Advanced microfluidic packaging for molecular diagnostics

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Abstract

STMicroelectronics has teamed up with Boehringer Ingelheim microParts GmbH to develop the In-Check™ Lab-on-Chip microfluidic disposable cartridge. In-Check™ is a ST proprietary platform dedicated to the in-vitro molecular diagnostics, e.g. biological analysis based on nucleic acid targets such as DNA, RNA. Its first generation has been released to commercial applications such as virus or bacteria borne infectious diseases. The second generation described herein will further enhance the platform customer experience by means of an innovative design of its disposable component. Indeed the new format represents a substantial step forward in the system integration and easy-of-use. This advanced microfluidics package enables fully automated application protocols such as analyte and reagents input, management and disposal. In-Check™ cartridge is a plastic-based self-contained embodiment which integrates a variety of design elements and components, including liquid loading and waste reservoirs, connecting micro-channels, two sets of valves one set to tight-seal biochemical reactors and the other set for fluid routing, hydrophobic membranes, fluorescent read-out window. Such microfluidic platform married with integrated Micro Electo Mechanical System (MEMS) device and software algoritms provides a highly flexible system to run complex biological assays as RNA Reverse Transcription (RT), DNA Polimerase Chain Reaction (PCR), Probe Hybridization and Detection. This paper will present the disposable product concept, key components and functionality together with the design and manufacturing challenge.

Key words: Microfluidic, MEMS, Molecular Biology, Cartridge, Lab-on-Chip, Microarray, PCR, Bio-chip

Introduction

Since its inception in the late 70s the Ink-Jet technology has been one of the most successful innovation story, disrupting established markets and enabling new applications and businesses. In more recent years Ink-Jet technology, by far the most mature and pervasive MEMS-based product in the market, has been leading the way to a host of new microfluidic applications. Which have thrived on the large knowledge base in electronic-fluid interaction, system integration and packaging.

Among the most important microfluidic applications today, we can identify products in the Bio-Medical field, often divided into Delivery Systems (i.e. spray devices, micro/nano pumps) and Bio Chips (i.e. microarray chips, bio sensors, lab-on-chip).

Generally these products comprise a silicon-based core active device housed in a specialty microfluidic package, also referred to as cartridge.

Differently from most typical IC technologies, one underlying motif of these products is the fact that the microfluidic package is a key component of the micro-system, embedding critical features and functionalities of the application.

A classical microfluidic system partitioning includes **i/** core silicon device, **ii**/ electrical substrate, **iii**/ microfluidic cartridge. Integration, scalability and manufacturability of these three components are crucial for the commercialization of these product families.

In-Check™ is ST Lab-on-Chip platform targeting the in-vitro molecular diagnostics market.

With its first-generation product announced in 2008 this platform is now entering in the commercial phase and being employed in selected labs in Singapore and around the world.

From a system perspective the platform may be viewed as a stack of three layers wraping around a disposable product, i.e. the microfluidic cartridge. There is an application software interface with the user, an electronic control and read-out instrumentation, and application specific biochemical reagents, also refrred to as bioware.

ST has teamed up with Boehringer Ingelheim microParts GmbH to develop the In-Check™ second generation disposable product. This format

represents a substantial step forward in the system integration and easy-of-use. Indeed all the microfluidic steps in the application protocols are fully automated, thanks to the hybrid integration of the core silicon chip with a microfluidic cartridge.

In-Check™ cartridge is a plastic-based self-contained embodiment which incorporates a variety of design elements and components, including liquid loading and waste reservoirs, connecting micro-channels, two sets of valves one set to tight-seal biochemical reactors and the other set for fluid routing, hydrophobic membranes, fluorescence read-out window.

The manufacturing flow makes use of a variety of materials and processes, particularly suited for series production, such as injection molding, lamination, precision pick-and-place.

Following we will present the disposable product concept, key components and functionality together with the design and manufacturing challenges, with particular focus on the microfluidic cartridge and micro-system assembly.

Work-Flow and Functional Blocks

In-Check™ Lab-on-Chip platform is designed to address molecular diagnostics applications in a compact, portable and automated format. It combines in the same disposable cartridge nucleic acid amplification by PCR and detection by Microarray. Typical work-flow of a DNA-based diagnostic test is depicted in Fig.1. In current embodiment the first step, DNA extraction from a raw sample, is left offline. PCR reagents such as primers, enzyme, dNTP, buffer are added to the extracted sample and aliquoted to 20uL range and loaded into the PCR reaction channel. High temperature PCR protocol is applied with a typical profile of 30 to 40 cycles, 95 °C denaturation - 60 °C anneal - 70 °C extension steps each from 30 to 90sec long. High temperature, evaporation and environmental contamination considerations require this reaction to take place in a tightly sealed microreactor. The combined action of temperature and enzyme exponentially amplify the low-concentration target DNA input to level easily detectable by Microarray.

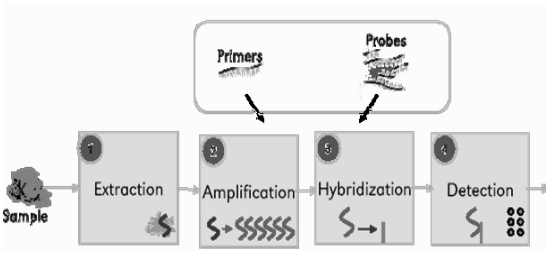

Fig. 1 Molecual diagnostic test work-flow

DNA Hybridization is indeed the subsequent step, whereas the amplified DNA product is first mixed with a hybridization buffer, then carried in to the hybridization chamber, where a spotted DNA Microarray will react by recognizing complementary perfect match in the hybridization mix. Typical Temp-Time protocol for this reaction is 50-60 °C by 30-90min. As the PCR reaction, the Hybridization reaction needs to be sealed tightly, although the conditions are not so extreme.

Before the DNA analyte detection takes place and information read-out, a washing step may be required. Washing buffer from the reservoir is flushed through the hybridization chamber to remove unbound DNA and excess fluorescent dye. Wash-to-Hybridization buffer ratio of 1-to-20 is necessary to leave a clean and readable Microarray.

Wash by-product is collected in a liquid-tight waste reservoir.

The current In-Check cartridge design enables two independent PCR reactions to run simultaneously, thus increasing the overall system complexity.

Fig.2 describes the micro fluidic schematic of the cartridge.

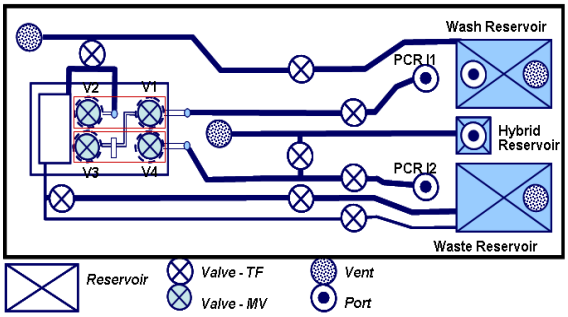

Fig.2 Cartridge fluidic schematic

Cartridge Architecture

The main product objective of the Lab-on-Chip packaging development is the design of a microfluidic disposable cartridge that integrates the described work-flow with no user intervention other than the initial manual loading of the analyte samples and buffers. Once the loading is completed the cartridge shall lock into a liquid-tight self-contained system.

The cartridge system-level architecture comprises three functional subsystems (Fig.3):

i/ MEMS Silicon Chip
ii/ Electrical Substrate
iii/ Plastic Microfluidic Embodiment

the latter being sometime itself referred to as plastic cartridge.

i/ MEMS Silicon Chip

The core of the system is a micro machined MEMS silicon chip. The active device shown in Fig.4 integrates the key functionalities of the

application. It carries two PCR reactors in the form of embedded horizontal channels coated with bio-compatible blocked dielectric layer, which can be accessed through RIE etched vertical inlets. Each reaction channel can accommodate 12uL reaction volume. Monolithically on the same chip a hybridization chamber with spotted microarray is integrated. The microarray probes are covalently grafted on an optically active Al/SiO_2 stack. Overall reaction volume is around 50uL

The device embeds Al resistor as temperature sensors and heaters, arranged in a quad layout to precisely control temperature uniformity and fast cycling during PCR.

The device is mass-produced at 8" wafer in the STMicroelectronics MEMS facility Italy.

ii/ Electrical Substrate

The MEMS chip is mounted on a PCB substrate 25mm x 75mm. PCB is made of 1 mm thick FR-4 and features 2 electrical layers cupper/ gold plated. It serves as precision mechanical support during the biochemical surface processing and microarray spotting. The chip is die-attached in a recessed cavity, opened on the backside to enhance thermal exchange, by epoxy glue cured a 140 ^{o}C. Low temperature Al wire-bonding encapsulated by a glob-top epoxy resin cured at 145^{o}C completes the sub-assembly process.

iii/ Plastic Microfluidic Embodiment

From a microfluidics/micromechanical stand-point this is the most complex subsystem.

According to the fluidic operating scheme of Fig.2 the following functional elements need to be incorporated into the cartridge design: i/ input ports, ii/ liquid reservoirs, iii/ routing micro-channels, iv/ valves, v/ vents, vi/ read-out window.

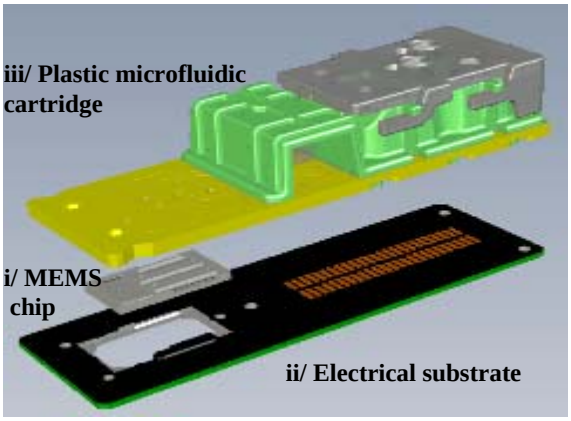

Fig.3 In-Check cartridge architecture

The disposable nature of the product and hence the cost constraint dictate for a 'passive' cartridge. E.g. no active pumping components are integrated. Indeed microfluidic actuation is provided either by capillary force or by external pressurized air supplied through hydrophobic port. Valves are micro-mechanical actuated.

The microfluidic functional elements have been either taken from BImP tool-kit or in most cases specifically developed.

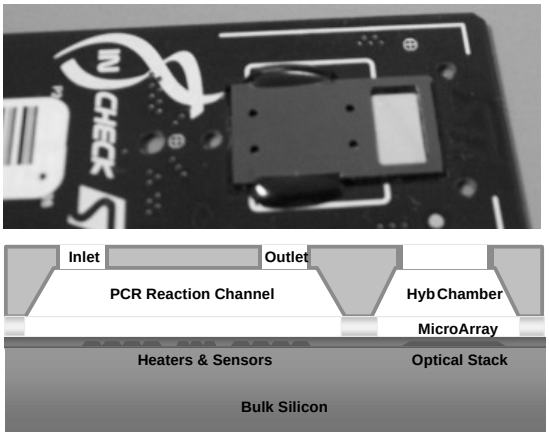

Fig.4 MEMS chip assembly and cross-section

i/ **Input Ports** are reverse conical-shape molded openings designed to match the micropipette tip used to load the reagents.

ii/ **Liquid Reservoirs**, namely hybridization, washing and waste reservoirs are filled with the reagents before the test run. Bubble-free loading is accomplished by proper shaping of inner walls. Injection molded Polycarbonate (PC) is the material of choice. After loading reservoirs are sealed by a top-lid which bears integrated hydrophobic vents for pneumatic actuation and inner pressure release.

iii/ **Routing Micro-channels** are a complex network of fluidic connections between reservoirs, input ports, and the active MEMS chip PCR and Hybridization chamber. Micro-channels are built on a single channel–plate layer. Injection-molded Cyclic-Olefin-Polymer (COP) is the material of choice. Channel dimensions can range from few tenths to few hundreds microns and are sealed by a laminated Polypropylene (PP) channel-film.

iv/ **Valves** design has proven to be a challenging one. Two different vale types have been developed (see Fig.2).

Membrane Valves (MV) are the four ones that interface directly the MEMS chip at the inlet/outlets of the PCR channels. They have to provide tight sealing during PCR cycle with temperature of 95^{o}C and inner pressure of 1.5bar (Fig.5).

Thin-film Valves (TF) are used as valves and by-passes on the micro-channels fluidic network. They are not meant to withstand high temperature or back-pressure and are integrated in the channel-plate / channel-film stack (Fig.5).

v/ **Vents ports** are used either as inner air venting port or as a pass-through air port for pneumatic liquid displacement. In both cases a

liquid-tight PTFE punched disks are welded on the PC structure (Fig.5).

vi/ **Read-out Window** is the cartridge section located directly on the MEMS chip microarray. Since the microarray is read by fluorescence emission, the main requirements are low optical absorption and extremely low auto-fluorescence. Such characteristics can be achieved by an optical grade COP material and optimized mold insert manufacturing to reduce surface roughness and material strain.

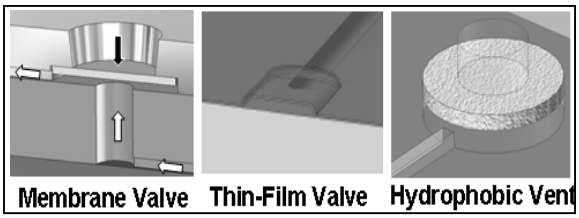

Fig.5 Microfluidics functional elements

Cartridge parts are assembled together by a combination of liquid glue, adhesive tape, rivets and mechanical snaps.

Thermal Design Considerations

Both PCR and Hybridization reactions require high temperature profile. PCR is particularly demanding because it operates up to temperature near to the water boiling point which develops high internal pressure, moreover in order to reduce overall test time fast temperature ramping up and down are desirable. The MEMS chip design allows the delivery of very high joule power density of about 20W/cm^2 that will heat up the bare die at a rate of 40 ^{o}C /sec. Cooling is accomplished by fan-forced air on the exposed chip backside, when unpackaged the rate is 8 ^{o}C /s.

On a fully assembled disposable product the cartridge adds substantial thermal mass to the MEMS chip thus modifying temperature profile and dynamic. Both steady- and transient -state have been characterized by 2-D ANSYS simulation and summary results are reported in Fig.6. Good temperature uniformity within the PCR reaction channels of less than 0.05 ^{o}C and a slower yet acceptable cooling-down rate of 4 ^{o}C /s is shown.

Another critical issue related to the operating temperature is the material mismatch. Indeed silicon thermal coefficient of expansion CTE is very low compared to the cartridge polymeric plastics, e.g. 3e-6 (Si) vs. 6.5e-5 1/^{o}C (PC). CTE mismatch in excess of 1:20 may occur that can lead to delamination. Silicon-plastic adhesion is one of the biggest challenges in hybrid packaging. Biological and chemical compatibility, manufacturability and other microfluidic requirements limit the choice of usable polymeric materials. The adhesion process must be carefully optimized. During the development phase of the In-Check cartridge a multidimensional DOE was undertaken. Liquid glue versus bi-adhesive tape were tested, plasma cleaning and surface blasting efficacy was taken into consideration. A summary of the DOE experiment is reported in Fig.7, hinting better adhesion force using liquid glue and little effect of either plasma or surface blasting.

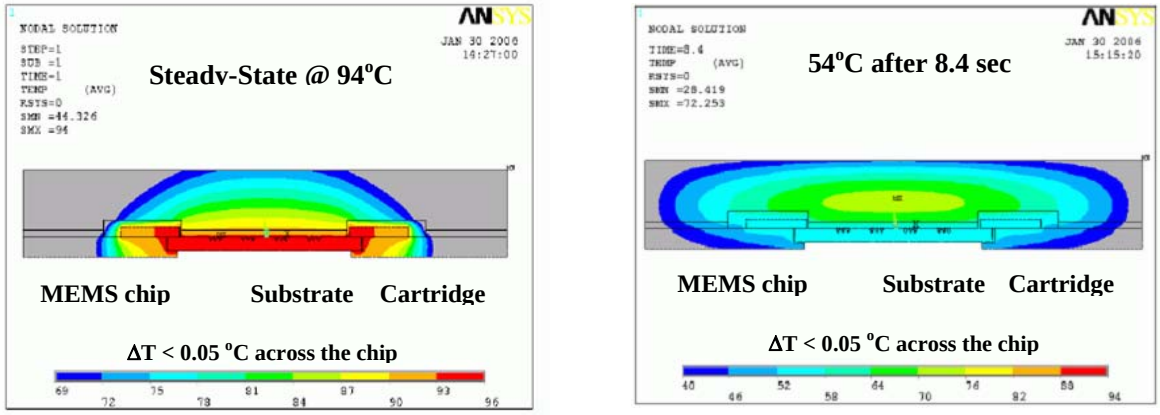

Fig.6 2-D ANSYS thermal simulation of the packaged system. High Temp steady state and cooling.

Material Compatibility

Unlikely classical IC packaging in microfluidic hybrid systems the package materials play a key role in the product functionality. Material properties selection and quality assurance is a crucial part of the development process. Extensive material compatibility verification must be undertaken during the course of the development. PCR reaction proven to be particularly sensitive. A bio-compatibility testing protocol was developed by which PCR yield from reaction tubes containing the material under testing was compared to the reference one.

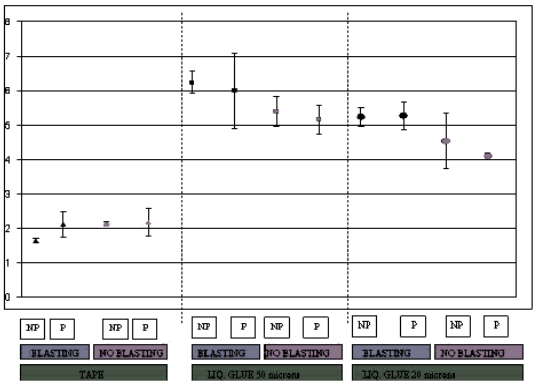

Fig.7 Adhesion force DOE

Functional Test and Design Verification

Due to complexity of the overall cartridge system, design verification of the main subsystems was carried out first. Two full cycles-of-learning were necessary to develop first functional prototypes.

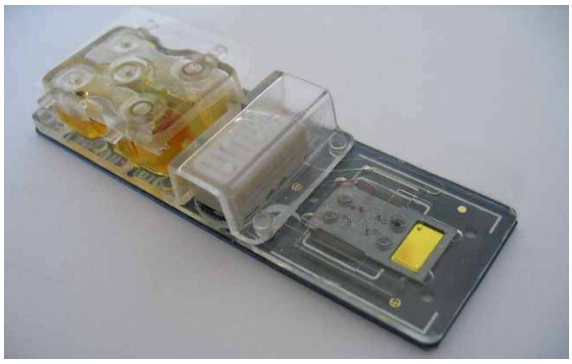

Fig.8 Assembled cartridge prototype after ink-colored microfluidic test

Fig.8 shows the cartridge status after ink-coloured reagent filling and complete microfluidic verification of reagents loading, PCR and Hybridization reaction, final washing and by-product waste collection. In Fig.9 reports a fluorescent picture of the microarray taken after a complete functional protocol, showing good detectable signals after DNA amplification and Hybridization to the specific Microarray probes and internal controls.

Conclusions

We have presented the latest advancement of the In-Check™ disposable product in the form of a self-contained easy-to-use microfluidic cartridge. Packaging and assembly technology of this hybrid silicon/plastic system are tightly linked to the product performance and its manufacturability. To-date achievements along with the faced challenges have been outlined.

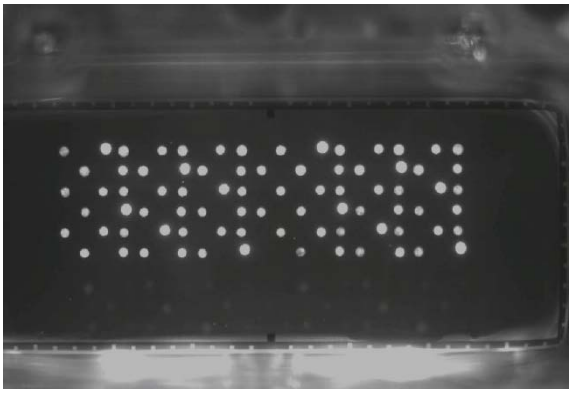

Fig. 9 Cartridge verification test. Microarray fluorescence picture after functional assay

Acknowledgements

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