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Evaluation of Silicon and Polymer substrates for fabrication of integrated microfluidic microsystems for DNA extraction and amplification

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***Abstract*—This paper is presenting two different alternatives for the DNA extraction and amplification that will be carried out by two competitive research projects developing bioanalytical microsystems with microfluidics. The first project will develop the microfluidics part on polymer material and the other one on silicon. The polymer approach is currently under development based on a modular microfluidic architecture aimed to simplify the process of designing and building such a microsystem device. A silicon alternative is about to start and is expected to decrease packaging costs of the microsystem allowing future manufacturability of the device.**

I. Introduction

DNA analysis nowadays requires pre-processing procedures to extract and purify the DNA from the samples taken from the patients. The aim of those pre-processing steps is to remove all protein and cellular material that might interfere with the subsequent PCR (Polymerase Chain Reaction) amplification of the DNA. It is needed to provide a yield of greater than 1μg of DNA at a concentration of 50-100ng/μl. In order to ensure high levels of reproducibility, many laboratories prefer to ensure that analysis uses standard concentrations of DNA, which necessitates arduous quantification of the DNA by measuring the optical density at 260nm or using picogreen. An efficient method of DNA extraction that produces pure, high-quality DNA is crucial to the success of the next step that is the PCR amplification and sequencing reactions. Nucleic acid extraction from biological materials has historically been and continues to be a laborious process, requiring time-consuming methods. Manual operations are labour intensive and are subject to errors in handling (a DNA extraction process requires about 20-40 minutes). The majority of kits for DNA extraction available in the market (Qiagen, Agowa, Dynal, Tepnel Biosystems, GenPoint, etc.) are supplied for manual processing of this operation. An automated robotic DNA extraction process requires a minimum of 25 minutes. Commercially available automated systems are based on automatic liquid handlers for DNA extraction (e.g. DNA/Magnetic Bead Extraction System AGE-96 - produced by Labcyte, Extragen 8C -produced by Talent s.r.l., Promega Wizard SV96 System, Roboseq 4200 PE liquid-handling robot – produced by MWG Biotech Ltd.). However these systems handle individual liquid quantities in the range of tens or hundreds of microliters, many of them use 96-well microtiter plate format, and are too expensive for many clinical laboratories to afford. The recent shift to solid-phase extractions on paramagnetic beads, silica or ion exchange resins has not only made DNA extractions more efficient, but these methods are also amenable to incorporation into microchip-based devices.

DNA extraction and amplification are usually pre-requisite steps that need to be implemented to provide a sufficient number of copies of the target gene sequences to enable visualisation using specific detection modules, and thus identification or characterisation of gene sequences. Conventional genetic analysis in clinical laboratories typically requires bench-top equipment and either manual or robotic transfer of liquids (e.g. 10-500uL) between tubes (or microwells in the case of microtitre plates) for separate steps of the process. Using conventional approaches, DNA extraction is most commonly implemented by initially rupturing the cells (cell lysis) in a buffer solution (e.g. a solution including SDS), then capture of the released DNA with either silica particles in a filter-type format, or silica-coated paramagnetic beads which can then be immobilised with a magnet. This allows all other cellular debris to be washed away, after which this "template" DNA can be eluted from the beads and resuspended in a liquid buffer ready for amplification using the polymerase chain reaction (PCR). PCR involves cycling the DNA through a series of temperatures using a programmable thermal cycler. Initially, the two strands of the template DNA duplex are separated by denaturation at ~95^{o}C, then short synthetic DNA "primer" sequences are annealed to the ends of the target section of template sequence (i.e. at a temperature usually between 50-60^{o}C), from which the Taq polymerase enzyme "zips" together the nucleotides present in the reaction mixture to build a new DNA sequence complimentary to template. By cycling the reaction through this process usually between 25 and 40 times, the number of available copies of DNA increases exponentially to yield a sufficient of DNA to enable detection and analysis. After sample preparation, conventional QC of PCR reactions involves analysis of an aliquot of each reaction using agarose gel electrophoresis, to ensure that a fragment of DNA of the expected size was amplified. A wide diversity of DNA analysis techniques is commonly used in clinical

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diagnostics laboratories, which makes any kind of standardisation extremely challenging. It would not be feasible to describe all of the techniques in the space available, and a comprehensive review of the techniques as applied to SNP analysis of the CFTR gene associated with cystic fibrosis has already been published [1].

Here we report on two separate research initiatives that are focused on the development of innovative, fully integrated, bio-microsystems for genetic analysis. These miniaturised systems will be low-cost, with embedded micro fluidics. The first research activity (Micro^{2}DNA) is developing the microfluidics part on polymer material while the second (BioMicro), will be based on silicon substrates. The two materials have very different characteristics, and while both are suitable for microfluidics applications, they require very distinct micromachining technologies. Both developments will include in their on-chip micro fluidic part sub-systems for extraction, purification and amplification steps, including temperature and flow monitoring sensors for the control of those sub-systems. The electronic detection part of the resulting microsystems is based on silicon material with integrated sensors and readouts for detection and controls. BioMicro's electronic detection is based on an innovative electronic DNA hybridisation detection sub-system using a patented optical real-time affinity biosensor.
Validation of the integrated micro-systems will be carried out with various genetic tests taylor made to each project.

Thus, the key steps that need to be made in order to enable the implementation of these reactions in an integrated lab-on-chip system as illustrated in Figure 1 are as follows:

- Storage of solutions and collection of waste solution in microreservoirs;
- Pumping of liquids using micropumps and control with microvalves;
- Release of DNA from tissue or other source (i.e. lysis of the cells)
- Mixing of liquids using micromixers;
- Precise temperature control (using microheaters and thermistors);
- Separation and purification (mirofilters and separation microcolumns);
- Detection (qualitative and/or quantitative concentration measurements).

These steps will be implemented in integrated systems fabricated in polymer or silicon (depending on the system), with consequent advantages and limitations for each type of material. Previous reports have reviewed generic aspects of microsystems based on polymer and silicon [2-6], however, the specific aspects relating to DNA extraction and amplification are the focus of the research presented here.

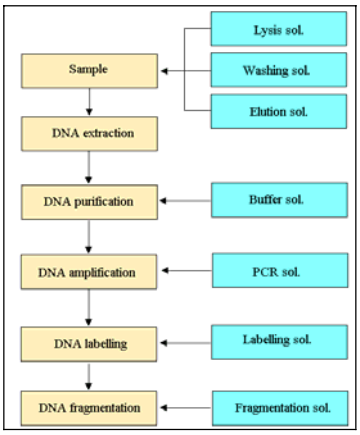


Figure 1: Schematic of the integrated microsystem for DNA extraction , purification, amplification, and fragmentation

II. DNA EXTRACTION AND AMPLIFICATION WITH MICROFLUIDICS

A. Polymer technology

While silicon and glass dominated the early years, a trend toward polymers as substrate material has been observed (for review see Zhang *et al* [7]). Plastic substrates are less expensive and easier to manipulate in mass production than silica-based substrates. Techniques such as plasma etching or reactive ion etching, laser ablation, imprinting, and injection molding are typically applicable to the fabrication of devices in plastic materials. In addition, a wide variety of plastic materials with different physical and chemical properties, can be applied to different microfluidic technologies. Recent works include laser ablation for rapid fabrication of elastomeric masters (poly(dimethylsiloxane), PDMS) for micro-contact printing, capable of patterning structures of 1μm. A method to fabricate 3D complex microstructures has also been presented, starting with a two-dimensional pattern, which is transformed in a free-standing 3D microstructure by connecting patterns on intersecting areas. 3D aligned microstructures have been fabricated by pressing a multilevel PDMS stamp on a substrate and applying a soft lithographic technique every time a new level of the stamp came into contact with the substrate surface, to produce complex patterns of aligned microstructures. Employing oxygen plasma oxidation of PDMS, irreversible bonds with glass, silicon, and other PDMS substrates can be achieved while the surface is rendered hydrophilic, capable of supporting electroosmotic flow (EOF). Hot embossing has also been employed in microchannel fabrication on PMMA. Sub-one-hundred-micrometer PMMA structures have been obtained by dry etching, imprinting of a surface-treated silicon master, and hot embossing techniques. Microchannels have finally been fabricated on stretched poly(ethylene terephthalate) (PET) films using laser ablation and parylene-C channels on polycarbonate substrates using vapor deposition to produce plastic CE chips.
Advances in polymer engineering have led recently to the development of a biochip device consisting of a plastic microfluidic chip, a printed circuit board (PCB), and a

Motorola eSensor microarray chip. The plastic chip includes a mixing unit for rare cell capture using immunomagnetic separation, a cell preconcentration/ purification/lysis/PCR unit, and a DNA microarray chamber.

Composite materials based mainly on plastic foils (especially PDMS) and different types of fibres (especially silicon carbide fibres) are being used. A draft of the biochip is presented in *Figure 2*

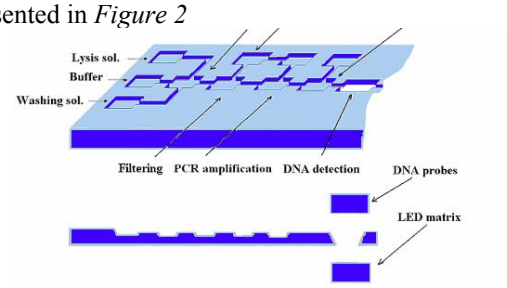


Figure 2: Microfluidic platform of DNA extraction and amplification biochip.

A modular technology for this biochip is under development in the Tyndall National Institute. A similar technology, without the use of metallized fibres, is reported in the literature [3].

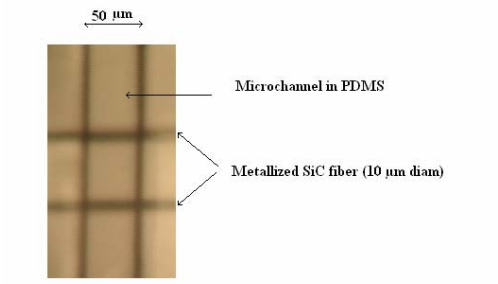
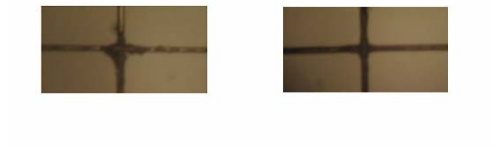


Figure 3: Microchannel in PDMS with metallized SiC fiber microelectrodes.

Silicon carbide fibre has several useful properties including very stable thermal and mechanical properties, compatibility with metallization (with both noble and non-noble type metals), compatibility with insertion in a PDMS layer with a metallized thickness between 1-400µm, and it is inert both chemically and biochemically. The interconnection of fibres has a low electrical resistance (see Figure 4a).



a) b)

Figure 4: Interconnection of fibres (a-contact resistance < 0.1 Ω; b-contact resistance > 20 M Ω).

B. Silicon Technology

Silicon technology comprises a collection of technologies used mainly for manufacture of active electronic components (micrprocessors, integrated circuits, discrete components, etc). It is a mature technology with well developed standard processes that involve the use of high temperatures, aggressive chemicals, etc., and is thus often incompatible with the manipulation of fragile bio-compounds like nucleic acids. The area on a silicon wafer needed for the fabrication of a device has a strong influence in total cost of the device when the silicon surface used is more than 1 sq. cm.

Thick film technology uses special inks to obtain conductive, resistive or dielectric layers, and cannot be used for circuit dimensions below 100 µm [8].

Photolithography using SU 8 photoresist is a subtractive technology that involves initial spinning of the polymer to create a uniform layer on a planar surface, followed by the use organic solvents to etch features on the substrate surface. It is very good for dimensions less than 100 µm, and can be used in combination with the silicon technology (or metallized glass or ceramic substrates). However, SU 8 processing is not appropriate for biochips comprising more than one layer, because the spinning of photoresist is only uniform on planar surfaces.

As a general remark, photolithographic processes do not make very efficient use of the materials, since most is removed during processing steps. Technologies used for polymer materials (moulding, hot embossing, etc.) provide the basis for increased productivities with lower costs, but these technologies are not applicable or difficult to apply to biochips having microelectrodes, actuators or multilayers with complex architectures.

C. Materials Data

The density, specific heat and thermal properties of a few materials are presented in the following table.

Material	Density (Kg/m3)	Specific heat (J/KgK	Thermal conductivity (W/mK)	Reference
Diamond	3140	520	1800	http://www.ioffe.rssi.ru/SVA/NSM/ Semicond/Diamond/thermal.html
Silicon	2320	700	148	http://www.el-cat.com/silicon_properties.htm
Silicon carbide	3100	750	120	http://www.accuratus.com/silicar.html
Polycarbonate	1200	1200	0.21	
COC	913	2680	0.18	
PDMS	970	1460	0.15	

Silicon has superior mechanical, thermal and electrical properties when compared with the plastic materials. In addition, silicon technology permits manufacture of any circuit element and actuator required for a biochip

microdevice. However, to date, manufacture of an integrated silicon biochip capable of DNA extraction, amplification, fragmentation and hybridisation has not been reported in the scientific literature. The likely reason for this may be due to the combination of technological challenges involved and the cost base of silicon substrates and processing.

III. Assay Development on Polymer and on Silicon Microsystems

The post PCR microfluidics layout of the chip will then have to be adapted to enable on-chip purification of the PCR product. On the polymer system, an electrically conductive polymer filter matrix will enable the PCR amplified product to be captured, purified and subsequently released in an appropriate buffer for detection. In contrast, a porous silicon matrix will allow the capture of PCR amplified DNA in the silicon system for the PCR product purification module. The captured target sequence will then be fragmented in the same chamber and then directed to the hybridization chamber.

Further simplification of the molecular biology protocol will be attempted in order to enhance the efficiency of the amplification step. The fragmented DNA will be labeled with Cy3 and hybridized to tiled array that will contain probes interrogating specific mutations (e.g. thalassaemia or cystic fibrosis). This will be followed by washes with solutions containing decreasing saline concentration.

The differences between the two approaches are summarized in the schematic in Figure 5 and the main issues to be addressed for post-PCR analysis are as follows:

- Different oligonucleotide immobilization approaches for the two different surface materials (polymer vs silicon)
- Comparison of detection platforms requiring DNA labeling against those not requiring DNA labeling
- Testing and evaluation of various hybridization assay configurations

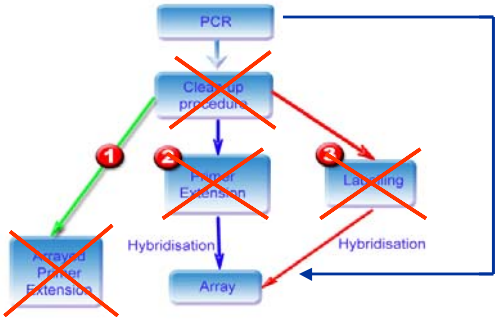


Figure 5: Differences with the Micro2DNA reaction scheme. From three different possibilities (1-3, dots labeled red), Micro^{2}DNA uses approach #2 or #3, while Biomicro will use a direct hybridization approach (blue line) thus simplifying the microfluidics part of the apparatus.

IV. Conclusion

A polymer biochip for DNA extraction and amplification based on a modular microfluidic architecture was compared with the future development of an integrated silicon-based system for genetic analysis. The main difference between the DNA extraction and amplification modules being developed in the two approaches, will be that for the one the modules will be fabricated in polymer using injection moulding or hot embossing, while for the other the modules will be fabricated on a low cost silicon substrate using silicon processing. This will result in very different technologies especially in terms of size (i.e. Si-based modules will be necessarily small to be compatible with low cost disposable devices, while polymer systems may be larger) and thermal behaviour (material properties will be very different). Integration of heaters, thermocouples, and other components will also require very different approaches for the two projects.

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