A CENTRIFUGO-MICROFLUIDIC CARTRIDGE WITH INTEGRATED DETECTION OPTICS TOWARDS AUTOMATED AT-LINE BIOPROCESS MONITORING OF IMMUNOGLOBULIN G

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ABSTRACT

This paper reports on a new microfluidic system for at-line bioprocess monitoring of human immunoglobulin G (IgG). This is the first report on the successful integration of supercritical angle fluorescence (SAF) detection with a centrifugal microfluidic "lab-on-a-disc" cartridge, enabled using novel SAF detection optics incorporated onto the microfluidic device. The microfluidic cartridge utilizes serial siphon valving to show for the first time sequential release of the sample, wash, and fluorescence-labeled solutions required to perform a quantitative IgG sandwich immunoassay. Using a custom, compact, benchtop instrument with integrated spinning motor and SAF optical components, a microfluidic, flow-through, IgG immunoassay on a Zeonor® surface was developed and successfully tested for IgG concentration monitoring.

KEYWORDS: Centrifugal, microfluidics, supercritical angle fluorescence, bioprocess

INTRODUCTION

While sample-to-answer systems are predominately focused on clinical diagnostic settings, the at-line monitoring of bioproduction processes is a key market area that remains relatively unexplored [1]. The monitoring of extracellular products (*e.g.*, therapeutic antibodies such as IgG) within the complex bioprocess matrix of a bioreactor is an essential and challenging task. Thus, the development of an at-line, sample-to-answer microfluidic system that can monitor bioreactor conditions is a vital step towards more optimized and cost-efficient bioprocess production. However, while the design and fabrication of effective and inexpensive microfluidic chips is becoming more widespread, the integration of these chips with hardware systems, especially signal detection systems, remains a challenge. Thus, system integration challenges remain in the design of an optically-integrated microfluidic analysis system.

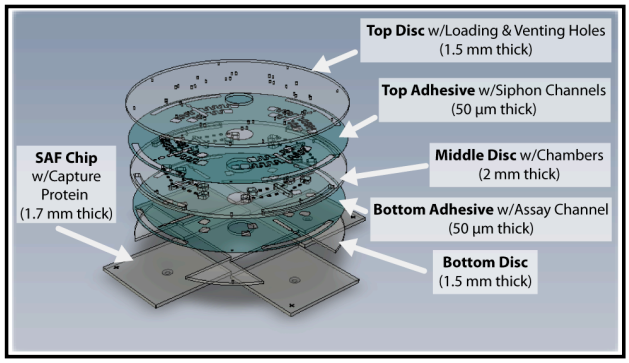

Figure 1: Diagram showing the various layers of the microfluidic disc. The disc layers were made of acrylic and adhesive, while the SAF chips were made of Zeonor®. The SAF chips were surface treated with APTES, coated with Protein A, and blocked with bovine serum albumin before assembly.

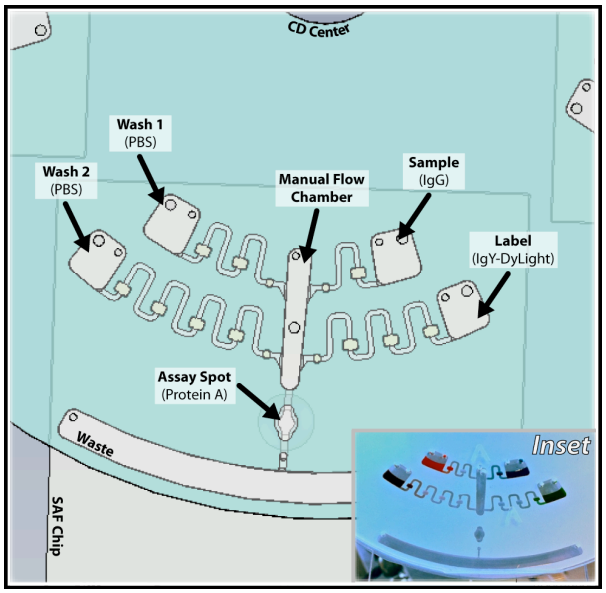

Figure 2: Schematic showing a close-up of one of four assay devices on the microfluidic cartridge along with a photo of the disc (Inset). The serial siphons are shown for each chamber. The manual flow chamber was used to process the IgG immunoassay.

THEORY

Fluorescence detection methods often require bulky and expensive optical hardware for implementation. The use of supercritical angle fluorescence (SAF) allows for simpler and less-expensive hardware while providing increased sensitivity over traditional, bulk fluorescence measurements. In brief, SAF works by extracting, focusing, collecting, and measuring the surface-confined emitted fluorescence that is optically trapped within a substrate via total internal reflection.

While there are many microfluidic technologies available, the centrifugal microfluidic platform is one of the more successful to date, and a variety of microfluidic functions have been demonstrated on the centrifugal platform including valving, metering, mixing, dilution, and particle handling. Centrifugal forces have also been shown to positively benefit active flow-through assays, especially for surface-based assays, by decreasing total assay time and increasing assay sensitivity.

In light of the advantages of SAF and centrifugal microfluidic technologies, this work, for the first time, integrates them to form a novel system capable of performing surface-based fluorescence bioassays. In particular, a sandwich immunoassay for bioprocess-monitoring of therapeutic IgG was used to demonstrate functionality of the integrated system.

EXPERIMENTAL

Centrifugal microfluidic cartridges were made of laminated acrylic and adhesive layers (Fig. 1), including a novel, injection-molded, optical SAF chip [2]. The siphon valves used were based on a previous report and adapted for this application (Fig. 2) [3]. An optimized aminopropyltriethoxysilane (APTES) surface treatment protocol was used to immobilize Protein A on the SAF chip surface (Fig. 3) [4]. The benchtop instrument, comprised of optical and centrifugal systems, was custom-fabricated using off-the-shelf components and controlled using National Instruments hardware and software (Fig. 4) [5]. The serial siphons were optimized first, and then assay data were obtained using the cartridge in a manual flow-through mode. Optical SAF measurements were taken using the benchtop instrument.

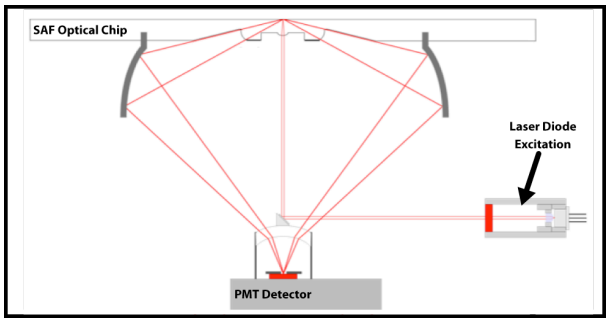

Figure 3: Diagram of the SAF detection principle showing collection of supercritical angle emission using the novel optical SAF chip.

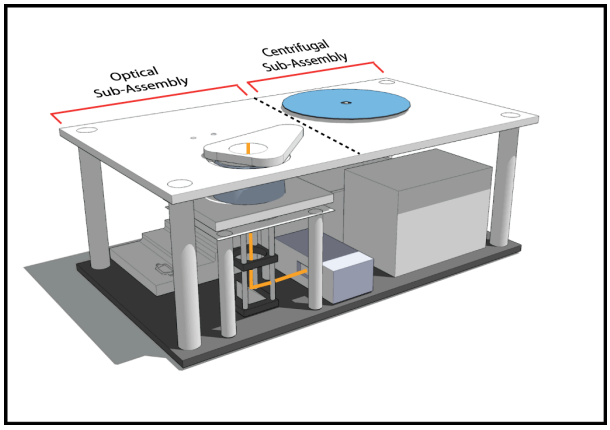

Figure 4: Schematic showing the benchtop hardware instrument with sub-systems labeled. The system requires a laptop for control of both the centrifugal and optical sub-assemblies. The system measures approximately 18 cm x 18 cm x 33 cm (H x W x L).

RESULTS AND DISCUSSION

SAF detection was successfully integrated onto the centrifugal microfluidic cartridge to enable sensitive and surface-confined fluorescence detection. The IgG monitoring assay was developed on the optical Zeonor® SAF chip and, using the developed cartridge and hardware, was demonstrated to meet the design specifications required for quantification of IgG in a bioprocess environment (Fig. 5). Specifically, the system exhibited an excellent, statistically significant response as measured within a dynamic range of two orders of magnitude (50 µg mL^{-1} to 500 ng mL^{-1} of IgG). The microfluidic IgG immunoassay was highly reproducible with a <10.5% coefficient of variation as calculated from 9 data points across 3 distinct prototype cartridges. It should be emphasized that such a low coefficient of variation is truly impressive for the prototype system presented, as this error represents a combination of the error in the immunoassay itself, the Zeonor® APTES surface, the prototype centrifugal cartridge, the prototype measurement system, and optical alignment between the latter two. In addition, serial siphons were shown to successfully gate the multiple reagents required. The results demonstrate that using an optimized surface chemistry in combination with centrifugal flow-through and SAF technologies yields a functional proof-of-concept immunoassay for quantifying IgG levels.

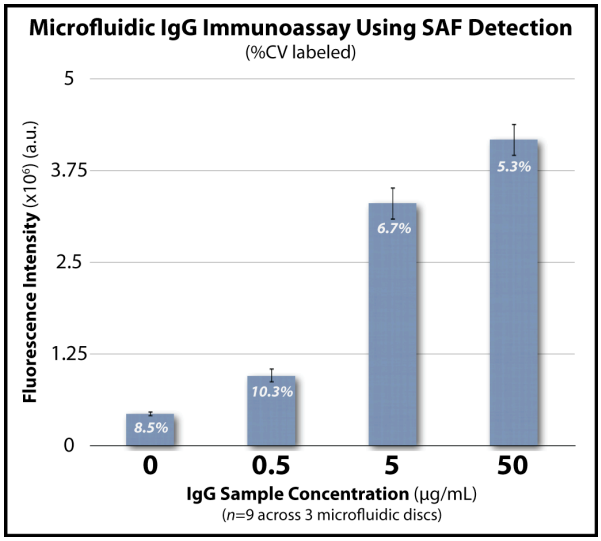

Figure 5: IgG quantification assay results. Three different IgG concentrations in addition to a blank were processed using the microfluidic disc in a manual flow-through mode and measured using the prototype SAF instrument (error bars are ± 1 std. dev.).

CONCLUSION

A surface-based, IgG bioprocess-monitoring assay was successfully demonstrated using a centrifugal microfluidic flow-through cartridge with integrated SAF detection. An APTES-based surface chemistry for immobilization of proteins in the microchannel on the Zeonor® SAF chip was shown, as was the instrument required to operate the cartridge. In addition, successful functionality of the serial siphons was shown for gating of the required reagents. Next steps include further incorporation of the developed IgG quantification assay with bioprocess sample preparation steps on the same microfluidic cartridge. Space on the microfluidic cartridge above the reagent delivery structures has been reserved for integration with these processes for further upstream liquid-handling operations. For the hardware instrument, a further reduction in the size of the prototype will facilitate improved portability, which can be achieved by integrating the optical and centrifugal subassemblies into a single system. Once these goals are achieved, manufacturing issues can be addressed such that a complete system can be realized for a commercial, at-line, sample-to-answer, therapeutic IgG bioprocess monitoring system.

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