FLUORESCENCE IN SITU HYBRIDIZATION (FISH) MICROFLUIDIC PLATFORM FOR DETECTION OF HER-2 OVER-EXPRESSION IN CANCER CELLS

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ABSTRACT

Detection of cancer biomarkers has become increasingly important for the early diagnosis, prognosis, and treatment of cancer. Genetic biomarkers like HER2 have been associated with many types of cancers including breast cancer and gastric cancer. Furthermore, Herceptin therapeutics has been found to be particularly effective for patients with an overexpression of HER2 to improve overall survival rates. Fluorescence in situ hybridization (FISH) has been commonly used for HER2 over-expression. However, it requires expensive reagents and skilled personnel. In this work, a novel, integrated FISH microfluidic platform capable of performing the entire FISH protocol automatically to diagnose HER-2 over-expression was reported. Experimental results showed that the developed platform could achieve comparable results when compared with the manual process.

KEYWORDS: Cancer biomarker, HER2, Herceptin, FISH

INTRODUCTION

Overexpression of the gene for human epidermal growth factor receptor 2, or HER2 gene, is an effective indicator of cancerations [1-2]. This condition refers to an abnormally high number of HER2 genes on chromosome 17, which results in an over-abundance of HER2 receptors on the cell surface, rendering the cell cancerous. Specifically, HER2 receptors can bind to other types of epidermal growth factor receptors to form dimers and activate intracellular signaling pathways that alter physiological control of the growth, differentiation, and angiogenesis, ultimately preventing cells from natural death and promoting cancer cell division.

Currently, FISH has been widely employed for the detection of HER-2 overexpression. FISH is a cytogenetic technique that uses fluorescently-modified DNA probes to detect and localize the presence or absence of specific DNA sequences on chromosomes. However, traditional FISH assays usually require a time-consuming process with expensive reagents to be performed by well-trained personnel. These requirements have thus limited the clinical application of FISH. Micro-electro-mechanical-systems (MEMS) and microfluidics provide integrated and miniaturized devices that include micropumps [3], micromixers, and microvalves and can be developed to perform the entire FISH protocol in an automated fashion while using less sample/reagent.

Toward improving the current FISH-based canceration detection, this work reports a new microfluidic chip that automatically performs the FISH protocol and reduces the consumption of biosamples and reagents (see Table 1). Breast cancer cell line immobilized on glass slides were first used as a model target. Experiment results demonstrated a high similarity between bench-top and microfluidic chip-based detection, indicating the potential of the developed microfluidic system as an effective platform for HER2 overexpression analysis.

Table 1: The detailed protocol of FISH for HER2 over-expression

Summary of FISH protocol steps on chip (cell line)	Time (min)	Volume (ml) / chip	Volume (ml) / bench
2× SSC pH5.3 (73°C)	3	1	30
0.0025% pepsin (37°C)	15	0.5	10
1× PBS (Room Temperature)	5	1	30
1% formaldehyde (Room Temperature)	5	0.5	5
1× PBS (Room Temperature)	5	1	30
70% 85% 100% Alcohol (Room Temperature)	3	1	30
Hybridization	960	0.002	0.002
2× SSC 0.3% NP-40 pH7.0~7.5 (75°C)	3	1	30
DAPI (Room Temperature)	1	0.002	0.002
Store chip (-20°C)	30		

EXPERIMENTAL

The microfluidic device was integrated with temperature and fluid control modules to efficiently perform the entire FISH assay in an automated fashion within a relatively short period of time while significantly reducing the amount of reagents and was designed to work with both immobilized cell lines and clinical tissue samples (Fig. 1). To demonstrate the microfluidic FISH assay, breast cancer cells immersed in a fix solution (methanol and acetic acid in a 3:1 v/v ratio) were first dripped onto a glass slide and then frozen for 30 minutes at -20 °C. The PDMS-based microfluidic device was then attached to the glass side. To start the FISH protocol, 2× SSC (pH 5.3) at 73 °C was first injected into the reaction area (see Fig. 1) by using micropumps (see Fig. 2) to simulate the slightly acidic condition in the stomach, followed by 0.0025% pepsin for decomposing membrane proteins. The cells were then sequentially washed by 70%, 85%, and 100% ethyl alcohol for dehydration. Then 2-μl probe solution that contain an orange-fluorescence probe specific for the HER2 gene and a green-fluorescence chromosome 17 enumeration probe (CEP-17) was added to begin the hybridization step for 16 hours. After washing away unbound probes, 4′, 6′-diamidino-2-phenylindole dihydrochloride (DAPI) solution was used to stain the nuclei. Finally, fluorescent images were acquired by using fluorescence microscopy and a digital imaging system while scanning the chip (see Table 1).

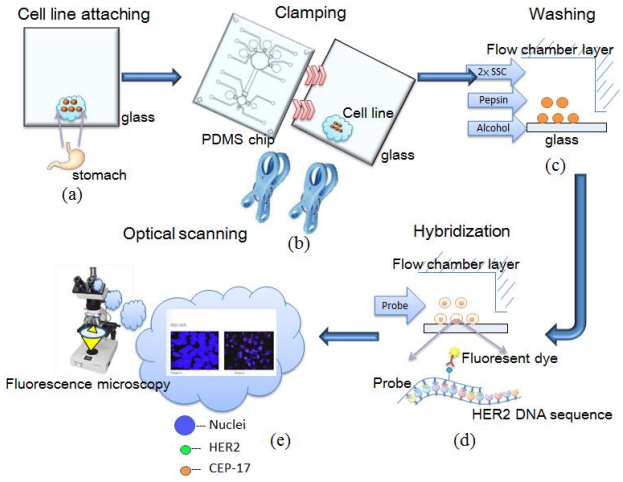


Figure 1: Schematic illustration of the microfluidic FISH protocol: (a) Drip breast cancer cells on glass; (b) Clamp the microfluidic chip and the glass with cell line together; (c) Transport 2× SSC pH5.3, pepsin , alcohol to the reaction area; (d) Hybridize the probe and HER2 gene; (e) Scan the fluorescent signal by fluorescence microscopy.

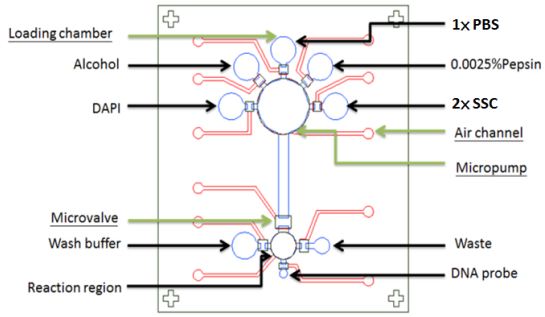
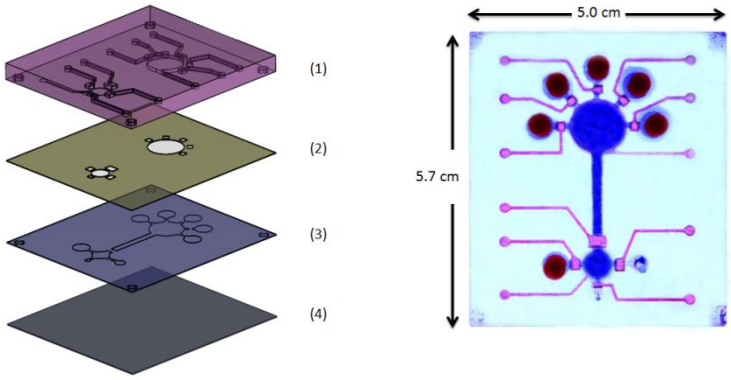


Figure 2: Schematic diagram of the FISH chip.

Figure 3 shows an exploded view of the FISH chip. This chip was capable of pumping reagents at as high as 35 μl/sec at -40 kPa (gauge pressure).Temperature and fluid control modules are very important to precisely control the variation of temperature and the motion of reagents. In this work, a dual-temperature controller, which was controlled by a designated computer program, was employed to deliver precise temperatures to the chip (Fig. 4).



(a) (b)

Figure 3: (a) Exploded view of the microfluidic FISH chip composed of (1) an air layer, (2) a middle layer, (3) a flow chamber layer, and (4) a glass layer;(b) A photograph of the chip. The dimensions of the microfluidic chip were 5.0 cm by 5.7 cm. Blue color indicated the flow layer and red color indicated the air layer.

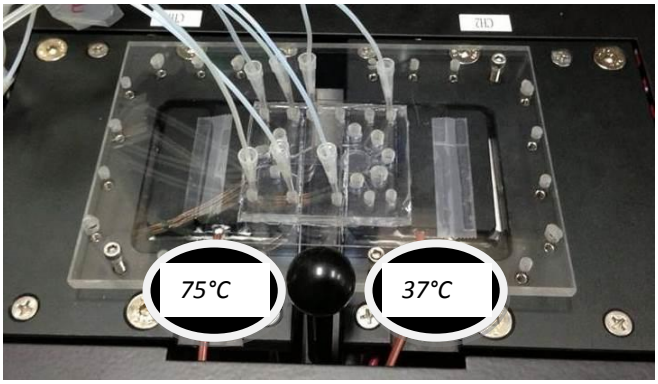


Figure 4: A photograph of the dual temperature controller. Left side was the reaction area usually maintaining 75°C during 2× SSC and hybridization. Right side was 37°C

RESULTS AND DISCUSSION

Upon the completion of the microfluidic FISH assay, HER2 over-expressions could be successfully observed in fluorescence images (Fig. 5). HER2 genes were indicated by the orange spots while the green spots signaled CEP-17 probes. HER2 overexpression was defined by the ratio between the numbers of fluorescence spots associated with the two genes (HER2/CEP-17); a ratio greater than 2.2 with 60 cells indicates HER2 overexpression. Furthermore, when single cell has more than 6 CEP-17 probes, the condition is referred to as polysomy. Notably, the entire process can be completed automatically within 20 hours. More importantly, the amount of reagents can be greatly reduced by approximately 80%. More testing about reducing the reaction time is undergoing.

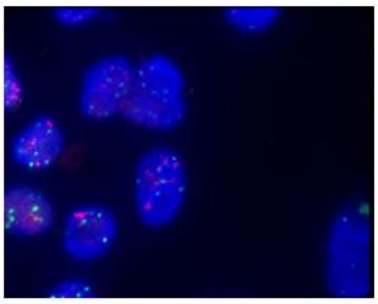
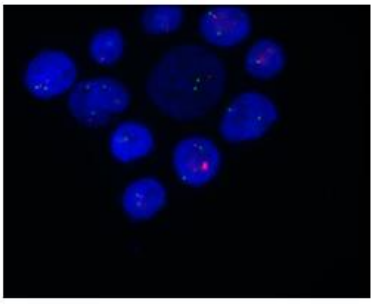
Breast cancer	Positive	Negative
		
HER2/CEP-17 ratio	Polysomy	1.067

Figure 5: Results as fluorescence pictures compared between positive and negative cases.

CONCLUSION

In summary, a new integrated microfluidic chip capable of automatically performing a FISH assay for the detection of HER2 gene over-expression in breast cancer cell line was presented in this work. This chip can be readily adapted for the detection of clinical tissue samples. With further optimization and improvements, this platform may be extended for other pharmacogenetic applications.

ACKNOWLEDGEMENTS

The authors want to express gratitude to Medical Technologist, Sean Lee in the Department of Pathology of CGMH for technical assistance. We also thank Dr. Yeh's lab in CGMH for supplying the necessary reagents.

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