CENTRIFUGO-THERMOPNEUMATIC WAX VALVE FOR CENTRIFUGAL MICROFLUIDIC PLATFORMS

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ABSTRACT

Valving concepts pose a key unit operation to automate biochemical assays on centrifugal microfluidic platforms. Wax valves are widely applied but require liquid dispensing of wax during fabrication and are triggered by temperature increase. For the first time we present a wax valve triggered by temperature decrease at the ease of a single pick-and-place fabrication step. Biochemical reactions at elevated temperature *e.g.* PCR may take place prior to valving by centrifugo-thermopneumatic liquid actuation.

KEYWORDS: centrifugal microfluidics, valving, centrifugo-thermopneumatics, off-the-shelf, PCR

INTRODUCTION

Centrifugal microfluidic platforms prove well-suited for the automation of biochemical applications [1, 2]. Valving, as an essential unit operation, may be controlled by rotation or by external means. In processing devices with temperature control for biochemical reactions, the intrinsic temperature control mechanism may be utilized to control wax valves. To date, wax valves open pathways upon temperature increase and require focused thermal energy for actuation. In contrast hereto, we introduce a novel centrifugo-thermopneumatic (CTP) wax valve to allow valving upon temperature decrease, which renders compatible with the global temperature actuation of an off-the-shelf PCR thermocycler (Rotor-Gene Q, QIAGEN, Germany).

FUNCTIONAL PRINCIPLE & FABRICATION

A U-shaped wax chamber in a microthermoformed COP cartridge [3] in-between a gas volume of a downstream structure and its air vent is filled with a wax bead by pick-and-place (Fig. 1) prior to sealing (Fig. 2A).

Figure 1: During fabrication of the centrifugal microfluidic COP cartridge, the CTP wax valve only requires a single and simple pick-and-place process of a solid wax bead into the wax chamber. Afterwards, the microthermoformed cartridge is sealed.

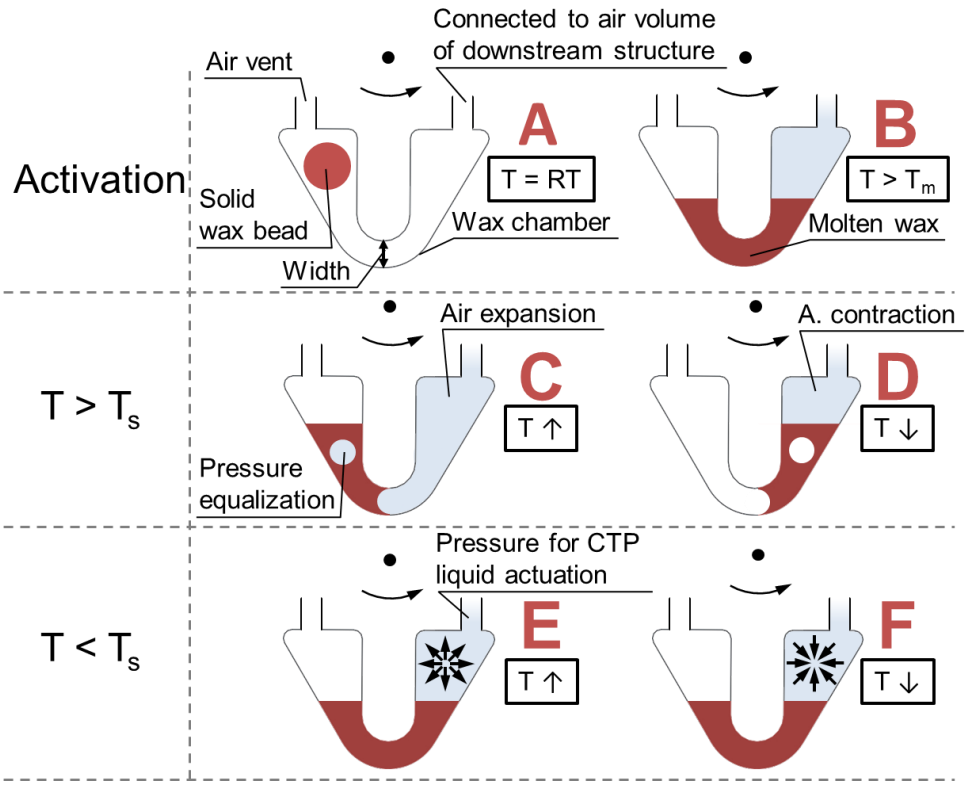

Figure 2: Stages of CTP wax valving. A: A solid wax bead is placed into a wax chamber during fabrication at room temperature RT (Fig. 1). B: Temperatures above the melting temperature T_m melt the wax and centrifugation transfers it radially outwards. C/D: Above the solidification temperature T_s, thermal expansion/contraction of air inside a connected downstream structure results in pressure equalization by bubbles leaving/entering through the molten wax. E/F: Below T_s, the air vent is closed by the solidified wax and temperature increase/decrease results in over/under pressure inside the downstream structure. Pressures can be used to CTP actuate a liquid connected to the air volume.

During processing, the valve is activated by exceeding the melting temperature T_m of the wax, which is then propelled radially outwards (Fig. 2B). Temperature changes above the solidification temperature T_s and resulting thermal volume expansions/contractions of air inside a downstream structure lead to pressure equalization by bubbles leaving/entering through the molten wax (Fig. 2C, D). At T_s, the wax is solidified and encloses an air volume inside the downstream structure. Further temperature decrease CTP actuates [4] liquid downstream, which is connected to the thermally contracted air volume (Fig. 2F).

EXPERIMENTALS

As proof-of-concept, the valve was placed in-between a PCR pre- and main-amplification for operation inside an off-the-shelf Rotor-Gene Q [4]. Different polar, COP-compatible wax materials were tested for a T_s below but close to the lower annealing temperature of 55 °C. Also, the wax chamber position, its width (Fig. 2A) and the amount of wax were examined. Ten cycles of 95 and 55 °C to simulate a pre-amplification, cool down to 42 °C and further to 36 °C for solidification of wax and CTP actuation of the PCR product into a downstream aliquoting structure, respectively, were executed.

RESULTS & DISCUSSION

A combination of polyethylene glycol (PEG) 6000 and 8000 (80/20 wt%) showed a suitable T_s of 50-52 °C. PEG 8000 thereby solidifies at 54 °C without closing the air vent. Its small solid particles support the solidification of PEG 6000 at T_s (Fig. 3), which by itself does not reproducible solidify (Fig. 3, Right).

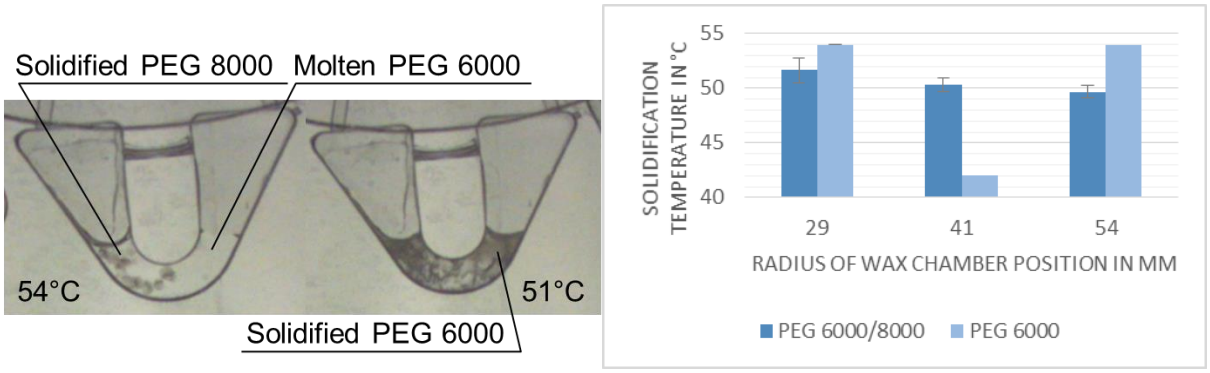

Figure 3: Left: Combination of PEG 6000 and 8000 (80/20 wt%). At 54 °C PEG 8000 solidifies without closing the air vent. Later during temperature decrease, PEG 6000 robustly solidifies at 50-52 °C supported by the present solid PEG 8000 particles. Right: Solidification of PEG 6000 with 8000 is robust (3 out of 3) compared to without 8000 (2/3 for 29 mm, 1/3 each for 41 and 54 mm). The slight decrease of T_s with the radius is caused by the processing device. Pressure equalization becomes easier with smaller radii (best for 29 mm) caused by the lowered centrifugal pressure of the molten wax inside the wax chamber (not depicted).

A wax chamber position on radius 29 mm, width of 1.5 mm and total PEG amount of 10 mg showed best pressure equalization during pre-amplification. The PCR product was successfully CTP transferred into the downstream aliquoting structure.

CONCLUSION & OUTLOOK

Applicability of wax valves is extended to applications, which require valving after elevated temperature. The centrifugo-thermopneumatic wax valve may be processed inside devices with global temperature control. The valve is expected to allow arbitrary thermal protocols prior to valving such as thermal lysis, PCR pre-amplification or thermally enhanced rehydration of pre-stored reagents. Its simple fabrication makes it a well-suited active valve for cartridges to be processed on off-the-shelf Rotor-Gene Q such as the recently demonstrated cartridge for forensic animal family identification *via* nested PCR [4].

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