Nanocomposites of Nanocrystalline Cellulose for Biosensor Applications

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ABSTRACT

In this work, we describe the use of a composite material made of a renewable source and metallic nanoparticles for biosensing applications. NCC is a product isolated from natural cellulose fibers, which is of approx. 100 nm long and 10 nm wide in size. We augmented the surface area and chemical affinity of NCC by optimally dressing it with gold nanoparticles (AuNP). The deposition of AuNP on NCC was controlled by using cationic Polyethylenimine (PEI). AuNPs were thio-funtionalized prior to enzyme immobilization and the enzyme (glucose oxidase) was conjugated on the composite by carbodiimide (EDC)/NHS coupling. Using UV-visible and fluorescence spectroscopy in the presence of a specific substrate we monitored the activity of the immobilized enzymes, and its efficiency was compared with that of the enzyme in free form. Our results using FT-IR and SEM show that thiol-AuNPs were attached to the surface of NCC. The enzymes activity were measured and quantified using different kit assays and proved that the enzymes were attached to the support and maintained their activity.

Keywords: biosensor, gold nanoparticles, glucose oxidase, nanocomposite, nanocrystalline cellulose.

1 INTRODUCTION

Enzyme based biosensors have emerged as a valuable technique for qualitative and quantitative analysis of a variety of target analytes in biomedicine and the pharmaceutical industry. They can be used for real-time diagnosis and monitoring of diseases, thus offering significant benefits over conventional analytical tools. Today few enzyme-biosensors are commercially available or being used to improve quality of health (e.g., diabetes management), while many are still in the development stage. There is a continuous need to improve and diversify technologies for immobilization of enzymes for the development of new formats, better economy, and performance.

Studies are focused on the development of new materials with good electronic properties, biocompatible, stable, and easily accessible for the analyte and large surface area for immobilization of enzymes. However, it is difficult to find a single material that pocess all these important characteristics. The development of composite materials has gotten increasingly interest as a way to achieve adequate sensitivity and stability for biosensors [1-3]. Nanocomposites are more attractive among other composite materials due to the synergistic properties originating from the components of the hybrid materials at the nanometric scale. New kinds of nanocomposites that can improve the analytical performance of the biosensors are highly desired to develop reliable enzyme-biosensors.

Nanocrystalline cellulose (NCC) is renewable, recyclable, biodegradable and nontoxic. NCC in particular has great potential as a natural biomaterial for the development of medical devices and applications in healthcare. A technological niche that could exploit NCC's unique properties is the field of biosensors [4,5], since it has a high aspect ratio and surface area and can be easily functionalized or bioconjugated, resulting in a rich source of new materials and platforms for diverse bioapplications, including enzyme immobilizations. Combining gold nanoparticles (AuNPs) with NCC will produce a nanocomposite material capable of immobilizing enzymes and creating a very active biocatalyctic support, suitable for biosensing applications. As proof of concept, we immobilized glucose oxidase (GOx) on the nanocomposites. This enzyme has been the subject of many studies for the detection of glucose using different immobilization systems, therefore it is an ideal benchmark system.

2 EXPERIMENTAL SECTION

Materials. Freeze-dried nanocrystalline cellulose (NCC) crystals were obtained from Alberta Innovates Technology Futures (AIFT). Gold Nanoparticles (AuNPs) were obtained from Nanocs. Branched-Polyethylenimine (PEI) 25kDa, Glucose Oxidase (GOx) from *Aspergillus niger*, 3-mercaptopropionic acid (3MPA, 99+%), N-hydroxysuccinimide (NHS), 1-ethyl-3-(3dimethylaminopropyl)carbodiimidehydrochloride (EDC), 2-(N-morpholino) ethanesulfonic acid (MES) and phosphate buffer saline (PBS, pH 7.2) were all obtained

from Sigma-Aldrich. Protein Assay was purchase from Bio-Rad and was used as received. Solutions were prepared using Millipore Milli-Q nanopure water (resistivity > 18 MΩ cm) and Free DNAsa water from Invitrogen. 400 mesh copper carbon TEM grids and Uranyl Acetate (Dihydrate, 99.6%) were purchased from Electron Microscopy Sciences.

2.1 Preparation of Nanocomposite

In brief, a mixture of 1 mL of freeze-dried NCC (1% stock solution) was stirred continuously with 2 mL of PEI (1% stock solution) for 1 h at room temperature (rt). The pH was adjusted to 1.5 with HCl (5%) and the mixture was centrifuged (20000 G for 20 min). The wet cake obtained was resuspended in 1 mL of Milli-Q water and 2 mL of AuNPs solution was added. The reaction was left under strong stirring for 48 h at rt. The NCC-AuNPs was collected by centrifugation (20000 G for 20 min) as a brown color wet cake.

2.2 Preparation of Thiol-Modified Nanocomposite

NCC-AuNPs wet cake was resuspended in 4 mL of 0.05 M of 3MPA in ethanol and incubated for 48 h at rt. The NCC-AuNP-S nanocomposite was collected by centrifugation (20000 G for 20 min) and washed with pure ethanol.

2.3 Covalent Immobilization of GOx on Nanocomposite

The GOx was covantly attached to thiol-fuctionallized nanocomposite by activation of the –COOH group using EDC and NHS. Thiol-fuctionallized nanocomposite (10 mg) was stirred for 2 h at rt in 1 mL of 50 mM MES and 500 mM NaCl pH 5 containing 2mM of EDC and 5mM of NHS. The excess of reactants was removed from the activated-nanocomposite by successive centrifugation and dilution with milli-Q water. The activated nanocomposite (6 mg) was suspended in 1 mL of 5 mg of GOx in a 100mM phosphate buffer with 500 mM NaCl at pH 7. The reaction was carried out at rt for 2 h. The GOx nanocomposite was retrive by centrifugation and washed with phospahte buffer.

2.4 Preparation of NCC colloidal suspensions

Colloidal suspensions of unmodified and modified NCC samples were prepared by dispersing the material in de-ionized water followed by sonication for 3 min. For our research work, 1 mg or 0.1 wt% suspension was used for FT-IR, UV-visible and scanning electron microscopy (SEM) and zeta potential measurements.

2.5 Scanning electron microscopy (SEM)

The morphologies of the nanomaterials were investigated by SEM using a Hitachi model S-4800 equipped with a field emission source and operating at an accelerating voltage of 30 kV. Sample preparation: a drop of the colloidal suspension was deposited on a carbon-coated copper TEM grid and the excess of suspension was wicked off using filter paper. The sample was then stained by depositing a drop of uranyl acetate solution (2 wt% in water) on the grid. The excess solution was wicked off using filter paper and the grid was dried at rt for at least 24 h prior to imaging.

2.6 Zeta potential measurements

The zeta potencial (ζ) of the nanomaterials (0.1 % w) was measured at different pH at 25 ^{0}C by a Malvern Zetasizer Nano-S (Malvern Instruments) fitted with a high-concetration zeta potential cell. The final value is an average of 3 repeated measurements for sample.

2.7 Spectroscopy

Fourier transform infrared (FTIR) spectra were collected from 4000 to 400 cm-1 for 32 scans at a resolution of 4 cm-1 using a Varian FTS 7000 FT-IR Imaging system with samples run as KBr pellets. UV/vis spectra were obtained using Agilent 8453 UV-Vis Spectrophotometer (300-900 nm). Solution spectra were obtained by measuring the absorption of dilute solutions in a quartz cell with a path length of 1 cm. Quantitative measurement of the amount of GOx immobilized onto the nanocomposite was carried out by determining protein concentration by using the Bradford method (Bio-Rad procedure). The BSA (Bio-Rad) was used as a standard protein.

3 RESULTS AND DISCUSSION

We show here that NCC provides an efficient strategy for immobilizing enzymes on surfaces. In recent years, PEI, an amino-rich cationic polymer, has been known to effectively interact with nanomaterials via physisorption [1,3,6]. This polymer has worked as a coat, controlling and enhancing the deposition of metal nanoparticles on the surface of nanomaterials. Therefore, we effectively coated the rod-like NCC with PEI. This was reflected by an increase in the zeta potential values from -51.2 ± 0.5 mV for NCC to +10 ± 0.9 mV for NCC-PEI (Table 1). Furthermore, the effect of the pH on the synthesis of NCC-AuNP composites was investigated by adjusting the pH of the reaction solution with 5%v/v HCl solution. The protonation degree of the -NH_3 group of PEI or the pH of the solution has a considerably influence the preparation of the NCC-Au nanocomposites. Under standard condition at

pH 9, there is no adsorption peak (Uv-Vis) from 400-800 nm as also seen with pure NCC. This suggested no evident attachment of AuNPs onto NCC coated surface that was confirmed by SEM images. However, as the pH of the solution decreases attachment of AuNPs is improved (Figure 1). Broad absorption spectra between 520-600 nm was observed with NCC-AuNPs compared to the sharp absorption at 520 nm of AuNPs solution. The broader and slightly red-shifted peak is probably because of the surface plasmon coupling between closely spaced nanoparticles as seen by others [7,8].

Sample	pH	Zeta Potential (mV)
NCC	5	-51.2 ± 0.5
NCC-PEI	9	10.0 ± 0.9
	6.5	65.9 ± 0.9
	4	57.2 ± 1.6
	3	58.4 ± 2.8
	1.5	56.6 ± 2.5

Table 1: Zeta Potentials at different pH of samples prepared. All samples were centrifuged and resuspended except for pure NCC.

The best Au deposition was observed when the pH of the solution was re-adjusted to 1.5 (Figure 1F). AuNPs were distributed along the NCC unlike the aconglomeration observed in the other samples. We believe that PEI chains become fully streched due to electrostatic repulsion between the protonated amine groups. This can facilitate the attachment of PEI molecules onto NCC surface and enhace Au deposition. In contrast, at high pH the polymer chains are uncharged, resulting in a compact cluster conformation, which inhibits PEI coating of NCC and Au deposition.

FT-IR measuraments were perfomred on the nanocomposite before and after the thiol- and GOx-fuctionalization of the nanocomposite. The absence of the characteristic peak at 2500-2700 cm-1 for streching of S-H indicates the formation of composite-S bond on the surface of nanocomposite. The additional peaks seen at 1655 cm-1 and 3369 cm-1 confirmed the binding of GOx. The sharp peak at 1655 cm-1 attributed to C=O stretching is known as the amide I band, and the peak at 3369 cm-1 is assigned to NH deformation (amide II band). The results are in agreement with the existing literature [9]. The amount of enzyme bound to the nanocomposite was determined using a standard curve of BSA protein and was found to be 1.24 ug/mL. Experiments to determine the loading capacity of the nanocomposite for the enzyme are underway as well as those to determine the enzyme activity.

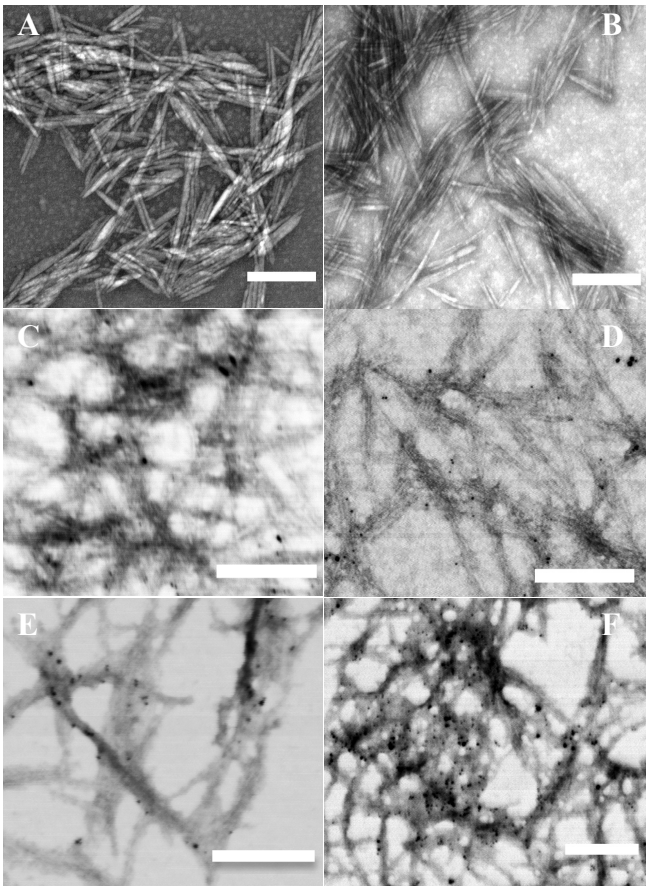

Figure 1: SEM of NCC (A), NCC-PEI pH 9 (B) and NCC-AuNPs: pH 6.5 (C), pH 4 (D), pH 3 (E), pH 1.5 (F). AuNPs deposited on matrix as black dots. Scale bar = 200 nm

4 CONCLUSION

We have synthesized NCC-Au nanocomposite in aqueous suspension by using PEI as a linking agent. The reaction conditions (i.e pH of the solution) for the assembly of NCC-Au nanocomposite were investigated in order to improve Au depostion onto NCC surface. The obtained nanocomposite was thiol-fuctionallized and demostrated that GOx enzyme can be covalently immobilized onto its surface. The assable of this matrix was confirmed by UV-vis, FT-IR and SEM studies. The resutls suggest that this biocampatible nanocomposite may prove to be a promising matrix for the immobilization of other enzymes and proteins with enhanced stability and activity for biosensor applications. Work is in progrees to determine the activity of GOx.

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