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

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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 mm^2) 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.

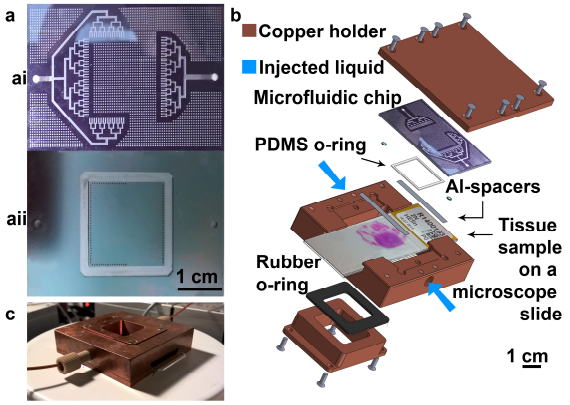

Figure 1: Microfluidic chip and setup for MA-FISH analysis. (a) The chip consisting of a bonded Pyrex-silicon stack. (ai) View on the Pyrex side of the stack. The flow is channelized via feedthrough holes to reach the face (aii). (aii) Silicon side of the chip stack. b. Exploded view of the setup. c. View of the upside-down assembly of the setup of (b) on a hot plate, interfaced with two syringe-pump tubings.

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 16x16mm^2 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.

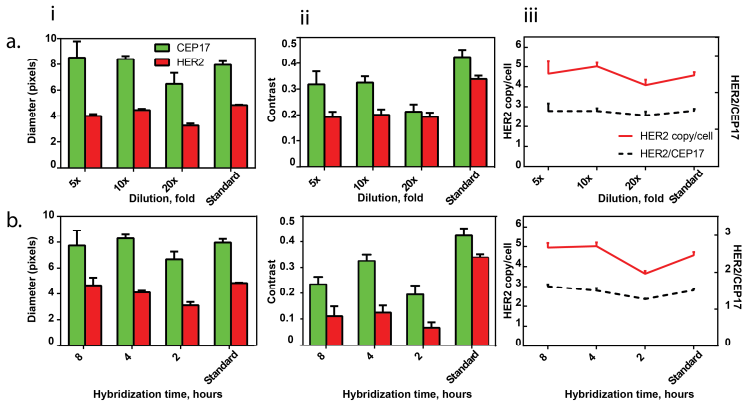

Figure 2: Optimization of the probe dilution (a) and hybridization time (b). (a) In green: dot signal radius (a.i) and contrast (a.ii) originating from the CEP17; in red: HER2 dot signal radius (a.i) and contrast (a.ii). (a.iii) Average count of red HER2 dots per nucleus of a cell (HER2/cell) for the three probe dilutions and the standard (full line, left axis) and the overall red HER2 dots and green CEP17 dots ratio (HER2/CEP17 ratio) (dashed line, right axis). (b) Similar presentation as in (a) for the optimization of the hybridization time.

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.

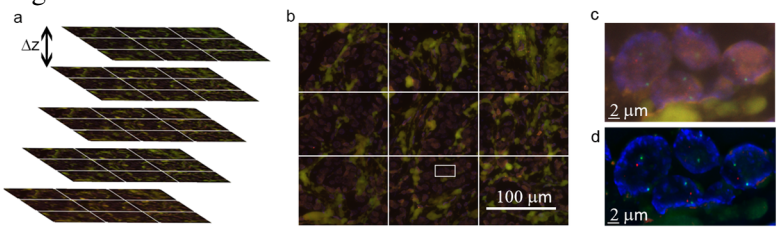

Figure 3. FISH signal presentation. (a) Evaluation of the MA-FISH signal is based on acquisition of a set of 3x3 mosaic z-stack images (b) The 9 images of one layer of the z-stack. (c) Zoom on a region of interest of (b). (d) Projected image of the 3-D region of interest in a single plane, following deconvolution of all layers of the z-stack.

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, giving potential contribution to the advancement of current trend towards personalized medicine.

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