A MICROFLUIDIC ARCHITECTURE FOR EFFICIENT REAGENT INTEGRATION, REAGENT RELEASE, AND ANALYTE DETECTION IN LIMITED SAMPLE VOLUME

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ABSTRACT
Biological assays generally employ interactions between analytes and specific reagents for detecting analytes in samples. The miniaturization and the integration of these assays on microfluidic devices enable rapid, multiplexed point-of-care tests using small amounts of samples and reagents. Here, we introduce a new microfluidic architecture that is only 6 µL in volume with an array of 8 parallel reagent and detection channels. This architecture "protects" the detection area against filling artifacts using valves, flow splitters and junctions, high and low flow resistances, and a bypass channel.

KEYWORDS: Microfluidics, Reagent Integration, Reagent Release, Analyte Detection

INTRODUCTION
The integration of small amounts of reagents in precise areas of microfluidics can be done using e.g. inkjet spotters, however, the dissolution of reagents in well-defined volumes of sample and in parallel microchannels is still a challenge. Inhomogeneous filling of channels holding reagents can lead to the formation of air bubbles and unreliable reagent concentration in areas for analysis, Fig. 1. Moreover, if excessive or insufficient sample passes through areas containing reagents, the apparent analyte concentration will be biased, Fig. 1b. Little work has been done to overcome these challenges [1, 2], and many applications still lack precise reagent distribution. We recently developed reagent integrators for a controlled release of reagents with a defined degree of dilution in self-powered microchips [3]. Using the same principle, we further developed an assembly of new class of microfluidic elements for precise release of small amounts of reagents and their homogeneous distribution in the detection channels.

THEORY
Flow splitters and flow junctions were designed to facilitate uniform sample flow through reaction channels. A delay channel with higher hydraulic resistance ($R_{\text{delay}}$) in relation to the bypass fast channel ($R_{\text{bp fast}}$), was designed after the reaction channels to direct a preferential flow into the bypass (Figure 2a). The bypass channel has also its own highly resistant delay channel ($R_{\text{bp delay}}$), which directs the sample flow primarily into the reagent and detection channels ($R_{\text{rd}}$). Figure 2b illustrates the electrical equivalent circuit of the microfluidic network. The resulting total hydraulic resistance ($R_T$) is:

$$R_T = R_{\text{inlet}} + \left( \left( R_{\text{bp delay}} + R_{\text{bp fast}} \right)^{-1} + \left( R_{\text{reaction}} + R_{\text{delay}} \right)^{-1} \right)^{-1} + R_{\text{outlet}} \quad \text{with} \quad R_{\text{reaction}} = R_{s1} + \frac{1}{2} R_{s2} + \frac{1}{8} R_{\text{rd}}$$

(1)

$$R_{s1} = R_{s4} \quad \text{and} \quad R_{s2} = R_{s3} \quad \text{where} \quad R_{s1, s2, s3, s4} \text{ are hydraulic resistances of branched channels.}$$

Figure 1. (a) Bio-analysis in microfluidics having integrated reagents, multiple channels and limited sample is exposed to nonuniform filling and reagent distribution due to air bubble formation and loss of reagents outside a reaction/detection area due to "overfilling" of the chip. (b) These effects can lead to wrong signal readings as illustrated in the graphs because the final concentration of reagents in the detection area may vary by having the sample flush out reagents to the outlet.
EXPERIMENTAL

The chip design is shown in Fig. 2a. The microfluidic chip comprises multiple 200-µm-wide reagent and 180-µm-wide detection channels (together termed "reaction channels"), a 20-µm-wide delay channel, and a bypass with a delay channel (20-µm-wide) and fast channel (100-µm-wide). All channels are typically 100-µm-deep. Microfluidic chips were sealed with Borofloat glass via anodic bonding. The pressure-driven filling of a colored liquid using a syringe pump (Nemesys) in the microchannels was monitored with a stereo microscope (Leica).

![Design layout (a) and electrical equivalent circuit (b) of a chip for bubble-free filling and precise distribution of reagents in a minimal volume of sample. Critical structures are flow splitters, flow junctions, a bypass channel and high and low flow resistances. The reagent and detection channels form 8 reaction channels.](image)

RESULTS AND DISCUSSION

Figure 3 shows successful pressure-driven filling of a single chip at 20 µL/min flow rate using a colored liquid. The delay valve helps liquids in the bypass and reaction channels wait for each other. Most of the colored liquid goes into bypass after the reaction channels are filled. The flow in the delay channel merges bypass through a delay valve.

![Images of (a) a silicon chip and (b) the pressure-driven filling of a colored liquid in critical parts of the chip (detection channels, left; flow junctions consolidating flow streams at the end of the reaction channels, right). (c) Snapshots showing the liquid filling the delay channel (arrow) until it merges with liquid from the bypass at the delay valve.](image)
Simulation of the resulting flow characteristics of the microfluidic network (Figure 4) supports the filling behavior. Initially, sample flows preferentially into the reaction channels as expected ($R_{\text{delay}} > R_{\text{reaction}}$). When the sample reaches the delay channel, it starts filling the bypass, maintaining a defined sample volume in the reaction channels. When volume of overfill is reached (~6.2 µl), the volume contribution of the bypass to the sample filling is much higher than that of the reaction channels: 0.32 µL/s and 0.01 µL/s, respectively (Figure 4b).

Figure 4. Simulation results comparing pressure-driven flow in the reaction channels (reagent and detection channels) and bypass. (a) The high resistance of the delay channel in the bypass initially favors filling in the reagent channels. (b) The 6 µL of the reaction channels fill preferentially after which flow occurs mostly through the bypass.

CONCLUSION
The architecture presented here ensures efficient filling with sub-microliter volume per reaction channel and is flexible and scalable. Chip production can be transferred to various materials, including polymers and is compatible with large-scale production. Therefore, the concept of this microfluidic network might offer wide range of applications in the field of point of care diagnostics and microfluidics in general.

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