

Blister pouches for effective reagent storage and release for low-cost point-of-care diagnostic applications

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ABSTRACT

Lab-on-a-chip devices are often applied to point-of-care diagnostic solutions as they are low-cost, compact, disposable, and require only small sample volumes. For such devices, various reagents are required for sample preparation and analysis and, for an integrated solution to be realized, on-chip reagent storage and automated introduction are required. This work describes the implementation and characterization of effective liquid reagent storage and release mechanisms utilizing blister pouches applied to various point-of-care diagnostic device applications. The manufacturing aspects as well as performance parameters are evaluated.

Keywords: Lab-on-a-chip, point-of-care diagnostics, microfluidics, reagent storage, blister pouches

1. INTRODUCTION

Microfluidics as a key enabling technology for many applications in the life sciences and diagnostics has made tremendous progress in recent years. Notably in this respect is the large activity in commercialization this technology is experiencing, translating academic results into commercial products [1,2]. In particular, the field of point-of-care (POC) diagnostics is one of the key drivers of this development [3]. The ability to translate complex protocols, such as those in molecular diagnostics, into an integrated sample-in-answer-out cartridge which does not need any significant hands-on time from the user and can be operated by relatively unskilled personnel, is one of the key arguments for the uptake of microfluidics technology in this field and a fulfillment of one of the earliest promises of the micro total analysis system (μ TAS) concept. While much of the academic work in microfluidics has been focusing on the development either of certain microfluidic “circuit elements” fulfilling functionalities such as mixing, droplet generation, or separation, many areas which are of great relevance for commercial products and indispensable for such integrated devices have not been addressed with the same intensity. Examples for such areas are fluidic interfaces [4], sample preparation [5] and reagent storage [6].

In particular the latter, reagent storage, has attracted significant interest for the development of commercial diagnostic cartridges. The reasons for this interest can be found in a variety of requirements arising from the application area:

- a) Many diagnostic assays employ different reagents. While these can be handled by pipetting robots in the case of central laboratory assays, the possibilities for such liquid handling is greatly reduced in benchtop or even portable POC systems.
- b) POC diagnostic tests are usually carried out by means of a disposable cartridge or cassette. In order to avoid contamination or carry-over issues, it is highly desirable to generate a system which does not have any fluidic or pneumatic interfaces between cartridge and instrument. Such a general system architecture would also negate the need for washing steps of any system components in between subsequent patient assays.
- c) The overall instrument requirements and ultimately the resulting instrument cost can be significantly reduced if only mechanical or optical/electrical (for detection) interfaces between the cartridge and instrument have to be implemented.

For the storage of reagents required to perform the diagnostic assay, two main application cases have to be distinguished: 1) dry reagent storage and 2) liquid reagent storage. The first case mainly applies to reagents such as polymerase chain reaction (PCR) master mixes which can be lyophilized or antibodies which can be air dried. In this paper, we will concentrate on a solution for the storage of liquid reagents using blister, as this covers a wide range of physical as well as chemical parameters.

2. TECHNOLOGICAL ASPECTS

2.1 Blister manufacturing

Blister can be defined in contrast to pouches or film packs as having a self-sustaining shape even without being filled with a reagent. For this reason, the blister material has to have a sufficient thickness to sustain the final shape while still being soft enough to eventually be compressed for blister emptying. Blister typically consist of two components: the structured blister dome which defines the volume of the blister, and the blister bottom film which is used to enclose the reagents. Compound films, consisting of a material stack typically made out of polypropylene (PP), aluminum and another PP layer (alternatively polyethylene terephthalate (PET) or polyamide (PA)) with a typical thickness of between 75 and 150 μm are well suited for blister dome formation. In order to shape the blister dome, and thus define its volume, a thermoforming process [7, 8] is used. The film is heated to a temperature above the glass transition temperature of the polymer and is then, either by using vacuum, pressure or a mechanical stamp, brought into its final shape which it retains after cooling back down to room temperature. The advantage of the thermoforming process is its rapid cycle time which, in the case of the blister dome production, is of the order of a few seconds. Limitations arise from the elastic limit of the compound film which defines a maximum extension before breakage. Specifically, the integrity of the aluminum film has to be secured in order to keep the barrier functionality of the blister intact. Additionally, the feature fidelity or minimum feature size is comparatively poor when compared to other polymer replication technologies such as injection molding or hot embossing [9]. However, this is not critical in the case of blister formation as the dimensions are typically of the order of mm. Figure 1a shows an exemplary computer-aided design (CAD) drawing of a 200 μl blister, Fig. 1b a thermoforming stamp for 150 μl blisters and Fig. 1c a set of completed blisters of different volumes.

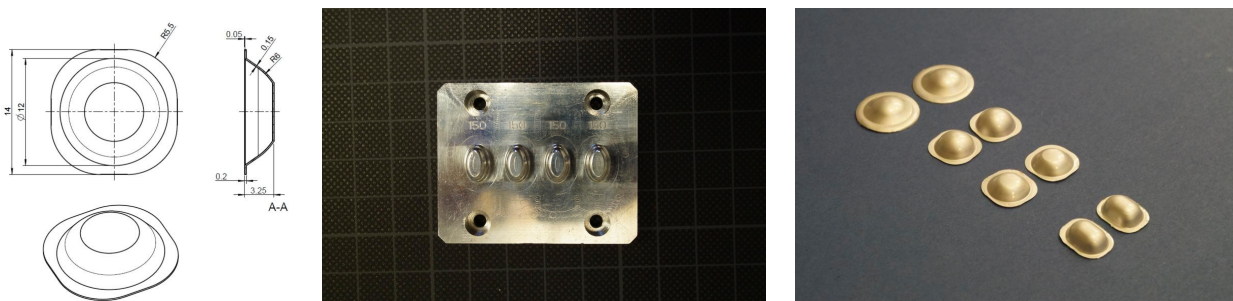


Fig. 1: a) CAD drawing of a 200 μl blister, b) thermoforming tool with 4 plungers, and c) finished blisters with different volumes.

The next step is the filling of the blister with the desired reagents. This is usually performed using a single channel or in production a multichannel, pipetting robot. The filling speed has to be chosen as to avoid splashing. The exact filling volume is achieved by balancing two surface tension effects: a) at the interface between the polymer foil and the liquid reagent a meniscus is formed, which, due to the normally hydrophobic nature of the film, generates a convex liquid surface with a contact point below the sealing plane of blister film. b) The surface tension of the liquid itself which creates an overall convex liquid surface across the blister diameter, with a maximum liquid level height above the sealing plane of the blister film. In order to achieve an air-free filling of the blister, these two effects have to be balanced, with an equilibrium value depending on the reagent used as well as the filling temperature.

Once the blister dome has been filled with the reagent, it is sealed with the bottom film. This process is usually a heat-welding process in which the polymer layers of the blister dome and the bottom film are welded together. As this process is very fast (<3 s), no noticeable temperature increase occurs in the liquid itself, so even temperature sensitive reagents such as antibody solutions for immunoassays can be encapsulated using this method.

2.2 Blisters as cartridge components

As with practically all elements in microfluidics, blisters cannot be seen as stand-alone components without an overall system consideration. In the case of blisters, this concerns the design of the blister housing, the blister mounting itself, blister protection structures and, most importantly, the mechanism of breaching the blister.

There are two main mechanisms to dispense the liquid from a blister: a) so-called “frangible seals” (or “preferential breach”) and b) piercing. In the case of frangible seal blisters, the sealing between the blister dome and the bottom film is structured in such a way that it bursts at a defined location at a defined pressure. Such blisters have been described, for

example, in [10] and [11]. This approach faces two main challenges. Firstly, on the manufacturing side, the process parameters for the blister sealing have to be tightly controlled, as small variations in the sealing process (force, temperature, material, geometrical tolerances) lead to large variations in burst pressure. The second challenge comes from the fact that upon bursting, the outflow of the blister is very non-uniform, with a large spike directly after rupture of the blister, followed by a rapid decrease in flow rate. This flow-rate variation can be somewhat alleviated by including a flow-restrictor structure [12] in the microfluidic design. However, this adds to the dead-volume of the circuitry as well as using real-estate on the cartridge which, especially in case of multiple reagents, can be a serious constraint. For this reason, we have opted to use blisters in conjunction with a piercing structure which is implemented in the microfluidic device. Fig. 2a shows the cross-section of such a needle structure, Fig. 2b an actual image of the microfluidic device.

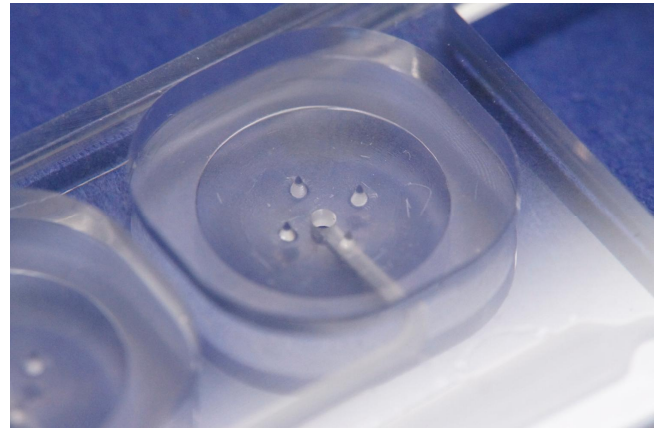
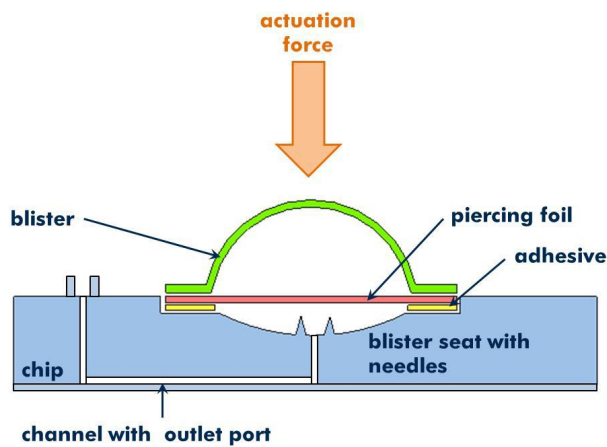


Fig. 2: a) Schematic cross-section of the blister mounted onto a piercing structure, and b) actual image of a piercing structure.

As these needles can be made as an integral part of the injection-molded cartridge, they do not need a separate assembly step but are an integral part of the manufacturing process. A force acting upon the blister will lower the whole blister onto these needles, puncturing the bottom film. The big advantage of this approach is a high degree of reproducibility and a comparatively broad parameter range during manufacturing, as only the material of the blister bottom, the piercing foil (which can be specifically chosen for easy penetration) and the radius of curvature of the needle structures have an influence on the opening force. Furthermore, for most blisters, multiple needles are used, creating a fairly homogeneous flow out of the blister.

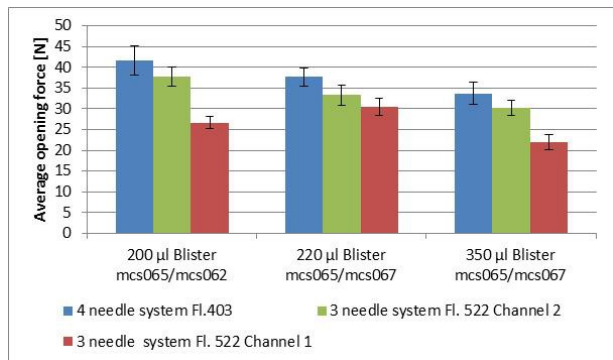
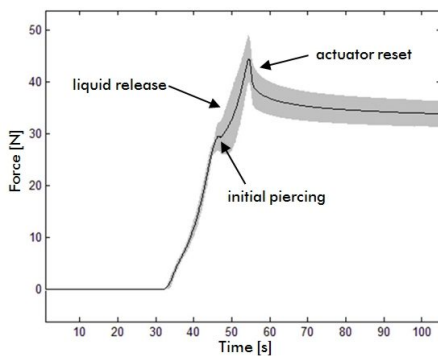


Fig 3: a) Force curve of blister actuation, and b) opening forces depending on blister volume and channel geometry.

An example of a force-time diagram for the opening process can be seen in Fig. 3a. An electrically driven actuator presses on to the blister which is pierced at $t = 45$ s. After the initial piercing, the actuator continues to press on to the blister until it reaches its maximum force upon complete blister clearance. The actuator keeps pressing on the blister in order to prevent a liquid backflow due to the elastic nature of the blister film material. It can be observed that, if the actuator is kept depressed, the blister relaxes somewhat and draws liquid back into the area underneath the blister. This can be prevented by keeping the actuator depressed until the end of the protocol. Fig. 3b shows typical variations of the

actuation force depending on the blister volume and microfluidic geometry. The general trend is an increase in actuation force with decreasing blister volume which can be explained with an increasing “stiffness” of the smaller blisters and a noticeable positive correlation of the actuation force with the hydrodynamic resistance of the microstructure (Channel 2 has a lower hydrodynamic resistance compared to Channel 1). The error bars are as a result of the variation over 10 measurements.

Another important parameter is the actual dispensed volume from the blister. While the blisters have a defined geometrical volume, due to the fact that the blister film will collapse during compression and create folds, not all of the volume initially contained within a blister will actually be dispensed, even if the actuating plunger is kept depressed throughout the dispensing process. Fig. 4a shows the absolute actual amount of dispensed reagent, and Fig. 4b the amount relative to the nominal volume. These measurements show that there is a practical lower limit for blister volumes, as the retained volume fraction increases below a threshold of about 100 μl .

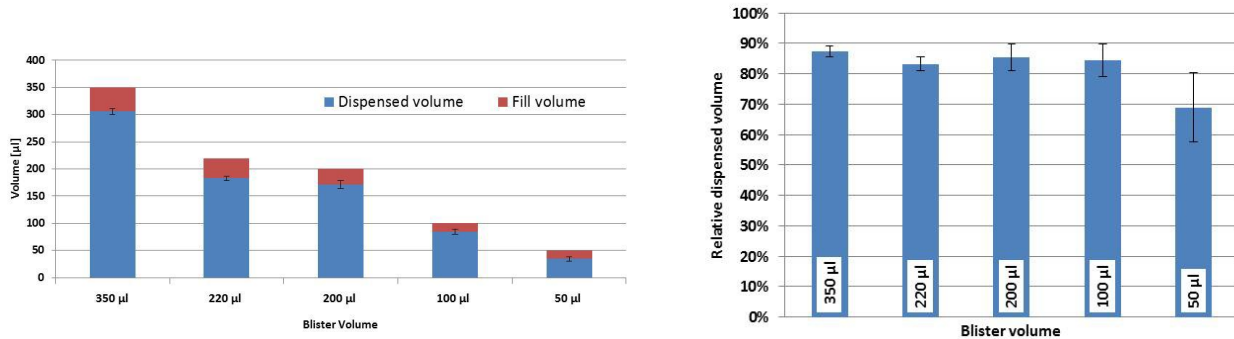


Fig. 4: a) Actual dispensed reagent volume from different blisters, and b) fraction of the nominal volume dispensed.

3. APPLICATION EXAMPLES

Some practical application cases are shown in Fig. 5a-c. Fig. 5a shows a cytometry cartridge which allows the detection of magnetically labelled cells in a complex matrix such as whole blood without sample preparation by using a sensor based on the GMR (gigantic magnetoresistance) effect. The blister contains the magnetic beads for labelling the cells. It can be noted that a barrier is included around the blister for protection. In Fig. 5b, a cartridge for a POC blood count chip for hematology applications is shown, as reported on previously [13,14]. In this case, the blister contains lysis buffer and a staining reagent. Fig. 5c shows the possibility of integrating a large number of blisters with different reagents on to a device. In this case, seven different reagents are stored on a cartridge which performs an analysis of tumor-associated μRNA clusters.



Fig. 5a-c: Application examples for diagnostic cartridges employing blisters.

4. CONCLUSIONS

In this paper, we present design, manufacturing, performance and application information on blisters for liquid reagent storage in microfluidic devices. They represent an important component for microfluidics-enabled POC diagnostic

devices, especially in the case of sample-in result-out systems. They supersede the need for external manual or automatic addition of liquid reagents into a cartridge during the implementation of an assay. This enables the removal of any liquid or pneumatic interfaces between instrument and cartridge, thereby reducing instrument complexity as well as contamination risk. The presented blisters have to be treated as part of a complete system, namely in conjunction with an actuation and breaching mechanism. Based on our experiments, the use of piercing structures to open the bottom film of the blister is preferable over blisters having a frangible seal, as the dispensing of the liquid is more homogeneous and no additional flow regulating structures have to be employed.

This technology is yet another building block for microfluidics as an enabling technology for POC molecular diagnostic tests which is most likely to become part of the much sought-after “killer-application” in microfluidics [15].

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