Topic No. 6.03 Protein Analysis & Characterization Abstract Reference No. 0465
Oral or Poster

Microgel-templated droplet ELISA

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We demonstrate amplified affinity assays performed on monodisperse microgel particles which template uniformly sized droplet formation without microfluidics systems. The signal from the assay is immobilized on each particle, and therefore, particles can be transferred back to an aqueous phase for quantification using standard flow cytometers.

Digital enzyme linked immunosorbent assays (ELISA) allow measurement of biomarkers down to individual molecules, leveraging sub-nanoliter compartmentalization and signal amplification. However, these technologies require tailored and often expensive microwell devices or microfluidic droplet generators for compartmentalization, and custom optical analysis systems to characterize low levels of fluorescence. The need for specialized and relatively costly equipment (e.g. Quanterix Simoa system) has impeded the adoption of digital ELISA technologies for biomarker discovery or clinical diagnosis.

Our transformative approach addresses several challenges to build accessible digital ELISA platforms by (1) leveraging a solid support of hydrogel particles to generate highly monodisperse droplets by simple pipetting (Fig.1,3), (2) accumulating amplified signal on individual particles while preventing cross talk, like current digital assays, by compartmentalization into droplets, and (3) quantifying the accumulated signals on each particle with standard flow cytometers. The base technology for droplet formation around a solid support can provide a pioneering platform for a host of emerging Lab-on-a-Particle analysis technologies.

Spherical biotinylated polyethylene glycol (PEG) hydrogel particles were prepared using previously published droplet microfluidic technologies and could be stored long-term [1] (Fig.2a,c). By simple pipetting and agitation, these particles template the formation of highly monodisperse droplets (Fig. 3a-b) within 45 seconds (19k particle-drops/s). This batch approach can be easily scaled to larger quantities of particle drops for a larger dynamic range and higher resolution.

As a proof-of-concept assay, varying concentrations of horseradish peroxidase (HRP) labeled streptavidin were incubated with the biotinylated particles, excess washed, and emulsified with tyramide conjugated Alexa Fluor 488 to amplify the signals of particle-bound HRP molecules. Tyramide is converted by HRP into short-lived radical intermediates, which covalently link to tyrosine residues on nearby proteins (Fig.1). The compartmentalization of the particles in an emulsion prevents activated tyramide intermediates from cross-talking with neighboring particles. The emulsion was subsequently disrupted, and the accumulated tyramide signals, which remained bound on the gel particles, were analyzed by a flow cytometer (Fig.4). Overall, we demonstrated increased sensitivity with the amplified biotin-streptavidin affinity system (~700 HRP enzymes per particle) compared to unamplified biotin-streptavidin detection. We are now expanding to a cardiac Troponin I digital immunoassay.

We envision the microgel particles can be fabricated at a central site and widely distributed to speed the adoption of highly sensitive immunoassays. The entire workflow upon receiving the pre-made particles will be performed using basic mixing operations and standard benchtop laboratory equipment without microfluidic chips or pumps, and the results of reactions can be analyzed with widely-available flow cytometers at high throughput. This platform has great potential for democratizing ultra-sensitive immunoassays, advancing the discovery of extremely rare biomarkers which can redefine disease diagnosis.

Word Count: 480

References:

1. J. M. De Rutte, J. Koh, and D. Di Carlo, Advanced Functional Materials, 2019, p. 1900071

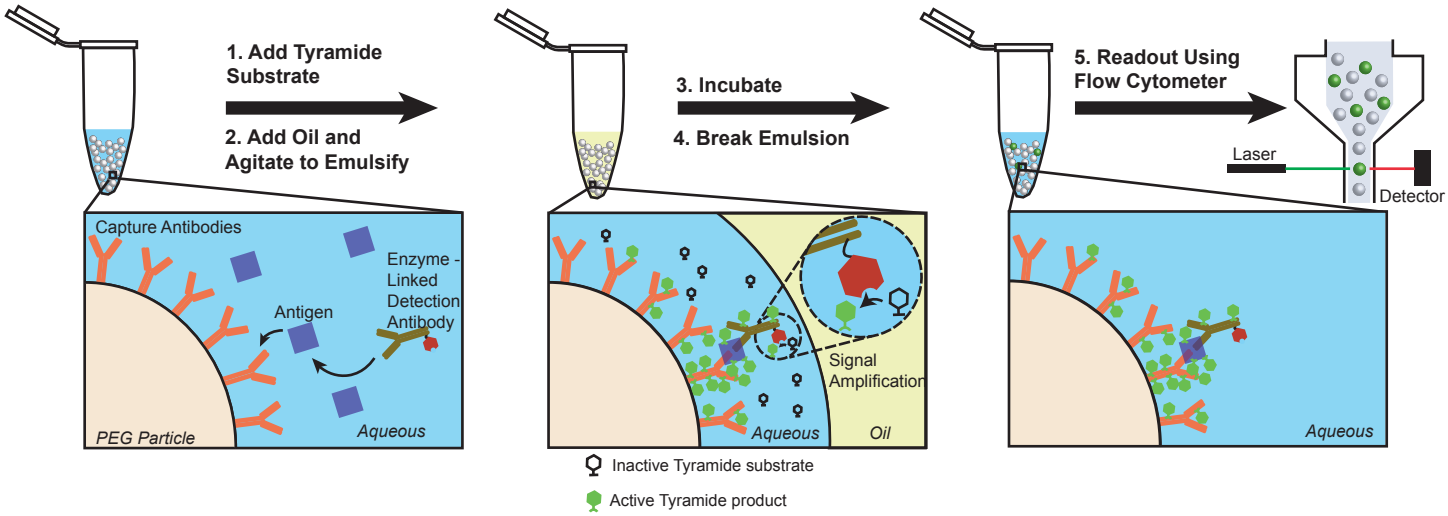

***Figure 1**: Workflow for the device free, droplet ELISA platform.*

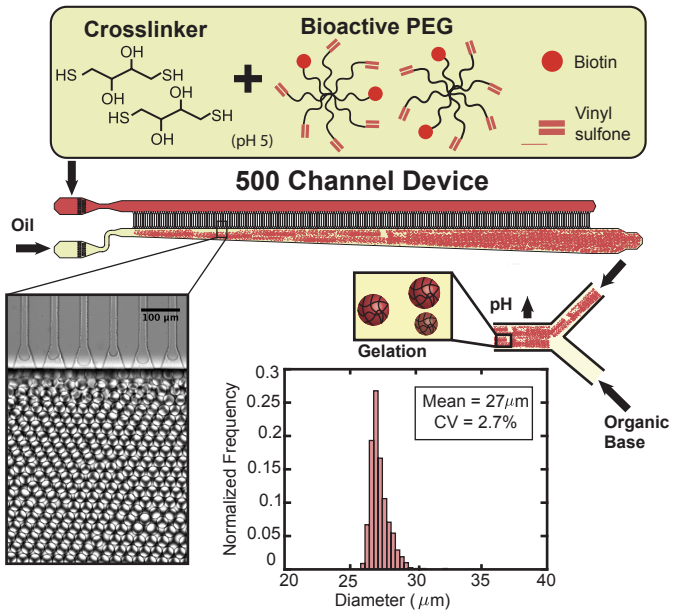

***Figure 2:** Highly monodisperse PEG hydrogel droplets are made at high throughput using step emulsification followed by pH modulation to induce gelation. Insert shows brightfield image of droplet generation via this process and histogram of droplet size distribution.*

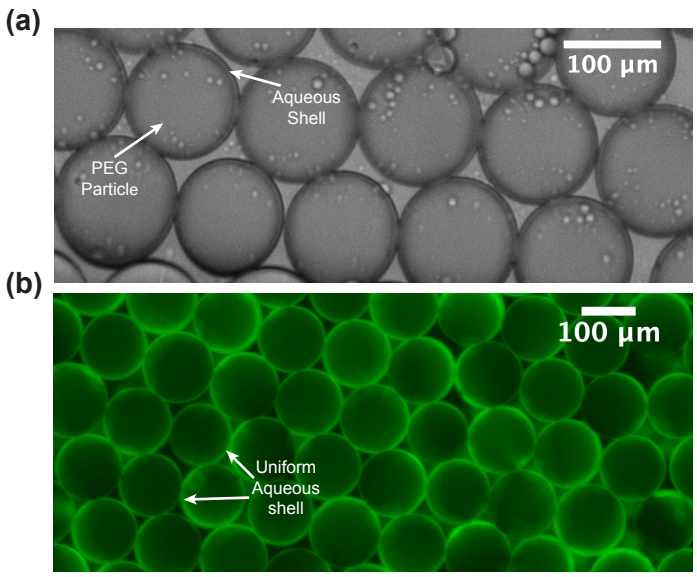

***Figure 3:** PEG hydrogel templated emulsions. (a) Highly monodisperse emulsion droplets created by pipetting aqueous solution with hydrogel particle templates. (b) fluorescent images of uniform aqueous layers around hydrogel particles.*

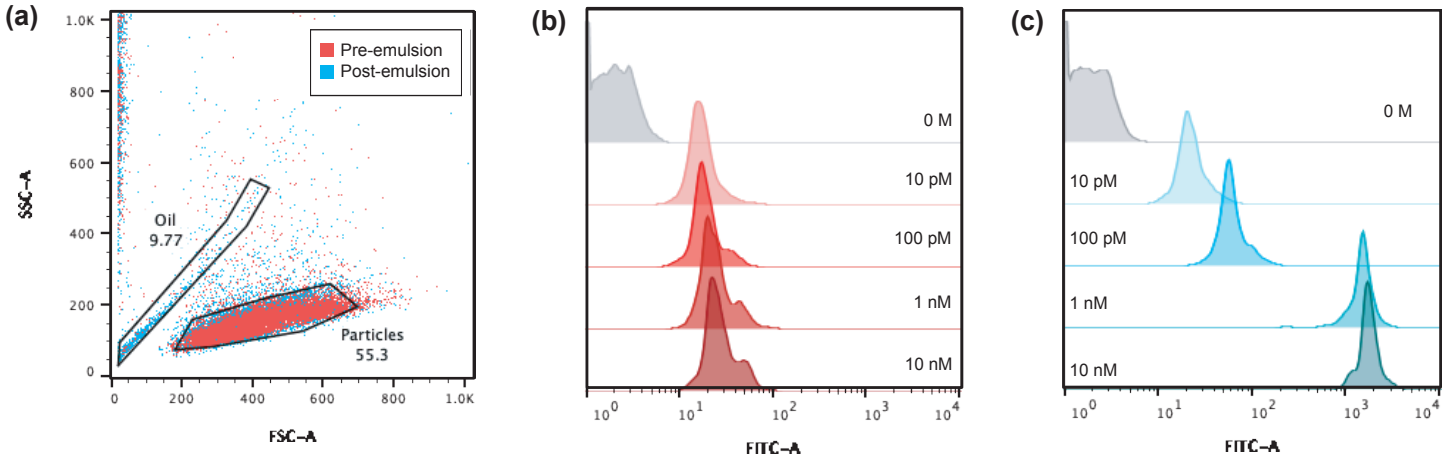

***Figure 4**: Proof-of-concept assay performed with streptavidin - HRP binding. (a) Forward and Side Scatter plots showing particles before (red) and after emulsifying and subsequent transfer back to aqueous solution (blue). (b) Histogram showing negative control for tyramide based assay using varying concentrations of streptavidin bound to PEG particles. (c) Histogram showing dose-response curves for tyramide based assay with varying concentrations of HRP-streptavidin bound to PEG particles showing a dynamic range. 10,000 particles were analyzed for each condition*