Preparation of mesoporous Nb_2O_5 film with tailored pore size and applicable way to immobilize enzyme

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An ordered mesoporous Nb_2O_5 film was prepared and a novel biosensor was developed based on the easy and effective immobilization of horseradish peroxidase (HRP) on it. This film is of interest due to their high surface area, electrochemical activity and ease of fabrication. Nb_2O_5 is environmentally benigh, with for example Nb_2O_5 particles being used as component part of bioceramics which have been manufactured for implantation purposes. Furthermore, such mesoporous Nb_2O_5 films exhibit highly ordered pore structures and very narrow pore size distributions which make it possible to design the selectivity of these substrates to protein immobilization, such as size and charge, by varying the pore diameter and by functionalizing support.

Mesoporous Nb_2O_5 films were prepared via mixed inorganic precursors strategy [1] and N_2 sorption curves indicate that the film has a high surface area (~150 m^2/g) and a narrow pore size distribution which centers at 5.0 nm. Then the biosensors were achieved by immersing this mesoporous Nb_2O_5 films (500nm thick, pore size 5nm) in horseradish peroxidase solution at 4□ for 3 days. Cyclic voltammetry and amperometric measurements were preformed using a CHI-660A electrochemical workstation (CHI Instruments Co. USA) and all experiments were carried out in a three-electrode cell

Fig.1 shows the representative Transmission Electron Micrograph (TEM) of the scratched mesoporous Nb2O5 film. Combined with the X-ray diffraction data, this ordered hexagonal pattern can be assigned to the [110] plane of the two-dimentional hexagonal mesostructure (space group, *p6m*).

Fig.2 (inset lines) depicts the cyclic voltammogram of the biosensor before and after addition of H_2O_2 in pH 7.0 phosphate buffer solution. The oxidation and reduction peaks produced by blank electrode might be attributed to the reversible redox reaction between Nb(V) and Nb(IV). The addition of H_2O_2 to the solution results in a dramatic increase in the reductive current and a concomitant decrease in the oxidation current. However, the blank Nb_2O_5 film-modified electrode without enzyme only had a little response. These phenomena illustrate that Nb_2O_5 can directly promote or mediate electron transfer between the protein redox site and the electrode surface without the addition of any redox mediators or promoters.

The effects of mesoporous Nb_2O_5 film with different thickness and various pore-diameter on biosensor response are investigated to find the optimum substrate for protein immobilization. As a result, we select mesoporous Nb_2O_5 Film of 500 nm thickness and 5 nm pore size for this study. The response time of biosensor is less than 30s and the linear response of the sensor to H_2O_2 was from 0.1μM to 0.1mM with the detection limit of 10nM at a signal-to-noise ratio of 3 (Fig.2). When not in use, the biosensor was stored in the refrigerator at 4□ and retained 90% of its initial current response after 30 days.

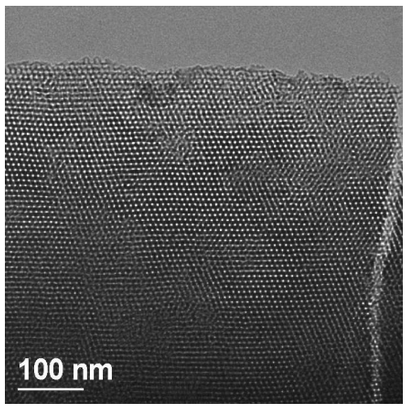

Figure 1, the representative Transmission Electron Micrograph (TEM) of the mesoporous Nb_2O_5 film

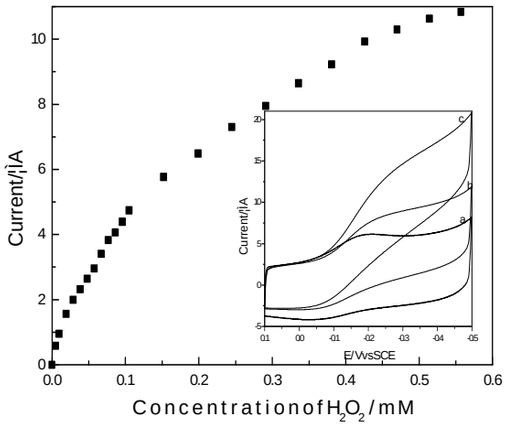

Figure 2, the calibration curve of the biosensor. The inset shows cyclic votalmmograms of the biosensor in the absence(a), in the presence of 5.0μM (b) and in the presence of 0.03 mM H_2O_2(c) at a scan rate of 100 mVs^{-1}, applied potential of -300mV vs SCE.

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References

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