Supporting Information

Green Electrochemical Sensing Platforms: Utilizing Hydroxyapatite derived from Natural Fish Scales as a Novel Electrochemical Material for the Sensitive Detection of Kidney Injury Molecule 1(KIM-1)

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Experimental details

Extraction of hydroxyapatite (HAp) powders from fish scales by heat treatment:

Fish scales were collected/extracted from fresh *Black Carp* (a common freshwater fish) and were washed with water several times in order to clean the scales. These were then air-dried at 50 ºC over-night and calcined by a two-step procedure: 400 °C for 2 hours to remove organics and then 700~1100 °C for 4 hours to obtain relatively pure HAp powders.

The phases of the extracting products were identified by powder X-Ray Diffraction meter (XRD; D/ruax 2550PC) using CuKα radiation (λ=1.5418Å). The diffractometer was operated at 40 kV and 100 mA at a 2θ range of 10-80º with a step size of 0.02º. The microstructures of these samples were observed by Field Emission Scanning Electron Microscope (FESEM; NOVA NANOSEM 230).

Electrochemical determination of KIM-1:

Commercial KIM-1 (also known as Recombinant Hunan TIM1/HAVCR, a DNA sequence encoding the mature form of human KIM-1 extracelluar domain AAC39862.1 (Ser 21-Gly 290) with a C-terminal polyhistidine tag was expressed and purified) was purchased from Sino Biological Inc. KIM-1 is a 100 kDa, type I transmembrane glycoprotein member of the TIM family of immunoglobulin superfamily molecules. This gene family is involved in the regulation of Th1 and Th2 cell mediated immunity. Human KIM-1 is obtained commercially and is reported to be synthesized as a 359 amino acid (aa) precursor that contains a 20 aa signal sequence, a 270 aa extracellular domain (ECD), a 21 aa transmembrane segment and a 48 aa cytoplasmic domain. The suppliers note that ECD contains one V-type Ig like domain and a mucin region characterized by multiple PTTTTL motifs. The mucin region undergoes extensive O-linked glycosylation. The KIM-1 stock solution (50 μg/ml) was prepared with sterile water and kept at −20 ºC~ −70 ºC. Concentration of this stock solution was determined via UV-Vis.

The detection of KIM-1 by the proposed modified carbon paste electrode was carried out by linear sweep voltammetry (LSV) and cyclic voltammetry (CV) over the potential range of −0.2 to 0.8 V (vs. SCE), by using an Electrochemical Working Station (CHI-660d, China) under ambient conditions. The 0.2 M phosphate buffer solution (PBS) was used as supporting electrolyte and purged with pure nitrogen for 30 minutes to remove the dissolved oxygen. A conventional three-electrode system consisting of the CMCPE working, SCE reference and platinum wire counter electrodes in a 10 mL glass sample cell were utilized for the determination. Human urine was collected from one of the authors and used on the same day.

Table 1. 2θ and interplanar spacing (d) values for (211), (300), (112) and (002) planes (corresponding to the strongest four diffraction peaks) in each calcined sample. The lattice constants for them were obtained by cell refinement [a]

T(°C)	$2\theta_{211}$	$2\theta_{300}$	$2\theta_{112}$	$2\theta_{002}$	d_{211}	d_{300}	d_{112}	d_{002}	XS[b]	a	c	v
700	31.725	32.852	32.150	25.837	0.2818	0.2724	0.2782	0.3445	39.9	0.9431	0.6890	0.5308
900	31.734	32.877	32.149	25.855	0.2817	0.2722	0.2782	0.3443	74.9	0.9430	0.6882	0.5300
1100	31.740	32.890	32.147	25.850	0.2816	0.7209	0.2871	0.3444	51.4	0.9427	0.6884	0.5298

[a] These data were collected by Jade 5.0 software. [b] Mean crystal size (XS) was evaluated by Scherrer equation.