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Poly(DL-lactic acid)/triblock PCL–PDMS–PCL copolymers: synthesis, characterization and demonstration of their cell growth effects in vitro

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Abstract

This paper describes the copolymerization of DL-lactic acid (DLLA) and triblock polycaprolactone–poly(dimethylsiloxane)–polycaprolactone (TEGOMER). The physical properties of poly(DL-lactic acid)/triblock PCL–PDMS–PCL (PDLLA/TEGOMER) copolymer were determined by IR, ^{1}H NMR and DSC. T_g values of copolymer decreased with increasing TEGOMER unit content. Degradation of PDLLA/TEGOMER copolymer films was investigated in phosphate buffered saline at pH = 7.4 and at 37°C. The copolymers degraded to half of their original molecular weight values in 40 days. The morphology of the films during degradation was examined by scanning electron microscopy (SEM). Cell growth experiments using Swiss 3T3 fibroblasts demonstrated that PDLLA/TEGOMER copolymer matrices allow the attachment and growth of cells.

Keywords: Degradation; Poly(DL-lactic acid); Copolymer; Cell growth

1. Introduction

In recent years, biodegradable polymers have been the focus of intensive research, due to their prospective utilization in biomedical and pharmaceutical applications [1–5]. Among them, much work and therefore progress has been made in the characterization and use of synthetic aliphatic polyesters such as polylactic acids [6–10]. Polylactic acid has high biocompatibility, good degradability, high tensile strength and is nontoxic. In addition, it has been approved by the US Food and Drug Administration for several applications in the human body. Current research has been focused toward modifying existing biodegradable polymers by copolymerizing with other nontoxic absorbable monomers [11–14]. Extensive efforts are being made to prepare copolymers from lactic acid and ϵ-caprolactone with unique structures which have good properties suitable for biomedical applications mainly in tissue engineering as a polymeric scaffold, degradable sutures and drug release systems [15–17]. Through modification of the polymerization process, it is possible to prepare polyesters of defined molecular weight, narrow molecular weight distributions, functional groups containing random, block, graft copolymers. The modification of polymers through the addition of small amounts of siloxane polymers have also received increasing attention in the last decade [18]. Copolymerization of poly(DL-lactide) with an elastomer may lead to a large variety of materials ranging from reinforced thermoplastics to thermoplastic elastomers. For example, rubbery materials may be useful for the design of soft implants and microcapsules used in oral drug delivery. The present work combines the copolymerization of DL-lactic acid (DLLA) with triblock polycaprolactone–poly(dimethylsiloxane)–polycaprolactone (TEGOMER) and the subsequent characterization of the resulting copolymer.

2. Experimental

2.1. Materials

Triblock PCL–PDMS–PCL copolymer was supplied by Th. GoldSchmidt A.G. of Germany, under the name TEGOMER (TEGOMER H–Si 6440). The molecular weight (M_n) of TEGOMER is 6500 ± 600 g mol^{-1} with PCL end blocks, $M_n \cong 2000$ g mol^{-1}. TEGOMER has the following molecular structure:

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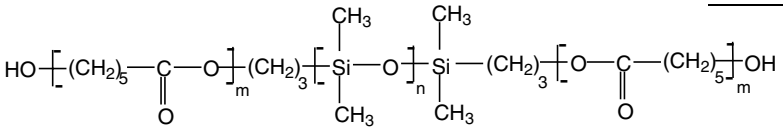
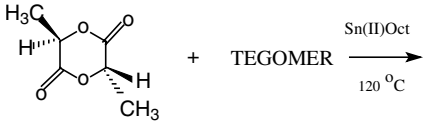


n / m = 30 /18

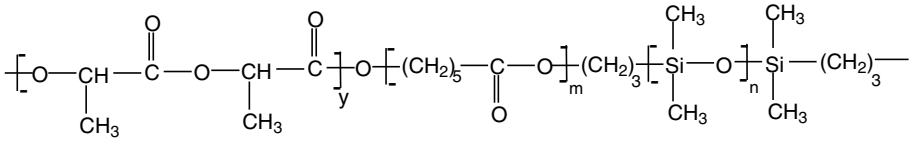
DL-Lactide (DLLA) was obtained from Polysciences, Inc. It was recrystallized from dry benzene and dried under vacuum at room temperature before use. Stannous octoate (Sigma Corp.) was used as received.

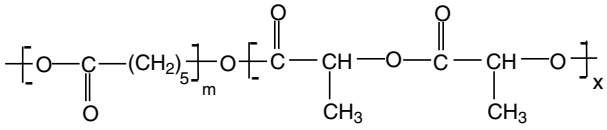
2.2. Synthesis of poly(DL-lactic acid)/triblock PCL–PDMS–PCL copolymers

Solvent free polymerization were carried out in sealed tubes. A representative polymerization is described below (Scheme 1 (synthesis of PDLLA/TEGOMER copolymers)). A solution of catalyst, stannous octoate, in dry chloroform (mole ratio, monomer to catalyst, M/I = 1000) and 4.13 g of (DLLA + TEGOMER) mixture were added to the tube. The feed ratios, TEGOMER/DLLA ranged from 0 to 0.43. The solvent was removed in vacuo, and the tube was sealed and immersed in a silicone oil bath at 120°C. At the end of the polymerization (20 h), the tube was cooled. Purification was performed by dissolving the reaction mixture in a small amount of chloroform and dropping into an excess of methanol. The characterization results of PDLLA/TEGOMER copolymers are summarized in Table 1.



DLLA





PDLLA / TEGOMER copolymers

Scheme 1.

Table 1
The characterization of the PDLLA/TEGOMER copolymers

Sample no.	DLLA/TEGOMER feed composition (%)[a]	Copolymer composition (%)[b]	Yield (%)	$M_n \times 10^{-4}$ (g mol^{-1})[c]	M_w/M_n[c]
1	100/0	100/0	83	11.0	2.13
2	98/2	–	62	3.54	1.82
3	95/5	94/4	72	3.22	1.61
4	87/13	90/10	60	3.52	1.77
5	80/20	70/30	61	2.69	1.59
6	70/30	63/37	63	2.69	1.45

[a] Molar ratio of monomer to catalyst (M/I) = 1000. Polymerization were carried out in the bulk at 120°C for 20 h.
[b] Using ^{1}H NMR.
[c] Using GPC.

2.3. Characterization

2.3.1. Polymer characterization

The molecular weights of polymers were determined by gel permeation chromatography (GPC) using Waters styragel column HT6F and Waters 410 differential refractometer detector. THF was used as the eluting solvent at a flow rate of 1 ml/min and monodisperse polystyrene standards were used to calibrate the molecular weights.

^{1}H NMR spectra of the copolymers were obtained on a Bruker AC 200 L spectrometer at 200 MHz. The spectrum was taken in deuterated chloroform at 20°C. The composition of copolymers was calculated from the ratios of absorbance at 0.07 and 5.15 ppm.

The infrared (IR) spectra were recorded on Perkin–Elmer 983 IR spectrometer. Polymer solutions (0.01 ml) with a concentration of 5% (w/v) were poured on to NaCl cell and chloroform was evaporated.

Differential scanning calorimetry (DSC) analyses of the polymers were obtained using a DuPont DSC 910 Model device. Samples were run under a nitrogen atmosphere from −140°C to +140°C with a heating rate of 10°C/min. Transition temperature were taken from the second heating run.

Scanning electron microscopy (SEM) was performed using a JEOL-JXA 840 A SEM. The specimens were prepared for SEM by freeze-fracturing in liquid nitrogen and applying a gold coating of approximately 300 Å on an Edwards S 150 B sputter coater.

2.3.2. Degradation

The in vitro degradation studies were performed at 37°C in phosphate buffer saline (pH = 7.4, PBS). Polymer films were prepared by solvent casting from chloroform with a total polymer concentration of 5% (w/v). Solutions were poured into Teflon moulds (diameter = 22 mm, height = 10 mm). Following solvent evaporation, polymer films were dried to a constant weight in a vacuum oven at room temperature. Dry films were placed in vials containing 15 ml PBS. The vials were incubated at 37°C for various periods of time. The buffer solution was replaced every 60 h. After incubation, the film was washed extensively with water and dried at 30°C in vacuum until a constant weight was reached. The degree of degradation was estimated from the mass loss and molecular weight loss by GPC.

2.3.3. Sponge fabrication and characterization

Porous sponges were formed from PDLLA/TEGOMER copolymer (87/13) using a particulate leaching technique [19]. The polymer was dissolved in chloroform to yield a solution with a concentration of 3.5% (w/v). The ratio of polymer/salt was 0.08 for this study. One ml of polymer solution was loaded into Teflon molds described above, packed with 0.44 g sodium chloride particles sieved to a size between 200 and 400 μm. The solvent was subsequently allowed to evaporate and the entrapped salt particles were removed by immersing the films in distilled water for 48 h. Sponges (1 mm in thickness) were lyophilized to remove residual water. The porosity of the devices was determined using mercury porosimetry (Autopore II 9220, Micromeritics).

2.3.4. Cell culture

Swiss 3T3 fibroblasts were grown in RPMI 1640 medium containing 10% fetal calf serum, L-glutamine, 100 IU/ml penicillin and 0.1 mg/ml streptomycin (Biological Industries, Israel). Cells were passaged at a ratio of 1:3 upon reaching confluency. Experiments were performed with cells from passages 8–10.

2.3.5. Cell growth experiments

These were performed in six-well plates (Techno Plastic Products, Switzerland). For these experiments, the biopolymers that were molded into 22 mm diameter, 1 mm thick sponges formed and lyophilized as described above, were placed into 100% ethanol for 30 min for sterilization. Ethanol was subsequently removed from the matrix by rinsing in sterile PBS for 2 h. In parallel experiments, round glass coverslips of 22 mm diameter (sterilized with a flame) were utilized after being coated with fibronectin (1 mg/ml) or gelatin (1% in H_2O) solution or were used uncoated.

For cell seeding, the cells growing on cell culture plates were suspended using trypsin–EDTA solution (Biological Industries, Israel) and counted using a hemocytometer. A 500 μl of cell suspension containing 50,000 cells was placed onto the biopolymers or glass discs prepared as described above. These were placed into six-well plates and allowed to attach to the materials for 4 h. The effects of cell culture treated plastic on the growth of cells was observed by placing the 500 μl cell suspension directly onto the six-well plates. At the end of this incubation, 2 ml of culture medium was added to each well and cells were grown in a CO_2 incubator for another 24 h.

The amount of cells present on the surfaces of the materials were quantified using a neutral red uptake assay [20]. Briefly, the cells were washed once with sterile PBS and incubated with PBS containing 2% FCS and 0.001% neutral red for 90 min. Following this incubation, a brief wash with 4% formaldehyde in PBS, enabled the cells to be fixed. The dye which was taken up into the cells was solubilized in 2 ml of 50% EtOH and 1% acetic acid in H_2O. The level of the dye was measured spectrophotometrically at 540 nm.

2.3.6. Tissue characterization

For SEM examination, samples that were seeded with 3T3 fibroblasts were fixed by immersion in 4% formaldehyde in PBS for 1 h and subsequently dehydrated by placing them in 50, 70, 90 and 100% ethanol/water solutions. The dehydrated samples were placed in liquid nitrogen until the SEM examination, which was performed on the same day. Before the SEM measurements, matrices were attached to

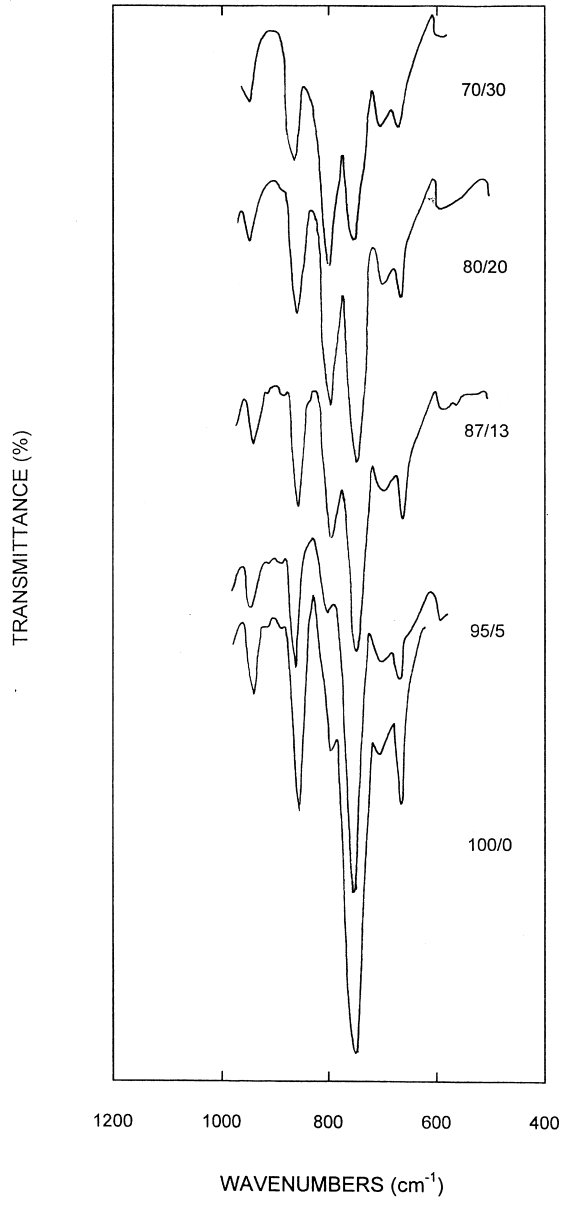


Fig. 1. The IR spectrum of the PDLLA/TEGOMER copolymer series.

mounts with carbon adhesive tabs and sputter coated with gold.

3. Results and discussion

In this work, copolymers of DL-lactic acid with triblock PCL–PDMS–PCL were synthesized. Table 1 shows the feed and copolymer compositions and the results of molecular weight measurements. In total, six samples were prepared and characterized. In Table 1, sample 1 represents a homopolymer of DL-lactide. The yield is 83%. It has a rather high molecular weight, $M_n = 1.1 \times 10^5$ g mol^{-1}. The copolymer compositions were calculated from the ratios of integral intensities of the methine proton of the lactic acid repeat units at 5.15 ppm to the methyl protons of polysiloxane repeat units at 0.07 ppm in the ^{1}H NMR spectra. It can be seen in Table 1 that, both the yield and the molecular weight decreases with increasing TEGOMER content in the copolymers. The increase in TEGOMER content also decreases the polydispersity of copolymers.

3.1. IR analysis

IR spectroscopy was also used to investigate the composition of the copolymers. Samples were analyzed as films on NaCl cells. Fig. 1 displays a portion of IR spectra of the poly(DL-lactic acid) and its various copolymer series with TEGOMER. The peaks of interest are the Si–CH_3 deformation peak at 800 cm^{-1} that is the characteristic peak of the PDMS component of copolymer [21] and the C–H bending peak at 750 cm^{-1}. In the IR spectrum of the copolymer with 17% TEGOMER content, the Si–CH_3 deformation peak appeared. As the amount of TEGOMER content was increased, the intensity of this peak increased. Its partner at 750 cm^{-1} is apparent but reduced in intensity, indicating the decrease of the ratio of poly(DL-lactic acid) in the copolymer. All five IR spectra have characteristic ester absorption bands at 1760 cm^{-1} [22] and asymmetrical and symmetrical methyl bending vibration bands at 1450 and 1375 cm^{-1} [23], respectively.

3.2. Thermal analysis

Fig. 2 shows the effects of copolymerization on the thermal properties, as characterized by DSC. The glass transition temperature (T_g) is strongly affected by copolymerization. The T_g of pure PDLLA was observed at 47°C. Although two glass transition temperatures for a block copolymer would be expected, these copolymers exhibited single T_g, which change with copolymer composition. Copolymerizing with TEGOMER results in a decrease in T_g values. This observation demonstrates clearly that PDLLA/TEGOMER is a random copolymer [14]. DSC analysis of pure TEGOMER indicated the formation of one-phase morphology with only PCL melting point around 55.8°C, as shown in Fig. 2. The T_g of the siloxane component could not be observed, probably due to the small block length of PDMS in TEGOMER. The crystalline melting peak of TEGOMER disappeared entirely after copolymerization, producing a complete amorphous polymer.

3.3. Degradation

Degradations of PDLLA/TEGOMER copolymer films (87/13) were investigated in phosphate buffer saline at pH = 7.4 and 37°C. The molecular weight and mass losses for the sample are shown in Fig. 3. These results indicate that polymer degradation occurs predominantly via random chain scission by simple hydrolysis of the ester bond linkage and the monomer diffuses out of the polymer bulk into water [7]. The appearance of the PDLLA/TEGOMER copolymer sample

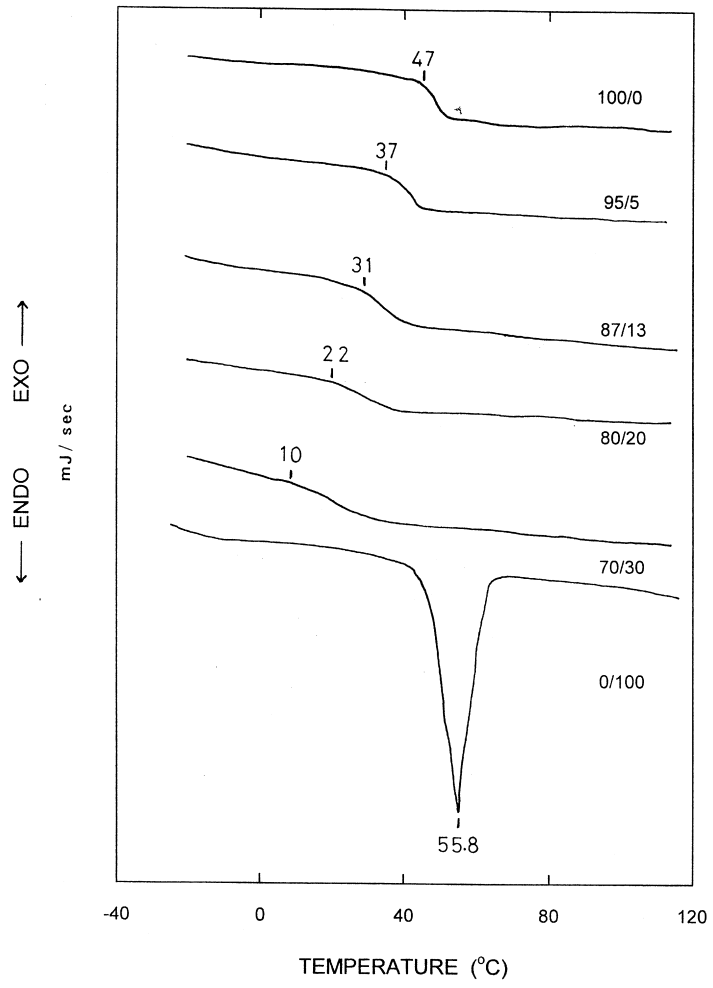


Fig. 2. DSC thermograms for PDLLA, TEGOMER and PDLLA/TEGOMER copolymers at different compositions.

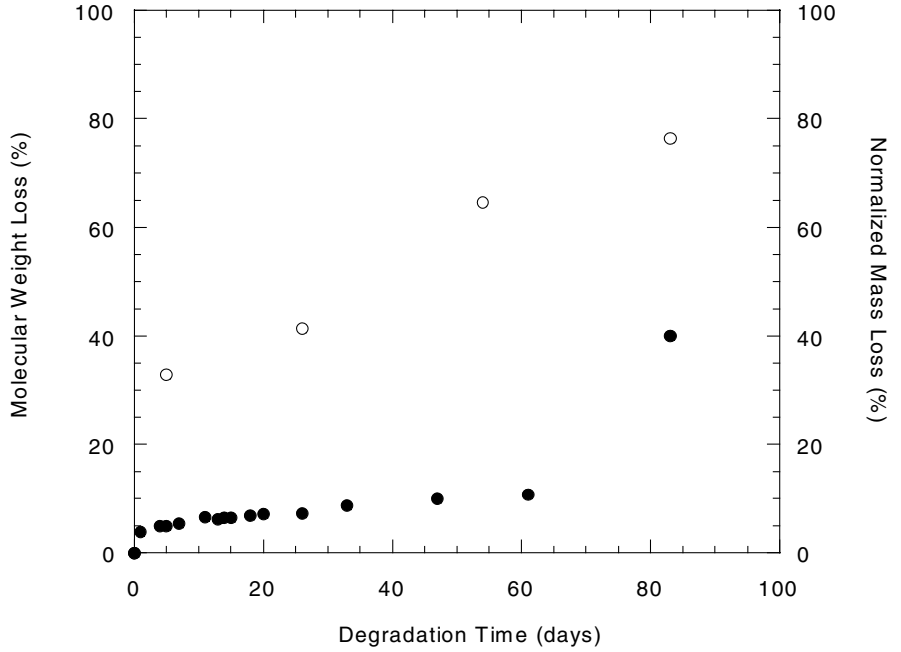


Fig. 3. Normalized mass and molecular weight loss: (●) mass loss; (○) M_w loss.

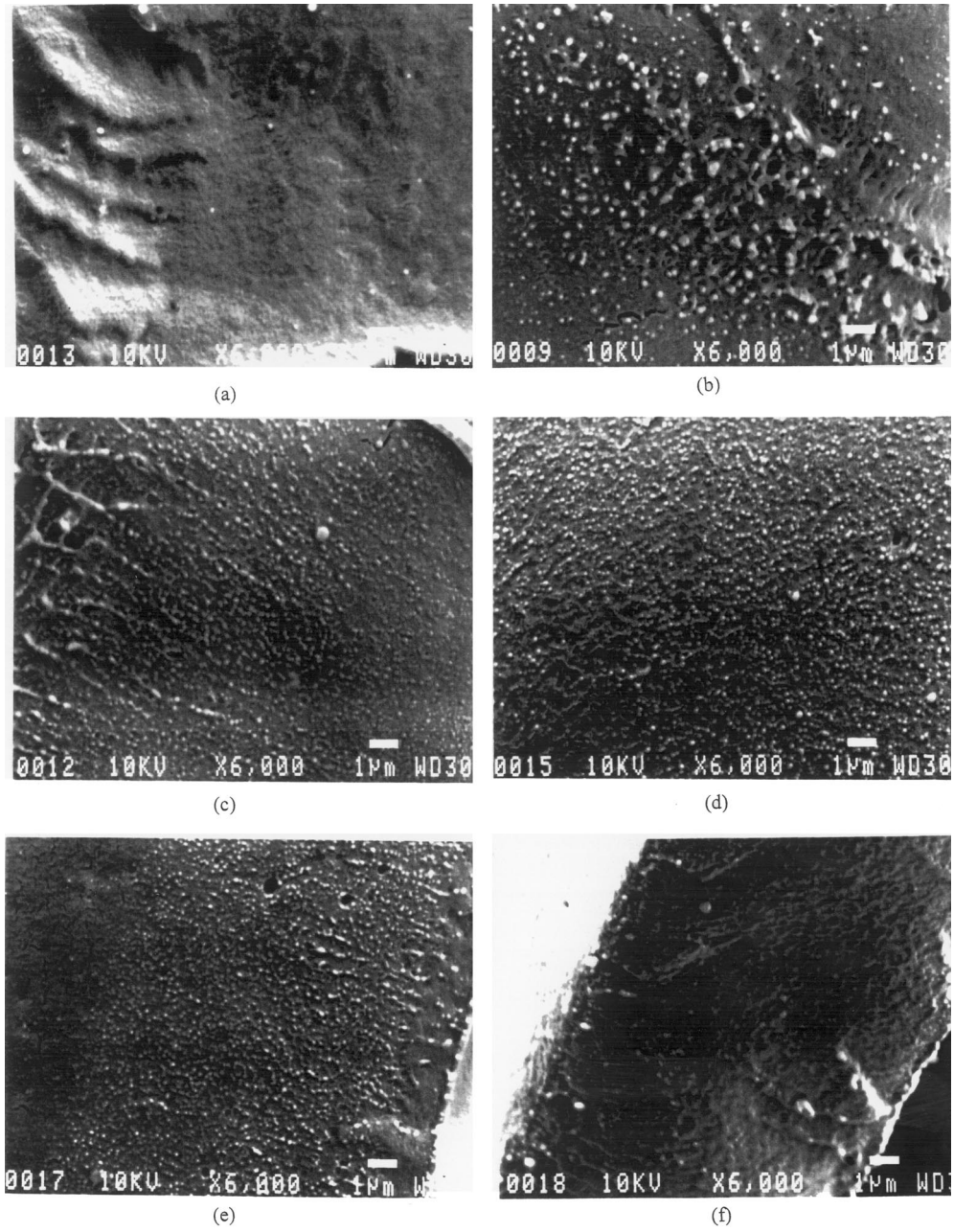
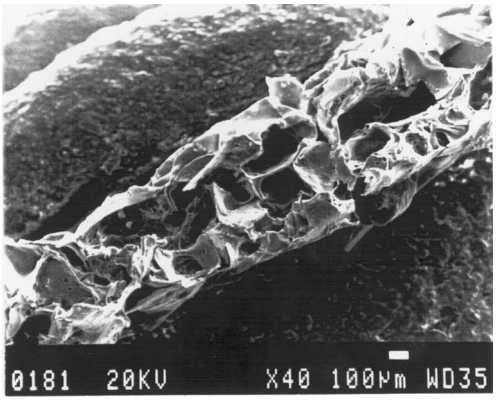


Fig. 4. SEM micrographs of PDLLA/TEGOMER (87/13) copolymers: (a) after synthesis; (b) 1 day; (c) 6 days; (d) 11 days; (e) 20 days; and (f) 40 days after degradation.

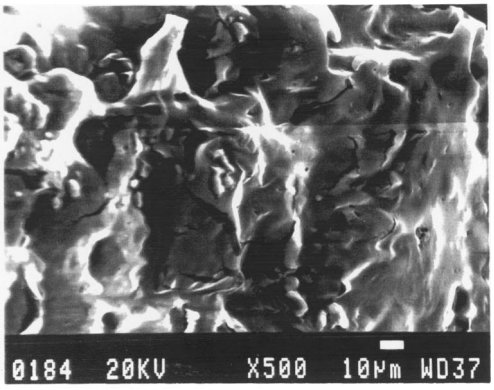
changed from a slightly translucent material to a white brittle material over the course of 80 days. At this time, the sample was fragmented very easily so needed to be handled very carefully. Mass loss was not simultaneous with loss of molecular weight indicating bulk erosion of the polymer sample. Once the average molecular weight of the sample decreases to a certain level, chains become soluble and mass loss is observed [1,7,9]. The mass loss of the PDLLA/TEGOMER copolymer sample remained steady through 60 days. After 60 days the molecular weight of the sample reduced below the critical value where the polymer would be soluble in the PBS and mass loss was observed.

3.4. Morphology studies

Fig. 4 summarizes the fractured surface morphology of PDLLA/TEGOMER copolymer (87/13) during degradation. As seen in Fig. 4a, the polymer film displayed a very dense structure prior to degradation. After 1 day of degradation, a well-defined erosion zone was observed (Fig. 4b). The erosion zone revealed longitudinal cracks, voids and very granular structure. As time progressed, the spherical structure appeared throughout the entire film. It was very organized and homogeneously distributed through the surface (Fig. 4c and d). These spheres had a very small



(a)



(b)

Fig. 5. (a) SEM micrographs of PDLLA/TEGOMER (87/13) copolymer matrix after 24 h of cell seeding. (b) A closer view of cells on PDLLA/TEGOMER (87/13) matrix. The original magnifications and size bars are shown in the photomicrographs.

size, less than 1 μm in diameter. We believe that these spheres could be the structure of small oligomers. Twenty days after the start of the degradation experiment it was possible to see the eroding zone; however, it was characterized by cracks rather than a fully spherical structure (Fig. 4e). The morphology of the film was more fragile. The size of the spheres became smaller. Finally, after 40 days of degradation, surface porosity was seen, but these pores were considerably small in size. After 40 days of degradation the polymeric films were broken into small pieces and hence were not able to continue the SEM measurements.

3.5. Cell interactions

SEM examination of PDLLA/TEGOMER copolymer samples 24 h after cell seeding is shown in Fig. 5a and b. A highly porous structure can be seen in the cross-section of PDLLA/TEGOMER copolymer devices. The porosity of the sponges was determined using mercury porosimetry and mean porosity was found as 80 ± 1%. Fig. 5b indicates that some cells adhered to these matrices.

The neutral red uptake assay enables a quantitative comparison of cell numbers between different cell seeding or proliferation conditions. In the assay, the absorbance measured at 540 nm is directly proportional to the viable cell number. As seen in Fig. 6, the highest cell numbers were observed when the cells were incubated for 24 h on tissue culture treated plastic, fibronectin or glass. The cell number on the biopolymer is lower and comparable to gelatin coated glass plates, indicating that it supports cell attachment. The 540 nm absorbance at the end of 24 h compared to the beginning of the incubation was shown to be increased by

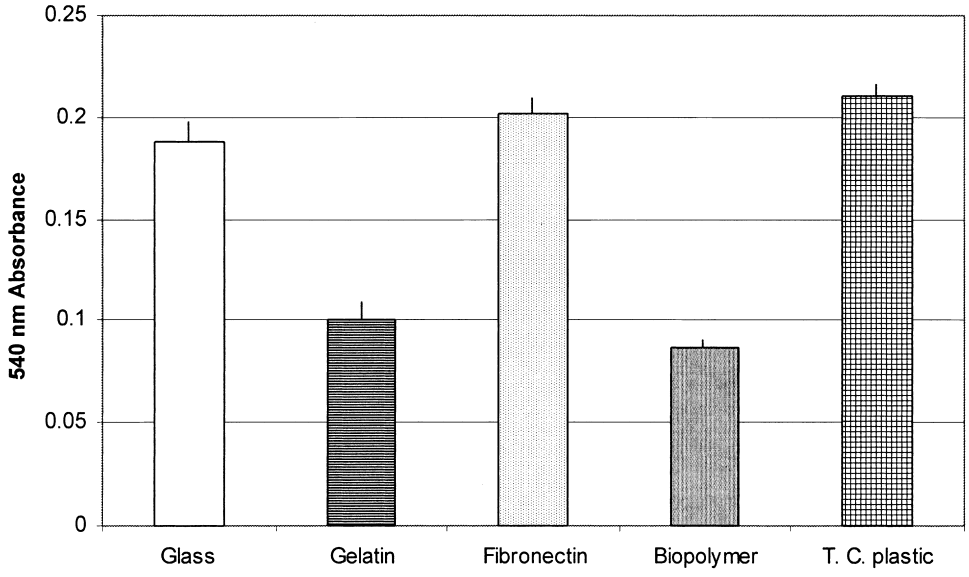


Fig. 6. Cell growth experiments performed by the neutral red uptake assay. All materials were seeded with 50.000 Swiss 3T3 fibroblasts. Changes in cell numbers 24 h after seeding are quantified by measuring the cellular uptake of the dye at 540 nm. (Biopolymer: PDLLA/TEGOMER Copolymer; T.C: plastic: tissue cultured treated plastic.)

12% (data not shown), indicating that the biopolymers also support cell growth.

It can be concluded that this biopolymer supports the attachment and growth of Swiss 3T3 fibroblasts, although less efficiently than some materials used in cell culture.

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