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Hydrogel beads-based sensing technology for measurement of insulin growth factor 1 (IGF1) in saliva

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Summary:

We report a hydrogel beads-based sensing technology for the measurement of a protein biomarker, insulin growth factor 1 (IGF1) with a limit of detection of 0.03 ng/mL and dynamic range of 0-1.6 ng/mL. We showed that concentrations of IGF1 were successfully measured in human saliva samples using hydrogel beads. In comparison to the gold standard method, enzyme-linked immunosorbent assay (ELISA), the use of beads was beneficial because the measurements were not influenced by the dilution factor of samples and the buffer used for sample dilution.

Keywords: Hydrogel, beads, enzyme-linked immunosorbent assay (ELISA), proteins, saliva

Background, Motivation and Objective

Salivary proteins can serve as biomarkers of diseases and health conditions. For example, salivary insulin growth factor 1 (IGF1) can be a biomarker of skeletal maturity, which can be useful for orthodontic treatment planning [1]. The concentration of salivary IGF1 in adults and adolescents is <0.9 ng/mL and ~4 ng/mL, respectively [2]. The state-of-the-art method for the measurement of proteins is enzyme-linked immunosorbent assay (ELISA) [3], which can require sample preparation and optimization of (diluent, block and wash) buffers for each protein/ sample, is tedious and time-consuming.

Our motivation is to devise technologies for accurate, easy, and rapid detection of biomarkers in body fluids. Our objective is to determine the utility of our hydrogel beads-based sensing technology [4] for measurement of salivary IGF1 and benchmark against ELISA.

Hydrogel beads-based sensing technology

As shown in Fig. 1, proteins were covalently captured by the reaction between the isothiocyanate reactive group and amines in the polyethylene glycol (PEG) hydrogel and proteins, respectively. Proteins were pre-concentrated because the volume of the hydrogel was lower than the sample. Equally, interferents were removed because the hydrogel was designed to serve as a molecular weight cut-off filter. When the hydrogel was exposed to light, fluorophores labelled proteins were released. The released proteins were selectively captured by antibodies and quantified by fluorescence.

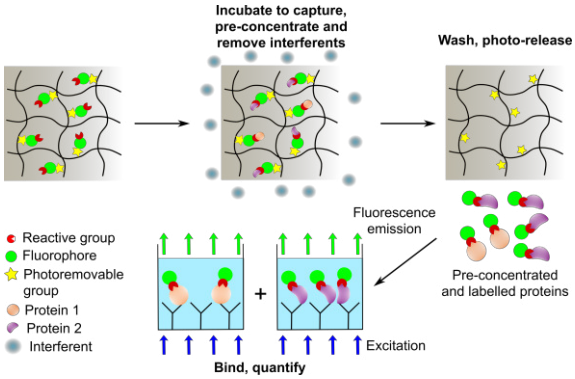

Fig. 1. Measurement of proteins using hydrogel.

Experimental

Each hydrogel bead was formed by polymerizing 4 µL solution, containing a crosslinker as well as active and inactive monomers with their chemical structures shown in Fig. 2 (a).

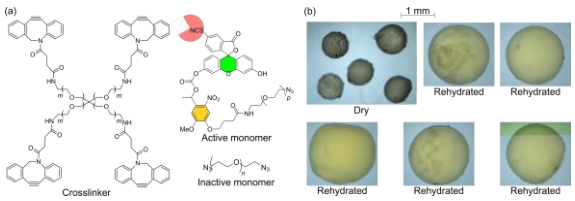

Fig. 2. (a) Chemical structures of the crosslinker and monomers used to make PEG hydrogel and (b) images of dried and rehydrated beads

Fig. 2 (b) shows that the diameters of dried and rehydrated beads were 0.49±0.03 mm and 1.03±0.46 mm, respectively. 20% (w:v) hydrogel beads were used because they were suited for pre-concentration of IGF1 and removal of

macromolecular interferent, mucin, which is present in large excess in saliva [4].

4 beads were incubated with 200 µL sample for 60 min while shaking, washed in 10 mM phosphate buffered saline (PBS, pH 7.4), immersed in 50 µL PBS, and then exposed to 365 nm light for 10 min. The released proteins were pipetted in a microtiter plate well coated with IGF1 antibody. The solution was left in the well for 30 min and then the well was washed with PBS containing 0.05% Tween-20. The sensing performance of the beads was compared against an ELISA kit (DuoSet DY291, Bio-Techne).

The wells were coated with IGF1 antibody by leaving 100 µL of 4 µg/mL antibody prepared in 50 mM bicarbonate buffer (pH 9.6) in wells overnight and then washing the wells with PBS containing 0.05% Tween-20. PBS containing either 1% bovine serum albumin (BSA, buffer 1) or 5% Tween-20 (buffer 2) was used to dilute samples, prepare biotinylated detection antibody and streptavidin labelled horseradish peroxidase enzyme for ELISA, and block wells after immobilization of capture antibody.

Saliva samples were collected from healthy individuals visiting the University of Birmingham Dental Hospital with approval from the local research ethics committee (ethics code 24/SW/0100, project code DRTB002.2029). Samples were stored at −80 °C prior to use.

Results

The calibration curves of IGF1 using ELISA and beads are provided in Fig. 3. The ELISA calibration curves were non-linear over the entire range of the protein concentration. In contrast, the beads calibration curves were linear for up to 1.6 ng/mL IGF1. The limit of detection (LOD) for IGF1 using the beads was 0.10 ng/mL and 0.03 ng/mL when the protein was prepared in PBS with 1% BSA and 5% Tween-20, respectively. Thus, the LOD and dynamic range of beads-based sensing technology is sufficient to measure IGF1 in saliva of adults.

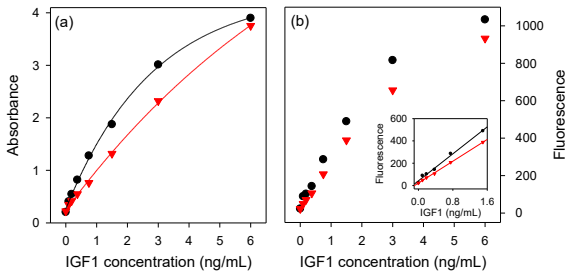

Fig. 3. Calibration curves of IGF1 in buffer 1 (black) and buffer 2 (red) using (a) ELISA and (b) beads.

Table 1 lists IGF1 concentrations measured using beads and ELISA in undiluted saliva using buffer 1, showing good agreement.

Tab. 1: Comparison of salivary IGF1 concentration determined using beads and ELISA

Method	IGF1 concentration (ng/mL) in sample			
	1	2	3	4
Beads	0.31 ±0.06	0.26 ±0.06	0.32 ±0.06	0.21 ±0.06
ELISA	0.28 ±0.03	0.25 ±0.02	0.29 ±0.03	0.20 ±0.02

Fig. 4 (a) shows that for each saliva sample, when measured using beads, the concentration of IGF1 was comparable irrespective of the sample dilution factor and buffer type. In contrast, as shown in Fig. 4 (b), these factors had a strong effect on the measurement of IGF1 concentration using ELISA. Thus, our conclusion is that hydrogel bead-based sensing technology is more robust than ELISA for the measurement of salivary IGF1.

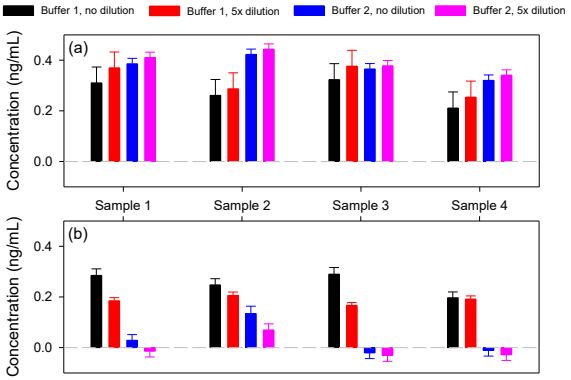

Fig. 4. Concentration of salivary IGF1 determined using (a) beads and (b) ELISA for different sample dilution factors and buffers used for sample dilution.

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