3D Structures based on carbon materials and conducting polymers for electroresponsive cell cultures

Estructuras 3D basadas en materiales de carbono y polímeros conductores para cultivos de células electroresponsivas

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Objectives and Novelty

The next generation of therapies for cancer or neural diseases require the development of structures that mimic cellular scenarios ruled by interconnectivity, three-dimensionality, porosity, stiffness, biocompatibility and, in the case of electroactive cells: conductivity. These structures, known as scaffolds, can simulate conditions and environments that occur in living organisms, allowing the study of specific mechanisms. However, most of the scaffolds reported in the literature do not match all those requirements simultaneously. Carbon nanotubes (CNTs) possess outstanding properties as their high electrical conductivity or tunable biocompatibility that have rocked them as excellent interface nanomaterial with biological tissue. However, their main limitation stems from the formation of 3D structures without incorporating other additives. Conducting polymers (CPs), confers three-dimensionality similar than conventional insulator polymers at the same time that maintain the inherent properties of the CNTs. The present work summarizes three innovative methods tailored to manufacture scaffolds composed of CNTs and the conducting polymer poly(3,4 ethylenedioxythiophene) (PEDOT) for electroactive cellular culturing. All the methods are based on the principle of polymerizing the PEDOT polymer, *i.e* chemical oxidative and electrochemical polymerizations. The aim of this PhD thesis is the development of conductive and porous materials and their correlation with maturation and proliferation of electroactive cells as astrocytes C8-D1A, neuroblastome SH-SY5Y and primary cardiomyocytes.

Results

Vapour phase polymerization (VPP) is the first technique that was developed to manufacture porous scaffolds. The reaction is a chemical oxidative polymerization that take place in presence of carbon nanotubes thanks to iron (**III**) catalyst, forming the PEDOT polymer around the tubes (see **Figure 1a**). The reaction take place in a vapour phase within a Schlenk sealed tube, placing the EDOT monomer at the bottom and using crystal sugar grains as porous generator (see **Figure 1**). The polymerization occurs within the interstices of the sugar grains, doping the CNTs with the conducting polymer. Subsequently, after the cleaning process, the scaffold presents a controlled porosity with porous size below 250 μm and above 45% of emptiness, corroborated by scanning electron microscopy (SEM) and micro-computer tomography analysis (see **Figure 1c**). The formation of this 3D structures represents a huge improvement in the methodology since PEDOT, in absence of CNT, collapses by itself when forming the 3D macrostructure. This methodology allows the modulation of time and temperature, having a remarkable impact in the compositions and correlatively on the mechanical properties. Moreover, PEDOT/CNT materials presents very high conductivity, improving previous insulator polymer/CNT scaffolds. Finally, the scaffold biocompatibility was evaluated using C8-D1A astrocytes culture. Astrocytes are involved in neurogenesis and commonly studied in neural models. The scaffolds present excellent attachment of the cells and growth corroborated by immunofluorescence assay, LDH and SEM (see **Figure 1c**). VPP represents a very powerful and versatile methodology that allows its use with other conducting polymers such as polypyrrole (PPy). This method has also been expanded to PPy/CNT scaffold and their interactions, properties and formation of these porous three-dimensional structures were also studied.

In the second methodology, the reaction is carried out in a three-electrodes chamber throughout a chronoamperometry reaction where the scaffold template is used as a working electrode (**Figure 1**). This methodology is known as the electropolymerization (EP) and avoids the use of oxidants or strong reaction conditions. Compared to the previous work, EP possesses an additional step of nucleation on the electrode surface. The role of CNTs as nucleation agent of the conducting polymer was deeply studied. The presence of CNTs assists the three-dimensional polymerization and helps the formation of larger macrostructures (>1 μm). While PEDOT scaffolds present a smooth surface and do not present three-dimensionality, PEDOT/CNT scaffolds possess internally connected pores and a rough surface topography. Moreover, the presence of brush-like structures along the topography confirms their compositional differences (**Figure 1d**). Besides the morphology, the presence of CNTs improves the conductivity of the device, confirmed by impedance analysis and four-point probe measurements. These PEDOT/CNT scaffolds were used as supporting materials for endogenous differentiation of neuroblastoma SH-SY5Y cell line without the addition of any chemical, confirmed by the use of different immunoassay labelings and SEM morphology evaluation. Apart from the PEDOT polymer, this methodology allows the manufacturing of PEDOT derivative scaffolds, that are not very well reported in literature. We also explored scaffolds formed with PEDOT copolymerized with a bis malonyl functional EDOT monomer (bisPEDOT) in the presence and absence of the CNTs, forming mixed 3D structures.

Conclusions

Innovative 3D materials are required in the field of tissue engineering for addressing model diseases or body implants. The main problem observed with conductive materials is their poor processability, which restricts the manufacturing of real three-dimensional structures. Basically, in this PD thesis this main disadvantage was addressed, by developing new manufacturing methods for the formation of scaffolds

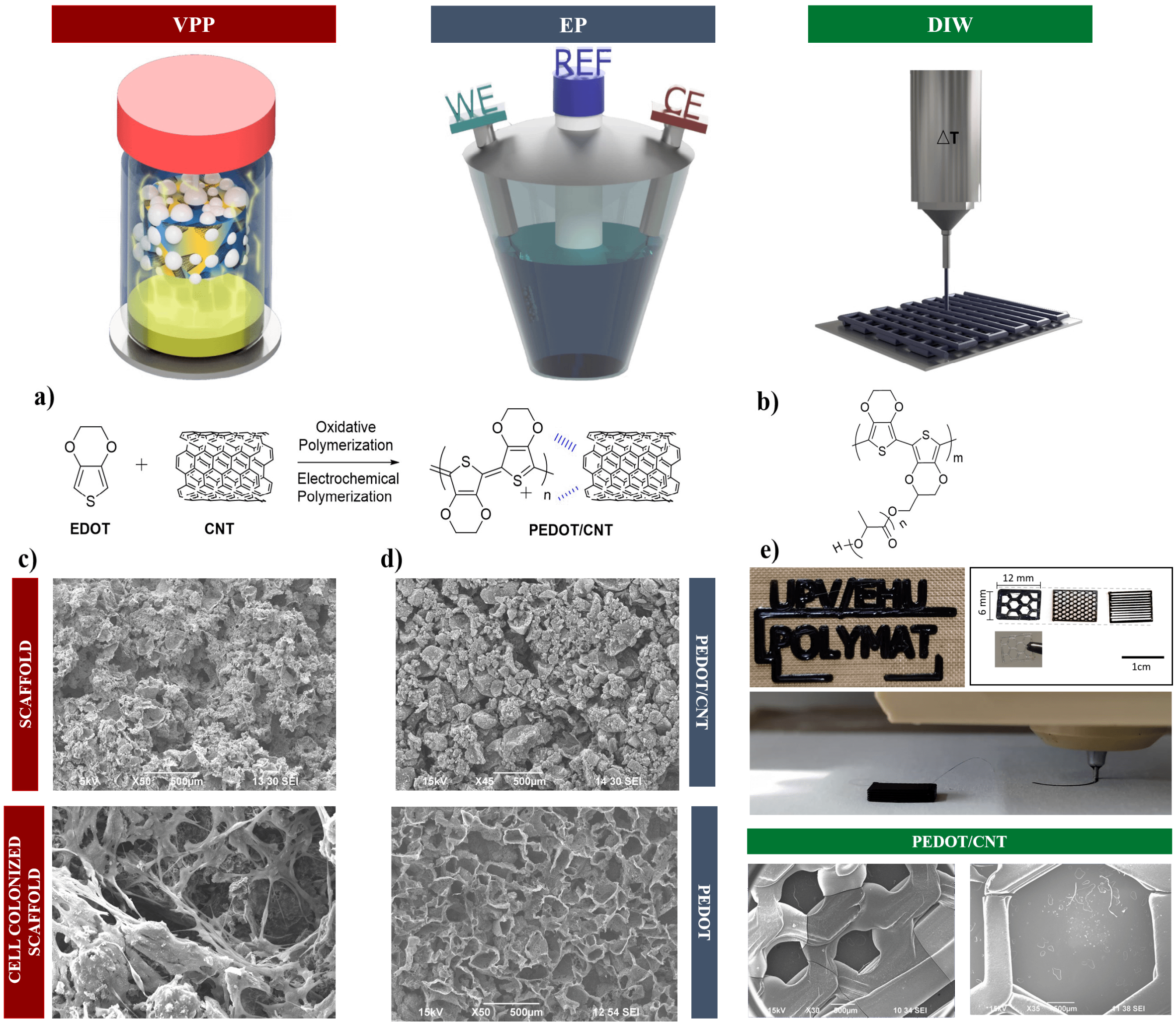

Figure 1. Schematic representation of the three methods: VPP, EP and 3D printing by DIW. a) Reaction of polymerization for VPP and EP methods, b) PEDOT-*graft*-PLA polymer designed and synthesized for 3D printing, c) SEM images of a porous scaffold (top) and the same porous scaffold colonized with C8-D1A cells (bottom), d) SEM images comparing PEDOT/CNTs (top) and PEDOT scaffolds (bottom) manufactured by EP and e) 3D printed PEDOT-*graft*-PLA structures (top) and SEM images of two different hexagonal patterns of different sizes (bottom).

Moreover, the design of a family of tailored conducting and biocompatible PEDOT-*graft*-poly(L-lactic acid) (PLA) co-polymers for direct ink writing (DIW) was also conducted. The macromonomer used was synthesized by ring opening polymerization in bulk. Copolymers synthesized with different PEDOT percentages were characterized and evaluated throughout complex viscosity for 3D printing. Finally, the most suitable composition was 3D printed, forming different shapes with high resolution (above 200-300 µm) (**Figure 1e**). Lastly, these patterns were used for cell growth and maturation of neonatal cardiac myocytes co-cultured with fibroblasts. CNTs were also incorporated within the polymer matrix, improving stability and conductivity.

composed of conductive materials as PEDOT and CNTs. The materials herein manufactured possess controlled porosity, tailored conductivity and suitable mechanical properties for implants and 3D cellular culturing of electroactive cells. In this regard, all the scaffolds presented good biocompatibility and functionality with cells throughout maturation and differentiation.

Related Publications

[1] Dominguez-Alfaro A, Alegret N, Gomez IJ, Mecerreyes D, Prato M. 2D and 3D Immobilization of Carbon Nanomaterials Throughout PEDOT Derivative Co-Polymerization. Polymers 2021; 13(3):436

[2] Domínguez-Alfaro A, Gabirondo E, Alegret N, León C, Hernández R, Vallejo-Illarramendi A, Prato M, Mecerreyes D. 3D Printable Conducting and Biocompatible PEDOT-graft-PLA co-polymers by Direct Melting Extrusion. Macromol Rapid Commun 2021; 2100100

[3] Dominguez-Alfaro A, Alegret N, Arnaiz B, Salsamendi M, Mecerreyes D, Prato M. Towards Spontaneous Neuronal Differentiation of SH-SY5Y Cells using Novel 3D Electropolymerized Conductive Scaffolds. ACS Appl Mater Interfaces 2020; 12(51):57330–57342

[4] Alegret N, Dominguez-Alfaro A, Mecerreyes D. Conductive Polymers for Tissue Engineering, in Redox Polymers for Energy and Nanomedicine 2020, The Royal Society of Chemistry, 2021; pp 383–414.

[5] Dominguez-Alfaro A, Alegret N, Arnaiz B, González-Domínguez JM, Martin-Pacheco A, Cossío U, Porcarelli L, Bosi S, Vázquez E, Mecerreyes D, Prato M. Tailored Methodology Based on Vapor Phase Polymerization to Manufacture PEDOT/CNT Scaffolds for Tissue Engineering. ACS Biomater Sci Eng 2020; 6(2):1269–1278.

[6] Alegret N, Dominguez-Alfaro A, Mecerreyes D. 3D Scaffolds Based on Conductive Polymers for Biomedical Applications. Biomacromolecules 2019; 20(1):73–89.

[7] Alegret N, Dominguez-Alfaro A, González-Domínguez JM, Arnaiz B, Cossío U, Bosi S, Vázquez E, Ramos-Cabrer P, Mecerreyes D, Prato M. Three-Dimensional Conductive Scaffolds as Neural Prostheses Based on Carbon Nanotubes and Polypyrrole. ACS Appl Mater Interfaces 2018; 10(50):43904–43914.

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Full Thesis can be downloaded from *www.ehu.eus/es*

Antonio Dominguez-Alfaro obtained his Industrial and Chemical Engineer degree from the University of Huelva in 2015. His PhD was carried out at the research centers CIC biomaGUNE and POLYMAT in the University of Basque Country (UPV/ehu), and it was defended in 2021. He worked under the supervision of professors David Mecerreyes and Maurizio Prato. The PhD work was based on the development of porous materials composed of carbon nanotubes and conducting polymers for biomedical applications. Besides, during his PhD he carried out an internship at Rice University (Houston, Texas, in 2018). In 2021, Dominguez-Alfaro joined the Biomolecular Nanotechnology Lab led by Aitziber Cortajarena, and he is working in the development of new conducting hybrid materials.