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Catalyst-Free Plasma-Assisted Copolymerization of Poly(ε-caprolactone)-poly(ethylene glycol) for Biomedical Applications

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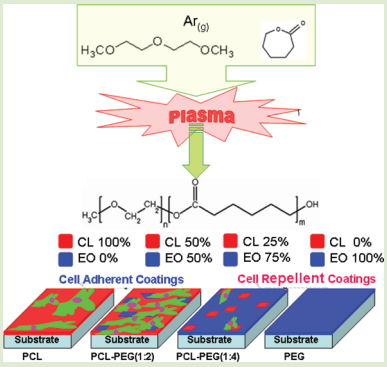
Supporting Information

ABSTRACT: Catalyst-free ring-opening polymerization (ROP) strategy was developed to overcome the disadvantage of incomplete and expensive removal of catalyst used during the multistep wet chemical processes. Nano-sized biocompatible and low molecular weight poly(ε-carolactone)-poly(ethylene glycol) (PCL-PEG) copolymer coatings were deposited via a single-step, low-pressure, pulsed-plasma polymerization process. Experiments were performed at different monomer feed ratio and effective plasma power. The coatings were analyzed by XPS, as well as MALDI ToF. Ellipsometric measurement showed deposition rates ranging from 1.3 to 3 nm/min, depending on the ratio of the PCL/PEG precursors introduced in the reactor. Our results have demonstrated that plasma copolymerized PCL-PEG coatings can be tailored in such a way to be cell adherent, convenient for biomedical implants such as artificial skin substrates, or cell repellent, which can be used as antibiofouling surfaces for urethral catheters, cardiac stents, and so on. The global objective of this study is to tailor the surface properties of PCL by copolymerizing it with PEG in the pulsed plasma environment to improve their applicability in tissue engineering and biomedical science.



Over the past few decades, the usage of poly(ε-caprolactone) (PCL) has significantly increased in the different fields of research portend the recognition of this highly versatile resorbable polymer, particularly in the field of biomaterials, biomedical, and tissue engineering.[1] The hydrophobic character and high degree of crystallinity of PCL limits its rate of degradation and, hence, it is less biocompatible in the presence of soft tissues. Therefore, to modify the above-mentioned PCL properties, PEG can be suitable to copolymerize with PCL because of its hydrophilicity, immunogenicity, nontoxicity, and lack of antigenicity.[2] A literature survey shows that PCL-PEG copolymers have been prepared by the ring-opening polymerization (ROP) of ε-caprolactone (ε-CL) using PEG[3] and amine-functionalized PEG[4] as macroinitiators with stannous octoate [$Sn(Oct)_2$] as catalyst. Furthermore, a few new catalysts such as zinc,[5] calcium,[6] aluminum,[7] yttrium tris(2,6-di-*tert*-butyl-4-methylphenolate) [$Y(DBMP)_3$],[8] and stannous chloride[9] complexes were also developed for the synthesis of amphiphilic PCL-PEG copolymers. However, the copolymers obtained using organometallic catalysts are not directly suitable for biomedical applications if the metal contaminant is not completely removed from the resultant polymer.[10] In recent years, the application of metal-free strategies to perform ROP reactions has been developed for the synthesis of polymers which provide several advantages over those that require metals to initiate the process, the most obvious being the absence of the costly removal of metal impurities from the resultant polymers and the end products. Using wet processes, such as hydro gel, spin-cast, and so on, metal-catalyst-free ROP of ε-CL was shown by utilizing tertiary amines in the synthesis of chitosan-graft-poly(ε-CL),[11] or by nucleophilic phosphines at high temperature anhydrous conditions[12] and mild organic acid (i.e., tartaric acid).[13] To summarize, there has been a limited number of studies reported on the preparation of catalyst-free PCL-PEG copolymers for biomedical applications.

Herein, we address the new strategy for the development of low-pressure RF plasma-based single step, solvent-, and catalyst-free dry chemical synthesis for the preparation of PCL-PEG copolymers for the biological applications. PCL-PEG copolymers were synthesized by simultaneously introducing ε-CL and diethylene glycol methyl ether (DEGME) monomer vapors in an inductively excited radio frequency (13.56 MHz) discharge (Figures 1a, S1a, and S1b). The snapshot of plasma polymerization process in the RF plasma reactor is shown in Figure 1b. For a short residence time in the reactor ($\tau \sim 200$ ms) and optimal specific energy conditions (P_{pk}: 25W; DC: 4%; P_{eff}: 1 W; total flow rate: 20 sccm; 0.8 eV/molecules), the fragmentation of the two precursors is minimized, and therefore, the retention of the ethylene oxide (EO) and caprolactone (CL) functionalities is ensured as shown by XPS analysis (Figure 2a–d). The C–O/C–C ratio obtained from

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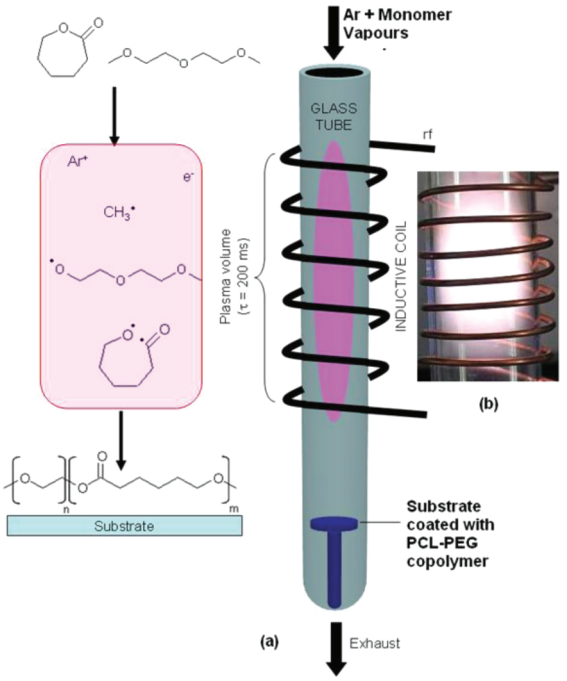


Figure 1. (a) Schematic view of the experimental setup consisting of a low pressure ICP glass reactor used to synthesize PCL-PEG copolymers from precursor vapors. Precursors are activated in the plasma volume (τ is the residence time in the plasma region) and a thin copolymer film is obtained downstream on the substrate. A hypothesized simple mechanism for the formation of PCL-PEG copolymer is also shown and (b) snapshot of plasma polymerization process.

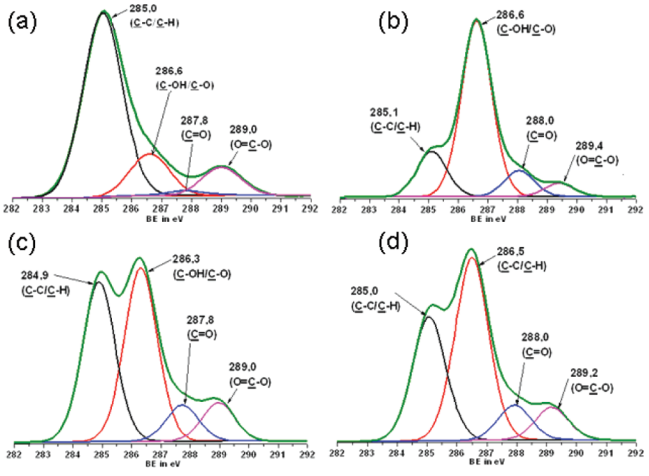
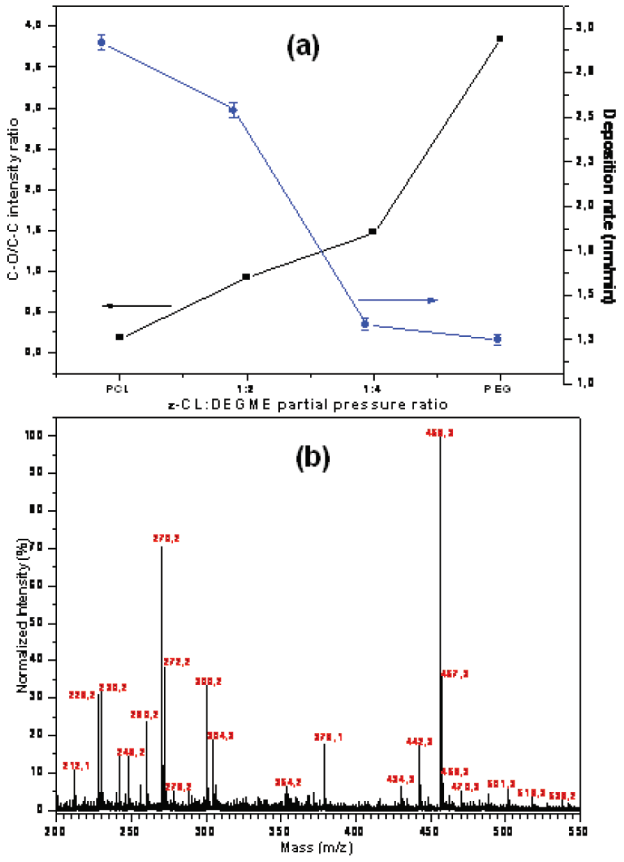


Figure 2. C1s XPS spectra for plasma polymerized (a) PCL, (b) PEG, (c) PCL-*co*-PEG (1:2), and (d) PCL-*co*-PEG (1:4). The ratio in parentheses corresponds to the monomer ratio introduced in the plasma. The deconvolution and attribution of XPS spectra is also shown.

XPS is a good indicator for the copolymerization of PCL and PEG, as shown in Figure 3a. The deposition rate of the nano coatings was calculated by ex situ ellipsometry measurements and plotted versus ε-CL/DEGME partial pressure. The results show that the deposition rate of PCL-PEG copolymers decreases by increasing the DEGME content in plasma. This observation suggests that ε-CL is more reactive in the plasma phase, as compared to DEGME due to the ring strain in ε-CL



(c)

m/z	Normalized Intensity (%)	Number of PCL units (m/z = 114)	Number of PEG units (m/z = 44)	-CH_2- (m/z = 14)
456.3	100	4	0	0
270.2	70	2	1	0
300	33	1	4	1
228	31	2	0	0
260.2	24	1	3	1
379	18	1	6	0
442	17	3	2	1

Figure 3. (a) Variation of the C−O (286.5 ± 0.1 eV)/C−C (285.0 ± 0.1 eV) ratio determined from C1s XPS spectra and deposition rate calculated from ellipsometry vs ε-CL/DEGME partial pressure ratio (error bars designate standard deviation on the mean of three measurements), (b) MALDI ToF mass spectrum (210−550 Da) of plasma polymerized PCL-PEG (1:2) coating dissolved in α-matrix. The ionization source used was a N_2 laser (λ = 337 nm), and (c) molecular weight of the most representative ion fragments measured by MALDI ToF and estimated number average degrees of polymerization.

molecules. Based on the C−O/C−C ratio, one can conclude that the surface hydrophilicity of the copolymer coatings is increased by increasing the DEGME content in the plasma.

The surface hydrophilicity of the different plasma polymerized coatings have been performed and determined by water contact angle (WCA) and to test the stability of the coatings, the coated silicon wafers were soaked in water for 30 min and quickly dried by air before measuring the WCA (see Figure S2). Our results show that, the PCL-*co*-PEG coatings deposited at P_{eff} = 1 W exhibited an excellent stability against soaking in water. The MALDI-ToF mass spectrum of the PCL-

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PEG (1:2) coatings dissolved in α-cyano-4-hydroxycinnamic acid (α-matrix; Figure 3b). The most intense ions detected have molecular weight ranging between 210 and 460 Da. The specific peaks of the copolymer chains are separated by m/z shifts of 114, 44, and 14 Da corresponding to one caprolactone unit ($C_6H_{10}O_2$), one ethylene oxide unit (C_2H_4O), and one methylene unit, respectively. An examination of the polymer ion signals having normalized intensities higher than 15% is reported in Figure 3c. Based on MALDI-ToF analysis, one can conclude that the average copolymer formula is approximately $(PCL)_4$-$(PEG)_6$. This observation is what we can expect from a low pressure plasma process. Indeed, in the gas phase, the mixing of the two monomers is quite homogeneous at the molecular level and, thus, the number of repeating unit should be very low. Therefore, we can conclude that we have obtained very homogeneous copolymers at the molecular level by this plasma polymerization process and, as a consequence, that it is not possible to obtain block copolymer.

Finally, the biological response to the plasma deposited PCL-PEG copolymer surfaces was investigated in vitro using the human ovarian carcinoma cell line (NIH:OVCAR-3). A 2 mL aliquot of cell suspension with a density 1×10^5/well was injected into each well and they were incubated at physiological conditions for 58 and 120 h. Cells deposited on PEG and PCL-PEG (1:4) coatings do not adhere and proliferated on the surfaces (Figure 4a). This cell repellent behavior is due to a high retention of ethylene oxide (EO) functionalities. There exist a huge number of references which explain the antifouling effect of the EO groups, for example, by providing a molecular basis for a water barrier layer, which avoids the protein adsorption on the surfaces. However, cells deposited on PCL-PEG (1:2) surfaces were well proliferated and even a better adhesion was obtained as compared to PCL homopolymers. In this case, the introduction of the ethylene oxide functionalities in the copolymer enhanced the overall rate of cell migration, cell proliferation, and adhesion properties.[14,15] The fluorescent images of cytoskeleton stain demonstrated the HBMEC adhesion and proliferation on the plasma deposited surfaces after 72 h of incubation. For cells on the PCL-*co*-PEG (1:2) coatings were experienced some stretching across the cytoplasm resulting in flattening of the cells (Figure 4b). Cells on the PCL-*co*-PEG (1:2) coated glass surfaces were well spread and developed the network required for better cell adhesion as compared to the PCL homopolymers. Cell repellent behavior of the plasma deposited pDEGME homopolymer coatings was observed due to the retention of the ethylene oxide functionalities (Figure 4c). Optical microscopy images of cell adhesion and proliferation on the plasma-polymerized coatings, which were incubated for 24, 48, and 72 h, are in agreement with above-mentioned results (Figure S3).

Cell to polymer surface interactions were examined with an SEM. At an accelerating voltage of 15 kV, images were obtained at different magnifications in the central region of each polymer surface after 24 h of endothelial fibroblast cell culture. The morphology of the cells seen on the SEM images was analyzed for the PCL-*co*-PEG (1:2) and pDEGME polymers (Figure 4d,e). The SEM image of the PCL-*co*-PEG (1:2) polymer surface shows that cells were well proliferated and have experienced flattening on the polymer surface which is in agreement with the fluorescent images of cytoskeletal stain. The cell adhesion was not observed on pDEGME coating, which was also in agreement with our previous cell adhesion tests. In

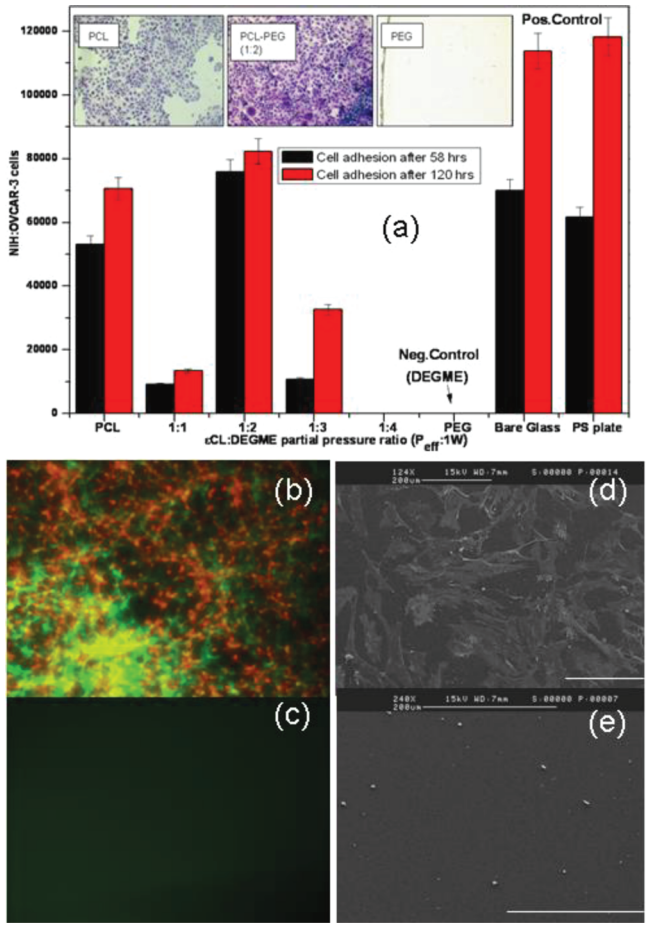

Figure 4. (a) Cell adhesion properties of NIH:OVCAR-3 on PCL, PEG, and PCL-PEG copolymers incubated for 58 and 120 h. Optical microscopy images (900 × 600 μm) of stained human ovarian carcinoma cells (NIH:OVCAR-3) seeded for 120 h on plasma-polymerized coatings and on bare glass and polystyrene (PS) culture plate taken as positive controls (inset picture; error bars designate standard deviation on the mean of three measurements), fluorescent images of cytoskeleton stain demonstrated human bone marrow endothelial cells (HBMEC) adhesion, and proliferation on the plasma-polymerized coatings after 72 h of incubation: (b) PCL-*co*-PEG (1:2), (c) pDEGME (optical magnification: 60×) and SEM images of cell to plasma deposited polymer surface interactions, (d) PCL-*co*-PEG (1:2), and (e) pDEGME (scale bar: 200 μm).

the recent studies for the advancement in regenerative medicine and modern cancer research, the key advantages of 3D culture over the traditional 2D culture have been discussed intensively and conveyed that the tissue engineered scaffolds are better alternative to mimic 3D ECM environment for promising and reproducible clinical applications.[16] In the present study, we have shown that the plasma processes are the promising tool to develop nano-sized polymer coatings for tailorable 2D cultures which can, therefore, in the near future, be used to modify 3D biocompatible (nano) scaffolds for cell culture and further clinical studies.

In conclusion, we report for the first time a catalyst free strategy to obtain nano-sized biocompatible PCL-PEG copolymer coatings, which was successfully developed by using a low pressure pulsed plasma copolymerization. The latter being a dry process as compared to the conventional multistep wet chemical techniques overcome, therefore, the disadvantage of incomplete removal of catalysts used in the wet

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processes. Our results show that as the C−O/C−C ratio for the plasma deposited coatings increased the cell adhesion decreases due to the increase in the density of the ether groups. Coating stability test showed that the plasma polymerized coatings, deposited under 1 W plasma power conditions, were stable after soaking with water. From the MALDI ToF analysis, we have confirmed the formation of the randomly distributed low molecular weight copolymers which were prepared by the single step plasma copolymerization process. Our results show that the copolymer coatings are characterized by a good retention of monomer functionalities and PCL/PEG mixing that is close to molecular. Cell adherent or repellent PCL-PEG copolymer coatings can be obtained by varying the monomer ratio in the plasma process. We have demonstrated that plasma copolymerized PCL-PEG coatings can be tailored in such a way to be cell adherent, convenient for biomedical implants, such as artificial skin substrates, or cell repellent, which can be used as antibiofouling surfaces for urethral catheters, cardiac stents, and so on. In the present work, we have illustrated the importance, efficiency, and utility of low temperature and low pressure single step pulsed plasma copolymerization process as compared to the conventional wet chemical techniques, which has a wide range of applicability in the tissue engineering and biomedical science.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

Experimental procedure, schematic of plasma reactor configuration, thin film characterization methods, and details for cell adhesion and proliferations. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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