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Efficient in situ growth of enzyme-inorganic hybrids on paper strips for the visual detection of glucose

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ABSTRACT

A visual colorimetric microfluidic paper-based analytical device (μPAD) was constructed following the direct synthesis of enzyme-inorganic hybrid nanomaterials on the paper matrix. An inorganic solution of $MnSO_4$ and KH_2PO_4 containing a diluted enzyme (glucose oxidase, GOx) was subsequently pipetted onto cellulose paper for the in situ growth of GOx@$Mn_3(PO_4)_2$ hybrid functional materials. The characterization of the morphology and chemical composition validated the presence of hybrid materials roots in the paper fiber, while the $Mn_3(PO_4)_2$ of the hybrid provided both a surface for enzyme anchoring and a higher peroxidase-like catalytic activity as compared to the $Mn_3(PO_4)_2$ crystal that was synthesized without enzyme modulation. This new approach for the in situ growth of an enzyme-inorganic hybrid on a paper matrix eliminates centrifugation and the dry process by casting the solution on paper. The sensing material loading was highly reproducible because of the accuracy and stability of pipetting, which eventually contributed to the reliability of the μPAD. The self-assembled natural and artificial enzyme hybrid on the μPADs specifically detected glucose from a group of interferences, which shows great specificity using this method. Moreover, the colorimetric signal exhibited detection limitation for glucose is 0.01 mM, which lies in the physiological range of glucose in biological samples.

1. Introduction

The synchronized development of point-of-care tests (POCTs) in academic/research labs and their industrial commercialization is justified by numerous advantages, especially the simplicity and low cost of implementing the mass production of such tests (Anderson et al., 2011; Price, 2002). The abundance, economy, and biodegradability of cellulose paper classifies it as an ideal material for building POCT analytical devices, which are called microfluidic paper-based analytical devices (μPADs) (Li et al., 2012; Shah et al., 2013; Yetisen et al., 2013). Apart from the fabrication of the paper device, the signal reading is another key element that determines the overall cost of the assay (Fu et al., 2010). If expensive or centralized equipment is required to analyze the reaction, the POCT potential and cost of the assay will be compromised. Based on this point of view, the visual detection, particularly colorimetric-based detection, rational meets the criteria of POCT because the presence of a target is proportional to the color change, which can be directly observed by the naked eye (Dungchai et al., 2010; Jokerst et al., 2012). Moreover, the POCT potential of colorimetric-based detection is strengthened by the efficient development of smartphones that have powerful cameras and image analysis applications (APPs)(Komatsu et al., 2016; Lu et al., 2015; Park et al., 2016).

The effective immobilization of the enzyme on the sensing surface is a key factor in building a colorimetric μPAD as it takes full advantage of the sensitivity and specificity of the biological enzyme (Josten et al., 1999). Enzyme immobilization was previously achieved by pure adsorption, covalent conjugation (Huang et al., 2008), or polymeric entrapment (González-Sáiz and Pizarro, 2001). However, the stability of the attachment and loss of its activity changes with the orientation during the immobilization process. Likewise, mass transfer limitations on the solid supports challenge the sensitivity of enzyme-based detection. Zare et al.(Ge et al., 2012) initially reported the concept of protein-inorganic hybrid nanoflowers, wherein the nanomaterial is fabricated by the addition protein to a metal ion solution, thus avoiding the use toxic chemicals and extremely harsh conditions. In order to build proteomic analysis and sensing, HRP@Cu_3 $(PO_4)_2$ (Lin et al., 2014a), α-chymotrypsin@Cu_3 $(PO_4)_2$ (Lin et al., 2014b), DNA@Cu_3 $(PO_4)_2$ (Hu et al., 2014), and GOx-HRP@Cu_3 $(PO_4)_2$(Sun et al., 2014) were synthesized in a solution by the mixture of biomolecules and an

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inorganic solution to form precipitates. In a typical experiment, an inorganic solution such as $CuSO_4$ is added to phosphate-buffered saline (PBS) that contains proteins or enzymes and is then incubated at 25 °C for 3 days (Ge et al., 2012; Huang et al., 2015). After incubation, the precipitate is separated by centrifugation, washed with distilled water, and dried in a vacuum oven at 60 °C for several hours. Clearly, the bulk solution reaction proceeds for several hours or days and requires further centrifugation to harvest the hybrid material. The enzyme activity can be preserved, though the enzyme may leak from the solid substrate following multi-step centrifugation and drying, which is a challenge for the generally easy, stable, reproducible, and precise enzyme loading. Moreover, the enzyme would not be fully incorporated into the hybrid. Recently, the protein immobilization efficiency was characterized by Liu et al. (2017). It is of shock to see that 40.24% enzyme remains in bulk solution which likely been discarded. Apart from synthesis the enzyme-inorganic hybrid in bulk solution Ariza-Avidad et al. (2015), tried to synthase NFs in the presence of cellulose paper. In their study, the filter paper was placed in the solution containing GOx, HRP, and $CuSO_4$ in 10 mL PBS at 37 °C for three days. Then the paper was rinsed and dried at room temperature before biological assay. Although centrifugation process was avoided, the synthesis efficiency in terms of reagent usage and reaction time have not been significantly improved.

The present study aims to directly grow of enzyme-inorganic hybrid on a solid surface, not only getting rid of long reaction time and centrifugation process, but also minimize the usage of enzyme. To get the aim, a manganese sulfate ($MnSO_4$) and enzyme-containing phosphate solution was drop-casted onto cellulose paper to produce the hybrid nanomaterials in a reaction that was completed for a total of 5–10 min at room temperature. The enzymes, GOx, not only function as an organic part to modulate the growth of the inorganic nanostructures but also hold their intrinsic properties to specifically catalyze glucose. Conversely, $Mn_3(PO_4)_2{\cdot}3H_2O$ petals function as an artificial peroxidase and as an effective enzyme-embedding matrix because of its biocompatibility and insolubility. The capabilities of the natural and artificial enzyme hybrid-embedded μPADs were validated by the colorimetric detection-based testing of glucose. The efficient growth of an enzyme-inorganic hybrid on paper is an innovative approach for the effective loading of enzymes on a sensing surface.

2. Experimental section

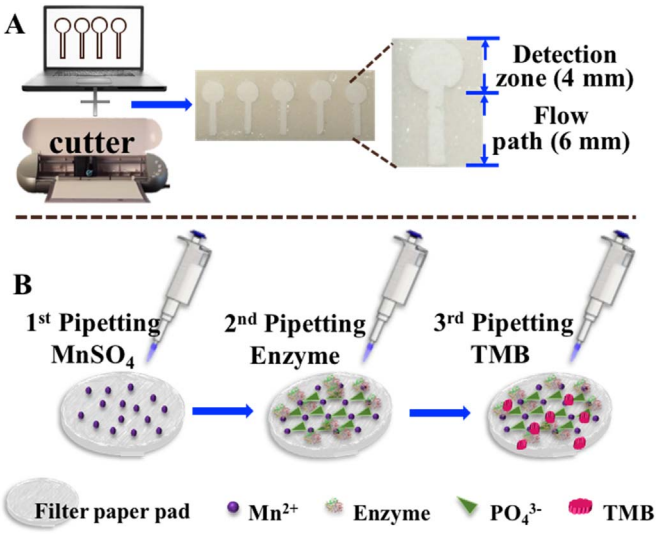
2.1. Materials and apparatus

All other chemicals used in the present study were of analytical grade or better. The glucose oxidase, 3,3,5,5-tetramethylbenzidine (TMB), manganese sulfate ($MnSO_4$), and potassium dihydrogen phosphate (KH_2PO_4) were purchased from the Aladdin Chemical Reagent Co., Ltd., China. Whatman quantitative filter paper (Grade 1, pure cellulose paper) was obtained from Sigma-Aldrich. Polyvinyl chloride (PVC) sheets, which were coated with a biocompatible highly polymer pressure-sensitive adhesive, were purchased from Shanghai Goldbio Tech Co., Ltd., China. All solutions were prepared with deionized (DI) water produced by the PURELAB flex system, ELGA Corporation.

The X-ray diffraction (XRD) patterns of the enzyme-inorganic hybrid were obtained with a XRD-7000 (XRD, Shimadzu XRD-7000) with Cu-Kα source radiation. The morphology and microstructure of the materials on the cellulose paper were investigated by field emission scanning electron microscopy (FESEM, JEOL-7800F). The chemical composition of the synthesized materials was characterized by X-ray photoelectron spectroscopy (XPS, Escalab 250xi, Thermo Scientific).

2.2. Fabrication of the paper chips

Paper cutting is a low-cost approach for fabricating a paper-based analytical device because of its efficiency with regard to time and batch production potential (Islam et al., 2015). Single μPAD was fabricated based on previous procedures published in literature. In brief, paper strips were designed and crafted by a desktop cutter (Silhouette Portrait, Silhouette America, Inc.). The long tail portion (length = 6 mm) is a flow path to guide solution-diffusion, while the round end of the paper strip is a detection zone (diameter = 4 mm) that senses materials will be loaded (Scheme 1A). The paper strip was then laminated on a PVC adhesive sheet in preparation of enzyme-inorganic hybrid nanomaterials growth and the subsequent colorimetric detection.



Scheme 1. In situ synthesis of enzyme-inorganic hybrid nanomaterials on paper chips (A) paper strips were designed and crafted by a desktop cutter; (B) sequentially casting a manganese sulfate ($MnSO_4$), enzyme-containing phosphate solution and chromogenic substrate 3,3,5,5-tetramethylbenzidine (TMB) onto the paper pad. (For interpretation of the references to color in this figure, the reader is referred to the web version of this article.)

2.3. In situ synthesis of enzyme-inorganic hybrid nanomaterials on cellulose paper

The hybrid nanomaterials were synthesized by sequentially casting a manganese sulfate ($MnSO_4$) and enzyme-containing phosphate solution onto the cellulose paper (Scheme 1B). Initially, the enzymes were solved in potassium dihydrogen phosphate (KH_2PO_4, 0.067 M, pH 7.4). The in situ synthesis of the hybrid was initialized by the addition of 4 μL $MnSO_4$ (0.1 M) onto the detection zone of the paper chip, followed by the administration of 4 μL of glucose oxidase (GOx, 1 mg/mL) onto the same place where $MnSO_4$ was casted. The paper chip was then dried at room temperature. Finally, peroxidase substrate TMB (2 μL, 2 mg/mL) was drop-casted onto the detection zone of the μPADs to prepare the paper chip for glucose detection. The visual blue color induced by glucose sample was captured by smartphone-camera. The photography was analyzed by ImageJ software (NIH, USA) and the pixel intensity was retrieved. The glucose induced colorimetric changes (Δ intensity%) was calculated as follow:

$$\Delta\text{intensity}\% = [(In_{control} - In_{sample})/In_{control}] \times 100\% \quad (1)$$

where $In_{control}$ is the signal from μPAD with TMB only, In_{sample} is the pixel intensity of enzyme-inorganic hybrid embedded strip after sample testing.

For comparison, manganese phosphate was also synthesized on paper cellulose using the same process without the presence of enzymes. Moreover, paper immersed into a bulk solution was also prepared as control group. In brief, one piece of circle filter paper (diameter = 4 mm) was immersed into a bulk solution containing 100 μL $MnSO_4$ (0.1 M) and 100 μL GOx (1 mg/mL in KH_2PO_4, pH = 7.4) for 15 min at room