Electronic Supplementary Material (ESI) for Analyst.

Online Methods for:

Using the inherent biochemistry of the endothelin-1 peptide to develop a rapid assay for pre-transplant donor lung assessment

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Microchip fabrication

Microchips were fabricated in-house at the Toronto Nanofabrication Centre (University of Toronto, Toronto, ON) using precoated (5 nm chromium, 50 nm gold, and AZ1600 (positive photoresist)) glass substrates purchased from Telic Company. Standard contact lithography was used to pattern the sensing electrodes and followed by Au and Cr wet etching steps and removal of the positive photoresist etchant mask. SU-8 2002 (negative photoresist) (Microchem Corp.) was then spin-cast (4000 rpm, 40 s) and patterned using contact lithography to create the 5 μm circular sensing apertures. Microchips were diced in-house using a standard glasscutter and washed with acetone (Caledon Labs), isopropyl alcohol (Caledon Labs), and then O_2 plasma etched using (Samco RIE System (Samco)).

Biosensor electroplating

Gold electrodes were electrodeposited at room temperature using a Bioanalytical Systems (BASi) epsilon potentiostat with a three-electrode system featuring a Ag/AgCl reference electrode (BASi) and a platinum wire auxiliary electrode. Gold apertures on the glass microchips served as the working electrode and the biosensors were deposited using 50 mM $HAuCl_4$ (Sigma-Aldrich) using D.C. potential amperometry at 0 mV for 30 seconds.

Biosensor functionalization

Synthetic ET-1 peptide (Sigma-Aldrich) was placed on freshly prepared gold electrodes (20 µL probe solution volume) in a humidity chamber and the deposition was allowed to occur overnight at room temperature. Electrodes were thoroughly washed with dH_2O then backfilled with 1 mM MCH (Sigma-Aldrich) for at least 2 hours. Electrodes were thoroughly washed before proceeding to hybridization experiments.

Hybridization

Hybridization reactions were carried out using 20 µL of 1 µg/mL ET-1 polyclonal antibody (ab48251; Abcam) in PBS (Invitrogen) or STEEN Solution™ (XVIVO Perfusion) for 30 - 60 minutes at room temperature. Following hybridization, electrodes were washed thoroughly and prepared for electrochemical measurements.

Electrochemical measurements

All electrochemical measurements were performed on a Bioanalytical Systems (BASi) epsilon potentiostat with a three-electrode system featuring a Ag/AgCl reference electrode (BASi), a platinum wire auxiliary electrode, and the biosensing electrode serving as the working electrode. Electrodes were incubated in 2.5 mM $[Fe(CN)_6]^{3-}$ and 2.5 mM $[Fe(CN)_6]^{4-}$ (Sigma-Aldrich) for 30 seconds then scanned using differential pulse voltammetry from 0 mV to 400 mV.

Enzyme-linked immunosorbent assay (ELISA)

High-binding, 96-well Costar® plates (Corning Life Sciences) were coated with 10 µg/mL of synthetic ET-1 peptide in PBS, overnight, at 4°C. Plates were thoroughly washed then blocked with a solution of 1% (w/v) of BSA (Sigma-Aldrich) in PBS for 60 minutes at room temperature and shaken at 500 rpm. For the ET-1 assay, a preincubation of various concentrations of synthetic ET-1 peptide and 1 µg/mL of ET-1 antibody was carried out in a separate reaction tube for 45 minutes prior to being added to the ELISA plate for 45 minutes at room temperature and 500 rpm. Plates were subsequently washed and incubated with streptavidin-HRP (Cell Signaling) for 30 minutes at room temperature and 500 rpm. Following washing, 3,3'5,5'-tetramethybenzidine (TMB) (Cell Signaling) was added to each well for 5-15 minutes and protected from light. To stop the reaction, an equal volume of 1.0 N H_2SO_4 was added to each well and the absorbance at 450 nm was read using a (Spectramax M2 (Molecular Devices)).

ET-1 analysis assay (EAA)

Varying concentrations of synthetic ET-1 peptide and 1 µg/mL of ET-1 antibody were combined in a separate reaction tube for 45 minutes prior to being added to the biosensing microchip at room temperature. ET-1 concentrations were

calculated by extrapolating x-values (anti-ET-1 antibody concentrations) from experimentally derived y-values (% available surface) based on the equation of the line from a standard curve of anti-ET-1 concentration dilutions. The calculated anti-ET-1 concentration was then subtracted from the actual, added, anti-ET-1 concentration to derive the endogenous ET-1 peptide concentration bound to anti-ET-1 antibodies in solution.

Statistical analysis

Statistical calculations and analysis were carried out using Prism 6 (GraphPad) and SPSS (IBM) software. For all statistical calculations, a P-value of less than 0.05 was considered statistically significant.