes are directly comparable to those for the control, being around three times higher than those noticed for membranes composed solely of Ch.

The Ch/PCL membranes studied showed results similar to those reported by Hoque et al. [34] for porous PCL-based scaffolds. According to these authors, enhanced cell attachment on PCL scaffolds may have positive effects on cell proliferation, which, in their case, was also promoted by the uninterrupted supply of nutrients to the cells inside the porous structures evaluated. In addition to Hoque et al. [34], Cruz et al. [35] showed that PCL blended with Ch had improved water sorption capability, which is a desirable

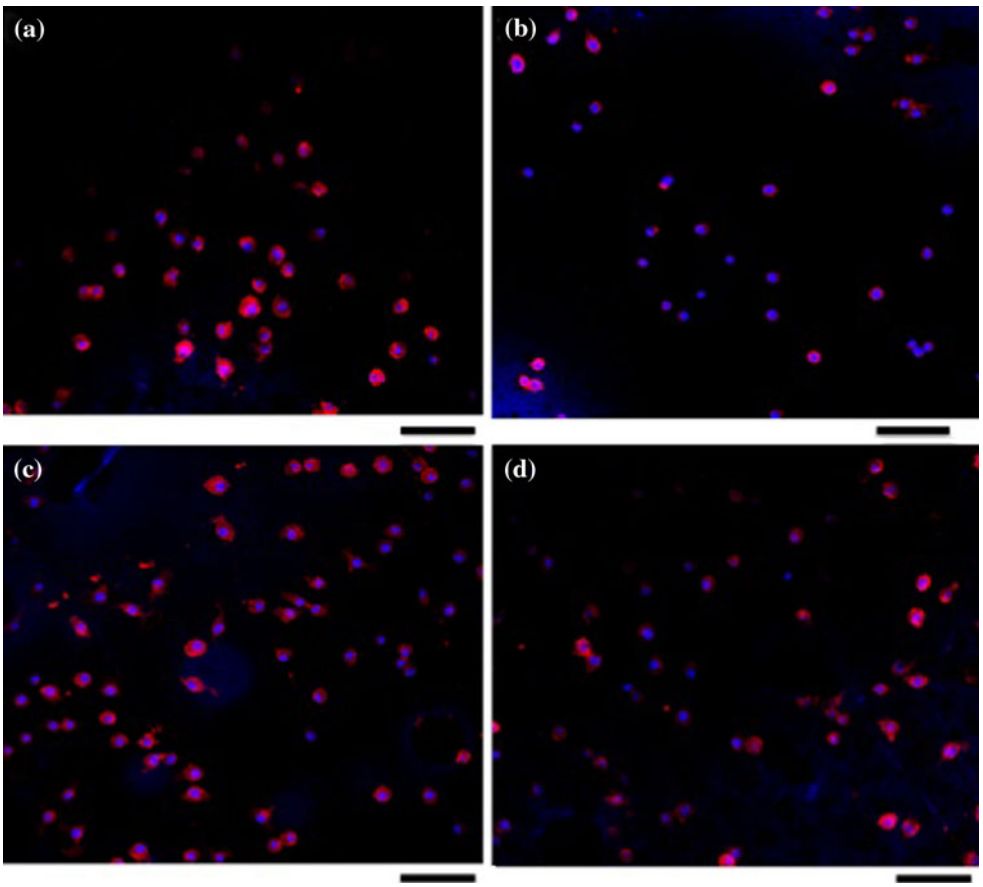

Fig. 5 Cell proliferation (L929) after 3 days on the surface of Ch/PCL membranes prepared in the absence (**a**, **b**) and presence (**c**, **d**) of 0.05% PF68 observed through confocal microscopy with DAPI and phalloidin staining. Cell nuclei are shown in *blue* and cytoplasm, in *red*. Membrane formulations: (**a**, **c**) Ch/PCL 80:20 (%w/w); (**b**, **d**) Ch/PCL 90:10 (%w /w). The *bar* dimension is 50 μm

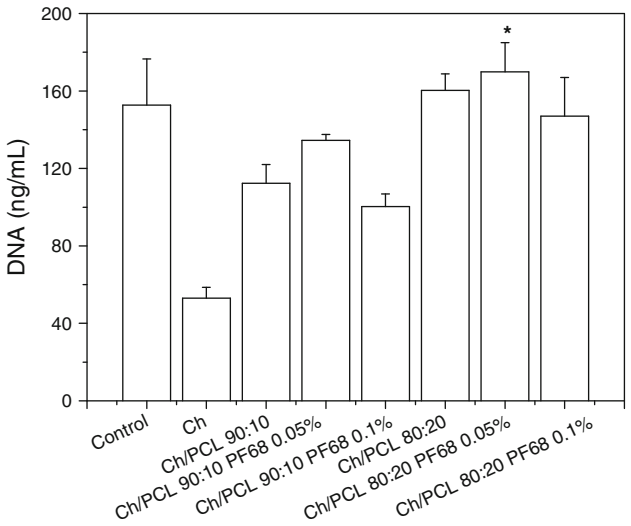

Fig. 6 Cell proliferation (L929) after 3 days on the surface of membranes prepared under different conditions, assessed through DNA extraction analysis. Groups were compared to an independent paired *t* test ($p < 0.05$)

property in many biomedical applications (the substrates must be able to supply the adequate transport of nutrients from the surroundings to the growing cells as well as have the ability to evacuate cellular wastes).

Therefore, it is possible to infer from the results obtained that as isolated Ch membranes were not fully satisfactory for fibroblasts culture, the inclusion of relatively large amounts of PCL in the membrane formulation possibly favor the surface topography and hydrophilic/hydrophobic balance of the Ch matrix, allowing greater cell adhesion and proliferation. Mixing PCL with Ch may give the additional advantage of a longer scaffold duration than with the use of Ch alone, since PCL has an in vivo reabsorption time of 3–4 years [35].

The presence of the surfactant during membrane preparation possibly caused a reduction in PCL crystallinity, making the mixture more uniform, which in turn promoted the proliferation of L929 fibroblasts. Despite the fact that all membranes were exhaustively washed before sterilization to remove the added surfactant, higher residual concentrations of it could have been retained in the structure of

the formulations prepared with 0.1% PF68, resulting in the mild cytotoxicity levels pointed out by the indirect analysis method. In the case of assessment through direct exposure of the cells to the membranes, the fibroblasts were probably able to adapt to the amounts of PF68 slowly released from the membranes unlike in their direct exposure to the membrane extracts.

Conclusions

When observed by scanning electron microscopy, the Ch/PCL membranes showed a fairly good distribution of PCL particles throughout the Ch matrix. When exposed to SBF, the membranes showed Ch matrix loss and advanced degradation of the PCL particles after 60 days. However, the degraded materials showed only minor changes in transition temperatures according to the thermal analysis (DSC). A reduction in melting enthalpy was observed, probably because the materials became more amorphous after incubation in SBF. The membranes prepared in the presence of PF68, in general, showed a greater ability to absorb FBS than those composed solely of Ch, but not to absorb PBS and SBF, which were taken up in similar amounts. Despite the improvement of the membrane degradation rate with addition of surfactant, the process was more intense for samples obtained employing only Ch. The DMA analysis showed that the Ch/PCL membranes with PF68 had lower storage modulus than samples without PF68. The blend membranes showed higher elongation and mechanical resistance than Ch membranes, but the addition of PF68 decreased the materials' tension resistance. Different Ch/PCL proportions did not result in a more significant decrease in L929 and Vero fibroblasts viability than the positive control (latex); however, a higher cell proliferation was detected in samples with 20% of PCL prepared in the presence of 0.05% PF68, indicating that these membranes may have potential use as wound dressings.

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References

1. Hench LL, Polak JM, Xynos ID, Buttery LDK (2000) Mater Res Innov 3(6):313
2. Dallan PRM, Moreira PD, Petinari L, Malmonge SM, Beppu MM, Genari SC, Moraes AM (2007) J Biomed Mater Res B 80B(2):394. doi:10.1002/jbm.b.30610
3. Marreco PR, da Luz Moreira P, Genari SC, Moraes AM (2004) J Biomed Mater Res B 71B(2):268. doi:10.1002/jbm.b.30081
4. Rodrigues AP, Sanchez EMS, da Costa AC, Moraes AM (2008) J Appl Polym Sci 109:2703
5. Malheiro VN, Caridade SG, Alves NM, Mano JF (2010) Acta Biomater 6(2):418. doi:10.1016/j.actbio.2009.07.012
6. Mao JS, Liu HF, Yin YJ, Yao KD (2003) Biomaterials 24(9):1621. doi:10.1016/s0142-9612(02)00549-5
7. Mi FL, Shyu SS, Wu YB, Lee ST, Shyong JY, Huang RN (2001) Biomaterials 22(2):165
8. Cruz DMG, Coutinho DF, Mano JF, Ribelles JLG, Sanchez MS (2009) Polymer 50(9):2058. doi:10.1016/j.polymer.2009.02.046
9. Malheiro VN, Caridade SG, Alves NM, Mano JF (2010) Acta Biomater 6(2):418. doi:10.1016/j.actbio.2009.07.012
10. Alves NM, Mano JF (2008) Int J Biol Macromol 43(5):401
11. Hejazi R, Amiji M (2003) J Control Release 89(2):151. doi:10.1016/s0168-3659(03)00126-3
12. Jayakumar R, Prabaharan M, Reis RL, Mano JF (2005) Carbohydr Polym 62(2):142
13. Alves NM, Leonor IB, Azevedo HS, Reis RL, Mano JF (2010) J Mater Chem 20(15):2911. doi:10.1039/b910960a
14. Rinaudo M (2008) Polym Int 57(3):397. doi:10.1002/pi.2378
15. Khor E, Lim LY (2003) Biomaterials 24(13):2339. doi:10.1016/s0142-9612(03)00026-7
16. Custodio CA, Alves CM, Reis RL, Mano JF (2010) J Tissue Eng Regen Med 4(4):316. doi:10.1002/term.248
17. Madihally SV, Matthew HWT (1999) Biomaterials 20(12):1133
18. Zhang H, Neau SH (2001) Biomaterials 22(12):1653
19. Li SM, Chen XH, Gross RA, McCarthy SP (2000) J Mater Sci Mater Med 11(4):227
20. Vert M, Li SM, Spenlehauer G, Guerin P (1992) J Mater Sci Mater Med 3(6):432
21. Sarasam A, Madihally SV (2005) Biomaterials 26(27):5500. doi:10.1016/j.biomaterials.2005.01.071
22. Flickinger MC, Drew SW (1999) Encyclopedia of bioprocess technology: fermentation, bio-catalysis and bioseparation, vol 5. Wiley, New York
23. Chisti Y (2001) Crit Rev Biotechnol 21(2):67
24. Kokubo T, Kushitani H, Sakka S, Kitsugi T, Yamamuro T (1990) J Biomed Mater Res 24(6):721
25. ASTM International (D882) (2002) Standard test methods for tensile properties of thin plastic sheeting, West Conshohocken, USA
26. ASTM International (F813 - 07) (1995) Standard practice for direct contact cell culture evaluation of materials for medical devices, West Conshohocken, USA
27. International Organization for Standardization (ISO) 10993–5 (2009) Biological evaluation of medical devices—Part 5: tests for in vitro cytotoxicity, Geneve, Swisszerland
28. Hansen MB, Nielsen SE, Berg K (1989) J Immunol Methods 119(2):203
29. Schweikl H, Schmalz G (1996) Eur J Oral Sci 104(4):412
30. Zhang XD, Yang DZ, Nie J (2008) Int J Biol Macromol 43(5):456. doi:10.1016/j.ijbiomac.2008.08.010
31. Kweon H, Yoo MK, Park IK, Kim TH, Lee HC, Lee HS, Oh JS, Akaike T, Cho CS (2003) Biomaterials 24(5):801
32. Mano JF (2008) Macromol Biosci 8(1):69. doi:10.1002/mabi.200700139
33. Caridade SG, da Silva RMP, Reis RL, Mano JF (2009) Carbohydr Polym 75(4):651. doi:10.1016/j.carbpol.2008.09.011
34. Hoque ME, San WY, Wei F, Li SM, Huang MH, Vert M, Hutmacher DW (2009) Tissue Eng A 15(10):3013. doi:10.1089/ten.tea.2008.0355
35. Cruz DMG, Coutinho DF, Martinez EC, Mano JF, Ribelles JLG, Sanchez MS (2008) J Biomed Mater Res B 87B(2):544. doi:10.1002/jbm.b.31142