Journal of Chromatography A, 1466 (2016) 29–36

Contents lists available at ScienceDirect

Journal of Chromatography A

journal homepage: www.elsevier.com/locate/chroma

Hollow silica microspheres for buoyancy-assisted separation of infectious pathogens from stool

Shannon E. Weigum [a,b,*], Lichen Xiang [a], Erica Osta [b], Linying Li [c,d], Gabriel P. López [c,d,e]

[a] Materials Science, Engineering, and Commercialization Program, Texas State University, San Marcos, TX 78666, USA
[b] Department of Biology, Texas State University, San Marcos, TX 78666, USA
[c] Department of Biomedical Engineering, Duke University, Durham, NC 27708, USA
[d] NSF Research Triangle Materials Research Science and Engineering Center, Durham, NC 27708, USA
[e] Center for Biomedical Engineering, Department of Chemical and Biomedical Engineering, University of New Mexico, Albuquerque, NM 87131, USA

ARTICLE INFO

Article history:
Received 15 June 2016
Received in revised form 9 August 2016
Accepted 1 September 2016
Available online 4 September 2016

Keywords:
Bioseparation
Sample preparation
Analyte concentration and isolation
Buoyancy cell separation
Glass microbubbles

ABSTRACT

Separation of cells and microorganisms from complex biological mixtures is a critical first step in many analytical applications ranging from clinical diagnostics to environmental monitoring for food and waterborne contaminants. Yet, existing techniques for cell separation are plagued by high reagent and/or instrumentation costs that limit their use in many remote or resource-poor settings, such as field clinics or developing countries. We developed an innovative approach to isolate infectious pathogens from biological fluids using buoyant hollow silica microspheres that function as "molecular buoys" for affinity-based target capture and separation by floatation. In this process, antibody functionalized glass microspheres are mixed with a complex biological sample, such as stool. When mixing is stopped, the target-bound, low-density microspheres float to the air/liquid surface, which simultaneously isolates and concentrates the target analytes from the sample matrix. The microspheres are highly tunable in terms of size, density, and surface functionality for targeting diverse analytes with separation times of ≤2 min in viscous solutions. We have applied the molecular buoy technique for isolation of a protozoan parasite that causes diarrheal illness, *Cryptosporidium*, directly from stool with separation efficiencies over 90% and low non-specific binding. This low-cost method for phenotypic cell/pathogen separation from complex mixtures is expected to have widespread use in clinical diagnostics as well as basic research.

© 2016 Elsevier B.V. All rights reserved.

1. Introduction

Separation of disease-related cells and microorganisms from complex biological mixtures is a critical first step in many analytical applications ranging from clinical diagnostics to environmental monitoring for food and waterborne pathogens [1]. Existing separation methods can be loosely grouped into two categories; those that rely upon physical differences among cells (i.e. size, shape, density, or electrophoretic mobility); and those that rely upon differences in chemical composition (i.e. protein or other cell surface markers) [1,2]. The most common physical separation methods include size-dependent micro/ultrafiltration and density-based centrifugation using Percoll® or Ficoll-Paque® density gradients [1,3]. These methods typically have the advantage of being label-free and non-destructive which allows for subsequent cell culture or growth; however, they depend upon a large physical difference between target and non-target materials, limiting the ability to discriminate between closely-related populations [2]. Affinity-based techniques, such as magnetic-activated cell separation (MACS) and fluorescence-activated cell separation (FACS) overcome these limitations through the use of target-specific antibodies to impart higher selectivity for the particular cell type or pathogen of interest [2,4,5]; however, high reagent and/or instrumentation costs continue to limit their use in many remote or resource-poor settings. Even microfluidic and lab-on-a-chip platforms for cell separation remain limited in their widespread clinical adoption due to complexity and cost.

Recently, a few studies have emerged describing a buoyancy-activated cell sorting (BACS) technique that uses low-density hollow glass microbubbles for separation of CD4+ T-cells and circulating tumor cells from whole blood, and even cancer stem cells from heterogeneous cell populations [6–8]. Advantages of BACS include (i) low material cost of the glass microbubbles, which are an industrial additive used in plastics and coatings available

* Corresponding author at: Department of Biology, Texas State University, San Marcos, TX 78666, USA.
E-mail address: sweigum@txstate.edu (S.E. Weigum).

http://dx.doi.org/10.1016/j.chroma.2016.09.002
0021-9673/© 2016 Elsevier B.V. All rights reserved.

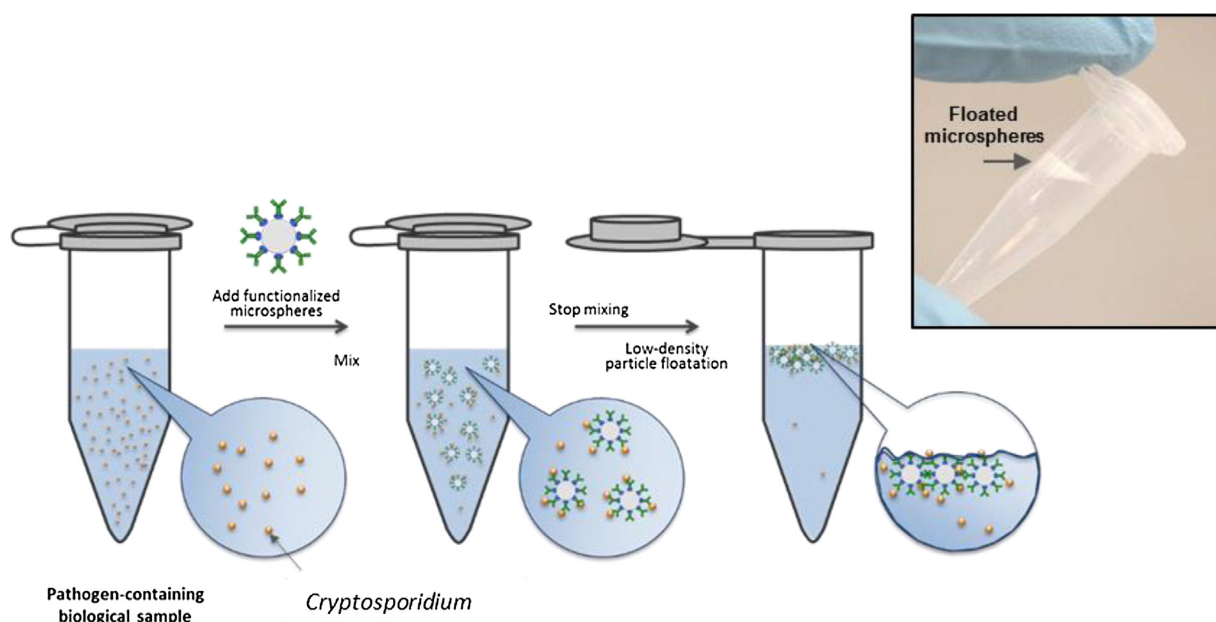

Fig. 1. Schematic of generalized "molecular buoy" concept for pathogen isolation thorough buoyancy-assisted cell separation. Photograph of floated microspheres in buffer visually demonstrates separation of the hollow silica microspheres from the bulk solution.

in kilogram quantities, and (ii) the simplicity of the floatation-based separation, which eliminates the need for any external instrumentation like a centrifuge or magnet. In addition, surface functionalization of glass microbubbles can take advantage of the well-established silica-based chemistries for direct conjugation of proteins, antibodies, or other affinity ligand [9].

In the current study, we describe for the first time a BACS process for the isolation of an infectious disease pathogen from perhaps the most complicated biological sample type, stool. A schematic of this "molecular buoy" method for isolation of *Cryptosporidium*, a protozoan parasite that causes diarrheal illness, is illustrated in Fig. 1. Here, a complex biological sample is incubated with functionalized hollow microspheres and mixed in order to facilitate target capture of the analytes. When mixing is stopped, the target-bound low-density microspheres migrate to the air/liquid interface where they float at the surface, which simultaneously isolates and concentrates the target analytes from the sample matrix.

We chose *Cryptosporidium* as our initial target pathogen since passive fecal floatation is a technique that has long been used for the recovery of parasitic eggs and oocysts in stool prior to microscopic examination and diagnosis in humans and pets [10–12]. The conventional stool floatation method relies on the differences in the specific gravity of the eggs/oocysts, fecal debris, and the floatation solution [11]. Some common floatation solutions include modified Sheather's solution, zinc sulfate and magnesium sulfate with a specific gravity in the range of 1.18–1.27 (specific gravity of water is 1.0) [11]. Parasitic eggs/oocysts that have a lower specific gravity (between 1.05 and 1.15) will, over time or under centrifugal force, float to the surface while heavier debris will sink to the bottom [11]. Drawbacks of this technique include low recovery rates, the remaining need for centrifugation, and the constraint to organisms that have an inherently low specific gravity, such as eggs and encapsulated cysts, which can have gas or air pockets that lower their overall density. Using the molecular buoy approach, we aim to overcome these restrictions and generate a high affinity, density-based separation technique that uses low-cost materials, eliminates any external instrumentation or centrifugation, and can be universally applied to isolate a variety of clinical pathogens and biomarkers.

2. Materials and methods

2.1. Materials and reagents

We obtained the H20 and H50 hollow silica microspheres, with an epoxy silane surface treatment and a density of 0.2 g/cm^3 and 0.5 g/cm^3, respectively from 3 M (H20/H50 glass bubbles; 3 M, St. Paul, MN). Buffers, glycerol, protein G, and L-cysteine were all purchased from Thermo Fisher Scientific (Waltham, MA). Phosphate buffered saline (PBS) was prepared in deionized water using BupH^{TM} Modified Dulbecco's PBS (#28374) buffer packs with a final concentration of 8 mM sodium phosphate, 2 mM potassium phosphate, 0.14 M sodium chloride and 10 mM potassium chloride at a pH of 7.4. *C. parvum* oosysts (Iowa isolate, #P102C) and negative control stool were purchased from Waterborne Inc. (New Orleans, LA). Antibodies included an unconjugated anti-*C. parvum* monoclonal mouse IgG3 (clone BEL 0126, #64526, Novus Biologicals, Littleton, CO), an anti-*C. parvum* directly conjugated to Alexa-Fluor® 488 (clone BEL 0126, #2402-3007AF488, Bio-Rad AbD Serotec, Raleigh, NC) and a goat-anti-mouse (GAM) IgG conjugated to Alexa-Fluor® 488 (#A11001, Invitrogen, Carlsbad, CA).

2.2. Microsphere size and floatation kinetics

The H20/H50 glass microspheres were suspended in PBS at a final concentration of 1.0 mg/ml. A wet-mount slide using ~10 µl of the microsphere solution was prepared for imaging on an EVOS FL Auto Cell Imaging System (Thermo Scientific, Waltham, MA) using an Olympus UPlanApo 10× objective (0.4 NA). The diameter of at least 5000 microspheres was measured directly from transmitted light microscopy images using ImageJ open-source software [13]. ImageJ particle analysis criteria applied to binary, thresholded images included a minimum circularity of 0.85 in order to eliminate non-solitary or broken microspheres from the size analysis. Histograms with a bin size 4 µm were prepared in SigmaPlot 12.5 (SyStat Software, Inc., San Jose, CA), along with all other graphs.

Additional size fractionation of the H20 microspheres was achieved using a stacked set of wire mesh test sieves (VWR, Radnor, PA) with standard mesh sizes of 140, 200, 270, and 500 that corre-

Download English Version:

https://daneshyari.com/en/article/5135969

Download Persian Version:

https://daneshyari.com/article/5135969

Daneshyari.com