MINIATURIZED HYBRID PUSH-PULL PERFUSION PROBE FOR SAMPLING WITH HIGH SPATIAL AND TEMPORAL RESOLUTION

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ABSTRACT

We present a newly designed and fabricated miniaturized hybrid push-pull perfusion sampling (μPPPS) probe with integrated droplet generator to capture concentration variations of target analytes in 'chemical memory'. The development of this tool will ultimately enable the monitoring of neurotransmitters with unprecedented temporal (~1 s) and spatial (<100 μm) precision, while requiring an absolute minimum of sample volume. The μPPPS probe is essential to gain deeper understanding of the fast dynamics in the release of neuromodulatory substances, such as acetylcholine or glutamate.

KEYWORDS: Microdialysis, Push-pull perfusion sampling, droplets, Neuroprobe.

INTRODUCTION

Traditional microdialysis probes suffer from 3 major problems; (1) they are only able to record changes in neurotransmitters in the range of several minutes, (2) have a rather large sampling area in the order of 1 mm^2 or bigger, and (3) are rather large and cause severe damage during insertion [1]. We have previously developed a microfabricated probe with integrated electrodes for electroencephalography or ion-selective electrochemical recordings [2]. Here, we present a newly designed and fabricated miniaturized hybrid push-pull perfusion sampling (μPPPS) probe with integrated droplet generator to capture concentration variations of target analytes in 'chemical memory'. The development of this tool will

ultimately enable the monitoring of neurotransmitters with unprecedented temporal (~1 s) and spatial (<100 μm) precision, while requiring an absolute minimum of sample volume. The μPPPS probe is essential to gain deeper understanding of the fast dynamics in the release of neuromodulatory substances, such as acetylcholine or glutamate.

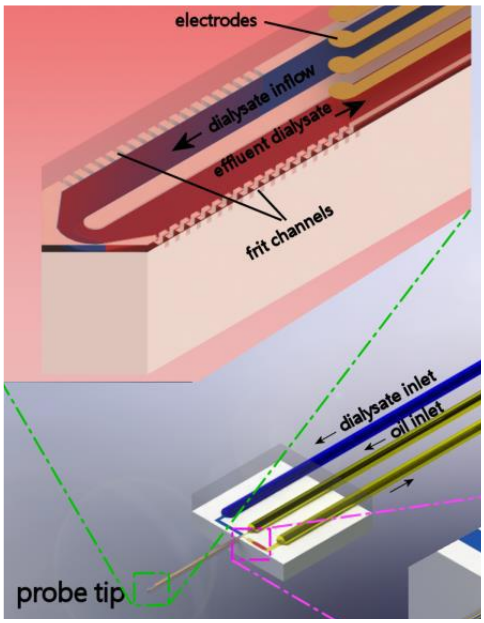

Figure 1. Artist impression of the micro-fabricated hybrid push-pull perfusion sampling probe (μPPPS). Insets: details of the probe tip and droplet generator.

EXPERIMENTAL

This μPPPS probe, as schematically shown in fig.1, is fabricated from silicon using a series of deep reactive ion etching steps, while a top cover from glass is thinned using chemical mechanical polishing. The resulting needle (fig. 2) has a 140x140 $μm^2$ cross sectional area, thereby minimizing damage compared to existing probes when inserted into, e.g., 5 mm sized brains of mice. Inside the tip of the needle (fig. 2A), small frit channels (3x20

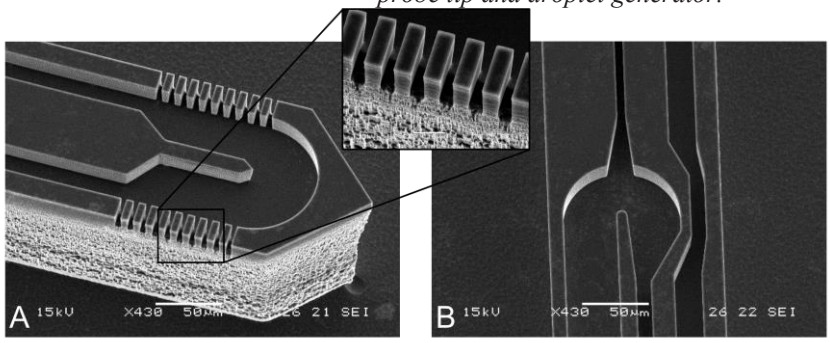

Figure 2. SEM photos of a μPPPS. A: tip of the needle, with the dialysate entry and return channel. Inset: frit channels through which the sampling takes place. B: droplet generator inside the needle. The microfluidic channels in this device are 20 μm deep.

μm^2) connect the extracellular fluid to a U-shaped inner dialysate flow channel. Halfway the shaft of the needle, a droplet generator is included (fig. 2B) to create a water-in-oil emulsion to prevent dispersion. The use of droplets, as previously demonstrated by Kennedy et al.[3], improves the temporal resolution to monitor the molecules preceding a migraine attack or a single cognitive process in real-time.

Detection of neurotransmitters is based on a fluorescent detection method using Amplex red/Amplite assay kits. A dedicated setup (fig. 3) has been developed, capable of measuring the fluorescent marker reliably down to 20 nM inside a 50 μm inner diameter fused silica capillary.

RESULTS & DISCUSSION

FEM simulations of the recovery rate using this geometry indicate that 70-85% is possible at a dialysate flow rate of 0.1 μL/min (fig. 4). Using the μPPPS probe installed in the setup (as shown in fig. 3) we have conducted initial in vitro tests by sampling a step-wise concentration increase of fluorescein from 0 to 1 mM (fig. 5). By capturing the fluorescein solution in droplets using n-decane with 2% Span 80 as the continuous phase, we show a response time of 2 s. It should be noted that, although the solution is stirred, this response time is due to both sampling and equilibration of the vessel.

CONCLUSION

In summary we show that we can significantly increase temporal and spatial resolution in neurotransmitter sampling using this novel miniaturized hybrid neuroprobe. To demonstrate the use of these devices in neurological research, we are currently working on the first in vivo tests to monitor glutamate levels in mice while triggering a cortical spreading depression.

ACKNOWLEDGEMENTS

Funding from the personal VENI grant of M. Odijk (13607), by TTW is gratefully acknowledged.

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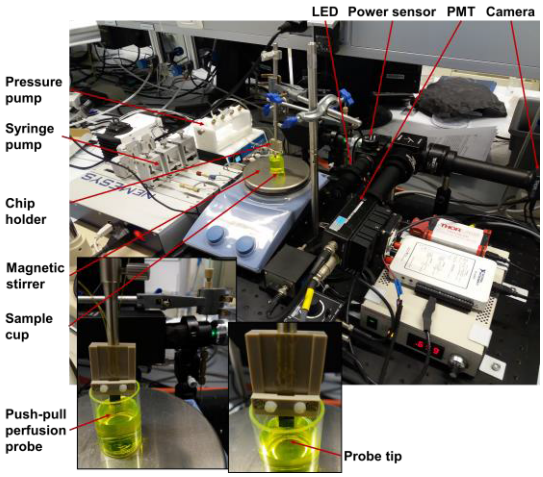

Figure 3. Experimental setup consisting of flow control hardware (pressure and syringe pump) and an optical detection system (PMT, camera and LED source).

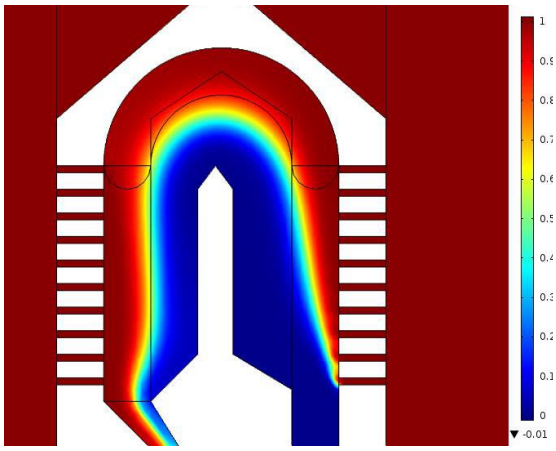

Figure 4. Simulated concentration profile in the tip of the needle. A dialysate flow rate of 150 nL/min was used. Under these conditions, the recovery rate is 62%.

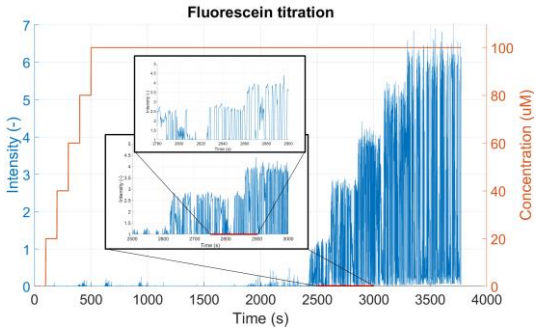

Figure 5. Sampling of fluorescein sodium salt at 0.2, 0.4, 0.6, 0.8 and 1.0 mM concentrations. Dialysate and oil flow rates were set to 0 and 150 nL/min.