Cytometry PART A
Journal of the International Society for Advancement of Cytometry

ISAC
International Society for Advancement of Cytometry

Affinity-Bead-Mediated Acoustophoresis: A Novel Tool in Cytometry

Martin Wiklund*

ACOUSTOPHORESIS combined with biofunctionalized capture beads—a method called affinity-bead-mediated acoustophoresis—can be used for highly selective isolation of CD4+ lymphocytes from peripheral blood progenitor cell products (PBPCS). This opens possibilities for improved hematopoietic stem cell transplantation.

Acoustophoresis is a method based on the migration of suspended particles or cells in an acoustic field caused by the acoustic radiation force. When implemented in a flow-through setting, acoustophoresis can be used for separation of particles from a fluid medium, or separation of particles having different size and acoustic properties (1). Over the last 10–15 years, acoustophoresis applied to microfluidic devices has been rapidly developing (2), primarily due to its simplicity and low cost relative alternative methods. For cytometry purposes, acoustophoresis-based technology was recently (in year 2010) implemented commercially for improving cell alignment, as an alternative to the standard sheath-flow alignment [Attune® NxT Acoustic Focusing Cytometer, Life Technologies™ (3)].

In most research studies so far, flow-through-based acoustophoresis have been used for the direct separation of cells. In these studies, the cell property determining the separation outcome is typically cell size and/or density and compressibility (1). An alternative strategy is to use an affinity-based separation principle relying on the interaction/binding between a ligand and a receptor. This strategy has previously been used together with acoustophoresis for example, enhancing the speed and sensitivity of latex agglutination tests (Fig. 1a) (4) and bead-based fluorescence assays (Fig. 1b) (5), and for specific extraction of molecular compounds from phage libraries (Fig. 1c) (6) and other complex samples (7). These assays are reviewed in more detail in Refs. 8–10. Common for the methods described in Refs. 4–10 is the use of affinity-specific beads for capturing/concentrating different small analytes (such as molecules and viruses) that are not directly influenced by the acoustic forces. However, it is also possible to isolate whole cells bound to affinity-specific beads (Fig. 1d). This approach is more difficult as the beads and cells often have similar sizes and acoustic properties. Thus, both bound and unbound cells are to some extent influenced by the acoustic field. For that reason, it is important to select capture beads with distinct acoustic properties differing from the properties of the cells to be isolated and the suspension medium.

In this issue of Cytometry A (page 933), Lenshof et al. demonstrate acoustophoretic isolation of specific cells by the use of 4.5 μm magnetic Dynabeads®. Such magnetic beads are denser than cells and will therefore respond faster to the acoustic field compared to cells of similar size, but also compared to polymer beads of similar size. Although previous studies of affinity-bead-mediated acoustophoresis for capturing molecules or viruses used polymer beads (4–7), the denser magnetic beads used by Lenshof et al. actually turned out to be more suitable for acoustic cell isolation. The trick used by the authors to optimize their separation was based on tuning the acoustic property of the suspension medium. Using a Ficoll wash buffer (slightly denser than cells) instead of a standard phosphate buffered saline (PBS) buffer (less dense than cells), they created an "acoustic impedance barrier" preventing

Department of Applied Physics, Royal Institute of Technology, Stockholm, Sweden

Received 8 August 2014; Accepted 19 August 2014

*Correspondence to: Martin Wiklund, Department of Applied Physics, Royal Institute of Technology, KTH – Albanova, SE-106 91 Stockholm, Sweden. E-mail: martin@biox.kth.se

Published online 2 September 2014 in Wiley Online Library (wileyonlinelibrary.com)

DOI: 10.1002/cyto.a.22565

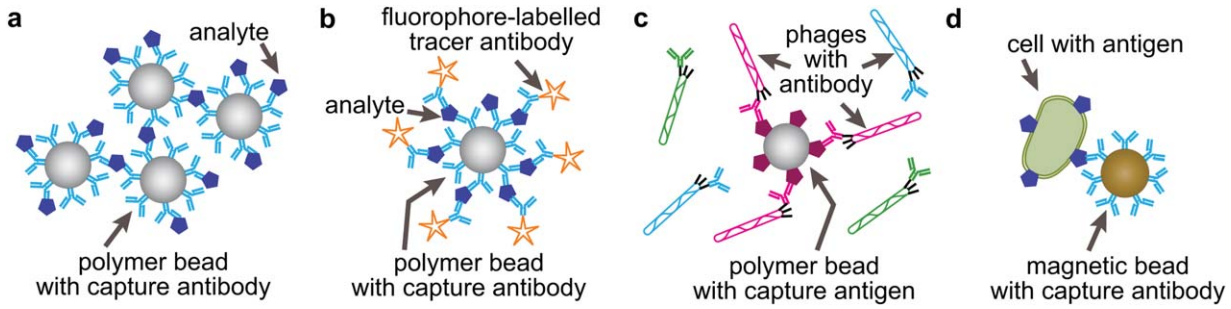

Figure 1. Schematics of different affinity-specific bead assays that have been used with acoustophoresis. (**a**) The latex agglutination assay (4). (**b**) The bead-based fluorescence assay (5). (**c**) The bead-based phage display assay (6). (**d**) The bead-based cell assay used by Lenshof et al. in this issue of Cytometry A (page 933), where the antigen of interest is the CD4 molecule. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

unbound cells to enter the separation zone (the center part of the channel), see Figure 2. This trick would not have been possible if standard polymer beads were used. In addition, the choice of magnetic beads also allowed for comparing their acoustic method with standard magnetic cell sorting techniques.

The affinity-bead-mediated acoustophoresis method presented by Lenshof et al. was used to selectively isolate CD4+ lymphocytes from peripheral blood progenitor cell products. This choice of bioapplication to their acoustic method is motivated by the need for simpler, faster, and more specific methods for isolating or depleting specific cells from complex clinical samples. For example, in hematopoietic stem cell transplantation, PBPCs are often used for treatment of patients suffering from hematological diseases. However, a common problem when transplanting stem cells is the occurrence of graft-versus-host disease caused by an immune response against nonmalignant recipient cells. For that reason, it is of outmost importance to develop methods for highly specific isolation or depletion of stem cells or lymphocyte subsets. As shown by Lenshof et al. in their paper, this can be realized using a single platform technique based on acoustophoresis.

One of the most interesting aspects for cytometry in the work by Lenshof et al. is the extra degree of freedom obtained by tuning the density of the wash buffer. Thus, by carefully selecting the acoustic properties of the capture beads and the suspension medium relative the properties of cells to be separated, simultaneous affinity-bead-mediated acoustophoretic sorting of multiple cell populations would be possible in the future. This is not possible with current magnetic separation technology. In addition, when implemented correctly—including temperature and power control (11)—acoustophoresis is today a proven safe method not compromising cellular viability or function (12). The biocompatibility of the device and method used by Lenshof et al. was tested using a set of different relevant assays: Colony-forming hematopoietic progenitor cell assays, lymphocyte proliferation assays and lymphocyte cytokine secretion assays. Altogether, their measurements confirmed that neither progenitor cell function nor CD4+ T-lymphocyte function is affected by acoustophoresis. This result is important for the safe acoustophoretic processing of real clinical samples in various future applications of the presented method.

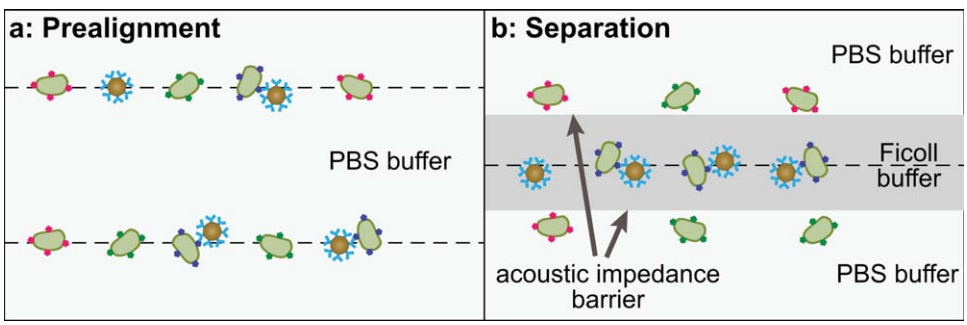

Figure 2. Schematic illustration of the acoustophoretic separation principle used by Lenshof et al., using the bead-based cell assay shown in Fig. 1d. (**a**) Both bead-bound cells, unbound cells and beads without bound cells are first prealigned into two acoustic nodes (dotted lines). Here, the sample is suspended in a lower-density PBS buffer. (**b**) Further downstream, the bead-bound cells and beads without bound cells are driven to a single acoustic node (dotted line) inside the higher-density Ficoll buffer (darker area), while unbound cells are prevented to enter the Ficoll buffer due to the "acoustic impedance barrier" between the two buffers. Since the magnetic beads have a density significantly higher than the Ficoll buffer, a bead-cell complex may penetrate this barrier, in spite of the fact that unbound cells cannot. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

Literature Cited

1. Lenshof A, Magnusson C, Laurell T. Acoustofluidics 8: Applications of acoustophoresis in continuous flow microsystems. Lab Chip 2012;12:1210–1223.
2. Bruus H, Dual J, Hawkes J, Hill M, Laurell T, Nilsson J, Radel S, Sadhal S, Wiklund M. Forthcoming Lab on a Chip tutorial series on acoustofluidics: Acoustofluidics—Exploiting ultrasonic standing wave forces and acoustic streaming in microfluidic systems for cell and particle manipulation. Lab Chip 2011;11:3579–3580.
3. Available at: https://www.lifetechnologies.com/se/en/home/life-science/cell-analysis/flow-cytometry/flow-cytometers/attune-acoustic-focusing-flow-cytometer.html. Accessed 14 July 2014.
4. Grundy MA, Moore K, Coakley WT. Increased sensitivity of diagnostic latex agglutination tests in an ultrasonic standing wave field. J Immunol Methods 1994;176:169–177.
5. Wiklund M, Toivonen J, Tirri M, Hänninen P, Hertz HM. Ultrasonic enrichment of microspheres for ultrasensitive biomedical analysis in confocal laser-scanning fluorescence detection. J Appl Phys 2004;96:1242–1248.
6. Persson J, Augustsson P, Laurell T, Ohlin M. Acoustic microfluidic chip technology to facilitate automation of phage display selection. Febs J 2008;275:5657–5666.
7. Augustsson P, Persson J, Ekström S, Ohlin M, Laurell T. Decomplexing biofluids using microchip based acoustophoresis. Lab Chip 2009;9:810–818.
8. Wiklund M, Hertz HM. Ultrasonic enhancement of bead-based bioaffinity assays. Lab Chip 2006;6:1279–1292.
9. Augustsson P, Laurell T. Acoustofluidics 11: Affinity specific extraction and sample decomplexing using continuous flow acoustophoresis. Lab Chip 2012;12:1742–1752.
10. Wiklund M, Radel S, Hawkes J. Acoustofluidics 21: ultrasound-enhanced immunoassays and particle sensors. Lab Chip 2013;13:25–39.
11. Svennebring J, Manneberg O, Wiklund M. Temperature regulation during ultrasonic manipulation for long-term cell handling in a microfluidic chip. J Micromech Microeng 2007;17:2469–2474.
12. Wiklund M. Acoustofluidics 12: Biocompatibility and cell viability in microfluidic acoustic resonators. Lab Chip 2012;12:2018–2028.