Molecular Stamping

European Commission Research & Innovation HiPerDART

SuNS MICROARRAY AS NOVEL BIOSENSOR FOR CLINICAL DIAGNOSTICS

Pisanelli B., Dattilo D., Chetta M., Carducci F., De Ceglia G.*

Molecular Stamping srl, via Sommarive 18, 38123 Povo (TN)

** Corresponding author: bpisanelli@twof.com, tel. (+39) 0461-314138*

Since their introduction just over a decade ago, DNA microarrays have already demonstrated an enormous potential for producing valuable insight into disease states. In their many forms, these multiplex devices can provide millions of data points which can be useful for patient prognosis and diagnosis [1, 2]. Microarray fabrication and production is a very labor-intensive process and requires attention to both biological and physical resources. Furthermore, its applicability to clinical diagnostics presents important limitations, mainly due to the dramatic fluctuation of the data among different experimental repetitions.

Here we present an innovative approach for probe design and microarray fabrication, which aims to produce a higher standard of clinical microarray technology. The probes have been designed maximizing the surface density by concatamerizing oligos in the z-direction with respect to the array plane (fig.2); in this way the probes act as signal amplifiers for ultrasensitive detection of specific nucleic acids, with the improvement of the assay sensitivity, through the increasing of the signal-to-noise/signal-to-background ratios (SNR-SBR), and consequently of the assay workflow (e.g. hybridization time and sample amount).

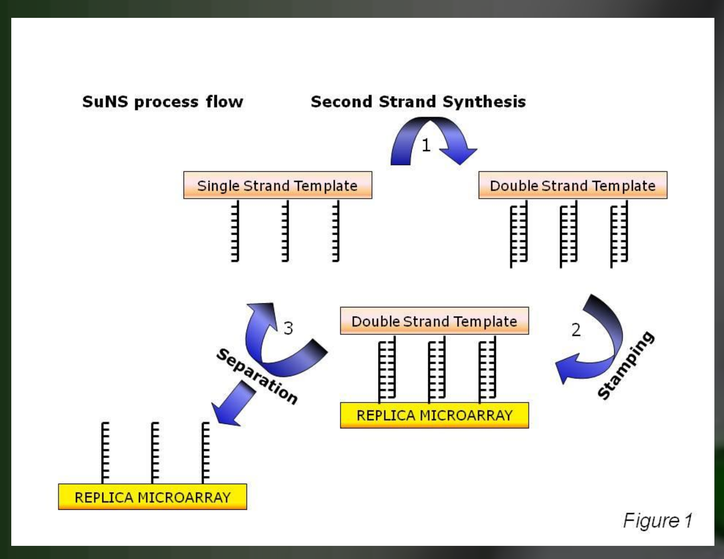
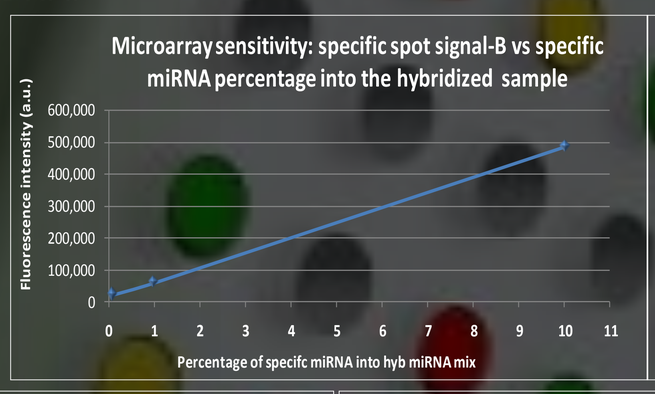


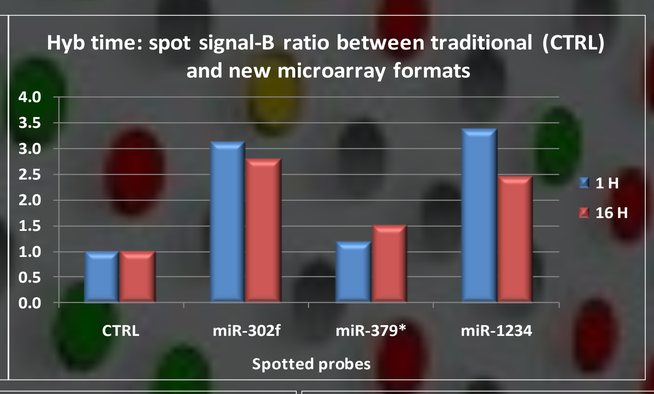
Figure 1

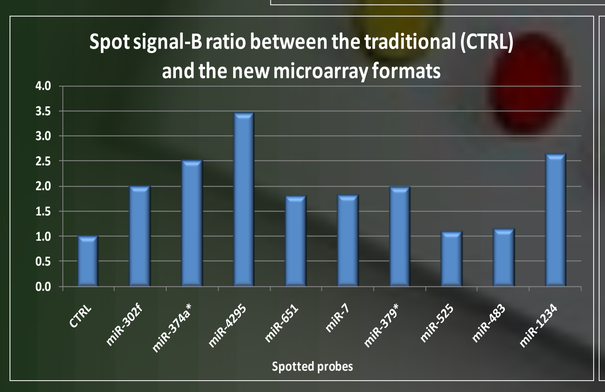
The microarray fabrication involves a novel printing technology (SuNS, Supramolecular Nano-Stamping, fig. 1), able to print single-stranded DNA features from a master microarray to new surfaces [3, 4, 5]. Using SuNS, a single microarray can be copied several times, producing its mirror images without losing high performance features in the array (feature morphology and probe uniformity) and allowing to scale up and dramatically reduce the costs of microarray fabrication, thus making DNA microarrays accessible not only to laboratory geneticists but also to health-care providers for routine diagnostics.

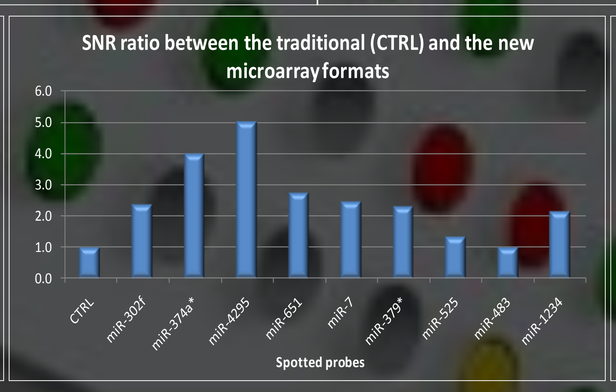
The design of the first SuNS microarray platform has been conducted to create a new tool for determining spatial and temporal patterns of microRNA expression in human tissues. As the number of identified microRNAs has increased rapidly, in conjunction with the importance of yield insight into the biological functions of these small molecules as post-transcriptional modulators of gene expression, the need for a tool that allows for the parallel detection of microRNA expression has become relevant.

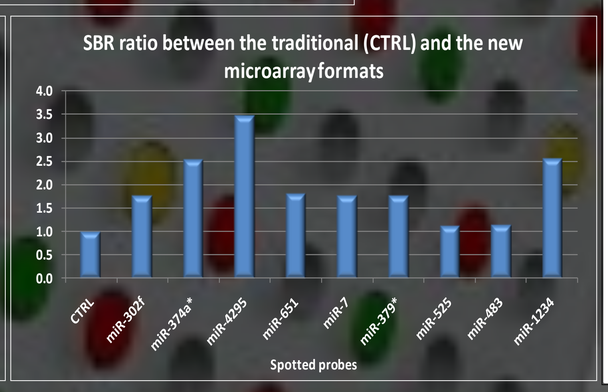
The first step of the microarray fabrication by SuNS involved the spotting of its master microarray, whose characteristics have been evaluated and will be compared with the SuNS microarray stamped from it. The spotted prototype contained 9 human microRNAs (7, 302f, 374a*, 4295, 651, 379*, 525, 483, 1234), whose features in terms of spot signal, signal-to-noise-ratio and signal-to-background ratio have been compared with those obtained from traditional spotted 60nt probes (CTRL). The comparison highlighted the improvement of all parameters for 7 out of 9 probes (especially the SNR, which increased 2 to 5 times compared to the CTRL), while microRNAs 525 and 483 showed comparable results with the traditional spotting approach. The microarray has also been tested in terms of sensitivity: each single miRNA, mixed and hybridized within a mix of 9 sequences, showed a detection limit of 0.1%,using 0.1 µM total hybridized DNA. The same result has been obtained diluting the same mix into 2 µg labeled human gDNA.











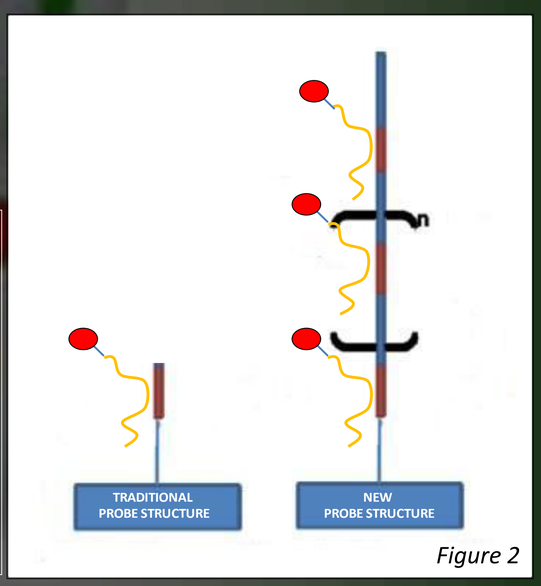


Figure 2

The SuNS approach for microarray fabrication has also been proposed within the European "***High Performance Diagnostic Array Replication Technology***" (HiPerDART) project, which aims to develop a clinical microarray platform as prognostic predictor that has clinical usefulness in the management of stage II and III of colorectal cancer, the third most common cancer worldwide after lung and breast (Eschrich et al, 2005; Barrier et al, 2006; Lin et al, 2007). To validate the microarray, the information of genomic expression in tumors will be complemented with two additional data sources: genomic expression in histologically normal colonic mucosa, resected from patients with colorectal cancer and germline polymorphisms and copy number variation.

Although these data showed only a proof of concept of the new platform, which needs to be optimized and fully validated for clinical purposes, the promising results in terms of probe features and assay sensitivity, together with a fast and robust fabrication process that aims to lower the production costs of the chips, make the SuNS microarrays well poised to become an important element in the clinical diagnostics market.

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