

Poly (ϵ -caprolactone)-Poly (ethylene glycol) Copolymer Coatings Developed by Low Pressure Inductively Excited PECVD for Tailored Cell Adhesion

Sudhir Bhatt¹, Jerome Pulpytel¹, Raphael Lis², Massoud Mirshahi², Farzaneh Arefi-Khonsari¹

*1*Laboratoire de Génie des Procédés Plasmas et Traitement de Surface, Université Pierre et Marie Curie, ENSCP11 rue Pierre et Marie Curie, 75231 Paris cedex 05, France

*2*UMRS 872, Centre de Recherche des Cordeliers, Faculté de Médecine Paris VI, 15 rue de l'Ecole de Médecine, 75006 Paris, France

Abstract: Poly (ϵ -caprolactone)-poly (ethylene glycol) (PCL-PEG) copolymers have great potential applications in the fields of nanotechnology, tissue engineering, pharmaceuticals and medicinal chemistry. In the present work we have developed, for the first time, amphiphilic biodegradable PCL-PEG copolymer coatings by catalyst free ROP of ϵ -caprolactone (ϵ CL), in presence of 2-Methoxyethylether or 2-(Vinylxy)ethanol. A low pressure inductively excited RF (13.56MHz) discharge was used operating in the pulsed mode with Argon as the carrier gas. Experiments were performed at different ϵ CL/PEG monomer feed ratio and effective power. PCL-PEG copolymer coatings were characterized by FTIR-ATR analysis. The copolymer coatings were stable after 72 hours of washing with water. SEM-FEG, XPS, XRD and AFM were also carried out on the coatings. Human ovarian carcinoma cell line (NIH:OVCAR-3) were cultured in physiological conditions and were seeded in a microplate which was loaded with autoclaved coated glass cover slips for 24 hours. The cell adhesion to the surface was determined by using an inverted microscope. The results show that by gradually varying the ϵ -CL/PEG partial pressure ratio of the monomers from 100% to 25%, the cell adhesion follow the same trend. Thus, PCL-PEG plasma copolymers are promising materials that can be used to control cell adhesion.

Keywords: PCL-PEG copolymer, Plasma co-polymerization, Cell repellent coatings.

1. Introduction

A clear upward trend in Poly (ϵ -caprolactone) (PCL) usage in research over the past decade signifies the recognition of this highly versatile resorbable polymer, particularly in the field of biomaterials and tissue engineering [1]. The days of its utilization as drug-delivery devices have been surpassed by the realization that PCL may be processed into composite structures with superior mechanical and biocompatible properties compared to the polymer on its own. PCL and its copolymers collectively provide a promising polymer platform for the production of longer term degradable implants which may be easily manipulated physically, chemically and biologically to possess tailorable degradation kinetics to suit a specific anatomical site. PCL having FDA approval and CE Mark registration makes it universally acceptable for the *in vitro* and *in vivo* applications. PCL is a hydrophobic, semi-crystalline, mechanically compatible, biodegradable and bio resorbable polymer which possesses exceptional blend-compatibility. However,

owing to the high degree of crystallinity and hydrophobicity, PCL degrades rather slowly and is less biocompatible with soft tissues, which restricts its further clinical application. Therefore, the modification of PCL is required. Poly (ethylene glycol) (PEG), for its hydrophilicity, nontoxicity and absence of antigenicity and immunogenicity, can be selected to be copolymerized with PCL, forming PCL-PEG copolymers. Thus, their hydrophilicity, biodegradability and mechanical properties can be improved.

Human ovarian carcinoma cell line (NIH:OVCAR-3) were cultured in routine experimental conditions and were seeded in a microplate which was loaded with autoclaved coated glass cover slips for 24 hours. The cell adhesion to the surface was determined by using an inverted microscope. The biological responses to the plasma co-polymerized glass surface of different PCL-PEG copolymers were investigated *in vitro*. Our results showed that surface hydrophilicity improved with the increased PEG segments in PCL-PEG copolymers and that

NIH:OVCAR-3 cell adhesion was inhibited by increased PEG contents. To our knowledge, for the first time, amphiphilic biodegradable PCL-PEG copolymer coatings were developed by catalyst free ring-opening polymerization of ϵ -caprolactone (ϵ -CL) in the presence of 2-Methoxyethyl ether (DEGME) or 2-(Vinylloxy) ethanol (DEGVE) in a low pressure inductively excited radio frequency (13.56MHz) discharge in the pulsed mode to avoid fragmentation of the two precursors used. Experiments were performed at different ϵ -CL/PEG monomer ratio by varying the partial pressure of ethylene oxide monomer and effective power (P_{eff}).

Plasma co-polymerized PCL-PEG coatings have unique practical advantages including (i) confirmative ultra thin film deposition, (ii) good adhesion to the substrate material, (iii) formation of chemically and physically durable surfaces, (iv) highly substrate independent treatments and (v) catalyst free, single step, low cost, less time consuming and environmentally benign process. Furthermore, while the chemical structure of a thin surface layer can be changed significantly, the bulk properties of the substrate remains unchanged.

Materials

ϵ -CL, the cyclic ester monomer and ethylene oxide monomers (DEGVE & DEGME) were purchased from Sigma Aldrich, France and used in this study without further purifications. For this study polished silicon wafers (100) purchased from Silitronix, France were used. They were ultrasonically cleaned in acetone bath for 30min and rinsed with ethanol for 30min. The cleaned wafers were air dried and then used for FTIR-ATR and XPS analysis. Glass cover slips (0.5cm^2) were purchased from Warner Instruments, France and which were used for cell adhesion and proliferation analysis.

Preparation and characterization of plasma co-polymerized surfaces

A low pressure inductively excited radio frequency (13.56MHz) tubular reactor plasma system bearing dimensions of 4 cm in diameter and 50 cm in length was used to carry out deposition of PCL-PEG copolymer coatings. All the coatings were deposited on Si wafer and glass cover slip substrates for 20

min. The P_{eff} was either 1W or 0.25W. Argon was used as a carrier gas. The schematic of the experimental set up has been given else where [2].

Results and discussion

FTIR-ATR Analysis of PCL-PEG copolymer coatings

The functional groups present in the coatings deposited onto Si wafer substrates were examined using FTIR-ATR spectroscopy. The spectra for both the cyclic ester monomer and ethylene oxide monomer are shown in figure 1. The spectral analysis of pulsed plasma deposited PCL [3] and PEG [4] homo and co-polymer coatings are shown in the figure 2. All the coatings were deposited at 1W effective power. We observed an absorption band at 1729 cm^{-1} which is attributed to the C=O stretching vibrations of the ester carbonyl group, 1177 and 1240 cm^{-1} are attributed to the characteristic C–O–C stretching vibrations of the repeated $-\text{OCH}_2\text{CH}_2$ units of PEG and the $-\text{COO}-$ bonds stretching vibrations, respectively. The absorption band at 3442 cm^{-1} is assigned to terminal $-\text{OH}$ groups in the copolymer coatings. All the C–H stretching bonds are centered at 2946 and 2867 cm^{-1} . All these signals indicate that the PCL-PEG copolymer is probably formed.

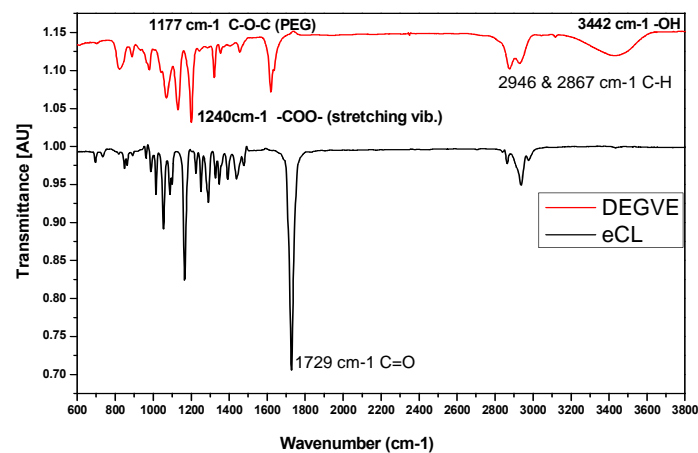


Figure 1. FTIR-ATR Spectra of ϵ -CL ($M_w=114.14$) & DEGVE ($M_w=132.16$) monomer. (Measurement volume was $40\ \mu\text{L}$ each).

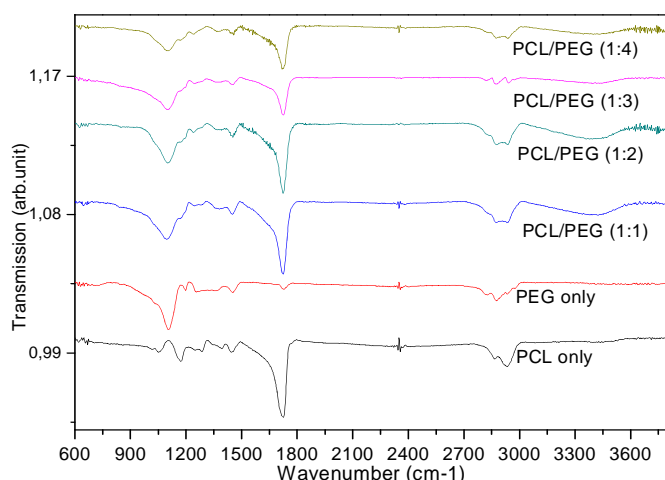


Figure 2. FTIR-ATR spectra of PCL and PEG homo & copolymer coatings deposited at pulsed plasma effective power $P_{\text{eff}} = 1\text{W}$.

Co-polymer synthesis in the pulsed plasma discharge

In the present study, the temperature of the monomers was optimised and hence exhibited linear correlations with the flow rate of carrier gas. Co-polymerization in the pulsed plasma environment was accomplished with various ratios of ϵ -CL monomer to PEG monomer which can act as a micro initiator for the ROP. An active hydroxyl group at one end of ethylene oxide monomer acted as an initiator (because of its nucleophilic character) and induced acyl-oxygen cleavage of cyclic ester to form PCL-PEG copolymers. The co-polymer synthesis in the pulsed plasma discharge is shown in figure 3.

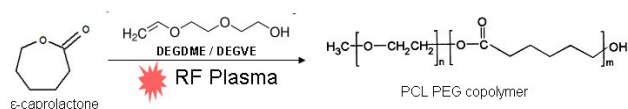


Figure 3. PCL-PEG co-polymer synthesis in the pulsed plasma discharge.

Human ovarian carcinoma cell (NIH:OVCAR-3) adhesion and proliferation on PCL-PEG homo & co-polymer coatings

Cellular adhesion tests have been performed using Human ovarian carcinoma cell line (NIH:OVCAR-3) which were obtained from ATCC and cultured in the RPMI-1640 supplemented with 1% (v/v) antibiotics (10,000 U/ml penicillin-G sodium, 10 mg/ml streptomycin), 2mM L-glutamine and

10%FBS. Cells were expanded by routine cell culture technique in 25 cm² cell culture flasks at 37 °C under 5% CO₂ atmosphere in the incubator for 24 hours.

The autoclaved plasma co-polymerized glass surfaces were placed into the bottom of 24well cell culture polystyrene micro plates. One millimeter of cell suspensions with a density 1×10^4 /well was injected into each well and they were incubated at physiological conditions. After 58 and 120 hrs of culture, the glass surfaces were washed in 1X PBS three times and were stained by using RAL 555 kit and given a final wash. The adherent cells on the surface were determined by using an inverted microscope (Nikon Instruments, Europe) and images of 900 $\mu\text{m} \times 600 \mu\text{m}$ were captured on 0.5cm² glass cover slips by using the CCD camera to determine cell adhesion. The adherent cells were counted by using indigenously developed MATLAB based image processing program with the help of which an average SD as low as 0.4% was observed. The biological response to the plasma co-polymerized glass surfaces deposited by varying ϵ -CL/PEG partial pressure ratio of the monomers was investigated *in vitro*.

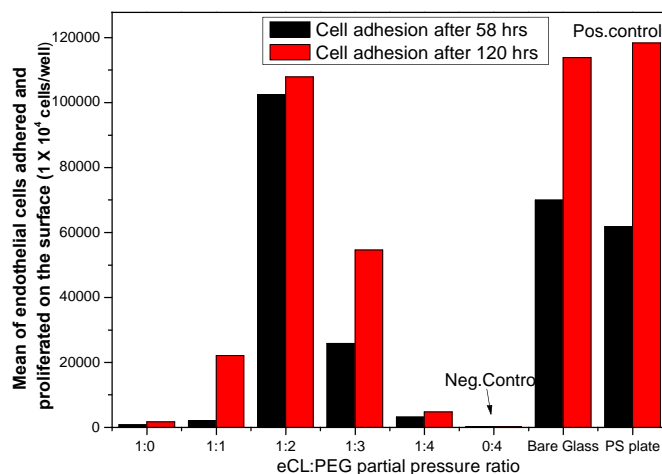


Figure 4. NIH:OVCAR-3 cell adhesion and proliferation on plasma coated glass surfaces at effective RF power $P_{\text{eff}}=0.25\text{W}$.

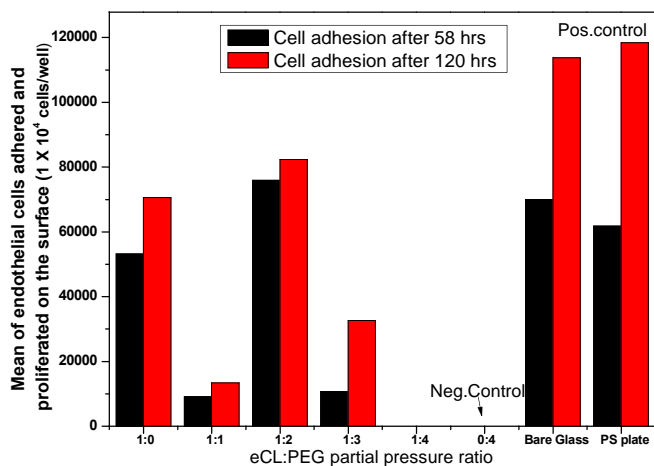


Figure 5. NIH:OVCAR-3 cell adhesion and proliferation on plasma coated glass surfaces at effective RF power $P_{\text{eff}}=1\text{W}$.

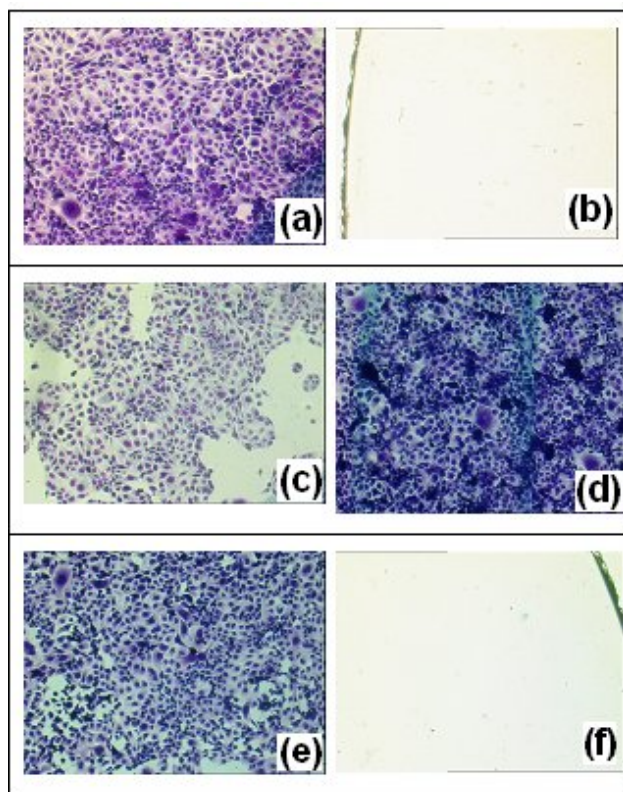


Figure 6. After 120hrs of culture, inverted microscopic images of NIH:OVCAR-3 cell adhesion and proliferation on plasma coated glass surfaces deposited at effective RF power $P_{\text{eff}}=1\text{W}$ for different $\epsilon\text{-CL/PEG}$ partial pressure ratio of the monomers (Image size: $900\ \mu\text{m} \times 600\ \mu\text{m}$). (a) 1:0, (b) 0:1, (c) 1:1, (d) 1:2, (e) 1:3, (f) 1:4.

As shown in figure 4 & 5, very low cell adhesion was observed on PCL coating at low effective power ($P_{\text{eff}}=0.25\text{W}$) and it increased with the increase in the effective power ($P_{\text{eff}}=1\text{W}$). As expected there was no cell adhesion on PEG coatings and they are also

stable after 120hrs of the cell culture. We have observed that as $\epsilon\text{-CL/PEG}$ monomer ratio gradually decrease from 100% to 25% (1:1 to 1:4) by varying the partial pressure of ethylene oxide monomers the cell adhesion decreased. Polystyrene plate and PEG coating were considered as positive and negative control respectively. Inverted microscopic images of cell adhesion and proliferation on co-polymer coatings at $P_{\text{eff}}=1\text{W}$ for different $\epsilon\text{-CL/PEG}$ partial pressure ratio of the monomers are shown in figure 6.

Conclusions

- Amphiphilic, biodegradable, bioresorbable and biocompatible PCL-PEG copolymer coatings were successfully developed by single step plasma co-polymerization process.
- Catalyst free ring opening polymerization of cyclic ester was performed by using ethylene oxide monomers as an initiator in the RF plasma.
- FTIR-ATR ($600\text{-}4000\ \text{cm}^{-1}$) spectral analysis of PCL-PEG copolymer coatings confirmed presence of ester and ethylene oxide groups.
- Surface hydrophilicity improved with the increased PEG segments in PCL-PEG copolymers and that Human ovarian carcinoma cell (NIH:OVCAR-3) adhesion was inhibited.
- Thus, PCL-PEG plasma copolymers are promising materials that can be used to control cell adhesion.

References

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