HAR Microfluidic Device to Concentrate Microalgae

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Summary

In this project the efforts were geared towards building and testing a high aspect ratio (HAR) microfluidic device to concentrate microalgae in a continuous flow microfluidic chip. This project was made possible through funding from USDA, SBIR phase-I grant to Phycal, Inc.

Algae are a promising candidate for large-scale production of biofuels, an important source of renewable energy. However, a significant portion (20-40%) of the cost in the traditional processing comes from concentrating (dewatering) the algae from the dilute growing concentrations (~0.1 wt%). A continuous flow microfluidic dewatering chip has been designed using an innovative, patented lateral displacement array (LDA) design. The LDA consists of densely packed vertical posts that 'bump' particles of certain size towards the center of the chip thereby concentrating them at the center. Chips with such LDAs consisting of round or triangular posts, patterned by X-ray lithography into SUEX or molded by hot embossing into polycarbonate were used to fabricate microfluidic LDA devices. Initial microfluidic tests show that both taller posts and triangular shape result in lower flow resistance. Also using lower flow rate requires lower pump pressure to push the algae solution through the LDAs.

Introduction

Microalgae are a potential candidate for large-scale production of biofuels, an important source of renewable energy [1]. A significant portion (20-40%) of the cost in the traditional processing comes from concentrating (dewatering) the algae from the dilute concentrations (~0.1 wt%) at which they grow [2]. A continuous flow microfluidic dewatering chip has been suggested using an innovative, patented lateral displacement array (LDA) design [3]. The post array in these LDAs consists of a channel filled with specifically arranged and densely packed vertical posts which are displaced by ~0.5µm in successive rows (Fig.1). This arrangement is such that particles above a certain critical diameter flowing through the channel is bumped to one side of channel in the direction the posts are being displaced (Fig. 1). When using a mirror image along the center line, particles are displaced towards the chip center from both sides and are concentrated there, Fig.1b [4-8]. Designing an outlet at the center end of the LDA will allow extraction of pre-concentrated algae solution. Simulation of this design shows that these arrays can be run continuously without getting clogged.

In a joint effort, partners Phycal, Princeton and LSU-CAMD evaluated LIGA based fabrication techniques to build these arrays of high aspect ratio (HAR) posts with moderate cost, high precision and tight tolerances in polymer materials [9]. In this summary we will discuss the fabrication of high aspect ratio microfluidic LDA chips, integrating them to build a device, and characterization of the device in fluidic experiments using both deionized (DI) water and dilute algae solution.

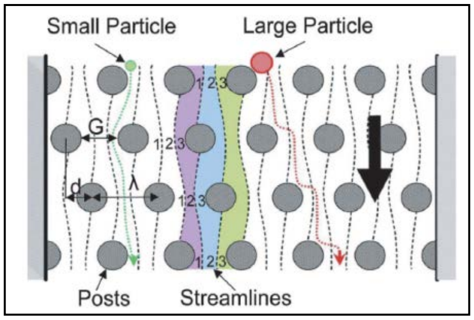


Fig. 1a: Schematic illustration of the ‘bumping’ of particles towards the center of LDA device [8].

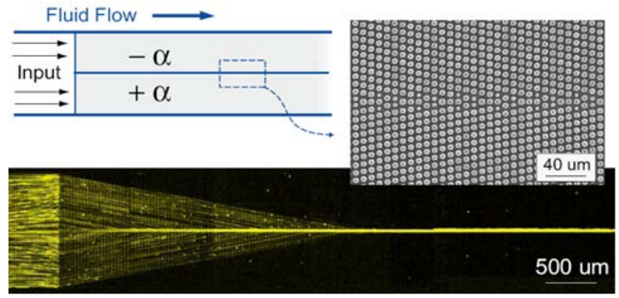
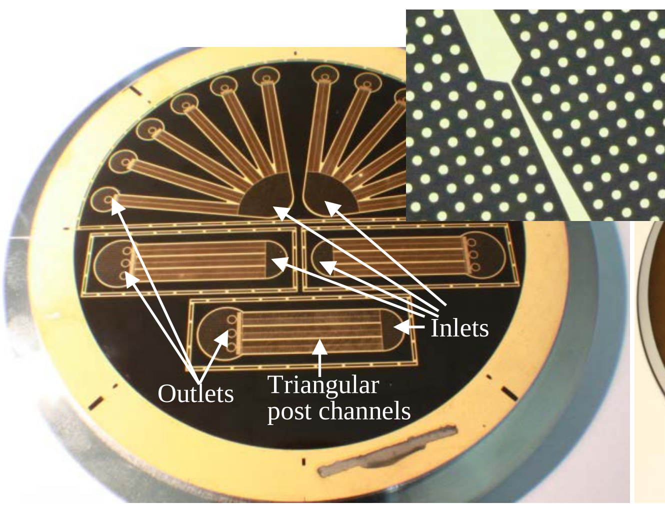


Fig. 1b: Simulation picture illustrating the operation [9] (courtesy: SBIR proposal Phycal).

Fabrication and Assembly

Fabrication of the LDA devices employed two different techniques. In one a nickel mold insert was used to mold chips and in the other approach, chips were fabricated by direct lithography of SUEX resist using graphite X-ray mask, Fig.2a. The X-ray mask also shows the layout of the chip, which has 3 different types of LDA channels, in first (top left and right) single input feeds 6 LDAs and each of them have separate output, in second (below first left and right) single input feeds 3 LDAs and each of them have separate outputs. Both of these have round posts of diameter 15 µm which are separated by 10 µm gaps. Third design is at the bottom of the mask and is similar to the previous one except that the LDA posts are triangular in shape with 15µm long sides of the equilateral triangles and 10 µm gaps. It is known that sharp corners will get rounded and these can be accounted for in the design along with a process bias of 1-2µm. Due to corner rounding the triangle sides are ~ 13µm leaving larger gaps. Schematic of a LDA/ single array subunit design is shown in Fig. 3.



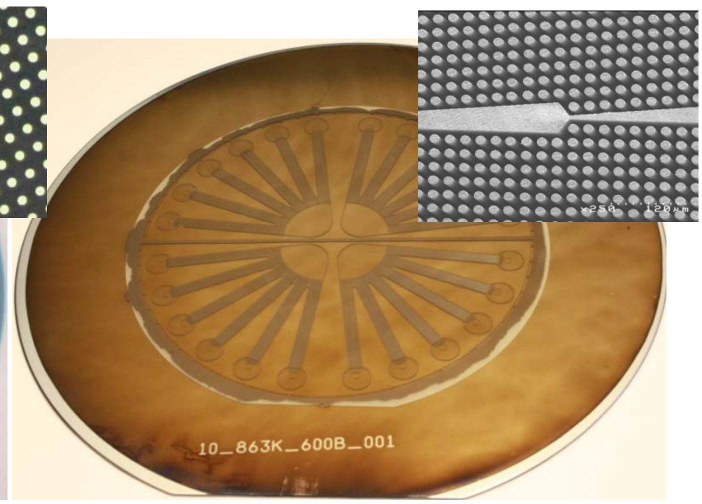


Fig. 2a: X-ray mask showing the layout of the chip[11] and inset shows higher mag view of gold plated patterned SU-8

Fig. 2b: 6” Si wafer with patterned PMMA as mold plating template and inset shows higher mag view of PMMA structures (Wafers courtesy of Microworks and KIT)

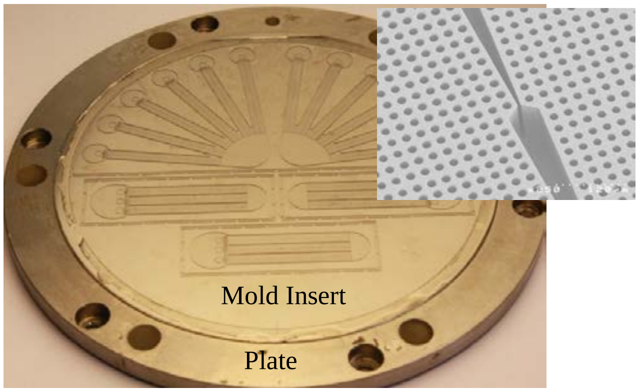


Fig. 2c: Ni mold insert with inverse LDA pattern and inset shows details of portion of single array subunit

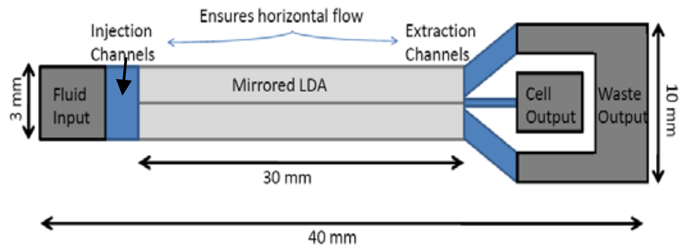


Fig. 3: Single array subunit, design [9] , (design from SBIR proposal, Phycal)

Two X-ray masks with opposite tone were fabricated. One for making a mold insert using positive resist (PMMA) coated onto a standard Si wafer substrate (Fig. 2b), and the other to expose negative resist, SUEX. X-ray masks were fabricated by optical lithography, patterning 40-50µm thick SU-8 resist on graphite substrate, and filling the developed areas by electroplating ~30um thick gold, Fig.2a. The robust mold insert, Fig. 2c, was prepared by using Si wafer, with conductive layer (TiO_x), and 100µm thick PMMA layer patterned by X-ray lithography followed by thick Ni plating (> 6mm, done at Applied Microswiss) [9]. The electroplated Ni mold insert was machined and polished at the back to make it flat/parallel to the Si wafer, followed by removal of Si. Then it was machined at the edges for mounting on to the platen of the Hex-2 molding machine, Fig. 2c. The mold insert was chemical-mechanical polished (CMP) to improve the rough front/molding surface (resulted due to plating into conductive, rough TiOx seed layer) and also to remove the burrs at the edge of the holes. Fig. 4a shows the rough surface as well as rough edges of holes still filled with PMMA before CMP. The inset shows smooth surfaces after CMP but still some rough edges.

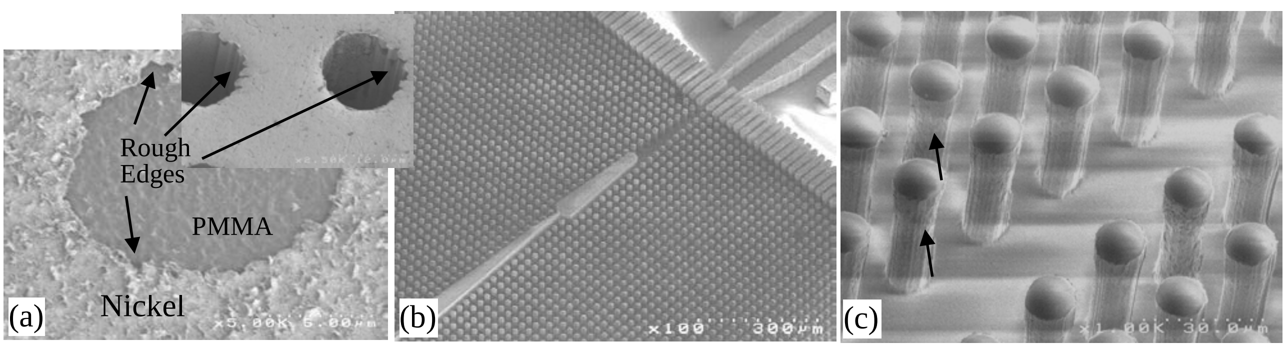


Fig.4: (a) Mold insert hole with rough edge before CMP, inset shown is after CMP (b) molded PC chip posts & exit channels (c) magnified view of posts showing narrowing of posts column due to pullout effect [11]

These rough edges made the demolding extremely difficult and even though we were able to mold/demold the chips into polycarbonate (PC) without almost any loss of posts, Fig. 4b, there were still issues as detailed in Fig. 4c showing rounding of the top of post due to reflow (resulting from extreme demolding conditions) and thinning of the posts due to pullout effect. Pullout was also apparent from the fact that the depth of the holes in mold insert was measured to ~70 µm, while the height of the molded, thinner posts was measured to be ~100 µm. The heights of the 50 µm posts as well as wider channel structures on the molded chips was about 70 µm and thus comparable to the depths in the mold insert. The taller small posts can result in sealing issues such as bending of the posts or leakage. It is expected to minimize or even eliminate these molding issues by removing the burrs at the edges employing an electropolishing approach for HAR structures recently reported by S. Kissling et. al. [12].

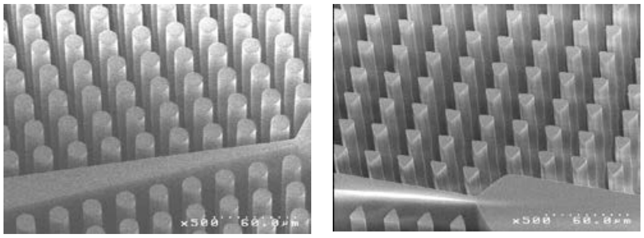


Fig.5: LDA channels with (left) triangular and (right) circular posts [11]

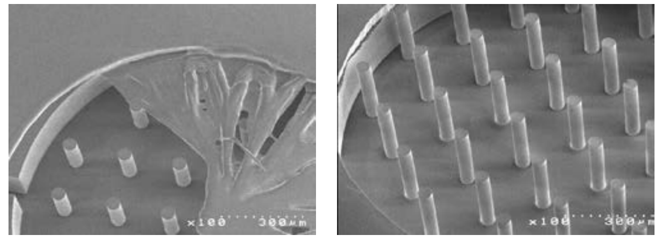


Fig.6 Inlet and outlet holes of patterned SUEX cover (left) with obstruction, (right) clean [11]

The processing parameters and fabrication details for direct X-ray lithography in SUEX resist are described in more detail elsewhere [9,10,14]. In brief, first a 100 µm thick SUEX sheet on a PET 'substrate' was UV flood exposed (or patterned with a mask) and subsequently a 100-350 µm thick resist sheet was laminated onto it. A PLB (post lamination bake) cross-linked the substrate and ensured tight sealing of a cover PET on the resist. Next, patterning by X-ray lithography and PEB (post exposure bake) followed along with development. Upon process completion an optional cover sheet (100µm SUEX) was laminated onto the structured chips and optionally patterned by aligned UV-lithography to define inlet and outlet holes, Fig. 3. We had fabricated chips with heights of the structures ranging from ~100 – 350 µm. An SEM micrograph with typical post structures is shown in Fig. 5. We had observed the issue of obstruction/skin in the inlet/outlet holes in the patterned cover, Fig. 6a. These were addressed and solved by changing the processing steps to fabricate the chips with cover. Fig. 6b shows a typical inlet/outlet in such chips. In these chips we patterned the substrate for inlet/ outlet holes followed by bonding of second SUEX layer and its patterning and lastly applying the cover and flood exposed it without development. Some of the fabrication efforts/results are shown in Figs. 7 and 8. The completed LDA chip with the cover is shown in Fig. 7a; and Figs 7b & c show the close up of the LDA at the entry (Fig. 7b) and exit (Fig. 7c) showing 15/50µm diameter posts along with inlet channels at the entry for pre-filtering and extraction channels at the exit. Fig. 8a exhibits the 350µm tall posts of diamter 50µm in the outlet as well as inlet areas, which supports the cover, shown in Fig. 8b. The present limitation of processing/fabricating these chips with 500µm tall structures in SUEX is documented by the collapsed 15µm tall post arrays, which were intact after developing until the last step of drying, shown in Fig. 8c [13,14].

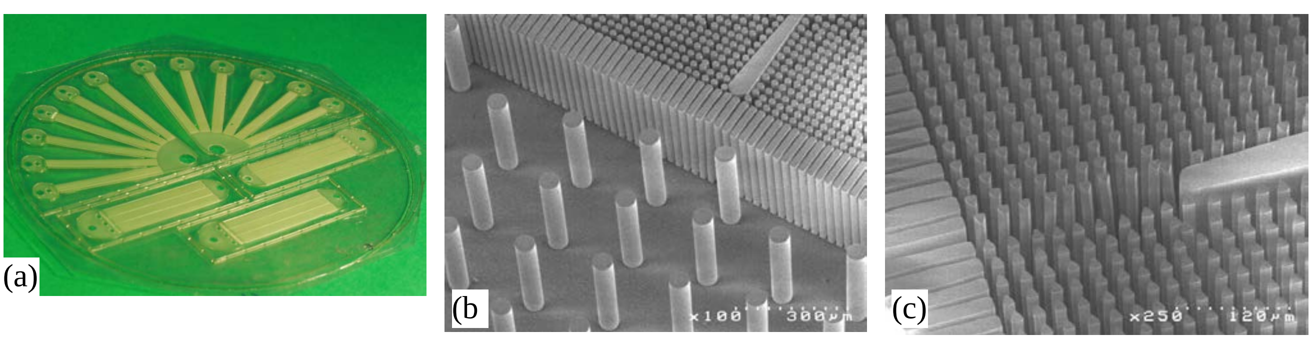


Fig. 7: (a) LDA chip; (b&C) close up of LDA structures at entry & exit showing 15/ 50µm post along with channels.

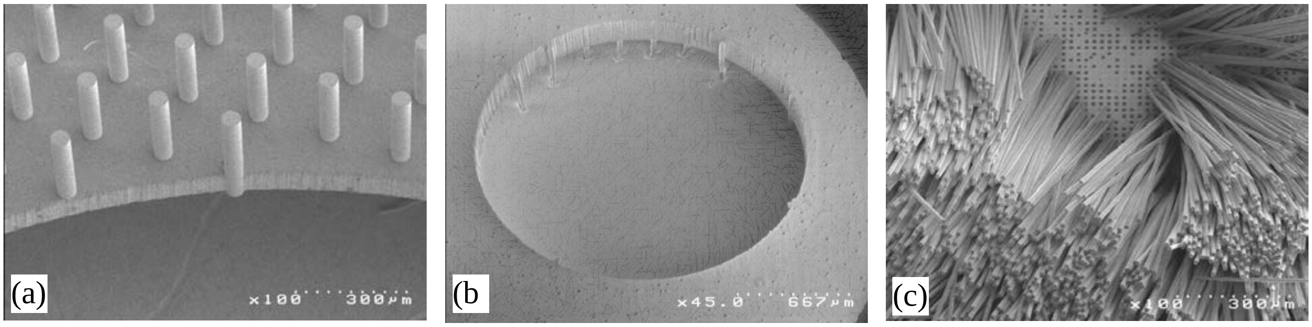
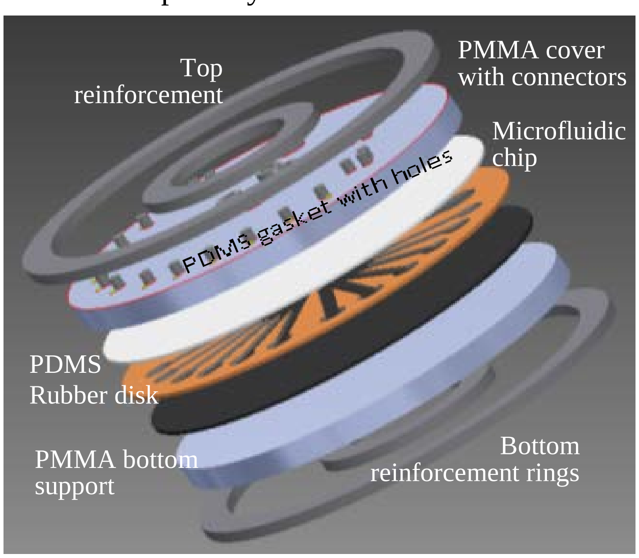


Fig. 8: (a) 350µm tall posts of diameter 50µm in outlet/inlet areas to support the cover sheet; (b) Inlet hole, for 6-LDA, covered with SUEX cover sheet (in picture at bottom); (c) 500µm tall post arrays collapsing during drying.

Fig.7a, shows a chip that was used as is for fluidic testing. Connections to tubes and pumps have been realized as illustrated in Figs 9 & 10. In order to connect the chips to the tubes without a permanent fixturing, a lid was designed and fabricated with the holes of the inlets and outlets on the chip matching those on the lid. This lid (PMMA cover with connectors, Fig. 10a) was micromilled into a 4mm thick PMMA disk. Subsequently, the fluidic testing device was assembled, schematic shown in Fig.9, by using the LDA chips OPEN (SUEX or PC chips without SUEX cover) or closed (SUEX chips with patterned cover). In order to obtain a good sealing betweeen the chips and the lid, a ~0.8mm thick PDMS disk was fabricated and the holes were drilled into it after mounting on the PMMA lid. Then the lid along with the PDMS gasket is placed on the chip aligning the holes of the lid to those of the cover on the chip. The back side of the device was also supported by a thick PMMA (~4mm) disk. In order to achieve a uniform pressure across the chip to prevent any leakage at the inlet or outlet, a ~0.8mm PDMS/rubber plate was used on the back side between the chip and the thick PMMA disk. Then this whole setup was sandwiched between a set of two steel rings with a set on top and bottom of the assembly, as shown in Fig.9. The clamps were now placed on to these steel rings to hold the assembled stack together, Fig.10b. Further, the clamps were tightened to apply a gentle pressure on the assembled device to ensure a good sealing between the chip and PDMS and/or lid especially at inlet and outlet areas without clogging the openings or channels.



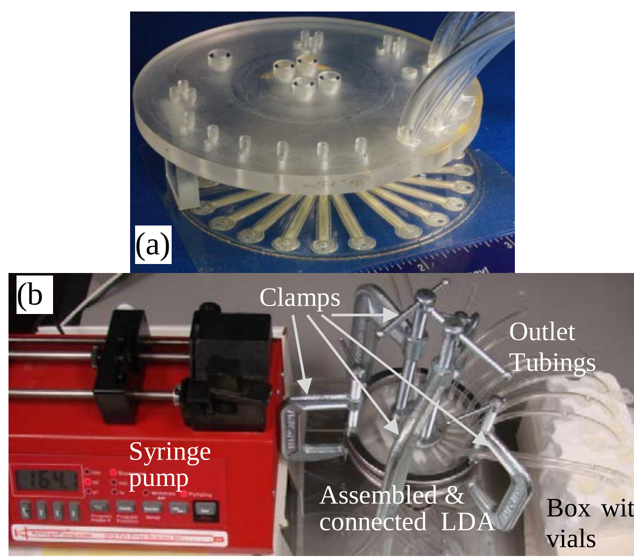


Fig.9: Schematic of the assembly of the device [11]

Fig.10: Setup for characterization of the device [11]

Connecting tubing to the inlet ports on the LDA chip and to a syringe pump filled with DI water or dilute microalgae solution and the outlet ports (for concentrated microalgae solution and waste water) to vials completes the setup, Fig.10b. Additionally, a pressure sensor was installed between the syringe and the inlets (not shown in the figure) to measure the pressure at which the microalgae solution was flowing across the LDA. Initially, before starting the syringe pump, all the tubing and the LDA chips were primed with the solution and it was ensured that pressure was almost zero. Then the syringe pump was started at a preset flow rate and the measurements from the pressure sensor were taken/ recorded.

Microfluidic Tests

Three types of chips, SUEX resist patterned by X-ray lithography with and without cover and molded, uncovered PC chips, were characterized using DI water and dilute microalgae solution. First, pressure measurements for DI water flow were performed and results are presented in Table 1 and Fig. 11. The pressure versus time measurements (Fig. 11) shows the expected results of rapid climbing of pressure in the first few minutes (<5) until a steady stream of water was pouring from the outlet. Then the pressure stabilizes at this level. The minor increase or decrease of the pressure observed is within the sensitivity range (0.5psi) of the sensor. Further, these first results with respect to using two different covers SUEX and PDMS shown in Figs. 11a and 11b were not conclusive with respect to which one will have a lower back pressure. This could be attributed to the fact that while assembling the device and/or applying the PDMS cover, due to the manual clamping some of the posts may bend and buckle thereby producing more resistance and causing higher back pressure. Also there are a number of defects in the mask and consequently in the post arrays producing slightly different flow conditions in each LDA. From Figs. 11a & 11b it can be concluded that as expected the 6 LDAs connected to one inlet produce lower back pressure compared to 3 LDA units for the same inlet flow rate due to the larger LDA volume available for the same sample volume flowing into the LDAs. This is also confirmed from Fig. 11c where the identical LDA produces a larger back pressure when using higher flow rates.

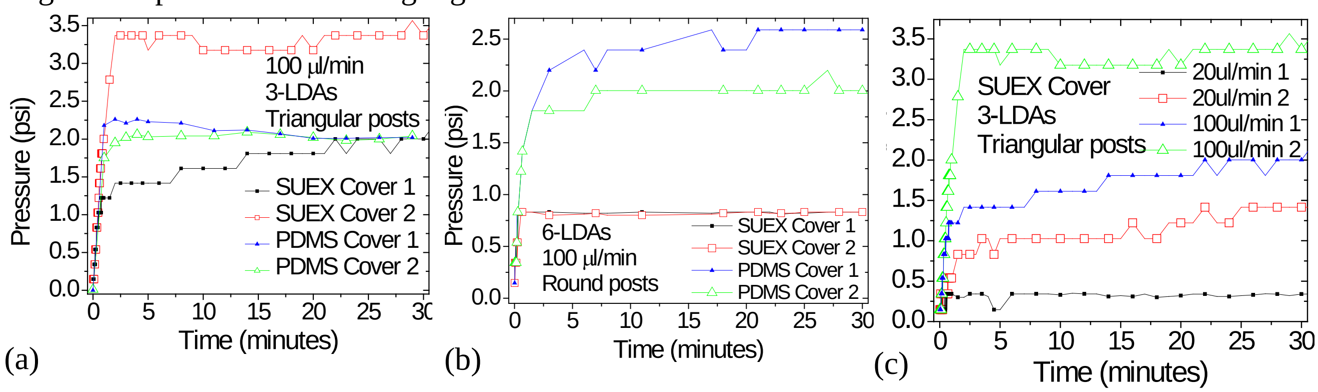


Fig. 11: The comparative results of pressure vs time for 2 different type of LDA arrangment on the chip using DI water (a) 3-LDAs with triangular posts and SUEX/PDMAS cover; (b) 6-LDAs with round posts and SUEX/PDMS cover; (c) 3LDAs with triangular posts and 3 different flow rates.

Figure 12a shows a SEM micrograph of the inlet area with in-flow filter. All structures are clean and standing straight. However, when the same area is inspected after flow tests with DI water (Fig. 12b) clogging of in-line filter as well as post arrays is observed along with some structure bending possibly caused by clamping the PDMS lid too tightly. A critical issue seems to be fouling from material sticking to the SUEX surfaces. ‘Bio-fouling’ is known from another epoxy-based resist, SU-8 [15] and suggests surface modification for example Parylene deposition to prevent fouling.

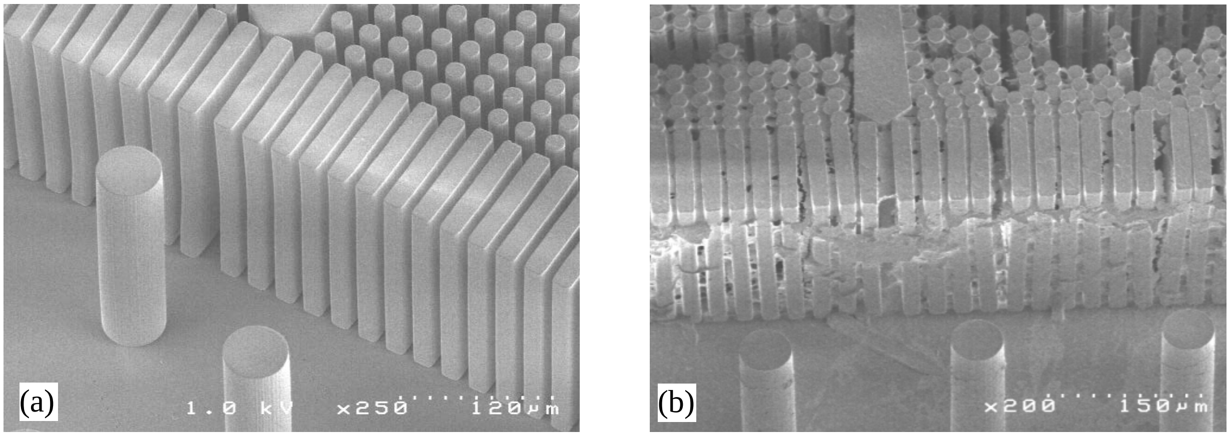


Fig. 12: SEM micrograph of inlet area of chip (a) before the test and (b) after flowing DI water for 30 mins.

In Table 1 results from pressure measurement using DI water in 250μm tall SUEX structures with PDMS cover for 3-LDAs with circular and triangular posts are summarized. As expected, LDA with triangular posts have lower back pressure due to more open space between posts from corner rounding during the fabrication process. For example, considering four posts completely enclosed inside a 40 μm side square, then for circular posts ~56% open space is available whereas for traingular posts the open space will be ~72% for the design values case and even larger due to rounding of corners for the actual structure.

Table 1: Pressure measurement for DI water [11]

Type of Chip	**Type of Post**	**Flow rate (μl/min)**	**Pressure (psi)**
SUEX 250 μm tall post with PDMS cover	circular	20	4.29
		100	14
	trian-gular	20	1.09
		100	4.91

All the readings were taken at the end of 30 mins

Results from back pressure experiments with dilute microalgae solution showed similar trends to tests with DI water, for example higher back pressure for higher flow rates and lower back pressure for triangular post arrays.

Another set of tests used microalgae solution for pre-concentration experiments. Observing algae flow under a microscope indicates that algae seem to appear more likely at the outlet for pre-concentrated solution. However, due to the small algae size (3-5μm) and high magnification needed to see them, the limited depth of focus will not allow proper inspection across the entire channel height. For a more quantitative analysis algae staining is required so that contributions from algae across the entire height are visible.

Preliminary measurements were also performed to determine the concentration of the algae solution after passing through the LDA using a hemocytometer. Concentrated algae sample collected in vials after passing through an LDA with triangular posts at a flow rate of 100μl/min was compared with solution injected into the chip. The area in the hemocytometer used for counting the algae per unit volume was 1mm x 1mm and the depth was 0.1mm. The pre-concentrated algae sample generated ~200 counts per $0.1mm^3$ volume compared to only ~5-10 counts for the initial solution, roughly a ~20x increase. This first result confirms that the chip indeed performs its function. However, compared to the expected (design) value of 40x algae increase/volume the efficiency is still too low likely caused by partial obstruction in the outlet area (Fig. 6a) and also by structural defects within the post areas due to the imperfect fabrication process.

Conclusions and Future Work

Fabrication of LDA devices by two different methods, direct x-ray lithography in SUEX negative resist and hot embossing/molding in polycarbonate, has successfully been demonstrated. The very challenging post array design is limiting the aspect ratio of molded structures to about 7:1 and defects from processing (esp. burr on the edges of the holes cause pulling) pose issues with sealing. For lithography patterned LDAs in SUEX aspect ratios of about 30 are routinely possible after process optimization. Pushing to heights of 500µm (aspect ratio of 50) remains problematic as posts tend to stick and collapse after development.

Cover sheet lamination and lid patterning is possible but need further process parameter optimization for high yield. Fluidic testing was successfully done with a setup allowing dissemble and inspection of structures afterwards. Bio-fouling is indicated by clogging of fine structures and needs to be compensated for before routine use with algae solutions or other bio samples is possible.

Next efforts focus on improving the design and making a new mask with fewer defects, developing concepts for stacking of LDA chips in order to increase throughout, and optimize the fabrication of fully sealed LDA chips with openings for easy measurements. With the initial efforts looking at algae dewatering applications the work done so far suggests that other applications requiring less throughput/volume flow may be better suited for these kind of continuous-flow separation chips. In the future we will look into using similar designs for cell separation of whole blood samples and similar applications.

Acknowledgements

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