Chemically Responsive Photonic Crystal Hydrogels for Selective and Visual Sensing of Thiol-Containing Biomolecules

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ABSTRACT: Intracellular thiols (e.g., cysteine, homocysteine, and glutathione) play critical roles in biological functions. Glutathione is the most abundant cellular thiol which is important for preserving redox homeostasis in biosystems. Herein, we demonstrated the fabrication of responsive photonic crystals (RPCs) for selective detection of thiol-containing biomolecules through the combination of self-assembly of monodisperse carbon-encapsulated Fe₃O₄ nanoparticles (NPs) and in situ photopolymerization. Typically, the polyacrylamide-based PCs were prepared by a cross-linking agent containing disulfide bonds. Interestingly, the specific chemical reaction between the disulfide bonds and thiol-containing biomolecules leads to the decrease of the cross-linking degree for the RPCs, triggering the swelling of the hydrogel and increase of the NP lattice spacing. The reduced glutathione (10⁻⁶ to 10⁻² mol/L) can be determined by measuring the diffracted wavelength or visually observing the structural color change. Moreover, the RPCs can be used to detect different kinds of thiol-containing biomolecules by a simple color variation due to different reaction rates between disulfide bonds and different thiol-containing biomolecules. This study provides a facile yet effective strategy for visualized determination of the thiol-containing biomolecules.

1. INTRODUCTION

Intracellular biothiols [e.g., cysteine (CYS), homocysteine (HCY), and glutathione (GSH)] play vital roles in physiological and pathological processes.¹ GSH is the most abundant nonprotein thiol and is important to maintain the optimum redox status of biosystems. For example, GSH protects cellular functions by removing reactive oxygen species through a reversible redox process. The ratio of reduced GSH and oxidized glutathione (GSSH) is a pivotal indicator for monitoring the oxidative stress and the cellular redox environment inside the cells.² More importantly, abnormal levels of GSH have been reported to be a diagnostic indicator for various diseases such as Alzheimer’s disease, cancer, cardiovascular disease, and others.³ Therefore, the sensitive and efficient biothiol detection has attracted great attention over the past decades.

Recently, the contents of biothiols in biological samples can be determined by various means, including fluorescence spectroscopy,⁴ circular dichroism spectroscopy,⁵ electrochemical methods,⁶ liquid chromatography,⁷ colorimetry,⁸ surface-enhanced Raman scattering,⁹,¹⁰ and enzymatic recycling assay.¹¹ Although various techniques have achieved good results, they often suffer from time-consuming, trained personnel, expensive instrumentation, or inconvenience, limiting their practical applications. Therefore, it still remains a big challenge to develop a facile strategy for biothiol detection in a sensitive, low-cost, and efficient manner.

Responsive photonic crystals (RPCs) are materials with periodically refractive indices. The photonic band gaps of RPCs can be tuned by the hydrogel swelling and deswelling in response to environmental variation, such as temperature,¹² electric field,¹³ light,¹⁴ metal ions,¹⁵ organic solvent,¹⁶ pH,¹⁷ biomolecules,¹⁸–²⁵ and others. The structural color provides an easy yet efficient method to indicate the lattice spacing of PCs and can be visually discerned by the naked eyes. However, physically RPCs do not have better selectivity because the photonic band gaps of physically RPCs can be altered by different kinds of organic solvents or biomolecules. Based on the specific reaction, the photonic band gaps of chemically RPCs cannot be altered by the organic solvents or biomolecules. Therefore, chemically RPCs have better selectivity than physically RPCs.

Herein, we demonstrated the fabrication of disulfide-based RPCs that can react with reduced GSH, triggering a diffraction wavelength shift. The RPCs were prepared using a cross-linking agent containing disulfide bonds by the combination of different thiol-containing biomolecules.
self-assembly of carbon-encapsulated magnetic nanoparticles (NPs) and in situ photopolymerization. The reaction between disulfide bonds and GSH decreases the cross-linking degree of hydrogel, which triggers the swelling of the hydrogel and increase of the magnetic NPs spacing. Based on the variation of the cross-linking degree in the RPCs under different GSH concentrations, the RPCs can be used to detect the concentration of GSH by measuring the diffracted wavelength or visually observing structural color variation. Moreover, different kinds of thiol-containing biomolecules can be detected by a simple color change due to different reaction rates.

2. RESULTS AND DISCUSSION

2.1. Fabrication of RPCs. Monodispersed carbon-encapsulated Fe₃O₄ NPs were synthesized by the one-step solvothermal method. In Figure 1a, the scanning electron microscopy (SEM) image for Fe₃O₄ NPs sample shows that the sample is composed of monodisperse carbon-encapsulated Fe₃O₄ NPs with a diameter of 130 ± 5 nm. The transmission electron microscopy (TEM) image in the inset of Figure 1a indicates that the Fe₃O₄ NPs have an obvious shell structure and are composed of numerous iron oxide nanocrystals. In our previous reports, we have shown that the Fe₃O₄ NPs can form one-dimensional (1D) chain structure under external magnetic field by the electrostatic repulsive and magnetic forces between Fe₃O₄ NPs. The RPCs film was prepared by adding acrylamide (AM), cross-linker N,N’-cystaminebis(acrylamide) (CBIS), and N,N’-methylenebisacrylamide (BIS). The periodically ordered structures of Fe₃O₄ NPs can be immobilized in the hydrogels by self-assembly of Fe₃O₄ NPs and UV photopolymerization (Figure 1b). As shown in Figure 1c,d, the RPCs display a vivid green color, which results from the strong diffraction of visible light by the periodic structures. The reflection peak of the RPCs was recorded and shown in Figure 1d. The position of the maximum reflection peak of the RPCs follows the Bragg’s law

\[ \lambda_{\text{max}} = 2n_{\text{eff}}d \]

where \( n_{\text{eff}} \) is the effective refractive index of the material, \( \lambda \) is the diffraction wavelength, and \( d \) is the center-to-center spacing of colloidal particles. Thus, the diffraction wavelength and resulting structural color can be changed by controlling the lattice spacing of the NPs in the RPCs.

2.2. RPC-Based GSH Sensing. The disulfide bond cross-linked RPCs have the stable chemical properties in the phosphate buffered solution (PBS, pH = 6.8). Structural color of the hydrogels does not change and has a diffraction peak at 538 nm within 6 months. The disulfide bond is susceptible to the disulfide–thiol exchange reaction. Based on the specific reaction, the disulfide bond cross-linked RPCs were fabricated and used to detect GSH through the change of structural color. As shown in Figure 3, when being exposed to the GSH solution, the disulfide bond in the RPCs can generate two thiols, and the cross-linking degree was thus decreased, triggering the swelling of the hydrogels and increase of NPs spacing. After the RPCs film was added into different concentrations of GSH solution respectively for 20 h, the structural color of the RPCs film changed from green to yellow and then red gradually as the GSH concentration increases from \( 10^{-6} \) to \( 10^{-2} \) mol/L, as indicated in Figure 2. Meanwhile, the corresponding reflection peak shows an obvious red shift.

Figure 1. (a) SEM image of the carbon-encapsulated Fe₃O₄ NPs. Inset in (a): TEM image of carbon-encapsulated Fe₃O₄ NPs of the core–shell structure. (b) SEM image of the linearly ordered structure in RPCs by self-assembly of Fe₃O₄ NPs. (c) Photograph of the RPCs at a fixed detection angle (90°). (d) Reflection spectra of the RPCs at a fixed detection angle (90°).
from 538 to 658 nm. The reaction process was under an N$_2$ atmosphere in order to prevent the oxidation of GSH. Generally, the intracellular GSH concentration in the human body will be in a range of 1–10 mM/L. Interestingly, the lowest GSH concentration of detection based on the RPCs can be 1 μM through the structural color change with 17 nm red shift. To investigate the GSH response speed, the RPCs film was immersed in 10$^{-5}$ mol/L GSH solutions in a real-time manner (Figure 4). The reflection peak shows a gradual red shift within 20 h, and 16 nm shift of diffraction wavelength can be observed after 1 h. While the reflection peak shows 17 nm red shift of diffraction wavelength after 20 h under 10$^{-2}$ mol/L GSH stimulus (Figure 2). The diffraction wavelength shift can be attributed to the gradual decrease of cross-linking degree with the continuous reaction. The factors of influencing reaction rate are the kinetics constant, concentration of the analyte, and porosity of RPCs. As increasing the enough reaction time, the photonic crystal hydrogels shows an obvious structural color change and a large shift of reflection peak. Therefore, the choice of reaction time (20 h) contributes to the naked eye detection of RPCs.

Furthermore, the RPCs can be used for detecting the other types of thiol-containing biomolecules (Figure 5). For example, the reflection peak of RPCs shifted from 538 to 592 nm after immersing in CYS solution (10$^{-3}$ mol/L) for 20 h. After reaction with GSH (10$^{-3}$ mol/L), the reflection peak of RPCs shifted from 538 to 621 nm. Furthermore, the reflection peak of RPCs shifted from 538 to 602 nm after the reaction with HCY (10$^{-3}$ mol/L). Figure 5f displays the evolution of the diffraction wavelength with the reaction. Based on the variability of reaction rate between disulfide bonds and different thiol-containing biomolecules, the cross-linking degree of RPCs decreased differently after the reaction with GSH, CYS, or HCY solution, respectively, causing the hydrogels to swell and the lattice spacing to increase differently. Therefore, the RPCs can be used to differentiate thiol-containing biomolecules through the structural color change.

2.3. Selectivity of RPCs. Because of different interactions between the polymer and molecules, the diffraction wavelength of physically RPCs can be shifted to different positions when being immersed in the different solutions. Usually, physical interaction-based RPCs do not have better selectivity because the solvent or biomolecules can affect the photonic stop band. While the chemical structure of reactive PCs changes after reacting with analyte molecules, and the other disturbed solvent or biomolecules can be removed after immersing PC films to the deionized water. Therefore, chemical PCs have better selectivity than physical PCs. The selectivity of the RPCs is shown in Figure 6. In our case, the RPCs were respectively dipped in the aqueous solution of 10$^{-3}$ mol/L bovine serum albumin (BSA), glycine, lysine, glutamine, and urea, glucose, or a mixture of urea, glucose, and BSA for 20 h and then immersed to the deionized water. No significant difference in the reaction of RPCs shifted from 538 to 658 nm. Therefore, the RPCs can be used for detecting other types of thiol-containing biomolecules. As increasing the enough reaction time, the photonic crystal hydrogels shows an obvious structural color change and a large shift of reflection peak. Therefore, the choice of reaction time (20 h) contributes to the naked eye detection of RPCs.

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After reacting with $10^{-3}$ mol/L GSH for 20 h, the RPCs were dipped in the PBS solution at an O$_2$ atmosphere for 10 h. However, the position of reflection peak did not change. This demonstrates that the RPCs are irreversible and cannot form the disulfide bond again under the O$_2$ atmosphere.

3. CONCLUSIONS

In summary, we have demonstrated a facile yet efficient strategy for the fabrication of RPCs for thiol-containing biomolecules sensing through the rapid self-assembly of magnetic NPs and in situ photopolymerization. Based on the specific reaction between the disulfide bond in the hydrogels and thiol-containing biomolecules, the RPCs can be used to selectively detect thiol-containing biomolecules concentration by the change of structural color. Moreover, RPCs can be used to differentiate thiol-containing biomolecules by a simple color change. This approach provides a new opportunity for the preparation of visualized chemosensors for thiol-containing biomolecule sensing.

4. EXPERIMENTAL SECTION

4.1. Materials. Ethylene glycol (purity > 99%), ferrocene (purity > 98%), glucose (purity > 98%), hydrogen peroxide (H$_2$O$_2$, 30% water solution), glutathione (reduced, purity > 98%), acetone (purity > 99%), glycine (purity > 98%), lysine (purity > 98%), acrylamide (AM, purity > 98%), and urea (purity > 98%) were purchased from Sinopharm. BSA (purity > 96%) was purchased from Aladdin. CBIS (purity > 99%), glutathione (purity > 98%), cysteine (purity > 98%), homocysteine (purity > 90%), BIS (purity > 99%), and 2-hydroxy-4′-(2-hydroxyethoxy)-2-methyl-propiophenone (purity > 98%) were purchased from Aldrich. The chemicals were used directly without further purification.

4.2. Synthesis of Monodisperse Carbon-Encapsulated Fe$_3$O$_4$ NPs. Fe$_3$O$_4$ NPs were synthesized by a previously reported procedure. First, ferrocene (0.35 g) was added into acetone (35 mL) to form a transparent solution. Then, H$_2$O$_2$ solution (1.9 mL) was dropped slowly to the solution and stirred for 25 min. Subsequently, the mixture was placed into a stainless steel autoclave and heated to 200 °C. After 72 h, the stainless steel autoclave was cooled to room temperature, and the products were then washed for three times using acetone and dried at 65 °C in a vacuum oven for 3 h.

4.3. Preparation of RPCs. Typically, 0.05 g of AM, 0.0001 g of BIS, and 0.0015 g of CBIS were added in 0.2 mL of ethylene glycol. It generated a transparent solution for 10 min by sonication. Then, 0.009 g of the as-prepared Fe$_3$O$_4$ NPs (diameter: 130 ± 5 nm) was added to the above solution, followed by sonication for ~15 min. Subsequently, the suspension was placed between two clean glass slides to form a green film under external magnetic field. Finally, the green films were polymerized for ~15 min under UV irradiation and immersed in deionized water for removing ethylene glycol.

4.4. Characterization. SEM investigation was performed on a Sirion 200 FEI microscope at an acceleration voltage of 10 kV. TEM investigation was performed on an FEI Tecnai G220 microscope with a CCD camera (Gatan USCA4000, Gatan).
operated at an acceleration voltage of 200 kV. A Canon IXUS 105 digital camera was used to get photographs of the RPCs films. Reflection spectral characterizations of the RPCs were collected by a fiber optic spectrometer (USB4000, Ocean Optics).

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**Notes**
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