3D Printing Techniques in the Manufacture of Microfluidic Devices for Generation of Microbubbles

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Abstract

The microfluidic devices used for the generation of monodisperse microbubbles have particular characteristics besides the obvious size of their structures. These odd features are related to the feed system of the channel systems that make up the devices, ranging from microscopic scale to the macroscopic scale that constitute the feed duct. The most commonly used technique is photolithography. With its use it is possible to obtain channels of triangular, rectangular and half-cane sections of microscopic dimensions with the scale of tens of micro meters with great precision. However, extremely precise and highly complex techniques are needed to adapt feed ducts to the structure of the device, that is, starting from the macroscopic scale to a microscopic scale without allowing leaks or any kind of imperfection in the coupling process. The technique of production of these devices using 3D printing avoids this adaptation process, since in a single step the device is ready to use, and there is no need for adaptive procedures. The 3D OBJET EDEN 250 was used to manufacture the devices using the transparent Vero Clear RGD810 resin, which allows the visualization of the internal structure of the channels that make up the device. The results are promising. It was possible to generate microbubbles with a polydispersity index of 0.55% with the 3D printed devices.

Keywords

Microbubbles, 3D Printer, Microfluidics devices

Introduction

Microfluidics deals with the flow of liquids into micrometer sized channels. To be considered a microfluidic device, at least one channel dimension should be in the range of 1.0-10.0 µm. Microfluidics can be considered as a science that studies the behaviour of fluids in microchannels. A microfluidic device is a set of microchannels embossed or shaped into a material (glass, silicon or polymer, such as PDMS, for Polydimethylsiloxane [1-3]. The micro-channels forming the microfluidic device are connected to ducts to supply them with the desired features.

The microchannels attached to the microfluidic device are connected to the outside by perforated inputs and outputs in the device, as an interface between macro and microscale.

Liquids (or gases) are injected and removed from the microfluidic device through these orifices (via piping, syringe adapters or even single holes) to external active systems (pressure controllers, pressure syringes or peristaltic pumps) or through passive forms (e.g., hydrostatic pressure).

The most commonly used process for the production of microfluidic devices is based on photolithographic methods, derived from the well-developed semiconductor industry.
Various materials, such as polymers (PDMS), ceramics (glass), semiconductors (silicon) and metal are used in the production of these devices. This manufacturing is currently possible due to the development of specific processes: Deposition and electrodeposition, engraving, gluing, injection molding, embossing and soft lithography (especially with PDMS) [4-7].

Access to these materials has allowed designing microfluidic devices with specific optical characteristics, biological or chemical compatibility.

Currently, PDMS and soft lithography have been used due to the ease of use, and the rapid manufacturing process. These techniques allow researchers to quickly prototype and test their models instead of wasting time on laborious manufacturing protocols [8,9].

Microfluidic technology: Constructions of a microfluidic device

The simplest current microfluidic device consists of microchannels moulded into a polymer which is deposited on a flat surface (such as a glass slide). The polymer most commonly used for molding microfluidic devices is PDMS. PDMS is a transparent, biocompatible product (very similar to the silicone gel used in breast implants), inexpensive deformable elastomer. It is easy to mould and glue on glass. For all these reasons, it is appreciated by the researchers [2,8].

Manufacture of a simple microfluidic device using photolithography

The manufacture of a microfluidic device begins with the design of microfluidic channels through dedicated software such as AUTOCAD or LEDIT. Subsequently, it is transferred to a photomask namely glass plates coated with chrome or plastic films for the most common models. The microchannels are thus printed with opaque UV ink (if the substrate is a plastic film) or engraved on chromium (if the substrate is a glass plate) [3].

Figure 1 and Figure 2 shows the manufacturing steps of a microfluidic device using photolithography.

Adapted from: https://www.elveflow.com/microfluidic-tutorials/microfluidic-reviews-and-tutorials/microfluidics-and-microfluidic-device-a-review/

The stages presented in Figure 1 are, (1) The resin is spread on a flat surface (usually a silicon wafer) of the desired thickness (which determines the height of the microfluidic channels) (2) The resin, protected by the photomask with the microchannel pattern, is then partially exposed to UV light. Therefore, in the case of a negative resin such as SU-8 type, only the parts representing the channels are exposed to UV light and cured, the other mould parts being protected by the opaque areas of the mask. (3) The moulding is developed in a solvent which writes resin areas that have not been exposed to UV light. (4) We then obtain a microfluidic template with a resin replica of the photomask standards (future microchannels “emboss” in the mould). The height of the channels is determined by the thickness of the original resin spread on the plate. Most of the time, the moulding is then treated with Silane to facilitate the release of microfluidic devices during the moulding steps.
Figure 2 presents the additional steps to the manufacturing of a microfluidic device using photolithography: (1) The moulding step allows bulk production of microfluidic devices from a molding. (2) A mixture of PDMS (liquid) and crosslinking agent (to cure PDMS) is poured into the molding and heated to an elevated temperature. (3) Once the PDMS is hardened, it can be removed from the molding. We get a replica of the microchannels in the PDMS block. (4) To allow the injection of fluids for future experiments, the inflows and outlets of the microfluidic device are punctured with a PDMS punch the size of future connecting tubes. Finally, the face of the block of PDMS with microchannels and the glass slide are treated with plasma. (5) Plasma treatment allows PDMS and glass bonding to close the microfluidic chip.

After performing the procedures, the device is now ready to be connected to microfluidic reservoirs and pumps using microfluidic tubing. The Tygon tube and the Teflon tube are the most commonly used tubes in the microfluidic connections.

The manufacturing of microfluidic devices as seen, requires many steps and is a meticulous and complex process, despite being able to produce devices with high operational capacity.

**Manufacturing of microfluidics devices using 3D printing**

The manufacturing process of microfluidic devices using 3D printing is a viable alternative for the manufacture of these devices due to its operational simplicity [10-13].

The whole manufacturing process happens in a single step, avoiding complex consecutive procedures to enable the use of the devices.
Initially the project is designed through computer aided design software such as AUTODESK FUSION 360®. This tool provides an interface for the creation of the 3D model to be produced using a 3D printer [11,12].

As mentioned earlier, an important feature of the presented technique is to obtain the operational design in a single step, eliminating complex procedures for fixing the feed ducts to the device (from the microscale to the macroscale). This operation has a high degree of complexity to avoid micro-leaks in the connections between the microchannels and the feed ducts [10-12].

Starting the device design in 3D using the AUTOCAD FUSION 360 tool, the 3D model is encoded in STL format to be sent to 3D printing. The printer used was the OBJET EDEN 250 with the transparent resin VERO CLEAR RGD810 which has the advantage of providing an internal view of the channels allowing the study of the dynamic behaviour of the fluids inside the device [11,12].

Figure 3 shows two microfluidic device designs created using the AUTOCAD FUSION 360 software.

Figure 4 shows the internal structure of the channels of the device (1) With their respective dimensions. The OBJET EDEN 250 printer is able to print internal channels of circular sections up to 300 µm in diameter, in this particular case the channel dimensions were 1.5 and 1.0 mm respectively.

Figure 5 shows the internal structure of the channels of the device (2) With their respective dimensions. The OBJET EDEN 250 printer is able to print internal channels of circular sections up to 300 µm in diameter, in this particular case the channel dimensions were 0.3 mm. As it can be seen, the manufacturing of the devices by 3D
printing happens in a single step without complementary procedures that makes this technique extremely simple.

Figure 6 shows the device (1) Manufactured according to 3D printing, showing the degree of transparency of the resin used in its manufacture. The transparency of the device is a key feature for the study of the dynamic behaviour of fluids within the device.

Once printed the device needs to undergo a process of cleaning the internal channels due to the injection of waxy carrier by the printer. This support allows the microchannels to be printed, preventing them from being tamponade during the manufacturing process.

Unclogging the channels is done in two stages: The first step, involves immersing the device in a 10% solution of caustic soda for 24 hours. This procedure is done to dissolve the support that fills the microchannels. The second step consists of the residual cleaning of the support, it is performed with the use of metallic dental wires of diameter compatible with the geometry of the channels, in the particular case a wire with a diameter of 300 µm was used. An additional procedure may be required using a syringe to inject the caustic soda solution by pressure to remove resilient carrier residues.

Results and Discussion

Production of microbubbles using the device manufactured by the 3D printer

The dynamic process of production of microbubble is based in microfluidics, form bubbles by a process of compression due to the instability at the interface of
The bubble size is associated with the physical properties of the liquid flow rates of gas and liquid phase, the size and profile of the channels, besides the speed of strangulation. This, in turn, is dependent on the rate of fluid flow and pressure of gas should be increased or decreased. For a given gas pressure, there is a maximum flow of fluid above which the gas flow is blocked, there is no formation of microbubbles as well as the indiscriminate increase in pressure induces an atomization (spray) of the liquid phase. Thus the formation mechanism of the bubble is significantly influenced by the variation of these parameters, particularly the viscosity of the liquid. The ordered control of these variables plays a key role in their size.

The choice of the T-junction device occurred due to its simplicity and high efficiency demonstrated by several of papers published in specialized journals. Using the device manufactured according to 3D printing in the T-junction configuration, it was possible to produce microbubbles with a Polydispersity Index (PI) of less than 1% \([6,14]\).

Figure 7 shows the image of a microbubble generated by the device 2 moving within the microchannel of internal diameter of 300 µm.

Table 1 shows that the population of microbubbles has a low Polydispersity Index (PI), being around 0.55% which characterizes a monodispersion. The Standard Deviation \((\sigma_1)\) was ± 0.4 µm and the Mean Diameter \((M_D)\) was 73.3 µm.

In order to test the normality of the data, the test was carried out, taking into account the number of elements of the sample. As the number of sample elements was greater than 50, the Kolmogorov-Smirnov test was used.

Due to the parametric nature of the data, a subsample of 20 elements of the total population of microbubbles was chosen for the t test to evaluate the differences between the means of the two groups.

Figure 9 shows the normal distribution of the sub-sample used to perform the normality test (Table 2).

The mean diameter \((M_D)\) of the sub-sample microbubble population was 73.2 µm, standard deviation \((\sigma_2)\) was ± 0.4 µm, and the Polydispersity Index \((PI_2)\) was 0.55%, coinciding with the standard deviation \((\sigma_1)\) and the Polydispersity Index \((PI_1)\) of the total sample, which confirms the monodisperse character of the microbubble population generated by the device.
Based on the results obtained it is considered that the microfluidic devices manufactured according to the 3D printing technique met the proposed goal of generating monodisperse microbubbles with a Polydispersity Index 0.55%. Moreover, with this technique it becomes possible to manufacture the devices in a simple cost-effective way, dispensing complementary procedures, which makes it operationally feasible [20].

However, the OBJET EDEN 250 printer cannot manufacture micro fluidic devices...
with internal structures smaller than 300 µm since this was the maximum resolution achieved so far.

Professional high-resolution printers such as the PHOTONIC PROFESSIONAL GT HIGH-RESOLUTION 3D manufactured by NANOSCRIBE can circumvent this limitation by producing microfluidic devices in Nano, micro and mesoscale. Its technology is regularly used to manufacture microscopic optical components and microfluidic elements such as filters or mixers on chips.

Acknowledgements

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References


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Table 2: Shows the data of the sub-sample used to perform the normality test.

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| Mean Diameter ($M_D$) [µm] | 73.2  |
| Standard Deviation ($\sigma_D$) [µm] | 0.4   |
| Polydispersity Index (PI) [%] | 0.55  |


