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Insights on Polymers for Microfluidics Applied to Biomedical Applications

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For the past two decades, microfluidics grew from microelectronics into a promising and synergetic field at the interface of biology and chemistry wet sciences on one side and microelectronics and optics dry-engineering on the other. This technological advent is a key enabler for personalized, democratized and miniaturized medical devices, as demonstrated by the various Lab-on-Chip and Point-of-Care microfluidic systems for genomic and proteomic applications and already several companies have launched and deployed innovative sample-to-answer assays in hospital with impressive rapid and fully integrated diagnosis performances (Cepheid, Abaxis and GenePOC, among many others). Due to fundamental characteristics of fluid mechanics at the microscale, microfluidics benefits from advantages that allows operational tasks of impressive potential such as handling multiple fluids in complex and parallel manner and enhanced diffusion and kinetic performance. Using these, proof-of-concept was achieved on single-nucleotide polymorphisms, genomic free polymerase-chain-reaction (PCR) detection at attomolar level, genomic pathogen identification of antibiotic resistance stains and others microbial susceptibility theranostics microfluidic systems [1-4]. Such systems can use body fluids such as blood and diluted sputum for diagnosis of septicemia and respiratory infectious investigations, or saliva and sweat for genomic, proteomic and metabolomics assays [5-8]. Beyond medical diagnostics, microfluidic technology has also impacted engineering in many other fields: environment; energy, biology, agronomy, cosmetic, IT and military.

More recently, some of the most promising developments were reported in microfluidics applied to cellular biology with platforms such as cytometry, cell-based assays, cellular biosensors, 2D and 3D innovative culturing platforms [9]. In particular, cellular microfluidic technology is transforming biological and medical research and is especially well poised to

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accelerate the development of stem cell-derived therapies and enhanced oncology detection – for example with microfluidics systems for counting and removal of circulating-tumour-cells [10,11]. Microfluidic technology can also integrate advanced cell culture capabilities with mechanical stimulation, to create advanced cell culture systems that most accurately mimic the physico-chemical-mechanical microenvironment of the body (e.g. lung cells that are exposed to air and media while cyclically stretched to mimic breathing behavior), devices now termed Organ-on-Chip, Human-on-Chip or Disease-on-Chip [12]. First demonstrated in 2010 with a Lung-on-chip system developed by D. Ingber at the Wyss Institute (FIG. 1), these microfluidic devices have now extended into liver-on-chip and gut-on-chip models [13-17]. Such devices could represent an important opportunity for drug development in pharmaceutical research and industry, as better in-vitro models can better predict efficiency and toxicity of potential targets drastically reduce the cost and time of drug development and promote a path for the advent of new and personalized drugs. They could also have the potential to help the shortage of organs donors due to immunologic issue, by enabling the development of autologous functional, cellularized artificial organs.

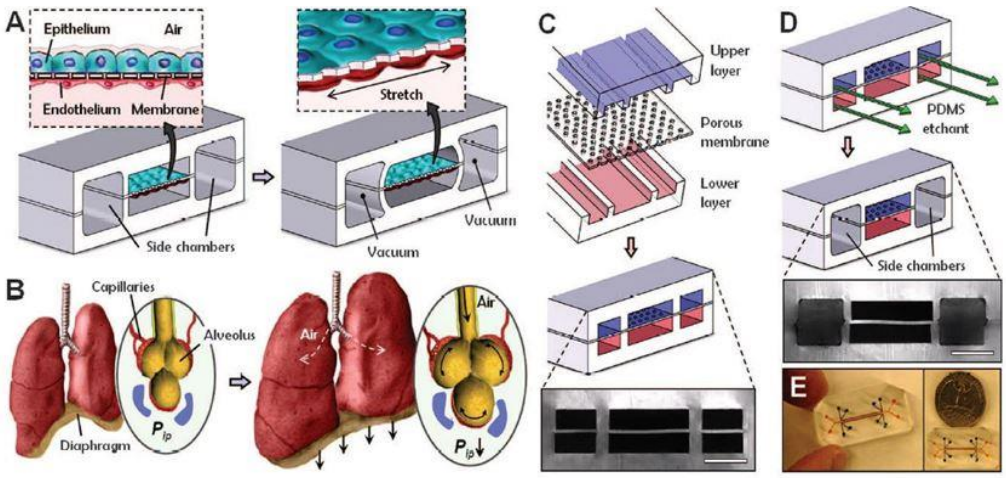

FIG. 1. **Biologically inspired design of a human breathing lung-on-a-chip microdevice. (A) The microfabricated lung uses compartmentalized PDMS microchannels to form an alveolar-capillary barrier on a thin, porous, flexible PDMS membrane. The device recreates physiological breathing movements by applying vacuum to the side chambers and causing mechanical stretching of the PDMS membrane forming the alveolar-capillary barrier. (B) During inhalation in the living lung, contraction of the diaphragm causes a reduction in intrapleural pressure, leading to distension of the alveoli and physical stretching of the alveolar-capillary interface. (C) Three PDMS layers are aligned and bonded to form two sets of three parallel microchannels separated by a 10-eMS membrane. Thmbrane containing an array of through-holes with an effective diameter of 10 µm. Scale bar, 200 µm. (D) After bonding, PDMS etchant is flowed through the side channels. Selective etching of the membrane layers in these channels produces two large side chambers to which vacuum is applied to cause mechanical stretching. Scale bar, 200 µm. (E) Images of an actual lung-on-a-chip microfluidic device.**

By essence, microfluidics is a highly multidisciplinary area, and as such, suffers from the need of multidisciplinary training and the challenges that arise from researchers' difficulties in understanding each protagonist's paradigm. More specifically, biomicrofluidics needs to accommodate the challenges of microfluidic design and assembly as well as the specific requirement of biological work. For the development of biomicrofluidics systems, these can be divided into three areas of concerns:

1. Biological constraints: The ideal biomicrofluidic material would match the requirement of high biocompatibility, low reagent adsorption (for most precise control over the chemical extracellular environment), physiologically relevant mechanical properties and ideally should be easily modified by surface treatment (e.g. for protein patterning).

2. Manufacturing constraints: Currently, key assembly steps (e.g. plasma bonding, thermal bonding) are incompatible with living conditions of cells and tissues, constraining biological protocols to be performed after full assembly of the microfluidic system. This increases the risk of bubble formation upon introduction of liquid into the microfluidic system, enhances waste and experiment length by requiring seeding of cells in fully assembled system. For bio-microfluidics, the final assembly of the system should be performed once all elements, including living organisms, are in place.

3. Accessible prototyping: Rapid prototyping of microfluidic chips must be at a low level of investment (capital cost, training, maintenance, and consumables) to promote innovation in the life sciences. Indeed, biomedical innovations and the related pre-clinical research require intense workloads for protocol validation and robust data acquisition [18,19] once the biomicrofluidic prototype is created. It is thus critical for the prototyping step to be as reproducible, rapid, and streamlined as possible.

Currently, the large majority of polymer-based microfluidic activities use the soft silicone-based elastomer polydimethylsiloxane (PDMS). However, this material has four important properties which limits its adequacy for biomicrofluidic systems: 1) evaporation, sorption, and gas permeability, 2) leaching-out of uncrosslinked oligomers, 3) hydrophobic recovery and 4) bonding methods incompatible with cell culture protocols and biomolecules [20]. The compliance issue is particularly true considering cell culture experiments that require accurate control over shear force on the cell-monolayer, and the inability to account for mechanical deformation bias data analysis and subsequent data interpretation [21]. Oxygen permeability in PDMS is three orders of magnitude higher as compared to polystyrene (the polymer used in cell culture flasks and petri dishes), and may in fact produce a hyperoxic microenvironment leading to cellular stress [22,23]. Water vapor resulting from the permeable PDMS also leads to problematic shifts on volumes, concentrations, chemical balances and changes in the medium osmolarity [24]. PDMS is also largely prone to bulk absorption of hydrophobic compounds: Regehr et al. have shown significant depleted estrogen levels in culture media, leading to inhibition of protein-1 activator [25]. Absorption not only affects fundamental cell culture condition (and physiology) on the chip, but also represent a significant hindrance for drug discovery and high-throughput screening applications, where minute amounts of drug targets are available and exact concentration of reagents reaching the cells are paramount to assess its potential. The hydrophobic recovery of PDMS over time represent a significant challenge for long-term culture, as hydrophobic surfaces have poor biocompatibility and prevent strong cellular adhesion. Finally, the bonding methods of PDMS (plasma, thermal or partly-cured) are incompatible with immersed environments and/or physiological conditions necessary for proteins, cells and tissues. As a result, this material is often at the root of many technical difficulties found when developing microfluidics systems for biological applications. Yet, the ease of use of PDMS, its rapid prototyping capabilities (2h to 4 h) and its affordable cost ($50/kg to 200/kg) still makes it the most common material for prototyping in academia.

It is thus clear that the choice of PDMS as the material for biomicrofluidic device can lead to significant complications at the initial design, development and validation stage. It is also important to bring attention to the complications associated with

later stages, when bringing prototypes to mass production. PDMS molding at low volume uses a casting method, in which a liquid monomer, mixed with a curing agent, is poured over the master mold and left to cure. This method can be later adapted for mass production only by liquid injection molding, which commonly used to create round and circular products. However, for mass production of planar elements, as found in microfluidic circuits, methods such as roll-to-roll and hot embossing and thermoplastic materials are favored. Therefore, a microfluidic device developed and validated with PDMS will need to be adapted to a new material and production method, rendering all results obtained with the PDMS interface obsolete. A change of material and assembly method will affect all surface treatments, valving, flow control and thus the overall performance of the microfluidic device, and represent a non-trivial, time-consuming and in most cases counter-productive step in R&D of microfluidic devices. Finally, it is worth noting that PDMS, and more particular its most popular formulations (Sylgard 184 and RTV-615), are not certified as medical grade material by the United States Pharmacopeial (UPS) convention (FIG. 2).

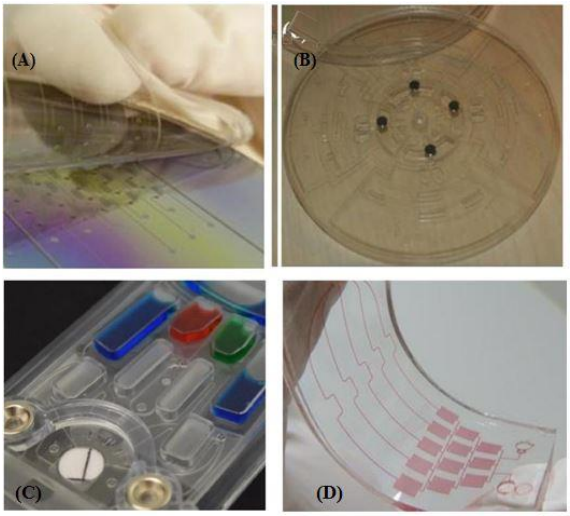

FIG. 2A. **Easy microfluidic device fabrication of Flexdym^{TM} foil from an SU–8 master mold can be obtained in a 30 s process, with an isothermal, low-pressure (<1 bar) press. (B) Flexdym centripetal Lab-on-Disc (LoD) for complete molecular assay (cellular lysing, metering, clarification, PCR amplification, amplicons digestion, and microarray hybridization functions and units were implmented)-others microbeads, parlyne-coated magnetic discs, dried DNA buffer reagents and spotted DNA array were integrated prior final assembly . (C) Hybrid Flexdym/PCO centripetal microfluidic blade for genomic colorimetric detection of *E. coli*. (D) Monolithic Flexdym/Flexdym dilutor-like microfluidic network (25 × 75 mm device) (filled with a red food colorant for ease of visualization).**

Currently, at the noticeable exception of UV-curable polyurethane-methacrylate (PUMA), polyimide and cyclic olefin polymers (Zeonex and select grades of TOPAS), few medical grade (Class VI) polymers are used for microfluidic research [26-29]. Others standard polymers, such as polystyrene (PS) and polymethylmethacrylate (PMMA) polymers are also viable alternatives, but to the best of our knowledge, use of medical material grades in biomicrofluidic applications have not been reported in literature. These materials are attractive for development of biomicrofluidic devices as they are amenable for rapid thermoforming processes such as injection molding, roll-to-roll and hot-embossing techniques. However, their implementation as replacement for PDMS in biomicrofluidic prototyping have several major drawbacks: 1) the requirement for large initial investments in equipment (>$200k), 2) the need of essential skills in polymer science and molding technologies, and 3) the use of solvent-based and others thermally and mechanically-assisted bonding approaches, which are

still incompatible with biological entities [30,31]. The rigidity of most of those polymers also renders them incompatible for use in organ-on-chip devices, which requires elasticity to stretch cells during culture, and its unwanted influence on cellular physiology is well established.

The choice of the material for biomicrofluidic devices can not only impact operation of the device and its successful implementation in biomedical laboratories, but also streamline the translation of a biomicrofluidic prototype into a commercial product. Until recently, choices were made to prioritise either ease of use (PDMS) or mass production (thermoplastics). We briefly present here our recent results on a new, transparent, and flexible Class VI thermoplastic elastomer material, Flexdym^{TM}, that can be used with fast and low-cost microfabrication methods. Indeed, prototypes can be created with a rapid 30s isothermal, low-pressure (<1 bar) and non-vacuum-assisted thermoforming process, and was demonstrated with various microfluidic prototypes including an integrated microfluidic CD-like genomic assay and hydrophilized microfluidic capillary pumps systems (FIG. 2) [32,33]. The fabrication process can be performed outside clean room facilities, and since the material is a thermoplastic, it is compatible with high-volume manufacturing technologies.

Due to its material formulation and its related mechanical and rheological properties, Flexdym^{TM} can bond to various substrates, enabling the creation of hybrid (polymer, glass or Si)/Flexdym^{TM} or monolithic Flexdym^{TM}/Flexdym^{TM} microfluidic devices. Most significantly, the bonding method (pressure-free or low-pressure, at temperatures down to room temperature), is entirely compatible with living entities (cells, proteins, etc.). The bond obtained can be temporary or permanent, and can sustain fluid pressure from 0.5 up to 7 bars depending on sealing-time and substrate materials. Assembly is also simplified by the fact that the material can be obtained directly as sheets or rolls of various thicknesses (0.45, 0.75 and 1.3 mm) (BlackHoleLab Inc., Paris, France), and doesn't require pre-compounding steps (unlike PDMS). Flexdym^{TM} can be cut and drilled with scissors, punches and laser. Compared to PDMS, Flexdym^{TM} shows very minimal sorption of rhodamine dye, and biocompatibility was confirmed by live-dead cell assay. Mechanical properties are similar to PDMS and adequate for cyclic stretching of cells. The surface can also be stably hydrophilized over long periods of time (~ 22° contact angle) [33]. The microfabrication approach of Flexdym^{TM} can be compared to the thermal molding of a "slow" adhesive polymer formulation, too slow to be bonded onto an inappropriate anti-sticking mold surface, but highly efficient to be bonded on a broad range of others polymer surfaces. It bridges the gap between PDMS and current thermoplastic candidates in terms of the benefits for research and product development, combining the advantages of those two material categories in a unique material. To the best of our knowledge, this is the first thermoplastic elastomer material that can be used for fast and reliable fabrication and assembly of microdevices while maintaining a high and stable hydrophilicity.

Current prototyping techniques and materials available in microfluidics are often not ideal for the development of biomicrofluidic systems, where physiological conditions are incompatible with assembly methods. In addition, biomicrofluidic systems are bound to grow in complexity, as reported by Potkay on the yet unsolved challenge of the artificial microfluidic lungs, which require the assembly of hundreds of microfluidic foils for the fabrication and testing of clinically-relevant prototypes [15]. The new material, Flexdym^{TM}, can integrate biological assembly in the fabrication of the biomicrofluidic devices, demonstrates similar mechanical and superior sorption and hydrophilicity properties to the more common PDMS, and can be accommodated for high-volume production. Therefore, our work provides a seamless pipeline of

microfabrication and bonding from very fast prototyping to high-throughput technologies, bringing clear benefits for microfluidic development and production, and catalyzing innovation in biomedical research.

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