The application of 3D printing to study microfluidic architecture for 'on-chip' mixing systems for SRCD and UV spectroscopy

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ABSTRACT

This paper details methodologies that have been explored for the fast proofing of on-chip architectures for Circular Dichroism techniques. Flow-cell devices fabricated from UV transparent Quartz are used for these experiments. The complexity of flow-cell production typically results in lead times of six months from order to delivery. Only at that point can the on-chip architecture be tested empirically and any required modifications determined ready for the next six month iteration phase. By using the proposed 3D printing and PDMS moulding techniques for fast proofing on-chip architectures the optimum design can be determined within a matter of hours prior to commitment to quartz chip production.

Keywords *3D printing, fast proofing, micro pumps, pdms chips*

1   INTRODUCTION

Circular dichroism (CD) is the differential absorption of left- and right-handed circularly polarized light. It is a form of spectroscopy used to determine the optical isomerism and secondary structure of molecules, and to study a wide variety of chiral materials in solution, particularly biologically important molecules such as proteins, nucleic acids, carbohydrates, lipids and drugs. It is an ideal technique to investigate protein/ligand binding interactions, such as those involved in signal transduction of normal and tumour cells, without the need for labelling or immobilizing any of the components. The benefit of carrying out such experiments using synchrotron radiation is that the light available is several orders of magnitude higher in intensity than that available using conventional CD instruments, thereby providing a much higher signal-to-noise ratio over a wide wavelength range (140-700 nm). The combination of high photon flux and small spot size at the Diamond Light Source (Didcot, Oxford, UK) will substantially reduce the measurement time for CD time-resolved experiments and the amount of chiral material required.

There is however an ever-growing need for different architectures to be used within the microfluidic flow cells that are used with these spectroscopy techniques which cater for a variety of analyte mixing and flow requirements.

Measurements are made by transmission of light through the complete cell, thus the flow cells are typically constructed from quartz or borosilicate glass to ensure minimal light absorption from the cell imaging windows, and fluidic channels of <100 µm to minimise scattering as the light passes through the fluid medium. These exacting requirements can mean that the process of manufacturing a new cell with bespoke microfluidic architecture may take up to six months. Only at this point can the cell be tested in order to observe whether the desired mixing, a notable challenge as a result of the low Reynold's number flows in micro-scale flows [1], and other flow characteristics predicted from CFD modelling have been achieved by the new design.

A number of rapid prototyping techniques have been employed previously for the construction of microfluidic systems. Ranging from casting patterns from laser printed acetate transparencies [2] to scoring polymeric surfaces with a sharp blade [3]. The most widely employed method is the patterning of silicon and glass wafers with photoresist and casting from elastomeric resin requires costly photomasks and precision lithographic mask alignment tools [4]. This process is also time consuming and requires specialist processing equpment.

For these reasons the authors decided to explore techniques that could yield more simplistic structures that could be used to mimic a final quartz cell in order to review microfluidic flow characteristics without the protracted lead time. The chips described in this paper cover devices completely produced by 3D printing, including the integration of a truly microfluidic comercially available pump, and the use of 3D printing to produce moulds and patterns to cast elastomeric microfluidic structures.

This paper will detail the development of these proposed techniques to convey their suitability for reviewing the rapid and low-cost evaluation and iteration of microfluidic channel geometries. Ultimately permitting the flow through novel microfluidic devices to be interrogated thoroughly and the design optimized prior to making the significant financial and, most crucially, time commitment to the production of a UV compatible flow cell for presentation to the synchrotron beamline.

2   CHIP DEVELOPMENT

2.1   Direct 3D Printing of Flow Chips

The first approach developed by the authors was a process route that involved 3D printing a complete flow cell chip with 3 dimensionally patterned channels contained within. The chip was produced using a commercial SLA system (Viper Si2 from 3D Systems, Cal. USA) from colourless Accura Si60 UV-cured resin. This approach required careful orientation of the chip during construction in order to minimise the supporting structures required within the channels, and then careful and laborious clearing of uncured resin from the channels using manual and ultrasonic agitation in an isopropyl alcohol (IPA) bath. An example of early chips produced this way can be seen in figure 1.
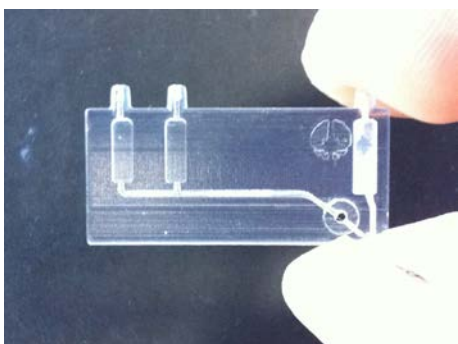

Figure 1: 3D printed chip with integrated flow channels

The channels in these chips were 300 μm diameter and contained reservoirs, ports and positioning for an UV compatible window. The small scale of the channels offers the benefit of requiring minimal support structures. The viscosity of the resin, however, poses considerable challenges for purging uncured resin from the channels.

The SLA printing process relies upon the selective curing of a photosensitive resin, which upon reaching a critical exposure energy (E_c) will solidify. The cured resin is then built up in layers to form complex 3D solid geometries. Jacob [5] demonstrated that knowledge of this and the penetration depth (D_p) of the incident light for the resin enables precise control of the solid layer depth (critical depth C_d) and subsequently the layer thickness through the expression:

$$C_d = D_p\ln(E_{max}/E_c)$$

The photopolymerisation reaction will however continue to partially occur within the resin in regions exposed to energies below E_c. These areas of partial curing produce a 'print-through' or 'overcure' effect which is typically equivalent to $3 \times C_d$, or 3 layers at z build resolution. This leads to poor control of the curing of undercut surface geometries (Figure 2) further impacting on

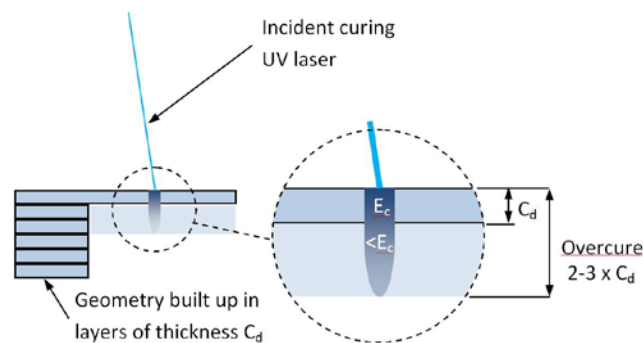the potential for reducing the scale of directly 3D printed chips with embedded channels.

Figure 2: 'print-through' in regions of undercut surfaces leads to poor geometric control of internal channel features

The standard commercial high-resolution settings of the Viper Si2 SLA process is capable of z layer thickness of 50 μm. Engineers at Aston University have been successful in producing features with a z step resolution of 25 μm, but the overcure effect of the laser still subsequently limits the enclosed channel geometries to a minimum vertical channel height of > 70 μm.

In addition to the simple flow-channel designs, others were fabricated via the same process but with integrated pumps. A review paper [6] concluded how there were no truly microfluidic reliable on-chip pumps available. To address this the authors worked with Kikuchi (Japan) to integrate their comercially available 'stand alone' microfluidic pump (figure 3) into a 3D printed microfluidic chip. The Kikuchi M07ST-ES2-10 piezoelectric diaphragm pump has a 250 nl dead volume, can be controlled to operate between 1 and 350 HZ and the manufacturers claim they are capable of a flow rate of up to 3 ml/min [7].

Figure 3: The 7 x 7 mm micro pumps

By printing the manifold for the pump directly onto the chip there are no additional dead volumes typically produced when using macro, off-chip pumps with fluidic connectors and pipework. Figure 4 shows one of the 3D printed flow chips with its integrated micro pump as it pumps a blue-dye water solution from a reservoir.
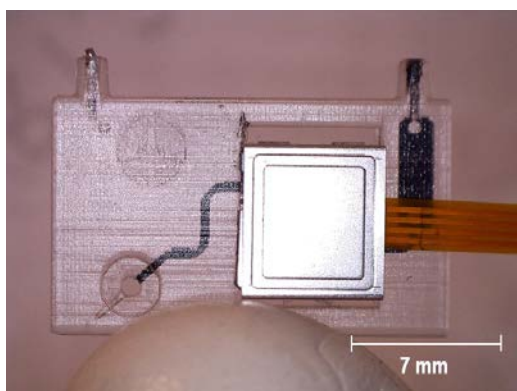
Figure 4: chip with integrated micro pump

Characterization of these pumps is ongoing along with the development of other drive technologies, including smartphone applications to control them. Additional research is also being carried out into surface coatings and treatments that can be applied to improve the wettability of the 3D printed resins in order to negate the requirement for elevated hydraulic pressures to initiate flow.

2.2   Modular 3D printed flow elements

For completeness an approach is being explored to 3D print modules with shaped cavities for 3D mixing (figure 5) and jigs to hold flat quartz windows (figure 6) through which the analyte may be imaged. Thereby offering a modular solution to prototyping the flow channel geometry whilst facilitating preliminary testing on the synchrotron beamline. It is expected that this could be an approach to additionally reduce lead times for producing working beamline compatible devices for CD development tests.

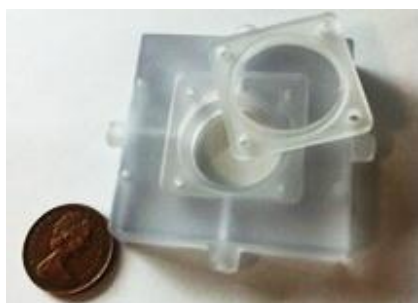Figure 5: 3D printed chips with shaped internal cavities

Figure 6: 3D printed frame for flat Quartz windows

2.3   Moulding from a 3D printed pattern

An approach used to great effect for microfluidic device patterning is that described by Bonyar *et al* [8,9] and Cormina *et al* [10] wherein an open mould is 3D printed with embossed fine channel and flow features (figure 7 shows a CAD image of a typical mould design). This mould is then used to cast the microfluidic channels from a conformal elastomer such as PDMS (polydimethylsiloxane) and the channels are then closed by clamping or bonding to a rigid substrate. This methodology is reported as benefitting from a cycle time of the order of 3-5 hours, including the 3D printing process [8]. This approach also takes advantage of the versatility and precision afforded by 3D printing with features as little as a single layer in height (approx. 25 μm), and enables the investigation of finer channel structures without the associated difficulties of clearing uncured material.

Beyond this a single mould can be used to produce numerous copies of a chip design with different surface treatments to compare flow behaviours, and permits the cost-effective production of single-use devices in order to minimise potential complications arising from sample contamination.

Figure 7: 3D CAD of a printed mould

Figure 8: (left) a mould housing was used to position the pattern, (middle) the pattern for the moulding and (right) the final PDMS cast flow-chip prior to trimming

Further advantages of this process lie in the material properties of PDMS. The low viscosity in its liquid state enables it to be used to replicate micro-scale geometries very precisely, it is optically clear, and it can be permanently bonded to glass through plasma or corona discharge treatment [11,12]. Finally the PDMS surface can be temporarily altered from its native hydrophobic state to a more hydrophilic nature through oxygen plasma treatment [ref 11,13,14].

The authors used this process to develop a meso-scale flow cell, with a square 500 × 500 μm profile serpentine channel. The cast chip was clamped between two machined polycarbonate jigs complete with hydraulic connection points to enable fluid to be introduced to the chip.

3   MIXING CAPABILITIES

The purpose of the chip was to demonstrate the persistence of laminar flow conditions through the gradual mixing of two concurrent fluid flows. The chip was manufactured from Sylgard 184™ (Dow Corning, USA) PDMS elastomer resin which was vacuum degassed prior to pouring and cured for 60 minutes at 100 ^{o}C prior to release. The initial flow channel was designed in a serpentine pattern with each waveform being 500 x 500 μm x 36 mm in order to mitigate the effects of the innate hydrophobicity of surface of the PDMS.

The flow chip inlets were connected using 1 mm PVC tubing to two 5 ml syringes (Becton Dickinson, USA) each loaded with a dyed distilled water solution. Pigments of yellow and blue were selected in order to provide a clear visual indication when mixing of the flow had started and completed. The outlet port was similarly connected using 1 mm PVC tubing to waste. To ensure balanced flow the two syringes were loaded with the same volume of fluid, the connective tubes were of identical length and the contents of the syringes were dispensed same rate using a dual syringe pump (KD Scientific 789210). The subsequent flow was imaged at 30 fps using a 16 MP camera (Sony NEX 5n) camera equipped with a 10 × magnification macro lens.

The initial trials using PDMS flow-chips have proven promising. The process allowed different channel designs to be investigated along with different flow rates. Figure 11 demonstrates an example where the fluids pass through the channels at a combined flow rate of (a) 1.5 ml/min without mixing and a flow rate of (b) 0.15 ml/min results yields a complete mixture of the fluids at exit.
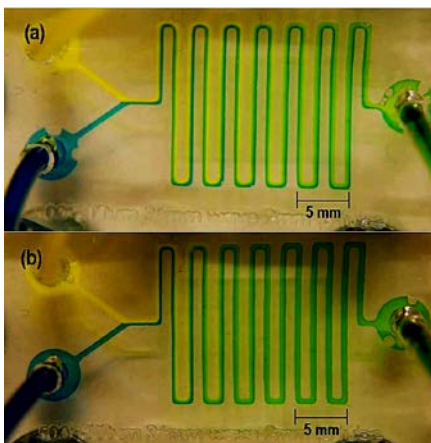

Figure 11: Mixing study within PDMS chip showing flows of (a) 1.5 ml/min and unmixed at exit and (b) 0.15 ml/min and fully mixed at exit

4   CONCLUSIONS

The work described here shows great promise in developing techniques for the fast-proofing of on-chip geometries prior to embarking on fabrication methods that are expensive in both time and financial cost. Although the directly-printed microfluidic channel methods still require much improvement the PDMS technique described offers considerable advantages. The channel geometries produced in this way are limited in comparison to direct printing as they are effectively two dimensional unless multiple layers of patterned PDMS features are employed. In this way they have much in common with those photolithographically produced from structural resists [4]. This method is currently unable to compete with photolithographic methods in terms of feature and channel resolution. However, the fabrication routes and a standardised manifold arrangement has been determined which can allow the design, fabrication, evaluation and iteration of new on-chip architecture in hours for what previously would have taken many months.

REFERENCES

[1] L. Capretto, W. Cheng, M. Hill, and X. Zhang, Top. Curr. Chem. 304, 27–68, 2011.

[2] N. Bao, Q. Zhang, J-J. Xu, and H-Y Chen, Journal of Chromatography A. 1089, 270-275, 2005.

[3] J. Do, J-Y. Zhang, and C. Klapperich, Robotics and Computer-Integrated Manufacturing, 27, 245-248, 2011.

[4] G. Whitesides, and A. Stroock, Physics Today 54(6), 42, 2001.

[5] P. Jacobs, Rapid Prototyping & Manufacturing: Fundamentals of Stereolithography, Society of Manufacturing Engineers, 1992.

[6] N. Nam-Trung, Fluids Eng 124(2), 384-392, 2002.

[7] Kikuchi pump manual supplied with devices

[8] A. Bonyár, H. Sántha, B. Ring, M. Varga, J. Kovács, and G. Harsányi, Procedia Engineering, 5, 291–294, 2010.

[9] A. Bonyar, H. Santha, M. Varga, B. Ring, A. Vitez, and G. Harsanyi, Int. J. Mater. Form. 7, 819-196, 2014.

[10] G. Cormina, A. Suska, and D. Fillipini, Lab Chip 14, 424-430, 2014.

[11] Y. Berdichevsky, J. Khandurina, A. Guttman, and Y-H Lo, Sensor Actuator B. 97, 402–408, 2004.

[12] K. Haubert, T. Drier, and D. Beebe, Lab Chip 6, 1548–1549, 2006.

[13] D. Bodhas and C. Khan-Malek, Sensors and Actuators B. 123, 368-373, 2007.

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