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On-demand generation and removal of alginate biocompatible microvalves for flow control in microfluidics

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ABSTRACT

This paper describes, for the first time, the use of alginate hydrogels as miniaturised microvalves within microfluidic devices. These biocompatible and biodegradable microvalves are generated *in situ* and on demand, allowing for microfluidic flow control. The microfluidic devices were fabricated using an origami inspired technique of folding several layers of cyclic olefin polymer followed by thermocompression bonding. The hydrogels can be dehydrated at mild temperatures, 37 °C, to slightly open the microvalve and chemically erased using an ethylenediaminetetraacetic acid disodium salt (EDTA) solution, to completely open the channel, ensuring the reusability of the whole device and removal of damaged or defective valves for subsequent regeneration.

1. Introduction

Lab on a Chip (LOC) it is a multidisciplinary area of science that covers chemistry, physics, engineering and biotechnology, claiming the miniaturisation of devices for fluidic handling and target detection. The driving force behind this miniaturisation is to enhance the performance gained by down-scaling analytical systems and to integrate multiple components into a single device [1].

LOC devices offer many advantages compared to other traditional analytical platforms; for example, the reduced dimensions of microfluidic components allow for the manipulation of small volumes of fluids which leads to less reagent consumption, reduced costs and less waste generation. The reduced dimensions of these devices allow for temperature to be controlled and changed quickly because of the low thermal mass and large surface to volume ratio of microfluidics, which facilitates heat transfer and enables the creation of portable devices for *in situ* testing. Moreover, the ability to couple multiple channels together, facilitates high sample throughput on multicomponent devices and so decrease analysis times [2]. It is this potential of integration of multiple components which leads to the Micro Total Analysis System concept, where the sampling, fluid handling, detection and report of the results take place in a single run.

The current state-of-the-art for microfluidic devices is based on flow systems that employ traditional pumping, valving and mixing components. These systems are generally expensive, difficult to integrate into a microfluidic device and, most of the times, can only be controlled from external sources, as for example solenoid valves [3,4]. Nevertheless, research is focussed on improving these microfluidic components with novel valves such as “Quake” PDMS micro-valves [5], “Doormat” valves [6] and check valves [7] among others, recently appearing in literature. These valves have been proven to be effective and cheaper but still need to be designed within the microfluidic device and/or integrated after the device is ready. The on demand, *in situ* generation of microfluidic valves, and their subsequent removal after use, has not been reported yet. Therefore, a novel field of microfluidic research to develop novel flow systems that are cheaper and easier to fabricate by employing these types of microvalves is emerging.

An intriguing alternative for microvalve integration within microfluidic devices is the use of smart materials for fluid handling and flow control [8]. In particular, hydrogels are network polymer chains that are highly water absorbent and posses a substantial degree of flexibility. The ability of hydrogels to absorb water arises from hydrophilic functional groups attached to the polymeric backbone, while their durability comes from the cross-links between

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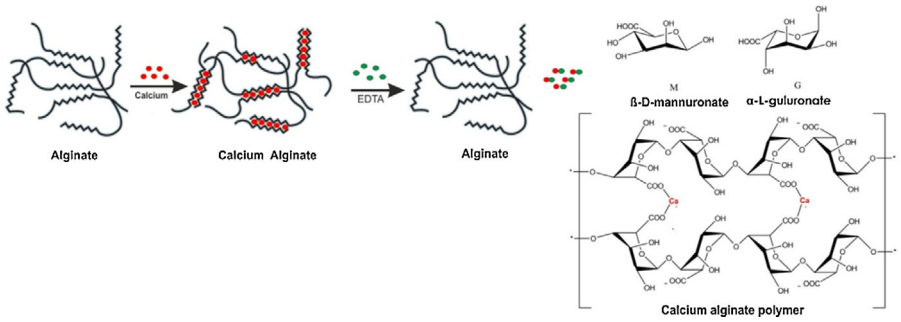

Fig. 1. Schematic of the calcium alginate polymer formation and chemical structure of the calcium alginate formation.

the network of polymer chains of the hydrogel [9]. A particular class of polymers can be identified as stimuli-responsive materials due to their ability to undergo volumetric changes in response to physical and chemical changes in their local environment [10]. These materials are able to change these properties, in a controllable manner, and has subsequently resulted in these hydrogels being used as microvalves in microfluidic devices (*e.g.* polyacrylamide gels). The chemistry used to synthesise hydrogels, and their integrated stimuli responsive compounds, is as diverse as their potential applications.

Calcium alginate is a water-insoluble hydrogel formed from linear copolymers of anionic polysaccharide (water soluble) and calcium cations that can chelate carboxylate groups and create crosslinkages between chains. The gelling properties of alginate depend strongly upon its monomeric composition, block structure, molecular size and concentration of polymer and calcium ions [11]. Alginate polymers are one of the most extensively used biomaterials in science due to their biocompatibility and biodegradability [12] and are extremely versatile biopolymers with a variety of technical applications in biomedical [13], pharmaceutical [14] and extensively within the food industry as a powerful thickening, stabilising, and gel-forming agent. Surprisingly, the microfluidic applications of alginates are rarely reported, with rudimentary usage such as for reagent storage within chips and for the fabrication of microcapsules in drug delivery systems [15].

Recent advances in microtechnology for biomedical applications, with products making it to market [16], have increased the necessity to integrate stimuli responsive materials with biocompatible capabilities within microfluidic devices, therefore gels obtained from natural polymers are good candidates [17]. In particular the use of smart materials as actuators, with innocuous chemical characteristics, will create an opportunity for the generation of novel microfluidic devices for biological applications.

This study demonstrates, for the first time, the use of calcium alginate hydrogels as miniaturised valves within microfluidic devices, as good alternatives to conventional hydrogel microvalves. These biocompatible and biodegradable microvalves can be generated *in situ* and on demand allowing for microfluidic flow control. As calcium alginate is dehydratated at room temperature (syneresis process), it can be thermally actuated at mild temperatures, slightly unblocking the channel and, in turn, restoring the flow rate. The alginate hydrogel can also be chemically erased from the main channel simply using an ethylenediaminetetraacetic acid disodium salt (EDTA) solution, ensuring the reusability of the whole device.

2. Experimental

2.1. Materials

Sodium alginate and dehydrated calcium chloride were purchased by Sigma-Aldrich (St Louis, MO, USA) and were used to prepare calcium alginate hydrogel. Ethylenediaminetetraacetic acid disodium salt (EDTA) was purchased by Merck (Darmstadt, Germany) and was used to chemically erase the calcium alginate hydrogel. All the solutions were prepared using deonised (DI) water from a Milli-Q water purification system (Millipore, Milford, MA).

Isopropyl alcohol (IPA) was purchased by Panreac Química S.L.U. (Spain). Rolls of 100 μm thick of COP films (ZeonorFilm®) were obtained from Zeon Chemicals (Düsseldorf, Germany). This material was chosen because of its desirable properties such as transparency, biocompatibility and resistance to acid and base.

Accura Amethyst was purchased from (3D Systems) for the fabrication of the luers by stereolitography technique (Viper SLA systems, 3D Systems) and were fixed to the device with screws.

The flow in the microfluidic device was controlled using a WPI SP120PZ syringe pump (Shangai, China). Alternatively, solutions were injected with a Harvard Apparatus model 11 elite syringe pump (Holliston, MA, USA). A MFCS™-EZ pressure driven flow controller (Fluigent, Paris, France) was used for the controlled injection of solutions. A SLG-0075 flow sensor (Sensirion CMOSens©, Switzerland) was connected for the monitoring of solutions inside the microfluidic device.

2.2. Chip fabrication

Microfluidic devices were designed and fabricated by the *Origami* technique [18] by rapid prototyping using the FC-8000-60 cutting plotter from Graphtec (Irvine, CA). The 3D design was cut into several 2D layers using the cutting plotter, assembled by folding and stacking of layers and then bonded by thermocompression [19].

Stereolithography 3D-printed interconnectors for fluidic handling were in-house fabricated and fixed to the device with screws [www.microliquid.com]. These interconnectors are connected to ICO + 3, 1 mL syringes (Novico Medica, Barcelona, Spain) and/or to male PMMA luers (Chipshop, Jena, Germany) with Tygon 0.8 × 2.4 mm tubes (Colmer Palmer, Vernon Hills, USA).

2.3. Hydrogel preparation

For the hydrogel preparation, typically water solutions of 4% of calcium chloride and 1% of sodium alginate were prepared [20]. When the sodium alginate is added to a calcium chloride solution, the calcium ions replace the sodium ions in the polymer and jellifies. The “egg-box model” for the formation of alginate polymers in the presence of alkaline metals was first described by Rees et al. [21] see Fig. 1.The model describes that divalent cations, such as Ca^{2+}, are coordinated with in the cavities of alginate chains [22]. A second alginate strand can also connect at the calcium ion, forming a link in which the calcium ion attaches two alginate strands together. The result is a chain of calcium-linked alginate strands that form a solid polymer.