Silk film for developing a colorimetric µPAD methanol biosensor

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Silk fibroin (SF) is a fibrous protein found in the cocoons of *Bombyx mori* silkworm. It is known as a versatile biomaterial due to its intrinsic high stability to temperature and moisture changes as well as its biocompatibility rendering its application in not just in sensing but also in drug delivery and tissue engineering. Assam is one of the largest producers of silk including *Bombyx mori* silk in India, therefore the success of SF for developing platforms for commercial sensors will enhance the economics of the state.

An important characteristic of the SF protein is its ability to be prepared into different types of scaffolds, such as fibers, films, or hydrogels. By tuning the secondary structure of SF, different properties of these formats can be also tuned. The secondary structure of SF mainly consists of two molecular conformations: silk I, which is water soluble and in a non- crystalline metastable form, with dominant structures of α helix and random coils; and silk II, which is water insoluble and highly stable and consisting of β- sheets. This unique structure consisting of silk I and silk II contributes to create a conductive environment for stabilization of the enzymes and microbial cells.

While developing enzyme-based sensors, stability is the vital concern because of the labile nature of enzymes. Microfluidic paper-based analytical devices (µPAD) have been used for development of low-cost, portable sensors; however, paper-based platforms suffer from coffee-ring effect. In the present paper we addressed these issues by introducing SF film for immobilizing enzymes and chromogenic reagent (ABTS) and wave-design microfluidic channels in the chromatographic paper for developing a hybrid silk µPAD for methanol detection following peroxidase reaction. The activity of alcohol oxidase (AOx), used as biorecognition element, could be wholly retained in non-dissolvable SF film (pore size < 3.5 microns) until 40 days of storage at room temperature (RT) by reducing oxygen permeability to the film. Similarly, ABTS in dissolvable SF film was protected from air-oxidation even up to two months of storage at RT. Furthermore, the detection approach exploited purple color as a high contrast response signal generated from ABTS di-cation formed from the reaction of SF protein with the ABTS radicals generated from the substrate-dependent peroxidase reaction. The wave-designed microfluidic channels could significantly reduce the coffee ring effect in the detection film. The developed biosensor required only a Smartphone to capture the emergence and enhancement of purple color in the detection SF film of the device with the methanol concentration. The bi-enzyme SF films prepared by co-immobilizing AOx with peroxidase (HRP) offered high sensitivity to the µPAD device with a detection limit (LoD) of 1±0.05 mM and a dynamic range of 1 mM-2 M for methanol. The complete study of this stand-alone hybrid µPAD biosensor for methanol will be described in the presentation.

References

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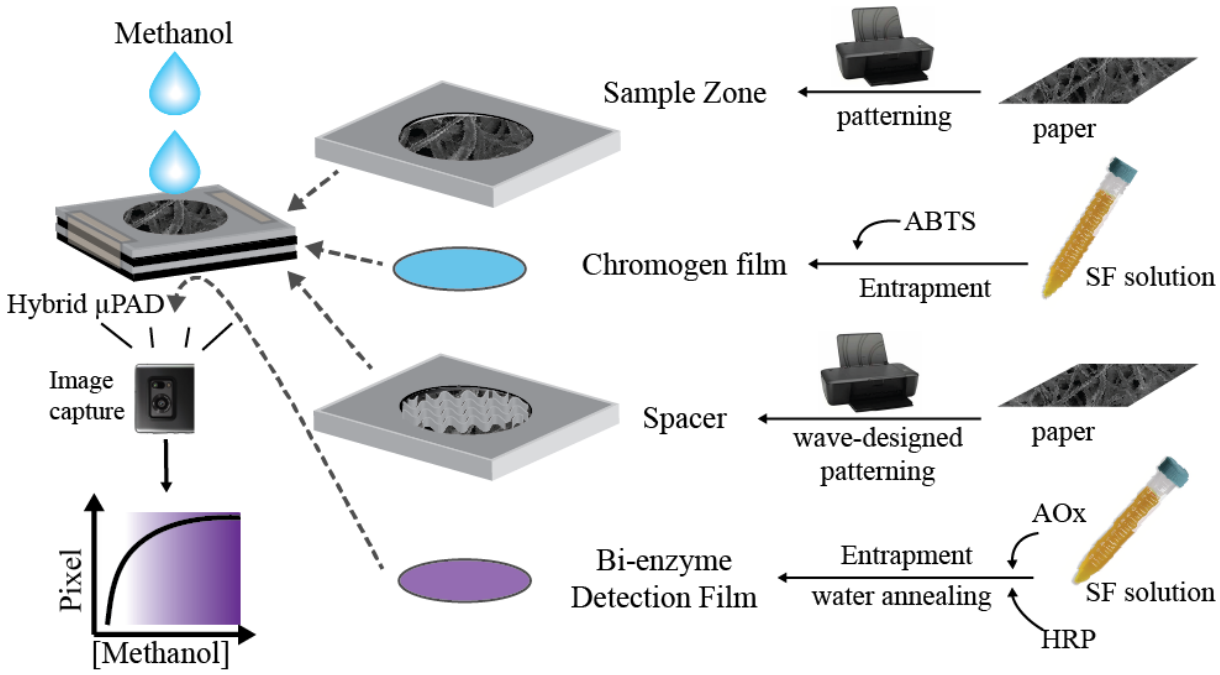

Figure 1: The schematic representation of the different section of the silk µPAD and its usage for methanol detection.

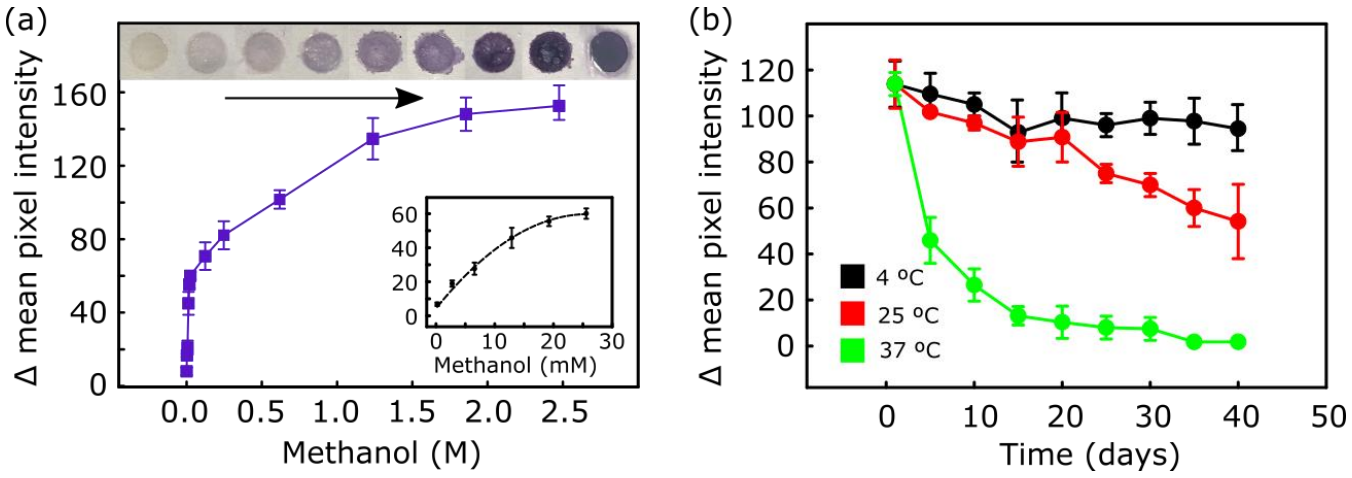

Figure 2: (a) Performance of the hybrid µPAD methanol biosensor; (b) Stability study of the µPAD at different storage temperature over 40 days.