3D-printed autonomous microfluidic chip for multistep point-of-care bioassays

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Here, we present a radically novel method for fabricating 3D passive microfluidics in one piece that is capable of accommodating multistep bioassays and actuating fluids within the channels without any external devices. Furthermore, this 3D printed (3DPed) platform allows on-chip pre-treatment of sample and the incorporation of semi-quantitative readout, next to being easily scalable and cost-effective. As such, it represents a very attractive concept for point-of-care (POC) testing in low resource settings that comply with the WHO guidelines to be affordable, sensitive, specific, user-friendly, rapid, equipment-free and delivered to those who need it (ASSURED)[1].

POC testing is crucial for healthcare in developing countries, but remains difficult to achieve. Lateral flow devices (LFD) persist as the most promising[2],[3], although they offer solutions only partially since they fail to accommodate complex bioassays that are often needed to reach desired sensitivity[4]. Sparked by the success of LFDs, many attempts were made to automate a timed assay sequence in 'passive microfluidics' driven solely by capillary flow. These approaches are based on defining 3D flow paths within stacks of porous membranes that are individually functionalized with hydrophobic/hydrophilic patterns and assay reagents.[5–7] The resulting interlayer contact issues led to unpredictable or defective devices.[7]

To fabricate monolithic passive microfluidics with a 3DP process based on digital data, a polymer powder was combined with liquid binders (Fig.1A). The 3DPed material was characterized using scanning electron microscopy, SEM(Fig.1B), revealing considerable macroporosity that resulted from remaining interparticle space. For testing the biocompatibility of the 3DPed platform, a sandwich ELISA targeting IgE was used as a model system (Fig.2A). The ELISA was performed in different formats, being microtiter plates, 3DPed discs, PDMS chips containing 3DPed plugs and fully 3DPed channels (the latter depicted in Fig.2B). A standard ELISA protocol was successfully downscaled to comply with requirements for microfluidics POC platform by substantially reducing incubation time and reagent volumes of different steps (Fig.2A). The modified ELISA (total assay time 30 min) was eventually implemented in 3DPed channels (Fig.2C), demonstrating (1) biocompatibility of the build material with the bioassay (i.e. low non-specific interaction) and (2) compatibility of selected ELISA with the flow system.

Finally, a completely integrated microfluidics device was designed after extensive characterization of flow rates for all buffers (Fig.2D), resulting in a 3DPed microfluidics device (Fig.2E) that can accommodate complex bioassays in completely autonomous fashion and as such can fill the gap between lateral flow tests and laboratory based ELISA.

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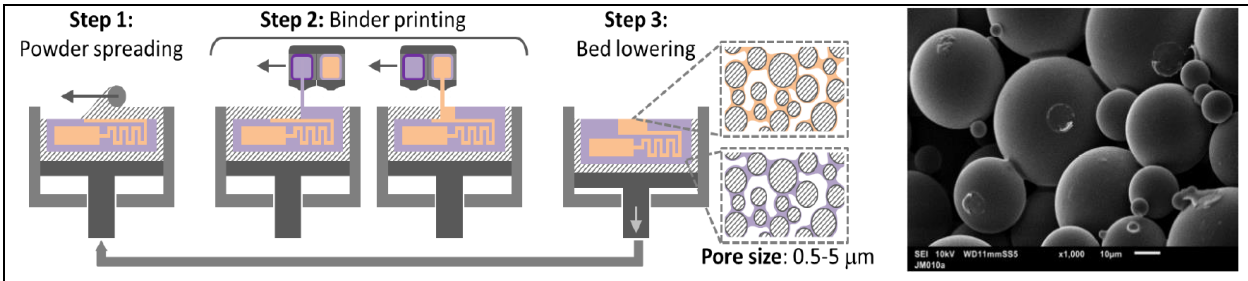
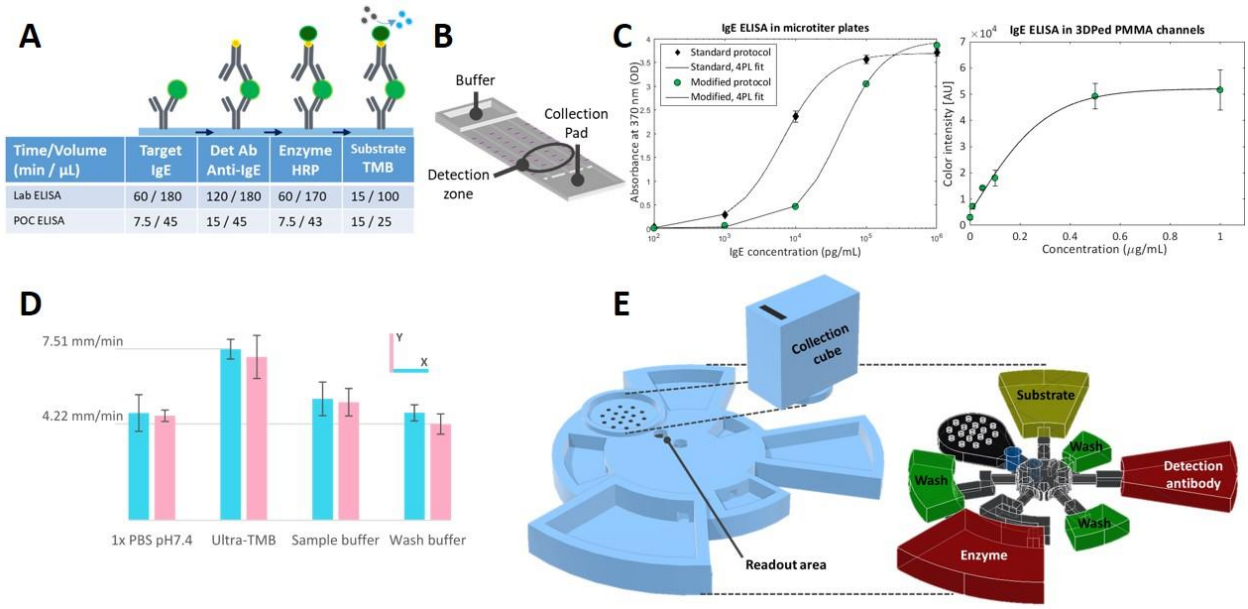


Figure 1: (A) 3DP process based on powder (hatched) and two types of binder (purple and orange). Object construction occurs by alternatingly spreading a layer of powder (Step 1) and inkjet printing object slices using a binder liquid (Step 2). The binder additives control the surface chemistry inside the printed porous object and are used to create capillary flow channels and install mechanisms for chemical amplification and readout. (B) SEM image of 40 µm sintered polymer powder. During the printing process, powder is melted by the binder and re-solidifies again at the bead interfaces, forming necks.



Time/Volume (min / µL)	Target IgE	Det Ab Anti-IgE	Enzyme HRP	Substrate TMB
Lab ELISA	60 / 180	120 / 180	60 / 170	15 / 100
POC ELISA	7.5 / 45	15 / 45	7.5 / 43	15 / 25

Figure 2: (A) Schematic overview of IgE sandwich ELISA components, with horseradisch peroxidase-based (HRP) readout and 3,3',5,5'-tetramethylbenzidine substrate (TMB). The incubation times and volumes per step are shown in the table for laboratory ELISA and POC ELISA; (B) IgE ELISA on autonomous 3DPed channels. Reagents were added in the buffer tank, and wick trough the porous material towards the detection zone and the collection pad. The signal is quantified based on RBG image analysis; (C) Calibration curves for IgE sandwich ELISA, performed in (left) microtiter plates and (right) fully 3DPed PMMA channels. The standard protocol was modified by both reducing incubation time and volume while maintaining the functionality of the assay. Error bars show standard deviation, n=3; (D) Flow rates for all buffers in 3DPed channels were determined in X- an Y-direction to design the integrated device. (E) Complete integrated chip capable of sequentially flowing reagents over the readout area. Sequence timing is achieved by varying channel lengths and the use of diodes to ensure unidirectional flow.