MICROFLUIDIC MODULES FOR ISOLATION OF RECOMBINANT CYTOKINE FROM BACTERIAL LYSATES

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ABSTRACT
The portability and personalization of health-care diagnostics and treatments benefits from advancements and applications of micro and nanotechnology. Modularization and miniaturization of standardized biochemical processes and tests facilitates the advancement and customization of analyte detection and diagnosis on-chip. The goal of our work here is to develop modular platforms for on-chip biochemical processing of synthesized biologics for a range of on-demand applications. Our report focuses on the initial development, characterization and application of microfluidic size exclusion/gel filtration and ion exchange protein concentration modules for cytokine isolation from spiked cell extracts.

KEYWORDS: Microfluidic, chromatography, protein synthesis, portable, biochemistry

INTRODUCTION
This work reports the development of microfluidic modules for on-chip purification of synthesized recombinant biopharmaceuticals. Recently, reactors for cell-free protein synthesis have been demonstrated over a range of scales from liters [1] to femtoliters [2]. Our group has demonstrated microscale reactors for cell-free protein translation [3] and enzymatic reactions [4]. Here we present the fabrication and testing of microfluidic modules that incorporate (1) microporous barriers for retaining chromatography beads, and (2) bifurcating channels for lateral distribution of fluid samples over the high surface area chromatography bed. These two features facilitate facile loading of devices with size-matched chromatography media and minimize dispersion across the channel width, respectively.

EXPERIMENTAL
Fabrication and assembly. Silicon masters are created using photolithography and deep reactive ion etching (DRIE). Briefly, silicon dioxide-coated wafers are used as starting material. Conventional photolithography steps are used to define the microfluidic architecture and are followed by a silicon-dioxide RIE etch process to selectively remove SiO₂ from the wafer, defining the hard etch mask for the DRIE process. A modified Bosch process is then used to transfer the pattern into the wafer to produce a ~130 μm tall silicon-based master for replicate manufacture of PDMS-based microfluidic chromatography modules. Standard PDMS replicate molding processes utilizing silastic tubing embedment and plasma-bonding are used for module production. Size exclusion (G-25 Sephadex, 978-0-9798064-7-6/$20©14CBMS-0001 2378 18th International Conference on Miniaturized Systems for Chemistry and Life Sciences October 26-30, 2014, San Antonio, Texas, USA
≤ 70 μm) and ion exchange (diethylaminoethanol (DEAE) sepharose) modules are packed with their respective size-matched chromatography media.

Characterization and implementation. Flow was achieved by using glass fluid columns affixed to the microfluidic inlet ports attached to a 1 mL micropipette tip. The fluid columns provide a pressure head for gravity-induced flow; pressure head heights were 7.5 cm, 10 cm, 20 cm, and 30 cm. Column fractions (5 μl) were collected into a 384-well plate, analyte absorbance was measured on a microplate reader. The concentration of protein samples were determined with the Bradford assay and spectroscopic absorbance measurements at 280 nm using bovine serum albumin (BSA) as a protein standard. Conventional protein agarose gel electrophoresis (SDS-PAGE, 10% miniprotein BioRad TGX gels) was used to observe the relative abundance and size distribution of proteins present in sample and product volumes. Enzyme-linked immunosorbent assay (ELISA) was used to determine the presence of the cytokine, granulocyte-macrophage colony-stimulating factor (GM-CSF) retrieved through the cleanup process of cytokine-spiked cell culture extracts.

RESULTS AND DISCUSSION

Our results demonstrate the characterization and implementation of chromatography-based microfluidic devices. Typical steps for protein purification include size exclusion and ion exchange. The size exclusion module shown in Figure 1 was used for desalting protein products. Column efficiency was determined by measuring elution peaks of small molecules; red 40 (MW = 496.42 g/mol) was used for flow and elution characterization. Elution peaks for red 40 are shown for pressure head heights of 7.5 cm, 10 cm, 20 cm, and 30 cm with corresponding flow rates (figure legend). Elution peaks of microfluidic size exclusion show a mean retention time of 115 min (7.5 cm), 86 min (10 cm), 46 min (20 cm), and 23 min (30 cm) for the respective pressure head heights (cm). A perfectly symmetrical peak holds a 1.0 symmetry value. The microfluidic desalting module exhibits absorption peak symmetry values between 0.8 and 1.8 for red 40 and are within ideal ranges [5].

An ion exchange module packed with the anion exchange media, diethylaminoethanol (DEAE) sepharose beads (Figure 2), permits on-chip concentration of proteins in microfluidics. As demonstrated by the increase in fluorescence of ion exchange beads during enhanced green fluorescent protein (EGFP) capture (0:00-0:45 hr:min), proteins are continuously collected through weak binding interactions, then
eluted (0:51-0:54 hr:min) with a high salt buffer to provide a concentrated protein solution (Figure 3). The post elution image (0:57 hr:min) shows no residual fluorescence. The inset graph (elution image (0:54)) shows a characteristic peak of EGFP fluorescence during elution off the ion exchange device. Elution data for each 5 μl fraction (150-220) is shown. Figure 4 shows protein abundance of ion exchange fractions with flow-through collection and elution fractions from a microfluidic ion exchange fractionation of E. coli cell extract spiked with human granulocyte-macrophage colony-stimulating factor (GM-CSF). The presence of GM-CSF was confirmed through SDS-PAGE gel electrophoresis (Figure 5) and ELISA. We envision that by coupling on-chip microfluidic cleanup modules with cell-free protein synthesis reactors, the deployment of scaled up cytokine reactors for on demand synthesis and delivery of biopharmaceuticals will be possible.

CONCLUSION

The realization of on-chip protein capture, fractionation, desalting and buffer exchange may be achieved through modular microfluidic platforms. Scaling down the biochemical purification process affords a range of possibilities for on-demand synthesis and delivery of biologics, and for processing minute samples for discovery-based pharmacosynthesis. By developing and characterizing plug-and-play modules for standardized purification processes, a variety of clean up sequences can be tailored for molecular and process specific applications, remotely and on demand.

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REFERENCES


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