New Semi-Biodegradable Materials from Semi-Interpenetrated Networks of Poly(ε-caprolactone) and Poly(ethyl acrylate)

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Semi-degradable materials may have many applications. Here poly(ethyl acrylate) and poly(ε-caprolactone) were combined as semi-interpenetrated networks, and thoroughly characterized in terms of final composition, interactions between components, wettability, and mechanical properties. PCL modulates the mechanical properties of the PEA elastomeric network. Cultures of fibroblasts and adipose-tissue derived stem cells showed excellent biological performance of the materials. The results are relevant for applications seeking materials leaving a permanent supporting skeleton after the partial degradation, as in patches for cardiac regeneration or in abdominal wall meshes.

1. Introduction

Cardiovascular diseases are one of the main causes of death in the developed world. One of them is myocardial infarction, which results from a coronary artery occlusion and decreases the supply of nutrients and oxygen to the portion of heart muscle irrigated by such artery. It often leads to an irreversible cell death within the affected part of the ventricle and the subsequent formation of non-functional scar tissue.[2,3] In spite of the progresses in pharmacological, interventional, and operative therapies, the prognosis for heart diseases is pessimistic and these therapies do not achieve sufficiently satisfying results.[3] Therefore, therapies on the horizon focus on cardiac tissue regeneration to counter the inevitable decline of heart function.[4] The direct transplantation of cells into the dysfunctional myocardial area has proved to be inefficient due to substantial cell loss.[2,3] Combination of cells with polymeric scaffolds ex vivo, generating an engineered tissue, which is next implanted, may improve over the results of the pure cell grafting. Here, the role of the scaffold may be manyfold: to improve survival of the transplanted cells, to promote the organized three-dimensional growth of tissue, to mechanically support heart function, to deliver active angiogenic factors, etc.[3,5]

Among the most currently used materials for cardiac tissue engineering studies are poly(glycolic acid) (PGA),
poly(lactic acid) (PLA), and their copolymers. The polymers of these polyester families are biodegradable, and have been successfully used in humans in many different applications. However, they lack the needed elastic properties for the applications in the heart, and their Young moduli do not match that of cardiac tissue.\[5,6\] Therefore, alternative polymers or their combinations are being continuously developed and proposed.

Degradable materials are intended as temporary implants, which have the advantage of obviating subsequent surgery to remove them once the tissue has regenerated; their degradation rate must match that of tissue regeneration, and they may further serve as drug delivery systems.\[7,8\] This represents an ideal for regeneration. Actually, the quality of the regenerated tissue may be insufficient, and further support is needed after the complete resorption of the implant. Non-degradable materials are then of interest, supplying a long-term support to the mechanical properties of the tissue.\[9\] To benefit from the advantages of both kinds of materials, some developments combine them as semi-degradable implants.\[9–11\] These materials, it is hoped, would, for some developments combine them as semi-degradable benefit from the advantages of both kinds of materials, while the other component is included in the interstices of one of them consists in a crosslinked polymer network, a semi-IPN is a two-component polymer mixture in which the stiffness and mechanical properties of the final material can be modulated with the mass fractions of both components.

2. Experimental Section

2.1. Preparation of PCL, PEA, and PCL-i-PEA films

Poly(\(\varepsilon\)-caprolactone) (Polysciences, \(M_w = 43\,000–50\,000\) Da) polymer, ethyl acrylate monomer (EA; Sigma–Aldrich, 99% purity), and 1,4-dioxane (Scharlab, 99.8% purity) as a common solvent were mixed in different proportions at room temperature (Table 1). After a 24 h stirring, PCL pellets had completely dissolved, and ethylene glycol dimethacrylate (EGDMA; Sigma–Aldrich, 98% purity) was incorporated at a concentration of 2 wt% with respect to EA always, as a crosslinker for PEA. Benzoyl peroxide (BPO; Fluka) and \(N,N\)-dimethyl-p-toluidine (DMT; Sigma–Aldrich, 99% purity) were employed as redox initiation system, added at a concentration of 1 and 0.33 wt% with respect to EA, respectively. The solutions were stirred, injected between 1 mm-spaced glass plates and allowed to polymerize at room temperature for 2 h. Low molecular weight substances were eliminated by washing the films with ethanol at 37 °C. Materials were dried at room temperature for 24 h followed by vacuum for 8 h.

Separately, pure PCL films were prepared as reference materials (Table 1) by melting the polymer at 80 °C for 5 h between glass plates and cooling at 40 °C for 1 h, and subsequently at room temperature until equilibration. Pure PEA polymer films were obtained by the same redox polymerization process, with the same amounts of dioxane, initiator, and crosslinker as above.

The nomenclature employed to label the composite and pure materials (Table 1) makes reference to the mass proportion of both components had all EA units reacted, all PCL chains remained in the blend, and dioxane completely evaporated. Thus, percentages of 0–26 wt% of PCL were nominally present in the blends.

2.2. PCL Extraction

Samples were weighed before the PCL extraction (\(m_0\)). After that, the PCL component of the semi-IPNs was extracted in a soxhlet for 16 h with acetone, which swells the PEA network and dissolves PCL. Samples were weighed before the PCL extraction (\(m_0\)). After that,
samples were transferred to ethanol at 50 °C and allowed to cool. Afterwards, samples were dried at room conditions for 24 h, followed by drying under vacuum at 60 °C for 8 h. Rinsed samples were weighed \(m_0\) and the mass loss was characterized through

\[
\text{Mass loss (\%)} = \frac{m_a - m_i}{m_0} \times 100
\]

All experiments were done in triplicate.

### 2.3. Density Measurements

A Mettler Toledo AX205 balance (Mettler-Toledo, Inc., Columbus, OH, USA; sensitivity of 0.01 mg) with a density kit was employed. Density of the samples \(\rho\) was calculated by Archimedes’ principle, weighing each replica in air and immersed in n-octane (Sigma–Aldrich, ≥98% purity), as:

\[
\rho = \frac{m_a - m_i}{m_2 - m_i} \times \rho_0
\]

where \(\rho_0\) is the density of n-octane \((0.703 \text{ g cm}^{-3})\), \(m_a\) is the sample weight in air, and \(m_i\) is the weight of the sample immersed in n-octane. The results given are the averages of three replicates.

### 2.4. Thermogravimetric Analysis (TGA)

The thermal decomposition of the materials was analyzed in an SDT-Q600 (TA-Instruments, Inc., New Castle, DE, USA) equipment. Samples of 5–10 mg were tested at a heating rate of 10 °C min\(^{-1}\) between 30 and 700 °C in a nitrogen atmosphere. The mass of the sample, \(m\), was recorded as a function of temperature to obtain the decomposition thermograms. The plots corresponding to these results are available, but are not included in the paper because of space reasons.

### 2.5. Fourier Transform Infrared Spectroscopy (FTIR)

The surface composition of the samples was determined in a Thermo Nicolet Nexus Infrared Spectrophotometer (Thermo Fischer Scientific, Inc., Waltham, MA, USA) in the attenuated total reflection mode (ATR). Spectra were obtained as the average of 200 scans. The rest of semi-IPNs started at a higher temperature \((\sim 80^\circ C)\) and subsequently measuring scans were carried out from \(-80^\circ C\) to \(-60^\circ C\). Since no fall in \(E\) occurred between \(-80^\circ C\) and \(-20^\circ C\) in the 26/74 sample, which could be attributed to the main relaxation of PCL, the measurements of the rest of semi-IPNs started at a higher temperature \((-60^\circ C)\). The scans were stopped at \(-50^\circ C\) to avoid the melting of PCL in the device.

### 2.6. Wettability

The surface wettability of the materials was analyzed in a Dataphysics OCA device (DataPhysics Instruments GmbH, Filderstadt, Germany). Contact angles were measured with 3 μL extra pure water (Sigma–Aldrich) droplets deposited on the surface. Ten water droplets per composition were assayed.

### 2.7. Differential Scanning Calorimetry (DSC)

Calorimetry measurements were performed in a Mettler Toledo 823e DSC (Mettler-Toledo, Inc.) with samples weighing approximately 10 mg. The samples were first cooled from room temperature to \(-80^\circ C\), and then they were subjected to three scans at 10 °C min\(^{-1}\) under a 60 ml min\(^{-1}\) nitrogen flow as follows: a first heating scan from \(-80^\circ C\) to \(100^\circ C\), a cooling scan from \(100^\circ C\) to \(-80^\circ C\) and a second heating scan from \(-80^\circ C\) to \(100^\circ C\), during which data were recorded. In order to analyze the thermal events during the scan, the thermograms are expressed in terms of the melt power and the product of the temperature rate times the sample mass.

### 2.8. Dynamic Mechanical Spectroscopy (DMS)

Dynamic mechanical spectroscopy (DMS) measurements were carried out in a DMS210 analyzer (Seiko Instruments, Inc., Chiba, Japan). Samples of dimensions \(20 \times 5 \times 0.5–1.2 \text{ mm}^3\) were cooled from room temperature to \(-80^\circ C\) and subsequently measuring scans were carried out from \(-80^\circ C\) to \(70^\circ C\) at a heating rate of \(2^\circ C \text{ min}^{-1}\) at a frequency of 1 Hz. Since no fall in \(E\) occurred between \(-80^\circ C\) and \(-20^\circ C\) in the 26/74 sample, which could have been attributed to the main relaxation of PCL, the measurements of the rest of semi-IPNs started at a higher temperature \((-60^\circ C)\). The scans were stopped at \(-50^\circ C\) to avoid the melting of PCL in the device.

Storage moduli at \(37^\circ C\) were compared with predictions from Takayanagi’s mechanical block model for bi-phasic materials in order to assess phase continuity in the materials.[28,29] This model considers a disperse phase, of volume fraction \(\omega_C\), in a continuous matrix of volume fraction \(1 - \omega_C\), and uses the solubility of the pure components to determine the predicted modulus of their bi-phasic mixtures as a function of \(\psi\).

### 2.9. Tensile Stress–Strain Tests

Samples with dimensions of \(40 \times 5 \times 0.5–1.2 \text{ mm}^3\) were stretched in the tensile mode in a SCM3000 95 (Microtest SA, Madrid, Spain) mechanical testing instrument until fracture. The PCL samples were assayed in a Dy34 device (Adamel LHomargy Division D’instruments SA, Paris, France) because the force needed to stretch these samples was higher than 15 N, the maximum value allowed for the Microtest SCM3000 95. The deformation rate was 1 mm min\(^{-1}\) in all cases. The results of three samples per composition were averaged.

From the curves obtained, the Young or elastic modulus, \(E\), of the materials was calculated as the slope of the initial linear part of a stress-strain plot, until strains of 0.05.
2.10. Accelerated Degradation Test

An accelerated degradation test was carried out with the samples immersed in a 5 m NaOH (extra pure, Scharlab) aqueous solution at 37 °C. The pH of the solution was 12.83. The mass loss was monitored at different times up to 52 d. Samples were assayed in triplicate.

2.11. MITT Cytotoxicity Assay

Samples of 7 mm-diameter were cut for in vitro assays and sterilized with a 25 kGy dose of gamma irradiation in a 60Co source (Aragogamma, Barcelona, Spain) before use. Cytotoxicity experiments were performed by indirect contact with commercial 1929 mouse fibroblasts from subcutaneous connective tissue, areolar, and adipose (Sigma–Aldrich) in their 13th passage. In order to obtain the extracts, samples were immersed overnight in a Dulbecco’s modified Eagle’s medium (DMEM; high glucose (4.5 g L⁻¹) Invitrogen), supplemented with 10% fetal bovine serum (FBS; Fisher) and 1% penicillin-streptomycin (P/S; Fisher), 0.1 g of sample per ml of medium. Complete medium was used as positive control and extract of latex was employed as negative control. In parallel, 1929 fibroblasts were seeded in number of 104 cells on the bottom of each well in a 96-well plate with the medium described above and incubated in a humid atmosphere with 5% CO₂ at 37 °C for 24 h. Next, this culture medium was replaced by the extracts of the different samples, or of latex in the negative controls. The medium was changed by fresh medium in the positive controls.

The MITT assay was performed with an MITT-based toxicity assay kit (Sigma–Aldrich, M5655) after 24, 48, and 72 h. At each time, the medium in the wells was removed and the cells were incubated in 100 µl of a 90% DMEM and 10% MITT (1 mg ml⁻¹) mixture for 2.5 h in the dark in 5% CO₂ at 37 °C. Next, the MITT solution was replaced by 120 µl of isopropanol and plates were shaken for 1 min. One hundred microliters of each well were transferred to a new 96-well plate to read the absorbance at 550 nm in a Victor Multilabel Counter 1420 (Perkin Elmer, Waltham, MA; USA). Three replicates of each material were tested.

2.12. Cell Cultures

To evaluate cell colonization and proliferation on the different materials, cultures with L929 fibroblasts and adipose tissue-derived stem cells, ADSCs, in their 10th passage were carried out. ADSCs (provided by the Hôpital Georges Pompidou, Paris) were isolated from fat biopsies of the right thoracic wall of female Rambouillet sheep, as described in ref[19]°. The studies were carried out in compliance with the rules of the International Organization of Standardization, ISO 10993/EN 30993 (Biological evaluation of medical devices, part 5). Three covers of each material were tested for each cell type. Polystyrene covers were employed as positive controls.

Samples were placed in 48-well plates. 2 × 10⁴ cells per well suspended in 20 µl of medium were seeded on the upper-surface of each sample and incubated for 30 min at 37 °C and 5% CO₂ in order to favor the initial adhesion on films. Next, DMEM medium supplemented with 10% FBS and 1% P/S was added in the case of fibroblasts culture, and minimum essential medium (α-MEM; Fisher) supplemented with 10% FBS, 1% L-glutamin (Lanza) and 0.02% Flasmoct™ (Invitrogen) for adipose cells culture, 500 µl per well. Samples were incubated up to 6 d. Culture medium was renewed each 2 d.

2.13. Immunocytochemistry

Cell cultures were analyzed after 2 and 7 d. One sample of each composition was rinsed with phosphate buffer (PB 0.1 M), fixed with 4% paraformaldehyde (Panreac) in PB for 20 min, and rinsed again with PB. Permeabilization and blocking was performed using 10% FBS and 1% Triton X-100 (Aldrich) in PB for 1 h at room temperature. Next, samples were incubated for 1 h in Phallacidin Bodipy FL (1:200, Invitrogen) in 1% bovine serum albumin (BSA; Aldrich, 35% purity) solution with PB at room temperature and in the dark. Samples were rinsed in PB and then stained for 10 min with 4',6-diamidino-2-phenylindole (DAPI, 1:5 000). Finally, PB was employed to rinse the samples. Samples cultured for 7 d were also stained using mouse monoclonal primary antibody anti-vimentin (Sigma–Aldrich) in a 1:200 proportion with blocking and permeabilizing solution at 4 °C in the dark. After 15 h, samples were rinsed with PB and incubated with Goat anti-mouse Alexa Fluor 555 secondary antibody (Invitrogen) at a 1:200 proportion with blocking and permeabilizing solution, for 1 h at room temperature. Finally, PB was used to rinse the samples. They were examined to collect fluorescent images under a Nikon ECLIPSE 80i fluorescence microscope (Nikon Instruments, Inc., Amsterdam, The Netherlands) and a Olympus FLUOVIEW FV1000 confocal laser scanning microscope (CLSM).


The morphology of L929 fibroblasts and ADSCs after culture on the substrates was analyzed by scanning electron microscopy (SEM). After 7 d of incubation, culture medium was removed and samples were rinsed with PB. Fixation of the samples was carried out with a 3% glutaraldehyde (GA; Electron Microscopy Science, 25% purity) solution in PB for 1 h, followed by a rinse with PB. Post-fixation was performed with 1% osmium tetroxide in PB, along with a smooth shaking for 1 h at room temperature, and followed by four rinses with distilled water. Then, samples were dehydrated through a series of graded ethanol (30, 50, 70, 96, and 100 °C) for 4 °C for 10 min each. Finally, samples were sequentially desiccated with hexamethyldisilazane (HMDS; Sigma–Aldrich) at proportions of 1:2, 1:1, and 1:0 with absolute ethanol at 4 °C for 10 min. HMDS was allowed to evaporate at room temperature overnight. After sputter-coating samples with gold, they were examined in a Hitachi S-4800 SEM at 25 kV and 15 mm of working distance.

3. Results and Discussion

3.1. Composition and Phase Structure of the New Materials

The combination of such dissimilar materials as are PEA and PCL, by the methods here described, naturally raises a
number of questions as to the final compositions that can be achieved by this process of IPN fabrication, to the extent of EA monomer incorporation to the PEA network, to the miscibility or phase-separation of both components.

For the purpose of achieving a mixture of these two polymers a redox polymerization of EA was chosen, which takes place at a reasonable rate with a very short induction period, a lower energy of activation (40–85 kJ mol\(^{-1}\)) and under mild conditions\([31,32]\). Thermal and photochemical initiations of the polymerization were discarded because they required longer times and would not guarantee the homogeneity of the mixture during the reaction. Because of the preparation method, the materials obtained are a two-component mixture, the PCL component being a linear soluble polymer, and the PEA component being in the form of an insoluble crosslinked network. After extraction of the soluble PCL component, residual weights could be compared with the nominal sample compositions, Figure 1a. There is a linear relationship between the mass extracted and the nominal composition, but the fractional loss is greater in each case than the nominal PCL mass fraction. This is interpreted as indicating that a part of the PEA polymer was not incorporated to the PEA network, remaining as a sol fraction, which was extracted along with the PCL. This soluble fraction of linear PEA chains increases with the PCL content, which seems to indicate that increasing amounts of PCL solution in the reacting mixture represent a growing obstacle for the incorporation of all EA monomers into the PEA network. On the whole, these results show that it was possible to obtain a significant proportion of PEA as an insoluble fraction, so that the materials were authentic semi-IPNs.

The densities of the materials are shown in Figure 1b. The homopolymers PCL and PEA have densities of 1.145 and 1.130 g cm\(^{-3}\), respectively, consistent with previous published values\([27,33]\). The densities of the different semi-IPNs depend on composition more or less linearly; within the bounds of experimental dispersion no significant excess volume can be assessed. This is consistent with a bi-phasic nature of the materials.

Fourier transform infrared spectroscopy (FTIR) spectra of the materials showed no significant differences between the absorbances of the samples (results not shown). All of them showed peaks from 3000 to 2800 cm\(^{-1}\) due to the symmetrical and asymmetrical CH\(_2\) and CH\(_3\) bond stretching, and a strong peak between 1800 and 1650 cm\(^{-1}\) due to the presence of C=O stretching vibration of carbonyl bonds. A small absorbance peak appeared in the region from 1600 to 1400 cm\(^{-1}\), attributed to C=C aromatic stretching vibrations of BPO, being more pronounced for the 0/100 sample, as expected for its higher fraction of initiator. Finally, an overlapping of the peaks due to C—O vibrations between 1300 and 1100 cm\(^{-1}\), and C—N vibrations between 1220 and 1020 cm\(^{-1}\) could be observed. The C—O peaks, as well as that of C=O, show no differences between samples, since these groups appear in both PEA and PCL monomeric units. Nevertheless, the peaks due to the C—N bonds seem more noticeable for the 0/100 sample, as occurs with the C=C ones. This is due to the higher concentration of the redox initiator pair (DMT and BPO) in this sample. These molecules contain aromatic rings and remain anchored to PEA polymeric chains. The C—N bond, distinctive of amines, belongs to the tertiary amine of DMT in greater concentration in PEA samples.

The thermal decomposition up to 700 °C of the pure polymers and the semi-IPNs blends is characterized by a single weight loss step, which occurs in the temperature range of the two pure components, between 300 and 450 °C in PEA, and slightly shifts toward higher temperatures, being 50 °C above in pure PCL (results not shown).

![Figure 1.](image) a) Loss of mass of the semi-IPNs after extraction of PCL, referred to the sample mass, as a function of the nominal PCL mass fraction in the semi-IPNs, \(\omega_{\text{PCL}}\). b) Experimental density, \(\rho\), of the semi-IPNs as a function of \(\omega_{\text{PCL}}\).
Further insight into the phase structure of the materials could be gained from differential scanning calorimetry. The normalized heat flow thermograms (Figure 2) show the glass transition of amorphous PCL segments, the glass transition of PEA, and, finally, the melting of PCL crystals at around 60 °C. The glass transition of amorphous PCL segments appeared around 60 °C, as reported in the literature.\(^{[21]}\) It was discernible only in the PCL curve, associated with a specific heat capacity jump of 1.16 \(\times\) 10\(^{-1}\) J g\(^{-1}\) K\(^{-1}\). The glass transition of PEA occurs at around –10 °C (Table 2) and was noticeable in the PEA and in all the semi-IPNs’ thermograms. With higher PCL content the glass transition temperature of PEA decreases slightly, as does the specific heat capacity jump at \(T_g\), since the fraction of material able to undergo conformational motions at this temperature decreases as a consequence of lesser PEA: thus, the value obtained for the 26/74 sample, divided by that of PEA homopolymer, gives a mass fraction of 73.8% of this component in the IPN, which comes very close to the nominal composition.

The endothermic peak due to melting of PCL crystalline phase appears at approximately 57 °C, as expected.\(^{[21]}\) The results of crystallinity are shown in the inset in Figure 2: the crystallinity of the semi-IPNs increases linearly with the PCL mass fraction in them, and extrapolates neatly to the value of pure PCL. This means that the PCL phase in the materials crystallizes as it would as pure, without any hindrance from the PEA phase.

Wettability of the different materials was also analyzed. Water contact angles on the different semi-IPNs are similar to that on pure PEA, around 100°, but different from that on PCL, around 80° (Figure 3). This suggests that on the surface of the samples, and for these compositions, PEA envelopes the PCL phase.

These results establish the materials as bi-phasic semi-IPNs, in which little or no modification of the properties of the pure components occurs as a consequence of mixing. Further conclusions about the distribution of both phases were obtained analyzing the mechanical properties exhibited by the materials.

### 3.2. Mechanical Properties and Degradation

Dynamic-mechanical thermograms at 1 Hz for the storage modulus, \(E'\), and the loss tangent, \(\tan \delta\), are seen in Figure 4a. The pure PCL curve shows a gradual decrease in \(E'\) from –60 °C and its associated peak in \(\tan \delta\) is characteristic of the main, \(\alpha\), relaxation process in the amorphous part of PCL, associated to its glass transition. The \(E'\) and \(\tan \delta\)

<table>
<thead>
<tr>
<th>Sample (PCL/PEA)</th>
<th>(T_g) PEA [°C]</th>
<th>(\Delta c_p) PEA [J g(^{-1}) K(^{-1})]</th>
<th>(T_m) PCL [°C]</th>
<th>(\Delta h_m) PCL [J g(^{-1})]</th>
</tr>
</thead>
<tbody>
<tr>
<td>0/100</td>
<td>–11.17</td>
<td>0.382</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>7/93</td>
<td>–10.48</td>
<td>0.463</td>
<td>57.21</td>
<td>3.96</td>
</tr>
<tr>
<td>14/86</td>
<td>–11.90</td>
<td>0.304</td>
<td>56.86</td>
<td>8.92</td>
</tr>
<tr>
<td>20/80</td>
<td>–12.27</td>
<td>0.305</td>
<td>56.98</td>
<td>13.19</td>
</tr>
<tr>
<td>26/74</td>
<td>–12.64</td>
<td>0.282</td>
<td>57.56</td>
<td>17.44</td>
</tr>
<tr>
<td>100/0</td>
<td>–</td>
<td>–</td>
<td>57.62</td>
<td>62.30</td>
</tr>
</tbody>
</table>

Figure 2. Normalized heat flow thermograms of the materials, in units of specific heat capacity, as a function of temperature. Inset: mass fraction of crystalline phase in the materials, \(\omega_c\), as a function of the PCL mass fraction in the samples.

Figure 3. Contact angles of water on the surface of the different materials, as a function of the PCL mass fraction in the material.
thermograms of pure PEA in this temperature range show the main α relaxation associated to its glass transition. The tan δ thermograms of the semi-IPNs show the relaxations of both component polymers, as corresponds to a bi-phasic material. The α relaxation of the amorphous PCL phase in the semi-IPNs is very much depressed, and the materials keep moduli of the order of $10^9$ Pa, typical of glasses, up to $-10^\circ C$, when the relaxation of PEA chains starts to occur (see the peaks in tan δ). The last drop in $E'$, at around $60^\circ C$, corresponds to the melting of PCL crystalline phase in the semi-IPNs. Thus, at low temperatures the mechanical properties of the semi-IPNs are dominated substantially by the vitreous phase of PEA and the PCL transition does not have a noticeable effect. The effect of the presence of the PCL phase on the α relaxation of the PEA phase is significant: it is shifted to lower temperatures, and the modulus values attained after the relaxation increase from 0.68 MPa (as expected for pure PEA\textsuperscript{155}) to 20.86 MPa for the 26/74 semi-IPN at 37$^\circ C$ (Figure 4b). The mobility of the PEA chain segments thus greatly depends on the presence of interpenetrated PCL chains, which have a restrictive effect on it, leading to a stiffening of the material. It becomes thus possible, by changing the relative amounts of both components, to tune the mechanical properties of the semi-IPNs at physiological temperature within a wide range of values, achieving moduli considerably greater than those typical of elastomers, which may be of interest for applications.

A similar conclusion can be derived from stress–strain tensile experiments. The curves obtained at room temperature are displayed in Figure 5a. PCL has a much higher tensile modulus, and a much lower yield strain, as corresponds to a semicrystalline polymer. The tensile Young moduli $E$ were obtained from the initial slopes of these curves, and are represented in Figure 5b. The same stiffening effect of PCL as was observed in the dynamic-mechanical data is here found again; for PCL mass fractions between 0 and 0.26 the tensile modulus increases from 0.4 to 6.6 MPa, whereas for pure PCL the value is 220.5 MPa. These tensile Young moduli, $E$, as well as the storage moduli, $E'$, derived from the dynamic-mechanical experiments at 37$^\circ C$, were compared with the moduli predicted by Takayanagi’s bi-phasic model, which determines the moduli of bi-phasic polymer blends starting from the sole moduli of the pure components and their volume fraction in the blends. In Takayanagi’s model, a parallel arrangement of two elements is combined in series with a third element (see inset in Figure 5b); one of the two elements in parallel has the properties and volume fraction of the disperse phase, whereas the other two elements (in gray color in Figure 5b, inset) represent the continuous matrix. In this way, the disperse phase, of volume fraction $w$, works in parallel (deforms with the same strain) with a fraction $(1 - \lambda)$ of the continuous matrix, and both work in series (deform with the same stress) with a fraction $\lambda$ of the continuous matrix. Thus, the case $\lambda = 0$ delivers a pure parallel arrangement of both phases, which corresponds to the co-continuity of the phase structure.

Both PCL and PEA were introduced in the equations as continuous phases, with several constant values of the parameter $\lambda$ over the whole composition range. The curves shown in Figure 4b for $E'$ show that neither with PEA nor with PCL as a continuous phase was it possible to fit the experimental points with uniform values of $\lambda$ over the composition range; the same happened with the stress–strain tensile moduli $E$. An independent fit of this parameter...
to match the experimental moduli for each sample composition gives the data shown in the insets to Figures 4b (dynamic-mechanical moduli) and in 5b (stress–strain moduli): the volume fraction of material working in series with the PCL phase rapidly drops to very low values, making clear that a high degree of phase co-continuity is present already for mass fractions of PCL equal to or higher than 0.2, and that the behavior of the semi-IPNs with \( \omega_{\text{PCL}} \) larger than 0.3 would correspond to a pure two-parallel block arrangement in Takayanagi’s model, i.e., to perfect phase co-continuity. The results of both the dynamic-mechanical and the tensile moduli are astonishingly coincident in this respect.

In degradation experiments, the samples were found to lose almost entirely their PCL content in 52 d (Figure 6). PEA samples did not degrade significantly, as expected for cross-linked acrylic elastomers, although a slight weight loss could be detected, likely on account of those chains not incorporated to the network. The mass fraction lost in the semi-IPNs corresponded quite closely to their PCL mass fraction.

3.3. Biological Performance

As completely novel materials, the semi-IPNs were tested for cytotoxicity, and for the behavior on them of seeded fibroblasts and adipose-tissue-derived stromal cells, in view of the multiple possible applications of this last cell type. None of the materials gave indications of cytotoxicity: the results of the MTT test on Figure 7 show the gradual increase in the optical density with culture time, proving that L929 fibroblasts proliferate well on all materials and suggesting that none of them is cytotoxic. There was no significant difference among samples of different
compositions. The positive control showed the same trend, although with more metabolic activity due to a larger amount of viable cells. No semi-IPN showed after any time an absorbance below 60% of that of the positive control. Meanwhile, the negative control shows low absorbance values, as corresponds to its cytotoxic character.

Cell adhesion on PEA, PCL, and semi-IPNs materials was studied in the fluorescence microscope and details were observed by CLSM. Figure 8 and 9 show staining for nuclei, actin cytoskeleton, and vimentin for fibroblast cultures at 2 and 7 d. At Day 2, the cells are seen isolated and round-shaped on PEA and PCL substrates (Figure 8a and c), whereas on the semi-IPN they are much more numerous and with a well-developed actin cytoskeleton, corresponding to a more spread morphology (Figure 8b). This differential early adhesion behavior of the cells may be due to the phase-structure of the semi-IPN materials. The co-continuity of this structure causes an alternation of softer (PEA phase) and stiffer (PCL phase) nanodomains, to which cells are sensitive. At Day 7, cells have proliferated so much on all materials that no significant differences are appreciated (Figure 8d–f). Still, CLSM details show that the cell morphology, as defined by the arrangement of the actin filaments and the cytoskeletal protein vimentin, is

Figure 8. Fluorescence microscope images of L929 fibroblasts cultured for 2 and 7 d on (a, d) 0/100 (PEA), (b, e) 26/74 PEA-i-PCL, and (c, f) 100/0 (PCL) samples. DAPI stain for nuclei (blue), phallacidin stain for actin (green), and vimentin stain for cytoskeleton (red, after 7 d culture). Scale bar represents 50 μm.

Figure 9. CLSM images of L929 fibroblasts cultured on: a) 0/100 (PEA), b) 26/74 PEA-i-PCL, and c) 100/0 (PCL) samples for 7 d. DAPI stain for nuclei (blue), phallacidin stain for actin (green), and vimentin stain for cytoskeleton (red). Scale bar represents 10 μm.
Figure 10. Fluorescence microscope images of ADSCs cultured for 2 and 7 d on (a, d) 0/100 (PEA), (b, e) 26/74 PEA-i-PCL, and (c, f) 100/0 (PCL) samples. DAPI stain for nuclei (blue), phallacidin stain for actin (green), and vimentin stain for cytoskeleton (red, after 7 d culture). Scale bar represents 50 μm.

Figure 11. SEM images of (a–d) L929 fibroblasts and (e, f) ADSCs cultured on 26/74 PEA-i-PCL and 0/100 (PCL) samples for 7 d. Scale bar in (a, b, e, f) represents 100 μm, and in (c, d) represents 60 μm.
different, cells being larger and more spread on the PEA and semi-IPN substrates than on pure PCL (Figure 9), with elongated multipolar morphology.

The same qualitative findings could be ascertained with the much larger ADSCs. Figure 10 shows again that 2-d concentration of cells was much lower on the PCL substrate (Figure 10c) than it was on either the PEA (Figure 10a) or the semi-IPN materials (Figure 10b), and that cells completely covered all materials at Day 7 (Figure 10d–f), exhibiting extended bi- or multipolar morphology and expressing vimentin after 7 d.

SEM observations (Figure 11) support these findings: L929 fibroblasts appear more rounded and isolated on the PCL substrate, and ADSCs adopt a spread morphology, attributable to the phase-separated alternating morphology of the materials.

Summarizing, both cell types adhered and proliferated on the surfaces of the different materials, with the semi-IPNs showing marked differences as regards early time adhesion and cell morphology, attributable to the phase-separated alternating morphology of the materials.

4. Conclusion

It was possible to blend the immiscible polymers PEA and PCL in the form of semi-interpenetrated networks, by a process of redox polymerization of the EA monomer in the presence of a PCL solution, and the proportions of both polymers could be varied from 0 to 0.26 PCL weight fraction. These materials are macroscopically homogeneous and their wettability is dominated by the PEA component. They possess a phase-separated structure, with a high degree of co-continuity for PCL mass fractions above 0.2. This is reflected in the moduli dependence on composition, giving rise to the possibility of tuning their stiffness within an ample range of values to meet the demands of applications. Moreover, the materials are partially degradable, leaving upon degradation a stable PEA continuous skeleton, which could be of help in tissue engineering applications to support mechanically newly formed tissue.

The semi-IPNs are completely non-cytotoxic, and fibroblasts and adipose-tissue-derived stromal cells attach to them and proliferate in an excellent way. The phase-separated nature of the materials may be the reason why early adhesion and spread of the cells is much more encouraged on the semi-IPN surfaces than on any of both PEA or PCL. These findings encourage further search on methods to obtain three-dimensional porous structures (scaffolds) for regenerative purposes made from these materials.

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