THE SELECTION OF NANOMATERIALS FOR CYSTATIN C (CYS C) LATERAL FLOW ASSAY DETECTION

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INTRODUCTION

Acute kidney injury (AKI) is a life-threatening condition and is the outcome of long-term chronic kidney disease (CKD) or the occurrence of cardiovascular events. (Chawla et al., 2014) Roughly 3 million people suffer from CKD in UK with over 3000 transplants taking place annually and over 5000 people awaiting one. Around 100,000 deaths are related to AKI and almost 30,000 people in the UK are undergoing dialysis. AKI also affects 1 in 5 people admitted to hospital as an emergency which is even more deadly than heart attack. Roughly 19 people in UK will develop kidney failure per day and every single day one patient will die awaiting kidney transplantation. (Waldum-Grevbo, 2015) Glomerular filtration rate (GFR), a parameter which measures how much blood is filtered by kidney each minute, is used to monitor renal disease at an early stage and higher GFR indicates better kidney function. GFR is measured based on biomarkers like creatinine and Cys C. (Di Nicolò, 2018)

The clinical range of Cys C is from 0.33 mg/L to 10 mg/L. Companies like Randox have Cys C clinical range identified between 0.4 – 10 mg/L, with the healthy range from 0.57 mg/L to 1.05 mg/L. (Ak, Wz, R, & Wm, 2013) Additionally, Cys C concentration (conc.) between 0.7 mg/L and 1.7 mg/L could suggest CKD stage 2 and 3. (Kimura et al., 2009) (Ogawa et al., 2008) EUROLyser has the clinical range of Cys C between 0.33 mg/L and 8.0 mg/L. Hospital turnaround time is usually a day or so and therefore measuring Cys C accurately at home is the objective of this research. To improve the sensitivity and specificity via point-of-care, lateral flow (LF) is used and optimised.

MATERIALS AND METHODS

Nanomaterials selection (gold nanoparticles (GNP), gold nanoshells (GNS), nanorods) are conducted for LF assay test line sensitivity.

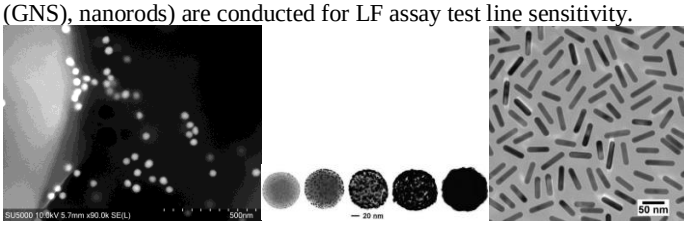

Figure 1 Left) 40 nm gold nanoparticles under SEM; Middle) 150 nm gold nanoshells (Oldenburg, 2019); Right) 55 nm x 15 nm gold nanorods (Joshi, Yoon, Hardin, Emelianov, & Sokolov, 2013).

The comparison between LF and standard calibrators like Roche or ELISA would indicate the specificity of the LF test. Additionally, scanning electron microscope (SEM), dynamic light scattering (DLS), and UV-vis are used for antibody and gold nanomaterials' conjugate characterization. Test line intensity was recorded by Lumos (Leelu reader, Lumos Diagnostics).

RESULTS & CHARACTERIZATION

DLS is used for size measurement of antibody gold nanomaterial conjugates. UV-Vis is used for observing the peak absorption shifting when conjugating different concentrations of antibodies to gain knowledge on the light absorption properties to add control and knowledge to the work. The SEM is utilised to determine the size of conjugates in their dry formats. Different concentrations of antibodies are used for conjugations and this may result in different performances when detecting Cys C (Figure 2). Left shows 0.167 mg/ml antibody and right illustrates 0.1 mg/ml antibody used for GNS conjugation for Cys C (0 to 0.1 mg/L) detection.

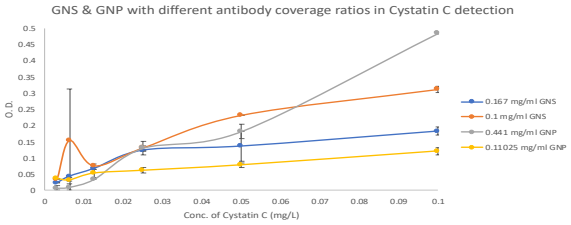

Figure 2 Detection of Cys C from 0.003125 mg/L to 0.1 mg/L with GNS conjugated antibody's concentrations as 0.167 mg/ml and 0.1 mg/ml; GNP conjugated antibody's concentrations as 0.441 mg/ml and 0.11025 mg/ml.

DISCUSSION

The maximum coverage of antibodies on the surface of gold nanomaterials could boost the detection sensitivity. As the maximum loading of the antibodies could make sure antibody performs its maximum binding effect when encountering its paired antibody on the test line. From Fig 2, the coverage ratio of the antibody on the surface of gold nanomaterials would enhance different Cys C detection capability. DLS could find the maximum coverage ratio of the antibody on the gold nanomaterials surface thus giving the optimized Cys C detection antibody concentration for nanomaterials' conjugation.

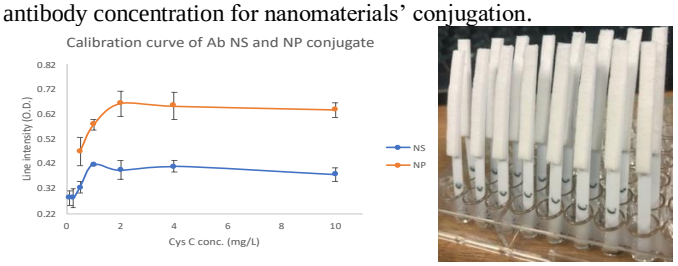

Figure 3 Left) Detection calibration curve of Cys C from 0 to 10 mg/L; Right) Half-moon signal suggests strong binding between paired antibodies, together with undistinguishable signals between higher Cys C conc.

Notably, higher concentrations from 2 mg/L onwards as noted in Fig 3 shows the hook effects of Cys C detection as optical density (O.D.) is not distinguishably differentiable. Tackling hook effects would be beneficial for quantitative Cys C detection.

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