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Reagentless colorimetric biosensing platform based on nanoceria within an agarose gel matrix

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ABSTRACT

A composite consisting of cerium oxide nanoparticles (nanoceria) and an oxidative enzyme co-entrapped in an agarose gel has been developed for the reagent-free colorimetric detection of biologically important target molecules. The oxidase, immobilized in the agarose matrix, promotes the oxidation of target molecules to generate H_2O_2 that subsequently induces changes in the physicochemical properties of nanoceria exhibiting a color change from white/light-yellow into intense-yellow/light-orange without any requirement for additional colorimetric substrates. By utilizing the unique color-changing property of nanoceria entrapped within the agarose gel, target glucose molecules were very specifically detected over a wide linear range from 0.05 to 2 mM, which is suitable to measure the serum glucose level, with excellent operational stability over two weeks at room temperature. The biosensor also exhibited a high degree of precision and reproducibility when employed to detect glucose present in real human serum samples. We expect that this novel nanoceria-based biosensing format could be readily extended to other oxidative enzymes for the convenient detection of various clinically important target molecules.

1. Introduction

Colorimetric biosensors have attracted enormous recent attention since they enable rapid visual detection of target molecules without the requirement of any sophisticated instrumentation or expert skill, which are quite necessary for realizing point-of-care testing (POCT) applications (Miranda et al., 2011; Song et al., 2011; Zhao et al., 2007). Because color changes can be read out even by the naked eye, colorimetric biosensors are generally simple, practical, and cheap to use (Pu and Liu, 2009). With the aim of transforming detection events into color changes, a number of novel materials have been developed and evaluated as colorimetric signal transducers.

Among them, noble metal nanoparticles (gold and silver) or conjugated polymers (polythiophenes and polydiacetylenes) have been intensively utilized in colorimetric assays, producing rapid and sensitive responses to target molecules (Jung and Park, 2015, Jung et al., 2016; Ho et al., 2008; Lee et al., 2007). However, they generally require surface modification with biological receptors and often produce false-positive signals by the relatively small alterations in the reaction environment. Natural enzymes such as horseradish peroxidase or alkaline phosphatase have also been employed for colorimetric biosensors because they can catalyze various colorimetric reactions in the presence of substrates and display good specificity and sensitivity for target molecules (Elandalloussi et al., 2004; Kim et al., 2011). In spite of their good performance, natural enzymes have critical limitations such as instability due to denaturation by harsh conditions (temperature and pH) and time-consuming and expensive procedures for preparation, purification, and storage. Therefore, over the past few decades, researchers have made intense efforts to develop artificial enzymes that overcome these limitations for a wide range of applications (Nelson and Cox, 2005). For example, chemical complexes such as cyclodextrin, porphyrin, hemin, hematin, and specially designed biomolecules in the type of nucleic acid and protein have been studied in place of natural enzymes (Bonar-Law and Sanders, 1995; Chen and Que 1999; Fiammengo and Jäschke 2005; Fruk and Niemeyer 2005; Joyce, 2004; Murakami et al., 1996). Recently, diverse nanomaterials have also been found to possess intrinsic enzyme-like activities (Wei and Wang, 2013; Shin et al., 2015). In contrast to natural enzymes, nanomaterials with enzyme-like characteristics, called nanozymes, are inherently

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stable in harsh conditions and easily mass-produced by chemistry-based scale-up processes, and these advantages make them promising candidates as alternatives to natural enzymes in analytical applications.

Nanoceria have recently been recognized as one of the promising nanozymes for the construction of colorimetric biosensors since they catalyze the fast oxidation of organic dyes to generate colored products without the need for additional oxidizing agents (e.g., H_2O_2) (Asati et al., 2009). Their oxidase-like activity is due to the presence of two oxidation states, Ce^{3+} and Ce^{4+}, of the cerium element that produce oxygen vacancies, which provide high activity of the material to act as a catalyst for both oxidation and reduction reactions (Korsvik et al., 2007; Pirmohamed et al., 2010). Because nanoceria do not have the problem of enzyme instability, they have been widely investigated for the construction of more robust and reliable immunoassay and biosensors to detect DNA and other molecules (Asati et al., 2011; Kim et al., 2014; Njagi et al., 2008).

It is also noteworthy that the external addition of H_2O_2 triggers a unique color change of nanoceria from white/light yellow into intense yellow/orange since it induces the oxidation of the surface-exposed Ce^{3+} into Ce^{4+} species and formation of peroxide complexes at the nanoparticle surfaces, consequently producing the unique absorption behavior of the nanoparticles (Das et al., 2007). The color change of nanoceria is induced only by H_2O_2 without any dyes and the changed color of nanoceria can be returned to its natural color within a few days due to the time-dependent recovery of the ionic valence between Ce^{3+} and Ce^{4+}; as a result, several colorimetric assays to detect H_2O_2 and glucose with the aid of glucose oxidase have been developed based on these characteristics (Lang et al., 2014; Lin et al., 2012; Ornatska et al., 2011). Although these examples demonstrate the potential of nanoceria as novel colorimetric transducers to detect H_2O_2 in samples, further investigations are required to make them more compatible with other enzymatic systems and facilitate their easy separation and reuse with improved stability.

To accelerate and widen the practical utility of nanoceria-based colorimetric biosensing platforms, herein, we have developed a composite that consists of nanoceria and an oxidative enzyme simultaneously entrapped in an agarose hydrogel matrix. To the best of our knowledge, this is the first report of immobilized nanoceria as a colorimetric signaling probe within a hydrogel matrix, which may provide an advanced sensory format as well as a biocompatible protective carrier for the enzyme (Mateo et al., 2007). Importantly, the colorimetric assay based on the composite can be performed without any addition of chromogenic reagents, which are usually required in the conventional enzyme or nanozyme-based colorimetric biosensors. Various characteristics of the composite system, such as specificity, sensitivity, stability, reusability, and detection precision were investigated.

2. Experimental

2.1. Materials

Nanoceria (10–20 nm particle size), agarose (low melting temperature), glucose oxidase from *Aspergillus niger* (GOx), cholesterol oxidase from *Streptomyces sp.* (ChOx), β-D-glucose, arabinose, fructose, lactose, maltose, glycerol, triton X-100, uric acid (UA), ascorbic acid (AA), dopamine, sodium acetate, and human serum were purchased from Sigma-Aldrich (Milwaukee, WI). The average particle size of the nanoceria was checked by transmission electron microscopy (TEM) analysis. Hydrogen peroxide (35 wt%, H_2O_2) was purchased from Junsei Chemical Co. (Japan). All other chemicals were of analytical grade or higher.

2.2. Characterization of the materials

For the TEM analyses of the nanoceria in agarose gel (nanoceria_agarose) and free agarose without nanoparticles, the agarose gels were first deformed, and 5 μL from each was applied by drop casting the particle suspensions on a carbon-coated copper TEM grid (Electron Microscopy Sciences, USA) followed by drying at room temperature. All samples were observed using field emission TEM (Tecnai, FEI) with accelerating voltages up to 200 kV, which was then switched to scanning transmission electron microscopy (STEM) and energy dispersive X-ray spectroscopy (EDX) imaging modes. For scanning electron microscopy (SEM) analysis, the samples were freeze-dried for 2 days and analyzed with a field emission scanning electron microscope (Magellan 400). X-ray diffraction (XRD) and X-ray photoelectron spectroscopy (XPS) analyses were performed with an X-Ray Diffractometer (D/MAX-2500, Rigaku) and X-Ray Photoelectron Spectroscope (K-alpha, Thermo VG Scientific), respectively, using samples freeze-dried for 2 days.

2.3. Preparation of nanoceria_agarose and agarose gel_entrapped composite consisting of nanoceria and GOx or nanoceria and ChOx (nanoceria_GOx_agarose or nanoceria_ChOx_agarose)

To entrap nanoceria in the agarose matrix, nanoceria were first dissolved in sodium acetate buffer (100 mM, pH 5.3) at a concentration of 15 mg/mL and mixed with 2% (w/v) low-melting agarose at a 1:4 vol ratio. After mixing thoroughly, 15 mL of the nanoceria_agarose mixtures was poured into a petri dish (90 × 15 mm, SPL Lifesciences, Korea) and solidified at room temperature for 1 h. After the mixture completely became hard, the agarose gel was imprinted using a 200-μL pipet tip as a specific mold to make tablet-type nanoceria_agarose (~5.4 mm in diameter), which greatly fit in the well of a 96-well plate (Fig. S1). Nanoceria_GOx_agarose or nanoceria_ChOx_agarose was prepared by mixing nanoceria (15 mg/mL in sodium acetate buffer (100 mM, pH 5.3)), GOx (10 mg/mL in sodium acetate buffer (100 mM, pH 5.3)) or ChOx (5 mg/mL in sodium acetate buffer (100 mM, pH 5.3)), and 2% agarose solution at a 1:1:3 volume ratio, and the other procedures were the same as described above.

After the formation of nanoceria_agarose, nanoceria_GOx_agarose, or nanoceria_ChOx_agarose tablets, the tablets were washed with deionized water three times and directly used in the experiments. The amount of protein and nanoceria that leached from the agarose gel into the supernatant during the washing steps was measured using the BCA assay (PIERCE, Rockford, IL) and inductively coupled plasma atomic emission spectroscopy (ICP-AES) (model: Polyscan 60E, Thermo Jarrell), respectively. The final loading amount of GOx, ChOx, or nanoceria in the composite system was calculated from the difference between the amount initially added and the amount that leached into the solution.

2.4. H_2O_2 detection using free nanoceria and nanoceria_agarose

H_2O_2 detection was performed in a single tube by employing the following procedure. A mixture containing free nanoceria (1 mg) or nanoceria_agarose (five tablets containing approximately 1 mg nanoceria. See Supplementary Information) and H_2O_2 (100 μL, 100 mM) in 900 μL sodium acetate buffer (100 mM, pH 5.3) was incubated at room temperature for 10 min. After the reaction, the reacted tubes were directly used to obtain images representing the progress of the reaction. The absorption intensity of the sample solution was measured by transferring 200 μL of free nanoceria solution or solution containing one tablet of nanoceria_agarose into the well of a 96-well plate. The 96-well plate was subjected to spectroscopic measurements in scanning mode or at

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450 nm on a microplate reader (Synergy H1, BioTek, VT). For the assay in the 96-well plate, a mixture containing nanoceria_agarose (one tablet containing approximately 0.2 mg nanoceria) and H_2O_2 (20 μL, from zero to 300 mM) in 180 μL sodium acetate buffer (100 mM, pH 5.3) was added to each well of a 96-well plate followed by incubation at room temperature for 10 min. After the reaction, the 96-well plate was subjected to the same procedures as those used for in-tube detection.

2.5. Glucose detection using nanoceria_GOx_agarose

Glucose detection was performed in one tube as follows. A solution containing nanoceria_GOx_agarose (five tablets containing approximately 1 mg nanoceria and 0.65 mg GOx) and carbohydrates including glucose (100 μL, 100 mM) in 900 μL sodium acetate buffer (100 mM, pH 5.3) was incubated at 37 °C for 30 min. For the assay in a 96-well plate, a mixture containing nanoceria_GOx_agarose (one tablet containing approximately 0.2 mg nanoceria and 0.13 mg GOx) and glucose (20 μL, from zero to 1 M) in 180 μL sodium acetate buffer (100 mM, pH 5.3) was added to the wells of a 96-well plate and incubated at 37 °C for 30 min. After the reaction, the reacted tubes or 96-well plate was directly used to obtain images representing the progress of the reaction, and the other procedures were the same as those used for H_2O_2 detection.

Long-term stability of the glucose-sensing system was checked in aqueous buffer (100 mM sodium acetate, pH 5.3) at room temperature under static conditions. The initial and residual activities at predetermined data points were determined by measuring the absorbance at 450 nm, as described above. The relative activity (%) was calculated using the ratio of the retained activity to the initial activity of each sample. Reusability was measured after cycles involving the typical reaction with glucose (10 mM) and brief washing with aqueous buffer (100 mM sodium acetate, pH 5.3) to remove unreacted glucose in the sample. After regaining the initial yellowish color of nanoceria_GOx_agarose, the sample was reused for the measurement of residual activity.

2.6. Glucose detection in human serum

The initial glucose concentration of human serum was first determined with a glucose assay kit (Sigma-Aldrich). Predetermined amounts of glucose were then added into the human serum to make spiked samples, which represent normal, boundary, and high levels of blood glucose. Finally, the glucose concentration of each spiked sample (20 μL, 10-fold dilution) was determined by the same procedure described above. The recovery rate [recovery(%)=measured value/actual value × 100] and the coefficient of variation [CV(%)=SD/average × 100] were determined to evaluate the precision and reproducibility of the assays.

3. Results and discussion

3.1. Construction of the agarose gel_entrapped composite consisting of nanoceria and GOx

A composite consisting of nanoceria and GOx in an agarose gel was developed. The construction of the composite involves the thorough mixing of nanoceria with GOx in a 2% agarose solution for highly dispersed encapsulation within the agarose matrix, followed by gelation at room temperature for 1 h. We hypothesized that the composite obtained by entrapping nanoceria and the oxidative enzyme would serve as an efficient colorimetric biosensor capable of being used for the reagent-free detection of biologically important target molecules. Specifically, in the presence of the target glucose, the immobilized GOx in the composite would generate H_2O_2 by its catalytic action, which would induce the oxidation of the surface-exposed Ce^{3+} into Ce^{4+} species and formation of unique peroxide complexes at the surface of the nanoceria (Heckert et al., 2008; Scholes et al., 2006), producing a color change from white/light-yellow into intense-yellow/light-orange without any colorimetric substrate (Fig. 1). Although a number of metal oxide nanostructures have been found to exhibit interesting catalytic properties (Solanki et al., 2011), H_2O_2-mediated color changing property of nanoceria is quite unique and could efficiently eliminate the need for chromogenic substrate, consequently enabling reagentless colorimetric detection of glucose. Since the color-changing mechanism depends on the alteration of the ratio between Ce^{3+} and Ce^{4+} on the surface of nanoceria, electrically neutral agarose, which also provides an advanced sensory format as well as a biocompatible protective carrier for the co-entrapped GOx (Lim et al., 2010), would be one of the most appropriate hydrogel matrices. When we entrapped nanoceria within the conventionally-used alginate gel, there were no color differences of entrapped nanoceria after the typical reaction with glucose or H_2O_2 presumably because the negatively charged COO^- groups of alginate interact with the positively charged cerium ions and thus significantly hinder the color changes of nanoceria (data not shown) (Lim et al., 2010).

The successful entrapment of nanoceria in agarose gel was confirmed by XRD, TEM, and SEM analyses. The XRD patterns of nanoceria_agarose perfectly matched those of free nanoceria and showed clear peaks for the crystalline phase of CeO_2 (JCPDS: 00-043-1002), indicating that nanoceria were well entrapped in the agarose gel (Fig. S2). The crystal sizes calculated by the Scherrer equation were approximately 10 nm (Cullity and Stock, 2001). TEM images of the nanoceria_agarose also confirmed that highly crystalline nanoceria with an average size of approximately 10–15 nm were well entrapped in the agarose gel (Fig. 2). Elemental mappings of nanoceria_agarose confirmed that nanoceria were homogeneously dispersed within the agarose matrix (Fig. S3), possibly leading to an enhanced colorimetric response compared with that of free nanoceria, which are prone to aggregation when suspended in aqueous solution (Bondioli et al., 2005; Feng et al., 2006). SEM images also showed the successful loading of nanoceria in the agarose gel as well as the porous structure of agarose, which facilitates the transfer of glucose molecules through the gel matrix (Fig. S4). We also performed XPS analysis to demonstrate the differences in the electronic structures of the nanoceria_agarose before and after reaction with H_2O_2 (Fig. S5). The electron binding energy of the nanoceria_agarose decreased by ~0.38 eV after reaction with H_2O_2, presumably indicating electron transfer by the oxidation of Ce^{3+} into Ce^{4+} on the surface of the entrapped nanoceria.

3.2. Colorimetric detection of H_2O_2 using the agarose gel_entrapped nanoceria

We first examined the colorimetric responses of nanoceria_agarose by performing the representative color-changing reactions in the presence of H_2O_2, and the responses were monitored using absorption spectroscopy (Fig. 3). Free nanoceria were used in this experiment as a control. The results of these experiments show that the nanoceria_agarose enables clear color changes from white to yellow after the reaction with 10 mM H_2O_2, which is similar to the change observed with free nanoceria. The generation of the yellowish color was also verified by a broad increase in the absorbance in the range from 420 to 500 nm. Interestingly, nanoceria_agarose exhibited a higher absorbance enhancement than that of free nanoceria possibly due to the well-dispersed nature of nanoceria within the agarose matrix, whereas free nanoceria are

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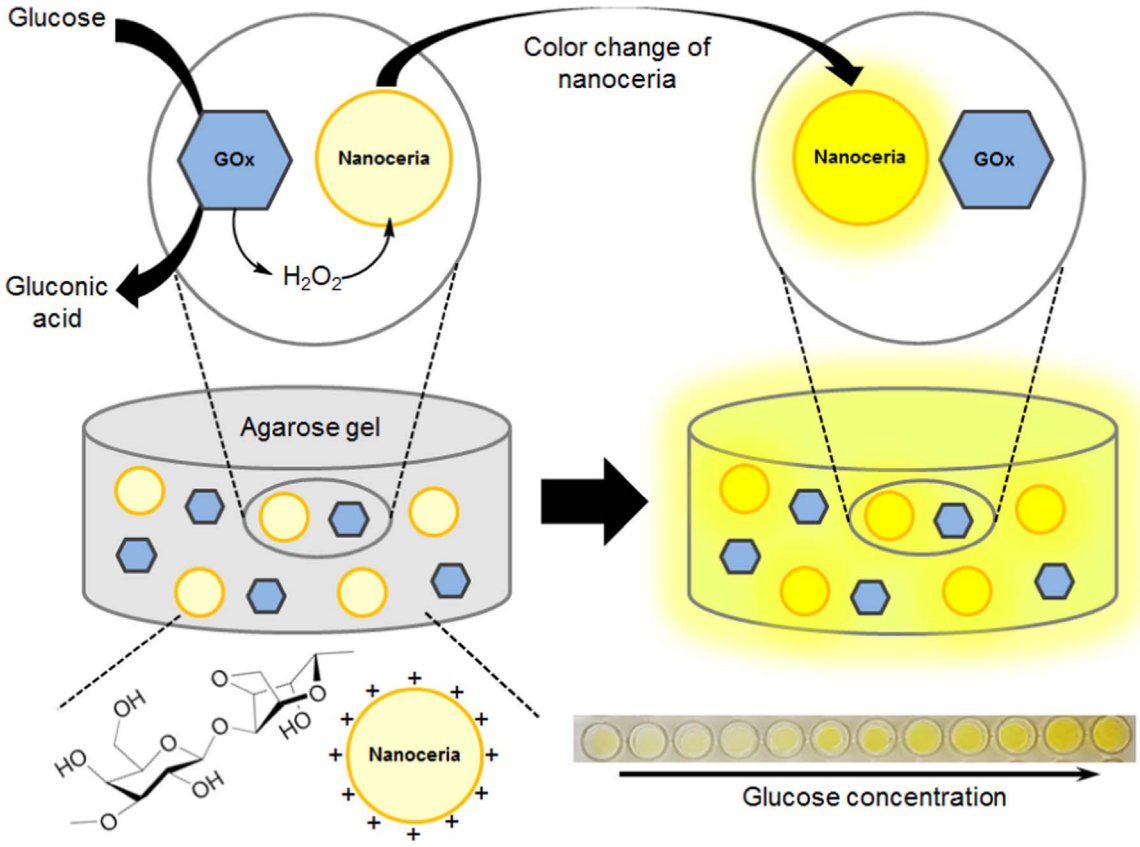

Fig. 1. Schematic illustration of the composite consisting of nanoceria and GOx in an agarose gel for the determination of glucose.

prone to aggregation (Bondioli et al., 2005; Feng et al., 2006). Based on the absorbance spectra of nanoceria_agarose, we analyzed multiple samples containing various concentrations of H_2O_2 based on visible images along with the corresponding absorption intensity at 450 nm (Fig. S6). Analysis of the dose-response curve for the nanoceria_agarose system indicated that the detection limit was as low as 5 μM in the linear range from 0.01 to 1 mM under optimal conditions (see Experimental). This level of sensitivity is sufficient to enable the coupling of the nanoceria_agarose system with any oxidase to create a versatile colorimetric sensor (Wu et al., 2009).

3.3. Colorimetric detection of glucose using the agarose gel_entrapped composite

The feasibility of the new strategy to detect glucose was demonstrated by employing nanoceria_GOx_agarose. Although the color of the composite became slightly yellowish due to the natural yellow color of GOx, the composite still underwent a vivid color change from light-yellow into intense-yellow/light-orange after the typical reaction with the target glucose (Fig. 4). To achieve the best sensing performance of the composite system, various reaction parameters including buffer system (pH, composition, and ionic strength), concentrations of nanoceria and GOx solution, assay time, and sample volume in each well of a 96-well plate were investigated to find the optimal reaction conditions for the detection of target glucose (Fig. S7). Under the optimized conditions, final loading amounts of nanoceria and GOx in an agarose gel after washing steps were determined to be about 18 wt% and 12 wt%, based on the analysis of nanoceria and GOx in the supernatant by using ICP-AES and BCA assay, respectively. Through the typical color-changing reaction, glucose was detected by observing the development of a vivid color and the corresponding absorption spectral change after 30 min (Fig. 4a, b). In contrast, no significant color change and absorption band development were observed from the negative control samples having the structural similarity with target glucose although they were employed even at 10-fold higher concentrations. Selectivity of nanoceria_GOx_agarose was also investigated by examining the interfering effects of three substances (UA, AA, and dopamine), commonly present in human blood, on the colorimetric detection of glucose (Fig. S8). In the experiment, certain amounts of UA, AA, and dopamine were added to glucose solutions, which were then subjected to the procedures for the determination of glucose using nanoceria_GOx_agarose. The determined glucose concentrations were compared with those from the same glucose solutions but without the interfering substance. As a result, there was no significant interfering effect of the substances observed on the colorimetric detection of glucose (Fig. S8). These results clearly demonstrate that the present biosensor shows excellent specificity only toward the target glucose. From the analysis of dose-response curve, the limit of detection (LOD) for glucose was determined to be as low as 20 μM in the linear range from 0.05 to 2 mM (Fig. 4c, d). These LOD and linear range values are among the best results of those recently reported describing various nanoceria-based colorimetric measurements of glucose (Table S1). The applicability of this novel nanoceria-based biosensing platform to other oxidative enzymes was also successfully verified by preparing nanoceria_ChOx_agarose and employing it to detect target cholesterol (Fig. S9).

3.4. Stability and reusability

Stability and reusability in glucose detection have attracted continuous attention since they could reduce the cost of the product over the long time (Kim et al., 2015). Because the entrapment

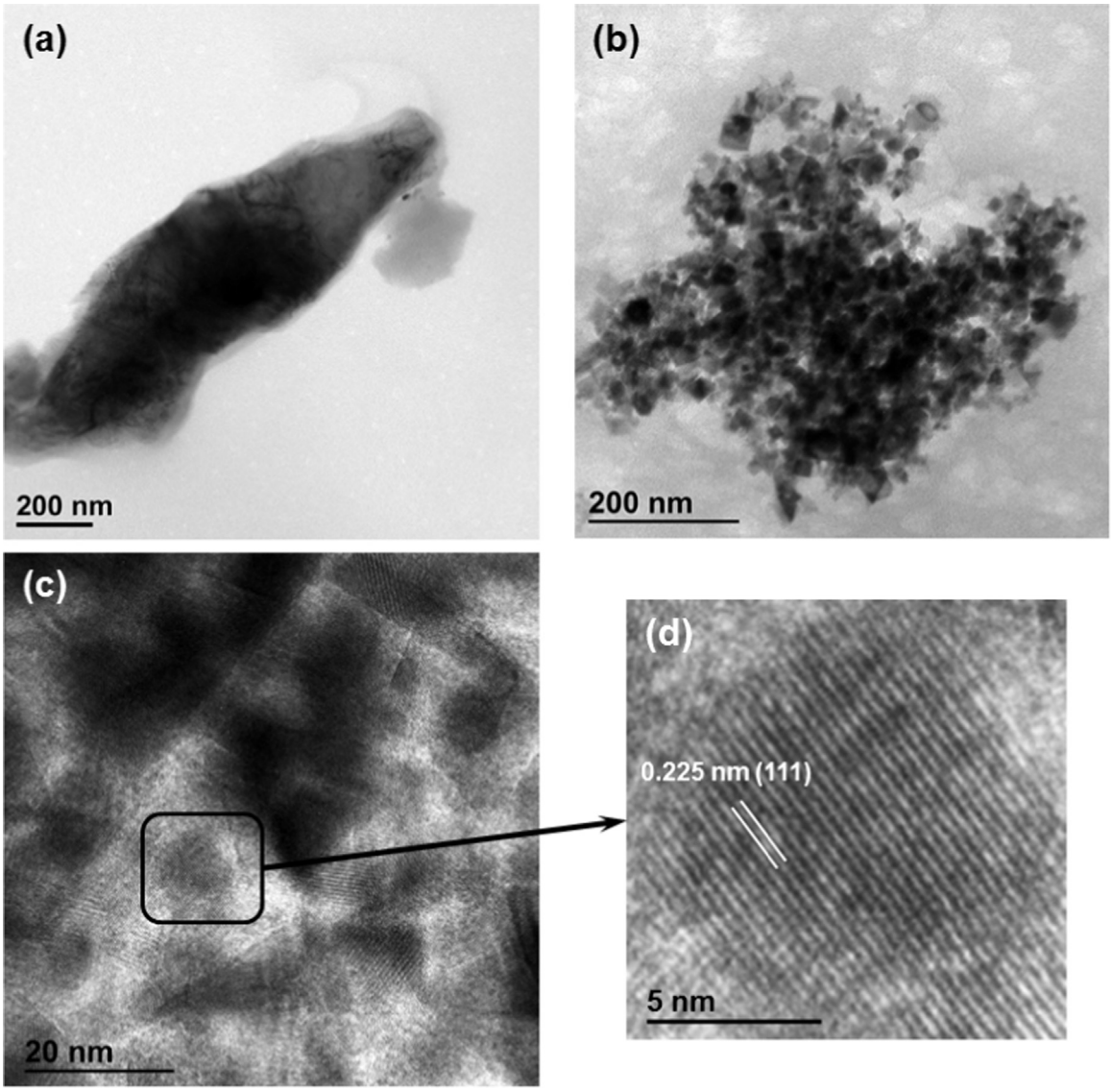

Fig. 2. TEM images of (a) free agarose gel, (b) nanoceria in agarose gel, (c and d) high-resolution TEM images showing the presence of crystalline CeO_2 nanoparticles in agarose gel.

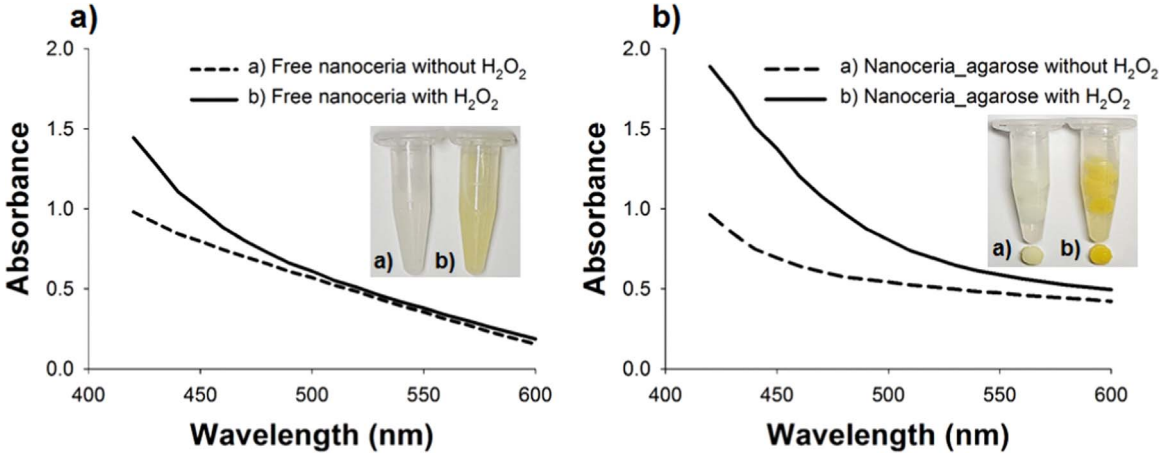

Fig. 3. Photographs of the color-changing reaction in the presence of H_2O_2 (10 mM) and the corresponding absorption spectra using (a) free nanoceria and (b) nanoceria_agarose.

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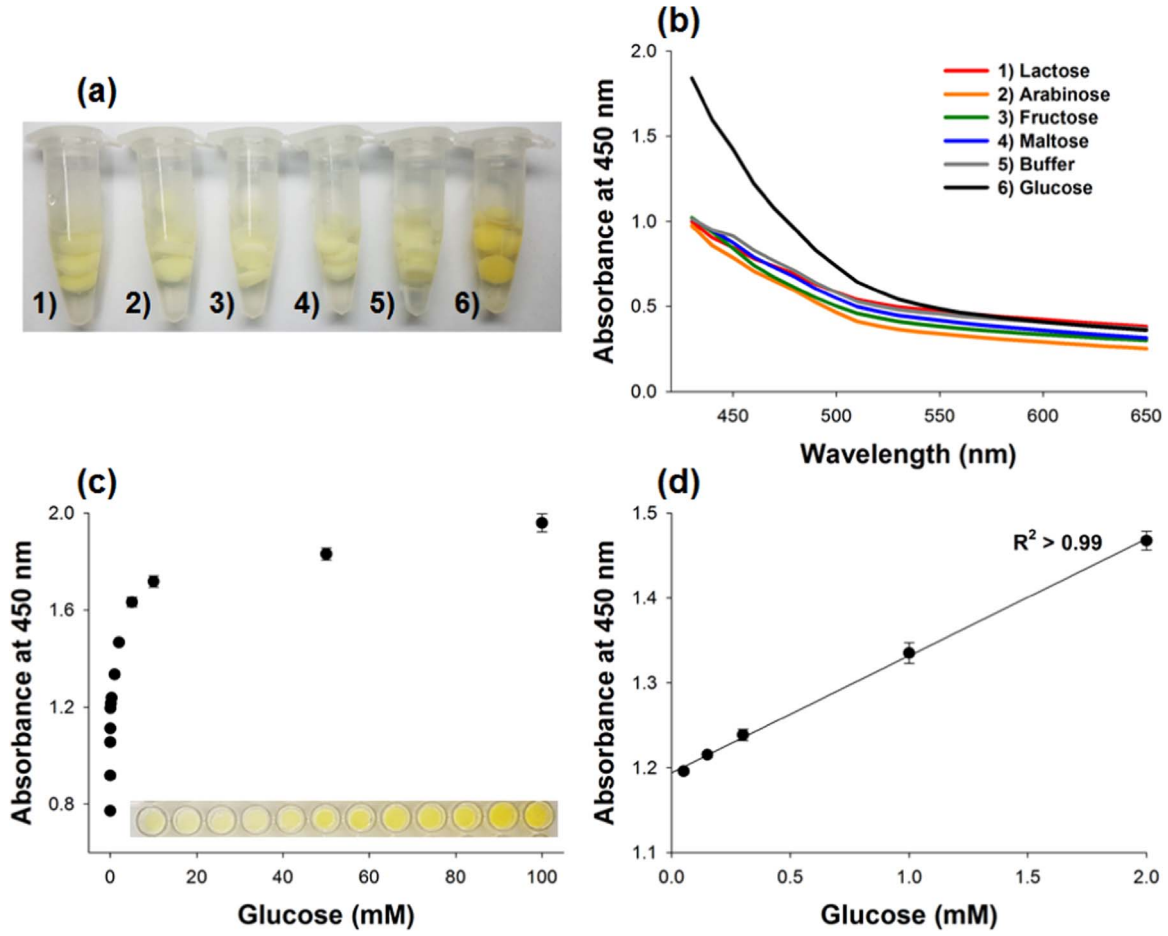

Fig. 4. Colorimetric detection of glucose using the composite entrapping nanoceria and GOx in an agarose gel. (a) Photographs for the colorimetric responses obtained from glucose and other molecules. (b) The corresponding absorption spectra. In the experiment, 10 mM glucose was used while 100 mM of other carbohydrates were used. (c) A dose-response curve and the real images for glucose detection and (d) the corresponding linear calibration plot. The error bars represent standard deviations obtained from three independent measurements.

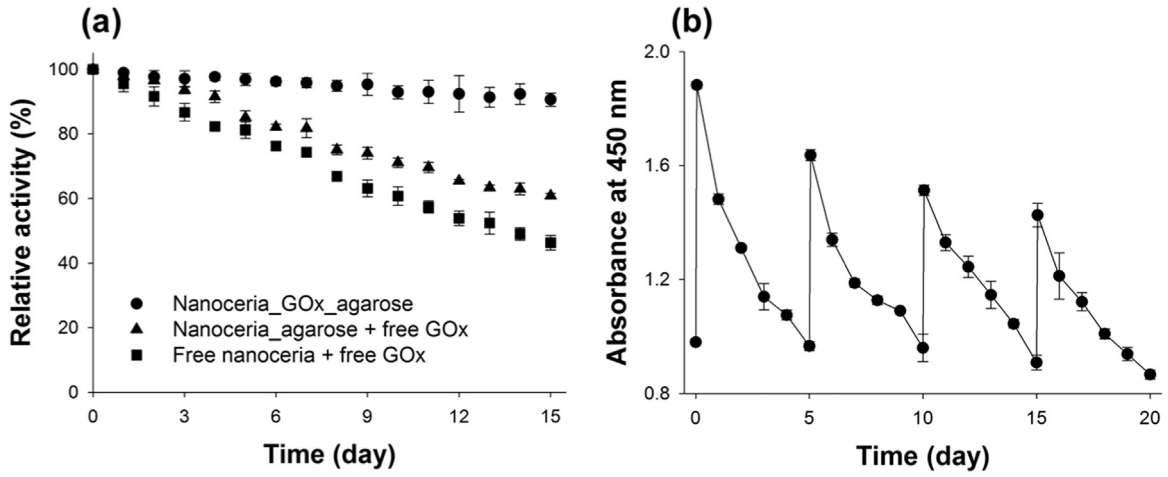

Fig. 5. Stability and reusability of the composite entrapping nanoceria and GOx in an agarose gel. (a) Long-term stability. (b) Reusability after cycles of glucose sensing and brief washing with aqueous buffer. After regaining the initial absorbance of the composite, the composite was reused for the measurement of the residual activity.

of an enzyme in agarose gel generally improves its stability by providing confined environments to limit enzyme unfolding and minimizing enzyme leaching from the matrix (Kunkel and Asuri, 2014), nanoceria_GOx_agarose was expected to have a high stability. To assess this feature, the residual activities of the nanoceria_GOx_agarose were determined along with those of free GOx incubated in the presence of either nanoceria_agarose or free nanoceria. During two weeks operation at room temperature under non-shaking conditions, no significant activity decrease was observed with the nanoceria_GOx_agarose, whereas free GOx incubated with nanoceria_agarose and free nanoceria displayed significant losses of activities (40% and 60%, respectively) (Fig. 5a). The new composite system was also expected to be reversible so that it could be reused for multiple cycles for the detection of

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Table 1
Detection precision of the nanoceria_GOx_agarose-based assay system for the determination of glucose levels in spiked human blood serum samples.

	Original amount[a] (mM)	Added (mM)	Expected (mM)	Measured[b] (mM)	SD[c]	CV[d] (%)	Recovery[e] (%)
Normal	2.28	0	2.28	2.40	0.112	4.7	105.3
Boundary		4	6.28	6.47	0.504	7.8	103.0
High		8	10.28	10.42	0.633	6.1	101.4

[a] Original glucose concentration in human serum was spectrophotometrically determined by glucose assay kit (Sigma-Aldrich).
[b] Mean of six independent measurements.
[c] Standard deviation of six measurements.
[d] Coefficient of variation.
[e] Measured value/Expected value × 100.

glucose, as free nanoceria were proven to be fully reversible by the spontaneous decomposition of the adsorbed peroxide species (Perez et al., 2008). As a result, the composite fully regained its initial yellowish color and corresponding absorption intensity at 450 nm up to 4 cycles, with each cycle involving a 5-days incubation at room temperature (Fig. 5b). However, the maximal absorption intensity after the reaction with glucose gradually decreased as the cycle number increased, indicating that the entrapped state of nanoceria within the agarose matrix would hinder the reversibility of nanoceria.

3.5. Diagnosis of representative stages of hyperglycemia

Finally, we evaluated the diagnostic capability of nanoceria_GOx_agarose using real human serum samples that contained representative levels of glucose corresponding to the normal, boundary, and high stages of hyperglycemia (normal: ≤ 5.6 mM, boundary: 5.6–7 mM, and high: > 7 mM) (Nair et al., 2014). The original amount of glucose in the serum samples was first determined with a glucose assay kit (Sigma-Aldrich, USA), and a predetermined amount of glucose was then added to establish the representative levels. As a result, the serum glucose levels were quantified with excellent precision, yielding CVs in a range of 4.7–7.8% and recovery rates of 101.4–105.3% (Table 1), verifying the excellent reproducibility and reliability of the method. These observations show that the reagentless colorimetric glucose assay using nanoceria within an agarose gel matrix is a promising analytical tool for diagnosing high levels of glucose in clinical settings.

4. Conclusions

The studies described above have led to the development of a composite system comprising nanoceria and GOx simultaneously entrapped in an agarose gel matrix, which can be employed for the reagentless colorimetric detection of the target glucose. The system displays excellent specificity, linearity, and sensitivity for glucose quantification, and it has a high stability along with reusability. The analytical utility of the new colorimetric biosensor was demonstrated by its use in diagnosing high levels of glucose through analyses of human blood serum samples. As a distinct advantage over the existing strategies, the proposed method enabled rapid visual detection of target molecules in a simple, convenient, and cost-effective manner. Therefore, this strategy could serve as a promising alternative to existing methods for the detection of several biomolecular markers including glucose, cholesterol, galactose, or even metabolic enzymes such as glutamate pyruvate transaminase (GPT) or glutamate oxaloacetate transaminase (GOT) by incorporating the appropriate oxidative enzyme corresponding to target molecule together with nanoceria into the agarose gel matrix. This technology creates new opportunities for using nanoceria-based hydrogel composites for the rapid, robust, and convenient identification of target molecules, thereby enabling efficient point-of-care detection in clinical diagnostics.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.bios.2016.08.113.

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