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A fluorescence *in situ* hybridization (FISH) microfluidic platform for detection of *HER2* amplification in cancer cells ☆

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ABSTRACT

Over-expression/amplification of human epidermal growth factor receptors 2 (HER2) is a verified therapeutic biomarker for breast and gastric cancers. *HER2* is also served as prognostic biomarker for gastric cancer because HER2 over-expression is associated with a 5–10% increase in cancer related death of gastric cancer. Cancer patients exhibiting HER2 over-expression can significantly improve their overall survival rates by taking the targeting drug Herceptin, which directly targets HER2. However, Herceptin has limited functions toward patients without HER2 over-expression and therefore it needs a highly specific and accurate detection method for diagnosis of HER2 over-expression. Currently, fluorescence *in situ* hybridization (FISH) technique is routinely employed to detect *HER2* amplification. However, it is a labor-intensive, time-consuming hybridization process and is relatively costly. Furthermore, well-trained personnel are required to operate the delicate and complicate process. More importantly, it may take 1–2 days for well-trained personnel to perform a whole FISH assay. Given these limitations, we developed a new, integrated microfluidic FISH system capable of automating the entire FISH protocol which could be performed within a shorter period of time when compared to traditional methods. The microfluidic FISH chip consisted of a microfluidic control module for transportation of small amounts of fluids and a hybridization module to perform the hybridization of DNA probes and cells/tissue samples. With this approach, the new microfluidic chip was capable of performing the whole FISH assay within 20 h. Four cell lines, two for non-*HER2* amplification and two for *HER2* amplification, and two clinical tissue samples, one for non-*HER2* amplification and another for *HER2* amplification, were used for verifications of the developed chip. Experimental data showed that there was no significant difference between the benchtop protocol and the chip-based protocol. Furthermore, the reagent consumption was greatly reduced (~70% reduction). Especially, only 2-μl usage for FISH deoxyribonucleic acid (DNA) probe was used, which is five-fold reduction when compared with the traditional method. It is the first time that the entire FISH assay could be automated on a single chip by using tissue samples. The microfluidic system developed herein is therefore promising for rapid, automatic diagnosis of HER2-related diseases by detecting the *HER2* gene with minimal consumption of samples and reagents and has a great potential for future pharmacogenetic diagnostics and therapy.

Abbreviations: Bio-MEMS, Bio-micro-electro-mechanical-systems; CEP17, Chromosome 17 centromere; CGMH, Chang Gung medical hospital; DNA, deoxyribonucleic acid; DAPI, 4′-diamidino-2-phenylindole; FISH, fluorescence *in situ* hybridization; HER2, human epidermal growth factor receptor 2; MEMS, micro-electro-mechanical-systems; NaSCN, sodium thiocyanate; PBS, phosphate buffered saline; PCR, polymerase chain reaction; PDMS, polydimethylsiloxane; PMMA, polymethylmethacrylate; RSD, relative standard deviation; SSC, sodium chloride–sodium citrate buffer; ddH_2O, double-distilled water; rpm, revolutions per minute

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1. Introduction

Detections of the HER2 over-expression become more and more important recently especially when HER2 over-expression is found to be associated with a 5–10% increase in cancer related death of gastric cancer (Hsu et al., 2011). Previous study indicated that cancer patients demonstrating HER2 over-expression can significantly improve their overall survival rates by taking the targeting drug Herceptin, which directly targets HER2 (Hsu et al., 2011). Since Herceptin is relatively costly and it has limited functions toward patients without HER2 over-expression, a highly specific and accurate detection method is needed to prevent unnecessary medical cost and drug abuse.

An abnormality in an individual's genome due to, for instance, exposure to a carcinogenic substance, can lead to a variety of genetic disorders or cancers. Traditionally, polymerase chain reaction (PCR) and reverse transcription polymerase chain reaction have been commonly used to detect human genetic disorders (Bruneau et al., 1990). However, these methods are inefficient for analysis of numerous genes in parallel. Alternatively, fluorescence *in situ* (FISH) hybridization is an important technique with high specificity for localizing mutant genes, or their respective mRNAs, within individual cells (Lichter et al., 1990; Pinkel et al., 1988; Vanneste et al., 2009; Langer-Safer et al., 1982), as well as cancers (Fox et al., 1995; Leversha et al., 2009; Nath and Johnson, 2000). FISH can allow for observations of genetic alterations, such as rearrangements and translocations, which are crucial indicators for certain cancers. FISH technique is routinely used to detect HER2 amplification (Young et al., 1995). However, it needs a labor-intensive, time-consuming process and is relatively costly. Furthermore, it also needs well-trained personnel to operate the delicate and relatively complicate process.

Recently, detection of cancer biomarkers by FISH has become increasingly important for the early diagnosis, prognosis, and treatment of cancer (Mitri et al., 2012). As mentioned above, genes such as *HER*2 have been associated with many types of cancers including breast cancer and gastric cancer. Furthermore, survival rates of patients demonstrating *HER*2/HER2 amplification/over-expression can be enhanced by Herceptin targeting therapeutics. However, FISH-based HER2 over-expression detection analyses require expensive reagents and skilled personnel. Also, in spite of long hybridization times and tedious washing steps, the technician may inadvertently contaminate the samples, thus compromising the accuracy of the analysis. Therefore, there is an urgent need to develop methods that can rapidly perform detection assays for over-expression of HER2 in an automated manner.

Micro-electro-mechanical-systems (MEMS) would be a solution for abovementioned issues. MEMS is a technique used to manufacture miniature systems that consist of microsensors, microactuator and micro-circuits (Li, 2002). Miniaturized systems have several advantages over their conventional counterparts, including size compactness, low power consumption, enhanced performance and reliability (Courtois and Blanton, 1999). Recently, a technology known as bio-micro-electro-mechanical-systems (Bio-MEMS) has been exploited for use in biosensing, drug delivery, biomedical engineering, and a variety of biomedical and chemical applications (Velten et al., 2005). Bio-MEMS technology is capable of producing microdevices comprised of low-cost materials that are associated with lower degrees of contamination due to their usage of lower reagent volumes and their disposability to a single use. Moreover, the miniaturized microfluidic systems developed by Bio-MEMS have demonstrated similar, or even superior, performance to their large-scale counterparts. Therefore, with Bio-MEMS technology, diverse processes and modules can be integrated into a single chip in order to reduce the amounts of samples and reagents, and power consumption. Furthermore, reaction times and overall costs could be dramatically reduced while simultaneously increasing sensitivity, throughput, portability, and potential for integration and automation (Byun et al., 2008).

Bio-MEMS and microfluidic-based technologies could meet these diagnostic demands. Recently, two Bio-MEMS and microfluidic-based systems have been reported for FISH assays. For instance, Mayer et al. employed an OncoCEETM microchannel technology to a pilot clinical trial for FISH detection of HER2 (Mayer et al., 2011). The adequate sensitivity and specificity of the OncoCEE^{TM} microchannel were demonstrated in this work. However, the sample should be subjected to complicated manual pretreatment step before applied to the OncoCEE^{TM} microchannel. Another Bio-MEMS and microfluidic-based system was reported by our group previously (Tai et al., 2013).This system integrated all manual steps into a microfluidic system so that a total automated fashion of FISH can be realized. However, the samples used in this study were cell lines only. In the hospitals, most of tissue samples of gastric cancer are paraffin sections which have undergone a series of dedicated steps in order to be used for FISH assays. Therefore, in this work, a new integrated FISH microfluidic platform aimed to automate the entire FISH assay for the detection of *HER*2 by using tissue samples is proposed. The integrated microfluidic system which is capable of performing the entire FISH protocol from tissue sample pre-treatment to DAPI staining to diagnose *HER*2 over-expression is demonstrated. It integrated several functional devices, including microvalves, micropumps, and reaction chambers that were capable of transporting, mixing, incubating, and storing multiple reagents and samples, on a single chip. Specifically, a fluid control module to control fluids transport, a temperature control system to regulate the temperature and a hybridization module to enhance the hybridization process were integrated to perform the whole FISH protocol. More importantly, the reagent consumption was significantly reduced (~70% reduction). To the best of our knowledge, this is the first work which could automate the entire FISH assay by using tissue samples.

2. Materials and methods

To verify the performance of the FISH chip, four breast cancer cell lines and two clinical tissue samples, including non-*HER*2 amplification cases as negative controls and HER2 amplification cases as positive controls demonstrating HER2 over-expression, were tested. The protocols for the cultured cells and tissue samples differed slightly, but both types of samples could be used with the chip designed herein. Therefore, the FISH microfluidic chip could be used with dual functions, which has never been demonstrated in literature.

2.1. Cell lines Preparation

In order to investigate whether *HER*2 genes could be detected on the FISH microfluidic chip, the breast cancer cell lines obtained from Chang Gung Medical Hospital [CGMH]-MDA-MB-468 (non-*HER*2 amplification case), MCF-7 (non-*HER*2 amplification case), SK-BR-3 (*HER*2 amplification case), and MDA-MB-361 (*HER*2 amplification case) were used. The cells were cultured in Dulbecco's modified Eagle medium supplemented with 10% fetal bovine serum and maintained in a 37 °C incubator. After trypsinization, suspended cells at a concentration of 5×10^6 cells/ml were washed twice with phosphate buffered saline (PBS) and spun at 1200 revolutions per minute (rpm) for 5 min, and the culture media supernatant was decanted. Then, cells were re-suspended in 1–2 ml Carnoy's fixative prior to application on the glass slide. Fixed cells (10 μl) were deposited on the glass slide and incubated at room