Supplementary Information: Nanochannel-based electrochemical assay for transglutaminase activity

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Received (in XXX, XXX) Xth XXXXXXXXX 20XX, Accepted Xth XXXXXXXXX 20XX
DOI: 10.1039/b000000x

Experimental Part:

Reagents: TGase from Streptovercillium sp. was purchased from Ajinomoto. N-Benzyloxy carbonyl-L-glutaminylglycine (CBZ) and the other reagents were from Sigma.

Preparation of electrodes: Mesoporous silica thin films were deposited under potentiostatic conditions on Au electrodes as previously described by Walcarius et al. Briefly, a sol mixture consisting of 4.4 mmol tetraethyl orthosilicate, 20 ml ethanol (95%), 20 ml aqueous solution of 0.1 M NaNO₃ and 1 mM HCl and 1.4 mmol CTAB was prepared under magnetic stirring. The sol was aged for 2.5 h under stirring before use. The Au electrode was then immersed in the precursor solution and electrodeposition was achieved under quasi-cyclic conditions by applying a cathodic potential of −1.3 V vs Ag/AgCl during 15 s. The electrode was then removed and raised with water to avoid deposition of nanoparticulated structures. The film-coated electrodes were then dried and aged overnight in an oven at 130 °C. To introduce reactive primary amino groups at the outer surface of the mesoporous silica thin film, the electrodes were dipped into a 0.28 M ethanolic solution of N1-(3-trimethoxysilylpropyl)diethylenetriamine during 3 h with gently stirring, and further exhaustively washed with an ethanol solution containing 0.1 M HCl under moderate stirring for 15 min.

Voltamperometric assay for TGase: The modified electrodes were dipped into 5 mL of 15 mM CBZ in 125 mM Tris·HCl buffer with 3 mM of CaCl₂ and 0.63 mM of EDTA, pH 8.0, at 25 °C. The mixture was stirred and 200 µL of TGase solution at different concentration in the same buffer were added. The electrodes were removed at scheduled times, washed with buffer solution and cyclic voltammograms were recorded in 0.1 M KCl solution containing 5 mM K₃[Fe(CN)₆]/K₄[Fe(CN)₆] (1:1).

Equipment and apparatus: Electrochemical experiments were performed with a FRA2 µAutolab III potentiostat/galvanostat, using a three-electrode system consisting of the film-functionalized Au surface as working electrode, an Ag/AgCl/KCl as reference electrode and a Pt wire as counter electrode. The surface morphology of the nanostructured electrodes was investigated by high resolution field emission scanning electron microscopy (FE-SEM) using a JEOL JSM-6335F microscope. AFM studies were performed with a SPM Nanoscope IIIa multimode microscope. Transmission electron microscopy (TEM) measurements were performed with a JEOL JEM-2100 microscope.

Fig. 1S A) Cyclic voltammograms recorded at the Au electrode coated with the amino-enriched mesoporous silica thin film upon different incubation times with 15 mM CBZ and 4 µU/mL TGase. B) Influence of the incubation time on the anodic peak current value.
Fig. 2S Calibration curve constructed for TGase concentration.

Fig. 3S Calibration plot constructed for Pb²⁺ determination.

Notes and references

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