Intrinsic oxidase activity of cerium oxide nanoparticles facilitate the detection of cancer biomarkers and cancer cells

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ABSTRACT

Nanomaterials exhibit unique size-dependent physical properties. Thus, identifying novel properties of these materials at the nanoscale, and their applications in energy, catalysis and medicine, among others, is an area of current and intense research. Cerium oxide has been widely used in catalytic converters for many years, but only recently its interesting nanoscale-derived properties have been elucidated. Among the most interesting properties that nanoceria (cerium oxide nanoparticles) possesses is its ability to behave as a potent antioxidant at pH 7, due to the reversible switching from two oxidation states (Ce^{+3} and Ce^{+4}). Recently, we reported that the antioxidant activity of nanoceria is pH-dependent, and this property can be used for the selective protection of normal tissue during cancer chemotherapy and radiotherapy. In the present study, we investigated the oxidase activity of nanoceria, which is also pH-dependent. In addition to this, we found that (i) at pH 4 nanoceria oxidized various organic substrates without the need of an oxidizing agent, (ii) nanoceria's oxidase kinetics can be modulated via the thickness of the polymer coating, and (iii) nanoceria can be used in immunoassays via the oxidation of sensitive dyes that facilitate detection of biological targets.

Keywords: nanoceria, cerium oxide nanoparticles, oxidase activity, immunoassay

Numerous catalytic properties have been recently identified in nanomaterials that are usually not found in these materials' bulk state.[1] The enhanced catalytic active of some of these nanomaterials have been utilized in a range of applications from energy storage to chemical synthesis and biomedical sciences.[2, 3] Although cerium oxide has been widely used in automobiles' catalytic converters, coatings for ultraviolet radiation protection and in fuel cells[4-7], only lately the unique properties of nano-scaled cerium oxide (nanoceria) have been elucidated. Specifically, nanoceria exhibits potent antioxidant activity at pH 7 that can be utilized in a series of multiple applications in the biomedical field.[8-12]

As nanoceria behaves as an antioxidant at physiologic pH, we hypothesized that it may have behave as an oxidant at slightly acidic pH. Specifically, we reasoned that the lower the pH the higher the oxidase activity of nanoceria would be, whereas at normal pH (pH 7) the oxidase activity will be less potent, as at this specific pH nanoceria exhibits enhanced cyclic autocatalytic antioxidant activity. Therefore, at acid conditions the oxidase kinetic parameters of nanoceria would be higher, therefore facilitating the oxidation of organic substrates (**Figure 1**). Furthermore, nanoceria's intrinsic oxidase activity can be utilized in the development of single-reagent immunoassays, eliminating the need of secondary antibodies, conjugated enzymes (i.e. horseradish peroxidase), and hydrogen peroxide (**Figure 1**).

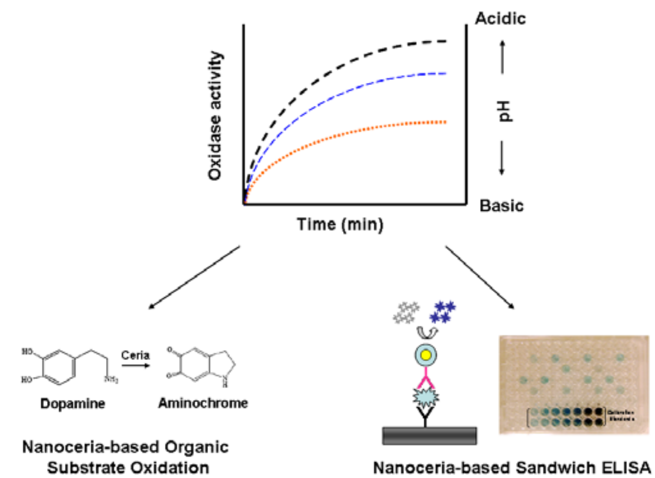
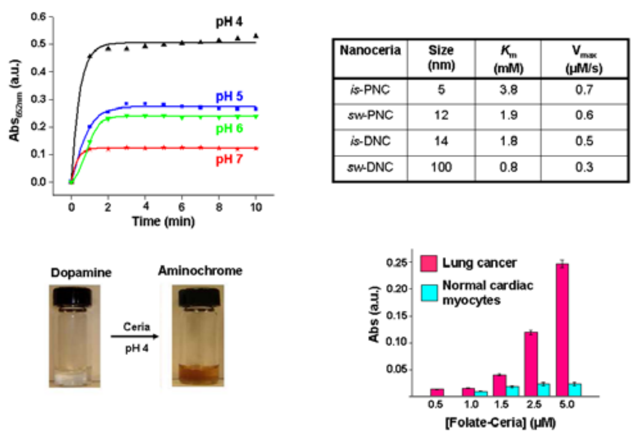


Figure 1. Proposed differential oxidase behavior of nanoceria and its' potential applications in organic chemistry and in single-reagent diagnostics.

In order to investigate nanoceria's oxidase activity, we synthesized four different preparations, using an *in situ* (is) and a stepwise (sw) synthetic protocol while coating the nanoparticle's core with either dextan (10 kDa) or polyacrylic acid (1.8 kDa). The resulting nanoparticles had diameters ranging from 5 to 100 nm, where the in situ method yielded smaller nanoparticles (**Figure 2**). We examined if polyacrylic-acid-coated nanoceria (*is*-PNC) can oxidize the organic substrate 3,3',5,5'-tetramethyl benzidine (TMB). Our results indicated that indeed *is*-PNC can oxidize TMB in a pH-dependent fashion, without the need of an additional oxidizing agent such as hydrogen peroxide (**Figure 2**). Specifically, when the pH was more acidic the enzyme kinetics were faster, in line with our hypothesis. Apart from TMB, we used another organic dye, 2-azinobis-(3-ethylbenzothizoline-6-sulfonic acid) (AzBTS), and dopamine, and similar results were obtained, indicating that nanoceria at acidic pH has an intrinsic oxidase activity. Notably, nanoceria was able to rapidly (within minutes) oxidize dopamine to aminochrome at low pH (**Figure 2**).



Nanoceria	Size (nm)	K_m (mM)	V_{max} (µM/s)
is-PNC	5	3.8	0.7
sw-PNC	12	1.9	0.6
is-DNC	14	1.8	0.5
sw-DNC	100	0.8	0.3

Figure 2. pH- and size-dependent oxidase activity of nanoceria. This enzymatic activity was used for the fast oxidation of dopamine and other organic substrates, as well as detection of lung cancer cells expressing the folate receptor through oxidation of TMB by folate-carrying nanoceria.

Next, we studied if the size and thickness of the nanoceria's polymer coating affects the oxidase activity of the nanoparticle. At pH, 4 the smaller the nanoparticle size and the thinner the coating was, the higher the Michaelis constant (K_m) and the V_{max} were (**Figure 2**). Interestingly, this trend was observed at other pH studied, indicating that the nanoceria's oxidase activity can be regulated by the nanoparticles' size, hence resulting in nanoparticles with custom-defined enzymatic activity. As nanoceria behaves as an oxidase in slightly acidic media, we utilized its activity in a modified immunoassay for the detection of cancer cells. Specifically, we conjugated an affinity ligand (folate) to nanoceria in order to facilate the nanoparticles' binding to cells that overexpress the folate receptor on their plasma membrane. For these studies, we used a lung carcinoma cell line that overexpress the folate receptor (A549), and normal cardiomyocytes which lack this receptor (H9c2). Our results indicated that only the lung cancer cells were able to oxidize TMB in a dose-dependent fashion, whereas the folate-receptor-negative cardiomyocytes nominally oxidized this substrate (**Figure 2**). Hence, this indicates that nanoceria can be used as a single specific reagent in cellular immunoassays, eliminating the need of enzyme-conjugated secondary antibodies and hydrogen peroxide that are typically used in common ELISAs.

Concluding, we have demonstrated that nanoceria possesses an intrinsic pH- and size-dependent oxidase activity.[13] Therefore, the use of nanoceria as a nanocatalyst for organic substrate oxidations is anticipated, eliminating the need of laborious procedures. Lastly, nanoceria's use as a single reagent in cellular immunoassays in the clinic, the molecular biology laboratory and the field is expected, resulting in cheaper and more durable detection modalities.

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