AirJump: Using Interfaces to Instantly Perform Simultaneous Extractions

Scott M. Berry,* Hannah M. Pezzi, Alex J. LaVanway, David J. Guckenberger, Meghan A. Anderson, and David J. Beebe

Department of Biomedical Engineering, University of Wisconsin—Madison, Madison, Wisconsin 53705, United States

ABSTRACT: Analyte isolation is an important process that spans a range of biomedical disciplines, including diagnostics, research, and forensics. While downstream analytical techniques have advanced in terms of both capability and throughput, analyte isolation technology has lagged behind, increasingly becoming the bottleneck in these processes. Thus, there exists a need for simple, fast, and easy to integrate analyte separation protocols to alleviate this bottleneck. Recently, a new class of technologies has emerged that leverages the movement of paramagnetic particle (PMP)-bound analytes through phase barriers to achieve a high efficiency separation in a single or a few steps. Specifically, the passage of a PMP/analyte aggregate through a phase interface (aqueous/air in this case) acts to efficiently “exclude” unbound (contaminant) material from PMP-bound analytes with higher efficiency than traditional washing-based solid-phase extraction (SPE) protocols (i.e., bind, wash several times, elute). Here, we describe for the first time a new type of “exclusion-based” sample preparation, which we term “AirJump.” Upon realizing that much of the contaminant carryover stems from interactions with the sample vessel surface (e.g., pipetting residue, wetting), we aim to eliminate the influence of that factor. Thus, AirJump isolates PMP-bound analyte by “jumping” analyte directly out of a free liquid/air interface. Through careful characterization, we have demonstrated the validity of AirJump isolation through comparison to traditional washing-based isolations. Additionally, we have confirmed the suitability of AirJump in three important independent biological isolations, including protein immunoprecipitation, viral RNA isolation, and cell culture gene expression analysis. Taken together, these data sets demonstrate that AirJump performs efficiently, with high analyte yield, high purity, no cross contamination, rapid time-to-isolation, and excellent reproducibility.

KEYWORDS: AirJump, exclusion-based sample prep, purification, extraction, air/liquid interface, paramagnetic particles

INTRODUCTION

The ability to extract or separate a target analyte (e.g., nucleic acid, protein, whole cell) from a complex background is an essential process that permeates throughout the life sciences and beyond. This technique is a critical prerequisite for many common analytical processes in diagnostics, biological research, biomarker discovery, forensics, and more. Unfortunately, conventional analyte isolation processes are time-consuming, expensive, and laborious, often becoming the bottleneck within the analytical process. Given the ubiquity of analyte isolation, simplifications streamlining this process have the potential to yield dramatic time and cost savings worldwide.

Recently, our group and others have developed a new paradigm for purification in which paramagnetic particle (PMP)-bound analytes are magnetically drawn through an interface (e.g., aqueous/air, aqueous/oil) in order to obtain a near-instantaneous separation of the analyte from the remainder of the unbound material.12 This collection of techniques, which we generally refer to as exclusion-based sample preparation (ESP), represents a significant simplification over “traditional” PMP-based separation techniques, which typically involve washing a PMP-bound analyte multiple times with buffer to remove the background from the bound fraction. In addition to streamlining the separation process, ESP offers functional advantages over traditional methods. Specifically, we have demonstrated that weakly or transiently bound protein complexes can be isolated intact with much higher efficiency than traditional methods, which are subject to loss during the dissociation-promoting washing process.13–15 Also, since ESP does not dilute or discard the original sample during isolation, this material can be “resampled” multiple times to extract different analytes sequentially.16

In this manuscript, we report a new type of ESP, termed AirJump. AirJump represents a further simplification of the ESP concept, in which PMPs are drawn directly out of a standard well plate and through a gap of air via a magnetic force applied from above. By “jumping” PMP-bound analyte through a liquid/air phase interface and narrow (∼1 mm) air gap, we achieve a purification efficiency higher than that observed with traditional “tube-based” washing, due in part to the lack of

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Figure 1. (A) Standard washing paradigm where PMP-bound analyte is magnetically pulled to the side of a tube and washed several times. (B) Mechanisms of carryover with standard method including tube residue and PMP-trapped material. Photo of AirJump components in disassembled (C) and assembled (D) states. (E) Schematic of AirJump operation; an elution plate is inverted and placed above a sample plate loaded with target analyte (blue) and nontarget material (green) (1). Upon application of a magnet, PMP-bound analyte “jumps” across the air gap and is deposited in the elution plate (2).

METHODS

2.1. AirJump Operation. The AirJump apparatus consists of a base with alignment posts, a standard 96-well plate (Falcon), elution wells configured as a strip or plate, and a magnet holder. The base and magnet holder are 3D printed from Accura 25 plastic (polypropylene-like) via stereolithography (SLA) (Midwest Prototyping). Strips of 8 elution wells are injection-compression molded in polypropylene (DTE Research & Design), while plates of 96 elution wells are 3D printed in Accura 25 (Figure 1C, D). To operate AirJump, a 96-well plate is placed on the base, and each well is loaded with 420 μL of solution containing sample, buffer, and PMPs (exact compositions discussed below). While this volume is somewhat flexible (±15%), it is important to select a total solution volume that completely fills the well, leaving a slightly convex surface extending above the top of the well plate. This is necessary to provide a “focal point” (the tip of the convex curvature) for the PMPs to aggregate prior to jumping. Accordingly, if solution volume is less than 420 μL, diluents may be added to increase the volume to this critical level. Once all wells are loaded, the alignment posts on the base are used to position the elution well strips or plates above the well plate, with the elution wells facing downward toward the well plate (i.e., sample and elution wells are positioned face-to-face). During assembly of the apparatus, care should be taken not to excessively jostle the AirJump fixture. To complete the setup, a holder containing an array of magnets (B333-NS2, K&J Magnetics) is placed above the elution well, such that the PMPs are pulled upward from the sample solution and into the elution wells, a process that typically requires 0.5–2 min for complete transfer. Once PMP transfer is complete, the AirJump apparatus is disassembled, and a multichannel pipet is used to resuspend the PMP-bound analytes (now located in the elution wells) in elution buffer (Figure 1E).

2.2. Operational Theory. To help explain the operational parameters of AirJump, we delve deeper into the underlying physics behind its operation. Neglecting gravity, which is insignificant at the scale of the PMPs, the PMPs will “jump” when the magnetic force applied to the PMP aggregate becomes greater than the surface tension force preventing the aggregate droplet from exiting the bulk sample liquid. As seen in Video S1 (Supporting Information), PMPs will aggregate at the top surface of the liquid until the aggregate size reaches a critical volume, after which the aggregate will traverse the liquid/air interface and collect in the elution well. Mathematically, the aggregate will jump when

\[ F_m > 2πrσ \]

where \( F_m \) is the force applied by the magnet on the PMP aggregate; \( r \) is the radius of the aggregate; and \( σ \) is the surface tension at the liquid/air interface. \( F_m \) is a function of a variety of variables including the shape and strength of the magnet, the material(s) between the magnet and PMP aggregate, the spacing between the magnet and PMP aggregate, the paramagnetic properties of the PMPs, and the radius of the PMP aggregate. While a few different types of PMPs were used throughout this manuscript, all PMPs were solid (nonporous) with a diameter ranging from 1 to 10 μm. While calculation of the exact values for \( F_m \) is complex and beyond the scope of this manuscript (information about the magnetic field of the specific magnets used in this study can be found at https://www.kjmagnetics.com/proddetail.asp?prod=B333-NS2), all variables influencing \( F_m \) (e.g., materials, spacing, magnet type) will remain constant during each experiment, with the exception of PMP aggregate radius, \( r \). Thus, we will develop a simplified, relative model enabling us to compare AirJump-specific parameters. Furthermore, \( F_m \) increases with \( r^2 \), while the surface tension force (the right-hand side of eq 1) increases linearly with radius. Therefore, while \( F_m \) is typically less than the surface tension force at the onset of PMP aggregation, \( F_m \) increases rapidly with PMP accumulation at the surface, eventually leading to an aggregate jump. If we simplify \( F_m \) to a generalized form, \( F_m = Cr^2 \), where \( C \) is a variable incorporating all the magnetic and material properties (which will be held constant in this instance), we can develop a straightforward
expression for the critical PMP aggregate radius, \( r_{\text{CRIT}} \) (a more detailed description of the variables influencing the force of a magnetic field on a PMP can be found in Shvetskaya et al.\(^{11} \)) and a detailed discussion on the movement of magnetic particles within droplets and across interfaces can be found in Long et al.\(^{18} \)). Using eq 1, setting the forces equal, and solving for \( r_{\text{CRIT}} \) we find

\[
 r_{\text{CRIT}} = \sqrt{\frac{2\pi\sigma}{C}}.
\]  

(2)

While eq 2 provides us with a relationship between critical PMP aggregate size and the surface tension of the liquid/air interface (which is directly related to the type of sample and buffer loaded into the sample wells), it also defines the potential for PMP loss during operation. Since the final aggregate of PMPs will likely not reach \( r_{\text{CRIT}} \), the maximum volume of the final PMP aggregate (which does not jump) will have a radius of \( r_{\text{CRIT}} \) or smaller. By calculating this loss volume, we will be able to select a total PMP concentration for our isolations that produce minimal residual PMP loss (i.e., by using a higher concentration of beads, this final remainder aggregate will contain a minimized (<15%) amount of the total target analyte, consequently maximizing yield).

2.3. Performance Characterization. Experiments were performed to characterize AirJump based on purity (using measurements of unintentional carryover) and PMP recovery yield (as measured by PMPs that were collected in the elution well relative to those that remained in the sample well following isolation). To measure carryover, a fluorescent dye (acridine orange) was spiked into buffers containing PMPs. Initially, we tested Protein A/G PMPs (Pierce, 50 \( \mu \)L of PMP slurry per 410 \( \mu \)L of sample) in PBS with 0.01% Tween 20 (PBST) and compared the carryover of one AirJump process to a traditional wash, as described by the manufacturer’s instructions for this PMP product (i.e., collect PMPs on the side of a tube with a magnet, remove liquid via micropipette, add fresh wash buffer, and resuspend). Protein A and Protein G are commonly used in immunoprecipitation processes to attach antibodies to substrates. Thus, PMPs such as the ones used in this experiment would be commonly used in protein isolations, making them a valid choice for performance characterization. Once AirJump was complete, the extracted PMPs were resuspended in 410 \( \mu \)L of PBST, and carryover fluorescence was measured with a Qubit Fluorometer (Life Technologies) and compared against a standard curve.

As suggested by eq 2, we hypothesize that solution surface tension will also affect AirJump performance since the size of the “jumping” PMP aggregates will change. Therefore, we prepared buffers with a variety of concentrations of Triton X-100 to measure the effect of surface tension on carryover. Each buffer was mixed with 25 \( \mu \)L of Dynabeads M280 Streptavidin PMPs per sample, which is a common PMP type and concentration used in many of our extraction experiments (streptavidin binds biotin very specifically and with extremely high affinity, and biotin can be conjugated to many types of biomolecules including nucleotides and proteins). PMPs were extracted from these solutions (420 \( \mu \)L per sample) via AirJump as previously described, and the extracted PMPs were resuspended in 420 \( \mu \)L of PBS. Fluorescence was measured using a Qubit Fluorometer (Life Technologies), and values were compared against standards to determine carryover percentage.

Similarly, PMP recovery was measured fluorescently by conjugating fluorescent secondary antibody (AlexaFluor 488-conjugated goat antimouse IgG, Life Technologies) to Protein G Dynabeads via mixing 15 mg/mL of PMPs with 1 \( \mu \)g of antibody per 1 mg of PMPs for 30 min at room temperature with shaking. Again, PMPs were extracted via AirJump, and the concentration of fluorescently labeled PMPs was measured with the fluorometer and compared against a standard curve (shown as Figure S1). These values were used to calculate the percentage of the PMPs that were successfully transferred to the elution well and the percentage that remained in the sample well.

2.4. AirJump Applications Overview. After conceptually demonstrating the ability of the AirJump to isolate PMPs from a sample with sufficiently high yield and purity, we demonstrated the suitability of AirJump for a variety of “real world” isolation applications. First, we use AirJump to immunoprecipitate (IP) a protein from a background of bacterial lysis. Additionally, this application also demonstrated a known advantage of ESP—the ability to isolate a low affinity target with higher yield than traditional wash-based protocols, which promote dissociation due to repetitive washes due to a combination of increased washing time, increased washing shear forces, and increased disruption of bound/unbound equilibrium. Following protein isolation, we isolate HIV viral RNA from plasma samples, a critical operation in the monitoring of HIV disease progression. With this application, we demonstrate the high level of precision and low limit of detection required for HIV viral load assays. Lastly, we simultaneously perform mRNA isolation from 96 separate cell cultures growing in a single well plate, thereby demonstrating the ability to isolate a complete 96-well plate of samples with no cross-contamination between wells, as measured by qRT-PCR. This final experiment is important to confirm that the “jumping” process does not generate cross-contaminating aerosols, as validated by highly sensitive qRT-PCR readout.

2.5. Immunoprecipitation of Low Affinity Proteins. We demonstrated the ability of AirJump to immunoprecipitate protein by isolating epitope-tagged green fluorescent protein (etGFP) from a background of E. coli lysate. Furthermore, the affinity of etGFP to a corresponding antibody (8RB13; generous gift of Dr. Richard Burgess) can be “tuned” by modulating the concentration of ammonium sulfate (AS) salt in the sample solution.\(^{1,3-2} \) We have previously demonstrated that another ESP technology, IFAST, can isolate proteins with low affinity interactions with much higher yield than traditional wash-based methods.\(^{3,14} \) Here, we confirm that this fundamental ESP advantage holds true for AirJump. Specifically, we created two experimental conditions with different affinities (a high affinity solution containing 2 mM AS and a low affinity solution containing 11 mM AS) and compared the performance of AirJump with three traditional washes as recommended by the PMP manufacturer (i.e., this protocol involves capturing the target protein on PMPs in a tube, pulling the PMPs to the side of the tube with a magnet, and washing the PMPs multiple times with buffer to remove background contaminants). Antibody was attached to PMPs by mixing 15 mg/mL of Dynabeads Protein G PMPs with 1 \( \mu \)g of 8RB13 antibody per 1 mg of PMPs for 15 min at room temperature. The quantity of recovered etGFP was measured by resuspending the isolated PMP aggregate in 10 \( \mu \)L of PBS, loading this solution into a 384-well plate, and measuring etGFP intensity with a fluorescent scanner (Typhoon Trio, GE Healthcare). Additionally, purity was measured by adding red fluorescent protein (RFP) to the lysate and fluorescently measuring the volume of RFP that was carried over during the AirJump isolation.

2.6. Isolation of HIV Viral RNA. The isolation of viral RNA from plasma samples is a critical step in the HIV viral load assay, an important test that indicates the effectiveness of antiretroviral therapy (ART). Typically, this RNA extraction is performed via expensive and complicated automated systems, which limits implementation in lower resource settings, including Sub-Saharan Africa,\(^{3} \) the epicenter of the HIV/AIDS pandemic. Here, we demonstrate the effectiveness of using AirJump to directly extract the viral RNA with sufficient repeatability and sufficient purity to quantitate with qRT-PCR. HIV viral particles (generous gift of Dr. Nathan Scherer) were spiked into HIV-negative serum at concentrations of 10, 100, 1000, and 10,000 copies per mL of serum. Samples were mixed with equal volumes of lysis buffer (Buffer MFL, Qiagen) and 10 \( \mu \)L of PMPs (Qiagen MagAttract Virus Isolation PMPs) and then mixed at room temperature for 5 min. PMPs were isolated via AirJump as previously described. Isolated PMPs were resuspended in 20 \( \mu \)L of elution buffer (Buffer MFL, Qiagen) and incubated at room temperature for 5 min. An amount of 10 \( \mu \)L of eluted RNA was loaded into a qRT-PCR reaction with 10 \( \mu \)L of one-step master mix (Fast Virus Master Mix, Life Technologies) and 1 \( \mu \)L of a primer/probe solution previously characterized by Rouet et al.\(^{27} \) (synthesized by Life Technologies). Samples were amplified for 50 cycles on Roche LightCycler 480 real-time PCR machine following the
master mix manufacturer’s recommended settings. Using a standard curve created with known viral RNA concentrations, we calculated the measured viral load based on the threshold cycle determined by the PCR reaction.

### 2.7. Isolation of mRNA from Cultured Cells

To demonstrate the ability to perform AirJump on an entire 96-well plate simultaneously without cross contamination, mRNA was extracted from two different cell types that were seeded in alternating wells (checkerboard pattern). Briefly, LNCaPs (lymph node derived human prostate adenocarcinoma cells) or PC3s (bone metastasis derived prostate cancer cells) were seeded into wells at a density of roughly 30 000 cells per well in 100 μL of RPMI 1640 media supplemented with 10% fetal bovine serum (FBS). After 24 h of culture, 300 μL of lysis buffer containing 1X RIPA supplemented with 150 μg of oligo-dT PMPs (New England Biolabs) was added to each well and mixed 10 times using a multichannel micropipette. These PMPs contain a series of thymidine bases that selectively capture the pol-A tails found on mRNA. After allowing 5 min for lysis and binding of the mRNA to the PMPs, AirJump was used to extract the PMPs from all wells for 2 min as previously described. After disassembly of the AirJump apparatus, a multichannel micropipette was used to resuspend each isolated cluster of PMPs in 30 μL of elution buffer (10 mM Tris buffer). After allowing 2 min for elution, the elution well plate was placed on top of the magnet array, and eluent was loaded directly into a 96-well PCR plate. In this instance, 2 μL of mRNA was mixed with 5 μL of Taqman Fast Virus One-Step Master Mix (a master mix with both RT and PCR capabilities, Life Technologies), 1 μL of prostate-specific antigen (PSA; a gene expressed by the LNCaP cells but not PC3 cells) primer/probe mix (Life Technologies), and 12 μL of water. qRT-PCR was performed following the master mix protocol on the Roche LightCycler 480, and gene expression was quantified using threshold cycle (Ct) values. qRT-PCR was also performed on a housekeeping gene (GAPDH, primer/probe mix also from Life Technologies) to confirm that viable mRNA was collected from the PSA-negative PC3 cells.

### 3. RESULTS AND DISCUSSION

#### 3.1. Performance Characterization

A comparison of an AirJump purification with a traditional wash process demonstrated that AirJump removes approximately 2-fold more background compared to the wash (p < 0.001, two-tailed Student’s t test; Figure 2A). The quantity of AirJump carryover was found to decrease significantly (p < 0.001) with increasing quantities of Triton X-100 detergent (Figure 2B), ranging from 0% to 1% (a concentration commonly found in lysis buffers). A more in-depth characterization of the interfacial tensions of these solutions can be found in Berry et al. Visually, we observed that the critical aggregate size was substantially smaller with the higher detergent concentration samples. Relatedly, a higher number of “jumping” PMP aggregates were observed with the high detergent concentration samples (i.e., the PMPs were transferred to the elution plate in many small aggregates with the high detergent concentration samples and one or only a few large aggregates with the low detergent concentration samples). In general, aggregating a critical jumping mass of PMPs required <1 to 30 s, with more frequent jumping occurring with higher detergent concentrations. The total time required to complete the AirJump process ranged from approximately 1 to 2 min. Lastly, we found that the percentage of the PMPs that was recovered ranged from approximately 1 to 2 min. Lastly, we found that the percentage of the PMPs that was recovered ranged from 0% to 1% (a concentration commonly found in lysis buffers).
largely be independent of purification due to nonspecific protein binding system, we demonstrated that AirJump can achieve very high purity (less than 0.5% carryover) and low PMP loss (less than 15% total loss). In summary, these characterization experiments demonstrate that, with some process optimization, AirJump can achieve very high purity (less than 0.5% carryover) and low PMP loss (less than 15% total loss).

3.2. AirJump Applications. Using our “tunable affinity” protein binding system, we demonstrated that AirJump can be used to recover more protein than a traditional washing-based protocol. When affinity is relatively high (as is the case with a 2 mM low salt buffer), AirJump recovers slightly more protein than the washing protocol (72% recovery vs 56% recovery, respectively; p < 0.01). However, when affinity is reduced by increasing buffer salt, this discrepancy increases (49% recovery vs 23% recovery, respectively; p < 0.001) (Figure 3A). The decreased recovery observed with the traditional IP method is caused by unwanted dissociation of the captured protein during the three washing steps. Previous results suggest that this dissociation is driven by a combination of increased isolation times (nearly instant with AirJump vs 5–10 min with traditional IP) and the perturbation of binding equilibrium caused by the addition of each wash buffer. Combined, these factors result in significant loss of the target protein, thus highlighting the potential advantage of AirJump when isolating lower affinity analytes. Furthermore, inadvertent recovery of background protein (spiked in RFP, in this case) was 20% lower with AirJump compared to the 3X washing protocol, confirming that purity is not sacrificed (it is slightly enhanced but not with statistical significance, p = 0.18) when using AirJump. Specifically, carryover of RFP decreased to 2.6% from 3.3% when using the 3X washing protocol. However, this difference was not statistically significant (p = 0.18), and it is unclear how much of this RFP background is due to nonspecific binding of the RFP to the PMP, which will largely be independent of purification method.

HIV viral RNA extracted from serum samples using AirJump was successfully quantified via qRT-PCR (Figure 3B). Samples ranged in concentration from 10 to 10,000 viral copies per 410 μL serum sample (consistent with the range encountered clinically). qRT-PCR of the AirJump extracted viral RNA demonstrated a high degree of accuracy and precision, particularly for samples containing 100 copies and above (measured results within 15% of expected results; coefficient of variation of 17%). At the lowest viral concentration (10 copies per sample), accuracy and precision were lower (measured values ranged from 18 to 49 copies). However, this viral concentration is below the reported limit of detection of many existing “gold standard” assays, thus some variation in the data is not surprising at this extremely low level (a negative “no template control” confirmed the specificity of these measurements). Rather, the data collected from these samples suggest that AirJump could be a potential viable sample preparation method within for HIV viral load quantitation in clinical settings.

Lastly, we demonstrated the high-throughput capabilities of AirJump by simultaneously extracting mRNA from 96 cell cultures with no measurable cross-contamination between adjacent samples. Within 15 min, mRNA was successfully isolated from 96 wells seeded with either PSA-positive LNCaP cells or PSA-negative PC3 cells (Figure 3C). Following AirJump, the mRNA was loaded directly into a PCR-compatible well plate for quantitation via qRT-PCR. A housekeeping gene common to both cell lines (GAPDH) was detected in all wells, regardless of cell type. However, when measuring PSA, a strong signal was observed from all LNCaP wells, but none of the PC3 wells, demonstrating the specificity of the AirJump method when performing high-throughput purifications. Given the high sensitivity of qRT-PCR (near single molecule), this experiment confirms the lack of cross contamination (via aerosol formation, misdirected PMP jumps, etc.) with relatively high certainty.

CONCLUSION

In summary, we have demonstrated Airjump as a suitable alternative to traditional analyte isolation processes. Airjump represents a substantial simplification and parallelization of separation techniques without any appreciable decrease in performance. In fact, Airjump performs significantly better with regard to low affinity analytes. Further, we have demonstrated the applicability of Airjump with three important application areas, including (1) isolation of low affinity proteins; (2) viral RNA isolation for HIV viral load quantitation; and (3) high-throughput gene expression analysis of cultured cells. In each of these application areas, AirJump performs proficiently, demonstrating its ability as a broad-purpose analyte isolation technology. Moving forward, we anticipate that such a technology could be truly transformative among the breadth of fields that rely upon analyte isolation to produce valid results.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acsami.6b02555.

Standard curve linking AirJump PMP recovery with fluorescence as well as additional cross-contamination data for the 96-well format AirJump (PDF)

Video of PMPs aggregating at the top surface of the liquid until the aggregate size reaches a critical volume, after which the aggregate will traverse the liquid/air interface and collect in the elution well (AVI)

AUTHOR INFORMATION

Corresponding Author

*E-mail: berry3@wisc.edu.

Notes

The authors declare the following competing financial interest(s): S.B., D.G., and D.B. hold equity in Salus Discovery LLC, which has licensed the Airjump technology. S.B., D.G., and H.P. are employed by Salus Discovery LLC, which has licensed the Airjump technology.

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