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Poly(3-hexylthiophene) porous materials for bio-photonics

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Introduction

Semiconducting polymers (SP) are attracting increasing attention in biophotonics, an emerging interdisciplinary field in biomedical science, for cancer phototherapies, photo-triggered drug release, bioimaging, and phototriggers of Reactive Oxygen Species (ROS) due to their intrinsic conductivity and optical properties [1].

In particular, intracellular ROS levels are very important for the overall lifecycle of a living cell. They play very important roles in the maintenance of the physiological functions of cells, while their unbalance is directly related to several different dysfunctions and pathologies [2]. For this reason, there is an intensive effort towards innovative, precise, reliable and not invasive tools to control the intracellular ROS levels, with high specificity and with spatial and temporal resolution.

For such purpose, SP are usually processed in the form of thin films (TF) and nanoparticles (NPs) whose performance is influenced by the π-conjugated semiconducting polymer structure. All those features clearly modulate the photophysical processes and ultimately determine their biophotonic applications [3]. In the case of subcutaneous biophotonic applications, a high part of the optical excitation, needed to be supplied for the activation of the photonic nanomaterials during the clinical treatment, is absorbed by the skin layers becoming necessary to develop nanomaterials with larger optical absorption area that facilitate the optical excitation [4].

Therefore, we present the development of porous nanomaterials with enlarged surface area that allows to enhance their final opto-electronic properties. To that aim, opto-active, electro-active and hydrolysable graft copolymers, made of poly(3-hexylthiophene) (P3HT) and poly(lactic acid) (PLA), P3HT-g-PLA, are synthesized and employed for the fabrication of porous thin films and nanoparticles. The efficiency of these copolymers for in-vitro applications can be controlled thanks to their dose-response effect to visible light excitation.

To this aim, this study includes the employment of these materials for in-vitro extracellular and intracellular stimulations in Human Umbilical Vein Endothelial cells (HUVECs). After assessing the polymers biocompatibility under illumination conditions, intracellular ROS production is monitored. ROS are effectively modulated with high specificity and yield, making it possible to propose these porous materials as highly efficient and biocompatible photoactuators.

Results and discussion

P3HT-g-PLA copolymers were successfully synthesized by chemical oxidative polymerization of P3HT and PLA, as determined by 1H NMRNon-porous thin films (SP-NFsolid) were fabricated by spin coating P3HT-g-PLA solutions over ITO-glass substrates and their morphology was analyzed by TEM. AFM results show that films prepared with the copolymers have a similar surface roughness (Ra=0.4 nm) than those ones prepared with the homopolymer P3HT used as control. Besides, all films possessed a similar thickness in the nanoscale (~ 200 nm). Likewise, solid nanoparticles (SP-NPssolid), with ~100 nm diameter, were synthesized by flash nanoprecipation. Subsequently, the

porous nanomaterials were obtained in a second step by PLA hydrolysis. P3HT-g-PLA films present a different morphology in comparison with the homologous non-porous ones. The surface roughness increased and pores with different sizes are observed as consequence of the hydrolysis of PLA present in those films, which was corroborated by 1H NMR analysis. Before PLA hydrolysis, non-porous materials show the characteristics peaks of P3HT and PLA, whereas after NaOH treatment, the PLA is totally hydrolysed exhibiting only the P3HT signals. Then, the optical absorption of the films and nanoparticles was determined by UV-vis spectrophotometry, showing an absorption peak at 480 nm due to the π-π^* electronic transition of P3HT chains in a flexible random-coil conformation. It is worth to mention that the PLA hydrolysis and pores formation do not produce any significant change in the UV-vis absorption spectrum, as desired for the photonic excitation.

Finally, the photo-electrochemical properties were determined by irradiating films and then nps, immersed in an aqueous electrolyte (0.1 M Phosphate Buffered Saline), with a LED (wavelength = 530 nm, power density = 110 mW/cm^2). Interestingly, the pore formation gives rise to a 4-fold increase of the photocurrent properties as comparing with non-porous materials, proving the enhanced opto-electronic properties of these nanomaterials to be further employed to enhance ROS production.

The viability and proliferation of HUVECs plated on porous thin films has been assessed via AlamarBlue assays, both in dark and light conditions (530 nm, 110 mW/cm^2). Regarding NPs, their internalization inside the cell cytosol has been assessed at different concentrations with confocal fluorescence microscopy, while the viability has been investigated using different illumination protocols also suitable for a future in-vivo application. Then ROS assays proved the high efficiency of the porous materials in the phototransduction process, producing at least a 2-fold increase in ROS production with respect to the solid material. This will make possible to decrease the LED power density to meet the needs for in-vivo applications. So, ROS tests provided an insight of the high potential of these porous copolymers to efficiently modulate intracellular physiological processes with high spatial and temporal resolution.

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