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Long-Term On-Chip Storage and Release of Liquid Reagents for Diagnostic Lab-on-a-Chip Applications

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***Abstract*—**A new concept for long-term reagent storage for Lab-on-a-Chip (LoC) devices is described. Here we present a polymer multilayer stack with integrated stick packs for long-term storage of several liquid reagents, which are necessary for many diagnostic applications. Stick packs are widely used in packaging industry for storing solids and liquids for long time. The storage concept fulfills two main requirements: First, a long-term storage of reagents in stick packs without significant losses and interaction with surroundings, second, on demand releasing of liquids, which is realized by pushing a membrane against the stick pack through pneumatic pressure. This concept enables long-term on-chip storage of liquid reagents at room temperature and allows an easy implementation in different LoC devices.

***Keywords*—**Lab-on-a-Chip, long-term storage, reagent storage, stick pack.

I. Introduction

MOLECULAR diagnostic LoC devices are cost- and time-saving options compared to conventional laboratory work flows. There are several possibilities to provide the required liquid reagents for diagnostic processing in LoC applications. Storing reagents on-chip in disposable LoC cartridges has several benefits, e.g. full automated processing, less misuse possibilities, reduction of (cross-) contamination risks, no costs of additional reagent storage and easy transportation. One of the major challenges is the long-term storage of liquid reagents in disposable LoC cartridges [1].

Since most LoC platforms are made of polymers, long-term storage of highly volatile reagents directly in cartridges is difficult due to low barrier properties of commonly used polymers (e.g. ABS, PC, PS, PP) [2]. Small fluid losses can change reagent mixtures in a way that bioanalytical reactions required for LoC-applications can be prevented. One possibility to overcome these circumstances is to store the whole LoC cartridge with integrated liquid reagents at low temperatures (e.g. -20 or 4°C). In this case the operator has to guarantee a continuous cold chain during transport and storage, which implicates disadvantages with respect to feasibility. Furthermore contamination of reagents stored in nearby chambers must be prevented to assure accurate diagnostic processing.

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There are promising concepts for long-term reagent storage, for example integrated glass ampoules, blisters or stick packs, which show high barrier properties based on their specific assembly. The challenge is to integrate these concepts together with a reliable opening mechanism on LoC platforms to ensure subsequent microfluidic processing.

Reagent storage in glass ampoules is demonstrated in [3], [4]. Liquid reagents are stored in glass capillaries, which are integrated on a centrifugally actuated LoC-disk. The glass ampoules are crushed by mechanical compression. Then, the released liquids are transferred via a filter membrane to other chambers through centrifugal actuation of the disk.

Concerning the storage of reagents in stick packs, a centrifugally-driven opening mechanism is shown in [5]. Here stick packs are integrated on a centrifugally actuated disk instead of glass ampoules. Delamination of peel seam begins at a defined hydrostatic fluid pressure, which can be adjusted by different rotation frequencies of the disk. The variation of different peel seam parameters during stick pack production enables releasing the liquid reagent at different frequencies.

For LoC platforms based on pressure driven microfluidic processing the stick pack reagent storage concept has to be adapted. In [6] a multilayer pressure driven microfluidic platform is presented. The polymer multilayer stack including a thermoplastic membrane is joined by laser radiation welding. The membrane can be actuated through pneumatic pressure or vacuum, which is needed for the integration of microvalves and for complex fluid management. Additionally it is possible to transfer liquid reagents in µl-scale out of chambers by actuating the membrane. The same principle can be used for releasing reagents out of stick packs. In this set up the stick pack is placed in a chamber in the multilayer stack. In initial state the elastic membrane, which is located above the stick pack, is unstressed. By supplying pressure to the pneumatic layer, the membrane begins to expand until it touches the upper side of the stick pack. Then the pneumatic pressure of compressed air is transferred to the stick pack, which leads to delamination of the peel seam. The expanding membrane pushes the liquid reagent out of the stick pack and is collected in an extra chamber for further microfluidic processing. In this work long-term on-chip storage of reagents in stick packs including pressure-driven opening mechanism for LoC-applications is investigated.

II. Methods and Materials

A. Stick Packs

Stick packs can be described as small tubular bags which can be used for storage of solid or liquid substances. They are widely used in the packaging industry and commonly consist of polyethylene composite films which are sealed at both ends by hot embossing or ultrasonic welding. High barrier properties are realized by an aluminum layer included in the composite film forming the stick pack. To ensure mechanical steadiness most stick packs have an outer third layer (e.g. PET), which protects the aluminum layer from damaging. Different applications of stick packs require different layer compositions, which can be realized easily by selecting convenient standard composite films. The stick packs used consist of a 3 layer film formed by a PET layer (12µm), an aluminum layer (20µm) and a PE layer (50µm) for inner sealing. Since barrier properties of the aluminum layer are sufficient for long-term storage of liquids, the inner sealing layer of PE can be identified as the main reason for leakage. In this work, stick packs have three different seams: a peel seam for reagent releasing and two sealed seams with cross and longitudinal alignment (Fig. 2). To prevent delamination of the sealed seam under pressure, different sealing parameters are chosen for each seam. The strength of peel and sealed seam can be controlled by sealing pressure and temperature during stick pack production. The footprint of stick packs used is 35mm x 15mm. The maximum reagent volume is 500µl. The thickness of filled stick pack is approximately 5mm. Relating to reagents commonly used in diagnostic applications, in this work stick packs are filled with water (H_2O), alkaline wash buffer (wb) and ethanol (EtOH) to make representative evaluations.

B. Multilayer Stack and Integration Concept

The stick pack including the reagent is placed in a polymer multilayer stack, which consists of a fluidic layer, an elastic membrane and a pneumatic layer. The multilayer stack can be joined by laser radiation welding at $\lambda = 1064$nm. The elastic membrane is black colored and forms the absorbing join partner, whereas the pneumatic and fluidic layers are transparent for laser light at the mentioned wavelength. Therefore it is possible to join the multilayer stack by laser radiation welding from the upper and bottom side. In Fig. 1 an exploded view of the multilayer stack is shown. To provide the reagents for further microfluidic processing, the liquids are collected in an extra reagent chamber after releasing.

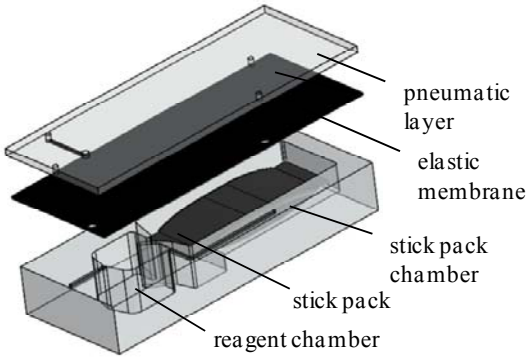

Fig. 1 Exploded view of polymer multilayer stack consisting of pneumatic layer, elastic membrane and fluidic layer with integrated stick pack

Since the multilayer setup has to withstand pressures up to 2 bars, the bonding of the polymer pneumatic layer, fluidic layer and the elastic membrane should demonstrate strong adhesion forces. In this work thermoplastic polyurethane (TPU) material is used as elastic membrane with a thickness of 100 µm. Pneumatic and fluidic layers consist of polycarbonate with thickness on the mm-scale. Bonding of these layers by laser radiation welding showed sufficient adhesion. Besides laser welding other bonding methods are possible.

C. Operation Mode

Fig. 2 shows the top view of the multilayer stack. Here the black elastic membrane is hidden due to reasons of illustration. In the red labeled area the elastic membrane is rigidly fixed to the pneumatic layer by laser welding, since the membrane should not reach into the reagent chamber or push against the left peel seam when pressure is supplied. In the remaining stick pack chamber the membrane is free to move.

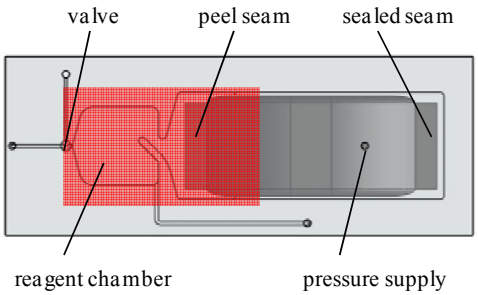

Fig. 2 Top view on multilayer stack (black elastic membrane is not shown)

To illustrate the operation mode of the elastic membrane, Fig. 3 shows three different states from side view. In initial state Fig. 3 a) the membrane is in its passive state. In Fig. 3 b) a pressure of $p = 1.9$ bar is supplied, which leads to expansion of the membrane. When the membrane contacts the upper side of the stick pack, hydraulic pressure is transferred to stick pack and the peel seam gets stressed mechanically. If the pressure supplied is sufficiently strong, resulting stress on peel seam leads to delamination (state Fig. 3 c)). Then the liquid

reagent is released and collected in the reagent chamber. Finally, providing reagents in stick packs for long-term storage is pressure-driven and no further actuation is required. In practice the multilayer stack including stick packs is aligned vertically, so due to gravitation liquid reagents (blue colored) are collected as shown in Fig. 3 c).

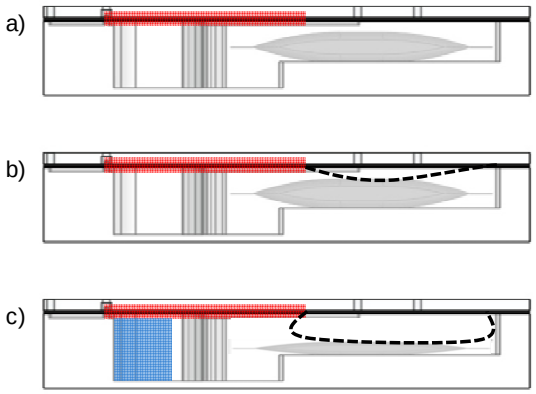

Fig. 3 Operation mode of multilayer stack: a) passive state b) actuation of membrane c) mechanical stress leads to delamination of peel seam and liquid reagent is collected in reagent chamber

III. Experimental Setup

The ability of stick packs for long-term storage and reliable opening behavior in the described polymer multilayer stack are a focus of this work. To investigate long-term storage, stick packs including different liquids (H_2O, wb, EtOH) were exposed to several thermal conditions for predefined periods. The mean reagent loss through permeation was calculated by the reduction of weight of 10 stick packs for each reagent, regarding the average empty weight of stick packs.

A. High Temperature Storage

Stick packs were stored at high temperatures for 35 days. One the one hand stick packs filled with water and wash buffer were stored at 70°C. One the other hand a lower storage temperature of 35°C was chosen for ethanol-filled stick packs due to its boiling temperature of 78°C. High temperature storage was made in a thermal oven (HORO, Hofmann GmbH). For each reagent 10 stick packs were tested.

B. Cumulative Storage

For accelerated aging stick packs (n = 10 for each reagent) were stored successively at 4 different conditions: 20 temperature shock cycles (-20°C/37°C à 20 min) in a thermo-shock chamber (TSS -70/130, CTS GmbH), low temperature storage at -80°C (Herafreeze HFU 686 Basic, Thermo Fisher Scientific) for 30 days, high temperature storage at 37°C for 35 days and low pressure storage at 600 mbar in an exsiccator for 72h.

C. Opening Delay

Opening behavior was investigated for stick packs stored at room temperature (n = 10 for each reagent) and for stick packs (n = 5 for each reagent) stored at different thermal conditions (cumulative storage) to prove reliable reagent releasing. The opening delay was measured manually with a stop watch. The mean value of opening delay was calculated for each reagent and storage conditions (room temperature and cumulative storage). To supply the required pressure of 1.9 bar for stick pack opening a pressure controller (DPI 515, Druck Ltd.) was used. Since the build-up of the polymer multilayer stack cartridges based on laser radiation welding for each stick pack is too complex for testing more than 50 stick packs, the multilayer stack including the membrane was clamped in a demountable test stand device with same dimensions. For this reason a high number of stick packs could be tested for reliable opening. However evaluation models joined by laser radiation welding were built up as well and showed similar results, which verifies the test stand.

IV. Results and Discussion

A. Storage Tests

Stick packs stored at high temperatures for 35 days show no significant weight losses. Reagent losses for storage of H_2O and wash buffer at 70°C are below 0.3% and for storage of EtOH at 37°C the loss is below 0.1%. For example, extrapolating the results for storage at high temperatures to a period of 2 years, resulting reagent losses remain below 5%, which should be good enough for most LoC-applications.

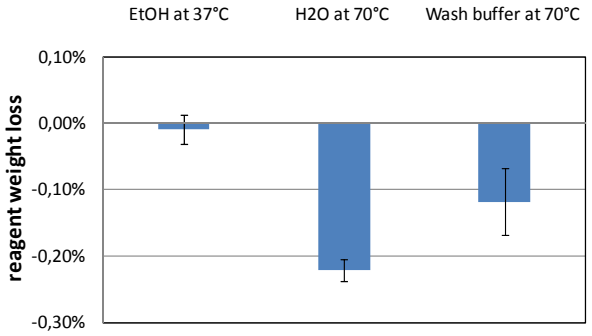

Fig. 4 Reagent mass loss after storage of 35 days at high temperatures for EtOH (37°C), H_2O (70°C) and wash buffer (70°C)

Beside storage at high temperatures, cumulative storage tests of stick packs with the same reagents were made for accelerated aging. Particularly storage at low pressure is essential to prove ability for air transport. Since the opening mechanism of stick packs is pressure-driven, the peel seam has to be robust against delamination through external pressure fluctuations. Fig. 5 shows that resulting mass losses of each reagent after cumulative storage are less than 0.1%. As expected, the highest mass loss of 0.08% was measured for ethanol-filled stick packs, whereas H_2O and wash buffer show similar results.

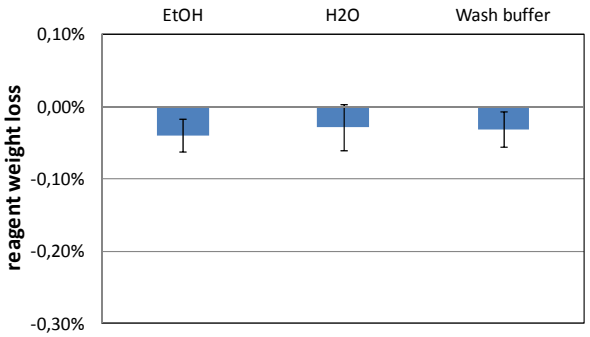

Fig. 5 Reagent mass loss after cumulative storage at different conditions for EtOH, H_2O and wash buffer: 20 temperature shock cylces (-20°C/37°C à 20 min), low temperature storage at -80°C for 30 days, high temperature storage at 37°C for 35 days and low pressure storage at 600 mbar

Compared to storage tests at 70°C it is obvious that high temperatures are the main reason for reagent loss in stick packs. However long-term storage of reagents in stick packs can be guaranteed. To which extent accelerated aging of the peel seam determines the opening mechanism is discussed below.

B. Opening Delay

In addition to the ability for long-term storage, stick packs with all three reagents were investigated for reliable opening. A pneumatic pressure of p = 1.9 bar was used for opening and releasing reagents. A repeatable opening of stickpacks for each reagent was shown. Results of opening delays for stick packs stored at room temperature and after cumulative storage for accelerated aging are shown in Fig. 6. The mean values of opening delay are different for each reagent. Concerning the storage at room temperature, stick packs filled with EtOH showed short opening delays ($\bar{t}_{EtOH}$ = 2,4 s), whereas opening delays of stick packs filled with water-based wash buffers were 4 times higher on average ($\bar{t}_{wb}$ = 9,7 s, $t_{max, wb}$ = 25 s).

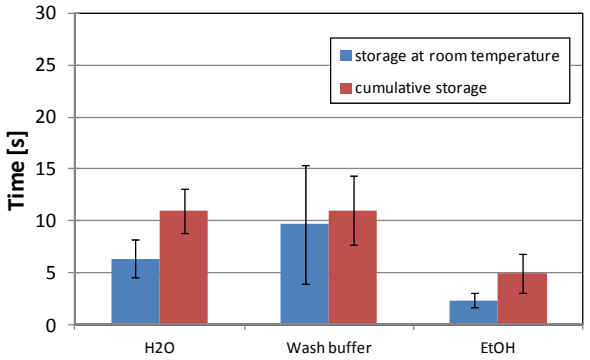

Fig. 6 Opening delay of stick packs filled with H_2O, wash buffer and EtOH with pressure p = 1,9 bar after storage at room temperature (n = 10) and cumulative storage (n = 5)

Stick packs after cumulative storage show a similar dependence of reagents to opening behavior. Here, accelerated aging of peel seams leads to a retarding delamination, which leads to increasing opening delays. Nevertheless, reliable opening of stick packs even after high thermal stress could be demonstrated.

V. Conclusion

In this work we presented a pressure driven, polymer multilayer lab-on-chip stack with integrated stick packs for long-term on-chip storage of liquids. Extrapolating the results of stick pack storage at high temperatures and accelerated aging, a long-term storage of liquids in stick packs over years is possible. Cumulative storage of stick packs including cycles of high thermal stresses show reagent losses below 0.1%. Even highly volatile liquids containing EtOH can be stored in stick packs with minimal losses. Furthermore, a repeatable pressure-driven opening of stick packs could be demonstrated. Accelerated aging of peel seam leads to retarding stick pack opening, but does not affect the reproducibility of opening behavior. The opening delays of stick packs stored at room temperature are assimilable to stick packs after cumulative storage and keep below 30s for each reagent. This concept enables on-chip storage of liquid reagents at room temperature and allows an implementation into different pressure-driven LoC devices. Then, manual pipetting of required reagents or providing a continuous cold chain is not necessary anymore, which brings massive advantages for the specific diagnostic work flow.

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