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Accepted Manuscript

ROYAL SOCIETY OF CHEMISTRY

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Journal Name

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ARTICLE

Preparation and validation of low cost microfluidic chips by shrinking approach

Cite this: DOI: 10.1039/xoxxoooooox

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Received ooth January 2012,
Accepted ooth January 2012

DOI: 10.1039/xoxxoooooox

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The present paper describes the production of microfluidic chips by an approach based on shrinkable biocompatible polymers (i.e. agarose) for the production of size controlled microfluidic channels. In addition, all steps of chip production were carried out by an inexpensive approach using low cost chemicals and equipment. The produced chips were then validated by producing monodisperse polymeric microparticles for drug delivery and hydrogel microfibers for cell embedding.

Introduction

Microfluidic systems have been applied in a wide range of areas such as molecular analysis, molecular biology, microelectronics, clinical diagnostics, and drug development.[1-5] Microfluidics had such and large success since there are many benefits resulting from the miniaturization of devices for use in these areas including, decreased time of analysis, reduced consumption of reagents and analytes, increased separation efficiency, decreased weight and volume and increased portability.[6-8] Moreover, microfluidics offers precise high throughput applications to pharmaceutical research for the production of novel functional materials. For instance, the so-called "Lab-on-a-Chip" devices have been used to screen and support complex pharmaceutical processes,[9,10] to study the interaction between cells and formulations[11,12] and the mechanisms of action of many drugs,[13] and finally, to produce micro sized droplets or particles for drug delivery[14-17] and cell encapsulation.[18-21]

Although microfluidic chips allowed a sharp reduction in the consumption of reagents and analytes, their costs remain rather high. Commercial standard microfluidic chips, are, indeed, very expensive, for instance, the price of a flow focusing (FF) device ranges between 110 and 130 € and the cost can dramatically increase if a specifically designed chip, with a tailored channel geometry is required for unique applications.

Typically, microfluidic devices are produced using a limited number of leading materials, namely: glass, polydimethylsiloxane (PDMS) and polymethylmethacrylate (PMMA).[6,22-25] In particular, PDMS is widely the most common polymer used for chips production because of its optical and mechanical characteristics, low toxicity, chemically stability and applicability to soft lithography procedures.[6,26] However, the experimental procedures required to the preparation of complete chips (i.e. with ports and connecting tubings) remain expensive and require specific facilities and equipment such a CAD software, photoresist materials, mask alignment devices, nanoports and plasma cleaner[27-29] and they are, therefore, only marginally applicable in teaching (high school, undergraduate and graduate programs), in developing regions of the world and (less importantly) in protocols requiring rapid evaluation of prototypes.

Recently, in order to answer to high cost relative to standard procedure for the preparation of microfluidic chips, few papers have reported about low cost approaches for the production of relatively simple devices. The group Michelle Khine[30] at School of Engineering, of California University, described an approach for the ultra-rapid direct patterning of polystyrene microfluidic chips by employing the shrinkage proprieties of thermoplastic sheets, commercially available "Shrinky-Dinks", a children toy. Unfortunately, in spite of the smart idea, the shrinkage behaviour exhibited by Shrinky-Dink is very variable from sheet to sheet making the approach very difficult to applications that required highly controlled channel size. Mandon and co-worker described an approach for the production of microfluidic chips integrating protein spots. The technology, called by the author ''Print-n-Shrink'', is based on the screen-printing of a microfluidic design onto Polyshrink polystyrene sheets very similar to Shrinky-Dink sheets.[31]. A very interesting simple and inexpensive fabrication method for (micro) fluidic chips has been described by Yang and colleagues.[32-33] They obtained a "Y-shaped" mixer device using very cheap materials, such as wooden coffee stirrers and food grade gelatin, to achieve an easy tool for teaching microfluidics to undergraduate and graduate students. The approach is very cheap and versatile but the large dimension of the channels (i.e. 0.5-1.0 cm width) are, in many cases, inappropriate for simulating real microfluidic experiments. Another promising development of low cost chips is represented by the paper-based microfluidics. This technology is an economic and effective way to perform simple operation, it can be quickly applied to a series of different areas of research, such as point of care and electrochemical sensor.[34-37] In spite of these advantages, paper-chips are not suitable for pharmaceutical research focused to the production of nano and microparticles.

To the best of our knowledge, a total low cost procedure to fabricate microfluidic devices for the production of drug delivery and tissue engineering systems is still lacking. Therefore, the present paper describes an approach for the production of chips, using extremely low cost chemicals and equipment. The procedure allows a precisely control of channel

geometry, down to 500 µm, employing shrinkable microhydrogels.

In order to fully demonstrate the potential of our protocol the microfluidic chips have been successfully validated by producing monodisperse polymeric microparticles for drug delivery and hydrogel microfibers for cell embedding.

Experimental

A. Materials and equipment

Delrin 100 BK 602 acetal resin was purchased from Du Pont de Nemours Italiana S.r.l., Milano, Italy; Araldite M Resin and Epoxy embedding medium kits were obtained from Sigma Aldrich, St. Louis, MO, USA, respectively containing Araldite Epoxy Medium, Araldite M Hardener 964, Araldite M Accelerator 960 and Epon_812 Medium, Hardener MNA, Hardener DDSA, Accelerator DMP 30; EMAX Epoxy EPI 114 resin and EMAX Hardener Black EPI 272 were purchased from Mascherpa S.P.A., Milan, Italy; Elastomeric polymers Sylgard 184 silicone elastomer kit and Silastic E RTV silicone rubber kit were obtained from Dow corning corporation Midland, MI, USA; Rhodorsil RTV 4407 A and B were purchased from Siliconi padova sas, Padova, Italy. RPMI 1640 and) fetal bovine serum, streptomycin and penicillin were purchased by Gibco, BRL, Milan, Italy. Agarose, ethyl acetate, triethyl citrate, glycerol, ethanol, PEG 200, PEG 600 and Carbopol were obtained from Sigma Aldrich, St. Louis, MO, USA. Sodium alginate IE-1105 (viscosity, 20.0–40.0 cP; pH, 6.0–8.0, C ¼ 1%, H_2O) was obtained from Inotech Biosystem International, Switzerland). Micromachining tool Micro miller MF 70 with 0.8 mm HSS drill bit Cat. N. NO 28 852 (Proxxon, Föhren Germany) and DREMEL 200 (purchased from Robert Bosch S.p.a., Milano, Italy) were employed. Plastic FEP (fluorinated ethylene-propylene) 1/16" OD, 0.75 mm ID and 3/16" OD, 2.25 mm ID Upchurch Scientific tubes was obtained from VWR International s.r.l. Milano, Italy; PVC 1/4 inch x 5/16 inch Nalgene tubing was purchased from Fisher Scientific SAS, Strasbourg, France; Timmer-Pneumatik tubes; H-PTFE-4/2 mm (OD/ID) was obtained from Timmer-Pneumatik GmbH, Neuenkirch1en, Germany; 10 ml syringes and 21 gauge hypodermic needles were purchased from Pic Solution, Como, Italy. Syringe pump KDS Model 100 Series was purchased by Kd Scientific Holliston, MA, USA.

B. Materials for template micromachining

Epoxy resins, EMAX and EPON, were respectively prepared as follows. EMAX was obtained mixing prepolymer and hardener 10:1 w/w ratio. EPON resin was obtained mixing Epon_812 Medium, DDSA, DPM-30 and triethyl citrate 5: 3: 1: 1 (w/w/w/w). All the mixture was degased in centrifuge at 1500 g for 5 min.

C. Microchip fabrication

The general procedure for the preparation of microfluidic chips by the "shrinking" approach is illustrated in Fig. 1. Firstly, according to the design of channel structures, the production of template was performed. To this aim, 50x50x8 mm plate was micromachined to produce squared interlinking microfluidic channels (0.8x0.8 mm). Different materials were tested as chip template, including Delrin (commercially available), PMMA or freshly prepared epoxy resins (EMAX or EPON), prepared as previously described. Masters were produced by replica molding technique employing an aqueous dispersion of agarose (2–4%, w/v, in water or water/ethanol mixtures). The dispersions were casted to templates and then, they were left to consolidate (gelify) for 3 h at 4°C. Finally, masters were slowly peeled from the templates leaving the micropatterns imprinted on agarose dispersion with a high fidelity of replication. Master shrinking was accomplished by immerging the masters into pure liquids or liquid mixtures, namely: ethanol, glycerol, PEG 200 and 600 or ethanol/glycerol mixture (50:50 v/v). After variable length of time (typically 24 h), masters were removed from the shrinking liquids and allow to further shrinking in air (from 2h up to 4 days).

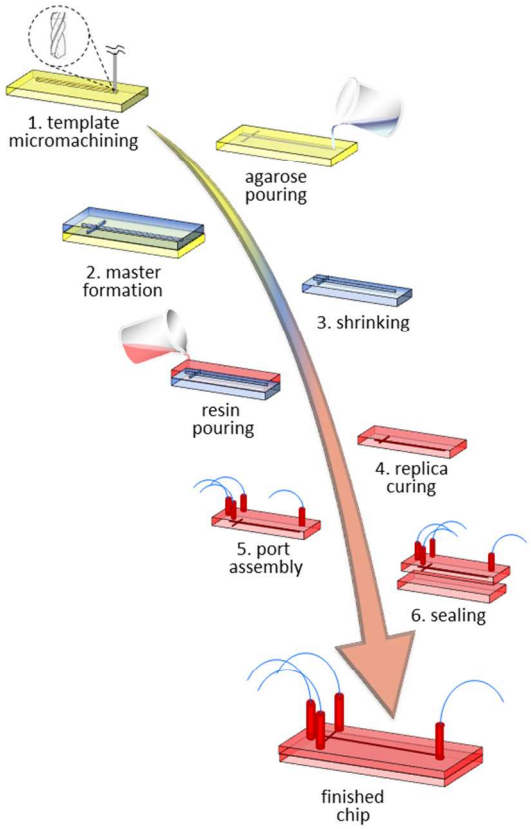

Fig. 1. General scheme depicting the main steps involved in the production of low cost microfluidic chip, by hydrogel shrinking approach.

Replica molding approach was also used to produce replicas: prepolymer mixtures were poured onto the agarose masters and, after consolidation in different conditions (ranging from 1-3 days, 30°C), replicas were released from master, and microstructures comprising interlinking channels were obtained. The replicas were produced by PDMS or epoxy polymers following the producer specifications.

Ports were accomplished by minor modifications of the procedures elsewhere described.[38-40] Briefly, for PDMS microchips ports, we punched holes, by Dremmel, in the replicas at the inlet/outlet, after, FEP tubes or hypodermic needle were placed in the holes. Small pieces of PVC tube with at least one planar surface were cut and a bit of prepolymer mixture around the periphery of the planar surface was putted. PVC tube pieces were placed centering the needles or FEP tubes to make a reservoir where prepolymer mixture were poured to achieve a rigid support. Finally, open microfluidic channels were sealed using a slab of partially cured master polymer to have irreversible sealing at RT.

D. Microchip geometrical and morphological analysis

During the production of the microchip, the morphological and dimensional characteristics of templates, masters and replicas

were determined by microscopic observations. Optical inverse and stereo microscopes were employed (Nikon Phase Contrast ELWD 0.3 and Nikon SMZ 1500, Nikon, Tokyo, Japan) and the obtained microphotographs were analyzed by EclipseNet version 1.16.5, Laboratory Imaging s.r.o. for Nikon B.V and ImageJ softwares.

E. Microchip validation: production of microparticles and microfibres

1. Preparation of monodisperse polymeric microparticles

Cellulose acetate (CA) microparticles were produced by solvent evaporation technique, employing a microfluidic chip with a flow-focusing (FF) geometry (Chip #1), following with minor modification the elsewhere described procedures.[41] Briefly, a 15% (w/v) CA dispersed in a mixture of ethyl acetate/ acetone 4:1 (v/v) was employed as oil phase (OP), whereas, a 0.5% (w/v) aqueous solution of Carbopol (CP), represented the water phase (WP). Thereafter, OP and WP were loaded into 10 ml syringes and both liquids were forced into the FF microchip by digitally operated syringe pumps. OP was injected into the central inlet at different flow rates, ranging between 80 and 250 µl/min while WP was injected into the two lateral inlets at a flow rate varying between 3.0 and 4.5 ml/min. With the combination of OP and WP used in the experiments, the FF system generated monodisperse droplets, that were left to consolidate by solvent evaporation at RT to obtain the solid CA microparticles.

2. Preparation of multifunctional microfibres

Human myeloid leukemia K562, were used for the production of multifunctional alginate microfibres. Cells were maintained in RPMI 1640 (Gibco, BRL, Milan, Italy) in 10% fetal bovine serum (Gibco, BRL,Milan, Italy) supplemented with 50 units per mL penicillin and 50 mg/ml streptomycin at 5% CO2. ‘‘Empty’’ and ‘‘multifunctional’’ (containing cells, microparticles or both) barium alginate microfibres were produced with a snake mixer (SM) microchip (Chip #2), following the below reported procedure. A sodium alginate solution (1.5–2.5%, w/v) and two sodium alginate suspensions were delivered via the three inlets of the snake mixer microchip at a flow rate comprised between 15 and 35 ml/min. The two suspensions contained different amounts (10–40 mg/mL) of either CA microparticles or cell suspensions (2–9 x 106 cells/ml). The output from the chip outlet was transferred via a FEP microtube into a $BaCl_2$ solution (1.0–2.0%, w/v) where the alginate stream was gelled to produce the final Ba-alginate microfibres. The obtained microfibres were examined by optical stereomicroscopy to determine the dimensions.

3. Geometrical and morphological analysis of microparticles and microfibres.

Dimension and morphology of microparticles and microfibers were evaluated by optical stereomicroscopy. Quantitative analyses were obtained by photomicrograph analysis software EclipseNet version 1.16.5. The mean diameter of microfibres (SD) was obtained by taking 9 measurements along the (10 mm) length of the samples at equal intervals, in triplicate.

Results and Discussion

General consideration

Nowadays, microfluidics represents a valid tool to minimize cost and maximize efficiency in many areas, including healthcare, physics, molecular biology and pharmaceutical sciences. The advantages offered by the use of microfluidics protocols are important both at laboratory procedures as well as industrial production. In this respect, the main goal of the current paper is to describe a produce for the complete production of microfluidic chips (i.e. including ports and connecting tubings) employing only very low cost materials and ready available instrumentation.

Microchip fabrication

Fig. 1 describes the main steps involved in the production of the chip: 1. template micromachining; 2. production of the master (by hydrogel consolidation); 3. master shrinking; 4. replica formation by resin pouring and curing; 5. port assembly; 6. microchip sealing.

As preliminary step, the design and drawing of 3 chips with different channel configuration were performed by a freeware program (Blender 2.69), namely: a flow focusing, a three inlet “snake”micromixer and a T-junction combined with a flow focusing. The templates were obtained performed by micromilling, employing a manual machine for “home working” (Fig. 2). Notably, such milling machines are inexpensive and easily available in hardware stores with a cost of approximately 300 euros. Micromilling, being based on the mechanical removal of the substrate materials, is a very convenient approach when compared to other microfabrication methods such as photolithography dry and wet etching. In addition, micromilling is very versatile, being suitable for the fabrication of complicated and multi-level microchips.[42]

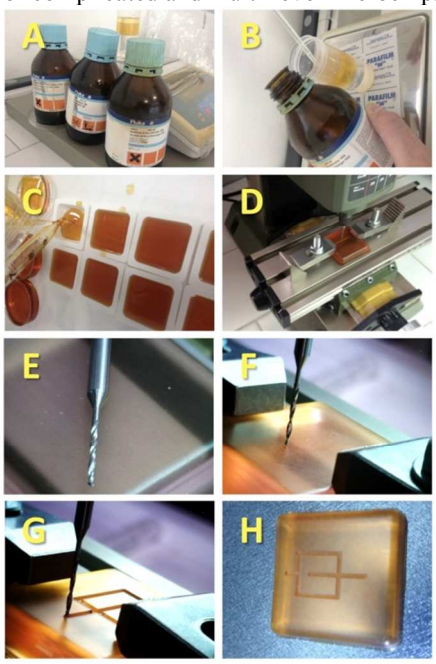

Fig. 2. Production of epoxy resin templates. Epoxy resin slabs preparation (A-C) and successive micromachining (D-G). Constituents of the epoxy resin, namely: Epon_812 (medium), DDSA (hardener) and DPM-30 (accelerator) (A); addition of the plasticizer (triethyl citrate) to the epoxy resin mixture (B); pouring of the uncured resin mixture into polystyrene weight boats (50x50x10 mm), the slab were left to cure for 24 h (C);. template production by micro milling using a micromiller MF 70 (D); 0.8 mm HSS drill bit (E-G) and final micromachined template (H).

The plates for micromachining were initially constituted of epoxy resin since this material is easily available as embedding polymer for the preparation of microscopy samples.[43] Liquid monomers of the medium were reacted at room temperature, by adding the hardener in the presence of the accelerator. In order

to obtain plates with adequate mechanical characteristics and workability, different media, hardeners and accelerators, were tested (specific details of the reactants are given in the experimental section); in order to reduce plate brittleness and scarce workability, the use of plasticizers, such as triethyl citrate and PEG 200, was also considered.
The analysis of the results reported in Table I demonstrates that mixture #5 displayed superior performances with respect to all other combinations of medium, hardener, accelerator and plasticizer; #6 showed indeed, a very good machinability together with a high fracture toughness, both on the channel walls as well as on plate surface (Fig. 3A).

Table 1. Mechanic characteristics of epoxy resin wafers tested for template production.

Batch	Medium (%,w/w)	Hardener (%, w/w)	Accelerator (%, w/w)	Plasticizer (%, w/w)	Characteristics
#1	E812 (60)	DDSA (30)	DMP-30 (10)	None	very hard and brittle
#2	E812 (60)	DDSA (20)	DMP-30 (10)	PEG 200 (10)	brittle
#3	E812 (50)	DDSA (30)	DMP-30 (10)	PEG 200 (10)	hard and brittle
#4	E812 (60)	DDSA (20)	DMP-30 (10)	TEC (10)	ductile, good workability
#5	E812 (50)	DDSA (30)	DMP-30 (10)	TEC (10)	extremely well workable
#6	E812 (50)	MNA (30)	DMP-30 (10)	TEC (10)	Brittle
#7	E812 (50)	DDSA (30)	AM960 (10)	TEC (10)	Very fragile
#8	AEM (50)	DDSA (30)	DMP-30 (10)	TEC (10)	Fragile
#9	AEM (50)	MNA (30)	DMP-30 (10)	TEC (10)	Brittle, poor workability
#10	AEM (50)	MNA (30)	AM960 (10)	TEC (10)	Brittle

E812: Epon_812 medium; AEM: Araldite epoxy medium; DDSA: dodecenylsuccinic anhydride; MNA: methyl nadic anhydride; DMP-30: 2,4,6-tris (dimethylaminomethyl) phenol; AM960: Araldite M Accelerator 960; TEC: triethyl citrate.

A further epoxy resin considered was the commercially available EMAX Epoxy EPI 114 resin; in this case, the resin was cured following the datasheet company indications; the hardened plates resulted very appropriate to micromachining in reason of their high plasticity and workability, resulting in smooth channel surfaces (Fig. 3B).

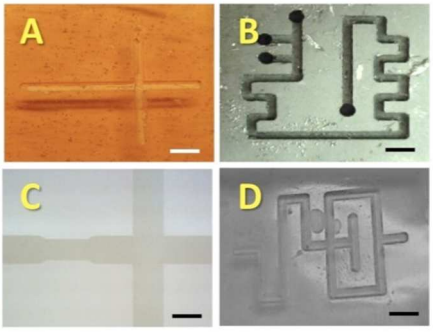

Fig. 3. Templates produced with different channel patterns and materials, namely: flow focusing pattern in epoxy resin (A), three inlet "snake"micromixer in EMAX resin (B), flow focusing pattern, with bottlenecking in delrin (C) and T-junction pattern combined with a flow focusing pattern in poly (methyl methacrylate) (D). Bars represent 4 mm panel A, B and D. In panel C bar represent 1 mm.

As further material for template production, two ready to use, commercially available plates were also considered, namely Delrin and PMMA. They were employed since these materials are commercially available, inexpensive and display high workability properties as demonstrated by the obtained templates depicted in Fig. 3C-D. The masters, intended as the positive replica of the templates, present relief structures (i.e. the positive of the channel geometry) on their surface. Therefore, masters were obtained by pouring an agarose solution on top of the templates, followed by the successive agarose gelling. After consolidation (typically after 3 h), gels were removed from the template and allowed to dehydrate. This process was intentionally designed to cause the progressive shrinkage of the master, therefore resulting in a substantial reduction of channel width and depth.
As first approach for dehydration, agarose masters were left to shrink in air at RT for 3 h. This treatment caused, as expected, a linear decrease of the dimension of the channels (see Fig. 4).
Unfortunately, the shrinkage resulted also in severe morphological unwanted effects. As clearly appreciable from Fig. 4 B, C the masters were highly distorted and channels and sidewalls were extremely wavy. To overcome this drawback, instead of using pure water, the agarose powder was solubilizes in a mixture of water/ ethanol (75:25 v/v), aiming to a reduction of the surface tension at the agarose gel/air interface which it was attributed as a cause of gel deformation. Following this procedure, the shrinking was rather pronounced, with about 60% reduction of the channel width in 3 h (Fig. 4 D). As appreciable from Fig. 4 E, at macroscopic level the resulting masters were satisfactory; conversely, the microscopic analysis of the channels revealed the deformation of the channel structure (see Fig. 4 F).

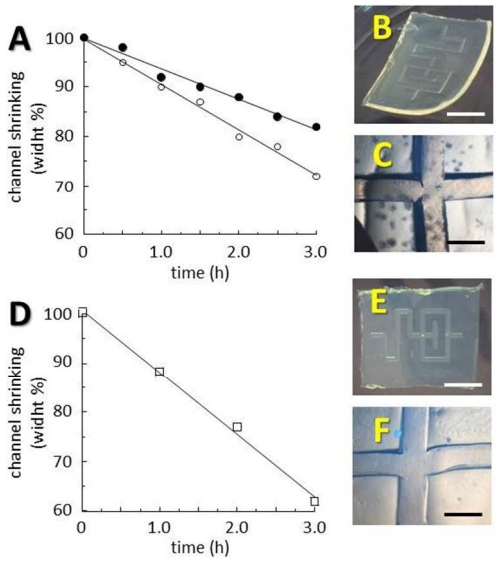

Fig. 4. Effect of master dehydration in air on channel size (i. e. width) and morphology. Masters were prepared with different amount of agarose dispersed in water (A-C) or in a water/ethanol mixture (75:25, v/v) (D-F). In the case of polymer dispersed in water different agarose concentration were tested, namely: 2% (closed circles) and 4% (w/v) (open circles). The stereo photomicrographs reported in panels B, C, E and F show the effect of 3 h shrinking on the general morphology of the channels. Bar corresponds to 1 mm.

With the aim to possibly reduce the deformation, an alternative dehydration approach was investigated. Agarose replicas were placed in water miscible solvents to obtain a progressive and controlled dehydration, as commonly employed for the preparation of microscopy samples.[44,45]
In Fig. 5, plots and pictures relative to this approach are reported. The master were treated with increasing concentration (from 25 to 100 %, v/v) of ethanol (Fig. 5 A, open triangle), glycerol (Fig. 5 A, closed triangle) or a mixture 50/50 % (v/v) of both solvents (Fig. 5 A, squares).

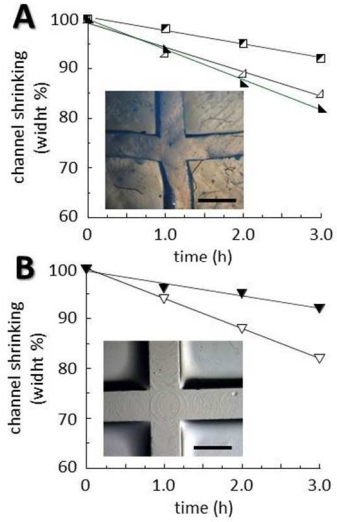

Fig. 5. Effect of master shrinking on channel size (i. e. width) and morphology. The dehydration of agarose master were carried out with the following liquids: ethanol (◣), glycerol (◿) and a mixture of ethanol/glycerol (50:50, v/v) (◩) (panel A) or PEG 200 (▼) and PEG 600 (▽) (panel B). Masters were prepared with 4% agarose (w/v) dispersed in water. The stereo photomicrographs reported in the insets of panel A and B show the effect of 3 h dehydration with ethanol and PEG 600, respectively. Bar corresponds to 1 mm.

The results reported in Fig. 5 B demonstrated that, after 3 hours, the dehydration led to a reduction of channel dimensions up to 82% of initial dimensions, but the morphology of the channel still remain unsatisfactory. Satisfactory results, in term of shrinking and morphology, were finally obtained by dehydrating the masters in PEG 600; the channel structures were well preserved maintaining a straight geometry (see inset of Fig. 5 B).
In reason of the excellent results obtained, in the first short term experiment with PEG 600, the effect of PEG 600 was investigated prolonging the length of the treatment period. In agreement, Fig. 6 reports the average data of master shrinking up to 96 h, showing as the channel width was reduced up to 60% of the initial dimension (namely from 800 to 505 µm). After the appropriate shrinking (i.e. depending of the final desired channel dimensions), the agarose master was used as a template for the production of replicas by soft lithography technique. [6,26] The duplication of the information (shape, morphology, and structure) present on the surface of the agarose masters were therefore obtained by different curable prepolymers, namely: PDMS and epoxy resins.
For instance, PDMS earned a high popularity for the fabrication of microfluidic devices, due to its numerous advantages including, ease of molding into (sub)micrometer features, relatively high chemical resistivity and low manufacturing cost.[46,47] In our experiments, 3 different types of PDMS were used, namely: Sylgard 184, Silastic e rtv and Rhodorsil rtv 4407; all polymers resulted in replicas with smooth and defined channels as well as planar surfaces around channels in agreement with our expectation (see Fig. 7 A).

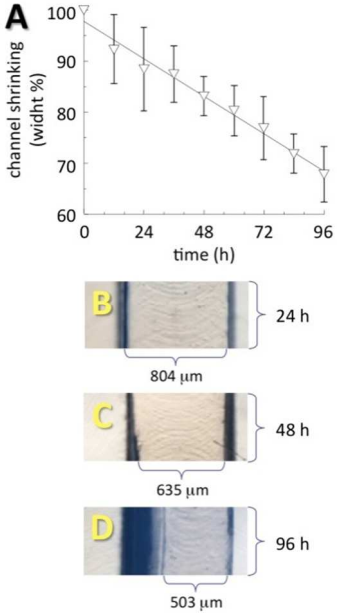

Fig. 6. Effect of long term master shrinking on channel size (i. e. width) and morphology. The dehydration was carried out up to 96 h exposure of the master to PEG 600. Masters were prepared with 4% agarose (w/v) dispersed in water. The stereo photomicrographs reported in panels B-D show the effect of shrinking on the general morphology of the channels. Data of graph represent the average of three independent experiments±SD.

Replicas were obtained with similar results (data not shown) also by the epoxy resin mixture #5 (see Table I for composition) used for epoxy template preparations.

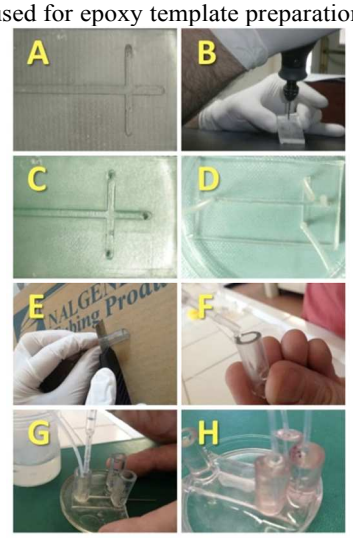

Fig. 7. General scheme depicting the main steps involved in the port assembly. Replica with flow focusing pattern produced with sylgard 184 (A); drilling of the holes in the replica at the inlet/outlet by Dremel 200 with a 0.5 mm tip (B, C); insertion of plastic FEP tubes (fluorinated ethylene-propylene, 1/16" OD, 0.75 mm ID) into the drilled holes (D); cutting of small pieces (1 cm) of PVC tube (1/4 inch x 5/16 inch) with planar surfaces (E); application of small amount of uncured PDMS mixture around the periphery of the tube (F); sticking of the tube on the replica, placing it centering the FEP tubes and pouring uncured PDMS mixture into the PVC tube, around the FEP tube (G); replica with the assembled ports (H).

Once masters were cured, the connection and interfacing (i.e. porting) were performed by two simply methods for PDMS and epoxy master, respectively. The port assembly for PDMS was performed following with some modification of the "reservoir approach" illustrated in Fig. 7. Notably, the procedure was entirely designed without the use of the costly commercially available ports (such as nanoports). The connections, produced with inexpensive commonly available materials in the laboratory, were robust and allowed an easy and resistant system to interface the microfluidic chips to the fluidic equipment.

Interfacing of epoxy polymer devices was performed by the "wall plug inspired approach".[38] In this case, hypodermic needles were inserted in the FEP tubes deeper than the interface between tube and hole in order to leverage the wall plug effect. This method permit to have tight junctions between FEP tubes and microchip inlet/outlet hallowing to work at high pressures avoiding the above described problems. Connection with pumps were achieved by "custom made" luer lock adapter[39] overcoming issues due to company standard dimension of adapters and their relatively high cost. In Fig. 8 is represented an example of connection of epoxy microfluidic chip to fluidic equipment.

As final step, microchannel sealing was carried out, employing an irreversible sealing method (Fig. 9).

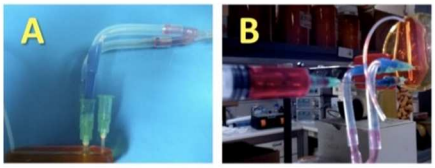

Fig. 8. Microfluidic chip with "wall plug inspired" ports and their connection with "custom made" luer adapter in epoxy resin microchip (B) and connection to syringe by "custom made" luer adapter of epoxy resin microchip (C). For more details on the construction of ports, refer to Chips and Tips (Lab Chip), http://blogs.rsc.org/chipsandtips/page/4/ and http://blogs.rsc.org/chipsandtips/page/5/.[38,39]

PDMS devices are usually sealed by exposure of the PDMS surface to an air/oxygen-based plasma or by treatment with piranha solution,[6;48] but these methods were not in agreement with our philosophy envisaging low cost and safety. In this respect, masters were sealed simply placing them onto a slab of partially cured PDMS or epoxy (depending on the master material).

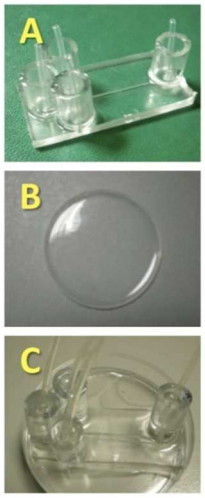

Fig. 9. Photographs of a flow focusing pattern produced with sylgard 184 replica with ports (A), circular PDMS slab (5 cm diameter, 2 mm thickness) (B); sealed PDMS chip after irreversible sealing of master with slab.

Carefully adjusting the curing time of the slab, an irreversible bonding between master and slab can be obtained. For example, Fig. 10 A shown a PDMS master placed onto a partially cured slab (1 h at 30°C), this condition did not result appropriated to accomplish the sealing, the texture of the slab resulted indeed low, hence, the master, sank in depth with consequent occlusion of the microchannels. In contrast, a slab cured for a longer period (12 h at 30°C) resulted too much consolidated, do not allowing the covalent bonding between slab and master (Fig. 10 B). Optimal results were obtained curing the slab for 8 h at 30° C, in this case the master sank only marginally into the slab (i.e. 100-200 μm), resulting in an irreversible sealing and leaving the microchannels open up (Fig. 10 C). Notably the sealing process, based on a partial curing of the slab, did not cause any deformation of the channels, as it was evident both at visual inspection or after optical microscopic observation.

Finally, the assembled microchips were validated by two typical application of microfluidics to pharmaceutics, namely: the production of polymeric microparticles as drug delivery systems and microfibers for cell embedding. To this aim the different liquids were pumped through the microchannels by syringe pumps or employing the technique described by Korczyk and co-workers.[49] This approach uses compressed air and a pressure transducer system to carry liquid inside the microchip.

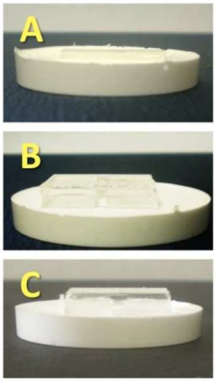

Fig. 10. Photographs of flow focusing pattern produced with sylgard 184 replica placed on Silastic E RTV slab. The replica was placed on the slab after 1 (A), 8 (B) or 24 h (C) of slab curing. Pictures show the different and progressive replica sinking depending on the curing time applied for the slab consolidation.

Microchip validation: production of polymeric microparticles as drug delivery systems and microfibers for cell embedding

Fig. 11 shows a typical PDMS microchip produced with the shrinking approach and the channel schemes of FF (# 1) and of SM (#2) chips that were respectively employed for the production of polymeric microparticles and microfibres.

1. Polymeric microparticles

It is well established that microfluidics offers a versatile route to nano- and micro-particles production.[50] It has been indeed demonstrated that the use of microfluidic chips result in many advantages with respect to conventional bulk methods; they include a better controllability and uniformity of particle characteristics.[51]

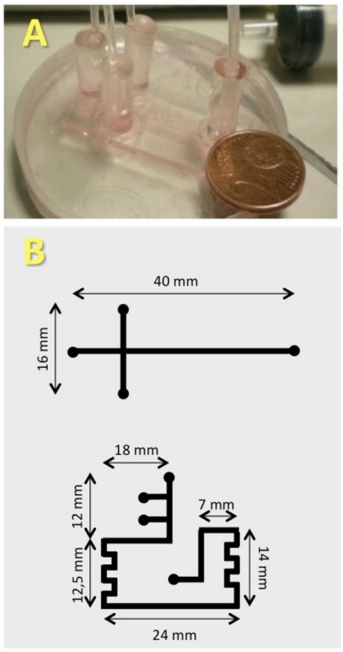

Fig. 11. Photographs of final assembled chip and schematic flow focusing pattern (Chip #1, above) and three inlet "snake"micromixer pattern (Chip #2, below) representation.

In this respect, chip #1 was validated by producing polymeric microparticles intended for drug delivery. The continuous preparation of monodisperse microparticles, constituted of the biocompatible polymer cellulose acetate, was accomplished by an emulsion−in liquid drying process.[52] The process follows a technique based on a combined method of microfluidic emulsification and subsequent solvent diffusion and evaporation. This method does not require toxic components (i.e. chlorinated solvents) and a time-consuming solidification process. The OP was a dispersion of CA in ethyl acetate/acetone that is a good solvent mixture for CA. The WP was an aqueous solution of CP as polymeric emulsifier and thickening agent. The presence of CP enhances indeed the stability of the O/W emulsion.

The OP and WP were separately introduced into the FF device by using a pressure transducer system able to carry liquids inside the microchip, resulting in the formation of monodisperse polymer droplets.

Once pumped into the chip, the OP and WP meet together at the junction of three inlet channels; the surface tension between liquids destabilizes the stationary jet of the OP generating a multiphase flow and, consequently, monodisperse droplets.[15,53] The produced CA containing droplets travel downstream through the main channel and are collected into water filled beaker maintained under magnetically stirring. In reason of the high solubility of acetone in water, once the CA droplets are in contact with the WP, a rapid diffusion of acetone into WP begins. Solvent diffusion leads to a relatively rapid consolidation of the droplets resulting in the formation of solid microparticles. Notably, it was possible to tune the size of the obtained particles by changing the flow rates of both the OP and WP, ranging between 0.080 - 0.250 and 3.0 – 4.5 ml/min, for OP and WP respectively. For instance, Fig. 12 reports a microphotograph of CA microparticles and size distribution obtained with the pumping rates of 0.1 and 4.0 ml/min for OP and WP respectively.

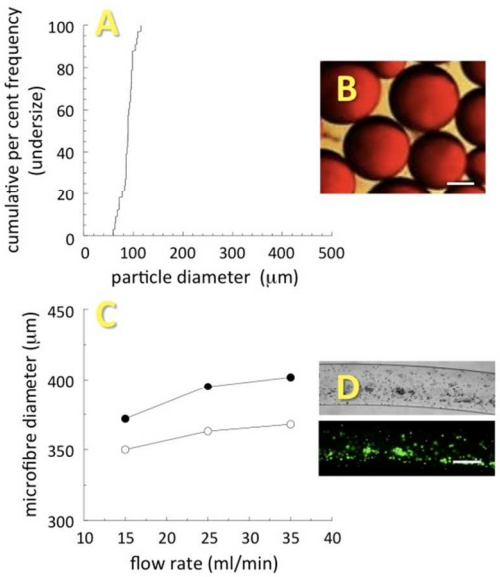

Fig. 12. Cumulative size distribution of CA microparticles prepared using Chip #1. Experimental conditions to prepare microparticles: WP flow rate: 4.0 ml/min; OP flow rate: 0.1 ml/min (A). Optical stereo photomicrographs of CA microparticles. Bar corresponds to 70 μm (B). Effect of flow rate on microfibre diameter produced by Chip #2 The microfibers were harvested in a gelling bath containing 2% (closed circle) or 4% (open circle) w/v of $BaCl_2$ (C). Optical stereo photomicrographs (above) and fluorescence microphotograph (below) of microfibres containing K562 cells (D). Bar represented 300 μm.

2. Multifunctional microfibres

As second example of validation for the produced microchip, alginate microfibres were obtained. Scaffolds in a fibrous form are indeed very appealing for tissue engineering approaches, since they can enable the guided cell growth, alignment and migration.[54] In this respect, we assessed the capability of chip #2 to produce microfibres to embed K562 cells. It is also worth highlighting that a fibre shaped carrier offers an important advantage over spherical shaped carriers since fibres can be easily located and removed from patients when either adverse effects are observed or after the cessation of function.

Notably, chip #2 is characterized by three inlets that allow to varying the content of microfibres, by adjusting the flow rates of independent pumps.

Using Chip #2, a sodium alginate solution and two sodium alginate suspensions were delivered via the three inlets. The two suspensions contained different amounts (10–40 mg/mL) of CA microparticles or cell suspensions (2–9 x 106 cells per mL). The output from the outlet of the chip was transferred via a FEP microtube into a $BaCl_2$ solution (2.0 – 4.0%, w/v) where the Na alginate flow stream was gelled to produce the final Ba-alginate consolidated microfibres. The Ba^{2}+ ions rapidly diffuse into the alginate suspensions causing the almost instantaneous ionic crosslinking, forming the typical egg box structure [55]. Notably, the particular shape of the microchannels allowed to achieve a homogeneous distribution of cells within the entire microfiber volume. Microfibre diameter could be tuned by adjusting the flow rate of alginate solution (data not shown) or using different concentration of Ba^{2}+ ions in the gelling bath (Fig. 12 C). Microfiber containing cells are shown in Fig. 12 D; the microphotograph confirmed the good morphological properties in terms of shape, dimension and surface characteristics. Moreover, the fluorescence photomicrograph recorded immediately after the microfibre preparation (reported

in Fig. 12 D) shows that the cells maintained a very high viability (>95%), indicating that the presented preparation strategy is highly biological compatible and suitable for the encapsulation of cells.

Conclusions

In conclusion, this work confirms that microfluidic technology can provide promising tools for research work and it describes the possibility to access microfluidic techniques using a very low cost procedure. The fabrication method herein described is simple and inexpensive and it could be suitably employed at high school or undergraduate level in developing regions of the world, providing microfluidic technology not only for education purposes, but also for diagnostics and research. We demonstrated the full functionality of the chips by producing polymeric microparticles and multifunctional fibres.

Notes and references

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1 G. M. Whitesides, Nature 442, 368 (2006).
2 A. Tripathi, J. Riddell , N. Chronis, Sens Actuators B Chem 186, 244 (2013).
3 J. Wang J, Talanta 56, 223 (2002).
4 R. Ehrnström, Lab Chip 2, 26N (2002).
5 I. Inoue, Y Wakamoto, H Moriguchi, K Okano, K Yasuda, Lab Chip 1, 50 (2001).
6 J. M. Ng, I. Gitlin, A. D. Stroock, G. M. Whitesides, Electrophoresis 23, 3461 (2002).
7 H. Becker, L. E. Locascio, Talanta, 56, 267 (2002).
8 H. N. Joensson, H. Andersson Svahn, Angew Chem Int Ed Engl 51, 12176 (2012).
9 B. Herranz-Blanco, L. R. Arriaga, E. Mäkilä, A. Correia, N Shrestha, S. Mirza, D. A. Weitz, J. Salonen, J. Hirvonena, H. A. Santos, Lab Chip 14, 1083 (2014).
10 L. Capretto, D. Carugo, S. Mazzitelli, C. Nastruzzi, X. Zhang, Adv Drug Deliv Rev 65, 11 (2013).
11 X.Y. Wang, C. Fillafer, C. Pichl, S. Deinhammer S, R. Hofer-Warbinek, M. Wirth, F. Gabor, Biomicrofluidics 7, 44127 (2013).
12 N. Annabi, S. Selimović, J. P. Acevedo Cox, J. Ribas, M. Afshar Bakooshli, D. Heintze, A. S. Weiss, D. Cropek, A. K. seini, Lab Chip, 13, 3569 (2013).
13 J. H. Sung, C. Kama, M. L. Shuler, Lab Chip 10, 446 (2010).
14 A. S Utada, E. Lorenceau, D. R. Link, P. D. Kaplan, H. A. Stone, D. A. Weitz, Science 308, 537 (2005).
15 P. Garstecki, I. Gitlin, W. Di Luzio, G. M. Whitesides, E. Kumacheva, H. A. Stone, Appl. Phys. Lett. 85, 2649 (2004).
16 W. C. Jeong, J. M. Lim, J. H. Choi, J. H. Kim, Y. J. Lee, S. H. Kim, G. Lee, J. D. Kim, G. R. Yi, S. M. Yang. Lab Chip 12, 1446 (2012).
17 W Engl, R Backov, P Panizza, Current Opinion in Colloid & Interface Science 13, 206 (2008).
18 S. Sakai, S. Ito, H. Inagaki, K. Hirose, T. Matsuyama, M. Taya, K. Kawakami, Biomicrofluidics 5, 013402 (2011).
19 F. L. Jaksch, S. Tay, Current Opinion in Biotechnology 25, 95 (2014).
20 S. Mazzitelli, M. Borgatti, G. Breveglieri, R. Gambari, C. Nastruzzi, J Cell Commun Signal. 5, 157 (2011).
21 S. Mazzitelli, L. Capretto, F. Quinci c, R. Piva, C. Nastruzzi, Advanced Drug Delivery Reviews 65, 1533 (2013).
22 P. N. Nge, C. I. Rogers, A. T. Woolley, Chem Rev 113, 2550 (2013).
23 X. Zhao, T. Dong, Sensors 13, 14570 (2013).
24 K. Ren, J. Zhou, H. Wu, Accounts of Chemical Research 46, 2396 (2013).
25 S.K. Sia, G. M. Whitesides, Electrophoresis 24, 3563 (2003).
26 Younan Xia and George M. Whitesides; Annu. Rev Mater Sci 28, 153 (1998).
27 J. C. McDonald and G. M. Whitesides; Acc. Chem. Res. 35, 491 (2002).
28 B. D. Gates, G. M. Whitesides, J. Am. Chem. Soc 125, 14986 (2003).
29 G. M. Whitesides, E Ostuni, S. Takayama, X. Jiang, D. E. Ingber, Annu. Rev. Biomed. Eng. 3, 335 (2001).
30 C. S. Chen, D. N. Breslauer, J. I. Luna, A. Grimes, W. C. Chin, L. P. Lee, M. Khine, Lab Chip 8, 622 (2008).
31 C.A. Mandon, K. A. Heyries, L. J. Blum, C. A. Marquette, Biosens Bioelectron 26, 1218 (2010).
32 C. W. Yang, E. T. Lagally, Methods Mol Biol 949, 25 (2013).
33 C. W. Yang, E. Ouellet, E. T. Lagally, Anal Chem 82, 5408-14 (2010).
34 C. Willyard, Nat Med 13, 1128 (2007).
35 D. A. Bruzewicz, M. Reches, G. M. Whitesides, Anal Chem 80, 3387 (2008).
36 A. W. Martinez, S. T. Phillips, E. Carrilho, S. W. Thomas, H. Sindi, G. M: Whitesides, Anal Chem 80, 3699 (2008).
37 D. D. Liana, B. Raguse, J. J. Gooding, E. Chow, Sensors 12, 11505 (2012).
38 L. Capretto, S. Mazzitelli, S. Focaroli, C. Nastruzzi, Chips and Tips (Lab Chip), http://blogs.rsc.org/chipsandtips/page/4/ (2010).
39 S. Mazzitelli, S. Focaroli, C. Nastruzzi, Chips and Tips (Lab Chip) http://blogs.rsc.org/chipsandtips/page/5/ (2009).
40 T. Das, D. Chakraborty, S. Chakraborty, Chips and Tips (Lab Chip) http://blogs.rsc.org/chipsandtips/page/6/ (2009).
41 Q. Xu, M. Hashimoto, T. T. Dang, T. Hoare, D. S. Kohane, G. M. Whitesides, R. Langer, D. G. Anderson Small. 5, 157 (2009).
42 P. C. Chen, C. W. Pan, W. C. Lee, K. M. Li, Int J Adv Manuf Technol 71, 1623 (2014).
43 J. H. Luft, J Biophys Biochem Cytol 9, 409 (1961).
44 K. G. Helander, J Microsc 145, 351 (1987).
45 T. Iwadare, H. Mori, K. Ishiguro, M. J. Takeishi, J Microsc. 136, 323 (1984).
46 A. Mata, A. J. Fleischman, S. Roy, Biomed. Microdevices 7, 281 (2005).
47 R. D. Lovchik, H. Wolf, E. Delamarche, Biomed Microdevices 13, 1027 (2011).

48 K. S. Koh, J. Chin, J. Chia, C. Chiang, Micromachines 3, 427 (2012).
49 P. M. Korczyk, O. Cybulski, S. Makulska, P. Garstecki, Chips and Tips (Lab Chip) http://blogs.rsc.org/chipsandtips/page/4/.
50 L. Capretto, D. Carugo, S. Mazzitelli, C. Nastruzzi, X. Zhang, Advanced Drug Delivery Reviews 65, 1496 (2013).
51 K. S. Huang, K. Lu, C. S: Yeh, S. R. Chung, C. H. Lin, C. H. Yang, Y. S. Dong, J Control Release 137, 15 (2009).
52 Thies, C., 1991. Formation of biodegradable drug-loaded microparticles by in-liquid drying processes. In: Donbrow, M. (Ed.), Microcapsules and Nanoparticles in Medicine and Pharmacy. CRC Press, Boca Raton, pp. 47–71.
53 P. Garstecki, H. A. Stone, and G. M. Whitesides, Phy. Rev Lett 94, 164501 (2005).
54 S. Mazzitelli, L. Capretto, D. Carugo, X. Zhang, R. Piva, C. Nastruzzi, Lab Chip 11, 1776 (2011).
55 L. Penolazzi, E. Tavanti, R. Vecchiatini, E. Lambertini, F. Vesce, R. Gambari, S. Mazzitelli, F. Mancuso, G. Luca, C. Nastruzzi and R. Piva, Tissue Eng 16, 141 (2010).