

Porphyrin-Containing Electrospun Nanofibers: Positional Control of Porphyrin Molecules in Nanofibers and Their Catalytic Application

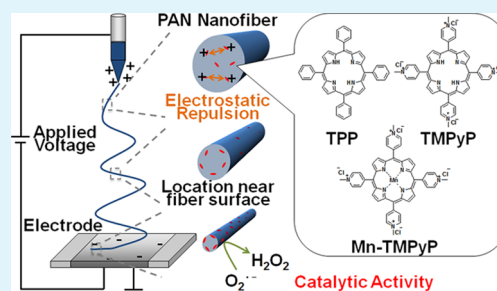
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Supporting Information

ABSTRACT: Polyacrylonitrile nanofibers containing a series of porphyrin molecules were prepared by an electrospinning method. We first succeeded in controlling the position of porphyrin molecules in the nanofibers by considering porphyrin characteristics and the electrospinning conditions. It was concluded that positive charge of cationic porphyrin, TMPyP, and higher applied voltages were effective to locate the porphyrin molecules on the polymer nanofiber surfaces because of the electrostatic repulsion among the molecules during the electrospinning process. The polymer nanofibers with cationic manganese–porphyrin (Mn-TMPyP) on their surface repeatedly showed superoxide dismutase (SOD) activity, which is a catalytic activity to work as antioxidant in various biochemical fields. The positional control of functional molecules in nanofibers demonstrated a new possibility of nanofiber applications.

KEYWORDS: nanofiber, electrospinning, porphyrin, superoxide dismutase activity



INTRODUCTION

Polymer nanofibers have received a lot of attention because of their unique properties, such as large specific surface area, low reflux resistance, and superior mechanical properties.^{1–4} Among several techniques to prepare polymer nanofibers, an electrospinning method^{5–7} that fabricates nanofibers through an electrically charged jet of a polymer solution/melt is capable of producing ultrafine polymer fibers with their diameters in the nanometer range (down to less than 30 nm^{8–10}). The electrospun polymer nanofibrous mats have great advantages in terms of the recovery and easy handling of the materials as well as lightweight, flexible, and inexpensive features that are common characteristics of polymeric materials; therefore, a number of applications are being explored, including filtration membranes, conductive fibers, optical and chemical sensors, catalyst systems, scaffolds for tissue regeneration, and immobilized enzymes.^{11–16}

Because not all the functional polymers give nanofibers by the electrospinning method because of their low solubility, viscosity, and entanglement of polymer chains, electrospinning from a mixture solution of functional molecule and conventional polymer possessing good spinnability is one of the simplest and most efficient methods to prepare functional polymer nanofibers. However, capability of such polymer nanofibers could be limited because the functional molecules generally locate randomly in the polymer nanofibers. Therefore, positional control of functional molecules in nanofibers is remarkably desired in various applications, especially in catalyst system and immobilized enzymes where functional molecules on the surface of nanofibers can mainly act as catalyst or enzyme. Though postfunctionalization including dip-coating, vapor-deposition, and ion-irradiation have been utilized for

surface coating of nanofibers,^{17,18} they required postprocessing that may damage on the nanofibers during the processes and were not easy to control the amount and thickness of functional molecules on the nanofiber surfaces. More simple and feasible ways are desired for practical application of nanofibers containing functional molecules. If positional control of functional molecules in polymer nanofibers, especially selective location of functional molecules on the nanofiber surfaces, is achieved by a conventional electrospinning method, it would be a great breakthrough for the novel polymer nanofiber preparation technique, leading to various applications.

Here we first demonstrate the positional control of functional molecules in polymer nanofibers to locate the molecules on the nanofiber surfaces. As functional molecules, we chose a series of porphyrin molecules due to their unique properties, such as electrical, optical, and catalytic characteristics, and their future applications.¹⁹ For example, we have reported that cationic manganese(Mn)–porphyrins possess both superoxide dismutase (SOD) activity and ONOO[–] elimination ability and are attractive candidates as antioxidant compounds for therapeutic agents for a variety of disorders associated with oxidative stress.^{20–23} If it is possible to easily locate the porphyrin on the nanofiber surface, the Mn–porphyrin loading nanofiber becomes useful in vivo nanobiomaterials, such as artificial kidney, artificial lung, and drug delivery system (DDS) with antioxidant ability.

First, in this study, distributions of two kinds of porphyrin molecules, 5,10,15,20-Tetrakisphenyl-21H,23H-porphyrin

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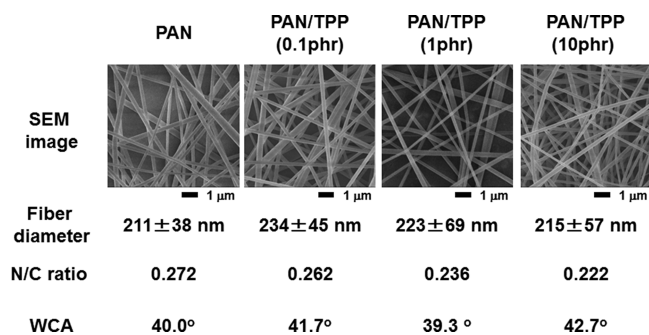


Figure 2. SEM images, fiber diameters, nitrogen/carbon (N/C) ratios, and water contact angles (WCA) of PAN/TPP nanofibers.

191 ± 28 nm, 215 ± 69 nm, or 243 ± 50 nm, respectively (Figure 3).

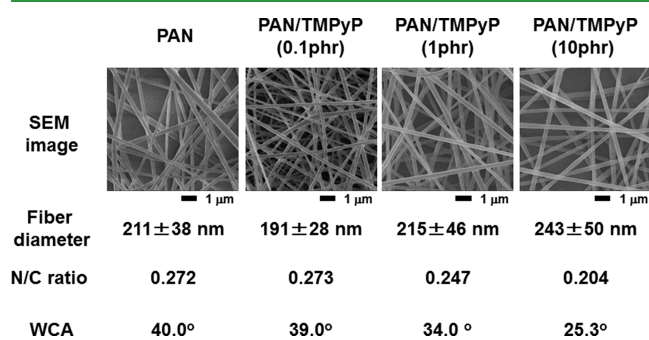


Figure 3. SEM images, fiber diameters, nitrogen/carbon (N/C) ratios, and water contact angles (WCA) of PAN/TMPyP nanofibers.

The surface properties of the electrospun nanofibers were characterized by X-ray photoelectron spectroscopy (XPS) and water contact angle (WCA) measurements. The nitrogen/carbon (N/C) ratios of the PAN/TPP nanofibers near the fiber surfaces were measured by XPS, as summarized in Figure 2. The PAN nanofiber without any porphyrin molecules showed relatively high N/C ratio (N/C = 0.272) because of high abundance ratio of nitrogen atoms in the chemical structure of PAN. Since TPP has lower abundance ratio (N/C = 0.091) than PAN, N/C ratios of PAN/TPP nanofibers gradually decreased by increasing the amount of TPP in PAN/TPP nanofibers. The WCA of PAN/TPP nanofibers were expected to increase due to the hydrophobic TPP molecules; however, the values were almost same to that of PAN nanofibers (Figure 2). These results represented that the TPP molecules located near the fiber surfaces but the amount of TPP on the nanofiber surface was limited. Figure 3 shows the N/C ratios and WCA of PAN/TMPyP nanofibers containing different amount of TMPyP. The N/C ratios of PAN/TMPyP nanofibers decreased in accordance with the increment of TMPyP amounts. The N/C ratio of the PAN/TMPyP-(10phr) nanofiber was 0.204, which was lower than that of PAN/TPP(10phr) nanofibers, though the TMPyP has twice higher abundance ratio (N/C = 0.182) than TPP. It means that more TMPyP molecules located near the surface of nanofibers than TPP molecules. This tendency was also supported by the WCA measurement results as follows: The WCA values of PAN/TMPyP nanofibers were lower than that of PAN nanofibers (40.0°), and the value reached down to 25.3° on the PAN/TMPyP(10phr) nanofiber due to the existence of hydrophilic TMPyP molecules on the nanofibers. On the other

hand, the WCA values of PAN/TPP nanofibers were almost similar to that of PAN nanofibers, indicating less TPP molecules located on the nanofiber surfaces. These experimental results indicated that TMPyP molecules were located near and on the nanofiber surfaces easier than TPP molecules in PAN nanofiber in the same electrospinning processes. It is interesting that the hydrophilic TMPyP preferred outside (near and on the surface) of the relatively hydrophilic PAN nanofibers though the electrospinning of the polymer solution was carried out under dry condition ($5 \pm 2\%$ RH) in the electrospinning apparatus. Therefore, we considered that the positive charge in cationic TMPyP helped electrostatic repulsion among TMPyP molecules in the polymer solution to locate the TMPyP molecules outside of nanofibers during the electrospinning process as shown in Figure 1.

Effect of Electrostatic Repulsion on the Position of Porphyrin Molecules in the Nanofibers. In order to verify the effect of electrostatic repulsion on the position of porphyrin molecules in the nanofibers, PAN/TPP and PAN/TMPyP nanofibers containing 1phr TPP or TMPyP were prepared from different applied voltages including 10, 20, and 30 kV. Figure 4 shows the SEM images of PAN/TPP and PAN/

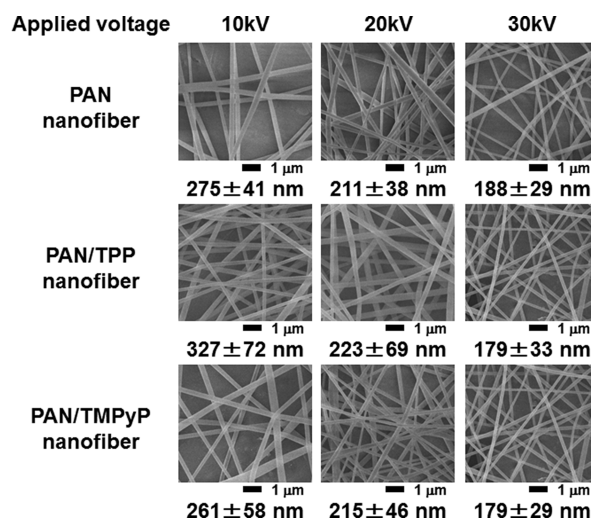


Figure 4. SEM images and fiber diameters of PAN, PAN/TPP, and PAN/TMPyP nanofibers prepared at the applied voltages of 10, 20, and 30 kV.

TMPyP nanofibers prepared from different applied voltages. As well as PAN nanofibers, the diameters of PAN/TPP and PAN/TMPyP nanofibers reduced by increasing applied voltages under same electrospinning conditions except applied voltage. It is known that the higher applied voltages stretched polymer chains more straightforwardly to yield thinner nanofibers.^{9,10} Both PAN/TPP(1phr) and PAN/TMPyP(1phr) nanofibers prepared from higher applied voltages had lower N/C ratios than those prepared from lower applied voltages (Figure 5a), indicating more TPP or TMPyP molecules located near the surface of nanofibers when the nanofibers were prepared from higher applied voltages. Similar phenomena were observed on the WCA measurements; that is to say, the PAN nanofibers containing hydrophobic TPP prepared from higher applied voltages showed higher WCA than the nanofibers prepared from lower applied voltages, and the PAN nanofibers containing hydrophilic TMPyP showed opposite tendency. Since there is little change in the PAN nanofibers without any

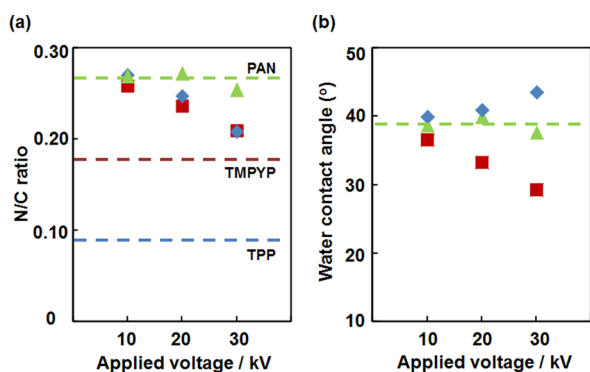


Figure 5. (a) Nitrogen/carbon (N/C) ratios and (b) water contact angles (WCA) of PAN (▲), PAN/TPP (◆), and PAN/TMPyP (■) nanofibers prepared at the applied voltages of 10, 20, and 30 kV.

porphyrin molecules by the applied voltages, diameters and other structural differences of nanofibers were considered to have no effect on N/C ratio and WCA. One possible reason why the nanofibers prepared from higher applied voltages had more porphyrin molecules near the nanofiber surfaces is the electrostatic repulsion among the porphyrin molecules during the electrospinning process. The polymer solutions were positively charged during the process because the high voltages were applied between the syringe needle (positive electrode) and the collector (negative electrode), therefore not only PAN and DMF but also TPP and TMPyP molecules may act repulsively during the electrospinning process. It is predicted that the porphyrin molecules, especially cationic TMPyP, may charge up easily because of their chemical properties; hence, the electrostatic repulsion among porphyrin molecules got stronger along with applied voltages to locate the molecules near the surface of nanofibers. Indeed, N/C ratio of PAN/TMPyP-(1phr) nanofibers prepared from 30 kV of applied voltages (N/C = 0.209) was close to that of TMPyP itself (N/C = 0.182) though the N/C ratio of PAN/TPPP(1phr) nanofibers prepared from 30 kV of applied voltages (N/C = 0.222) was still far from that of TPP itself (N/C = 0.091), suggesting the easily chargeable TMPyP molecules could be strongly influenced by increasing the applied voltages for the electrostatic repulsion during the electrospinning process. Thus, the positional control of porphyrin molecules in nanofibers, especially positional control of cationic TMPyP molecules on the surface of PAN nanofiber, was first succeeded in the electrospinning method from simple blend solutions. Now we are studying other electrospun nanofibers containing other functional molecules and attempting additional experiments such as cross-sectional TEM observation and energy-dispersive X-ray spectrometry to support the location of functional molecules in the nanofibers. The future work will be published elsewhere.

Catalytic Application of Mn-TMPyP-Containing Electrospun Nanofibers. Here we demonstrate one application for the positional control of functional molecules in polymer nanofibers. Among various applications of porphyrin molecules, we focus attention on the catalytic activities, in particular SOD activity of Mn-TMPyP, as mentioned above. SOD are enzymes that catalyze the reduction from superoxide radical anion ($O_2^{\cdot-}$) into hydrogen peroxide (H_2O_2) in cytoplasm and mitochondria.²⁷ In mitochondria, manganese superoxide dismutase (Mn-SOD) is the key enzyme for the protection of oxidative stress.²⁸ We have already reported that water-soluble

cationic Mn-porphyrins including Mn-TMPyP with SOD activity work as antioxidant in various biochemical fields.^{20–23} Functionalization of nanofibers with SOD active molecules has several advantages as follows: First, such nanofibers may be expected to show distinguished characteristics on the basis of their large specific surface area and low fluid resistance. Second, the nanofibers may have excellent processability applicable to various biomedical devices, such as artificial organs and DDS. In addition, the nanofibrous mats can be used repeatedly.

The PAN/Mn-TMPyP(1phr) nanofibers were prepared in a way similar to that of PAN/TMPyP nanofibers (for details, see Supporting Information). The applied voltages were chosen 10 and 30 kV to compare their SOD activities. The SOD activities of PAN/Mn-TMPyP nanofibrous mats were measured by a Cytochrome C method, which is the most typical method to evaluate SOD activities.^{25,26} Figure 6a represents the SOD

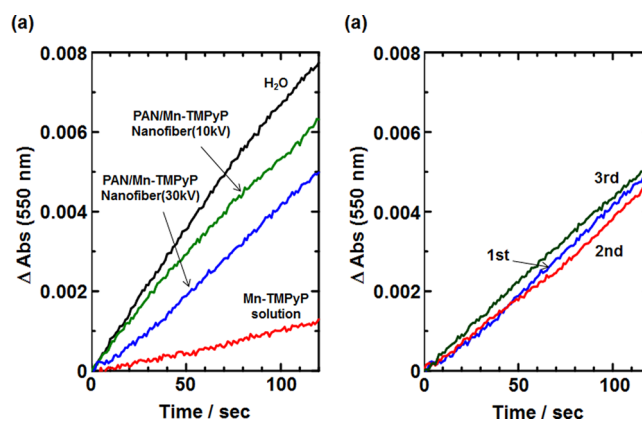


Figure 6. (a) SOD activity plots of PAN/Mn-TMPyP nanofibrous mats prepared from the applied voltages of 10 and 30 kV, Mn-TMPyP aqueous solution (3 μ M), and water. (b) Repeated SOD activity plots of PAN/Mn-TMPyP nanofibrous mat prepared from the applied voltages of 30 kV.

activity plots of PAN/Mn-TMPyP nanofibrous mats prepared from 10 and 30 kV of applied voltages, Mn-TMPyP aqueous solution as a positive control, and water as a negative control. The vertical axis of Figure 6 represents absorbance change at 550 nm, which is derived from ferro-cytochrome C; therefore, samples with higher SOD activity showed lower slope because most $O_2^{\cdot-}$ were reduced by the SOD active samples to prevent reduction of ferri-cytochrome C to ferro-cytochrome C (For the mechanisms of cytochrome C method, see Figure S1 in the Supporting Information). The PAN/Mn-TMPyP(1phr) nanofibrous mat in water (0.66 mg nanofibers/0.3 mL water, corresponding to the 0.3 mL of 3.0 μ M Mn-TMPyP aqueous solution) showed obvious SOD activities compared to water. The plots of PAN nanofibrous mat without any porphyrin molecules was almost same to that of water (data not shown), indicating that the SOD activity was derived from the Mn-TMPyP molecules in the nanofibers. Since it must be difficult to act as catalyst for Mn-TMPyP inside the nanofibers, the SOD activities were considered to be contributed from the Mn-TMPyP on the nanofiber surface. Then, the PAN/Mn-TMPyP nanofibrous mat prepared from 30 kV applied voltage showed better SOD activity than that prepared from 10 kV applied voltage. This result agreed with the previous study that more porphyrin molecules located on the nanofiber surfaces with the higher applied voltage during the electrospinning process. Here it is concerned about the elution of water-soluble Mn-TMPyP

molecules into water during immersion of nanofibers in water. It was, however, confirmed that no Mn-TMPyP molecules eluted from the nanofibers as described in the Supporting Information. Finally, the SOD activities of PAN/Mn-TMPyP nanofibrous mat prepared from 30 kV applied voltage were repeatedly evaluated via washing the nanofibrous mat with water after every measurement. As shown in Figure 6b, the PAN/Mn-TMPyP nanofibrous mat kept similar SOD activities at least three times after washing with water and replacing reagents. The SOD activities of PAN/Mn-TMPyP nanofibrous mats were inferior to Mn-TMPyP aqueous solution as seen in Figure 6a); however, the nanofibrous mats had advantages on their possible repetitive utilization with high durability.

CONCLUSION

A series of PAN/porphyrin nanofibers with ca. 200 nm diameters were prepared by electrospinning under various conditions to study the distribution of porphyrin molecules in the nanofibers. The cationic porphyrin, TMPyP, was easier to locate on the polymer nanofiber surfaces than TPP because of its electrostatic repulsion during the electrospinning process. Besides, it was revealed higher applied voltage locates more porphyrin molecules near the surface of nanofibers because of stronger electrostatic repulsion among the molecules. The PAN nanofibers containing 1phr Mn-TMPyP prepared from 30 kV applied voltage showed definite SOD activities derived from catalytic active Mn-porphyrins, suggesting that not a few Mn-TMPyP molecules located on the polymer nanofibers even though the nanofiber contains only 1phr Mn-TMPyP. Besides, the PAN/Mn-TMPyP nanofibrous mats kept comparable SOD activities after washing with water several times. This study demonstrated the possibility of the positional control of functional molecules in the electrospun polymer nanofibers for future applications. In particular, cationic Mn-porphyrins with SOD activity located on the nanofiber surface prepared from biocompatible polymers may provide a new way of radical scavengers as medical devices or therapeutic drugs for treating oxidative stress injuries.

ASSOCIATED CONTENT

Supporting Information

Experimental details of Cytochrome C method, preparation of PAN/porphyrin nanofibers, preparation and characterization of PAN/Mn-TMPyP nanofibers, and elution test of Mn-TMPyP from the nanofibers. This material is available free of charge via the Internet at <http://pubs.acs.org>.

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Notes

The authors declare no competing financial interest.

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