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# Photosensitive semiconducting polymer-incorporated nanofibers for promoting the regeneration of skin wound

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ABSTRACT

Photosensitive semiconducting polymer (SP) combined with light stimulation has shown the capability in promoting the proliferation of human dermal fibroblasts (HDFs). However, the high cytotoxicity of the used SP hindered its further application in bioactive scaffolds. In this contribution, we designed and synthesized a SP, poly (*N*,*N*-bis(2-octyldodecyl)-3,6-di(thiophen-2-yl)-2,5-dihydropyrrolo[3,4-*c*]pyrrole-1,4-dione-alt-thieno[3,2-*b*]thiophene) (PDBTT) with low cytotoxicity and strong absorbance in red and near-infrared region (600–1200 nm). The photosensitive SP was then applied in electrospun poly(ε-caprolactone) (PCL) nanofibrous scaffold and evaluated its proliferative effect on HDFs under the illumination from red light-emitting diode (LED) with high tissue penetration. After 9 days of continuous stimulation, the hybrid electrospun PCL/PDBTT nanofibers with low cytotoxicity showed excellent support for HDFs adhesion, proliferation and collagen secretion than neat PCL nanofibers and HDFs on the stimulated PCL/PDBTT nanofibers gained typical spindle morphology, indicating the well cell spreading on the stimulated PCL/PDBTT nanofibers. The incorporation of functional materials within synthetic biomaterials could be a novel way in improving the performance of engineered tissue constructs by providing multiple cues (*e.g.* electrical stimulation) to the attached cells.



## 1. Introduction

Human skin is the largest organ in the body, which serves as a protective barrier of our body to the outside world [1]. However, when skin gets injuries (such as burns, chronic wounds or excision), it will lose its protective functions against pathogens [2]. Therefore, skin wounds must be rapidly and efficiently mended. Although autograft remains the standard of wound care, insufficient availability of autografts complicates wound closure and increases the risk of infection in patients with large sized wounds [3]. The development of tissue engineered skin grafts, which usually incorporate collagen and other natural proteins, has gained popularity with its ability to assist in skin restoration *via* promoting cell adhesion, proliferation, differentiation, and delivering bioactive molecules [4–8]. However, the fragile natural proteins within tissue-engineered skin grafts limit their wide applications, due to the high cost and short shelf-life [9]. As a result, materials with low cost and good durability are highly desired for developing advanced skin grafts. Electrical stimulation (ES) was found to promote the proliferation of a variety of cells from skin, bone, heart and muscle [10–13] and ES can significantly increase the rate of wound epithelialization and proliferation of fibroblasts [14] by improving the expression of receptors for transforming growth factor-β (TGF-β) [15]. Recently, systems based on biodegradable and semiconducting polymers (SPs) constitute one of the most promising solutions to develop advanced materials that enable to deliver electrical stimulation to desired tissue and stimulate the proliferation and differentiation of various cell types [16–18]. Therefore, SPs that represents a class of smart polymeric materials hold great promises in fabricating highly selective, biocompatible and economic biomedical devices [19]. Specially, photosensitive SPs combined with light stimulation can realize temporally controllable and sustainable stimulus to achieve precise control in delivering stimulation to promote cell proliferation [20,21]. One of the photosensitive SPs, poly(3-hexylthiophene) (P3HT) has been incorporated in biodegradable nanofibers to promote the proliferation of HDFs under light stimulation. However, the high toxicity of P3HT greatly decreased the proliferation of HDFs in non-stimulated condition. Moreover, the max absorbance of P3HT is around 465 nm, while light around this wavelength will mainly absorbed by the main tissue chromophores (hemoglobin and melanin) [22], thus the effect of light on the photosensitive polymer will be greatly reduced [23].

In this contribution, we designed a photosensitive SP, poly (*N*,*N*-bis (2-octyldodecyl)-3,6-di(thiophen-2-yl)-2,5-dihydropyrrolo[3,4-*c*]pyrrole-1,4-dione-*alt*-thieno[3,2-*b*]thiophene) (PDBTT), with high stability and strong absorbance above 600 nm. We further electrospun the

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PDBTT incorporated PCL nanofibers to study the proliferative effect of PCL/PDBTT nanofibers on HDFs under red LED illumination. The low cytotoxicity of PCL/PDBTT nanofibers was confirmed by the MTT assay, and the proliferation of HDFs on the stimulated PCL/PDBTT nanofibers was significantly higher as compared to the proliferation of HDFs on the non-stimulated neat PCL nanofibers. Moreover, F-actin staining revealed the typical elongated morphology of HDFs on the stimulated PCL/PDBTT nanofibers, indicating the sufficient cell-to-cell communications on the stimulated PCL/PDBTT nanofibers. The results support that the designed photosensitive and conductive PDBTT could be applied as functional material with low cost and high durability in skin tissue engineering.

## 2. Materials and methods

### 2.1. Materials

Poly(ε-caprolactone) PCL (M.W. 80,000), chloroform, Sirius red, methanol and dimethyl sulfoxide (DMSO) were all obtained from Sigma-Aldrich, Singapore and used as received. Dulbecco's modified eagle's medium (DMEM), fetal bovine serum (FBS), penicillin-streptomycin solution and 4′,6-diamidino-2-phenylindole, dihydrochloride (DAPI) and Alexa Fluor® 633 phalloidin were all purchased from Life Technologies, Invitrogen, Singapore.

### 2.2. Synthesis of PDBT-alt-TT

To a Schlenk flask was added 3,6-bis(5-bromothiophen-2-yl)-2,5-bis(2-octyl-1-dodecyl)pyrrolo[3,4-*c*]pyrrole-1,4-dione (0.2 mmol), 2,5-bis(trimethylstannyl)thieno[3,2-*b*]thiophene (0.2 mmol), tetrakis (triphenylphosphine)palladium (4 mol% equivalent) and toluene (20 mL) under a nitrogen atmosphere. The flask was securely sealed and stirred for 72 h at 110 °C. After cooling to room temperature, the reaction mixture was poured into a stirring mixture of methanol (100 mL) and concentrated hydrochloric acid (8 mL) and stirred for 16 h. The precipitated product was collected by filtration and subjected to consecutive Soxhlet extractions with ethanol, ethyl acetate and toluene. The toluene extraction was concentrated and precipitated in ethyl acetate, the solid was collected and dried under vacuum to obtain the final yields. 1H NMR (400 MHz, $CDCl_3$, $\delta$): $\delta$ 0.86 (t, 12H), 1.10–1.47 (m, 64H), 1.88 (s, 2H), 3.98 (d, 4H), 7.52 (d, 2H), 7.22 (d, 2H), 8.63 (d, 2H).

### 2.3. Characterization of the yield semiconducting polymer

Gel permeation chromatography (GPC) was carried out at 160 °C using a Polymer Labs PL 220 system with a refractive index detector and 1,2,4-trichlorobenzene as the eluent. 1H NMR data was performed on a Bruker DPX 400 MHz spectrometer with chemical shifts referenced to tetramethylsilane (TMS). Cyclic voltammetry (CV) experiments were performed using an Echochimie Autolab potentiostat (model PGSTAT30) in 0.1 M tetrabutylammonium hexafluorophosphate in dry acetonitrile at a scan rate of 100 mV/s. An Ag wire electrode, a platinum wire, and a glassy carbon disk were used as the reference electrode, counter electrode, and working electrode, respectively. The glassy carbon disk was coated with a polymer thin film by using a PDBTT polymer solution in chloroform. The HOMO and LUMO energy level of the polymer were calculated from the onset oxidation and reduction potentials, respectively, using ferrocene as reference ($E_{HOMO} = 4.8$ eV) [24].

### 2.4. Electrospinning of PCL/PDBTT nanofibers

PCL and PDBTT with a weight ratio 15:1 were dissolved in chloroform–methanol (75:25 v/v) to obtain 10% (w/v) solution. The polymer solution was fed into a 3 mL standard syringe attached to a 27G blunted stainless steel needle using a syringe pump (KDS 100, KD Scientific, Holliston, MA) at a flow rate of 1.0 mL/h. A high voltage of 15 kV (Gamma High Voltage Research, USA) was applied when the polymer solution was drawn into fibers and collected on an aluminum foil-wrapped collector kept at a distance of 12 cm from the needle tip. The obtained PCL/PDBTT nanofibers were collected on 15 mm cover slips and aluminum foil. After drying overnight under vacuum, the nanofibers were used for the characterization and cell culture experiments.

### 2.5. Characterization

UV–vis spectrum of the PDBTT solution was measured using a Shimadzu UV-2501PC UV–vis-NIR spectrophotometer after dispersing the PDBTT in chloroform and methanol (75:25 v/v) mixed solution. The morphology of the electrospun nanofibers was studied under scanning electron microscope (JEOL JSM-5600, Japan) at an accelerating voltage of 10 kV, after sputter coating with gold (JEOL JFC-1200 fine coater, Japan). Diameters of the electrospun fibers were analyzed from the scanning electron microscope (SEM) images using image analysis software (Image J, National Institutes of Health, USA). The J–V characteristics of the PCL/PDBTT nanofiber was recorded using a calibrated San-Ei XES-151 S (Japan) class A solar simulator under standard conditions (1 Sun, 1.5 G). Before the test, the FTO plate having the photosensitive nanofiber was pressed against an aluminum (Al)-sputtered FTO using binder clips and connected to the stimulator with wires. Hydrophilicity was determined by contact angle assessment machine (VCA optima™, AST Products Inc., USA). The water contact angles for samples of pure PCL and PCL/PDBTT nanofibers were measured by the sessile drop method. Drops of distilled water were deposited onto the surface of the samples and the direct microscopic measurement of the contact angles was done with the computer software. The water droplet was 200 μL to prevent a gravitational distortion of the spherical profile. The result of each sample was obtained by averaging 10 tests. The mechanical properties of the electrospun PCL and PCL/PDBTT nanofibers were studied using a tabletop tensile tester (Instron 3345, USA) at a load cell capacity of 10 N. The specimens with dimensions of 10 mm breadth × 20 mm length and a thickness of 70–80 μm, were prepared and stretched at a crosshead speed of 10 mm $min^{-1}$. Six specimens of individual scaffolds were tested and the results were obtained.

### 2.6. Cell culture

Human dermal fibroblasts (HDFs) used for this study was purchased from American Type Culture Collection, USA. HDFs were cultured in DMEM supplemented with 10% FBS and 1% penicillin–streptomycin solution in a 75 $cm^2$ cell culture flask. Cells were incubated at 37 °C in a humidified atmosphere containing 5% CO2 for 6 days and the culture medium was changed once every 3 days. The 15 mm cover slips with electrospun nanofibers were placed in 24 well plate and pressed with a stainless steel ring to ensure complete contact of the scaffolds with the wells. The specimens were sterilized under UV light, washed thrice with 1× phosphate-buffered saline (PBS) and subsequently incubated in DMEM overnight before cell seeding. HDFs were grown to 80% confluency, detached by trypsin, counted by trypan blue assay using a hemocytometer and seeded on the scaffolds at a density of 10,000 cells per well.

### 2.7. Light stimulation

To study the proliferative effect of PCL/PDBTT nanofibrous scaffold on HDFs under light stimulation, illumination of HDFs on PCL and PCL/PDBTT was performed using red light-emitting diodes (LEDs) with an emission wavelength of 635 nm. Light stimulation study was carried out for 9 days and HDFs on PCL and PCL/PDBTT were irradiated for 1 h once every 24 h. After culturing the cells for 3 and 9 days, cells were rinsed with 1× PBS to remove unattached cells and 500 μL of freshly prepared methylthiazolyldiphenyltetrazolium bromide (MTT)