Rapid Prototyping using 3D Printing in Bioanalytical Research

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In bioanalytical research laboratories, 3D printing is no longer just a conception; it has become a useful tool for the fabrication of various analytical devices and custom labware in the past few years. Due to its fast design-to-object workflow, ease of learning, and the ability to make complex structures with sufficient resolution, 3D printing technology has shown its application in biomedical engineering, tissue scaffolding, surgical preparation, pharmacokinetics/pharmacodynamics, forensic science, and medical science[1, 2].

Microfluidics is one of the most represented areas of 3D printing with several review articles describing the latest improvements of the fabrication of novel 3D-printed microfluidic devices. These include the integration of these devices with electrodes, biosensors, and valves, and their applications in chemistry and biology [3-5], such as the analyses of cells and biomolecules as well as interfaces that enable bioanalytical measurements using cellphones [6]. Applications of 3D printing in other analytical devices have also been reported, such as 3D-printed paper spray ionization cartridge with fast wetting and continuous solvent supply features [7], 3D-printed supercapacitor-powered electrochemiluminescent for protein immunoarray [8], membrane module design with 3D printing technology [9], 3D-printed grinding device for reproducible preparation of nanospray tips [10], and 3D-printed platforms for solute delivery, separations and diagnostics [11].

There are a number of interesting examples in literature about the use of 3D printing in bioanalytical research, and we'll highlight just a few here. 3D printing is a promising technique for developing sample-to-device interfaces for limited-resource settings and point-of-care diagnostics. Jue *et al* demonstrated a 3D-printed interlock meter-mix device for metering and lysing clinical urine samples [12]. The 3D-printed static mixer contains elements designed to mix urine and lysis buffer that are injected into the device simultaneously. Rapid mixing within the first few static mixer elements was achieved. Gowers *et al* described a 3D-printed microfluidic device with integrated electrode biosensors for continuous monitoring of human tissue metabolite levels, such as glucose and lactate [13]. The 3D-printed microfluidic chip and 3D-printed electrode holder in this wearable device enabled a simple connection between the microdialysis probes and electrode biosensors. In addition, a soft 3D-printed elastomer was used to ensure a good seal between electrode holder and microfluidic chip. 3D-printed devices also have been used to increase efficiency during the drug-development process. Lockwood *et al* showed the parallel *in vitro* pharmacokinetic profiling of molecules by

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using a 3D-printed fluidic device [14]. The device contained multiple flow channels, and each channel was integrated with porous membrane-based insert wells. The membranes enabled small-molecule drugs to diffuse back and forth between flow channels and the insert wells. Multiple pharmacokinetic profiles were generated simultaneously by using this device and the volume consumption was reduced from liters to milliliters, in comparison with diffusion-based dynamic *in vitro* models.

In our laboratory, we are working to develop inexpensive disposable cartridges that address the entire bioanalytical workflow including sample collection, transportation/storage, sample preparation, and analysis. As an example of this approach, we have been investigating paper-spray MS, in which biofluids samples are deposited and stored on paper. Extraction and ionization are then carried out directly from the dried biofluid spot on the paper without additional sample preparation (CITE). We have begun using 3D printing to generate prototype sampling cartridge as well as various devices to facilitate the experiments. This equipment isn't necessarily complicated. It could be as simple as a piece of plastic to hold blood samples in a certain way while drying or more complex like a new disposable cartridge designed to perform automatic sample preconcentration.

In the past we would manufacture these objects by using a milling machine to carve the desired piece out of blocks of plastic. Advantages of the milling machine include its relatively low cost (a quality benchtop milling machine can be purchase for approximately US$1000) and the wide range of materials can be machined, including metal and plastics with good solvent resistance such as Delrin® and Teflon®. Machining parts was often time consuming, however, and required planning and foresight to work within the limits of what could be done with a milling machine. Parts also had to be machined one at a time. Paper spray cartridges made using a milling machine required an afternoon of tedious progress to cut out a slot for the paper using a narrow and fragile milling bit. As a result, only one or two cartridges would be made and would require cleaning between each sample. Recently, a service opened on campus that provided access to a number of different types and brands of 3D printers. Using a sufficiently high resolution 3D printer a cartridge with the desired dimensions can be printed in an hour. In addition, modifications to the design require only as much time as changing the 3D model and printing off new cartridges. This has allowed for rapid prototyping with multiple iterations and the ability to print off multiple cartridges to allow an entire experiment to be set up at once without the tedium of cleaning the cartridges between each analytical run.

In a recent experiment, for example, a special membrane had to be held against a small piece of paper while plasma wicked through from whole blood [15]. The membrane was prone to ripping so a special holder was designed capable of holding the membrane gently during the experiment. Initially the holder was machined from three pieces of plastic taking around 2 days to design and manufacture by hand. The experiment had to be modified, and the original holder no longer worked as desired. A second holder was produced using 3D printing. Using a free 3D modeling program, it took around 2 h to model the holder and two more hours to print five copies of the

holder to scale-up the experiment. This speed and ease of making copies has proved useful in a number of experiments.

In another example, a paper spray cartridge with integrated solid-phase extraction (SPE) was developed in our laboratory recently for the selective and sensitive detection of small molecule drugs in plasma [16]. The cartridge consisted of two parts that were assembled together, as shown in Figure 1a. Using a milling machine, it took us about a week to produce enough cartridges to analyze a batch of samples for quantitative analysis, in which dozens of samples needed to be prepared and tested at the same time. In addition, the milling process had to be done carefully to ensure reproducibility among cartridges. However, there is no such reproducibility issue in 3D-printed cartridges. Moreover, 3D printing speeds up the commercialization process of the SPE cartridge. In order to achieve an automatic high-throughput analysis, we redesigned the SPE cartridge to make it work in a Prosolia (Indianapolis, IN, USA) paper spray autosampler [Unpublished data]. The redesigned 3D-printed SPE cartridge could be printed within 2 h, costing only US$2. As shown in Figure 1b, the new cartridge has a smaller size in comparison with its prototype, the same position to apply spray solvent and spray voltage as a Prosolia paper spray cartridge, and is assembled from four parts with more complicated structure that would impossible to produce by a milling machine.

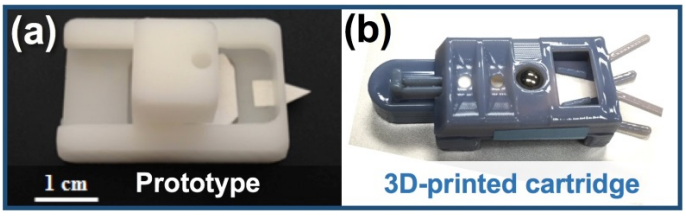

Figure 1. Paper spray cartridge integrated with solid-phase extraction. (a) prototype made by milling machine. (b) 3D-printing cartridge.

Advantages of 3D printing for fabrication of bioanalysis prototypes include reproducibility, high precision, ease of learning, fast building time, and low printing costs. However, there are some drawbacks to using 3D-printed devices. Depending on the quality required, 3D printers can range in price from a few hundred dollars to tens of thousands of dollars for machines capable of fine detail. In addition, depending on the desired end-use of the 3D-printed part, solvent compatibility of the material may need to be considered. For example, the primary 3D printer used in our work is an Objet® printer from Stratasys® (Eden Prairie, MN, USA). This type of printer uses two types of materials, a rigid photopolymer that makes up the structure and a soluble support material to fill any gaps during the build. We have found that even with thorough cleaning, peaks in the mass spectrum originating from the support material show up during analysis using the 3D-printed cartridges. Whether or not the material will leech contaminants that will interfere with analysis needs to be taken into consideration any time a 3D-printed sample makes direct contact with the sample. In addition, current materials for 3D printing have shown less strength and durability, and the choice of materials available to produce functional devices is limited. Optical transparency and

biocompatibility of the materials also need to be considered in some bioanalytical studies.

In conclusion, 3D printing has recently attracted attention as an alternative method to fabricate analytical devices. With the progress of 3D printing technology, such as more material choices, higher resolution and throughput, 3D printing has the potential to be utilized in more chemical and biological applications and change the perceived limitations in the experimental design for bioanalytical studies.

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