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# Fabrication of multipotent poly-*para*-xylylene particles in controlled nanoscopic dimensions

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ABSTRACT

In this study, poly-*para*-xylylene-based multifunctional nanoparticles (PPX-NPs) were fabricated. Based on the solubility characteristics determined for asymmetrically substituted poly-*para*-xylylenes in polar solvents, well-dispersed nanocolloids with a controllable size ranging from 50 to 800 nm were produced in solution by the displacement of the solvent (water). These size ranges were found to have acceptable cellular compatibility through examinations of cultured 3T3 fibroblasts and adipose-derived stem cells treated with the PPX-NPs. In addition, these nanoscale PPX-NPs exhibited versatile bioconjugation properties in that a variety of available functional groups can be adopted from their counterpart, thin-film poly-*para*-xylylenes, during the production of these nanoparticles. For instance, bifunctional PPX-NPs with maleimide and benzoyl moieties were produced to enable immobilization via a maleimide-thiol reaction concurrent with a photochemical reaction. A cleavable PPX-NP was also produced with a thiol-exchangeable surface property. Additionally, by performing electrohydrodynamic jetting of parallel polymer solutions of selected poly-*para*-xylylenes, Janus-type or multicompartment PPX-NPs were created. The PPX-NPs can potentially be used for various biomedical applications such as combined diagnostics and drug delivery, multiplexing of detection, multiple-drug loading, and the targeted delivery of biomolecules or drugs.



## 1. Introduction

Research on nanoparticle technology has recently undergone unprecedented growth, which has spurred its widespread application in biotechnology, specifically in medicine. The nanoscale colloids used in nanomedicine are multifunctional and perform the simultaneous diagnosis and treatment of a disease. Specifically, targeted therapy has also been demonstrated. A wide range of related topics have been reviewed [1–6]. Several challenges still exist for these nanocarriers, including the inability to cross biological barriers, the prolongation of the blood-circulation half-life, diagnostic sensitivity, treatment efficacy, and the heterogeneity and drug resistance of tumors [7,8]. Although surface-modification approaches have shown some success in overcoming these challenges, the toxicity caused by the modification step and the low availability of various particles are still limiting [9]. A general protocol for the modification of the surfaces of these nanocarriers is of great interest. The ultrathin polymer of poly-para-xylylene (commercially known as parylene, which is approved by FDA for many applications) [10] has been modified with a wide variety of functional side groups, and the functionalized molecules have been used to manipulate functional compounds [11], biomolecules [12–14] and cellular responses [15–18]. These side groups have shown promise as modifications on various substrate materials [19–21]. An encouraged idea of transferring the proven modification and immobilization method by using these functional polymers from conventional substrates [22,23] to particles or colloids has been performed, and there has been success in producing multifunctional patchy surfaces for microscale colloids [24]. However, this method results in particle agglomerates when the particle aerodynamic diameter is on the nanometer scale [25]. In the present study, a direct synthesis route to produce functional PPX-NPs is introduced.

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## 2. Material and methods

### 2.1. CVD polymerization and copolymerization

Polymers of benzoyl-, maleimide-, and dithiodipropanic acid that were functionalized with the poly-para-xylylenes were prepared by CVD polymerization from 4-benzoyl [2,2]-paracyclophane [26], 4-N-maleimidomethyl-[2,2]paracyclophane [14], and 4-(3-((3-methylamido)disulfanyl)propanoic acid)-[2,2]paracyclophane [27], respectively. Summarized schemes of the synthesis routes for these substituted [2,2]paracyclophanes and the functionalized poly-para-xylylene are also included in the supporting information. During the CVD process, the starting materials of substituted paracyclophanes were sublimated under vacuum at 100–110 °C and were transformed into the reactive species by pyrolysis, which was kept at 810 °C (4-benzoyl [2,2]-paracyclophane), 580 °C (4-*N*-maleimidomethyl-[2,2]paracyclophane), or 550 °C (4-(3-((3-methylamido)disulfanyl)-propanoic acid)-[2,2]paracyclophane). Subsequently, the radicals were transferred into the deposition chamber with a wall temperature of 90 °C, and the polymerization occurred on a rotating and cooled sample holder at 15 °C. To optimize the deposition, a constant argon flow of 20 sccm was used, and a coating pressure of 75 mTorr was maintained throughout the CVD polymerization. The copolymer of poly[(4-*N*-maleimidomethyl-*p*-xylylene)-*co*-(4-benzoyl-*p*-xylylene)-*co*-(*p*-xylylene)] was prepared from 4-*N*-maleimidomethyl-[2,2]paracyclophane and 4-benzoyl-[2,2]paracyclophane via CVD copolymerization using a dual-sourced CVD installation. A 1:1 feeding ratio (molar) of 4-*N*-maleimidomethyl-[2,2]paracyclophane to 4-benzoyl-[2,2]paracyclophane was maintained during the CVD copolymerization process. The pyrolysis temperatures were 580 °C for 4-*N*-maleimidomethyl-[2,2]paracyclophane and 810 °C for 4-benzoyl-[2,2]paracyclophane. The spontaneous copolymerization formed the poly[(4-*N*-maleimidomethyl-*p*-xylylene)-*co*-(4-benzoyl-*p*-xylylene)-*co*-(*p*-xylylene)] on the substrates placed on top of the rotating and cooled (15 °C) sample holder in the deposition chamber. The system pressure was also maintained at 75 mTorr during the entire CVD copolymerization process.

### 2.2. Particle fabrication

Monofunctional and bifunctional PPX-NPs were prepared using a solubility-induced precipitation method according to previously reported procedures [28]. Polymer solutions of poly-benzoyl-*para*-xylylene, poly-maleimidomethyl-*para*-xylylene, or poly[(4-*N*-maleimidomethyl-*p*-xylylene)-*co*-(4-benzoyl-*p*-xylylene)-*co*-(*p*-xylylene)] were prepared in THF or acetone 0.5% [w/w]. The concentrations of the polymer solutions were adjusted to values ranging from 0.05 to 3 mg/ml. Subsequently, the resulting polymer solutions were added to 8 mL of deionized water (15 °C) under continuous and vigorous stirring by ultrasonication. Finally, the nanocolloidal suspension was placed in a vacuum desiccator for 30 min to partially evaporate the solvents. The nanocolloidal suspension was concentrated to a desired concentration by removing the water under identical conditions. Janus-type PPX-NPs were prepared using an electrified co-jetting system containing a syringe pump (New Era Pump Systems, Inc., USA) and a high-voltage supply (Simco-Ion, USA). Polymer solutions of poly-benzoyl-*para*-xylylene and poly-maleimidomethyl-*para*-xylylene were stored in separate syringes (Terumo Medical Corporation, USA) that were placed side by side. A double-sided needle (Hamilton Company, USA) was connected by the tip of the two syringes and further attached to the cathode of the high-voltage supply. During the electrified co-jetting process, the flow rate was controlled with a step size of 10 μl/h, and the electron voltage was controlled in the range of 5–20 kV. Aluminum foil was connected to the anode as a collecting substrate.

### 2.3. Particle characterization

The compositions of the PPX-NPs were verified using a Spectrum 100 FT-IR spectrometer (PerkinElmer, USA) that was equipped with a liquid nitrogen-cooled MCT detector and an AGA grazing angle specular reflectance accessory (Pike Technologies, USA). The spectra of the PPX-NPs were recorded and compared with the thin-film forms of the polymers. The dimension and shape of the PPX-NPs were examined using a Nova$^{TM}$ NanoSEM (FEI, USA) that was operated at a primary energy of 5 keV and with a pressure of $5 \times 10^{-6}$ Torr in the specimen chamber. The SEM samples were prepared by drying the nanoparticle suspensions onto a piece of silicon wafer and coating them with a conductive layer before the SEM analysis. The TEM images of the PPX-NPs were obtained using an H-7650 TEM (Hitachi, Japan) with an accelerating voltage of 75 keV. The TEM samples were prepared by dropping a droplet of the nanoparticle suspension onto a carbon film that was deposited on one side of a copper grid (300 mesh, Electron Microscopy Sciences, USA), followed by natural drying. The size distribution of the PPX-NPs was examined using a Zetasizer Nano ZS dynamic light scattering system (DLS, Malvern, UK) at 25 °C with water as the dispersant. Molecular weights and polydispersity indices (PDI) of the polymers were determined by gel permeation chromatography (GPC) using a Viscotek module-350 system with polystyrene as standard and THF as eluent. Confocal microscopy images of the fluorescent-labeled PPX-NPs were recorded using a TCS SP5 confocal laser scanning microscope (Leica Microsystems, Germany). An Ar/ArKr laser (wavelength 488 nm) and a HeNe laser (wavelength 633 nm) were used to observe FITC-RRRGD and Alexa Fluor® 633 RGDYCC. The detection wavelength range for each fluorescence emission was confined to 505–525 nm for FITC and 665–675 nm for Alexa Fluor® 633.

## 3. Bioconjugation

### 3.1. Benzophenone cross-conjugation

Folic acid (5 mg/mL, Sigma–Aldrich, USA) was first conjugated to the benzoyl moiety of the bifunctional PPX-NPs via a photoimmobilization process according to previously reported prodedures [13,26]. The reaction was performed using 365-nm UV irradiation (OmniCure, S1500, USA) for five minutes. Dialysis was performed three times with DI-water to remove the unreacted folic acid.

### 3.2. Thiol-maleimide coupling reaction

The fluorescent tag of FITC-RRCC (2 mg/mL, Yao-Hong Biotechnology, Taiwan) was reacted with the maleimide group of the bifunctional PPX-NPs via a thiol–maleimide coupling reaction [14] at 25 °C in aqueous solution (pH 7.4) for 2 h. The immobilization of thiol-terminated biotin (10 mM, Nanocs, USA) was reacted with the maleimide group by using the same reaction conditions for anisotropic PPX-NPs. The resulting nanocolloidal solution was centrifuged (14,000 rpm, three times using DI-water as a resuspension agent) to remove unreacted FITC-RRCC or thiol-biotin.

### 3.3. Amine-NHS ester coupling reaction

The molecule of FITC-RRRGD (2 mg/mL, Yao-Hong Biotechnology, Taiwan) was reacted with the NHS ester group on the anisotropic PPX-NPs via the amine-NHS ester coupling reaction [16] which was performed at 25 °C for 4 h. The resulting nanocolloidal solution was centrifuged (14,000 rpm, three times using