chemistry proceedings

MDPI

Proceeding Paper

Combining Oligothiophene with Oligo-(D, L-Lactide) into a Complex, Branched Topology Toward a Functional Interface Aimed at Biomedical Applications [†]

Anca-Dana Bendrea [1,*], Natalia Simionescu [1], Elena-Gabriela Hitruc [2], Luminita Cianga [1], Ioan Cianga [1] and Mariana Pinteala [1]

[1] Centre of Advanced Research in Bionanoconjugates and Biopolymers, "Petru Poni" Institute of Macromolecular Chemistry 41 A, Grigore GhicaVoda Alley, 700487 Iasi, Romania; natalia.simoinescu@icmpp.ro (N.S.); lcianga@icmpp.ro (L.C.); ioanc@icmpp.ro (I.C.); pinteala@icmpp.ro (M.P.)

[2] Department of Physics of Polymers and Polymeric Materials, "Petru Poni" Institute of Macromolecular Chemistry, 41 A, Grigore-GhicaVoda Alley, 700487 Iasi, Romania; gabihit@icmpp.ro

[*] Correspondence: anca.bendrea@icmpp.ro

[†] Presented at The 28th International Electronic Conference on Synthetic Organic Chemistry (ECSOC 2024), 15–30 November 2024; Available online: https://sciforum.net/event/ecsoc-28.

Abstract: The focus of this study is on those properties of a new oligothiophene grafted with oligo-(D, L-lactide) side chains (**OTh-PDLLA**) that enable to establish its prospect as a biomedical material. In this vein, **OTh-PDDLA** self-assembling capability in various solvents was established by measuring particle size using dynamic light scattering. AFM investigation of the surface topography of the films obtained from several solvents, as well as the results of the interaction with normal human gingival fibroblast (NHGF) cells showed that **OTh-PDLLA** offers several ways to modulate topographically the films surface, which allows an easy adjustment of interactions with biological entities.

Keywords: biomaterial; grafted-conjugated polymers**;** oligothiophene; polylactide; bioelectrical interfaces

Citation: Bendrea, A.-D.; Simionescu, N.; Hitruc, E.-G.; Cianga, L.; Cianga, I.; Pinteala, M. Combining Oligothiophene with Oligo-(D, L-Lactide) into a Complex, Branched Topology Toward a Functional Interface Aimed at Biomedical Applications. *Chem. Proc.* **2024**, *6*, x. https://doi.org/10.3390/xxxxx

Academic Editor(s): Name

Published: 15 November 2024

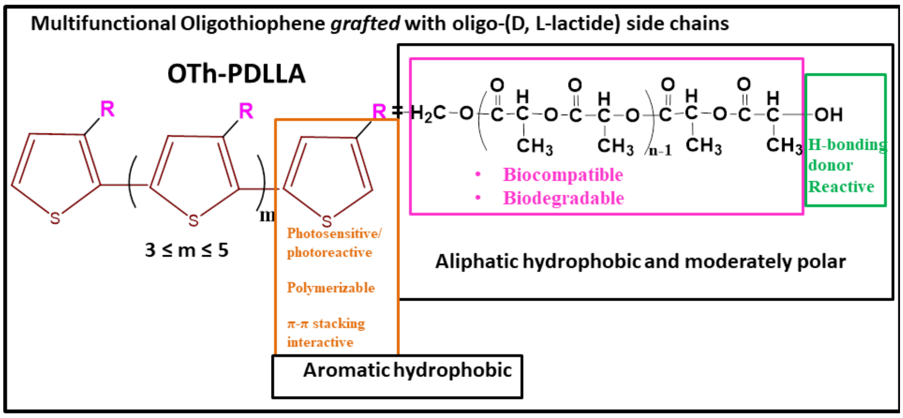
1. Introduction

Conducting, π-conjugated polymers (CPs), serendipitous discovered by Shirakawa's group 50 years ago [1] proved to be complex materials 'equipped" with appropriate tools for effectively bridging the interface between electronics and biology [2]. As such, their unique and efficient ion-to-electron conversion combined with their ability to work in physiologically relevant media, their mechanical biomimicry and biocompatibility already place CPs as the obvious member of synthetic polymers-based biomedical materials to directly interact and/or interfacing with complex biological system [3].

The progress in bioelectronics area requires for sophisticated materials that will assure not only the electrical communication with the biological entities, but also the conformability on non-flat surfaces and/or transience behavior [4]. One of the alternatives to such materials can be graft-CPs (or "hairy-rod" CPs) that has a "rod-*graft*-coil" architecture. Such topology offers, in addition to the freedom of various combinations of chemistries, also the opportunity to program, optimize and control, from the molecular design stage, the processing-structure-properties relationship and the self-assembly pathway [5]. In particular, conjugated polythiophene and its derivatives *grafted* with flexible, biocompatible and functional side chains showed their applicability as active surfaces for selective proteins adsorption [6], as excellent biocompatible and electroactive cellular matrices

[7–9]. Their applicability in cell imaging [10] or for sensing of plethora of bioanalytics [11–13] has been also exploited.

Considering of today importance of electroactive biomaterials' biodegradability in particular of those based on PTh, we recently, reported about synthesis and structural characterization of an oligothiophene grafted with oligo-(D, L-lactide) side chains (denoted as **OTh-PDLLA**) [14]. The environmentally friendly, poly (lactic acid) (PLA) is biocompatible, biodegradable, sustainable and non-cytotoxic aliphatic polyester approved by FDA to be used in medical science and biotechnology [15]. Engineered to function as an efficient bioelectronics interface, being also potentially biocompatible and bioerodible, **OTh-PDLLA** (Scheme 1) was synthesized by "grafting through" approach via *metal-free* photoinduced oxidative homopolymerization. The "grafting through" strategy allows for engineering both the rigid conjugated backbone and flexible side chains, while the photo-mediated polymerization of the Th-PDLLA macromonomer was an original, successful approach. Both, the structural dissymmetry and particular topology seems to induce unusual features of the new synthesized oligomer [14]. The present report focuses on the study of those properties of **OTh-PDLLA** that allow to establishing its suitability as a biomaterial. Thus, its capability for forming thin films, on either rigid or flexible supports, using for processing solvents with different polarities and variable concentrations and films surface properties were explored.



Scheme 1. Design criteria for the synthesis of the multifunctional **OTh-PDLLA.**

2. Materials and Methods

Particles size measurement was carried out by dynamic light scattering (DLS) using a Malvern Zetasizer Nano ZS instrument equipped with a 4.0 mW He–Ne laser operating at 633 nm and a detection angle of 173°. The intensity weighted mean hydrodynamic size (Z average) and the polydispersity factor were obtained from analysis of the autocorrelation function. Samples were used as prepared, without filtration at a two different concentration, c = 10 mg/mL and c = 1 mg/mL, respectively, in different solvents. The reported values represent the average of three measurements made for each sample, at 25 °C with an equilibration time of 5 min before starting each measurement. **The wettability** of all the surfaces was investigated by measuring the static contact angle using a DataPhysics OCA 15 (DataPhysics GmbH, Filderstadt, Germany) optical contact angle goniometer. The contact angle was determined under ambient condition within 5 s after placing 1 μL drops of deionized water on the sample's surface. The images of the water droplets were recorded and analyzed using the software provided by the manufacturer to determine the contact angle values. At least three measurements (five contact angle values) were performed at randomly selected locations across the sample's surface. **AFM micrographs** of the polymers films were taken in air, on a SPM SOLVER Pro-M instrument. A NSG10/Au Silicon tip with a 35 nm radius of curvature and 255 kHz oscillation mean frequency was used. The apparatus was operated in semi-contact mode, over a 10 × 10 μm² scan area, 256

× 256 scan point size images being thus obtained. The polymers films were prepared by drop casting on round glass slides and PLA films as substrates. For **the biocompatibility** evaluation MTS assay by the direct contact procedure adapted from ISO 10993-5:2009(E) was used. First, each specimen of the test samples was carefully placed into 24-well tissue culture-treated plate and sterilized by UV-radiation for 15 min and then incubated in complete cell culture medium (MEM α medium with 10% fetal bovine serum and 1% Penicillin-Streptomycin-Amphotericin B mixture) in 24-well tissue-culture-treated plates for 1 h. For the in vitro biocompatibility assessment (direct contact method), human gingival fibroblasts (HGF, CLS Cell Lines Service GmbH, Eppelheim, Germany) were seeded into 24-well plates and allowed to adhere, then samples were placed in the wells and incubated for 24 h and 72 h. After incubation, cell viability was determined using the CellTiter 96® AQueous One Solution Cell Proliferation Assay (Promega, Madison, WI, USA), according to the manufacturer instructions. Absorbance readings were performed on a FLUOstar® Omega microplate reader (BMG LABTECH, Ortenberg, Germany). Experiments were done in triplicate and treated cell viability was expressed as percentage of Control cells' viability (means ± standard deviation).

3. Results and Discussion

Designed as a multifunctional material, **OTh-PDLLA** has encoded in its structure all the "ingredients" that grants it to function as electroactive biointerface or as active layer in flexible and/or implantable transient electronics. As such, its capability for forming thin films, on either rigid or flexible supports compatible with biological milieu and the surface features with direct impact on biological events of the formed films, were investigated by contact-angle measurements, atomic force microscopy(AFM) or dynamic laser-scattering (DLS). Despite the fact that polymeric films obtained by drop-cast technique could show heterogeneities on the film thickness, we choose this method for the **OTh-PDLLA** thin films fabrication because it allows the slow solvent evaporation into a solvent saturated atmosphere, enabling the polymer's self-assembling. As such, **OTh-PDLLA** dispersions (10 mg/mL and 1 mg/mL) in chloroform (Chl) and in dimethyl sulfoxide (DMSO), respectively were casted on either rigid surface of the round glass slides or flexible, biocompatible and bioerodible PLA sheets. Regarding the solvents used in this study several aspects, like Hansen solubility parameters, Flory-Huggins solvent-polymer interaction parameters [16–19], as well as pharmaceutical nature and therapeutic properties of DMSO [20] were taken into consideration. Thus, while Chl could be considered a good solvent for both conjugated main chain and PDLLA side chains, DMSO is PDLA selective, having the lowest value of the interaction parameter [19] and the best fit of the HS parameters with those of PDLLA [16]. Indeed, all the surface features of **OTh-PDLLA** with direct impact on its performance as biomaterial, like surface chemistry, wettability, or morphology were found to be solvent and concentration-dependent. Thus, solvent polarity had more influenced on size and morphology of the oligomers 'self-assembled structures than on its wettability (Table 1, Figures 1 and 2). Unimodal DLS traces (not showed) and almost identical value of the hydrodynamic radii (Dh) of the formed particles were registered by DLS measurement of the **OTh-PDLLA** dispersions in Chl with different concentrations. As Chl is a mutual solvent for both constitutive parts of **OTh-PDLLA**, most probable their shape dissimilarity is inducing its self-assembling in this solvent. A mixed-population of particles, having 600 nm and 5100 nm, respectively was formed in the PDLLA selective solvent DMSO. The highest value, of 5100 nm could be due (associated with the presence of) either to particles clusters [21], or (with) to the presence of particles with non-spherical geometries [22].

Table 1. Hydrodynamic radii, water contact angle, surface roughness of **OTh-PDLLA** films and particles size in dry state measured by AFM.

Sample	Particle Size by DLS (nm)	Water Contact Angle (Degree) of Oligomer's Films Deposited on		[2] Roughness by AFM (nm)		Particle Size by AFM (nm)
		Glass [1] (78)	PLA [1] (82)	Glass (2.55)	PLA (36.5)	Glass
OTh-PDLLA DMSO, 10 mg/ml	600; 5100	83	80	53	73	Width = 600, height = 40
OTh-PDLLA CHCl$_3$, 10 mg/ml	947	85	-	15	-	600
OTh-PDLLA CHCl$_3$, 1 mg/ml	934	74		41	-	450

[1] Values of water contact angle for uncovered supports; [2] Calculated for 10 × 10 μm^2 area scanning;.

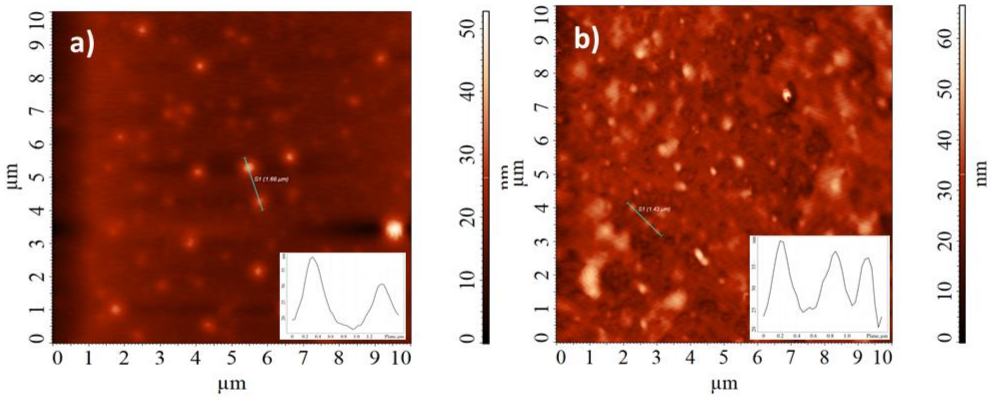


Figure 1. AFM images for **OTh-PDLLA** films obtained from its dispersion in Chl at c = 10 mg/mL (**a**) and c= 1 mg/mL (**b**).

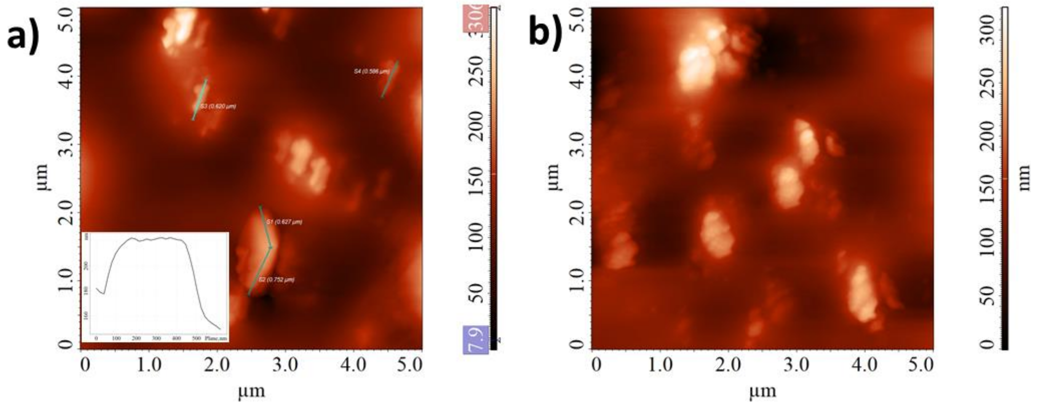


Figure 2. AFM images for **OTh-PDLLA** films obtained from its dispersion in DMSO at c = 10 mg/mL deposited on glass (**a**) and PLA film (**b**).

Following the dictum "seeing is believing" the AFM images confirmed that the self-assembled structures of **OTh-PDLLA** in Chl (Figure 1), "sensed" by DLS, are round in shape with average size of around 600 nm (for Chl solution concentration of 10 mg/mL) and 450 nm (for concentration of 1 mg/mL), arranged as a uniformly scattered network. A similar morphology was observed for PEDOT-co-PDLLA films obtained by the slowly cast of chloroform PEDOT-co-PDLLA (1:05, 1:25, and 1: 50) solutions on the gold mylar substrates [23].

A completely different, sophisticated morphology was noticed for the films of **OTh-PDLA** from DMSO on glass slides or on PLA sheets (Figure 2). Thus, depending on the nature of the used support, nanorods of 600 nm in width with bending propensity (Figure 2a) or twist supermolecular nanostructures (Figure 2b) were observed. Beside the inter-particles interaction on the one hand and the particles-support interactions on the other hand, there are other important aspects like solvophobic/solvophilic interactions, or the already mention shape dissimilarity of the oligomer constitutive parts, as well as the thiophene rings 'tendency for π-π stacking during aggregation, that could contribute to formation of the observed morphologies [5,18,24].

Due to its important role on cellular response, the film's wettability was evaluated by the measuring the water contact angle (WCA) outlined in Table 1. The WCA of bare substrates were also registered, the found values being consistent with those already reported in literature [25]. The investigated **OTh-PDLLA** films are quite hydrophobic as the average values of WCA are between 74 and 85°. The films obtained from DMSO and from Chl showed almost the same value of WCA (83° and 85°, respectively), which means that neither the polarity of the solvent nor the roughness of films had no influence on surfaces wettability. On the other hand, a decrease of films hydrophobicity with concentration decreasing was registered (Table 1). This concentration-dependent wettability is a very important feature from the prospect of the synthesized oligomer's applicability as biomaterial. In this scenario, the effects of the **OTh-PDLLA** films surface features on the normal human gingival fibroblasts (NHGF) viability were further evaluated. It has been reported that the attachment and proliferation of these cells are sensitive to the surface topography, as well to the surface hydrophobicity [26], having a maximum adhesion on surfaces with WCA values between 60 and 80° [27]

In the present report, the NHGF viability, at 24 and 72 h (Figure 3) was evaluated by MTS assay based on the mitochondrial activity of cells in direct contact with the material surfaces.

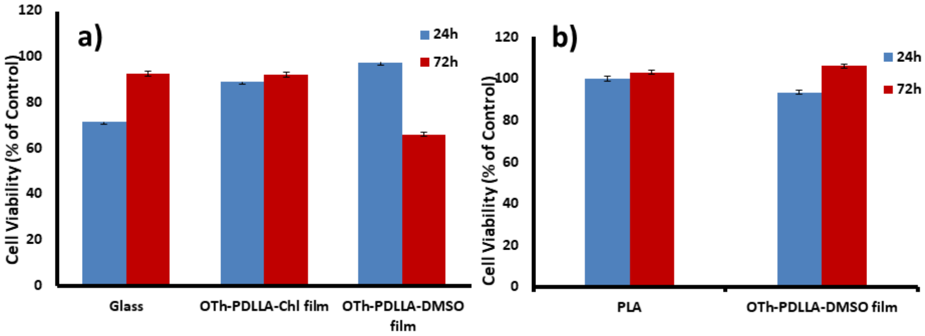


Figure 3. In vitro cell viability of NHGF seeded on **OTh-PDLLA** films obtained from its dispersion at c = 10 mg/mL deposited on glass (**a**) and PLA film (**b**).

The obtained data showed the good biocompatibility and non-cytotoxic effect of the **OTh-PDLLA**, highlighting the impact of the films topographic properties on cellular behavior. As such, keeping constant the concentration of **OTh-PDLLA** in the used solvents and also the rigidity of the films' support, the viability of NHGF after 24 h was higher for the **OTh-PDLLA**-coated glass slides by comparing to uncovered glass (Figure 3a). A slightly increase in the cell viability value for the DMSO-casted films compared with those obtained from Chl solution was noticed, as well. However, after 3 days of cells-polymers direct contact, the viability of the cells seeded onto the DMSO-casted films decreased considerably, while a very good viability, (around 90%) was registered for the cells seeded onto Chl-films. As the tested films have similar values of the WCA, the smoother Chl-films surface, measured directly from AFM topographic scans (Table 1), seems to support better the NHGF cells growth. Regarding the influence of the surface roughness of the films, it seems that this parameter is neutral if we take into account the cells viability seeded on either uncovered or **OTh-PDLLA-**covered PLA sheets (Figure 3b). Analyzing the NHGF viability for the DMSO-casted films it can assumed that the particles shape modulates the cellular behavior.

4. Conclusions

Understanding the structure –property relationship is the “key” to control morphology which is the functions origin of any (bio) material. In this context, the present study showed that **OTh-PDLLA** offer several ways to modulate films surface topography properties in order to adjust their interactions with biological entities as proteins or cells. Employing techniques like dynamic laser-scattering (DLS), contact- angle measurement and atomic force microscopy (AFM) changes in the in particle’s size, in wettability and films surface topography were investigated. Notably, keeping constant the nature of solvent but varying solution’s concentration the films roughness can be varied, while solvent polarity has more influence in films morphology than in their wettability. Using MTS assay the oligomer’s biocompatibility was confirmed, this being the first that advocate for **OTh-PDLLA** potential as electroactive biointerface or as active layer in flexible and/or implantable transient electronics.

Author Contributions: The manuscript was written through contributions of all authors. All authors have read and agreed to the published version of the manuscript.

Funding:

Institutional Review Board Statement:

Informed Consent Statement:

Data Availability Statement:

Conflicts of Interest: The authors declare no conflicts of interest.

References

1. Müllen, K.; Scherf, U. Conjugated Polymers: Where We Come from, Where We Stand, and Where We Might Go. *Macromol. Chem. Phys.* **2023**, *224*, 2200337.
2. Zeglio, E.; Rutz, A.L.; Winkler, T.E.; Malliaras, G.G.; Herland, A. Conjugated Polymers for Assessing and Controlling Biological Functions. *Adv. Mater.* **2019**, *31*, 1806712.
3. Inal, S.; Rivnay, J.; Suiu, A.-O.; Malliaras, G.G.; McCulloch, I. Conjugated polymers in bioelectronics. *Acc. Chem. Res.,* **2018**, *51*, 1368.
4. Morsada, Z., Hossain, M.M.; Islam, M.T.; Mobin, M.A.; Saha, S. Recent progress in biodegradable and bioresorbable materials: From passive implants to active electronics. *App. Mat. Today.* **2021**, *25*, 101257.
5. Hicks, G.E.J.; Li, S.; Obhi, N.K.; Jarrett-Wilkins, C.N.; Seferos, D.S. Programmable Assembly of π-Conjugated Polymers. *Adv. Mater.* **2021**, *33*, 2006287.
6. Bendrea, A.-D.; Fabregat, G.; Cianga, L.; Estrany, F.; del Valle, L.J.; Cianga, I.; Aleman, C. Hybrid materials consisting of an all-conjugated polythiophene backbone and grafted hydrophilic poly(ethylene glycol) chains. *Polym. Chem.* **2013**, *4*, 2709–2723.
7. Molina, B.G.; Bendrea, A.-D.; Lanzalaco, S.; Franco, L.; Cianga, L.; del Valle, L.J.; Puiggali, J.; Turon, P.; Armelin, E.; Cianga, I.; et al. Smart design for a flexible, functionalized an electroresponsive hybrid platform based on poly(3,4 ethylenedioxythiophene) derivatives to improve cell viability. *J. Mater. Chem. B* **2020**, *8*, 8864–8877.
8. Bendrea, A.-D.; Fabregat, G.; Torras, J.; Maione, S.; Cianga, L.; del Valle, L.J.; Cianga, I.; Aleman, C. Polythiophene-g-poly(ethylene glycol) graft copolymers for electroactive scaffolds. *J. Mater. Chem. B* **2013**, *1*, 4135–4145.
9. Maione, S.; Fabregat, G.; del Valle, L.J.; Bendrea, A.-D.; Cianga, L.; Cianga, I.; Estrany, F.; Aleman, C. Effect of the Graft Ratio on the Properties of Polythiophene-g-poly(ethylene glycol). *J. Polym. Sci. Part B Polym. Phys.* **2015**, *53*, 239–252.
10. Cianga, L.; Bendrea, A.-D.; Fifere, N.; Nita, L.E.; Doroftei, F.; Ag, D.; Seleci, M.; Timur, S.; Cianga, I. Fluorescent micellar nanoparticles by self assembly of amphiphilic, nonionic and water self-dispersible polythiophenes with “hairy rod” architecture. *RSC Adv.* **2014**, *4*, 56385–56405.
11. Molina, B.G.; Bendrea, A.D.; Cianga, L.; Armelin, E.; del Valle, L.J.; Cianga, I.; Alemán, C. The biocompatible polythiophene-g-polycaprolactone copolymer as an efficient dopamine sensor platform. *Polym. Chem.* **2017**, *8*, 6112–6122.

12. Molina, B.G.; Cianga, L.; Bendrea, A.-D.; Cianga, I.; Alemán, C.; Armelin, E. An amphiphilic, heterografted polythiophene copolymer containing biocompatible/biodegradable side chains for use as an (electro)active surface in biomedical applications. *Polym. Chem.* **2019**, *10*, 5010–5022.
13. Demir, B.; Yilmaz, T.; Guler, E.; Pinar Gumus, Z.; Akbulut, H.; Aldemir, E.; Coskunol, H.; Colak, D.G.; Cianga, I.; Yamada, S.; et al. Polypeptide with electroactive end groups as sensing platform for the abused drug 'methamphetamine' by bioelectrochemical method. *Talanta* **2016**, *161*, 789–796.
14. Bendrea, A.D.; Cianga, L.; Göen Colak, D.; Constantinescu, D.; Cianga, I. Thiophene End-Functionalized Oligo-(D,L)-Lactide as a New Electroactive Macromonomer for the "Hairy-Rod" Type Conjugated Polymers Synthesis. *Polymers* **2023**, *15*, 1094.
15. Davachi, S.M.; Kaffashi, B. Polylactic Acid in Medicine. *Polym.-Plast. Technol.* **2015**, *54*, 944–967.
16. Sato, S.; Gondo, D.; Wada, T.; Kanehashi, S.; Nagai, K. Effects of various liquid organic solvents on solvent-induced crystallization of amorphous poly(lactic acid) film. *J. Appl. Polym. Sci.* **2013**, *129*, 3.
17. Hansen, C.M.; Smith, A.L. Using Hansen solubility parameters to correlate solubility of C60 fullerene in organic solvents and in polymers. *Carbon* **2004**, *42*, 1591–1597.
18. Kim Tremel, K.; Ludwigs, S. Morphology of P3HT in Thin Films in Relation to Optical and Electrical Properties. *Adv. Polym. Sci.* **2014**, *265*, 39–82.
19. Orwoll, R.A.; Arnold, P.A. Polymer–Solvent Interaction Parameter χ. In *Physical Properties of Polymers Handbook*; Mark, J.E., Eds.; Springer, New York, NY, USA, 2007. https://doi.org/10.1007/978-0-387-69002-5_14.
20. Hoang, B.X.; Han, B.; Fang, W.H.; Tran, H.D.; Hoang, C.; Shaw, D.G.; Nguyen, T.Q. The Rationality of Implementation of Dimethyl Sulfoxide as Differentiation-inducing Agent in Cancer Therapy. *Cancer Diagn. Progn.* **2023**, *3*, 1–8.
21. Bendrea, A.-D.; Cianga, L.; Ailiesei, G.-L.; Ursu, E.-L.; Colak, D.G.; Cianga, I. 3,4-Ethylenedioxythiophene (EDOT) End-Group Functionalized Poly-ε-caprolactone (PCL): Self-Assembly in Organic Solvents and Its Coincidentally Observed Peculiar Behavior in Thin Film and Protonated Media. *Polymers* **2021**, *13*, 2720.
22. Bhattacharjee, S. DLS and zeta potential—What they are and what they are not? *J. Control. Release* **2016**, *235*, 337–351.
23. Da Silva, A.C.; Higgins, M.J.; Cordoba de Torresi, S.I. The effect of nanoscale surface electrical properties of partially biodegradable PEDOT-co-PDLLA conducting polymers on protein adhesion investigated by atomic force microscopy. *Mater. Sci. Eng. C* **2019**, *99*, 468–478.
24. Mishra, A.; Ma, C.Q.; Bauerle, P. Functional oligothiophenes: Molecular design for multidimensional nanoarchitectures and their applications. *Chem. Rev.* **2009**, *109*, 1141–1276.
25. Khakalo, A.; Filpponen, I.; Rojas, O.J. Protein adsorption tailors the surface energies and compatibility between polylactide and cellulose nanofibrils. *Biomacromolecules* **2017**, *18*, 1426–1433.
26. Aktas, O.C.; Metzger, W.; Haidar, A.; Açil, Y.; Gülses, A.; Wiltfang, J.; Marques Sacramento, C.; Nothdurft, F.P. Enhancing adhesion and alignment of human gingival fibroblasts on dental implants. *J. Cranio-Maxxillo-Facial Surg.* **2019**, *47*, 661–667.
27. Tamada, Y.; Ikada, Y. Effect of Preadsorbed proteins on cell adhesion to polymer surfaces. *J. Coll. Interf. Sci.* **1993**, *155*, 334–339.