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Fabrication of functional nanofibers through post-nanoparticle functionalization

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Abstract

A facile method has been developed to functionalize nanofiber surfaces with nanoparticles through dithiocaramate chemistry. Gold nanoparticles (AuNPs) and quantum dots (QDs) have been immobilized on nanofiber surface. These surfaces provide scaffolds for further supramolecular functionalization, as demonstrated through FRET pairing of QD-decorated fibers and fluorescent proteins.

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Supporting Information

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Keywords

Dithiocarbamate; nanoparticles; nanofibers; immobilization; supramolecular structures

1. Introduction

Engineering the surface functionality of nanofibers provides new systems for materials science (e.g., textiles^[1] and solar cells^[2]) and biomedicine (e.g., biosensor^[3-5] and drug delivery^[6]). There are a number of strategies for modifying nanofiber surfaces, including plasma treatment,^[7] co-electrospinning,^[8,9] chemical modification^[10] and surface graft polymerization.^[11,12] However, these methods have limitations in terms of functional group density, where the plasma treatment cannot effectively modify the surface of buried nanofibers^[7,13] and grafting polymer on the nanofiber surface typically generates a low density of functional groups.^[14]

Integration of nanoparticles (NPs) into nanofibers provides a promising approach to provide dense functionalization of nanofibers with desired properties. Through the appropriate choice of NP core, NPs can incorporate their unique physicochemical properties to nanofibers such as magnetic and fluorescent properties.^[15,16] NP-nanofiber composites feature enhanced functionality and improved performance for nanofiber alignment, solar cells, and photocatalytic applications.^[17, 18]

NPs can be introduced to the nanofiber structures through direct synthesis and assembly of NPs,^[19,20] covalent conjugation^[21] and supramolecular interactions (e.g., hydrogen-bonding and electrostatic interactions).^[22,23] Dithiocarbamate (DTC) chemistry provides an alternative fabrication strategy that addresses the construction challenges of NP-nanofiber composites through N-C-S₂ group formation to generate stable self-assembled NPs on nanofibers. DTC chemistry has been employed to stabilize ligands on gold/semiconductor NP surface.^[24-26] Compared to other strategies, DTC immobilization provides a simple and robust technique to functionalize surface with positively, neutral, and negatively charged NPs with high stability in organic solvent and buffer solution. Furthermore, the NP immobilization reaction via DTC can be conducted in water/alcohol system, which provides a facile and eco-friendly fabrication process.^[27]

Here we present a facile approach to functionalize nanofibers through post-functionalization with NPs using DTC chemistry, using NPs as “paint” to provide dense functional groups on nanofiber surface. In the NP-nanofiber composites, amine-functionalized nanofibers were activated in the presence of CS₂ to generate DTC groups on the nanofiber surface, allowing the immobilization of gold NPs (AuNPs) and quantum dots (QDs) by displacing ligands on the NPs surface with dithiocarbamate group (Scheme 1).^[26] The resulting NP-nanofiber composites were characterized by electron microscopy and energy dispersive X-ray spectroscopy. The QD-functionalized surfaces were further decorated non-covalently using a fluorescent protein, providing a prototype for sensor and responsive device design.

2. Results and discussion

PCL/PCL-TMSPEDA core/shell nanofibers were synthesized by electrospinning. (PCL: polycaprolactone; TMSPEDA: N-[3-(Trimethoxysilyl)propyl] ethylenediamine) to generate nanofiber mats on aluminum foil. The primary amine on nanofiber surface was then converted to a DTC structure in the presence of a CS₂ solution, resulting in capture of NPs from solution (Scheme 1). AuNP-TTMA (core diameter *ca.* 2 nm, coated with thioalkyl tetra(ethylene glycol)ated trimethyl ammonium (TTMA) functionalized ligands) was used as a representative NP to modify the nanofiber surface. After the immersion of the CS₂-activated nanofibers into the solution of AuNP-TTMA, DTC displaced the TTMA ligand on NP, covalently binding the NPs to the nanofiber surface.

The formation of AuNP-TTMA monolayer on nanofiber surface was characterized by scanning electron microscope (SEM), transmission electron microscopy (TEM) and energy-dispersive X-ray spectroscopy (EDS). Nanofibers exhibited submicron fiber diameters ($\sim 0.45 \mu\text{m}$) with cylindrical morphologies and smooth surfaces as observed *via* SEM (Figure 1a). However, the smoothness of the nanofiber surface decreased after the functionalization with AuNP-TTMA (Figure 1b). The changes in surface structural features were due to the self-assembly of NPs on nanofiber surface through DTC chemistry. AuNP-nanofiber composites were further characterized using TEM, where AuNPs on the nanofibers were correspond to the dark spots in the bright field TEM image. AuNPs were observed on the nanofibers under the CS₂ activation (Figure 1c and 1d) while very few AuNPs were shown in the absence of CS₂ (Figure 1e and 1f). The moderate coverage of AuNPs on nanofibers might be caused by insufficient amine groups on the nanofibers (Figure 1c, 1d and Figure S1). The immobilization of AuNPs on the nanofibers was further investigated by EDS analysis (Figure 2). The EDS spectrum showed the compositions of C, N, O, Si, S and Au in the AuNP-nanofiber composites. A significant amount of S and Au was detected in the composites, indicating the effective coating of AuNP on nanofibers through DTC-based process.

Beyond the properties provided by the particles, these functionalized materials provide scaffolds for further supramolecular functionalization. Supramolecular assembly has been used to generate nanocomposites for protein delivery,^[28] gene delivery^[29] and sensing,^[30] demonstrating the bio-friendly nature of the strategy. CdSe/ZnS core-shell QDs ($\lambda_{\text{max}} = 555 \text{ nm}$) featuring TTMA functionality (Scheme 1) were synthesized as previously reported.^[31] QDs were immobilized on the nanofiber surface *via* DTC chemistry as described above. The bright QDs on the nanofiber surface allowed the nanofibers to be visualized under fluorescence microscopy, demonstrating uniform distribution of QDs through the QD-nanofiber composites (Figure 3c). Further experiments showed that positive charge was not required for forming the QD-nanofiber composites, demonstrating the procedure is adaptable for a wide variety of NP functionality (Figure S2). The capability of NP-functionalized nanofibers for biomolecular association was demonstrated using Förster resonance energy-transfer (FRET) between green emitting QDs and red fluorescent proteins. Negatively charged red fluorescent protein, mCherry ($\lambda_{\text{max}} = 610 \text{ nm}$, pI = 6.23), was used to interact with cationic QDs functionalized nanofiber surface. The spectral overlap of the emission spectra of QDs and the absorption spectra of mCherry allowed the use of FRET to

monitor the QD-mCherry association (Figure 3a and 3b).^[32,33] To rule out the possibility of interaction between QD in solution and mCherry, the QD-nanofiber composites were washed thoroughly to remove the unbound QDs. After the immersion of QD-nanofiber composites into mCherry solution and followed by gentle wash, the green fluorescence of QD-nanofiber composites was quenched, and the red fluorescence of mCherry and FRET signals were observed (Figure 3d). The decrease of green fluorescence in the confocal images indicated the FRET efficiency and hence the supramolecular conjugation of QD-mCherry through complementary electrostatic interactions.

3. Conclusion

We have demonstrated a versatile approach to functionalize the surface of nanofibers through DTC-mediated functionalization with NPs. This method allows the fabrication of a monolayer of NPs on nanofiber surface, providing dense functional group coverage and adding the inherent unique properties of the NPs to nanofibers. This effective surface modification strategy provides a potentiality to create multifunctional nanofibers to achieve multiple objectives in biological and materials applications such as sensors, solar cells and photocatalysis. Further supramolecular interactions can be conducted on NP-nanofiber composites by engineering the surface ligands of NPs, which would provide a scaffold for multifunctional nanofibers.

4. Experimental Section

4.1. General

All chemicals used for the fabrication of NPs were purchased from Sigma-Aldrich, Fisher Scientific or Stream, unless otherwise specified. The chemicals were used as received. Dichloromethane (DCM) used as solvent for chemical synthesis was dried according to standard procedure.

4.2. Structural study and elemental mapping analysis

Each specimen was dehydrated by immersing in 100% ethanol for 30 min, infiltrated with *N*-butylglycidylether and finally embedded in epoxy resin. Then the specimens were sliced to 50-100 nm thickness using Ultramicrotome EM UC7 (Leica, Wien, Austria). The electro spinning fibers were observed under transmission electron microscopy (TEM; Tecnai G2, FEI, Hillsboro, OR, USA) at 120 kV. Bright field TEM, high-angle annular dark field scanning transmission electron microscopy (HAADF-STEM), and scanning transmission electron microscopy elemental mapping energy-dispersive X-ray spectroscopy analysis (STEM-EDS) were performed using Tecnai Osiris (FEI, Hillsboro, OR, USA) at 200 kV.

4.3. Synthesis of nanofibers

5% (w/w) polycaprolactone (PCL) (Mn 70,000-90,000; Sigma-Aldrich, St. Louis, MO) and 1.0% (w/w) *N*-[3-(Trimethoxysilyl)propyl] ethylenediamine (TMSPEDA) solutions were prepared by blending at a 1:1 ratio in 1,1,1,3,3,3-hexafluoro-2-propanol (HFP) (>99% purity; Sigma-Aldrich, St. Louis, MO) by stirring at room temperature for 24 h. Blended PCL-TMSPEDA solutions were then poured individually into separate 20 cc syringes, fitted

with a 20 gauge blunt-tipped needle, and electrospun using a DC high voltage power supply (Glassman High Voltage, Inc., High Bridge, NJ) at positive 25 kV, 20 cm needle-to-collector distance, 15 mL/h flow rate, for 10 min at an average relative humidity of 30%. These nanofibers were spun for 10 min to generate nanofiber mat on the aluminum foil.

4.4. Preparation of nanoparticles

The synthesis of AuNP-TTMA was followed the reported literatures with some modification.^[34] Briefly, AuNPs protected with 1-pentanethiol (C5) and TTMA-terminated thiol ligands were prepared following the reported procedure.^[35,36] NPs functionalized with TTMA moiety were prepared following the ligand exchange procedure.^[37] In a typical reaction, 10 mg of C5-protected NPs was first dissolved in 1 mL of dry dichloromethane (DCM). TTMA terminated thiol ligands (40 mg) were dissolved in 5 mL of organic solvent (DCM : MeOH = 4 : 1 v/v), and then added to the solution of C5-protected NPs. The reaction mixture was stirred at room temperature for 3 days. Finally, the organic solvent was evaporated and NPs were dissolved in MilliQ water. The aqueous solution of NPs was purified by dialysis for 3 days.

The synthesis of QD-TTMA was followed the reported literatures with some modification.^[29] Basically, hydrophobic CdSe/ZnS core-shell QDs were synthesized according to the reported procedure^[38,39] and the preparation of QD-TTMA was through ligand exchange process with slight modification.^[29] TTMA-terminated thiol ligands were placed in MeOH under ligand to QD ratio by weight (7: 1), and the solution was stirred at 37 °C for 4 days. The aqueous QD-TTMA solution was purified by dialysis.

4.5. Fabrication of NPs on nanofibers

NPs (i.e., AuNP-TTMA and QD-TTMA) were prepared in methanol (7 μ M in 1 mL), and triethylamine (TEA, 33 μ L) was added to the solution. The mixture was sonicated for 1 min and filtered using a syringe filter (diameter: 25 mm; porosity: 0.22 μ m; materials: PTFE (polytetrafluoroethylene)). The nanofiber samples (0.3 cm $\times$ 1 cm of aluminum foil) were immersed in the methanol (600 μ L), and CS₂ (20 μ L) was added to the solution. The mixture was sonicated for 30 sec - 1 min. After the sonication, the nanofiber samples were under the CS₂ activation for 5 min. After the activation, NPs solution (600 μ L) was added to the nanofiber immersed solution for a 30 min incubation. The nanofiber samples were washed with methanol, ethanol and finally dried under the nitrogen flow.

4.6. Cloning and expression of mCherry

Genetic engineering manipulation and protein expression were done according to previously reported methods.^[40] To construct pQE80-6 $\times$ His-mCherry plasmid, mCherry gene was sub-cloned from pCHERRY3 plasmid (Addgene ID 24659)^[41] into *Bam* HI and *Hind* III (downstream of 6 $\times$ His tag) restriction sites of pQE80 expression vector. To produce recombinant proteins, plasmid containing the respective genes were transformed into *Escherichia coli* BL21(DE3) strain. A transformed colony was picked up to grow small cultures in 50 mL 2 $\times$ YT media at 37 °C overnight. The following day, 15 mL of grown culture was inoculated into one liter 2 $\times$ YT media and allowed to grow at 37 °C until OD reaches 0.6 by using UV-Vis spectroscopy. At this point, mCherry expression was induced

by adding isopropyl- β -D-thiogalactopyranoside (IPTG; 1 mM final concentration) at 25 °C. After 16 h of induction, the cells were harvested and the pellets were lysed using a microfluidizer. His₆-tagged mCherry were purified from the lysed supernatant using HisPur cobalt columns. The integrity and the expression of native protein were determined by a 12% SDS-PAGE gel and absorbance spectra.

4.7. Confocal microscopy setting of QD-nanofiber composites and QD/mCherry-nanofiber composites

Confocal microscopy images were obtained on a Zeiss LSM 510 Meta microscope by using a 10 $\times$ objective. The settings of the confocal microscope were as follows unless otherwise specified: green channel of QD: λ_{ex} = 488 nm and λ_{em} = BP 505-530 nm; red channel of mCherry: λ_{ex} = 543 nm and λ_{em} = LP 585 nm.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgements

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Figure 1.

SEM images of (a) nanofiber and (b) AuNP-TTMA functionalized nanofibers. TEM images of nanofibers after the immersion in the AuNP-TTMA solution in the (c-d) presence and (e-f) absence of CS₂, where (d) and (f) were the cross-sectional TEM image.

Figure 2.

EDS mapping of the cross-sectional STEM images of nanofibers after the immersion in the AuNP-TTMA solution in the (a) presence and (b) absence of CS₂. High-angle annular dark field scanning transmission electron microscopy (HAADF-STEM) was used in this experiment.

Figure 3.

(a) Schematic illustration of FRET process between QD-TTMA and mCherry. (b) Spectral overlap of QD-TTMA and mCherry. Abs: absorption; EM: emission. Confocal images of (c) QD-TTMA functionalized nanofibers and (d) QD-TTMA/mCherry conjugates coated nanofibers. For each series of confocal images in (c) and (d), fluorescence images of QD ($\lambda_{\text{ex}} = 458 \text{ nm}$, $\lambda_{\text{em}} = \text{BP } 505\text{-}530 \text{ nm}$), mCherry ($\lambda_{\text{ex}} = 543 \text{ nm}$, $\lambda_{\text{em}} = \text{LP } 585 \text{ nm}$) and FRET signals ($\lambda_{\text{ex}} = 458 \text{ nm}$, $\lambda_{\text{em}} = \text{LP } 585 \text{ nm}$) were shown. Emission filters: BP= band pass, LP= high pass.

Scheme 1.

DTC-mediated immobilization of NPs to an amine-functionalized nanofiber.