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Enzyme-Mimetic Catalyst-Modified Nanoporous SiO_2−Cellulose Hybrid Composites with High Specific Surface Area for Rapid H_2O_2 Detection

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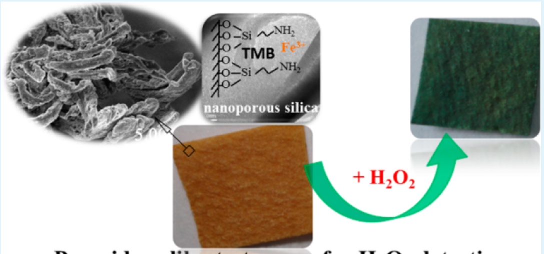
Supporting Information

ABSTRACT: Mesoporous silica−cellulose hybrid composites were prepared by surface sol−gel coating process on nature cellulose substance. The template CTAB (hexadecyl trimethyl ammonium Bromide) in the silica film can be removed by extraction to obtain high specific surface area (80.7 m^2 g^{-1}), which is 2 orders of magnitude higher than that of raw cellulose. In the following, the enzyme-mimetic catalyst and chromogenic agent were introduced onto the hybrid system. Just as the peroxidase, the resultant hybrid material exhibits extraordinary sensitivity for the H_2O_2 and shows an immediate and obvious color change. The detection limit is about 1 μmol L^{-1} by the naked eye.



Peroxidase-like test paper for H_2O_2 detection

KEYWORDS: *mesoporous silica, hybrid nanostructures, enzyme-mimetic catalyst, H_2O_2 detection*

INTRODUCTION

The rapid and accurate H_2O_2 determination is very important in many fields such as food, pharmacy, clinic, industry, and environmental protection.[1] Therefore, the development of efficient and sensitive methodologies for H_2O_2 detection is in great demand. Enzymes such as horseradish peroxidase (HRP) can catalyze the reaction of peroxidase substrate 3,3,5,5,-tetramethylbenzidine (TMB) in the presence of H_2O_2 to produce a blue color reaction, which have been employed to detect H_2O_2 molecule and also have a great potential for practical application in diagnostic kit for H_2O_2 and glucose.[2] However, most of these enzymes have their inherent problems such as the inactivation under harsh environment, digestion by proteases, time-consuming and expensive preparation.[3] To overcome these drawbacks of the natural enzymes, researchers have given intense efforts to develop the artificial enzyme mimetics. Recently, Yan and co-workers first reported that Fe_3O_4 nanoparticles showed peroxidase-like activity similar to HRP, opening the door to fabricate enzyme mimetics in the field of nanomaterials.[4] Up to now, various nanostructured materials including Fe_3O_4-based nanocomposites,[3,5,6] carbon nanotube,[7] polymer-coated CeO_2 nanoparticles,[8] metal or bimetallic nanoparticles,[9,10] V_2O_5 nanowires,[11] sheetlike FeS,[12] and hemin-graphene hybrid nanosheet[13] have been found with peroxidase- or oxidase-like activity. However, these processes employed nanostructured peroxidase-mimetic catalyst are considerably time-consuming and inconvenient because of the fussy reagents′ addition and separation, especially in the case of on-site inspection. Additionally, the chromogenic reaction will be too weak to be observed by naked-eye owing to trace amount of H_2O_2. Consequently, it is desirable to develop some novel strategies to design more sensitive sensors to detect the H_2O_2 rapidly and directly.

Cellulose is the most abundant renewable raw material in the world.[14] Natural cellulosic materials such as paper, cotton and cloth are produced from plant and widely used in our life. Therefore, it is an ideal choice to employ natural cellulosic substance as a scaffold to fabricate chemosensors.[15] Herein, a reversible cellulose based colorimetric H_2O_2 sensor was fabricated by immobilization of peroxidase-like Fe^{3+} onto mesoporous silica film precoated natural cellulose substance (e.g., cotton or commercial filter paper). The resultant enzyme-mimetic hybrid composite exhibits extraordinary sensitivity, giving an immediate and obvious color change in the presence of H_2O_2 in aqueous solution, and the detection limit is about 1 μmol L^{-1} by naked eye. Moreover, the peroxidase-like activity of the hybrid system can be retained after being recycled several times.

RESULTS AND DISCUSSION

Scheme 1 shows the fabrication process for the peroxidase-like Fe^{3+} modified silica-cellulose hybrid microfibers. First, mesoporous silica films functionalized with amine groups were coated onto the cellulose microfibers′ surface (cotton or commercial filter paper) by means of a surface sol−gel process[16] using TEOS (tetraethyl orthosilicate) + APTES (3-

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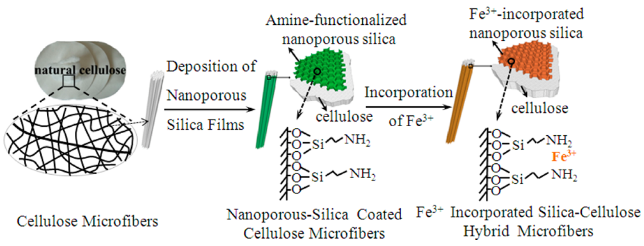
Scheme 1. Schematic Illustration of the Fabrication Process for Cellulose-Based Peroxidase-Like Catalyst



triethoxysilylpropylamine) as precursors and CTAB (hexadecyl trimethyl ammonium bromide) as template (see experimental sections for details). Next, the template was removed by ethanol extraction to obtain white nanoporous-silica coated cellulose microfibers (NP-Si-Cell) with high specific surface area. Finally, the Fe^{3+} was anchored onto the hybrid microfibers to get a yellow cellulosic product by the interaction between Fe^{3+} and amine groups (Fe-NP-Si-Cell).[17]

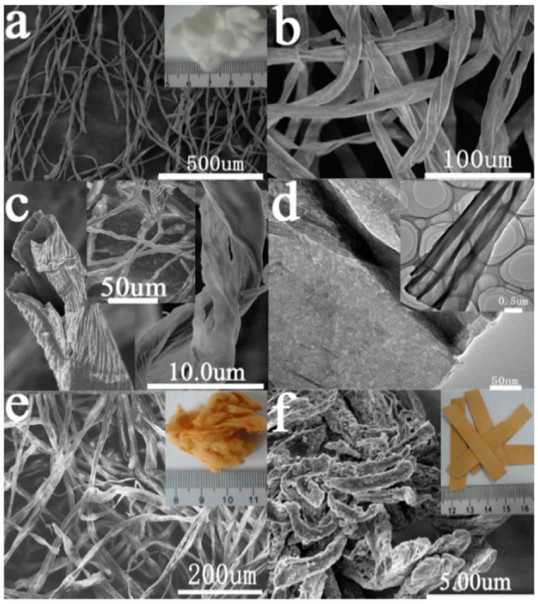


Figure 1. Electron micrographs of the samples. (a, b) Scan electron micrographs (SEM) image of NP-Si-Cell, inset is a photograph of the resulting material. (c) SEM image of the mesoporous silica fibers as a replica of cellulose after calcination. (d) Transmission electron microscopy (TEM) image of the mesoporous silica shell coated on the cellulose. (e) SEM image of Fe^{3+} incorporated silica-cellulose hybrid microfibers (Fe-NP-Si-Cell) made from cotton, inset is a photograph of the as-synthesized sample. (f) SEM image of Fe-NP-Si-Cell made from filter paper, inset is a photograph of the resulting sample.

Figure 1 shows the electron micrographs of samples at different stages. When cotton was used as raw material, it was found the obtained NP-Si-Cell remained its original fibrous structure after coating the mesoporous silica (Figure 1a, b; Figure S1 in the Supporting Information). Energy-dispersive X-ray (EDX) results indicated that the silica and the template (CTAB containing Br atoms) were both loaded onto the cellulose after coating (see Figures S2a, b in the Supporting Information) due to the appearance of Si and Br signals in the EDX spectra. After ethanol extraction, the disappearance of Br signal in the spectrum of EDX for the NP-Si-Cell (see Figure S2c in the Supporting Information) suggested that the CTAB was successfully removed by extraction. Additionally, it was found there was contrast at a cross-section of the NP-Si-Cell sample, indicating the silica was fully coated onto the cellulose surface (see Figure S3 in the Supporting Information). To verify the coated structure further, the resultant cellulose/mesoporous-silica hybrid material was calcined at 550 °C for 2 h in air to remove the original cellulose. After calcination, a little part of white powder was obtained (about 20% of original weight) and the EDX confirmed the white powder was silica (see Figure S4 in the Supporting Information). SEM and TEM images (Figure 1c, d; Figures S5 and S6 in the Supporting Information) clearly verified original morphology of cellulose was faithfully replicated by the silica film, and the cellulose microfibers were precisely copied as irregular hollow tubes or helical structure. This result agrees well with the previous reports about the replication of cellulose with other inorganic matrix such as TiO_2.[16,18] The TEM images also indicate that the cellulose has a perpendicularly uniform mesoporous SiO_2 shell (Figure 1d; Figure S6 in the Supporting Information). The thickness of the silica shell was estimated to be no more than 350 nm (see Figure S5 in the Supporting Information). The coated mesoporous silica can not only improve the mechanical character of the cellulose but also provide largely available surface for modification. After the Fe^{3+} modification, the white NP-Si-Cell became yellow, indicating the Fe^{3+} has been successfully assembled. The SEM images show that the morphology of the obtained Fe-NP-Si-Cell (inset of the Figure 1e) retains its original fibrous structure (Figure 1e and Figure S7 in the Supporting Information). To facilitate the application for Fe-NP-Si-Cell, the filter paper was also employed as raw material to prepare such cellulose based peroxidase-like catalyst system (Figure 1f, Figures S8 and S9 in the Supporting Information).

EDX for the Fe-NP-Si-Cell (Figure 2a and Figure S10 in the Supporting Information) further verifies that the iron has been successfully incorporated. The X-ray diffraction (XRD) pattern shows (Figure 2b.) that the cotton consists of highly crystalline cellulose. After mesoporous silica coating and Fe^{3+} incorporation, the crystal structure of the cellulose-based hybrid

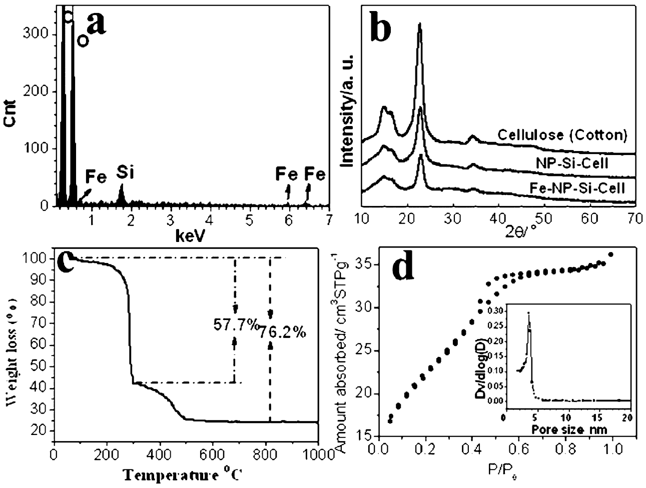


Figure 2. (a) EDX spectra for Fe-NP-Si-Cell. (b) XRD pattern for the samples. (c) TG curve of Fe-NP-Si-Cell. (d) N_2 adsorption and desorption isotherms of Fe-NP-Si-Cell, inset is the pore size distribution.

materials was still preserved, although the intensity of the XRD peaks became relatively weak due to the outer mesoporous silica shell. The composition in Fe-NP-Si-Cell was estimated by thermal-gravimetric analysis (TG) under O_2 atmosphere (Figure 2c and Figure S11 in the Supporting Information). As shown in Figure 2 c, the as-prepared hybrid material calcined under O_2 experienced three weight-loss stages. The first stage ranged from about 30−150 °C is attributed to desorption of adsorbed water. The second stage begins at 250 °C and finishes at 300 °C, indicating that the carbonization of cellulose takes place at 250 °C by dehydration.[19] The third stage takes place between 300 and 500 °C, which is attributed to the oxidation of the carbon derived from cellulose. TG results show that the mass percent of silica is about 20.4 wt % in NP-Si-Cell (see Figure S11 in the Supporting Information), the mass percent of silica + Fe_2O_3 (the residue of Fe^{3+}) in Fe-NP-Si-Cell is about 23.8%. Then the amount of iron in Fe-NP-Si-Cell is estimated to be 2.9%, which is much lower than that of other reported iron-based peroxidase mimetics.[3−5] Figure 2d and Figure S12 in the Supporting Information depict the N_2 adsorption−desorption isotherm and pore size distribution of the Fe-NP-Si-Cell and NP-Si-Cell respectively. According to the IUPAC classification, the isotherm was identified as type IV with and H2-type hysteresis loop, suggesting the mesoporous structure of the as-prepared hybrid material.[6] The NP-Si-Cell exhibits a rather narrow pore size distribution centered at 3.1 nm, which is in accordance with the TEM observation. The BET specific surface area of the silica-cellulose hybrid material (NP-Si-Cell) was measured to be 80.7 $m^2 g^{-1}$ and the pore volume was 0.06 cm^3 g^{-1}. After Fe^{3+} incorporation, the pore size distribution centered at 2.9 nm became narrower and smaller, without significant changes in BET specific surface area and pore volume (78.1 m^2 g^{-1}, 0.06 cm^3 g^{-1}), indicating the Fe^{3+} incorporation did not block the pore of the silica. Compared with the raw material (cotton) which possesses a surface area less than 0.8 m^2 g^{-1}, the surface area for the current silica−cellulose hybrid material is 2 orders of magnitude higher, thanks to the mesoporous structure of the surface silica shell.

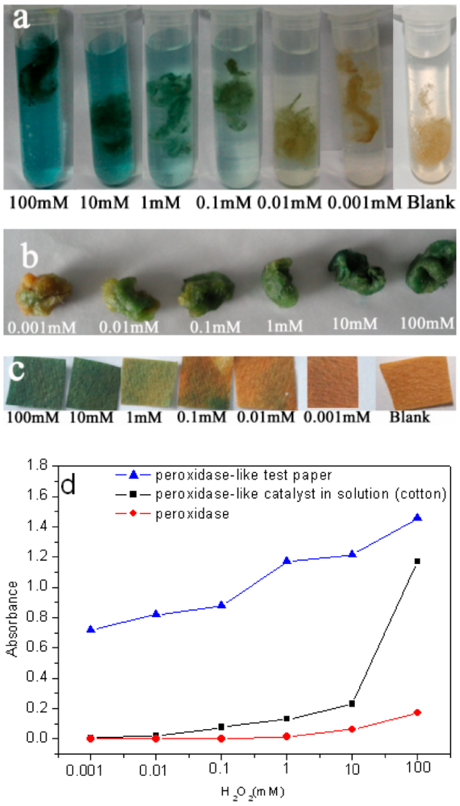


Figure 3. (a) Photograph of peroxidase-like catalyst induced color change in the presence of different concentration of H_2O_2 (4 mL of H_2O_2 including 200 μL of 5 mM TMB, 40 °C, pH 5.5−6.0, 10 mg of catalyst, 10 min). (b) Photographs of the Fe-NP-Si-Cell taken out from the different concentration of H_2O_2 solution after the reaction. (c) Color change of peroxidase-like test paper in presence of different H_2O_2 concentration. (d) Comparison of absorbance at 655 nm for different methods.

To examine the peroxidase-like activity of the Fe-NP-Si-Cell, the catalytic oxidation of peroxidase substrate TMB in the presence of H_2O_2 was tested. In the experiments, the Fe-NP-Si-Cell was immerged into the H_2O_2 solution containing TMB and held in water bath at 40 °C for 10 min. The results of these experiments show that Fe-NP-Si-Cell displays peroxidase activity which promotes specific optical responses of TMB (Figure 3a and Figure S13 in the Supporting Information). According to the previous reports,[6,20] the peroxidase-like activity of Fe-NP-Si-Cell was mainly attributed to the valence variations of Fe ions. In the presence of Fe-NP-Si-Cell, the H_2O_2 decomposes into several free radical species, such as HO. and HO_2., and then these free radical species oxidize TMB into blue colored products (see Scheme S1 in the Supporting Information). After reaction for 10 min, the color of the H_2O_2 solution turns into blue gradually due to the oxidation of TMB by Fe-NP-Si-Cell and the absorbance of the solution at 655 nm decreased with reducing H_2O_2 concentration (Figure 3c, see Figure S13 in the Supporting Information). Interestingly, during the catalytic process, the yellow hybrid composite (Fe-NP-Si-Cell) immerged in solution turned into green quickly before the solution became blue (Figure 3a, b). Surprisingly, when the concentration of H_2O_2 was reduced to below 0.01 mM and the color change of solution could not be distinguished obviously by naked eye owing to the trace amount of diluted colored substrate in solution, however, the color change of the Fe-NP-Si-Cell can be easily observed (Figure 3a, b). As is known, the mixture of blue and yellow produces green, which is the most sensitive color for human eyes. It is believed that the mesoporous silica shell on cellulose surface can trap and concentrate the oxidated TMB (the colored substrate) due to the high specific surface area, when the TMB is oxidated by the peroxidase-like catalyst on the surface of mesoporous silica.[6] To prove above point, the adsorption experiments for the oxidated TMB on the MCM-41 which pore structure and character are similar with that of the silica shell in Fe-NP-Si-Cell, were investigated. When the white MCM-41 powder (0.05g) was put into the solution containing the colored substrate (10 mL 0.5 mM oxidated TMB), the colored substrate was adsorbed on the MCM-41 quickly (within 15 s) with shaking, then the solution became clear and the white MCM-41 powder turned into light blue own to the colored substrate adsorption (see Figure S14 in the Supporting Information). Inspired by this phenomenon, we loaded the TMB on the surface of the hybrid composite made from filter paper (Figure 1f, Figure S9 in the Supporting Information) to get the enzyme-mimetic test paper (Fe-NP-Si-Cell-TMB) for H_2O_2 detection. In this case, it is anticipated the TMB on the surface of Fe-NP-Si-Cell can be quickly oxidated and captured by the hybrid system. As expected, the test paper underwent an obvious color change from yellow to green immediately after being dipped into H_2O_2 solution at room temperature (Figure 3c, d). The detection efficiency for the peroxidase-like test paper (within 3 s) at room temperature is higher than the colorimetric method with peroxidase in solution (several minutes) even at higher temperature (40 °C). Importantly, because of the high surface area for the peroxidase-like test paper, the color change could be clearly identified by naked eye inspection even down to 1 μmolL^{-1} H_2O_2 solution, which was much more sensitive than that of peroxidase-like catalyst or peroxidase in solution (Figure 3c, Figures S15 and S16 in the Supporting Information). To identify the role of mesoporous SiO_2 in the fast and sensitive response, we also introduced the solid SiO_2 layer on cellulose by sol−gel method without template. Interestingly, it is found the cellulose (test paper) was hard to be wetted by the water containing H_2O_2 due to the compact silica structure on surface (see Figure S17 in the Supporting Information), so the response time for the solid SiO_2 coated sample was longer (generally more than 30 s) than that of the mesoporous one. At the same, the sensitivity was also relative low (about 0.1 mM) because of the less available active sites. Additionally, when the used peroxidase-like test paper was stored in water for a long time (24 h), the blue oxidated TMB on surface probably could be further converted into colorless substance in atmosphere at room temperature. So, the hybrid composite with green color can be resumed and reused. It can be recycled for 10 times without losing the peroxidase-like activity, indicating a stable hybrid structure (see Figure S18 in the Supporting Information).

■ CONCLUSIONS

In summary, a concept to fabricate enzyme-mimetic catalyst modified with nanoporous SiO_2-cellulose hybrid composites for sensitive and efficient H_2O_2 detection was provided. The detection limit was ~1 μmolL^{-1} by the naked eye. The detection is time-saving (within 3 s) because of the effective surface capture. The currently developed methodology provides a new strategy for the detection of guest molecules for practical applications.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

Experimental details, scan electron micrographs (SEM) image of cotton, filter paper, silica coated cellulose of filter paper, cross-section of NP-Si-Cell, calcined NP-Si-Cell, TEM images of calcined NP-Si-Cell, Fe-NP-Si-Cell, EDX spectra for cotton, filter paper, nanoporous silica-coated cotton, calcined NP-Si-Cell, Fe-NP-Si-Cell, TG curve of NP-Si-Cell, N_2 adsorption and desorption isotherms of NP-Si-Cell, UV−vis spectra of the solution with different concentration of H_2O_2 catalyzed by peroxidase, peroxidase-like catalyst (Fe-NP-Si-Cell), test papers, the photographs of the oxidated TMB adsorption experiments on MCM-41. These 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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