Long term degradation of poly(ε-caprolactone) films in biologically related fluids

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Abstract

The aim of this work was to study the long term degradation behaviour of poly(ε-caprolactone) (PCL) films, potentially useful as substrates for tissue engineering, obtained by two different methods (compression moulding or casting in chloroform) in two biologically related media: phosphate buffered solution (PBS) and Dulbecco’s modified Eagle’s medium (DMEM). The films were characterized at different degradation times by differential scanning calorimetry (DSC), Scanning Electron Microscopy (SEM) and Fourier Transform Infrared Spectroscopy (FTIR). The molecular weight was determined by Gel Permeation Chromatography (GPC). Chemiluminescence (CL) was used to assess physical or chemical changes from the early stage of the degradation. A different behaviour is observed in samples immersed in PBS when compared with those treated in DMEM. In this medium, the degradation after one year and a half (18 months) shows that although the chemical structure has been modified, the layers become more fragile but maintain their consistency. A higher degradation rate is obtained for membranes obtained by casting with respect to those obtained by compression moulding.

Keywords: Degradation; Chemiluminescence; Biodegradable scaffold; Tissue engineering

1. Introduction

Poly(ε-caprolactone) (PCL) is a biodegradable, biocompatible and semicrystalline polymer with a melting point ($T_m$) between 59 and 64 °C, depending on the crystalline content, and a glass transition temperature ($T_g$) around −60 °C, yielding a rubbery state at room temperature [1]. Due to its slow degradation rate, PCL has not been utilized as frequently as other members of the aliphatic polyester family such as polyglycolide (PGA) and polylactide (PLA); limiting, up to now, its applications to drug delivery devices or commercial sutures (monocryl [2]). However, due to its good biocompatibility, ability to form compatible blends [3–6] and copolymers with a wide range of other polymers [7–10] improving its mechanical properties, processability and high permeability due to its rubbery characteristics, PCL has expanded greatly its field of applications. Most are related to tissue engineering, taking advantage of the longer times of degradation which can be decreased to adjust to the new tissue formation rate: cartilage [11,12], liver [13], bladder [14], vascular [15,16], skin [17], nerve [18], bone [19–21]… The requirements for these materials to act as substrate for the development of different types of cells are not only related to the correct adhesion, proliferation, preservation of the phenotype … but also to mechanical aspects, since these substrates should ensure the appropriate performance of the device until the new tissue generated is capable of restoring the original functionality.

Despite the increasing interest in this polymer and the great number of applications that may arise from this research, there
are few studies that focus on the degradation behaviour in fluids that resemble biological media. Moreover, the design of new grafts should not only be based on an adequate selection of the material but also on the capacity of previewing its possible behaviour once implanted in terms of degradation mechanisms and feasibility of its performance along time. The stability of biopolymers used as substrate for tissue engineering is an important field of research, with particular emphasis on their durability during service-life. However, most degradation studies have been designed for different applications not related with implantable devices. In this sense, media such as static and dynamic seawater [22, 23], distilled water [24], compost [25], soil [26], in general in the presence of different microorganisms, or different mineral fluids [27, 28] in particular phosphate buffered solutions [4, 29, 30] have been used. Most of these studies focus on the degradation after few weeks of immersion and, in many of them, accelerated degradation through different agents (enzymes, free radicals, temperature, alcalis) is considered. On the contrary, there are few studies about the degradation behaviour “in vivo” [31] or the mechanism involved [32]. Initially, PCL was used as a biodegradable packaging material as it could be degraded by microorganisms [33]. However, afterwards, it was confirmed that PCL could also be degraded by a hydrolytic mechanism under physiological conditions [31]. The degradation mechanism resembles that of other aliphatic polyesters such as PLA and PGA: hydrolytic cleavage of ester groups causes random chain scission.

Recently, chemiluminescence [34–37] has become a useful technique for the study of polymer degradation, oxidation mechanisms, kinetics and stabilisation efficiency. This fact is due to its offered advantages with respect to other well established techniques [38]. The degradation of polymer materials can be induced by exposure to various factors such as heat, oxygen, UV light, humidity, and microorganism, during processing or in service-life of the material under ambient conditions [39]. The chemiluminescence emission can be related to the hydroperoxide (POOH) content of a polymer which originates by the reaction of oxygen with radicals (P) formed in the macromolecular chain [40, 41]. The emission of chemiluminescence from polymer samples in an inert gas, such as nitrogen, is proportional to the hydroperoxide content formed during the processing of the material; this being related to its thermal oxidation history. In the presence of oxygen, the samples are highly oxidised in a diffusion-controlled reaction simultaneously to the emission, and the relative concentration of POOH enhances with respect to that under nitrogen. The rate of oxidation \( R \) increases under these conditions, and the bimolecular termination of peroxo radical will be large. Hence, the chemiluminescence intensity \( I_{\text{CL}} \) is significantly enhanced with respect to the emission produced under nitrogen. All these parameters commented here could be used to evaluate degradation during processing of poly-(e-caprolactone), and the long term degradation of poly-(e-caprolactone) films in biologically related fluids. This method has proved to be very sensitive and it is possible to detect very minor amounts of the oxidative defects caused by degradation. A sensitive instrument can measure minute changes in relative degrees of oxidation of a material at levels well below the detection limit of other techniques.

The aim of this work was to study the long term degradation behaviour of poly-(e-caprolactone) (PCL) films obtained by two different methods in two biologically related media. The membranes so obtained are being successfully used [42] as substrate for tissue engineering. The study of the degradation after prolonged periods of immersion (18 months) in a medium, DMEM, used in many cell cultures and its comparison to the results obtained in a phosphate buffered solution (PBS) should be an adequate tool to preview its possible behaviour in the presence of cells and once implanted.

2. Experimental part

2.1. Membrane preparation

The used poly-(e-caprolactone) was supplied by Aldrich as pellets purchased (Sigma–Aldrich Corporation, St. Louis, USA, \( M_w = 65,000 \) g/mol). The PCL films were prepared by compression moulding (F) or casting in chloroform (C). F films were prepared by compression moulding of a fixed amount of powder (0.5 g) in a Collin-200 press under the same temperature (100 °C) and pressure cycle (1 min, 0 bar, and 2 min at 20 ton). Also, the cooling rate, from 100 °C until room temperature, was controlled and maintained constant to assure the same crystallinity index in all the samples. Under such conditions, circular polymer films of 10 cm diameter and 100 ± 10 \( \mu \)m thick were obtained. C membranes were obtained by casting. PCL of 0.5 g was dissolved in 50 ml of chloroform, the solution was poured into a Petri dish and air-dried overnight. The films were subsequently dried by removing the solvent with an extensive treatment at 50 °C for 48 h yielding circular polymer films of 10 cm diameter and 100 ± 10 \( \mu \)m thick.

2.2. Degradation assay

The degradation assay was carried out by immersing circular pieces (1 cm) cut from PCL films, sterilized by 30 min UV irradiation, in two different media: phosphate buffered solution (PBS) and Dulbecco’s modified Eagle’s medium (DMEM, Sigma–Aldrich Corporation, St. Louis, USA) supplemented with 10% foetal bovine serum (BioWhittaker Europe, Belgium), 1 mM L-glutamine, penicillin (20,000 U/ml) and streptomycin (20,000 \( \mu \)g/ml) (BioWhittaker Europe, Belgium), under a CO2 (5%) atmosphere and at 37 °C. Four samples were extracted weekly in the first month, each 15 days in the second and monthly subsequently. The media were renewed every time samples were removed.

2.3. Characterization of the PCL films

Differential scanning calorimetry (DSC) was performed on a Seiko SSC/5200 thermal analyzer over the range 30–80 °C. The measurements were made at a heating rate of 5 °C/min
and the instrument was calibrated with an indium standard \((T_m = 429 \text{ K}, \Delta H_m = 25.75 \text{ J g}^{-1})\). The melting peak \((T_m)\) and the melting enthalpy \((\Delta H_m)\) were obtained, and the percentages of crystallinity \((\%X_c)\) were determined using the reference of 139.5 J g\(^{-1}\) for crystalline poly(e-caprolactone) \([43]\).

Chemiluminescence (CL) spectra of film samples were obtained using a CL400 ChemiLUME apparatus from Atlas Electric Devices Co. The PCL films (4 mm diameter) were held in aluminium pans in the sample temperature-controlled cell, under a continuous flow of dry nitrogen or oxygen (50 ml/min). Dynamic tests were performed by heating the samples at a heating rate of 10 °C/min, under constant flow of gas. In isothermal measurement the samples of material were preheated up with a pre-test ramp (10 °C/min) to the test temperature (120 °C) under nitrogen or oxygen. The films were covered by a lens focussing the emitted light from the sample to the water-cooled photon counting photomultiplier, which was previously calibrated using a radioactive standard provided by Atlas. The light emitted was measured as a function of time as the sample was heated. Fourier transform infrared (FTIR) spectra were obtained in a Nicolet Nexus spectrometer equipped with a Smart Golden Gate ATR accessory. Scanning Electron Microscopy (SEM) was performed in a JEOL JSM 6330F in samples previously covered with gold. The molecular weight was determined in tetrahydrofuran (THF) by Gel Permeation Chromatography (GPC) with a chromatography system consisting of a Waters 2695 Separation Module and a Waters 2414 RI detector. Molecular weights are relative to polystyrene standards (Waters). All GPC tests were made with 0.5% solutions (w/v) in THF at a flow rate of 1 ml/min.

3. Results and discussion

The samples obtained by compression moulding or casting have been shortened as \(F_{PBS}\) and \(F_{DMEM}\) or \(C_{PBS}\) and \(C_{DMEM}\) depending on whether they have been immersed in PBS or DMEM, respectively. No significant changes could be measured in the pH of the media (7.1 ± 0.1 for PBS and 8.4 ± 0.2 for DMEM) measured after each sample withdrawal when compared with the blank tubes containing only the medium. The degradation of the DMEM medium, detected by a colour variation, caused a significant diminution in the pH, down to 6.0; these samples were not considered for this study.

The measurement of the weight and dimensions of the membranes before and after immersion in the media showed no considerable variations. Once considered that no macroscopic differences can be detected, a closer characterization of the morphology has been carried out by Scanning Electron Microscopy. The examination of the surface by SEM demonstrates that both types of samples \((C\) and \(F)\) immersed in DMEM are affected, yielding a granulated morphology (Fig. 1). This type of morphology already appears after three months of immersion and increasing hole sizes are observed with degradation time. The cracks observed in the 18 month micrograph are vacuum-generated during the membrane examination under the microscope and gives an idea of their decreasing consistence after this time of degradation.
other hand, the samples immersed in PBS show a surface similar to those not degraded. The differences between samples C and F after a prolonged degradation period (18 months) in DMEM can be more easily appreciated at a lower magnification (Fig. 2) where, $F_{DMEM}$ membranes appear unaffected while the surface of $C_{DMEM}$ samples appears patchy with considerable gaps and cracks between the fragments.

The differences arisen by the immersion of the samples into PBS or DMEM can be noticed by the changes in some IR vibration bands after two months for the samples obtained by casting and four months for those prepared by compression moulding (Fig. 3). The first of the variations was the appearance of a wide band between 3600 and 2600 cm$^{-1}$, which could be attributed to the stretching vibration of O–H bands from COOH and OH groups as well as to the O–H stretching band. A second change can be observed in the 1100–1050 cm$^{-1}$ region where a doublet of bands increases greatly their intensity; this transformation could be explained by the contribution of the C–O band. Both the changes could be due to the ester bond cleavage yielding a carboxylic and an alcohol group as a consequence of the poly($\varepsilon$-caprolactone) degradation. No additional changes were observed after more prolonged immersion times.

In order to study the influence of processing in the morphology of the polymer, thermal analysis of initial poly($\varepsilon$-caprolactone) films prepared by compression moulding F and casting in chloroform C, was undertaken. The first feature to point out from this data is the observation that C samples exhibit higher values of crystallinity and that the melting point was observed at higher temperatures than the F ones (Table 1). This fact can be attributed to the gradual precipitation from solution in C, which allows the development of higher levels of crystallinity.

![Fig. 2. SEM micrographs of samples $F_{DMEM}$ and $C_{DMEM}$ immersed for 18 months.](image1)

![Fig. 3. FTIR spectra of the $F_{DMEM}$ and $F_{PBS}$ samples.](image2)
The DSC of $F$ and $C$ films degraded at different times in PBS and DMEM was undertaken. In both the media, the degree of crystallinity of $F$ samples increased with immersion time from an initial value of 61–77% after 18 months, which corresponded to the crystallization of amorphous regions. Besides, it was observed that the melting point slightly increased due to the degradation of the samples, and a broadening of the melting temperature interval was appreciated. For samples degraded in DMEM, $F_{\text{DMEM}}$, the thermograms during the second heating exhibited two melting peaks, which could be attributed to the presence of crystals with two different sizes (Fig. 4). In contrast, multiple melting peaks were not observed under the same conditions for $F_{\text{PBS}}$. This effect could be attributed to a higher decrease in the molecular weight, as a consequence of the ester groups’ hydrolysis, that was observed for $F_{\text{DMEM}}$ with respect to $F_{\text{PBS}}$. As a result, the formation of small domains with a very low degree of perfection would take place in $F_{\text{DMEM}}$, and partial melting of the defective crystals was observed.

A similar behaviour was observed for poly($\varepsilon$-caprolactone) films prepared by casting in chloroform, $C$. The degree of crystallinity increased with incubation time in PBS and DMEM media, although this enhancement of crystalline content during degradation after 18 months ($\Delta% X_c = 4$ — values calculated from the first heating — Table 1) was much smaller when compared with the $F$ samples ($\Delta% X_c = 15$). PCL is a semicrystalline polymer, consequently, the initial degree of crystallinity will play an important role for further crystallization during degradation in the incubation media. Since $C$ exhibited a higher value of crystallinity, developed during processing compared to $F$, it would result in higher restricted mobility of amorphous segment in $C$, which is necessary for reorganization and crystallization during degradation. As it was previously described for $F$, a different behaviour was observed in samples immersed in DMEM when compared with those treated in PBS, i.e. $C_{\text{DMEM}}$ exhibited two melting peaks attributable to different crystalline domains, which do not appear for $C_{\text{PBS}}$.

All these changes suppose an alteration in the structure which implies the degradation of the polymeric chain and, consequently, a decrease in the molecular weight of the material with the time of immersion, as can be detected in Figs. 5 and 6. In both the types of samples the degradation in DMEM provokes a considerable diminution in the molecular weight as well as an increase in the polydispersity, this effect being much more pronounced in samples $C_{\text{DMEM}}$. Besides, a considerable diminution in the proportion of the peak attributable to the initial mean molecular weight and the appearance of a new signal at lower molecular weights has been detected. These results agree with those observed by DSC and adjust with the degradation of caprolactone described by other authors [44]: two slopes in the molecular weight versus time profile, the first having a higher rate due to an autocatalytic cleavage of the ester bonds while the second exhibits a lower rate related to the formation of crystalline polymer due to the formation of shorter chains in the polymer degradation.

Chemiluminescence temperature-ramping tests under nitrogen and oxygen for initial compression moulded poly($\varepsilon$-caprolactone) samples ($F$) were undertaken. Under nitrogen,
no chemiluminescence emission was detected at temperatures below 65 °C, which corresponds to the melting temperature of the polymer determined by DSC. At temperatures above \( T_m \), the mobility of the peroxyl radicals is favoured and their bimolecular termination takes place, which is responsible for chemiluminescence emission in polymers, through the deactivation of the generated carbonyl moieties in the excited state. Even though chemiluminescence intensity is increasing, initial \( F \) showed weak emission in the whole range of temperature. Since in the absence of oxygen, the species responsible for the chemiluminescence correspond to the initial concentration of hydroperoxide generated during the processing of the material [45], it would indicate that no significant amounts of chemiluminescence-inducing species were produced by oxidation during film processing.

Under oxygen, a similar trend was observed in the CL profiles, and no emission was detected below the melting point. The chemiluminescence emission is enhanced with respect to that obtained under nitrogen, in the whole range of temperatures studied, since in such conditions the samples have been oxidised in a diffusion-controlled reaction simultaneously to the emission [46]. Macro-radicals react with the oxygen to give peroxy radicals; their concentration will be large and the bimolecular termination reaction of two peroxy radicals to give ketone products will be enhanced. Re-plotting the ramping temperature CL emission profile under oxygen in Arrhenius form yields two different temperature intervals: Region I and Region II. It would indicate that polycaprolactone undergoes thermal degradation by a two-step mechanism. The activation energies were calculated, the process of low activation energy predominates at low temperatures (range I: \( E_{\text{act} \ I} = 63 \text{ kJ mol}^{-1} \)) and that of high activation energy predominates at higher temperatures (range II: \( E_{\text{act} \ II} = 150 \text{ kJ mol}^{-1} \)). These results are in good agreement with other authors [47]. It has been reported that the thermal degradation of PCL showed a two-step mechanism. The first stage was attributed to the random chain scission and the second step corresponded to specific chain end scission.

The isothermal chemiluminescence analysis under oxygen at 120 °C was undertaken for initial \( F \), and samples degraded at different times in the two media considered, \( F_{\text{PBS}} \) and \( F_{\text{DMEM}} \). Initially \( F \) exhibited two chemiluminescence emission peaks (Fig. 7). A weak emission due to the thermal history of the film is observed at the onset of heating. Furthermore, a second peak is observed when the oxidation induction period is attained. In the induction period of oxidation, the rate of formation of peroxyl radicals is nearly similar to their rate of termination by recombination, and the CL signal is low. At the end of this period, the radical concentration increases due to autoacceleration of the oxidation, and the chemiluminescence emission is enhanced. The peak-top times \( (t_{\text{max}1}) \) and \( (t_{\text{max}2}) \) and their corresponding intensities \( (I_{\text{CL-max}1}) \) and \( I_{\text{CL-max}2} \) of chemiluminescence were determined and are summarised in Table 2.

For \( F_{\text{PBS}} \) and \( F_{\text{DMEM}} \) samples, a different evolution of the CL emission peaks was observed depending on the media and time of degradation. \( F \) initial sample showed the highest intensity of CL second peak. For \( F_{\text{PBS}} \), as the ageing time is increasing, the peak-top time \( (t_{\text{max}2}) \) is longer and a decrease in the intensity of CL is observed, Fig. 7. The chemiluminescence parameters are summarised in Table 2. A possible explanation could be based on the different oxygen diffusion in the initial and degraded samples of the material, i.e. during the ageing, degradation of amorphous phase takes place reducing the diffusion of oxygen in the polymer and the formation of new peroxides would be restricted. The physical spreading of the oxidation from an initial highly reactive centre to other particles would be more hindered [48,49]. As degradation process developed, a new increasing peak appears at shorter times \( (t_{\text{max}1}) \). This early emission could be due to the formation of new peroxides assumed to exist in the polycaprolactone. During immersion in PBS media, several kinds of peroxides are generated in different active sites of polymer. The different stability of the formed species will be related with their mobility which depends on the microenvironment and the molecular weight of the polymer segment. This explanation would be on
agreement with previous works which describe the possibility of different peroxy radical formation in polyolefins and PECT [50–52].

A different behaviour was obtained in the degradation of poly(ε-caprolactone) films in DMEM, \( F_{\text{DMEM}} \) when compared with the results before commented on \( F_{\text{PBS}} \) degraded samples, Fig. 8. In this case the disappearance of the initial high CL intensity is nearly completed in hours, being much faster than observed on the PBS degradation media. This indicates that the oxidation species formed in the polymer units are efficiently fragmented in the ageing conditions, and the CL decreases drastically. In contrast, the CL emission at short peak-top time (\( t_{\text{max}1} \)) is enhanced much faster in \( F_{\text{DMEM}} \) than in the \( F_{\text{PBS}} \) degraded samples, and higher intensities are detected after 18 months of treatment. The chemiluminescence parameters calculated for samples degraded at different times are summarised in Table 2. These results are in good agreement with those obtained by DSC and GPC, where a higher degradation as a consequence of the ester groups’ hydrolysis, for \( F_{\text{DMEM}} \) was observed with respect to \( F_{\text{PBS}} \).

The isothermal study of the chemiluminescence emission of PCL films prepared by casting in chloroform \( C \) and degraded for a period of 18 months in PBS and DMEM media \( C_{\text{PBS}} \) and \( C_{\text{DMEM}} \) was also undertaken. CL profiles versus time are plotted in Fig. 9. In contrast to \( F \) sample, initial \( C \) sample exhibited one peak at short times (\( t_{\text{max}1} \)) and \( t_{\text{max}2} \) was not observed. This fact would be related with the higher crystallinity developed during casting processing compared to compression moulding, as was determined by DSC. The higher crystallinity would restrict the mobility of amorphous segment where hydroperoxides are generated, and their bimolecular termination reaction, responsible for chemiluminescence emission, would be reduced. As it was previously described for \( F \) samples, the intensity of chemiluminescence enhanced with ageing time, and a different behaviour was observed in samples immersed in DMEM (\( C_{\text{DMEM}} \)) when compared with those treated in PBS (\( C_{\text{PBS}} \)). The former exhibited higher CL intensity, since the emission is related with the content of oxidative species, this would confirm that poly(ε-caprolactone) degraded faster in DMEM than in PBS media.

The faster degradation in DMEM can be justified by considering the complexity of its composition: inorganic salts, amino acids, vitamins... The possible enzymatic activity due to the presence of foetal bovine serum can be neglected owing to the previous treatment performed in this medium. However, although the enhanced degradation cannot be attributed to any specific molecules, the considerable differences between a medium composed by a phosphate buffer and another that includes a great variety of molecules must be stressed out. This supposes a closer degradation assay approximation with

Table 2

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<th>Degradation time</th>
<th>PBS media</th>
<th>DMEM media</th>
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<tbody>
<tr>
<td></td>
<td>( t_{\text{max1}} ) (min)</td>
<td>( T_{\text{CL-max1}} ) (mV)</td>
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<tr>
<td>0 h</td>
<td>66</td>
<td>80</td>
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<tr>
<td>14 h</td>
<td>9</td>
<td>114</td>
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<tr>
<td>7 days</td>
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<td>32</td>
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<tr>
<td>1 month</td>
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<td>119</td>
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<tr>
<td>5 month</td>
<td>12</td>
<td>103</td>
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<tr>
<td>6 month</td>
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<tr>
<td>9 month</td>
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<td>15 month</td>
<td>9.5</td>
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<tr>
<td>18 month</td>
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<td>183</td>
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the only difference of the presence of cells and the corresponding biochemical routes.

In this work we have used several techniques to examine the degree of degradation in the media described. In addition we have demonstrated the utility of chemiluminescence analysis as a fundamental tool in this characterization: its sensitivity to detect the very first stage of oxidation of polymers and the short periods of time required to obtain information about the physical or chemical changes in the early stage of the degradation, well before any defects become visible in the polymer or failure by fracture were produced.

Concerning the final destination of ester linkages generated by the hydrolytic chain scission, in a first step these fragments must be small enough to diffuse through the polymer bulk. Once implanted, the low molecular fragments and the small polymers particles are removed from the site of implantation by solubilisation in the body fluids or by phagocytosis, carried out by macrophages which degrade them intra-cellularly [53]. The products so generated are either metabolised via the tricarboxylic acid cycle or eliminated by direct renal secretion [54].

4. Conclusions

A much faster degradation behaviour is observed in Dulbecco’s modified Eagle’s medium (DMEM) when compared to that determined in phosphate buffered solution (PBS). This effect is more pronounced in samples obtained by casting.

![Fig. 8. F<sub>DMEM</sub> chemiluminescence intensity versus time at 120 °C under oxygen.](image1)

![Fig. 9. Chemiluminescence intensity versus time at 120 °C under oxygen of initial C and C degraded in PBS (C<sub>PBS</sub>) and DMEM (C<sub>DMEM</sub>) for 18 months.](image2)
The different characterization techniques confirm a degradation behaviour based on hydrolytic cleavage of ester groups causing random chain scission. The alteration of the polymer structure causes a progressive decrease in the molecular weight of the material and the generation of low molecular fragments that should be more easily metabolised by the host organism.

Chemiluminescence may be a characterization tool of great interest since it allows us to preview physical or chemical changes in the early stage of the degradation, before any defects become visible in the polymer or failure by fracture occurs.

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References