Microorganism-based assemblies of luminescent conjugated polyelectrolytes†

Libing Liu, Xinrui Duan, Huibiao Liu, Shu Wang* and Yuliang Li

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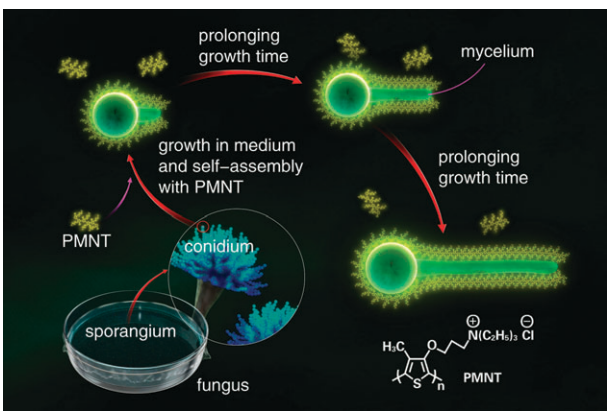
A novel approach was developed for the assembly of fluorescent conjugated polyelectrolytes into tubes on the micrometre scale of tunable length using fungi as living templates.

The development of self-assembled meso- and macroscopic materials is of current interest because of their unique structures and applications in photonics, electronics, catalysis, and sensors.[1,2] However, the synthesis of self-assembled materials with controlled size on the nanometre or micrometre scale in all dimensions continues to be a challenge. In this regard, by integrating biology into materials research, it is possible to use well-ordered biomolecules as construction tools for the preparation of supramolecular materials on the nano- and micrometre scales.[3–5] In nature, it is well known that many microorganisms can mediate metal ions for the production of inorganic materials, such as iron oxides by bacteria, siliceous materials by diatoms, and biogenic minerals by fungi.[6,7] By learning from nature, recently, microorganisms including viruses, bacteria and fungi have been used as living templates for directing the synthesis of inorganic materials, such as gold nanoparticles, magnetic microstructures, and semiconducting nanowires.[8,9] Microorganisms have several advantages as live templates for materials preparation. They are readily available, inexpensive and uniform in size. More importantly, they typically live under comfortable conditions of temperature, pressure and acidity, which make them ideal candidates for preparing materials in an environmentally friendly manner in comparison to high-temperature, high-pressure and caustic conventional techniques.[9a] To date, self-assembly mediated by microorganisms to fabricate well-ordered organic functional materials has however remained challenging.[10]

In recent years, one-dimensional (1-D) nano- and microstructures of conjugated polymers have attracted much attention due to their unique electronic and optical properties and their applications in sensing devices, solar cells, and optoelectrical nanodevices.[11,12] Although well-defined 1-D conjugated polymer structures have been prepared using hard templates, such as anodized alumina membranes and zeolite channels,[13] and using soft templates, such as DNA, surfactants, lipids and proteins,[14,15] few distinct strategies to date have yielded monodisperse conjugated polymer tubes of tunable length. In this paper, we demonstrate a novel approach using fungi to template the assembly of fluorescent conjugated polymers into tubes. In particular, it is possible to tune their length by controlling the growth of the fungi in the medium.

The conjugated polymer used here for the assembly is a water-soluble cationic polythiophene derivative (PMNT).[16] We hypothesize that the amphiphilic PMNT can accumulate on the surface of microorganisms by electrostatic attraction and hydrophobic interactions. In culture medium solution containing PMNT, the fungi grows as wires and PMNT simultaneously binds to their outer surface membrane to yield luminescent wires. Scheme 1 presents a schematic of the method for the assembly of conjugated polymer tubes with controlled length using fungi as a living template. In culture medium solution, the fungal conidium germinates to produce mycelia, of which the diameter is uniform and the length is proportional to growth time. The PMNT is integrated into the fungal mycelia through self-assembly. Thus, by controlling the growth time of the fungi, the length of conjugated polymer wire could be tuned.

To demonstrate the assembly of PMNT, *Aspergillus niger* (*A. niger*, CGMCCC, Code 3.0808) was selected as the living template.[17] *A. niger* is a filamentous Ascomycete fungus and one of the most common and easily identifiable species of the genus *Aspergillus*. The fungal cell wall is a complex structure mostly composing of chitin, glucans, polyphosphate and a small quantity of proteins, lipids and other biopolymers.[18] Thus one expects that the amphiphilic PMNT can bind to the



Scheme 1 Schematic illustration of the use of living mycelia of fungi as templates for the assembly of conjugated polymer PMNT into tubes with tuned length.

Beijing National Laboratory for Molecular Science, Key Laboratory of Organic Solids, Institute of Chemistry, Chinese Academy of Sciences, Beijing, 100190, P. R. China. E-mail: wangshu@iccas.ac.cn; Fax: +86 10 6263 6680; Tel: +86 10 6263 6680

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fungal cell wall through electrostatic attractions and hydrophobic interactions.

A conidiophore stock suspension was obtained by growing the fungus on a modified Martin agar medium slant at 26 °C for 4 days. The conidia were harvested from the surface by adding sterile Millipore water and scraping the surface with a sterile spatula. The spore concentration obtained was enriched by centrifugation. The final spore concentration was adjusted to about 3×10^5 spores mL^{-1} by dilution using modified Martin broth medium containing 10 μM PMNT. The spore solution was loaded into a sterile culture vessel at 26 °C. The growth of spore sample was observed under a fluorescence phase contrast microscope at 0, 4, 8, 16, 20 and 24 h, respectively. As shown in Fig. 1a–f, upper images are phase contrast images and lower ones are fluorescence images. From the images obtained, the lineal length of a hypha from spore to growth spot was 4 μm (4 h), 35 μm (8 h), 118 μm (16 h), 231 μm (20 h) with a hyphal diameter of about 3–5 μm and the growth speed was 7.8–14.2 μm h^{-1}. If the nutrition was enough to sustain vegetative mycelia of *A. niger*, the mycelial growth was unlimited. These results showed that the PMNT assembled into supramolecular tubes on the living fungal template, and different lengths could be obtained by controlling the growth of the fungus in the medium. As shown in Fig. 2, the binding of PMNT to mycelia resulted in a 25 nm red shift of its emission maximum. This red shift possibly results from the interpolymer π-stacking aggregation induced by the assembly of PMNT on the fungal cell wall.

To get more insight into the assembly behavior of PMNT on the outer surfaces of the fungus, microstructural characterizations of PMNT/fungus assemblies were performed using scan electron microscopy (SEM). The TEM image of the fungus

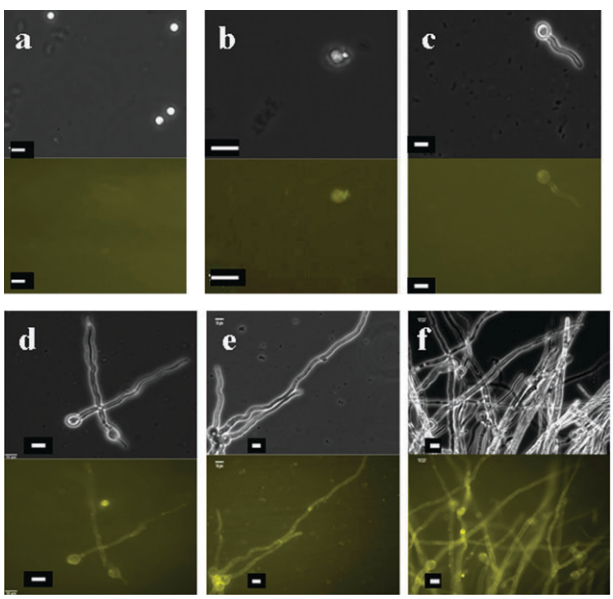

Fig. 1 *A. niger* grows from conidia to mycelia in modified Martin broth medium containing 10 μM PMNT for (a) 0 h, (b) 4 h, (c) 8 h, (d) 16 h, (e) 20 h and (f) 24 h. Upper images are phase contrast images and lower ones are fluorescence images. The false color is yellow and the type of light filter is D455/70 nm exciter, 500 nm beam-splitter, and D525/30 nm emitter. The scale bar is 10 μm.

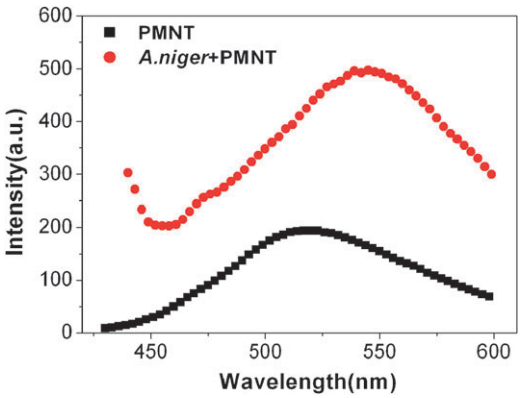

Fig. 2 The fluorescence emission spectra of PMNT itself and PMNT/mycelium assembly. The excitation wavelength is 425 nm.

itself (Fig. 3a) showed a large number of wires with a hyphal diameter of about 3–5 μm. Higher magnification SEM images of fungi (Fig. 3b) indicated that the fungal cell had a smooth outer surface. For PMNT/fungus assembly, the outer surface of the fungus was observed to be coated with a continuous and coarse layer of PMNT aggregates (Fig. 3c and d). It was noted that the different concentrations of PBS buffer, such as 100 mM, 200 mM and 500 mM, did not wash PMNT away from the hyphae, which showed that the PMNT binds to the fungal cell wall mainly by hydrophobic interactions.

In summary, we take advantage of the well-ordered structure of fungi as living templates to produce biological supramolecular materials by assembly. This method provides a new means by using a conventional microorganism for preparing highly ordered, environmentally friendly and economical 1-D conjugated polymer tubes on the micrometre scale. In particular, for the fungus/conjugated polymer assemblies, their length can be tuned by controlling the growth of the fungus in the medium. Microorganisms as general living templates for the universal preparation of a variety of well-ordered organic functional materials can be realized.

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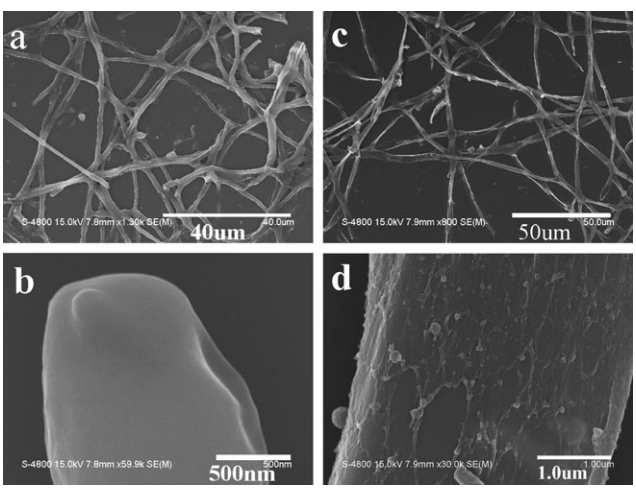

Fig. 3 (a) Lower magnification and (b) higher magnification SEM images of fungi. (c) Lower magnification and (d) higher magnification SEM images of fungi coated with PMNT.

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