Microfluidic diagnostics: time for industry standards

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> **"Arguably, standardization of materials selection, process development and tool design across the industry would have the largest impact on the successful launch of microfluidic diagnostics into the marketplace."**

Miniaturized, or microfluidic, diagnostics seem to have become lost somewhere on the way from the laboratory to the marketplace. Microfluidics are systems that move around nanoliter or smaller amounts of fluid in channels that are micrometers or smaller in size [1]. Such systems have been used to perform fast nucleic acid amplification tests (NAATs), ELISAs and miniaturized cell culture, in short amounts of time and with some multiplexing [2,3]. Then, why can laboratory directors not just sit back, push a few buttons on their 'push button' control instruments and have a coffee?

The basics of lab-on-a-chip (LOC), or micro total analysis system (μTAS) technology have been around for more than 20 years, yet few commercial applications of these technologies have emerged [4]. Although there are many common obstacles to commercializing any medical device or diagnostic, there are some unique problems in the case of medical microfluidics utilizing closed channel architectures. These include: a lack of standardization in materials selection and process development; a lack of on-chip sample preparation technologies; a need to consult with clinicians and clinical chemists throughout assay development; and a need to conduct assay development and chip design at the same time. Arguably, standardization of materials selection, process development and tool design across the industry would have the largest impact on the successful launch of microfluidic diagnostics into the marketplace.

There is a significant disconnect between the materials and methods currently used for bench demonstrations of microfluidics, and those required to make manufacturable and quality-controlled devices for clinical use. The fabrication tools and processes required to make robust microfluidic devices for molecular detection are similar to those required for the fabrication of microelectronic chips. In fact, the size scales in microfluidic devices are usually at least an order of magnitude larger than those in state-of-the-art computer chips. Indeed, many fabulous structures can be formed using the most common rapid prototyping technology, soft lithography with poly(dimethylsiloxane) elastomer (PDMS). By investing time and effort, some traditional biology laboratories have begun implementing some small-scale assays to increase the throughput of basic research. Missing, though, are the fabrication processes that can be automated and performed in line or as in line batch processes needed for mass production.

The materials used in the laboratory to create microfluidic devices are very well suited to the requirements of work at the bench, but do not lend themselves well at all to manufacturing scale-up. In most cases, complete redesign from the materials selection forward has to be completed to take a microfluidic process from the bench to a high-volume application. Since the performance of microfluidics is so heavily dependent on the materials and chemical properties of the microchannel walls, these kinds of changes are not trivial to make [5]. Even small changes in the composition of the channel wall can make a huge difference in the success of a PCR assay, for example. Complicating the issue is the fact that not all commodity

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plastics are compatible with all assays, and manufacturers are not compelled to disclose the amount and identity of every additive in the raw material. Most notably, additives in some common laboratory plasticware have recently been demonstrated to interfere with enzymatic reactions [6]. If these effects are observable in 96-well plate volumes, then they are expected to be more pronounced in plastic reaction chambers with much larger surface-to-volume ratios.

Some of the earliest work in µTAS was done in silicon and glass, but the workhorse material of the microfluidic research laboratory has been PDMS. PDMS is an inexpensive, clear elastomeric polymer with rubbery mechanical properties (it is above its glass transition temperature) at room temperature. In the laboratory, PDMS is mixed in small batches, poured onto moulds with microscale features, and cured at moderate temperatures for minutes to hours. Cast microfluidics can be cut into shape easily for experiments with a razor blade. Open PDMS channels are closed by adhering the channel-bearing component to a glass slide or a second, flat piece of PDMS. Inlets and outlets can be formed easily using punch tools; the tacky nature of PDMS allows for tubing to be inserted directly into holes in the material with minimal leaking. The ability to prototype rapidly makes PDMS an ideal material for testing new designs. When a PDMS part does not work in the laboratory, little troubleshooting is pursued; one just starts over with a new piece.

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The mechanical properties of PDMS change depending on the ratio of curing agent to prepolymer solution [7]. Many surface modifications are not permanent on PDMS due to its relatively high chain mobility compared with polymers that are below their glass transition temperature at room temperature [8]. Dimensional stability of channels under high pressure is also a concern for assays that require the delivery of precise amounts of reagent. Both of these properties are likely to be issues in applications that require long shelf lives. The gas permeability of PDMS is sometimes seen as a benefit, as it allows for culture of live cells inside chips without additional gas exchange mechanisms. It is possible that the gas permeability and robust reversible elastic behavior desirable for valving applications will mean that PDMS will remain relevant as a material for microfluidic diagnostics, but it is not likely to be a major substrate material in most future commercial devices.

As such, commercial products are either made of glass and are thus batch processed using chemical etchants, or are made using engineering thermoplastics. The use of engineering thermoplastics has many advantages, but most of these advantages are unrealized due to a lack of focus on these materials in the research laboratory [9]. Engineering thermoplastics are rigid polymers that are below their glass transition temperature at room temperature. Thermoplastics are not crosslinked, and thus can be melted and flowed into a mold under high pressure. Injection molding (injection of the molten polymer into a mold that is then cooled), compression molding (applying melting heat and pressure to the solid polymer) and hot embossing (heating to the glass transition and pressing features into a solid part) are all potential mass production methods for thermoplastic microfluidics. Injection molding requires the highest amount of capital investment, but is also the highest throughput. Once a process is in place, injection molded plastic parts can cost pennies to make, if the volumes are high. Injection molding is not feasible in a research setting, so most thermoplastic microfluidic development is done using compression molding or hot embossing. A company does not want to make a seven-figure investment in an injection molding machine until large customers are on the horizon. Outsourcing is difficult since very few injection molding contractors are set up to press parts in a clean or cleanroom environment, which is often necessary to comply with Good Manufacturing Practice.

Regardless of which molding technique is used, chip bonding remains an issue. Molded parts have open channels and require a cover be bonded on top to close them [10]. Some applications require chips with multiple layers, each having channels that interconnect perpendicular to the surface of the chip. These all require that bonding between the same or similar plastic parts be achieved with minimal leak rates. There are several methods to bond chips, not all of them suitable for high-throughput manufacturing. These include heat-sealing with a flat piece of the same material or a different material, solvent-assisted methods, and adhesive methods. Chip sealing remains an issue since these processes can alter the surface chemistry of the channels and interfere with the performance of assays.

Of the multitude of thermoplastics available that are moldable, there are a few commodity plastics that have emerged as suitable for medical microfluidic applications. These include poly(methyl methacrylate) (or PMMA), polystyrene, polycarbonate, and a variety of cyclic polyolefin materials. Polystyrene is the material used to make most tissue culture plates, and is therefore seen as a known material for assay development. PMMA, has good optical properties for fluorescence and UV detection modes, is relatively easy to seal to itself, and is available in grades suitable for both injection and compression molding. PMMA does not have a glass transition temperature high enough to perform PCR cycling on-chip. Polycarbonate does have a high glass transition temperature, but poor optical properties for fluorescent detection. The cyclic polyolefins seem to have the best combination of optical and mechanical properties, but are significantly more costly than the other polymers mentioned.

Is it time for companies and researchers making and testing microfluidic molecular diagnostics and the instruments that run them to choose a standard set of materials and focus new development efforts on them? If it is too early in the life of the industry to impose materials standards, it is not too early to put forth standards regarding the way that microfluidic diagnostics interface with the outside world. Agreed upon specifications for the

size, shape and location of inlets and outlets would be a good first step, followed by specifications for the overall outer dimensions of a chip. Standards do already exist (set forth by the Society for Biomolecular Screening) for well plates, and microfluidic inlets and outlets can easily be placed on this standard spacing for interface with current laboratory equipment. Additionally, there are standards for the size and shape of microscope slides [11]. These standards will make excellent starting points for standardizing microfluidic platforms.

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A push toward standardization has begun to come from many corners. There are several chip-making companies who are actively pursuing the goal of creating a 'catalog' of microfluidic components that can be assembled into the desired configuration by the end users [12]. Such catalogs of parts would enable scientists in the wet laboratory to develop new assays without huge investments of time, money and expertise. These prototype devices could then be used to demonstrate utility before larger investments are made.

Lack of chip standards requires each chipmaker to also be an instrument builder. It is fairly well accepted that in the clinic, microfluidic diagnostics will all eventually consist of a chip and a 'box'. The box is the automated instrument that introduces and/or pumps fluid through the chip, heats it up and cools it down, reads out the result and tabulates the data. A standard size and shape for diagnostic microfluidics would free chip and assay designers to think about new applications, at the same time enabling automation engineers to forge ahead with new turn-key operations. As the industry currently exists, almost all box building is being done by the chipmaker, and the box then becomes an integral part of the assay. Furthermore, it represents a burden moving forward, as it has to be repaired, maintained and upgraded as the chip designs are changed.

Is it possible to set standards for an industry that is not fully formed? The microelectronics industry in the USA started setting standards in the 1970s, arguably very early in its development. International standards followed in the 1980s.

In summary, several obstacles stand in the way of wide commercial application of microfluidic technology for diagnostics. Some of these obstacles are general to all medical devices and emerging technologies, but some are particular to the way in which microfluidics grew out of the academic laboratory. First, while PDMS is a fine material for research demonstrations, movement of PDMS-based technologies to scalable materials sets should be a priority. Second, for any particular diagnostic application, assay development and chip development need to happen simultaneously. Finally, for the future of microfluidic diagnostics of all kinds, some kind of industry standardization process needs to begin. These areas might not be the sexiest areas of microfluidics research, but the products of such efforts are likely to be the most enabling, and finally lead to the realization of real-time personalized medicine.

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