BIOMARKER CONCENTRATION AND DETECTION DIRECTLY ON PAPER
University of California, Los Angeles, USA

ABSTRACT
This is the first ever integration of an aqueous two-phase system (ATPS) and the lateral-flow immunoassay (LFA) within a paper-based device to simultaneously and seamlessly concentrate and detect biomarkers. This device revolves around a new phenomenon we discovered where the ATPS solution phase separates and concentrates the target biomarker as it flows through paper, reducing the time to result and eliminating the need for extraction. Our approach demonstrated a 10-fold improvement in the LFA detection limit of a protein with an assay time of 25 min.

KEYWORDS: paper-based diagnostics, aqueous two-phase systems, lateral-flow immunoassay; simultaneous concentration and detection

INTRODUCTION
This paper reports the first ever integration of an ATPS and LFA within a three-dimensional (3-D), paper-based device to simultaneously and seamlessly concentrate and detect biomarkers (Fig. 1). This device revolves around a new phenomenon we discovered where the ATPS solution phase separates and concentrates the target biomarker as it flows through a paper membrane. Our group is the only one to have previously combined ATPSs with LFA for improved detection of biomolecules [1,2], but in these experiments, the ATPS was first allowed to phase separate in a test tube before manually extracting the phase containing the concentrated biomolecule and applying it to LFA. In the method proposed here, rather than using the test tube for phase separation, the ATPS was added directly to the paper, and the paper was found to enhance the macroscopic phase separation process. This significantly reduced the time to result and eliminated the requirement for an extraction step, thereby improving the ease-of-use of the device. Furthermore, the target phase containing the concentrated biomarker automatically separated to the leading front of flow, which continued downstream towards LFA detection. Overall, our approach demonstrated a 10-fold improvement in the LFA detection limit of a model protein with an overall assay time of 25 min.
Figure 1: Unlike our previous proof-of-concept studies which required ATPS separation in a test tube followed by extraction and application to LFA, our new and improved device simultaneously concentrates and detects biomarkers on paper (gray box).

EXPERIMENTAL

Polyethylene glycol (PEG) 8000 and potassium phosphate salt (5:1 dibasic to monobasic ratio) were dissolved in Dulbecco’s phosphate-buffered saline (PBS). Dextran-coated gold nanoparticles were synthesized according to Min and coworkers [3] with slight modifications. To form dextran-coated gold nanoprobe s, the dextran-coated gold nanoparticles were conjugated to anti-transferrin antibodies. To visualize the two phases of the ATPS as they flowed in the paper, dextran-coated gold nanoparticles (purple) and Brilliant Blue FCF dye were added to PBS solutions. The LFA component of the paper-based device was slightly modified from our previous studies [2]. Specifically, the cellulose sample pad was replaced with a 5 x 20 mm fiberglass paper, which connected a nitrocellulose membrane containing the test and control lines. At the beginning of the sample pad, a 3-D paper well composed of multiple strips of fiberglass paper was used.

RESULTS AND DISCUSSION

As shown in Fig. 2a, when a drop of a PEG and salt ATPS is added to a fiberglass paper strip, the PEG-poor phase rapidly separates from the PEG-rich phase. We then investigated 3-D paper architectures, which further enhanced this phenomenon and enabled use of an ATPS with a 10-fold concentrating effect (Fig. 2b). Specifically, in the example in Fig. 2b, the phase separation occurs on the order of seconds, whereas macroscopic phase separation in a test tube would have occurred on the order of hours. We believe the 3-D architecture improves the phase separation process by increasing the cross-sectional area normal to the direction of flow. This allows more volume of sample to wick through the paper at the same time, allowing more PEG-rich domains to be retained by interactions with the paper while the PEG-poor domains coalesce more easily. The 3-D architecture also benefits from the gravitational force, which is a driving force for phase separation in a test tube. The results of an experiment comparing traditional LFA to our paper device are shown in Fig. 3. The detection limit of a model protein transferrin using traditional LFA was 1 ng/µL. When using our paper device, the detection limit was improved 10-fold to 0.1 ng/µL. This research has the potential to vastly improve disease diagnosis in resource-poor settings by being able to lower the detection limit, transforming the current state of healthcare through the development of next generation LFA tests.

Figure 2: (a) A mixed PEG-salt ATPS is added directly onto paper. The PEG-poor phase (purple dye) separates rapidly from the PEG-rich phase (blue dye). (b) The paper well improves the phase separation phenomenon and enables use of an ATPS with greater concentrating effects (darker purple front contains concentrated biomarkers).
Figure 3: Traditional LFA is compared to our combined ATPS and LFA paper-based diagnostic device. The detection limit of a model protein transferrin is improved 10-fold.

CONCLUSION

Our 3-D paper diagnostic is the first to demonstrate ATPS phase separation in paper, allowing for concentration of the target biomarker as the sample flows through the paper to the detection zone. This implementation improves the sensitivity of conventional LFA devices while maintaining ease-of-use and time-to-result. With 10-fold improvements in detection limit, our device has the potential to rapidly identify pathogens at lower concentrations that were previously undetectable with conventional LFA. This robust and portable device requires no electricity or sophisticated laboratory equipment and is ideal for point-of-care applications in resource-poor settings. Once fully developed, this platform technology has the potential to revolutionize the state of health care in resource-poor settings by providing rapid, accurate, and inexpensive diagnostics, leading to improved patient management, treatment, and outbreak prevention.

ACKNOWLEDGEMENTS

This work was supported by UCLA funds.

REFERENCES


CONTACT

* D.T. Kamei; phone: +1-310-206-4826; kamei@seas.ucla.edu