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Ultrasensitive sensor for detection of early stage chronic kidney disease in human

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ABSTRACT

A facile label free, ultrasensitive platform for a rapid detection of chronic kidney disease has been fabricated. Early intervention in patients with chronic kidney disease has the potential to delay, or even prevent, the development of end stage renal disease and complications, leading to a marked impact on life expectancy and quality of life. Thus, a potable electrochemical diagnostic biosensor has become an attractive option as electrochemical analysis is feasible to use for on-site detection of samples. In human, Cystatin C present in human body fluids is freely filtered by the glomerulus, but reabsorbed and catabolised by the renal tubules. Trace detectable amount is eliminated in urine, giving this molecular marker an edge over serum creatinine's disadvantages. A carboxyl functionalized multiwalled carbon nanotubes screen printed electrode was immobilized with papain (cysteine protease) where amino group of papain covalently bound carboxyl group on electrode surface by EDC (1-ethyl-3-(3-dimethylaminopropyl) carbodiimide) and NHS (N-hydroxysuccinimide) chemistry. The modifications on sensor surface were characterized by field emission scanning electron microscopy. Interaction between papain and chronic kidney disease specific biomarker, Cystatin C was detected by cyclic voltammetry and differential pulse voltammetry within 10 min. The sensor is highly specific to Cystatin C and showed negligible response to non-specific macromolecules present in urine. The sensitivity of the sensor was 1583.49 $\mu A\ cm^{-2}\ \mu g^{-1}$ and lower limit of detection of Cystatin C was found 0.58 $ng\ L^{-1}$ which presents as a promising platform for designing potable kidney disease detector.

1. Introduction

Chronic kidney disease (CKD) is the fourth most common non-communicable form of kidney malfunction, characterized by progressive loss of function of the renal tubules. Increasing evidences have established CKD to be a secondary syndrome associated with lifestyle disorders like hypertension, cardiovascular disease, diabetes and hormonal imbalances (Bello et al., 2005) with a prevalence rate of 1 in every 10 adults. Additionally, it has been observed that CKD as a preliminary manifestation may eventually lead to hormonal imbalances, thus making it a part of 'vicious cycle' (Thomas et al., 2008). Progression of CKD is categorized in stages I-V demarcated by the changes in glomerular filtration rate (eGFR < 60 mL min^{-1} 1.73 m^{-2}) and proteinuria (30 mg g^{-1} or higher) (Biljak et al., 2017; Almeida et al., 2015; Ghaderian and Beladi-Mousavi, 2014). Hospital based detection are tedious and currently possible for stage III-V CKD patient. Inability to curb the progression of CKD leads to acute kidney failure (stage V) (Neild, 2017) and mortality becomes a confirmed likelihood as administration of therapy (angiotensin inhibitors) is delayed (Biela, 2015; Levey et al., 2005). At present, there is a dearth of diagnostics that detect CKD patients at stages I and II using non invasive samples. Hence, the necessity arises to develop facile diagnostics for candidates destined for CKD progression.

Although, several biomarkers are reported for CKD such as creatinine (Cr), neutrophil gelatinase-associated lipocalin (NGAL), kidney injury molecule-1 (KIM-1), liver-type fatty acid binding protein (L-FABP) and Cystatin C (CysC), CysC has been identified as the most decisive "gold standard marker" for CKD progression (Uchida and Gotoh, 2002). CysC, a cysteine protease inhibitor, has been established as a constituent of urine of normal individual and has a tendency to be reabsorbed by healthy kidneys (Kazuo and Akiko, 2002). Elevated levels of CysC is innately associated with disease prognosis. As per CKD-EPI equation, CysC has been found to be statistically correlated with increasing Cr concentration and decreasing eGFR signifying deteriorating kidney functionality. Additionally, CysC is devoid of its association with variation in age, sex and dietary intake of individuals as

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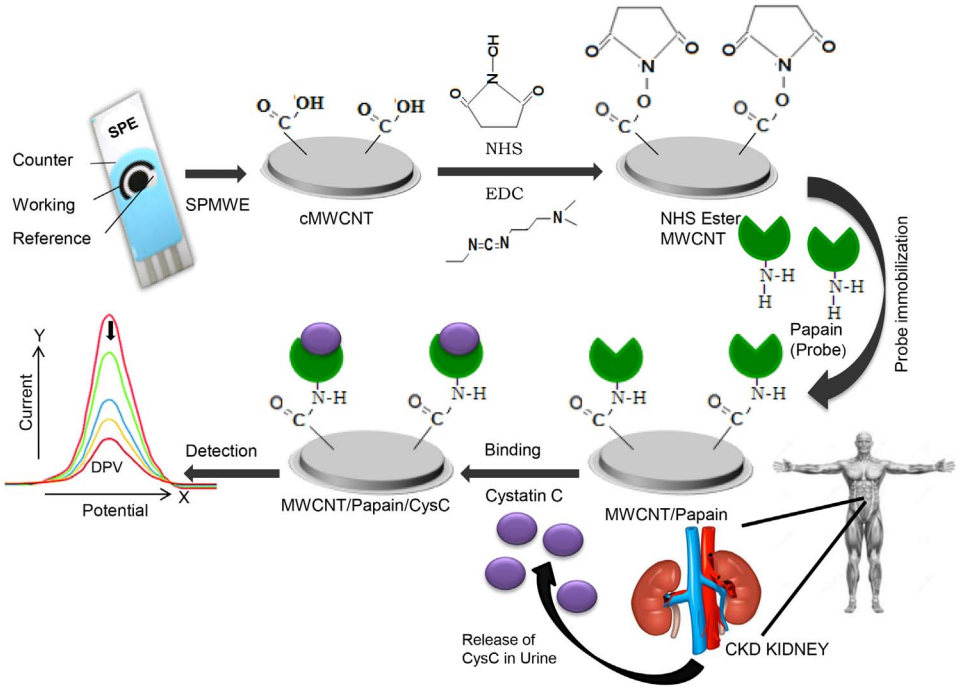

Scheme 1. Schematics of non-invasive strategy for detection of CKD biomarker CysC in human urine. Papain covalently immobilized on working surface of carboxyl activated SPMWE using EDC:NHS chemistry. SPMWE incubated with increasing concentration of CysC for 10 min at RT (27° C) was measured by electrochemical methods using 5 mM of potassium ferricyanide [$K_3[Fe(CN)_6]$] as indicator at pH 7.2. Electrochemical response of probe (SPMWE/papain) with CysC was measured by CV (−200 V to 800 V) and DPV (−400 to 600 mV).

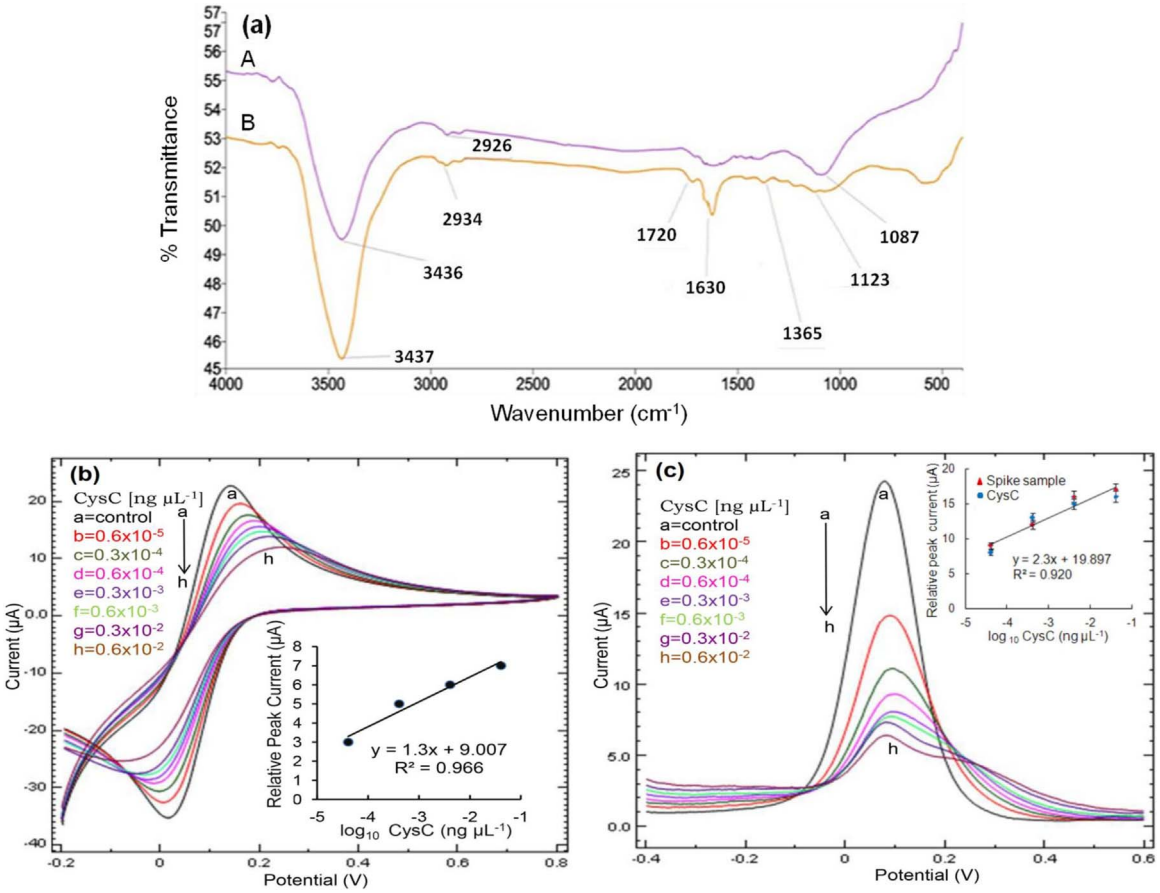

Fig. 1. (a) FTIR spectra of [A] cMWCNT and [B] MWCNT/papain for confirmation of covalent linkage between MWCNT and papain through amide bond using EDC-NHS chemistry at frequency 400–4000 cm^{-1}. **(b)** Electrochemical CV response study using 5 mM $K_3[Fe(CN)_6]$ in 0.1 M PB, pH 7.2, CV of SPMWE/papain as control (a) and binding with 0.6×10^{-5} ng μL^{-1} to 6.6×10^{-3} ng μL^{-1} CysC (b→h). [Inset] Plot shows relationship between relative I_p with lowest concentrations of CysC, where X-axis is relative peak current (µA) and Y-axis is $\log_{10}$ concentration (CysC). **(c)** DPV plot of biosensor using different CysC concentrations ranging from 0.6×10^{-5} ng μL^{-1} to 6.6×10^{-3} ng μL^{-1}. [Inset] Plot shows relationship between different concentration of CysC for the calculation of sensitivity and LOD, where X-axis is relative peak current (µA) with respect to control (SPMWE/papain) and Y-axis is CysC concentration in $\log_{10}$.