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Paper Bioassay Based on Ceria Nanoparticles as Colorimetric Probes

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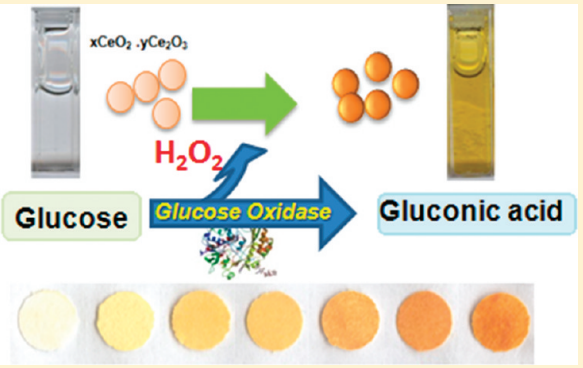
Supporting Information

ABSTRACT: We report the first use of redox nanoparticles of cerium oxide as colorimetric probes in bioanalysis. The method is based on changes in the physicochemical properties of ceria nanoparticles, used here as chromogenic indicators, in response to the analyte. We show that these particles can be fully integrated in a paper-based bioassay. To construct the sensor, ceria nanoparticles and glucose oxidase were coimmobilized onto filter paper using a silanization procedure. In the presence of glucose, the enzymatically generated hydrogen peroxide induces a visual color change of the ceria nanoparticles immobilized onto the bioactive sensing paper, from white-yellowish to dark orange, in a concentration-dependent manner. A detection limit of 0.5 mM glucose with a linear range up to 100 mM and a reproducibility of 4.3% for $n = 11$ ceria paper strips were obtained. The assay is fully reversible and can be reused for at least 10 consecutive measurement cycles, without significant loss of activity. Another unique feature is that it does not require external reagents, as all the sensing components are fixed onto the paper platform. The bioassay can be stored for at least 79 days at room temperature while maintaining the same analytical performance. An example of analytical application was demonstrated for the detection of glucose in human serum. The results demonstrate the potential of this type of nanoparticles as novel components in the development of robust colorimetric bioassays.



Colorimetric sensors on patterned paper or plastic were developed as the least expensive, user-friendly alternative to conventional analytical instrumentations for 'point-of-care' medical diagnosis, environmental monitoring, and food quality control.[1−4] These low-cost platforms have been integrated with both colorimetric[5,6] and electrochemical[7−9] detection systems. Such sensors are miniaturized and disposable and can be used for on-site analysis. Examples of paper bioassays include patterned paper fabricated by photolithography for detection of glucose and bovine serum albumin (BSA),[1] inkjet-printed sol−gel bioinks for detection of neurotoxins,[10] aptamer−nanoparticle-based lateral flow devices for detection of DNA sequences.[11]

In conventional colorimetric paper-based enzyme assays, an enzymatic reaction associated with a colorimetric process involving soluble reagents is performed onto a paper or plastic platform. Typically, the enzyme is immobilized onto the sensing platform. The level of target analyte is quantified by a visible color change detectable with the naked eye. Most strategies reported previously involve the migration of soluble dyes onto hydrophilic paper with well-defined hydrophobic regions to provide control of reagents and biological liquids. This type of assay has been reported initially by the Whitesides group for simultaneous detection of glucose and BSA in urine.[5] The assay is based on the enzymatic conversion of glucose by glucose oxidase to form H_2O_2 which reacts with horseradish peroxidase (HRP) and potassium iodide, resulting in a color change from clear to brown. Abe et al.[12] have developed a multianalyte paper-based microfluidic device by inkjet printing to quantitatively detect human serum albumin, glucose, and pH. Dungchai et al.[6] have proposed an alternative design in which multiple colorimetric indicators are used to improve the ability to visually discriminate between different analyte concentrations such as glucose, lactate, and uric acid. The assay is based on the oxidation of soluble indicators such as 4-aminoantipyrine, 3,5-dichloro-2-hydroxybenzenesulfonic acid, *o*-dianisidine dihydrochloride, and potassium iodide by H_2O_2 produced by analyte-specific oxidase enzymes. In another report, a biocompatible enzyme-based sol−gel ink was printed onto a colorimetric paper strip to create a bioactive surface. The sensor was used for the indirect detection of acetylcholinesterase (AChE) inhibitors, paraoxon, and aflatoxin B1, by measuring the residual activity of AChE on paper using the conventional Ellman's colorimetric assay.[10]

In addition to the use of soluble dyes, nanoparticle-based colorimetric bioassays have been reported, with some adapted to paper platforms.[13] Traditionally, these assays are based on gold nanoparticles and the detection principle relies on nanoparticle-induced aggregation or dispersion in the presence of the analyte.[13,14] Most colorimetric nanoparticle assays are DNA assays; few have been adapted to enzymatic reactions. An enzymatic paper "dipstick" for detection of enzyme inhibitors

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which incorporates a sol−gel/enzyme/gold nanoparticle composite with colorimetric detection has been developed on paper. This method is based on the growth of gold nanoparticles induced by the product of the enzymatic reaction.[16] Detection of glucose in urine samples using gold nanoparticles functionalized with glucose oxidase has been reported recently.[15]

In this paper, we describe a novel concept that utilizes cerium oxide (CeO_2 or ceria) nanoparticles as a novel chromogenic component for colorimetric paper bioassays. As opposed to procedures based on nanoparticle aggregation, here we take advantage of the color changes resulting from alterations in the redox state and composition of these nanoparticles in response to the analyte. We hypothesize that ceria can be used as a redox color indicator in the absence of soluble dyes and demonstrate the possibility to incorporate ceria nanoparticles and enzymes into a paper-based colorimetric format. We establish the analytical capabilities of this approach in bioanalysis for the detection of H_2O_2 and of oxidase enzyme substrates, using glucose and glucose oxidase as a model example.

Ceria has been traditionally used in catalytic applications in automotive combustion engines and solid oxide fuel cells. Relatively few applications of ceria in bioanalysis have been reported. Nanoparticles of ceria have recently been used in biomedicine because of their interesting catalytic and radical scavenging properties and their low toxicity.[17] It has also been found that ceria nanoparticles have oxidase-like activity in aqueous environments, being able to act as an oxidation catalyst.[18] Ceria exists as mixed valence state oxides of Ce^{3+} and Ce^{4+}; the two states can interchange in a redox environment. For example, H_2O_2 can act as both an oxidation and reduction agent and induce a switch between Ce^{3+}/Ce^{4+} states.[19] We have used this property to fabricate ceria-based electrochemical sensors for the detection of H_2O_2[20] and enzyme sensors that are able to operate in oxygen restrictive environments.[21,22] Perez et al. have used polymer-coated ceria nanoparticles to develop a ceria-based immunoassay in which these particles act as a catalyst to facilitate direct oxidation of organic dyes[18] and of a nonfluorescent substrate to its stable fluorescent product.[23] In this study, we demonstrate that ceria nanoparticles can be used as a colorimetric indicator to replace commonly used soluble dyes in oxidase enzyme assays. We then adapt this concept to a paper-based bioassay format.

Bioassay Concept and Detection Mechanism. Conventional colorimetric assays of oxidase enzyme substrates are based on an oxidase enzyme, HRP and an organic redox dye such as *o*-dianisidine, KI, or ABTS (2,2′-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid). The method involves two enzymatic reactions: oxidation of substrate by the oxidase enzyme to form H_2O_2, which in a second step, is coupled with HRP and the soluble redox dye to generate a color change (Figure 1A as an example for glucose detection). In previously developed paper-based enzymatic assays, the soluble dye (e.g., KI) migrates to the sensing spot by capillary action.[5,6] In the new colorimetric assay proposed in this work, redox active ceria nanoparticles are used as a chromogenic indicator for H_2O_2, eliminating the need for both the organic dye and the peroxidase enzyme. The functioning principle of this ceria-based assay for the detection of glucose is presented schematically in Figure 1B. The particles and the enzyme are coimmobilized onto a paper platform, and the only step needed to perform the analysis is the addition of the analyte. The visible color change from white-yellowish to dark orange in the presence of glucose is due to the change of the oxidation state

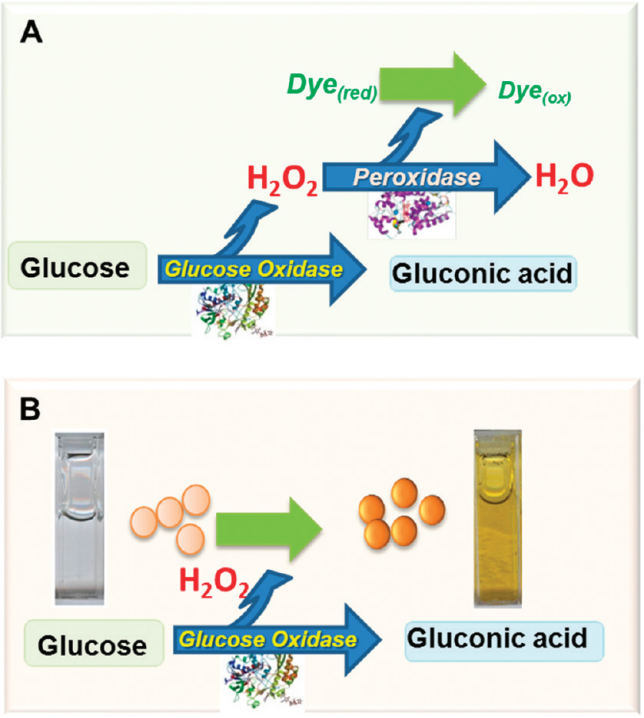

Figure 1. Schematic representation of the working principle of colorimetric assays for the detection of glucose: (A) Conventional assay involving the use of HRP and an organic dye. (B) The ceria-based assay described in this work.

and formation of surface complexes[19,24] onto the ceria nanoparticle surface, induced by the enzymatically produced H_2O_2. Hydroxylated Ce^{4+} forms a reddish-orange complex with H_2O_2, with a maximum absorbance at 465 nm. This interesting property, first discovered by Lecoq de Boisbaudran, has been used as a sensitive test for cerium ions,[25] but it has not been utilized as an analytical test for other compounds. To our knowledge, this present work is the first to report (1) the use of ceria nanoparticles as chromogenic indicators in an enzyme assay, (2) the immobilization of ceria onto paper, and (3) the integration of this concept to construct a paper bioassay for the detection of glucose for point-of-care diagnostics. Initial studies were designed to determine the concentration dependency of the nanoparticle assay as a function of H_2O_2 concentration. This was done with nanoparticles both in solution and immobilized onto the paper platform. Specific structural characteristics and physicochemical changes of the nanoparticles after exposure to H_2O_2 and the optimum operational parameters for achieving high sensitivity were evaluated. These investigations established the feasibility of the colorimetric detection on paper using these particles. Next, we coimmobilized glucose oxidase and ceria onto paper and determined the analytical performance of this assay for the detection of glucose in standard solutions and in human serum.

EXPERIMENTAL SECTION

Reagents. Cerium(IV) oxide nanoparticles 20 wt % colloidal dispersion in 2.5% acetic acid, 10−20 nm (289744) and 100 nm (544841) average particle size, glucose oxidase (G6641), glucose (G5767), aminopropyltriethoxysilane (APTS), chitosan,

glutaraldehyde, H_2O_2, and sodium phosphate were from Sigma Aldrich. The average particle size of the ceria nanoparticles was checked by scanning electron microscopy and dynamic light scattering. Human serum (S7023) was purchased from Sigma and used as received. Whatman filter paper (no. 1) was from VWR and used as received. All reagents were used without further purification, and all solutions were prepared with distilled, deionized water (Millipore, Direct-Q system) with a resistivity of 18.2 MΩ.

Detection of H_2O_2 with Ceria in Solution. Colorimetric detection of H_2O_2 with colloidal ceria nanoparticles was performed by mixing distilled water with colloidal ceria solution (0.2% w/v) and adding H_2O_2 (0.1 M in serial dilution) to obtain final H_2O_2 concentrations ranging from 0.01 to 0.5 mM. The absorbance was measured at 500 nm with a Shimadzu P2041 spectrophotometer equipped with a 1-cm path length cell.

Preparation of Ceria-Based Bioactive Paper. Round-shaped Whatman no. 1 filter paper were cut with a hole-puncher, soaked for 10 min in 3% colloidal ceria solution, and dried for several hours at 75 °C. After drying, the ceria paper was dip-soaked in 5% APTS in ethanol for 10 min and then dried for 10 min at 100 °C. This silane treatment stabilized the ceria nanopaticles onto the filter paper. These ceria paper strips were used for the detection of H_2O_2. To fabricate enzyme-modified ceria paper, freshly prepared silane−ceria papers were soaked in 1% chitosan solution (in 0.5% succinic acid) for 10 min and air-dried for 5−10 min. The paper was then treated with 5% glutaraldehyde for 1 min, dipped in water, and air-dried for 5−10 min. To immobilize glucose oxidase, 20 μL of 9 mg/mL enzyme (190.8 U/mL) were applied onto the ceria strips. The strips were then rinsed with phosphate buffer at pH 7.4 and air-dried. The diameter of the strips was 0.6 cm.

Detection of H_2O_2 and Glucose with Ceria-Modified Paper. The ceria-based paper assay was first optimized for H_2O_2 detection prior to the application of the detection of glucose. For detection of H_2O_2, the silane-modified ceria paper was immersed in solutions of varying concentrations of H_2O_2. For detection of glucose, the ceria papers with immobilized glucose oxidase were immersed in glucose solutions of concentrations ranging from 0.5 to 500 mM in 0.05 M phosphate buffer. The intensity of the color was measured and used to construct the calibration curve and to determine the analytical performance of the assay. For analysis of serum, samples were spiked with known concentrations of glucose and the intensity of the color was measured. The concentration of glucose in the serum sample was quantified using the standard addition method. Control experiments in the absence of ceria and enzyme were performed. The reproducibility of the paper bioassay was evaluated by measuring the color intensity of 11 enzyme-modified ceria paper strips that were fabricated following the same procedure. The storage stability was assessed by measuring the color intensity of identical enzyme strips stored in the same conditions (room temperature, refrigerator, and freezer) in response to 100 mM glucose at various time intervals.

Color Intensity Measurement. The color developed instantaneously for H_2O_2 and within 10 min for glucose. For quantification of the color, the ceria paper strips were scanned with a conventional office scanner. Images were analyzed using Adobe Photoshop. The colors of the paper were assessed using the eyedropper tool on Photoshop, which took an average of 31 × 31 pixels (or 961 pixels) close to the center of the papers, leaving out the edges. The area of individual papers was found to be approximately 2600 pixels. The blue color intensity was found to be the most sensitive color analysis option as blue is the complementary color to yellow/orange; e.g., the blue color channel is a "negative" image of the yellow channel. Other available color scale options were tested: gray, red, and green (two other channels of RGB color scheme), CMYK, and "Lab" color but the sensitivity of analysis was lower.

RESULTS AND DISCUSION

Spectroscopic Investigation of Ceria Nanoparticles for Colorimetric Detection of H_2O_2. Initially, we investigated if interaction of ceria nanoparticles, of 20 nm diameter, with H_2O_2 in solution induces a color change of the nanoparticles and if this color change is concentration dependent. The UV−visible properties of nanoceria treated with H_2O_2 have been observed by Das et al.[17] when investigating the neuroprotective properties of these particles in cell cultures, but the size and concentration dependency have not been studied or applied for analytical purposes. Figure 2 shows that a suspension of colloidal ceria (0.2% w/v), that is nearly colorless in the absence of H_2O_2, changes its color instantaneously to reddish-orange upon contact with H_2O_2. Development of the color indicates changes in the surface properties and chemical composition of the ceria nanoparticles. Addition of H_2O_2 can induce oxidation of the surface-exposed Ce^{3+} to Ce^{4+} species[26] and formation of peroxide complexes at the nanoparticle surface.[27] These processes and the ratio of the different ceria species are dependent on the nature, surface coating, and size of the particles. These can vary with the synthetic procedure and additives used to fabricate and stabilize the particles. In an aqueous environment, ceria can form $Ce(OH)_2^{2+}$, $Ce(OH)_3$, and $Ce(OH)_4$, and after exposure to H_2O_2, it can form surface-adsorbed hydroperoxide, superoxide (O_2^-), and peroxide (O_2^{2-}) species such as $Ce(OH)_2(O_2)$, $Ce(O_2)^+$, and $Ce(H_2O_2)^{3+}$.[27] To determine the nature of these changes in the commercial Sigma particles used in this work, we performed structural and physicochemical characterization of the nanoparticles in the presence and absence of H_2O_2. X-ray photoelectron spectroscopy (XPS) spectra of the 20 nm Sigma particles revealed the presence of both Ce^{3+} and Ce^{4+} species (Figure 1S, Supporting Information). After addition of H_2O_2, a small change in the Ce^{3+}/Ce^{4+} ratio was observed, with a slight increase in the Ce^{4+} concentration. The formation of adsorbed peroxide and hydroxylated species onto the ceria particles was confirmed by Fourier transform infrared (FTIR) spectroscopy (Figure 2S, Supporting Information).

The intensity of the color is linearly dependent upon the concentration of added H_2O_2, with a linearity range from 0.01 to 0.15 mM (Figure 2B inset). To demonstrate the importance of particle size on assay sensitivity, we studied two colloidal solutions consisting of 20 and 100 nm particles. Figure 2C shows the effect of particle size on the intensity of the color. An intense dark orange/brown color was obtained with the 20 nm particles, while for the larger particles only a slight color change was observed. This observation demonstrates that the size and surface area of the nanoparticle are critical parameters of this colorimetric assay; only small size ceria particles, having a higher surface to volume ratio, provide quantifiable signals. The sensitivity of the method can be further enhanced by increasing the surface area to volume ratio. In colloidal solution, the assay performed optimally at pH values close to neutral. At a pH higher than 8, the nanoparticles form large aggregates of hydroperoxide complexes that

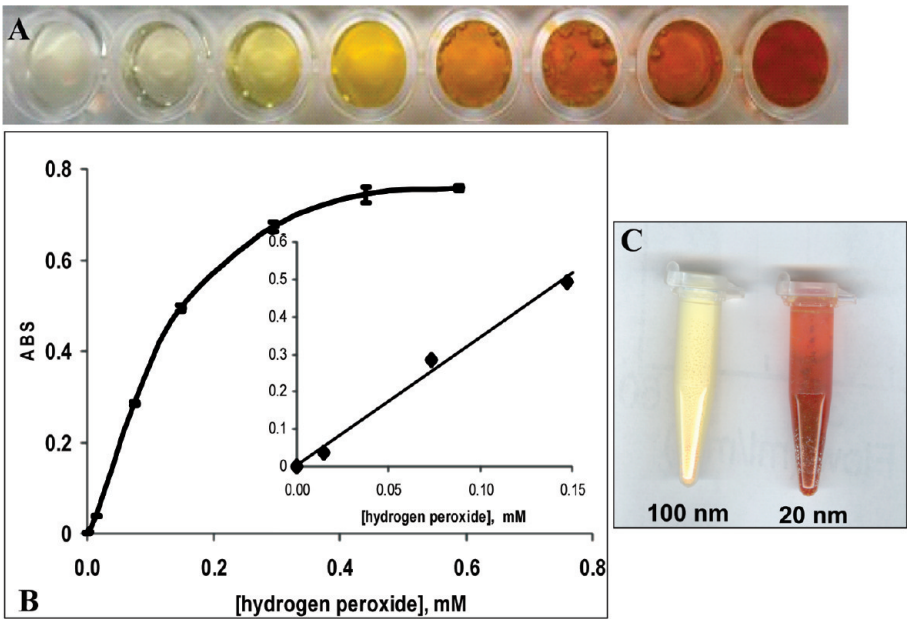

Figure 2. Colorimetric assay of colloidal ceria nanoparticle suspension in the presence of various concentrations of H_2O_2 at neutral pH: (A) Photographs of wells showing formation of color in a concentration-dependent manner from 0.01 to 0.6 mM H_2O_2. (B) Calibration curve and linear range (inset) for H_2O_2. The intensity of the color was determined spectrophotometrically by measuring the absorbance of the dark orange ceria complex with H_2O_2 at 500 nm. (C) Effect of particle size (20 and 100 nm ceria) in the presence of 110 mM H_2O_2.

precipitate in solution. A physiological pH of 7.4 and the 20 nm nanoparticles were used in this study to demonstrate the 'proof-of-concept' of the colorimetric paper-based bioassay for the detection of glucose.

Fabrication of the Ceria-Modified Paper: Immobilization of Ceria Nanoparticles on Paper and Detection of H_2O_2. To adapt this assay to paper format, we immobilized 20 nm ceria particles onto Whatman no. 1 filter paper. Preliminary studies to electrostatically attach ceria by simple adsorption onto the cellulose paper did not stabilize the particles; the binding was weak, and the particles leached rapidly from the surface. In future experiments the ceria particles were entrapped within a silica layer deposited onto the paper surface, as described in the Experimental Section. Silica sol−gel is an excellent immobilization matrix for use in colorimetric sensors because of its high stability and optical transparency. Effective stabilization of the ceria particles was achieved by silanization of the paper using aminopropyltrimethoxysilane (APTS) to form siloxane bridges with the OH-rich surface of the cellulosic fibers and the hydroxylated ceria through hydrogen-bonding. The alkoxy end groups of the APTS provide anchoring points for silane attachment to surfaces bearing hydroxyl groups,[28] enabling strong adhesion between the cellulose fibers and the ceria, effectively attaching the particles. The method provides an easy and efficient fabrication of ceria paper with good uniformity, surface coverage, and high mechanical strength. The APTS also provided amino functionalities for further grafting of glucose oxidase in the development of the glucose bioassay.

The immobilized ceria nanoparticles retained their color-changing properties in response to H_2O_2, even after silanization. The paper developed a dark-orange color instantaneously after applying H_2O_2 solution. The appearance of the color is due to changes onto the ceria nanoparticle surface as described above. The color is evenly distributed on the surface of the sensing strip, indicating uniform distribution and surface coverage of the nanoparticles onto the paper and also confirming the effectiveness of the immobilization process. No color change was observed in the absence of ceria for any of the H_2O_2 concentrations tested. Figure 3 shows a schematic representation of the ceria-modified paper, the response of this assay to H_2O_2 concentrations ranging from 0 to 500 mM, and the corresponding linear calibration curve with a linearity range from 2.5 to 100 mM H_2O_2 (Figure 3). The optical images and the calibration curve provide evidence that quantitative colorimetric detection of H_2O_2 is possible with this assay. In this format, the ceria paper can be used to determine H_2O_2 concentrations in the concentration range from 2.5 to 100 mM. Examples of applications include determination of extracellular H_2O_2 to monitor overproduction of H_2O_2 (e.g., to indicate inflammatory conditions near wound margin) and in water monitoring in environments where H_2O_2 is used as a disinfecting agent and it is present in high concentrations.

Interestingly, although the catalytic activity of ceria particles has been reported to be pH dependent when these particles were used to oxidize organic dyes,[18,23] our ceria-based paper assay did not show pH dependency to H_2O_2 over a wide range of pH. The effect of pH was evaluated by measuring the color intensity of the ceria paper to 100 mM H_2O_2 at pH values ranging from 2 to 10.5. The assay with immobilized ceria particles performed similarly over the entire pH range tested (Figure 4). We attribute this behavior to the stabilization of the ceria species through formation of hydrogen bonding with the immobilized silica layer.

Fabrication of the Ceria-Based Glucose Assay: Analytical Performance Characteristics for the Detection of Glucose. To demonstrate versatility of this assay in bioanalysis and impart selectivity, we biofunctionalized the ceria paper with glucose oxidase and used this assay for quantitative detection of glucose. Figure 1B shows the working principle of the ceria bioassay via detection of H_2O_2 produced by glucose oxidase in the presence of glucose, followed by changes in the ceria nanoparticle surface,

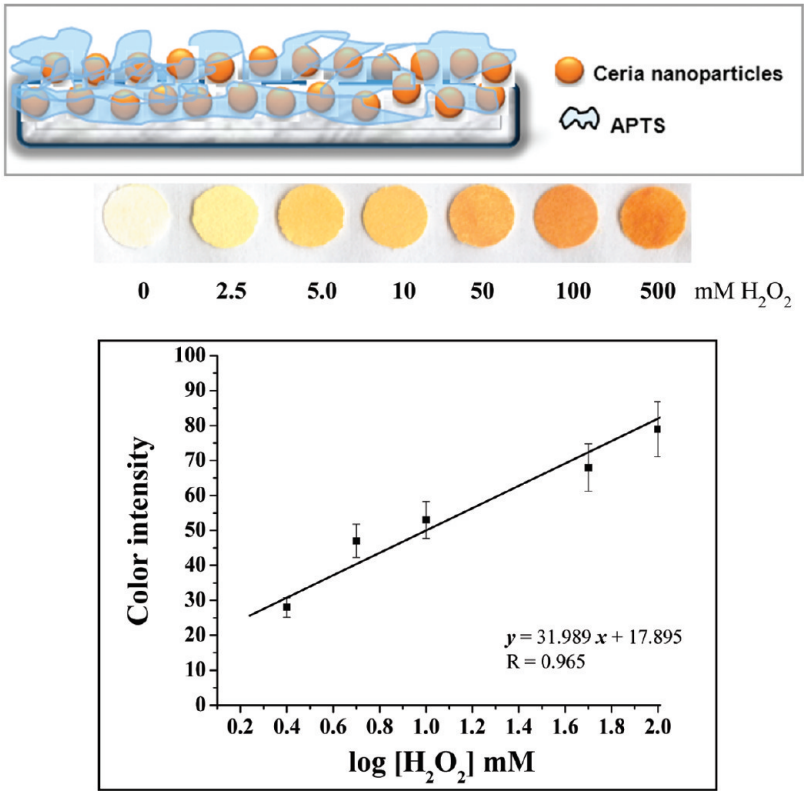

Figure 3. Ceria-based paper assay for the detection of H_2O_2. (A) Schematic of the paper with immobilized ceria nanoparticles in APTS. (B) Colorimetric responses of ceria strips to concentrations of H_2O_2 ranging from 0 to 500 mM. (C) Linear calibration curve for H_2O_2 (triplicate measurements of color intensity quantified using Adobe Photoshop).

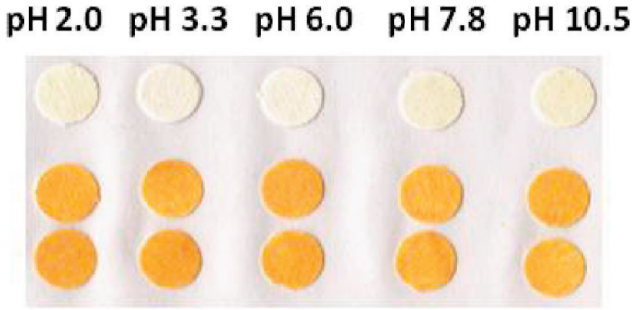

Figure 4. Effect of pH on the colorimetric response of the ceria paper to 100 mM H_2O_2 (top row: control with no H_2O_2, two bottom rows: response after addition of H_2O_2 in duplicate experiments).

with the development of the color from white-yellowish to dark-orange. To fabricate the glucose assay, glucose oxidase was immobilized onto the silanized paper, in close proximity to the ceria nanoparticles. The method was optimized to obtain a bioactive and stable enzymatic layer and provide good functionality, reaction rate, and sensitivity of the assay. Preliminary experiments were performed to entrap the enzyme within the APTS silica sol by depositing the enzyme simultaneously with the APTS onto the paper. However, using this procedure, no activity was detected onto the ceria paper upon addition of glucose, indicating that the enzyme was deactivated or leached during the process. In future experiments, the enzyme was covalently attached onto the silanized paper using glutaraldehyde. However, this procedure involving direct functionalization of the APTS paper with the coupling agent, followed by enzyme deposition, showed very little enzymatic activity. We found that deposition of a chitosan layer onto the silica paper, prior to covalent binding of the enzyme, provides a much more stable and biocompatible environment for the enzyme.

Figure 5A shows the multilayered sequence of the bioactive paper, consisting of ceria nanoparticles, silica, chitosan, and glucose oxidase. The amino groups of the chitosan provide enhanced binding sites for the enzyme, enhancing stability and bioactivity. In the presence of glucose, the ceria paper with immobilized glucose oxidase visually changes the color from white-yellowish to dark-orange in a concentration-dependent manner. As seen in Figure 5B, increasing concentrations of glucose result in an increase in the color intensity. No change in color was observed in the absence of enzyme, indicating that the color formed is due to the enzymatically generated H_2O_2. The response time of the assay after addition of glucose is 10 min. The linear range of the assay is between 0.5 and 100 mM (Figure 5C). The glucose assay performed similarly at pH values close to physiological conditions, between 6.5 and 7.5. The detection limit of the assay is 0.5 mM glucose, which is comparable to that reported with the paper assay developed by Martinez et al. based on HRP, glucose oxidase, and potassium iodide.[5,29]

Stability and Reproducibility. The reproducibility of the ceria-based paper assay with immobilized glucose oxidase was evaluated for 11 identical ceria strips prepared independently but following the same construction protocol. The colorimetric images of the 11 ceria strips tested are shown in Figure 3S, Supporting Information. The average color intensity after

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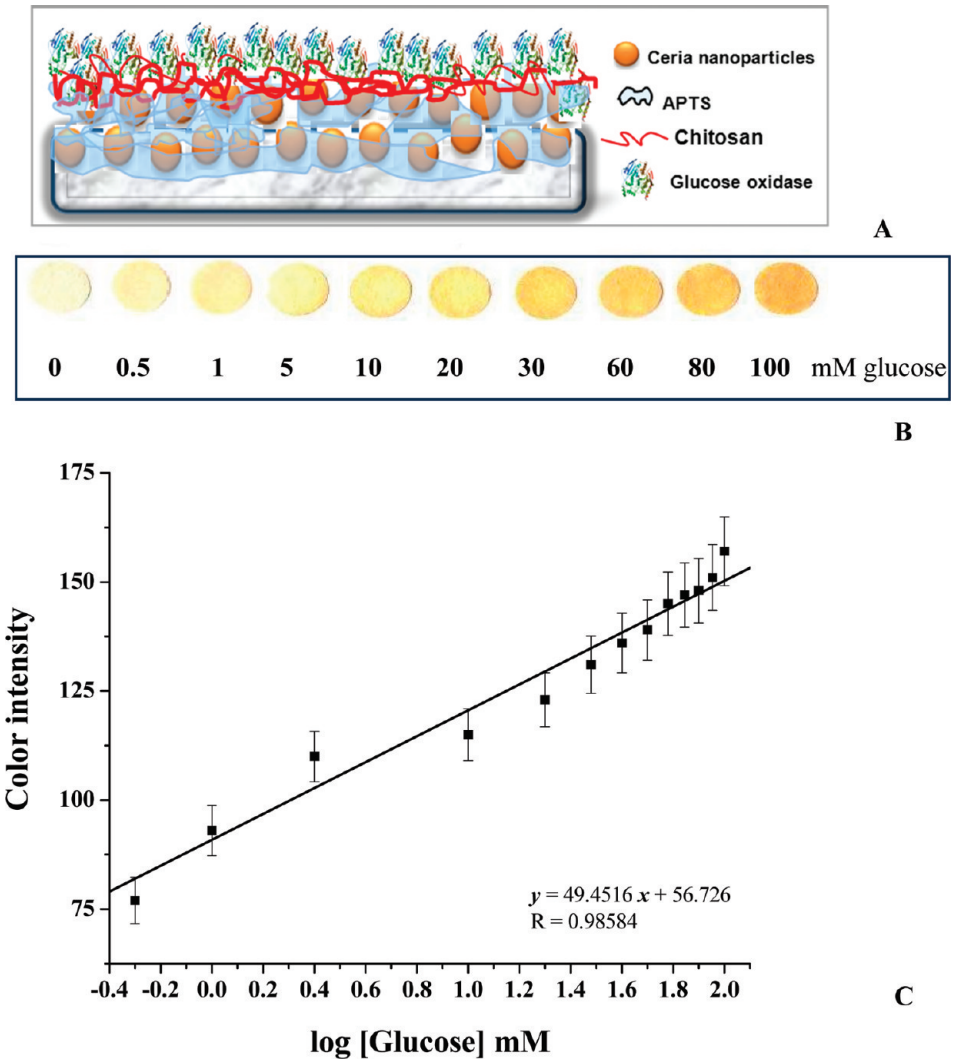

Figure 5. Ceria-based paper bioassay with immobilized glucose oxidase for the detection of glucose. (A) Multilayer sequence of the immobilization of glucose oxidase onto the silanazed paper. (B) Colorimetric response of the paper sensors to glucose concentrations ranging from 0 to 100 mM. (C) Linear calibration curve for glucose (triplicate measurements of color intensity quantified using Adobe Photoshop).

addition of 100 mM glucose was 102.2 (± 4.4) for $n = 11$ with a relative standard deviation of 4.3%. The storage stability of the glucose assay was evaluated by measuring the colorimetric response of the ceria papers stored in dried state at room temperature (RT), in the refrigerator and freezer. A concentration of 100 mM glucose was used in these tests, and each experiment was performed in triplicate. Figure 6 shows the storage stability data under the three conditions over a period of 79 days. The results demonstrate excellent storage stability of the assay, even when stored at room temperature. Ceria papers stored in the refrigerator showed no decrease in response after 79 days: the stability of the assay under these conditions was 100% with a coefficient of variation of 1.6%. The stability of the ceria papers stored at room temperature was 100% in the first 29 days and then decreased by 10% in the next 50 days.

Reusability. The ceria paper assay is fully reversible and can be reused for multiple cycles for the detection of both H_2O_2 and glucose. This is possible because of the spontaneous decomposition of the adsorbed peroxide species, which takes place gradually over several days at room temperature or within several minutes by slight heating. We first studied the reversibility of the assay after exposure to 88 mM H_2O_2. The ceria paper gradually regained its initial white-yellowish color at room temperature after a 10 days storage period in dry state. The decomposition process can be sped up by incubating the ceria papers at 100 °C in the oven; the assay fully regained its initial sensing properties after 40 min heat treatment. The paper can be reused for further applications for at least 10 assays, without significant loss of activity. Figure 7 shows sequential colorimetric responses of reused ceria discs after cycles 1, 3, 5, and 10 (the discs of light yellow color are the reused sensing paper and the ones of dark orange color are after exposure to the same concentration of H_2O_2). The ceria paper with immobilized glucose oxidase was reused for six consecutive cycles, with a coefficient of variation of 6.4% in between assays.

Analytical Application: Detection of Glucose in Real Samples. Performance of the ceria-based glucose assay was tested in human serum. According to the provider, Sigma Aldrich, the human serum was collected from a healthy patient, with no preservatives and no additives added. We can therefore assume that the serum sample contains the normal physiological concentration of glucose in a healthy person, between 3.6 and

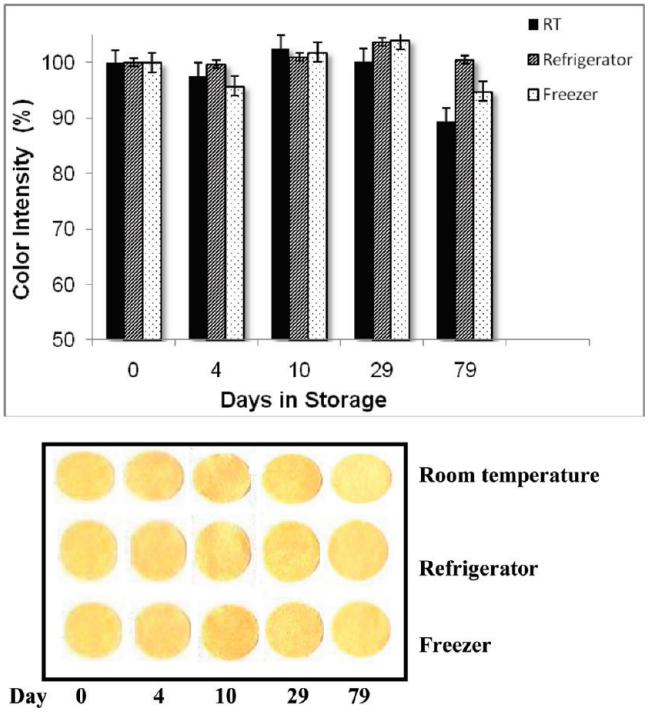

Figure 6. Storage stability of the ceria paper with immobilized glucose oxidase at room temperature and in the refrigerator and freezer (shown as % descrease with 100% being the response at day 0) and images of colorimetric responses under the three storage conditions.

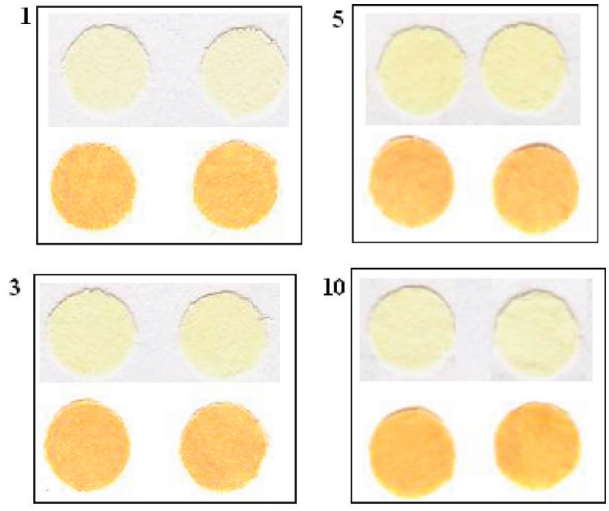

Figure 7. Reusability of the assay after reconditioning the ceria paper by heating at 100 °C. The picture shows colorimetric responses of reused ceria discs after cycles 1, 3, 5, and 10 (top row of light yellow color is the sensing paper after reconditioning, bottom row of dark orange is the colorimetric response after exposure to H_2O_2).

5.8 mM. To determine the glucose concentration in the serum sample, we have used the standard addition method to calibrate the assay. The concentrations of glucose added to the serum to construct the calibration curve ranged from 0 to 35 mM. Experiments were performed in triplicate for each concentration. Figure 8 shows the calibration curve for glucose in serum with

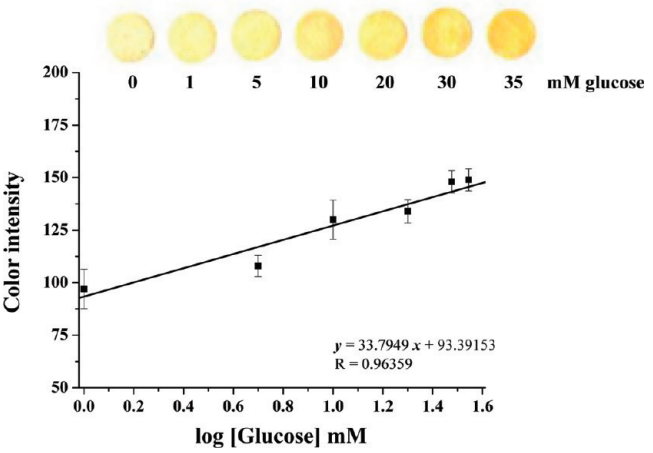

Figure 8. Colorimetric responses of ceria bioassay to glucose in spiked human serum and the corresponding linear calibration curve (average color intensity measurements of $n = 3$ ceria strips).

the corresponding colorimetric images for each concentration tested. The intensity of the color obtained with the ceria papers in buffer in the absence of glucose was 41(±3.79) for $n = 3$. Under the same conditions but after addition of serum, the intensity was 74(±5.69). This indicates that glucose is present in the serum sample. The concentration of glucose in serum determined from the calibration curve using the standard addition method was 3.71 mM. This indicates that the ceria paper performs efficiently in serum.

CONCLUSION

This study demonstrates that ceria nanoparticles can be used as quantitative chromogenic probes in bioanalysis, offering a promising alternative to commonly employed organic dyes. The sensing design takes advantage of changes in the physicochemical and optical properties of ceria nanoparticles in response to the analyte, to create an easy colorimetric readout without additional instrumentation. This study also provides a new nanoparticle platform for the fabrication of paper-based colorimetric bioassays. Significantly, the assay does not require addition of external reagents, because all the sensing components and reagents needed for detection are deposited onto the paper substrate. The assay eliminates the use of both the dye and the peroxidase enzyme in conventional colorimetric tests for glucose. Demonstrated here, as a feasibility study, is detection of H_2O_2 and glucose, but the method can be adapted for the detection of other molecules and other oxidase enzyme substrates. Another novel aspect described here is the patterning and stabilization of ceria nanoparticles onto paper using a silanization procedure and coimmobilization of glucose oxidase onto the same sensing platform to provide specificity. The assay shows sensitivity for detection of physiological glucose concentrations, and it is robust, inexpensive, and performs successfully in human serum samples.

ASSOCIATED CONTENT

Supporting Information. Additional information as noted in the text. This material is available free of charge via the Internet at http://pubs.acs.org.

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