DOI 10.5162/EUROSENSORSXXXVI/OT6.7

Hydrogel-based Sensing Technology for Quantitative Measurements of Low Concentrations of Proteins in the Presence of Interferents

Khalid Haliru[1], Ruchi Gupta[1]
[1] School of Chemistry, University of Birmingham, Birmingham, B15 2TT, UK

r.gupta.3@bham.ac.uk

Summary:
We report a hydrogel-based sensing technology for the measurement of proteins with a limit of detection of 0.003 ppm or 53 pM for an exemplar analyte, streptavidin, while being less laborious than the state-of-the-art method, enzyme-linked immunosorbent assay. Equally, we showed that the presence of high molecular weight interferents such as mucin at 100- and 1000-times higher concentrations than the exemplar analyte did not influence protein measurement.

Keywords: Hydrogel, sensing, pre-concentration, labelling, proteins

Background, Motivation an Objective

Finger-prick blood and saliva can be collected minimally- and non-invasively, respectively, and contain proteins that can serve as diagnostic indicators of diseases [1]. However, such proteins are present in low concentrations particularly in early stages of diseases [1] and hence are difficult to measure. The state-of-the-art method for the measurement of low concentrations of proteins is enzyme-linked immunosorbent assay (ELISA) [2], which is tedious and time-consuming. Our motivation is to develop sensing technologies for accurate, easy, and rapid detection of low concentrations of proteins in biological samples. Our sensing technology is based on a smart hydrogel [3] for protein pre-concentration, fluorescent labelling, and release as shown in Fig. 1. The released proteins can be selectively captured and detected by fluorescence (see Fig. 1). Using our hydrogel-based sensing technology, we can measure at least 0.01 ppm of proteins while being less laborious than ELISA.

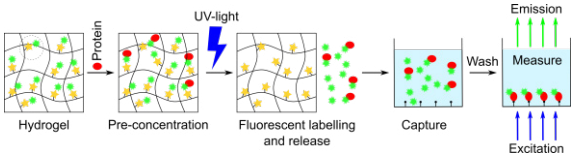

Fig. 1. A schematic showing the use of our hydrogel for pre-concentration, labelling and release of proteins for their selective capture and detection.

Description of the hydrogel

Our hydrogel is a co-polymer of acrylamide/ bis-acrylamide and methacrylamide attached to fluorescein isothiocyanate (FITC) *via* a light-cleavable (nitroveratryl, NVOC) group and a poly(ethylene glycol) (PEG) linker. The chemical structures of the active (FITC-NVOC-PEG_{3400}-methacrylamide) and inactive (acrylamide) monomers and bis-acrylamide crosslinker used to make hydrogels are shown in Fig. 2.

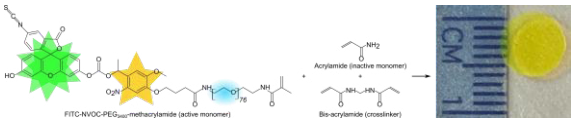

Fig. 2. Chemical structures of monomers and crosslinker used to make our hydrogels.

Herein, we investigated the suitability of our hydrogel-based sensing technology for quantitative measurements of proteins and for detection of proteins of interest (herein, streptavidin) in the presence of interferents, mucin and bovine serum albumin (BSA), found in abundance in saliva and blood samples, respectively.

Methodology

Hydrogel disks (shown in Fig. 2) were formed by APS/TEMED initiated polymerization of 29:1 acrylamide: bis-acrylamide and FITC-NVOC-PEG_{3400}-methacrylamide with a total monomer concentration of 5% (w:v). The molar ratio of FITC-NVOC-PEG_{3400}-methacrylamide and acrylamide monomers was 1 to 40. The hydrogel disks were stored in 10 mM phosphate buffered saline (PBS), pH 7.4 in darkness.

Hydrogel disks were immersed for 24 h in 10 mL solutions of streptavidin in PBS without and with either mucin or BSA. Subsequently, the disks were washed for 24 h in 10 mL PBS, im-

mersed in 200 µL PBS and irradiated with 365 nm light for 60 min to release proteins. The solutions containing released proteins were pipetted in wells of microtiter plates coated with biotin and left for 30 min. Wells were washed with PBS, illuminated with 470 nm light and the fluorescence emission spectra were collected.

To coat each well with biotin, we pipetted 300 µL of 1.5% (w:v) chitosan solution and oven dried at 75 °C for 2 h. We then added 200 µL of 20 mg/ml NHS-PEG-biotin for 3 h, washed with PBS, added 200 µL of 20 mg/ml of NHS-PEG-methyl for 3 h, and washed with PBS.

Results

The fluorescence emission spectra of streptavidin solutions obtained by irradiating hydrogel disks after immersion in different concentrations of the protein solutions are shown in Fig. 3. Fig. 3 confirms that our hydrogel-based sensing technology can allow quantitative measurements of proteins with a limit of detection 0.003 ppm or 53 pM as determined by three times the standard deviation of the intercept divided by the slope of the calibration curve.

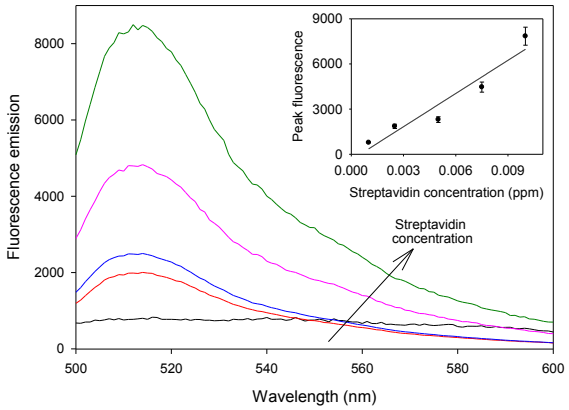

Fig. 3. Fluorescence spectra of solutions containing proteins released from our hydrogels after the hydrogels were immersed in streptavidin solutions of different concentrations for 24 h.

We then immersed the hydrogel disks in 0.005 ppm streptavidin solutions without and with an interferent, mucin. Fig. 4 shows that the presence of 100- and 1000-times more concentrated mucin than streptavidin had minimal effect on the fluorescence signal. This observation can be explained by considering that the molecular weight of streptavidin and monomeric mucin are 66 kDa and 640 kDa, respectively, which would mean that diffusion of mucin into the hydrogel would be much slower than for streptavidin. As a result, the ratio of concentrations of streptavidin to mucin in hydrogel disks would have been much higher than in solutions.

Fig. 5 shows that fluorescence of 1000x BSA was higher than buffer, suggesting that BSA released from hydrogels may non-specifically adsorb to wells of microtiter plates. Equally, Fig. 5 shows that the presence of 1000x BSA in streptavidin solutions reduced the fluorescence signal compared to streptavidin in buffer. This may be because proteins can diffuse and hence would compete for isothiocyanate groups in hydrogel disks. This means less streptavidin would be captured by hydrogel disks, resulting in reduced fluorescence signal.

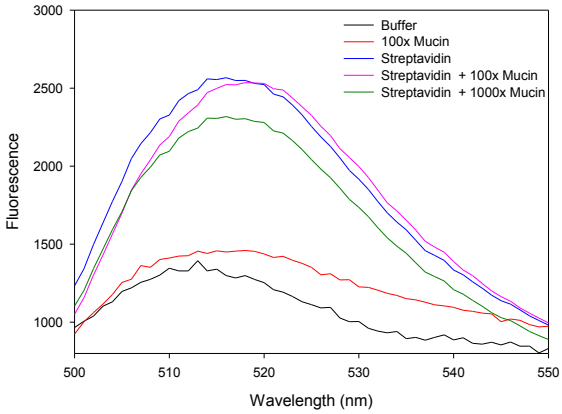

Fig. 4. Fluorescence of solutions containing species released from our hydrogels after immersion in different types of solutions for 24 h.

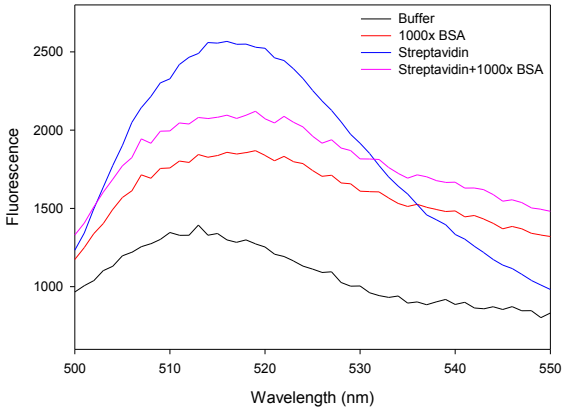

Fig. 5. Fluorescence of solutions containing species released from our hydrogels after immersion in different types of solutions for 24 h.

References

[1] S. Surinova, R. Schiess, R. Huttenhain, F. Cerciello, B. Wollscheid and R. Aebersold, On the Development of Plasma Protein Biomarkers, *Journal of Proteome Research* 10, 5-16 (2011), doi: 10.1021/pr1008515.

[2] S. Aydin, A Short History, Principles, and Types of ELISA, and Our Laboratory Experience with Peptide/ Protein Analyses Using ELISA, *Peptides* 72, 4-15 (2015), doi: 10.1016/j.peptides.2015.04.012.

[3] L. Kellermann, R. Gupta, Photoactive Hydrogels for Pre-concentration, Labelling, and Controlled Release of Proteins, *Analyst* 148, 4127-4137 (2023), doi: 10.1039/D3AN00811H.