A HOLOGRAM BASED MICROFLUIDIC SYSTEM FOR FIRST RESPONSE OF SPORE DETECTION

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ABSTRACT

A novel hologram based microfluidic system was developed for first response detection of spores. Protocols to integrate pH sensitive hologram film into a multi-layer disposable Poly(methyl methacrylate) (PMMA) microfluidic chip were successfully developed. Spore pre-concentration, sample incubation were implemented on-chip. The protocol was optimized to be able to detect spores within 20 mins (including fluid manipulation). The main advantage of such detection is label-free and real time monitoring.

KEYWORDS: Hologram, Spore detection, Microfluidics,

INTRODUCTION

Spores of Bacillus anthracis can be easily developed as a biological weapon which is usually in powder format. Breathing in spores may lead to lung inflammation, hemorrhaging and even tissue death (Figure 1). On-site rapid detection of spores is thus necessary which cannot be done through traditional detection approaches such as ELISA [1] and PCR [2].

Figure 1: Breathing in spores may lead to lung inflammation, hemorrhaging and even tissue death.

EXPERIMENTAL

Here we integrated poly[HEMA-co-EDMA-co-MAA] hologram film into PMMA microfluidic chip for the detection of powder format spores (B.Megaterium). Sample powders was collected with a customized swap bud into nutrient broth in a syringe attached to the microfluidic chip (Figure 2a). The chip consisted of two inlets (for spore sample and germination solution), four microvalves, one filtering chamber (or incubation chamber), one detection chamber where hologram film embedded and one waste chamber (Figure 2b). Through valve operation, spore samples were pre-concentrated by filtering, incubated with germination solution (metered through valve operations) and detected at detection chamber where hologram sensor located.

Protocol of pH sensitive film fabrication was modified [3] for integration into plastic chip to ensure the UV crosslinked, air-dried hologram films embedded with layers of fine grains of metallic silver were
firmly attached to the PMMA slides and works well after a few rounds of thermal bonding (~125°C) during chip fabrication. The prepared chips can be stored at room temperature without affecting their stability. When the developed gratings was illuminated with a white light source, the silver nanoparticle spacing worked as Bragg mirrors and the reflected light from the periodic gratings resulted in a narrow-band spectral peak whose wavelength is governed by the Bragg equation

\[ \lambda_{\text{peak}} = 2n_0d \sin(\theta) \]

(\( \lambda_{\text{peak}} \), wavelength of reflected light at maximum intensity; \( n_0 \), refraction index of medium; \( d \), spacing between two consecutive nanoparticle-based layers; \( \theta \), Bragg angle;) (Figure 3).

Figure 2:  a) Photos of the hologram detection system and microfluidic chip (75mmx50mm, filter membrane pore size – 0.4µm); b) Microfluidic chip with patterned hologram film

Figure 3: Reflected light from periodic gratings resulted in a narrow-band spectral peak governed by Bragg equation

Figure 4: Wavelength blue-shift associated with germination and outgrowth of spores in nutrient broth (95mM, Sigma 70112) and D-glucose (5 mM, Sigma G8270)
RESULTS AND DISCUSSION

Hence, the spacing changes ($d$) caused by chemical or biological event, i.e. pH change during spore metabolism here, can be detected by the change in the wavelength of the reflection hologram. Spore germination and metabolism can be real time monitored with UV-spectrophotometer by incubating spores in germination solution. The pH decrease of the incubation solution during spore germination & outgrowth (glucose to acid conversion and release of metabolic products) caused a shrink of the hologram film which led to a blue-shift of wavelength as shown in Figure 4. For a rapid detection of the presence of spores, a 6 nm blue-shift of wavelength was observed in 15 mins (Figure 4). Large blue-shift of wavelength (~30nm) was observed for overnight incubation (Figure 5).

CONCLUSION

Hologram film was successfully integrated into a multilayer PMMA microfluidic chip for fast and label-free detection of bacterial spore in powder format. A UV-spectrophotometer integrated fluid manipulation system was developed to facilitate the microfluidic based spore detection.

ACKNOWLEDGEMENTS

The project was kindly supported by the Singapore Technology Enterprise Commercialisation Scheme grant from Spring (Grant No. TI/TECS/POV/13/25). We would like to thank Chan Cong Zhi Leon (Singapore Institute of Manufacturing Technology, Singapore) for his help on the hologram detection.

REFERENCES


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Figure 5: A large blue-shift (30nm) and a color change from light red to yellow were captured for spores incubated overnight in nutrient broth.