IN VITRO SYNTHESIS OF PROTEIN IN THREE PHASE FLOW VIA MERGING OF DROPLETS OF PCR AND TRANSLATIONAL MACHINERY

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ABSTRACT
In this report we present a method for plug-based in vitro protein synthesis via merging of PCR droplets with protein translational machinery in three phase flow. This approach was performed using a monolithic microfluidic device with various channel sizes fabricated by a facile, cost-effective, non-lithographic and single step method.

KEYWORDS: Three phase flow, in vitro protein synthesis, multilevel microfluidics.

INTRODUCTION
Miniaturized in vitro protein synthesis is accepted as a powerful tool for identifying gene functions as well as for studying structure-function relationships of protein molecules. In particular, when simultaneous expression of the multiple proteins is in demand, in vitro protein synthesis technology offers an excellent throughput that can hardly reached by conventional in vitro methods. And it is demonstrated that microfluidic based system could be useful platform for protein expression in droplets. Dittrich et al. have encapsulated translation and transcription machinery along with green fluorescent protein (GFP) encoding gene in a droplet using a “Y” channel microfluidic device for in vitro protein expression [1]. While, Huebner et al. have used similar channel configuration to encapsulate single E. coli cells in droplets to express yellow fluorescent protein [2]. The microfluidic tube-like cartridge with preloaded nanoliter plugs is another attractive technique for screening of chemical and biochemical reactions [3]. This technique relies on multilevel microfluidic device to inject substrate of interest into the arrays of reagent plugs. For fabrication of such devices, multilayer photolithography is a prerequisite and requires use of sophisticated equipment and clean room atmosphere. Here, we present a facile single step fabrication method of microfluidic device with varying channel sizes which can be executed in a common laboratory with minimal facilities and no expensive photolithographic tools. By using this device, initially arrays of nanoliter plugs for pre-PCR cocktail was produced, followed by merging PCR droplets with protein expression machinery to express green fluorescence protein (GFP) after incubation at 37° C for 1 hour.

EXPERIMENTAL
The PCR reaction mixture comprised of 1 μM each of the primers, T7P 15 UP and T7 terminator as previously described[4], 2.5 U/μl of Taq polymerase and 250 μM of dNTP (New England Biolabs, Ipswich, MA). The target ORF of enhanced green fluorescence protein (EGFP) was amplified by using pIVEX2.3d EGFP as a template. The composition of standard protein expression mixture for in vitro protein synthesis is de-
scribed elsewhere [5]. Microfluidic devices were fabricated using either of polydimethylsiloxane (PDMS) (Sylgard 184, Dow Corning, Midland, MI) or low molecular weight perfluoropolyether (Low MW-PFPE), as depicted in Figure 1.

First, a framework for channel formation was assembled using commercially available tubing of different sizes (Upchurch Scientific, Oak Harbor, WA). A perfluoralkoxyalkane (PFA) tubing (OD 1.5 mm and ID ~508 μm) was connected to both ends of a polyetherether ketone (PEEK) tubing (OD ~510 μm). For the creation of side injection channels, fused silica capillaries were attached to the surface of the PEEK tubing. In parallel, a mixture of PDMS prepolymer and curing agent (10:1) was poured in a Petri dish and half-cured at 60°C for 45 min. While, in the case of using Low MW. PFPE as the substrate for fabrication, prepolymer was mixed with photo initiator and photocured for 1 min. After being placed on top of the half-cured polymers, the assembled tubing frame was completely covered with extra prepolymer PDMS and thermally cured for 2 hours and photo-cured PFPE for 9 min, respectively. Completely cured elastomers were cut carefully and the tubing structure was slowly pulled off.

RESULTS AND DISCUSSION

The optical pictures of T-junction microfluidic device made of PDMS or Low MW PFPE are shown in Figure 1 (C). It was confirmed that the diameter of the channel formed in the final devices was identical to the OD’s of the tubing used to make the framework, i.e. the diameters of side injection channel (365 μm) and the center channel (510 μm) was identical to the dimensions of fused silica capillary and PEEK tubing, respectively; on the other hand, the large channel connecting the central channel was found to have ID of 1.5 mm (Figure 1 (D)). The SEM image of tilted angle horizontal sectional view of microfluidic device shown in Figure 1 (D) clearly indicates the presence of various channel sizes in a single device. The large channels at both ends were used as a port for PFA tube cartridge to collect the droplets.

The channels were made fluorophilic by the procedures described elsewhere [3] to avoid adsorption of protein solution onto the walls of the PDMS device and to facilitate wetting of channels with fluorocarbon liquid (FC-77, 3M, St. Paul, MN) used as carrier liquid. Alternatively, the microfluidic device made of Low MW PFPE material was used without any prior treatment, as it wets easily with FC liquid when compared to PDMS device.
The schematic of the method used for protein synthesis in present work is shown in Figure 2. As an initial step, array of nanoliter droplets were produced and stored in a PFA tube cartridge for PCR. After PCR these droplets are merged with protein expression machinery to express green fluorescence protein. The merged droplets were collected in a PFA tube followed by incubation at 37°C for “1” hour for protein synthesis. A fluorescence microscope (TE-2000, Nikon, Japan) equipped with high resolution CCD camera was used to monitor the fluorescence from the expressed green fluorescence protein (EGFP). The average volume of each PCR droplet was found to be 47 nL (n=10, % RSD 3.11). The volume of droplet was measured using image analysis software (Image J, NIH, USA). While the volume of protein merged was about 1.25 times higher than the volume of the PCR with an average volume of 59 nL. Fluorescence image of expressed EGFP in PFA tube cartilage is shown in Figure 2.

CONCLUSIONS

In this paper, we demonstrate a substantially simple, single step method to fabricate monolithic, multilevel microfluidic device with minimal or nil sophisticated fabrication facilities. Apart from this, we have successfully demonstrated use of the fabricated microfluidic devices in protein synthesis in three phase flow. The presented method for protein synthesis holds tremendous potential in simultaneous expression of protein and study of protein function in short span of time with high throughput.

REFERENCES