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Three-dimensional printed sample load/inject valves enabling online monitoring of extracellular calcium and zinc ions in living rat brains

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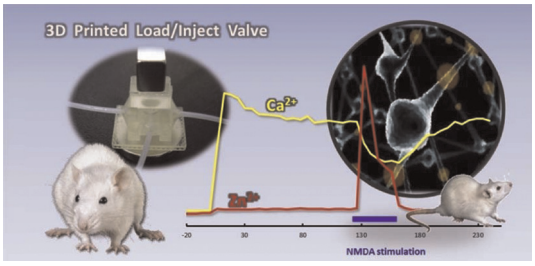
HIGHLIGHTS

- A 3D printed sample load/inject valve interfaced between MD sampling and an ICP-MS.
- The valve equipped a printed 5-μL sample loop can minimize the salt matrix effects.
- The dynamic variations of brain extracellular Ca and Zn were investigated *in vivo*.
- The physiological response to excitotoxic *N*-methyl-D-aspartate was also revealed.

GRAPHICAL ABSTRACT



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ABSTRACT

We have developed a simple and low-cost flow injection system coupled to a quadruple ICP-MS for the direct and continuous determination of multi-element in microdialysates. To interface microdialysis sampling to an inductively coupled plasma mass spectrometer (ICP-MS), we employed 3D printing to manufacture an as-designed sample load/inject valve featuring an in-valve sample loop for precise handling of microliter samples with a dissolved solids content of 0.9% NaCl (w/v). To demonstrate the practicality of our developed on-line system, we applied the 3D printed valve equipped a 5-μL sample loop to minimize the occurrence of salt matrix effects and facilitate an online dynamic monitoring of extracellular calcium and zinc ions in living rat brains. Under the practical condition (temporal resolution: 10 h^{-1}), dynamic profiling of these two metal ions in living rat brain extracellular fluid after probe implantation (the basal values for Ca and Zn were 12.11 ± 0.10 mg L^{-1} and 1.87 ± 0.05 μg L^{-1}, respectively) and real-time monitoring of the physiological response to excitotoxic stress elicited upon perfusing a solution of 2.5 mM *N*-methyl-D-aspartate were performed.

1. Introduction

Metal ions in the synaptic cleft and extracellular space are proposed to play a crucial role in regulating brain functions; however, their homeostasis and dynamic response to acute or chronic stimulation remain merely investigated [1–3]. To study dynamic actions of brain metal ions *in vivo*, microdialysis (MD) sampling offers a great opportunity by means of *in situ* sampling from living anesthetized/awake subjects as well as for further identification and quantification of chemical substances of interest [4–6]. Unfortunately, the direct hyphenating of an MD sampling device to the most sensitive elemental analysis instrument,

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namely the inductively coupled plasma mass spectrometry (ICP-MS), still requires an appropriate interfacing device for coupling MD sampling with ICP-MS measurement [7].

Since its introduction at the beginning of 1980, ICP-MS has become a widely used and the most sensitive instrument for (ultra-) trace element determination. However, one of the inherent limitations of its application is direct determination of trace elements in high salt content matrices. The interface to handle sample and reagent streams for the pretreatment (*e.g.*, mixing, dilution, derivatization, extraction, separation, elution) within flow injection analysis (FIA) systems has profoundly improved the convenience of modern analyses and increased innovation as a result of their versatile setups and ready multi-functional integration. During the evolution of FIA systems, from simple flow injection and sequential injection (SI) configurations to currently popular SI-lab-on-valve and lab-on-a-chip configurations [8,9], the valve components have always remained indispensable for precise manipulation of the continuous or segmented flowing streams, regardless of how they are actuated. Because commercial valve modules are usually limited to available geometries and dimensions, the dead volumes in the connectors, adapters, and tubing are usually larger than the total volume of the functional parts of the equipped valves. For the smaller volumes of samples (such as rat brain microdialysate) or reagents that are often preferred for manipulation in microfluidic devices, the ability to develop or integrate more reliable valve components into miniaturized analytical systems remains a stumbling block [10,11]. Additionally, for the high matrix content of samples that are often preferred for manipulation in preconcentration procedures [12], the development of direct determination procedure with many advantages in terms of high throughput, simplicity, small reagent consumption, and reduced risk of sample contamination is still a highly demanding task.

Three-dimensional (3D) printing has emerged recently as a powerful technique for manufacturing cost-effective, bespoke, complicated, and customer-oriented appliances that have previously required expensive equipment or sophisticated procedures [13,14]. Taking advantage of the ability to build devices layer-by-layer in a single manufacturing process, the in-laboratory construction of multifunctional reactionware for chemical synthesis and analysis [15–17], the preparation of initiator-integrated complex 3D microstructures for further surface modification and functionalization [18–20], and device prototyping to assist analytical procedures [21–26] have all been realized recently. Unlike conventional two-dimensional (2D) lithography, which repeats multiple manufacturing processes on multiple working planes to fabricate complicated devices, direct 3D printing fabrication provides the ability to construct miniaturized devices in a single printout, allowing the creation of all of the system's manifolds and components in multilayer working domains. By adopting this ideal additive manufacturing concept, in this study we employed a commercially affordable 3D printer to construct an adequate interface – a two-position sample load/inject valve – for constructing a FIA–ICP-MS system that can perform direct and continuous determination of trace elements in microdialysate samples.

2. Materials and methods

2.1. Chemicals

All chemical solutions were prepared by the water purified through a Milli-Q Integral water purification system (Millipore Corporation, Billerica, MA). Sodium chloride (NaCl; 38979) and *N*-methyl-D-aspartate (NMDA; M3262) were purchased from Sigma–Aldrich (St. Louis, MO). Nitric acid (HNO_3; 6901-05, J.T. Baker, NJ) was ultrapure grade. Stock solutions (1000 mg L^{-1}) of Ca, and Zn were purchased from J.T. Baker and subjected to serial dilution with 0.9% NaCl. The resin (PMG-0121, Rays Optics Inc.) compositions for the used 3D printer (MiiCraft®) were modified acrylate, modified acrylate oligomer, acrylate monomer, epoxy monomer, photo initiator, and additives but with the undisclosed ratios.

2.2. 3D printed valve fabrication

Here, we study the use of a commercial MiiCraft® 3D printer (US$2300) to manufacture a sample load/inject valve to online handle tiny amount of microdialysates. Our designed valve comprised two simple parts: a base (stator) and a rotor. The base contained a negative cone-shaped hole (67.5°) with the intersection of four channels (Fig. 1A). Two of the opposite channels were designed for connection to inlet and outlet tubing for the loading of MD samples; the other two were designed for the carrier stream to deliver the volume-confined microdialysate to the ICP-MS system for determination. Short pieces of polytetrafluoroethylene (PTFE) tubing (i.d.: 0.01 in; Alltech) were inserted into the four printed channels and fixed using epoxy glue. The rotor was positively cone-shaped; its two printed hollow channels within perfectly matched the holes on the base (Fig. 1B). One channel was straight and functioned as a bypass channel; the other, designed with a reverse U-shaped (with right-angled corners) geometry, functioned as a sample loop (its volume could be adjustable by changing the channel length and width). The detailed dimensions of our designed sample loop were provided in Supplementary data (Fig. S1). The construction of the device involved four steps: (i) computer-aided design (CAD) using "SolidWorks 2013 (Dassault Systèmes, Paris, France)" software; (ii) export of an stl file; (iii) digital slicing into multiple 2D layers; (iv) export for printing using "MiiCraft® Suite" software (v. 0.63). More detailed descriptions and operation parameters for the 3D printer are available elsewhere [18–20,26]. The as-prepared base and rotor were treated with a dry Teflon® spray (DuPont™) and then assembled with the aid of two permanent magnets; manual adjustment of the rotor position, to touch the two stoppers aligned on the base, allowed convenient control over the valve position for sample loading or injection (Fig. 1C and D).

2.3. Apparatus

The online monitoring system comprised the MD sampling device, the 3D printed sample load/inject valve, and the ICP-MS system. The MD sampling device contained a metal-free MD probe featuring a 4-mm-long, 500-μm-diameter polyarylethersulfone (PAES) membrane having a molecular weight cut-off (MWCO) of 20 kDa (8010435; CMA 20, CMA Microdialysis, Solna, Sweden), and a plastic 5-mL syringes (4606051V, B. Braun, Melsungen, Germany). The Agilent 7500ce ICP-MS system (Agilent Technologies, CA) featured platinum sampling and skimmer cones and a perfluoroalkoxy alkane (PFA) Micro-Flow nebulizer (PFA-100, Elemental Scientific, NE) fitted to the Scott-type PFA double-pass spray chamber. We connected the outlet tubing of the MD probe directly to the inlet tubing of the sample loading channel on the base; after sample loading was complete, we switched the rotor manually to the injection position for 10 s, and then passed a continuous carrier stream (0.5% HNO_3, v/v) through the bypass channel turned to deliver the loaded MD sample into the ICP-MS system, *via* an equipped concentric nebulizer, for time-resolved analyses at m/z 43 (Ca) and 66 (Zn). The detailed schematic representation of the operating system was illustrated in Fig. 2.