SmartHEALTH: a microfluidic multisensor platform for POC cancer diagnostics

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ABSTRACT

A universal microfluidic platform as a multisensor device for cancer diagnostics, developed within the framework of the EU project SmartHEALTH [1], will be presented. Based on a standardization concept, a microfluidic platform was realized that contains various functional modules in order to allow in its final setup to run a complete diagnostic assay on a chip starting with sample preparation to a final detection via a sensor array. A twofold concept was pursued for the development and standardization: On the one hand, a standard footprint with defined areas for special functional elements was chosen, on the other hand a toolbox-approach [2] was used whereas in a first instance different functional fluidic modules were realized, evaluated and afterwards integrated into the microfluidic multisensor platform. One main characteristic of the platform is that different kind of sensors can be used with the same fluidic chip. For the read-out and fluidic control of the chip, common fluidic interfaces to the instrument were defined. This microfluidic consumable is a hybrid system consisting of a polymer component with an integrated sensor, reagent storage on chip, integrated valves and metering elements.

Keywords: lab-on-a-chip, microfluidic platform, multisensory device, cancer diagnostics

1. INTRODUCTION

A universal microfluidic platform intended for the point of care (POC) diagnosis of various cancer markers for breast, cervical, and colorectal cancer will be presented. The underlying microfluidic technology is one of the enabling technologies allowing for the realization of the overall assay, whereas different sensor technologies are the core for a sensitive and specific diagnostic assay.

Within the SmartHEALTH project, an overall diagnostic approach for the cancer diagnostics in the field of breast, cervical, and colorectal cancer covers protein as well as nucleic acid based markers. In the field of breast cancer, the targets are protein markers, for cervical cancer protein and nucleic acid based markers, and for colorectal cancer nucleic acid based markers only. In case that no amplification of nucleic acid markers via PCR is necessary, a common platform for both marker types can be used. If PCR is necessary, two different fluidic platforms will be applied.

Besides different marker types, different sensors will be evaluated for the read-out of the reactions within the presented lab-on-a-chip platforms. The detection technologies for sensing comprise electrochemical sensing [3], transmission plasmon biosensors (TPB), based on the difference in light absorption of metal nanoparticles upon analyte binding [4], and a circular disk resonator sensor (CDR, a technology based on automotive pressure sensors) [5].

The aim was the development of an integrated diagnostic device with a common interface to an instrument allowing for the integration of various kinds of sensor technologies. Therefore, a standardization concept was chosen which defined the footprint of the device, areas for sample introduction, liquid storage, sample preparation, valving, and sensing. The footprint of the devices equals the geometry of a microscopy slide, allowing for the use of existing fabrication and

automation equipment in order to realize such devices in a cost-efficient manner already in small volumes, and secondly making the evaluation at the prototyping stage much easier since equipment to test the devices is widely available.

The overall assay was subdivided into different functional elements fulfilling different fluidic needs. This includes on-chip liquid storage, fluidic interfaces, plasma generation devices, sensing areas etc. All these modules were developed independently, and after successful evaluation integrated in the overall device. For the nucleic acid based tests, a PCR chamber, sample preparation on-chip to extract the nucleic acid – in this case RNA – out of the sample, and dry reagent storage were tested.

For the microfluidic sensing platform, the design life cycle will be presented resulting finally in an integrated cartridge, realized by means of mass fabrication methods, allowing for manufacturing costs comparable to various other kinds of molecular diagnostic tests.

It is important to stress that one major focus of the standardization is to enable a cost-efficient production of consumables even with small lot sizes for different kind of application simply due to the flexible use of a common fluidic platform and the simple exchange of the biological sensors themselves.

2. MATERIAL AND METHODS

2.1 Standardization concept

For the realization of the microfluidic multisensor platform, a standardization approach was evidently necessary due to several reasons: On the first hand, by defining footprint and fixed areas for interfaces to the user and the read-out instrument, a single instrument interface can directly operate different kinds of lab-on-a-chip platforms having standard interfaces but varying in the fluidic functions.

Secondly, the standardization adopts proven standards from routine laboratory automation equipment, thus allowing the use of existing instruments for first evaluation trials. Different functional elements like valves or the complete liquid handling can be operated by one common interface.

Thirdly, standardization keeps fabrication cost and particularly capital investment cost limited, since e.g. a single injection molding tool is sufficient for several devices and available fabrication and automation infrastructure can be used.

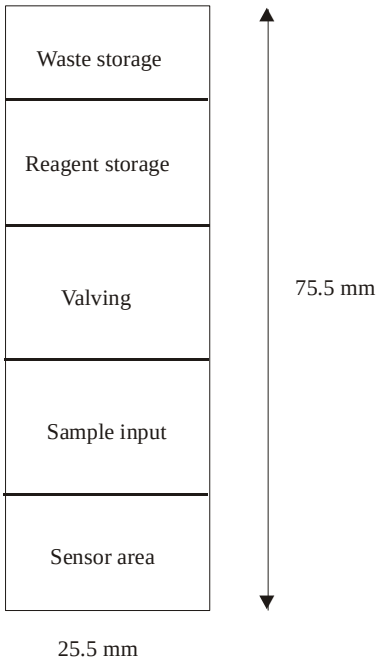

Fig. 1: General distribution of functional areas requiring interfaces to the outer world on the footprint of a microscopy

Finally, the platform should allow for various kinds of sensors, operated via a common fluidic pathway, differing in the sensor technology being applied. This makes it possible that also smaller numbers for different kind of tests and using different detection technologies can be realized at an affordable prize.

As footprint, the dimensions of a microscopy slide, 25.5 mm x 75.mm, was chosen, since laboratory automation, inspection systems and fabrication tools are directly at hand. On this footprint, the functional areas were distributed as shown in Fig. 1.

The most important interface is the sensor area, since at this point an optical, electrochemical, electrical, or magnetic interface needs to be located for the read-out. Preferably, an area at the rim of the chip should be chosen as this allows for enough space in the instrument as counter-interface, for e.g. contacts combining overlapping sensors with the instrument. From the fluidic logic scheme, the sensors are at the end of the flow path, which is directly reflected in the chip design. Furthermore, this facilitates the integration of the sensor plate and electrical contacts to the sensor.

The sample input should be as close as possible to the sensor to avoid unnecessary sample loss due to unspecific binding at the channel surface. On the other hand, if sample preparation is necessary, this will be directly placed in between sample input and the sensor area.

Valving is needed for nearly all reactions on chip and requires in any case a counterpart in the instrument. Due to the required area for the counterpart of the

on-chip valves in the instrument (e.g. stepper motors) the arrangement of the valves on chip has to be chosen carefully.

Finally, liquid storage, waste or dried stored reagents, need to be organized on chip as well. Since the modules may require an interface in the instrument to e.g. resuspend lyophilized reagents or to open diaphrams, this needs to be foreseen in the instrument as well. Furthermore, due to the required storage volumes, they influence the thickness of the chip as lateral dimensions are restricted.

Fig. 1 shows a possible distribution of the functional areas on chip. This was taken as a general footprint and was modified in non-critical areas whereas it was fixed for the critical positions like the sensor area.

2.2 Fluidic modules

The single fluidic process steps to be carried out on chip were defined and respective fluidic modules were realized and tested. This included active and passive mixing elements, membrane integration units, reaction chambers, fluidic interfaces, liquid storage, and valving on chip.

2.2.1 Mixing on chip

For the dilution of sample on-chip, two options for mixing were tested. In a first instance an array of passive diffusive mixers was realized that consisted of a double spiral set-up. The second mixing device contained an integrated stirrer bar and has to be operated via a magnetic mixer in the instrument. The passive mixer operated in sucking mode allows for a 1:1 and 1:2 mixing ratio of two fluids. The mixing ratio of the active mixer is controlled via predefining volumes either by including fixed volumes on-chip and completely emptying the chambers, controlling the amount of liquid via precise pumping, or the use of metering units on chip.

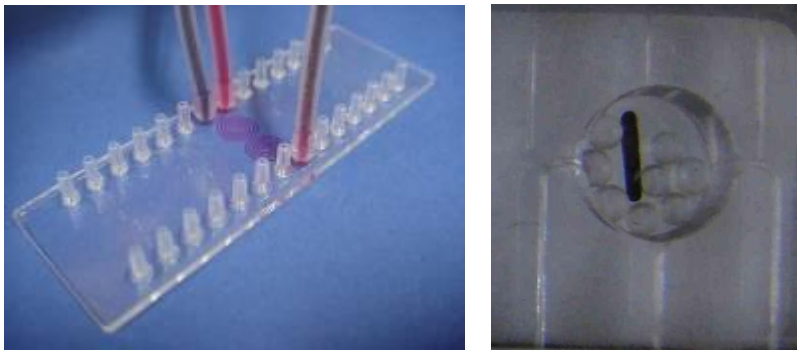

Fig. 2: Passively working mixer with a double spiral as mixing zone (left), active mixer with integrated stirrer bar.

2.2.2 Membrane unit

For separating plasma from the remaining sample, chips with integrated separation membranes were developed, see.Fig. 3. They contained input channels on the top side of the chip to fill the membrane cavity and the plasma was extracted through outlet channels on the bottom side of the chip by applying negative pressure on the outlet channels.

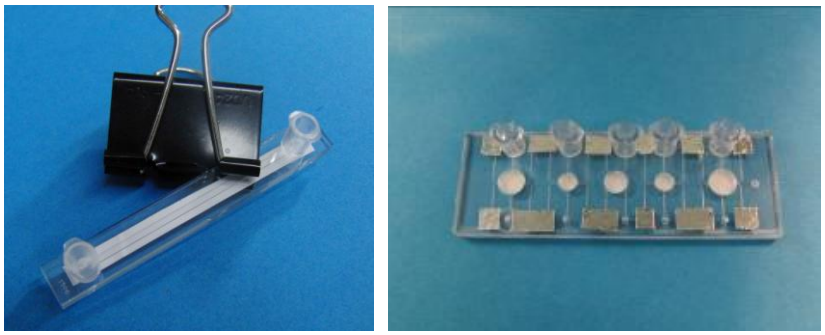

Fig. 3: Plasma separation chips with a channel design (left) and a design with round plasma separation elements (right).

2.3 Assay development platform

For the realization of the integrated chip, a flexible platform to test the different reagents being necessary to carry out the assays on-chip as well as their respective volumes is necessary.

For this approach, an assay development platform was realized allowing to apply the sample via two inlets and to use 6 different liquids to run the test. Fluidic interfaces were realized via integrated fluidic ports generated by the injection molding of the chips. The fluids were operated by applying negative pressure at the outlet port. The sensor was glued underneath a designated area via double-sided sticky tape. Waste remained on chip in a waste reservoir.

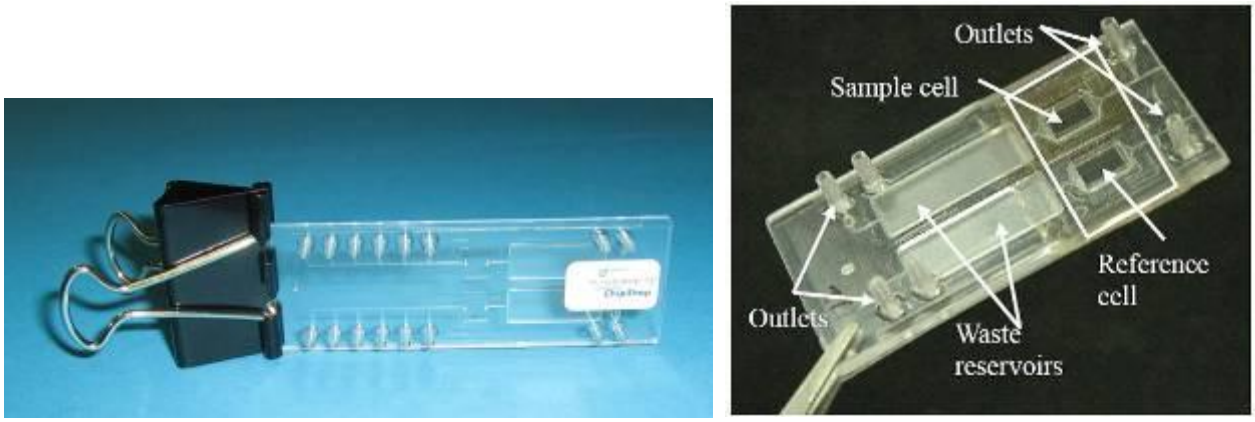

Fig. 4: Assay development platform (left) and adopted platform for the read out via the optical set-up for the TPB (transmission plasmon biosensor) read out.

2.4 Dried reagent storage

To minimize the different liquids to be externally provided, dried reagents are implemented in the cartridge. For this task, a master mix for the PCR including polymerases and HPV (human papilloma virus) primer was developed. This master mix was either pipetted into open or already closed chips and freeze dried afterwards. The freeze dried reagents were resolubilized on-chip and transferred to a conventional tube for off-chip PCR. As control, the same master mix and primer were used without this prior lyophilization step.

2.5 Sensor technologies to be implemented

As sensor technologies, in a first instance two different approaches are being used with the same fluidic platform. Firstly, electrochemical detection with electrodes deposited on a glass substrate and with immobilized biological sensor layers are integrated on the chip. Secondly, transmission plasmon biosensors are used, in which biomolecules are attached to a thin nanoparticle gold film on a glass substrate.

The glass substrates with different sensors with already immobilized biomolecules are integrated in the chip with the help of a double sided sticky tape.

2.6 Realization of the fluidic devices

All fluidic devices were realized in thermoplastic polymers. As polymers, either COC (cyclo-olefinic copolymer, tradenames “Topas” and “Zeonor”) or COP (cyclo-olefinic polymer, tradename “Zeonex”) were used. They were either structured by direct mechanical milling, using injection molded polymer wafers of different thicknesses.

Several of the devices were already realized using injection molding, which later on will be the preferred fabrication technology. The mold inserts were made either by direct precision mico-milling, see Fig. 5, or electroplated from a lithographically defined photoresist structure.

All structured chips were covered with a thin layer of a COP foil. Membranes for plasma preparation were directly integrated on chip either via gluing, ultrasonic or heat assisted welding, or a simple clamping mechanism. The sensor

chip, the electrodes or the gold film both with immobilized biosensors placed on a glass surface, was assembled with double sided sticky tape that already contained the microfluidic structures.

An important aspect of the project was the utilization of scaleable production technologies already at an early stage in order to evaluate high-volume, low cost fabrication technologies [6].

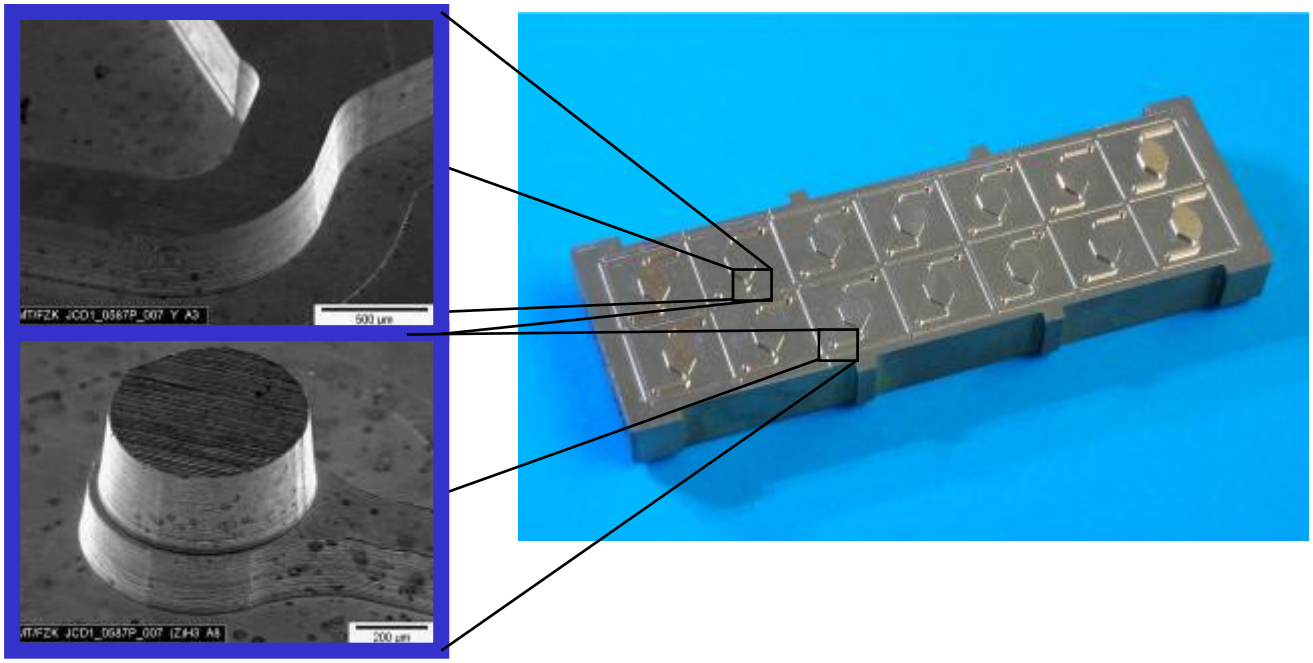

Fig. 5: Microstructured mold insert made in brass for the PCR and lyophilization evaluation platform (right) and details of the mold insert (left).

3. RESULTS

3.1 Fluidic and functional evaluation of single modules

With the individual fluidic modules, a functional evaluation was carried out. The mixing modules were evaluated under principle functional aspects and with respect to achievable mixing ratios. Both the double spiral and the active mixer performed well. The efficiency of the double spiral mixer increases with the flow rate, however only a limited mixing ratio (e.g. 1:2 or 1:3) could be achieved. For the desired 1:10 mixing ratio the active mixer demonstrated a better performance.

The plasma generation from full blood was carried out using membranes integrated in the fluidic chip. Both the channel approach with integrated membranes and the approach with a round cavity worked well. It needs to be stated that it is crucial to work with fresh blood and not to use high pressure. Sucking the sample through the membrane was successfully achieved without exceeding the pressure that is critical for the erythrocytes and would lead to breaking them. The transfer of the plasma to the next reaction vessels proved to be a critical design issue. E.g. the cavity approach worked with a larger volume underneath the membrane. However, this volume could not be fully emptied and resulted in a reduction of the yield. For the design of the integrated chip this was taken into consideration.

3.2 Assay development platform

With the help of this platform in the TPB sensor configuration, measurements could be done, the filling of the measurement chamber could be evaluated, material aspects were inspected, and the number of different reagents for the assays as well as their respective volumes could be defined.

For the measurements on-chip, a maximum of 4 different reagents were necessary, and it was decided to design the integrated chip with maximum 500 µl volume per reagent reservoir. Since COC is being used as material which is a

hydrophobic material, a surface treatment in order to hydrophilize the material improved the filling behavior of the chip and in particular in the measurement chamber.

Based on these evaluations, the integrated platform could be designed.

3.3 Dried reagents

The freeze drying of HPV (human papilloma virus) primer together with the polymerase worked well. Both methods to resolubilize the dried reagents on-chip, either active pumping and sucking of the liquid, or just heating up and waiting, worked successfully, with the resolubilization within a few seconds in case of the active fluid support or within 1.5 – 3 min depending on the lyophilization strategy.

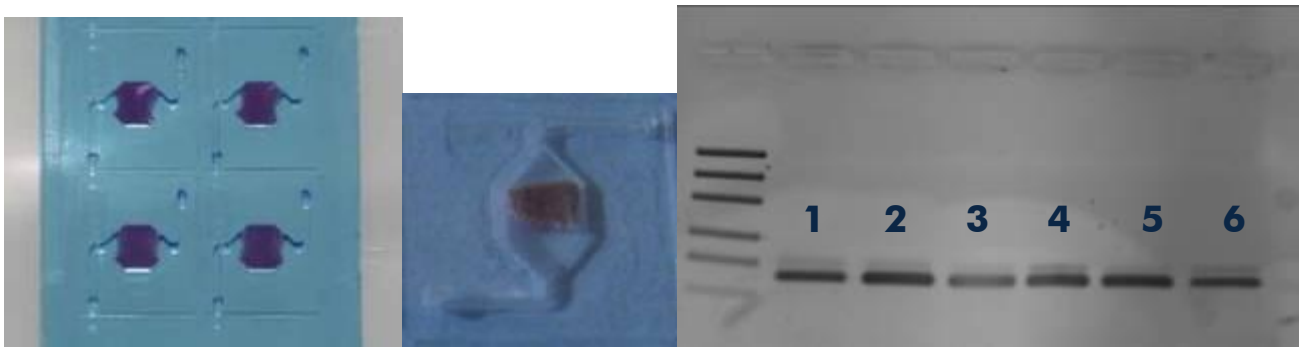

Fig. 6: Chip with liquid master mix before lyophilization (left), lyophilized reagent (middle), and results of a PCR with lyophilized HPV primer (right), whereas lane 1 and 3 are control lanes (without lyophilization of the same master mix) for lane 2 and 4.

It turned out that the lyophilization on chip had no influence on the PCR reaction itself.

3.4 Integrated chips

Based on the results with the single modules, a chip for the detection of various kinds of targets could be developed on which the different sensor technologies could be integrated. Since as well different targets as well as different sensor technologies could be matched with these chips, they were named General Detection Chips (GDC) in order to demonstrate their universal platform character.

A first General Detection Chip family allows for the integration of the EC and the TBP sensor. This chip family is used for the detection of pre-prepared sample materials like plasma, antigens or nucleic acid solutions to be tested with the sensors embedded on-chip. Liquid reagents could be successfully stored on chip, on the one hand due to the material choice (COC), on the other hand due to the tight separation of the liquids on chip via integrated valves. Nevertheless if reagents are stored on chip, they can be harmed by a humid atmosphere caused due to wet storage on-chip. This is critical for the functionality and the life time of the device.

Fig. 7 shows a member of the General Detection Chip family 1. The sample is introduced via a needle piercing a septum. Passing the valves, the sample flows over the sensor zone attached to the end of the chip. Finally it travels to the waste reservoir. After this, different reagents are floated over the sensor and the measurement is done. The reagent reservoirs are vented via piercing the septa with needles being part of the instrument.

This flow scheme proved to be well functioning. For the General Detection Chip family 2, the sample preparation needed to be implemented, and concerns with respect to the humid atmosphere arising from the liquid storage on-chip were implemented in these chips. In order to not waste the area necessary for fluidic functions for simple liquid storage and to avoid the humid atmosphere, the liquid storage was designed to be part of a second cartridge which is mounted on one chip end. The functions carried out on-chip are the following: the sample is inserted via a sample input port, sucked over the plasma preparation membrane and via a metering element, a defined volume of plasma is directed in the mixing chamber filled with a predefined volume of water. After mixing the component, depending on the sensor applied, the sensor area is flooded with buffer or with diluted sample first. After this, the different reagents are flooded over the sensors and the measurement takes place.

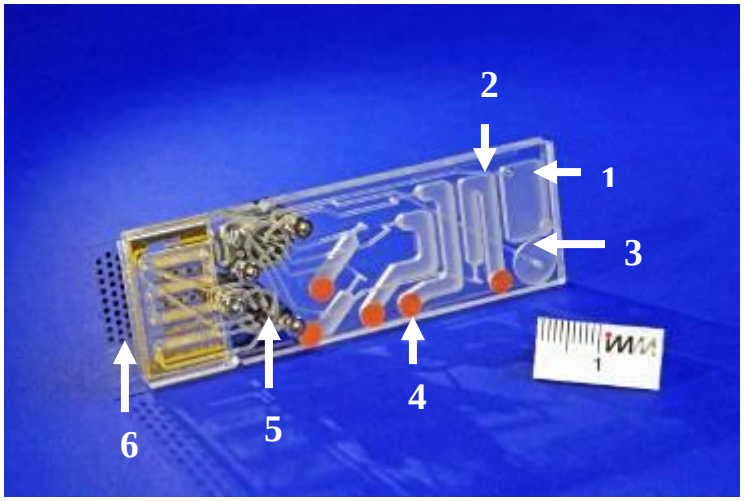

Fig. 7: Prototype of the General Detection Chip family 1 with integrated reservoirs for liquid storage (2), to be operated via piercing septa (4), a waste reservoir (1), a port to apply pressure (3), turning valves (5), and the integrated sensors on a glass chip (6).

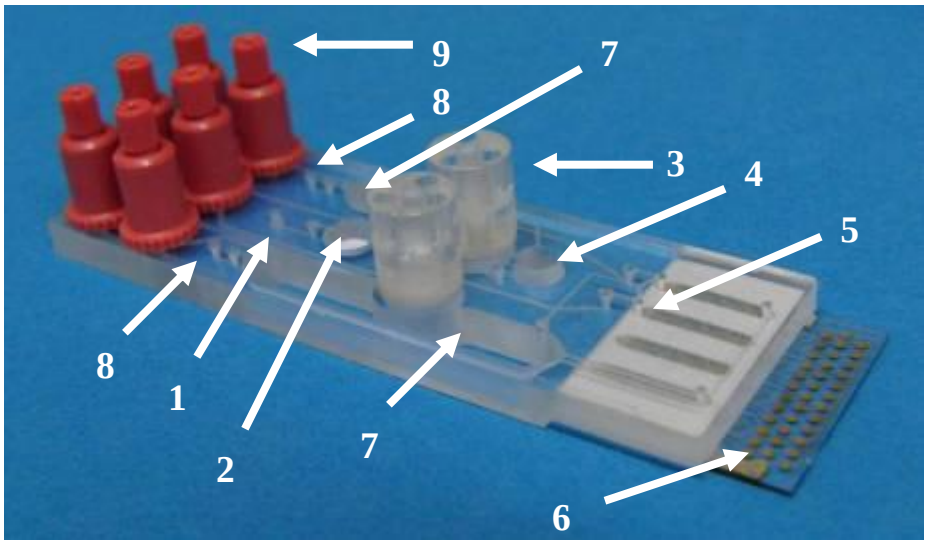

Fig. 8: Prototype of the General Detection Chip family 2 with integrated sample preparation. The chip contains a sample input (1), a plasma generation unit (2), turning valves (3), a metering and mixing chamber (4), a sensor zone (5), the glass plate with electrodes and immobilized sensors (6), waste reservoirs (7), sucking ports (8), and fluidic interfaces (9) via which the necessary reagents will be provided.

4. SUMMARY AND OUTLOOK

Within the SmartHEALTH project, a variety of individual fluidic devices have been developed and established including plasma preparation unit, mixing elements and assay development platforms, to allow for all necessary steps on-chip to realize the target fluidic system: a universal microfluidic platform to be used with various kinds of sensors. The established single fluidic modules were integrated in General Detection Chips allowing to cope not only with different kinds of samples (e.g. DNA and protein) but also with the different sensor technologies. Two General Detection Chip families have been prototyped, one with liquid storage directly on the same chips as the sensors and without sample preparation, the other with external liquid storage, and integrated plasma generation.

With the fluidic flow-scheme operating successfully, the platforms need to be further evaluated with the sensor and clinical test will be carried out with the electrochemical sensor and the respective instrument. Furthermore, for detecting

minor amounts of nucleic acids, the RNA marker for colorectal and cervical cancer, an integrated device combining several of the described functional elements and biological methods in addition with further fluidic modules for a RT-PCR step (reverse transcription) followed by a PCR will be one of the next steps within the SmartHEALTH project.

ACKNOWLEDGEMENT

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