FLUORESCENCE IN SITU HYBRIDIZATION (FISH) ENHANCEMENT USING MICROFLUIDIC FLOW FOR AN ACCURATE, FAST AND ECONOMICAL ASSESSMENT OF HER2 STATUS IN BREAST CANCER

Huu-Tuan Nguyen¹, Raphaël Trouillon¹, Seiya Matsuoka¹, Maryse Fiche², Laurence de Leval², Bettina Bisig² and Martin A.M. Gijs¹*

¹ Laboratory of Microsystems 2, École Polytechnique Fédérale de Lausanne, Switzerland
² Institute of Pathology, Centre Hospitalier Universitaire Vaudois and University of Lausanne, Switzerland

ABSTRACT

The fluorescence in situ hybridization (FISH) is the gold standard in human epidermal growth factor receptor 2 (HER2) status assessment in breast cancer. The dissemination of the technique is impeded by the cost of reagents and long experimental time. For overcoming these limitations, we have developed a new method for implementing FISH for HER2 assessment for tissue analysis based on microfluidic technology.

KEYWORDS: Microfluidics, Fluorescence In Situ Hybridization, Tissue Analysis, Breast Cancer.

INTRODUCTION

In standard FISH protocol, the probe solution is applied on a tissue slide, which is subsequently cover-slipped and incubated overnight. Hereafter, the slide is imaged under a fluorescent microscope and one counts red and green fluorescent dots in the tumor cell nuclei, corresponding to the positions of the HER2 gene and the reference centromere of chromosome 17, respectively. Widespread utilization of FISH is impeded mainly by a high cost of the probe solution and a long protocol time. Integration of FISH in a microfluidic system for cell analysis was first reported in [1], showing the possibility to implement FISH with less probe and time. In contrast, FISH for tissue analysis requires a much larger staining surface, thus challenging miniaturization. The first FISH analysis for tissue integrated in a microfluidic device [2] demonstrated staining of a 20 times smaller tissue surface (5x5 mm²) than that used in the standard protocol. Therefore it resulted in a lower throughput; also no improvement of the hybridization time was reported. Here we develop microfluidics-assisted FISH (MA-FISH), in which hybridization of the DNA probes with their target DNA strands was obtained by applying square-wave oscillatory flows of diluted probe solutions in a thin microfluidic chamber of 5 µl volume.

EXPERIMENTAL

A custom-made microfluidic tissue processor (figure 1), which has been developed in our laboratory [3] [4], was used for creating homogenous flow over a clinical tissue surface during the hybridization step inside a thin 16x16mm² size chamber that was formed by clamping mechanically a microscope slide carrying a tissue section against the microfluidic chip (figure 2). Optimization of the parameters, such as hybridization time and probe concentration, were based on analyzing adjacent slides originating from the same tumor, using an image processing program (Cell Profiler). The diagnostic outcome of the test was obtained by analyzing z-stacks of images, counting red HER2 probe-labelled dots and green centromere enumeration probe of chromosome 17 (CEP17) dots in clusters of 20 cells evaluated for 3 positions on each tissue slide.
RESULTS AND DISCUSSION

MA-FISH with 10x diluted probe in 4 h is an optimized condition that resulted in a large dot size with fair signal contrast, and a similar dot count with respect to the standard technique (figure 3). The efficacy of the test was confirmed on different tissue samples. The result of our imaging protocol, as applied to a MA-FISH slide that was hybridized using a 10x diluted probe solution during 4 h is illustrated in figure 4.

CONCLUSION

Comparing to the standard protocol, MA-FISH decreases the consumption of the expensive probe solution by a factor 5 and the duration of the hybridization step by a factor 4. The total duration of a test thereby decreases from two days to one. Moreover, the principle of this technique can be extended to other kinds of probe or tissue

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REFERENCES


CONTACT

* M.A.M Gijs; phone: +41 21 69 36923; martin.gijs@epfl.ch