Poly (ε-caprolactone) – poly (ethylene glycol) coatings deposited by catalyst free PECVD reactor for biological applications

A. Baitukha[1], F. Arefi-Khonsari[1], S. Bhatt[2], M. Mirshahi[3] and J. Pulpytel[1]

[1] *Université Pierre et Marie Curie, Laboratoire Interfaces et Systèmes Electrochimiques, Paris, France*
[2] *Frank Reidy Center for Bioelectrics, Old Dominion University, Norfolk, VA, U.S.A.*
[3] *INSERM, UMRS872, Paris France*

Abstract: Present research work is devoted to develop dense barrier and cell repellent coatings on collagen films for controlled drug release systems. Diethylene glycol monomethyl ether and ε-caprolactone were used as precursors for deposition at low pressure pulsed plasma enhanced chemical vapour deposition reactor. Drug release kinetics, biocompatability and cell adhesion are investigated *in vitro* experiments.

Keywords: pulsed plasma enhanced CVD, PCL-PEG copolymerization, drug delivery system

1. Introduction

Drug delivery systems (DDS) are intensively studied and developed for their application in medical treatment of various diseases [1]. By controlling drug release around treatment area over prolonged period of time it is possible to precisely maintain concentration of drug in therapeutic window and avoid overdoses as well as sub-therapeutic concentration of drug. Localized to the treatment area DDS allow to decrease overall dosage of drugs introduced into body and eliminate undesired side effects.

One way to deliver drug is to load it into biocompatible polymer matrix. Polymer is a crucial component in DDS, as the diffusion of drug through the polymer film defines kinetics of drug release [2]. The polymeric material has to be non-toxic, biocompatible, biodegradable, and with no immune reaction to it and its by-products, with no formation of biofilm on its surface and with good mechanical properties to be easily handled and mounted into the body [3].

Polyethylene glycol (PEG) is an excellent material with cell repellent properties, also it is FDA approved product to be used in medical and clinical practice [4]. However stability of the PEG coatings in aqueous environment is not good. Combining PEG with PCL and fabricating amphiphilic coatings is one way to fabricate functional and stable coatings [5].

In our work we use amphiphilic coatings deposited by low pressure pulsed plasma to incorporate drug onto collagen film surface, thus making more functional collagen films for biomedical application.

2. Experimental procedure

Biocompatible collagen films (supplied by Biom'UP, 10x10 cm^2, 100 µm thick) were used as substrates. Schematic of experimental setup is illustrated in Fig. 1.

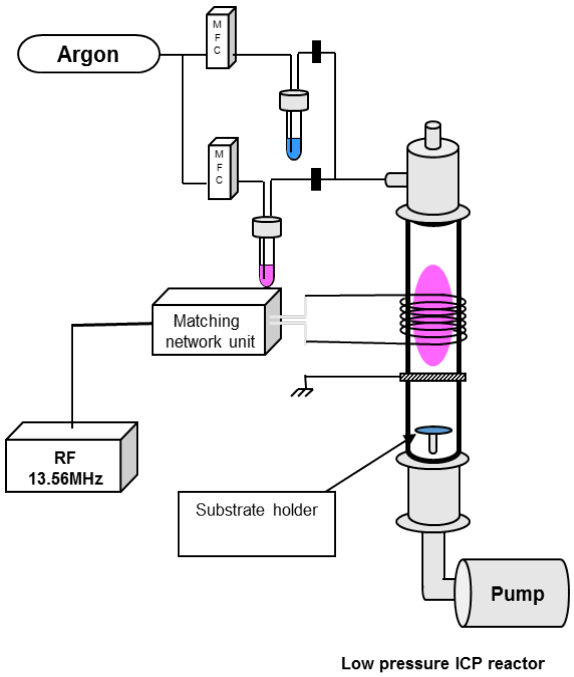

Fig. 1. Inductively coupled pulsed plasma enhanced chemical vapour deposition reactor for coating catalyst free poly(ε-caprolactone) - poly (ethylene glycol) films.

10 sccm of argon gas is fed through bubbling systems with diethylene glycol monomethyl ether (DEGME) and 7 sccm of argon gas through ε-caprolacton precursors, to make composition of DEGME to ε-caprolacton as 1:4. 13.56 MHz RF power supply is used to generate plasma at 0.5 mbar.

To fabricate DDS multi-layered structure is used (Fig. 2). The first layer deposited on collagen is dense PCL-PEG barrier layer (200 nm) deposited in continuous plasma mode (25 W). The second layer is drug dried from aqueous solution on the surface of barrier layer. The third

layer is dense barrier layer deposited at the same conditions as the first layer. The last top layer is a cell repellent PCL-PEG film deposited in pulsed plasma mode (25 W, 25 Hz pulses with 10% duty cycle).

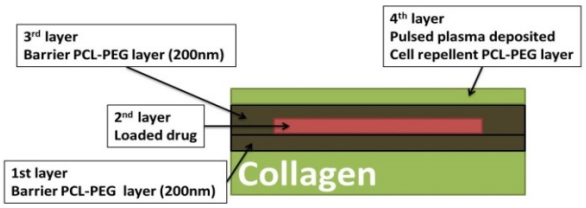

Fig. 2 Schematics of multi-layered DDS

ATR FTIR spectra were taken from films deposited on silicon substrates. Human ovarian carcinoma cell line (NIH:OVCAR-3) was used for measurements of cell adhesion *in vitro*. Uncoated glass and glass with pulsed plasma copolymerized PCL-PEG films were placed at the bottom of polystyrene well microplates and filled with 2ml of cell suspension with a density 10^5 cells/well. All samples were incubated at physiological conditions for 2, 24 and 48 hours.

Methylene blue (100 µg/cm^2) was used as a model drug. Concentration of drug released into PBS solution was measured using UV-VIS spectrometer (Ocean Optics HR4000).

3. Results and Discussion

Collagen is a porous material and allows medium liquid to diffuse through it. The first layer we coated on surface of collagen is deposited in continuous plasma mode to make a dense film, which acts as a barrier between drug and collagen. This prevents leaching drug into collagen film. After placing drug as a second layer, third layer is another dense PCL-PEG film to prevent leaching of drug into medium environment. In such a way drug is sandwiched and covered with barrier films on both sides. The top layer of DDS is deposited in pulsed mode with PCL-PEG 1:4. Under pulsed mode it is possible to regulate interactions of monomer with energetic species in the plasma and preserve chemical structure of monomer.

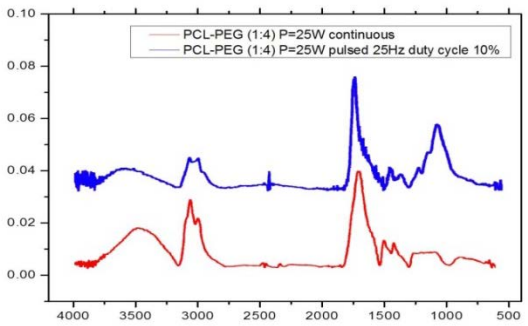

Fig. 3. FTIR spectra of PCL-PEG films deposited in continuous and pulsed plasma modes.

ATR FTIR spectra of deposited PCL-PEG films in pulsed plasma mode have absorption peaks around 1107 cm^{-1} related to C-O-C bond (Fig. 3), which comes from DEGME precursor. Surface of such coatings have properties similar to PEG film, which have excellent cell repellent properties. On the other hand continuous mode plasma facilitates fragmentation of the monomer and no C-O-C absorption peak is observed. This results in highly cross-linked dense coatings, which is used as a barrier film for DDS.

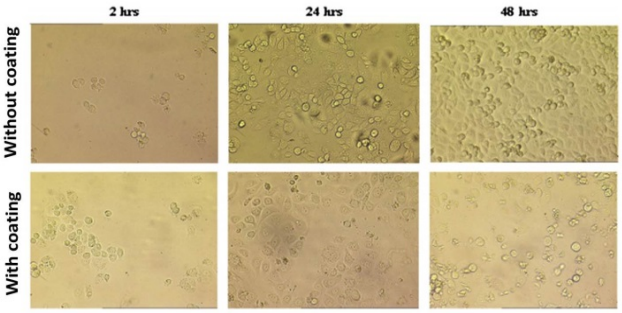

Fig. 4. Cell adhesion on glass surface without and with PCL-PEG coating.

Cell adhesion was evaluated from observing and counting adhered cancer cells in optical microscope images after incubating at physiological conditions (Fig. 4). Bare glass surface show proliferation and adhesion of seeded cancer cells, whereas due to the retention of ether groups on the film surface, pulsed plasma copolymerized PCL-PEG coatings prevents adhesion and growth of cells on its surface.

PCL-PEG coatings deposited under continuous plasma mode show good barrier properties. Methylene blue release profile of proposed multi-layered DDS is illustrated in Fig. 5.

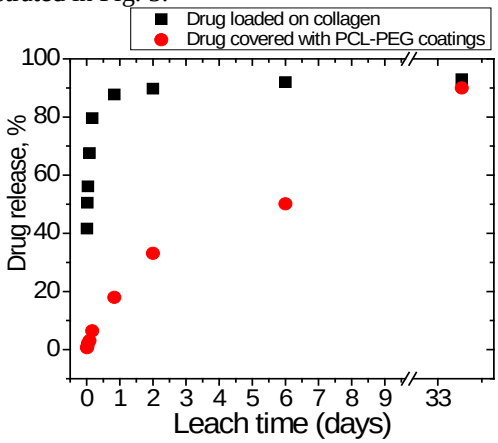

Fig. 5. Methylene blue release into PBS solution over time for uncoated and coated with 200nm thick PCL-PEG barrier films on collagen.

Samples with no barrier film release about 90% of loaded drug in the first few hours. Whereas protected by PCL-PEG barrier layer drug release is extended up to one

month. By varying thickness of the deposited barrier film it is possible to tune kinetics of drug diffusion through barrier layer.

4. Conclusion

In this research work catalyst free PCL-PEG coatings deposited in pulsed and continuous mode plasma were used to prepare multi-layered DDS. Proposed model of DDS on collagen films prevents migration, adhesion and growth of cancer cells on its surface, and by tuning the thickness of dense barrier films it is possible to control drug release kinetics and improve therapeutic effect by providing required concentration of drug around the treatment area. For the near future research, *in vivo* experiments are planned to carry out. DDS loaded with cisplatin will be tested on mice infected with mouse colon cancer CT26 and ovarian cancer cells OVCAR-3.

5. References

[1] Y. Zhang, H. Chan, K. Leong, Advanced Drug Delivery Reviews, 65, 104 (2013)
[2] D. Hsieh, CRC Press, (1988)
[3] O. Coulembier, P. Degee, J. Hedrick, P. Dubois, Progress in Polymer Science, 31, 8 (2006)
[4] S. Alconcel, A. Baas, H. Maynard, Polymer Chemistry, 2, 1442 (2011)
[5] R. Ferrari, C. Colombo, C. Casali, M. Lupi, P. Ubezio, F. Falcetta, M. D'Incalci, M. Morbidelli, D. Moscatelli, International Journal of Pharmaceutics, 453, 2 (2013)