REAGENT INTEGRATORS FOR THE CONTROLLED RELEASE OF PICOGRAMS OF REAGENTS IN SELF-POWERED MICROFLUIDIC CHIPS

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ABSTRACT

Microfluidic chips have the potential to revolutionize the field of in vitro diagnostics, owning to their ability to quickly and accurately analyze small volumes of samples and multiplexed analysis. The downscaling of diagnostic tests brings up new challenges for the deposition and distribution of small amounts of reagents inside a microfluidic chip. We introduce functional microfluidic elements, which we call reagent integrators (RIs), for a controlled release of picogram amounts of reagents with a defined degree of dilution. We demonstrate the impact of RIs on one-step immunoassays.

KEYWORDS: Microfluidics, Capillary System, Reagent Integration, Immunoassay

INTRODUCTION

A variety of microfluidic platforms for complex biological tests have been developed. However, little work has been done on the integration of reagents into microfluidics. Previous work ranged from simple methods releasing dried reagents from cavities [1] or porous pads [2] in actively pumped systems to discrete chemical release of droplets of picoliter volume using microactuators [3]. Nevertheless, many applications are still lacking a solution that allows precise release of small amounts of reagents and compatibility with self-powered microfluidics low-cost production. RIs are a new class of microfluidic elements, for the integration and tailored release of picogram amounts of dried reagents in capillary-driven microfluidic chips [5]. These chips combine the simplicity of a conventional lateral-flow strip test with the ability to precisely distribute reagents in a microliter sample.

THEORY

A RI splits and merges back a sample in parallel "reagent" and "diluter" microchannels to dissolve and dilute dried reagents in a controlled volume fraction of a sample, Fig. 1a. The level of dilution varies with the hydraulic resistance of the reagent channel in relation to the total resistance of the RI, a ratio defined as the dilution factor θ, Fig. 1b (left).

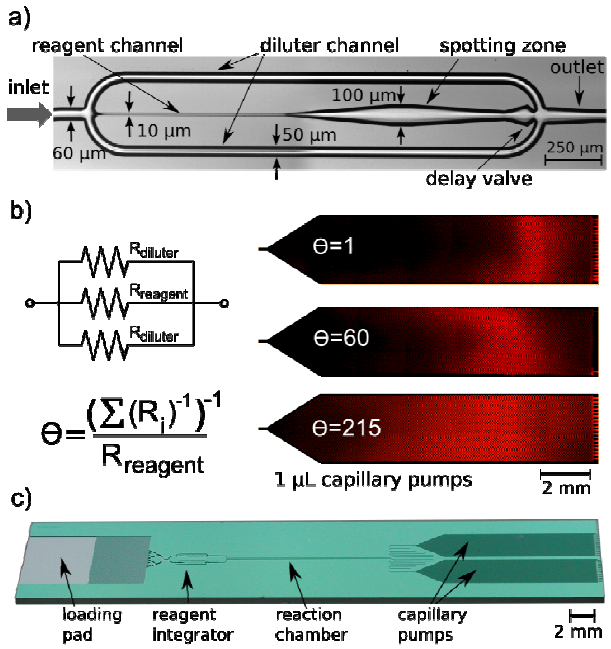


Figure 1: Design parameters and working principle of RIs. (a) Example of a RI etched in Si using standard lithography. (b) Electrical equivalent circuit of a RI and definition of the dilution factor θ (left). Influence of dilution factors on the distribution of 100 pg of horseradish peroxidase (HRP) in 1 µL of sample spiked with a fluorogenic substrate as visualized by the spread of the fluorescent product (resorufin) in the capillary pumps (right). (c) Microfluidic chip in Si for one-step immunoassays comprising several elements including two RIs.

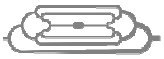
EXPERIMENTAL

Channel networks for microfluidic chips were patterned on a silicon wafer using two-layer lithography with a photoresist and a SiO_2 mask. Deep reactive ion etching (AMS-200SE, Alcatel Micro Machining Systems) was used to transfer the pattern into silicon with a depth of 20 µm in the reaction chambers and 60 µm elsewhere. Chip surfaces were cleaned with an air-based plasma and coated with 3-(2-aminoethylamino)propyltrimethoxisilane and methacryloxypropyltrichlorosilane from the wet and gas phase, respectively. Reagents were deposited into the spotting zone of the RI using an inkjet dispenser (Autodrop MD-P-705-L, Microdrop). Microfluidic chips were covered with a polydimethylsiloxane cover having lines of capture antibodies when immunoassay experiments were performed. The cover was then also passivated with a layer of bovine serum albumin. 15 µL of sample was pipetted onto the loading pad to start capillary filling of the microchannels. The reagent concentration and assay signal on the capture lines were observed with a fluorescence microscope (Eclipse 90i, Nikon).

RESULTS AND DISCUSSION

Fig. 1b illustrates how the dilution factor of a RI influences the distribution of 100 pg of HRP in a 1 µL sample filled in a microfluidic chip. The distribution of HRP is visualized by the fluorescence of resorufin, a product of the enzymatic reaction of HRP with Amplex Red® and H_2O_2, which both were present in the sample. The small footprint and low fabrication requirements of a RI eases the implementation into a microfluidic chip (Fig. 1c). Concepts for RIs having different θ are based on either restricting the flow through the reagent channel (Tab. 1, A) or increasing the flow through the diluter channels (Tab. 1, B). RIs of type A provide a large range of θ without increasing the footprint of the RI, whereas type B relaxes the critical dimensions needed for microfabrication. The key design criteria that were considered in designing RIs were: (1) all channels must fill by capillary action, (2) air bubbles are prevented, (3) low/no dead volumes are created, (4) deposition of reagents must require only limited accuracy, (5) dispensed reagents must not contaminate diluter channels and (6) the distribution of reagents in the sample exiting the RI must be homogeneous across the channel. Experiments with different types of RIs have shown that the reagent concentration profile across the channel after the RI is most homogeneous for RIs having a central reagent channel. A spotting zone with a diameter of 100 µm can be targeted by an inkjet dispenser and capillary forces suffice to entirely fill this zone with sample. Narrow channels of high capillary pressure placed after the spotting zone guide and hold spotted solutions in the reagent channels where they evaporate. Delay valves at junctions close to the outlet of the RI prevent formation of air bubbles due to the fast filling of liquid through the diluter channels compared to the reagent channel.

Table 1: Design strategies and related characteristics of RIs. RIs are represented using different scales, see footprint of RIs for comparison.

Concepts for RIs[a]	Θ	RI footprint /mm²	# Delay valves	Critical dimension /µm	Hydraulic resistance /10¹⁵ m⁻³	Comments
A 	7	0.9	0	30	1.8	- risk of shortcutting the central channel - likely to have air bubbles in the spotting zone
	27	0.9	0	5	1.9	
	∞	0.9	0	30	2.1	- release by passive diffusion - air bubbles enclosed
	60	0.9	1	10	3.1	- delay valve prevents shortcut of central channel - homogeneously filling spotting zone
	215	0.9	1	10	3.7	- reagents released in center of channel
B 	2	0.8	1	30	3.4	- RIs with uneven number of channels advantegous (more reliable and centered release of reagents) - reliability decreases with increasing number of valves - large footprint for high dilution factors - likely to have air bubbles in the spotting zone
	3	0.9	3	30	2.4	
	6	1.6	4	30	2.2	
	7	2.7	5	30	1.0	
	14	4.0	6	30	0.8	

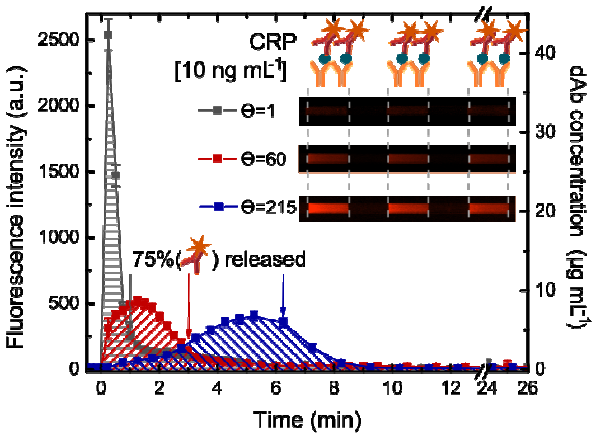


Figure 2: One-step fluorescence immunoassay using different RIs. Detection of 10 ng mL^{-1} CRP in human serum on microfluidic chips with different RIs using a sandwich immunoassay (C2-CRP-C6alexa). Release curves monitor the concentration of dAbs in the reaction chamber at different times and assay signals obtained with respective RIs.

Figure 2 shows the power of RIs as applied to immunoassays on one-step microfluidic chips [4, 5]. Labeled antibodies for the detection of 10 ng mL^{-1} of CRP in human serum are released in 1 min, 4 min or 8 min by varying θ from 1 to 215. A slow release of antibodies increases the chance of immobilization of CRP-antibody complexes on capture lines on the surface. As a result, the intensity of the assay signal is significantly enhanced (Fig. 2, inset). This method contrasts with conventional lateral flow tests in which the sample migrates along a nitrocellulose membrane and dissolves dried reagents in a manner that is very hard to optimize and control. Therefore, the release time of reagents and the sensitivity performance of lateral flow tests tend to be limited.

CONCLUSION

RIs provide an efficient solution for the tailored release of reagents in active as well as in passive systems. Chip production can be transferred to various materials, including polymers and reagents can be spotted into the chips using inkjet technology. Therefore, the concept of RIs is compatible with large-scale production and widely applicable in the field of point of care diagnostics and microfluidics in general.

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