



An off-the-shelf digital microfluidic platform for integrated extracellular vesicle isolation and detection

Murat Alp Güngen^{a,b,1}, Eylül Gulsen Yilmaz^{a,b,1}, Fatih Inci^{a,b,*}

^a UNAM-National Nanotechnology Research Center, Bilkent University, Ankara, 06800, Turkey

^b Institute of Materials Science and Nanotechnology, Bilkent University, Ankara, 06800, Turkey

ARTICLE INFO

Keywords:

Extracellular vesicles
Digital microfluidics
Point-of-care diagnostic

ABSTRACT

Extracellular vesicles (EVs) are membrane-bound nanoparticles that serve as minimally invasive biomarkers, yet their clinical translation is hampered by limitations in existing isolation and detection strategies. Here, we systematically benchmarked EVs secreted from HeLa cell cultures using ultracentrifugation, microfluidic ultra-filtration, and magnetic bead-based capture, the latter implemented both conventionally and on OpenDrop, an off-the-shelf digital microfluidic (DMF) platform. This comparative evaluation encompassed key performance metrics, including yield, purity, workflow complexity, and operational efficiency. Nanoparticle tracking analysis and scanning electron microscopy revealed that EVs isolated from DMF exhibited recovery rates and vesicle morphology on par with those obtained through conventional bead-based workflows, while requiring markedly reduced reagent volumes. Western blotting and flow cytometry confirmed robust expression of tetraspanin EV markers (CD63 and CD81), across a dynamic concentration range. On-chip ELISA performed directly on the DMF device demonstrated strong linearity across EV concentrations ranging from 5×10^6 to 1×10^9 particles/mL ($R^2 = 0.8935$), in close agreement with conventional benchtop measurements ($R^2 = 0.8388$). Together, these results establish the DMF platform used in this study as a versatile, and cost-efficient solution capable of integrating EV isolation and detection within a single, unmodified device. By uniting conventional laboratory workflows with miniaturized, digitally controlled operations, this work underscores the potential of accessible DMF technologies to advance portable point-of-care EV diagnostics in the future.

1. Introduction

Extracellular vesicles (EVs) are a heterogeneous class of membrane-bound nanoparticles released by virtually all cell types and enriched with a diverse repertoire of biomolecules, including proteins, lipids, nucleic acids, and metabolites (Amin et al., 2024; Rajagopal and Harikumar, 2018). By transporting these cargos between cells, EVs serve as key mediators of intercellular communication and contribute to essential physiological and pathological processes (Shirejini and Inci, 2022). Their involvement has been documented across major disease states—including cardiovascular dysfunction, neurodegeneration, and cancer—highlighting their relevance as biomarkers and therapeutic agents (György et al., 2011). Although earlier categorized into exosomes, microvesicles, and apoptotic bodies, recent ISEV guidelines recommend operational classifications based on size (exosomes or

extosomes) to improve clarity and reproducibility in EV research (Küçük et al., 2024). Because EVs circulate in a range of biofluids such as blood, urine, sweat, tears, and lavage samples, they provide accessible, minimally invasive windows into cellular health and hold considerable promise for precision diagnostics, longitudinal disease monitoring, and therapeutic development (Andreu and Yáñez-Mó, 2014; Derin and Inci, 2022). However, unlocking this potential depends critically on the ability to isolate EVs with high purity and molecular integrity.

Conventional EV isolation strategies—including ultracentrifugation, polymer precipitation, and membrane filtration—face persistent challenges that limit their compatibility with clinical and point-of-care (POC) contexts (Shirejini and Inci, 2022). Ultracentrifugation remains the de facto standard but requires prolonged spin times, expensive instrumentation, and is prone to co-isolation of protein aggregates and non-vesicular contaminants, leading to variable purity and low yield

This article is part of a special issue entitled: Microfluidic-based sensors published in Biosensors and Bioelectronics: X.

* Corresponding author. UNAM-National Nanotechnology Research Center, Bilkent University, 06800 Ankara, Turkey

E-mail address: finci@bilkent.edu.tr (F. Inci).

¹ Co-first authors.

<https://doi.org/10.1016/j.biosx.2026.100816>

Received 27 November 2025; Received in revised form 2 June 2026; Accepted 2 July 2026

Available online 9 July 2026

2590-1370/© 2026 The Authors. Published by Elsevier B.V. This is an open access article under the CC BY license (<http://creativecommons.org/licenses/by/4.0/>).

(Allelein et al., 2021). Polymer-based precipitation offers faster processing but often introduces polymer–protein or polymer–lipid contaminants that compromise downstream analyses (Atabay et al., 2025). Filtration-based approaches, including tangential flow systems, may suffer from membrane clogging, reduced recovery, and shear-induced vesicle deformation (Tauro et al., 2012). Collectively, these limitations underscore the need for isolation strategies that are faster, more specific, and more compatible with small sample volumes (Saylan et al., 2025). To this end, magnetic bead–assisted immunoaffinity capture has emerged as a compelling alternative, leveraging antibody-functionalized beads to selectively bind EV surface markers with high specificity and operational simplicity (Liu et al., 2016). All of these strengths make magnetic bead–based EV isolation a good starting point for next-generation point-of-care (POC) diagnostics.

On the other hand, microfluidic technologies have transformed the landscape of EV isolation and analysis by enabling precise manipulation of fluids at the micrometer scale within controlled physical and chemical environments (Gholizadeh et al., 2017; Guo et al., 2018; Havers et al., 2023; Zhang et al., 2022). Their ability to process microliter volumes with high efficiency and reduced reagent consumption positions them as powerful tools for clinical translation and POC testing (Abreu et al., 2022; Akceoglu et al., 2021; Chen et al., 2024; Sun et al., 2025). Microfluidic devices have exploited a variety of isolation mechanisms—including size filtration, deterministic lateral displacement, acoustic streaming, dielectrophoresis, and immunoaffinity capture—to enrich EVs with improved purity and throughput (Liang et al., 2017; Parlatan et al., 2023). Nevertheless, persistent obstacles such as channel clogging, device-to-device variability, fabrication complexity, and insufficient standardization continue to hinder widespread adoption (Havers et al., 2023; Meggiolaro et al., 2022). These challenges highlight the need for microfluidic platforms that are simpler, more robust, and more universally accessible (Derin et al., 2025).

Microfluidic isolation of EVs potentially improves purity and selectivity over conventional methods but faces trade-offs between throughput, recovery, and scalability due to low flow rates and limited volumes (Chen et al., 2024; Havers et al., 2023). Challenges such as clogging, biofluid variability, and co-isolation of contaminants complicate benchmarking, requiring metrics like particle-to-protein ratios and single-EV profiling (Dong et al., 2020; Hassanzadeh-Barforoushi et al., 2025). Still, practical limitations, including complex fabrication, device-specific operation, and cost, hinder standardization, driving efforts toward unified benchmarks and integrated platforms combining physical and affinity-based methods (Abreu et al., 2022; Li et al., 2025; Meggiolaro et al., 2023). Advanced systems achieve ~60–85% recovery, with hybrids showing up to 2× higher recovery and 8 times higher purity than conventional methods, while processing ranges from μL -scale chips (20–30 min) to mL-scale systems (10–50 min) (Arjmand et al., 2025; Kim et al., 2025; Lo et al., 2025). Integrated detection enables sub-pg/mL to fM sensitivity and multiplexing, with automated workflows achieving high reproducibility ($\text{CV} < 5\text{--}10\%$) and improved clinical utility, highlighting the need for standardized reporting for cross-platform comparison and translation (Dai et al., 2025; Sun et al., 2025; Tian et al., 2022).

Digital microfluidics (DMF), which manipulates discrete droplets on dielectric and hydrophobic coated surfaces using electrowetting-on-dielectric (EWOD) platforms, offers unique advantages over continuous-flow microfluidics (Peng et al., 2014). DMF platforms provide programmable droplet actuation, precise reagent handling, low sample consumption, and compatibility with magnetic bead manipulations—all without the need for external pumps. Using off-the-shelf DMF devices provides a rapid, low-risk route for implementing complex EV assays by enabling programmable droplet operations (dispense, split, mix, transport, and wash) without microchannels, allowing multi-step workflows such as immunomagnetic capture and on-chip detection on a single reconfigurable platform (Lo et al., 2025;

Sista et al., 2008). Operating at microliter-to-picoliter scales, DMF significantly reduces sample and reagent consumption while maintaining high sensitivity (Samiei et al., 2016; Xu et al., 2023). Recent custom-built DMF systems have demonstrated the feasibility of bead-based EV capture and miRNA analysis (Tong et al., 2024, 2025). However, most DMF devices described in the literature rely on bespoke fabrication, limited availability, or specialized components, and challenges in standardization similarly impede broad adoption (Bajo-Santos et al., 2023; Havers et al., 2023). These requirements restrict access to well-equipped research facilities with cleanroom infrastructure and microfabrication expertise, limiting reproducibility across laboratories and hindering clinical translation (Alistar and Gaudenz, 2017; Convery and Gadegaard, 2019). In contrast, off-the-shelf DMF platforms eliminate these barriers by offering a plug-and-play solution that requires no custom fabrication, operates without specialized training, and is available at significantly lower cost — thereby enabling broader standardization and facilitating adoption in resource-limited or clinical settings where bespoke systems would be impractical (Albin et al., 2024; Azad et al., 2025). Consequently, there remains a notable gap in the field: the lack of widely accessible, off-the-shelf DMF platforms capable of performing both EV isolation and downstream detection on a single device. On the other hand, OpenDrop, an open-source and commercially available DMF system developed by GaudiLabs, offers a potential solution to this gap (Alistar and Gaudenz, 2017). Featuring interchangeable open and closed cartridges and integrated magnetic pads aligned with designated electrodes, OpenDrop enables plug-and-play digital microfluidic operation without the need for custom engineering. However, its applicability to EV workflows—including isolation, processing, and immunodetection—has not been systematically evaluated.

In this study, we focus to address this unmet need by systematically evaluating the performance of an unmodified, off-the-shelf DMF platform (OpenDrop V4) for both magnetic bead–based EV isolation and on-chip ELISA detection. Importantly, this work serves as a technological validation of a commercially available DMF system for EV processing, rather than a clinical validation study. Consequently, further investigations involving clinically relevant biofluids, larger sample cohorts, and standardized validation protocols would be required to establish its applicability for diagnostic and translational biomedical applications in the future. EVs derived from HeLa cell cultures were isolated using ultracentrifugation, microfluidic ultrafiltration, and magnetic bead-based capture performed either conventionally or on the DMF device, enabling a comprehensive comparison across methodologies. We further demonstrate that the same DMF platform can perform on-chip ELISA for EV-associated markers, thereby establishing a versatile, multipurpose workflow on a single, commercially available platform. Importantly, the novelty of the present work lies not in the concept of DMF-based EV processing per se, but in demonstrating that a commercially available, off-the-shelf platform can achieve comparable analytical performance without requiring any custom fabrication, specialized components, or cleanroom access. Custom DMF systems, while powerful, are accessible only to laboratories with specialized microfabrication expertise and infrastructure. The OpenDrop platform, by contrast, is commercially available at a fraction of the cost, requires no cleanroom access, and can be operated with minimal training — factors that are critical for adoption in resource-limited settings and for broader standardization across laboratories. The contribution of the present work therefore lies in demonstrating that comparable analytical outcomes — including EV isolation, on-chip washing, and quantitative immunodetection across a dynamic concentration range — can be achieved on a platform accessible to a broad research community without any hardware modifications.

2. Materials and methods

2.1. Cell culture

Human cervical cancer cells (HeLa; ATCC: CCL-2) were maintained under standard culture conditions. Cells were grown in high-glucose Dulbecco's modified Eagle medium (DMEM) supplemented with 10% fetal bovine serum (FBS), 100 U/mL penicillin, and 100 µg/mL streptomycin. Cryopreserved stocks stored at -80°C were rapidly thawed in a 37°C water bath and immediately transferred to a 15-mL conical tube. The suspension was diluted with 5 mL of fresh complete medium and centrifuged at 2500 rpm for 5 min. After removing the supernatant, the cell pellet was resuspended in 5 mL of pre-warmed medium and seeded into a culture flask to initiate primary culture. Cells were incubated at 37°C in a humidified 5% CO_2 atmosphere. Culture medium was refreshed twice daily until cells reached approximately 85% confluence, at which point they were subcultured for routine maintenance or downstream experiments.

2.2. EV isolation studies

2.2.1. Ultrafiltration-based EV isolation

EVs were isolated using a microfluidic ultrafiltration chip described previously (Inci, 2022). Briefly, the chip integrates two sequential membrane filters with pore sizes of 200 nm and 50 nm, respectively (Liang et al., 2017). Cell culture supernatant was introduced into the system via connected tubing and first passed through the 200 nm membrane, which retained larger particulates. Nanoparticles smaller than 200 nm subsequently traveled through an intermediate micro-channel to the second membrane. EVs were captured at the 50 nm filter and collected from the outlet tubing. All flow operations were performed at a constant rate of 40 µL/min using a syringe pump.

2.2.2. Ultracentrifugation-based EV isolation

Ultracentrifugation—a widely used approach for EV purification, was employed as a reference method. On the fourth day of culture, 15 mL of conditioned medium was harvested from HeLa cell flasks. To remove cells, debris, and larger contaminants, samples were centrifuged at $20,000 \times g$ (14,000 rpm) for 15 min at room temperature. The clarified supernatant was then passed through a 0.22-µm filter to prevent microbial contamination and transferred to a clean tube. For EV precipitation, the filtered medium was ultracentrifuged at $100,000 \times g$ (33,200 rpm) for 70 min at 4°C using a P50AT2 rotor. The resulting pellet was gently resuspended in 1 mL of PBS and prepared for nanoparticle tracking analysis (NTA).

2.2.3. Magnetic bead-mediated EV isolation

In this method, 5 µL of resuspended capture beads were transferred into round-bottom microtubes, after which 500 µL of EVs isolated by ultrafiltration were added. The suspension was gently vortexed and incubated overnight at room temperature in the dark to facilitate EV binding. Following incubation, samples were washed with 1 mL of $1 \times$ Assay Buffer, and beads were collected either using a magnetic rack or by brief centrifugation. The supernatant was removed carefully to avoid disturbing the microspheres. Subsequently, 5 µL of the primary detection antibody (CD81-PE) was added, and samples were gently mixed and incubated for 60 min at 4°C in the dark. Beads were washed again with $1 \times$ Assay Buffer and collected as described above. The final bead pellet was resuspended in 350 µL of $1 \times$ Assay Buffer, protected from light, and analyzed by flow cytometry within 2 h of preparation.

2.2.4. DMF-based EV isolation

DMF isolation of EVs was performed using an OpenDrop V4 platform (GaudiLabs, Switzerland) equipped with an integrated magnetic panel. Prior to use, the surface of the open-format cartridge was cleaned with isopropyl alcohol and coated with 5 cSt silicone oil to facilitate droplet

actuation and mitigate surface biofouling (Kalyani et al., 2025). Prior to DMF-based processing, EVs were bound to magnetic beads using the immunoaffinity capture protocol described in Section 2.2.3. Briefly, EV-bound magnetic bead complexes were prepared off-chip by overnight incubation at room temperature, after which the bead-EV complexes were loaded onto the DMF platform for subsequent on-chip manipulation. For each run, 15 µL droplets containing magnetic bead-tagged EVs were dispensed onto the device and electrically grounded using the built-in grounding rod. After activation, droplets were transported across the electrode array to defined magnetic capture sites (the fifth pads from both sides on the upper third row in Fig. 4A) using a 200 V DC actuation voltage. The magnetic panel was then activated to immobilize the beads, and droplets were incubated for 15 min.

Following incubation, droplets were moved away—while maintaining magnetic activation—and removed by pipetting, leaving the bead-bound EVs retained on the electrode pads. Fresh droplets of EV suspension were subsequently added, grounded, and transported to the magnetic pads. The magnets were briefly deactivated to resuspend the bead-bound EVs, then reactivated to enable further capture. This process was repeated for four additional enrichment cycles. The final droplet containing the accumulated bead-associated EVs was collected and processed for downstream analyses.

To evaluate the feasibility of on-chip washing, EV-bound magnetic beads immobilized at designated capture sites were subjected to washing directly on the DMF platform. Briefly, fresh 15 µL droplets of either PBS or PBS supplemented with 0.1% Tween were dispensed onto the device, grounded, and transported via electrowetting actuation to the magnetic capture pads. Upon reaching the capture site, the wash droplets were merged with the residual droplet containing the immobilized beads, while the magnetic field was continuously applied to ensure stable bead retention and prevent displacement. Following a 2-min incubation period, the merged droplet was actuated away from the capture site and removed by pipetting, leaving the bead-bound EVs immobilized on the electrode pad. This washing procedure was repeated for a total of four cycles under each condition. Subsequently, the washed bead-bound EVs were collected and characterized using NTA to assess the effectiveness of the on-chip washing process.

2.3. Characterization of EVs

In accordance with MISEV guidelines, EV preparations were evaluated using complementary orthogonal methods to assess particle size, morphology, molecular markers, and analytical intactability (Welsh et al., 2024). EVs were characterized using four approaches: NTA, scanning electron microscopy (SEM), Western blotting, and flow cytometry.

- (i) **Nanoparticle Tracking Analysis (NTA):** Particle concentration and size distribution were quantified using a NanoSight NS300 system (Malvern Instruments Ltd., UK). Samples were diluted 10 – 10^3 -fold in PBS depending on particle density and analyzed at 25°C using a 488 nm laser. Five videos of 60 s each were recorded per sample, and viscosity was set to 0.92 mPa s. Particle diameters were calculated using the Stokes–Einstein equation.
- (ii) **Scanning Electron Microscopy (SEM):** For morphological assessment, EVs were immobilized on 0.5×0.5 mm silicon wafers, washed by sonication in ethanol and distilled water for 5 min, and dried with compressed air. After fixation in 2% paraformaldehyde (v/v), 10 µL of EV suspension was deposited onto the wafers. Samples were washed three times with ethanol, air-dried, and sputter-coated with 10 nm Au/Pd before imaging on an FEI Quanta 200 FEG microscope (USA).
- (iii) **Western Blotting:** EV-associated tetraspanins (CD63 and CD81) were examined by Western blot. Proteins were separated on 10% resolving and 4% stacking SDS–PAGE gels and transferred onto

methanol-activated PVDF membranes. Membranes were blocked with 5% non-fat milk in TBST (10 mM Tris, 15.4 mM NaCl, 0.1% Tween-20) and incubated overnight at 4 °C with primary antibodies against CD81 (Invitrogen MA5-33123, 1:1000) and CD63 (Invitrogen MA5-30187, 1:1000). After washing, membranes were incubated for 1 h at room temperature with HRP-conjugated goat anti-rabbit secondary antibody. Chemiluminescence was visualized using a ChemiDoc MP system (Bio-Rad, USA) following application of Luminol/Enhancer and Stable Peroxide Buffer (1:1, v/v).

- (iv) *Flow Cytometry*: Flow cytometric analysis was performed using a refined gating strategy to ensure accurate discrimination of bead-bound EVs. Single microsphere populations were identified using FSC versus SSC plots on a linear scale, while bead autofluorescence was evaluated through logarithmic FL3 and FL4 channels. EV-positive bead populations were subsequently detected based on FL2 fluorescence associated with specific surface marker binding.

2.4. ELISA experiments for EV detection

A modified bead-based ELISA workflow was employed to detect EVs without the use of conventional microplates. Briefly, 50 µL of EV suspension was incubated overnight at room temperature with 5 µL of rabbit anti-CD63 antibody-conjugated magnetic beads to enable primary capture. After incubation, 5 µL of mouse anti-CD81 antibody was added as a secondary detection antibody, and the mixture was gently agitated for 4 h. The use of a mouse anti-CD81 detection antibody in combination with an HRP-conjugated anti-mouse secondary antibody ensures species-specific signal detection. Because the capture antibody (anti-CD63) is of rabbit origin, the anti-mouse secondary antibody does not cross-react with the capture complex, thereby preventing non-specific signal contributions from unbound capture beads and ensuring that the measured signal arises exclusively from the target EV-bound detection antibody. Then, they were collected by centrifugation at 5000 rpm for 5 min followed by magnetic separation for an additional 5 min. The supernatant was discarded, and the pellet was incubated with 50 µL of HRP-conjugated mouse secondary antibody for 1 h at room temperature. Samples were then washed with 350 µL of wash buffer, centrifuged at 5000 rpm for 5 min, and placed on a magnetic rack for 5 min to remove unbound enzyme conjugates. For color development, the bead pellet was incubated with 50 µL of TMB substrate for 5 min. The enzymatic reaction was quenched by adding 50 µL of stop solution, and the absorbance of the resulting solution was measured at 450 nm using a microplate reader.

2.5. ELISA experiments for DMF

To demonstrate the low-volume advantages of DMF for biomedical assays, the ELISA workflow described above was adapted for execution on the digital microfluidic platform. Using the open-cartridge configuration, seven 10-µL droplets containing graded concentrations of HRP-tagged EVs (1×10^9 , 5×10^8 , 1×10^8 , 5×10^7 , 1×10^7 , and 5×10^6 particles/mL), along with a 10-µL EV-free control droplet, were dispensed onto the device surface and grounded using the built-in grounding wire. Droplets were positioned two electrode pads apart to prevent unintended coalescence.

Subsequently, 10-µL TMB substrate droplets were pipetted and grounded on electrode pads directly above or below the EV droplets on adjacent rows. After placement, the actuation voltage (200 V DC) was applied, and TMB droplets were transported to their assigned EV-containing pads. Upon co-location, TMB and EV droplets were merged, after which the actuation voltage was turned off. Color development was monitored by photographing the device at 1-min intervals. Following a 5-min reaction period, droplets were carefully retrieved from the cartridge surface and transferred into microplate wells

preloaded with stop solution for downstream absorbance measurement.

3. Results and discussion

3.1. Comparative analysis of isolation techniques

HeLa cell cultures (Fig. S1) were used as a model system for evaluating multiple EV isolation strategies and subsequent characterization workflows. Conditioned medium containing EVs and soluble proteins was processed using four complementary approaches: ultracentrifugation, microfluidic ultrafiltration, magnetic bead-based isolation, and magnetic bead-assisted capture on the DMF platform (Fig. 1). Collectively, these techniques enabled direct comparison of recovery efficiency, purity, and suitability for miniaturized, on-chip analytical workflows.

NTA was employed to quantitatively compare EVs isolated via ultracentrifugation, microfluidic ultrafiltration, magnetic bead-based capture, and the DMF platform, evaluating their effects on particle size and concentration (Fig. 2). EVs isolated by ultracentrifugation exhibited a mean diameter of 101.5 ± 16.4 nm and a mode of 51.5 ± 15.0 nm, consistent with the expected size range of small EVs (30–150 nm) (Lin et al., 2020). The particle concentration was $3.62 \times 10^8 \pm 1.37 \times 10^8$ particles/mL (Fig. 2A and B). The broad size distribution reflects partial co-isolation of small non-vesicular components, a recognized limitation of ultracentrifugation. On the other hand, microfluidic ultrafiltration yielded slightly larger vesicles (mean 168.2 ± 8.3 nm, mode 116.9 ± 35.3 nm) at a modestly lower concentration (2.55×10^8 particles/mL), likely due to selective membrane retention and shear stress within the microchannels (Fig. 2C and D) (Inci, 2022). Despite this, the narrow size distribution indicates a homogeneous population suitable for downstream applications. Magnetic bead-based isolation achieved the highest recovery, with an average concentration of $9.19 \times 10^{10} \pm 1.88 \times 10^{10}$ particles/mL and vesicles around a mean diameter of 107.3 ± 3.9 nm and a mode of 63.4 ± 28.5 nm, demonstrating efficient capture of small EVs with high reproducibility across replicates (Fig. 2E and F) (Oksvold et al., 2015). The DMF platform performed notably well, isolating EVs with a mean size of 205.1 ± 25.9 nm at a concentration of $1.43 \times 10^9 \pm 7.84 \times 10^7$ particles/mL (Fig. 2G and H) (Lo et al., 2025). Although slightly larger than vesicles obtained by ultracentrifugation or magnetic bead capture, the DMF yield exceeded that of conventional microfiltration and approached that of traditional bead-based methods, despite using minimal sample and reagent volumes. The larger mean particle diameter observed in the DMF-isolated fraction may arise from several factors. First, immunoaffinity-based capture can selectively enrich EV subpopulations expressing higher densities of specific surface markers, which may introduce a size bias relative to bulk isolation methods (Kowal et al., 2016; Théry et al., 2018). Second, transient vesicle clustering during magnetic enrichment and droplet manipulation may increase the apparent hydrodynamic diameter measured by NTA. Finally, since NTA reports hydrodynamic size rather than physical membrane diameter, vesicle-protein interactions or minor aggregation events may influence the measured size distribution. Notably, the mode values remained within the expected size range of small EV populations. Overall, the DMF approach enables efficient EV isolation while using substantially reduced reagent volumes, highlighting its potential for integrated and miniaturized EV analysis workflows. As shown in Fig. 2H, the mode values of DMF-isolated EVs remained within the expected size range, indicating that the elevated mean diameter reflects a minor subpopulation rather than a systematic shift across the entire population.

To evaluate the feasibility of on-chip washing within the DMF workflow, EV-bound magnetic beads immobilized at the capture sites were washed directly on the device using PBS or PBS supplemented with 0.1% Tween. Wash buffer droplets were actuated across the electrode array, merged with the bead-containing droplet under continuous magnetic activation, and subsequently removed, thereby completing a

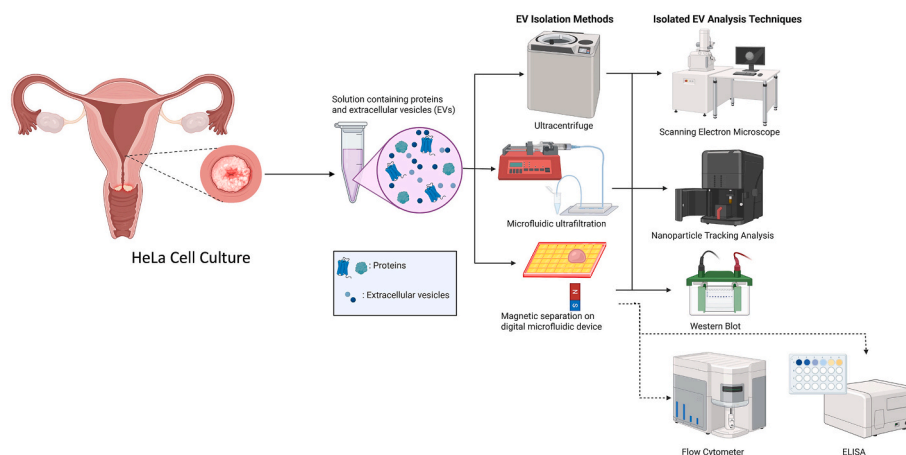


Fig. 1. Workflows for EV isolation methods evaluated in this study, including ultracentrifugation, microfluidic ultrafiltration, magnetic bead-based capture, and DMF-assisted magnetic bead isolation.

full wash cycle without disrupting bead retention. As shown in Fig. 2I, washing resulted in a substantial reduction in measured particle concentration compared with the unwashed condition ($1.43 \times 10^9 \pm 7.84 \times 10^7$ particles/mL). Following washing, particle concentrations decreased to $9.06 \times 10^7 \pm 2.45 \times 10^7$ particles/mL for PBS and $8.71 \times 10^7 \pm 1.06 \times 10^7$ particles/mL for PBS-Tween. These findings confirm that on-chip washing can be effectively implemented within the DMF platform, enabling the removal of loosely associated or non-specifically bound particles while maintaining stable retention of bead-bound EVs during droplet-based wash cycles.

As an additional characterization, flow cytometry histograms display the fluorescence intensity (FL2-A) profiles of EVs captured via magnetic bead-based isolation across concentrations ranging from 1×10^9 to 5×10^6 particles/mL (Fig. 3A–F). The number of detected events decreased proportionally with EV concentration, demonstrating a strong correlation between particle abundance and fluorescence signal intensity. This concentration-dependent trend confirms the sensitivity and reliability of the magnetic bead-based detection approach. Notably, histograms exhibited a tight, unimodal peak across all concentrations, indicative of a homogeneous EV population with minimal aggregation. These results underscore the high specificity of surface marker-mediated isolation and the reproducibility of the assay across a wide dynamic range.

Western blot analysis confirmed the presence of canonical tetraspanin markers, i.e., CD63 and CD81, across all EV isolation methods, validating the preservation of vesicle-associated surface proteins. However, differences in band intensity between isolation approaches need to be interpreted with caution, as the NTA quantifies particle number rather than vesicle-associated protein content. This distinction is particularly relevant for magnetic bead-based workflows, in which no elution step is performed prior to protein extraction. In such cases, EVs remain bound to the capture beads, and vesicle-associated proteins may become partially inaccessible due to steric hindrance at the bead-vesicle interface. Consequently, a fraction of these proteins may potentially remain attached to the bead surface or be inefficiently extracted, leading to an underestimation of protein abundance and reduced apparent signal intensity in Western blot analysis. In addition, the negative EV marker Calnexin was assessed, and it was not detected in any of the isolation conditions, indicating minimal cellular contamination and supporting the specificity of the EV preparations (Fig. 3G). In addition, SEM imaging further demonstrated morphological differences between isolation methods (Fig. 3H–K). As a result, ultracentrifugation and magnetic bead-based isolation produced predominantly spherical vesicle populations consistent with canonical EV morphology. Ultrafiltration samples exhibited greater heterogeneity with some aggregation, likely attributable to membrane retention effects during filtration. DMF-

isolated EVs also displayed some morphological heterogeneity, which may reflect selective enrichment of mixed EV subpopulations by immunoaffinity capture, as well as minor artifacts arising from residual silicone oil or surface-associated material inherent to the open-cartridge DMF format.

Collectively, these results demonstrate that both conventional and DMF-based affinity platforms enable consistent EV isolation. Complementary flow cytometry analyses corroborated a strong quantitative correlation between EV concentration and fluorescence signal, further confirming the robustness of these approaches.

3.2. On-chip ELISA detection of EVs

The DMF platform enables to isolate EVs and also provides the running of ELISA on the same system (Fig. 4A–E). Regarding analytical performance, we compared conventional ELISA with the DMF-based ELISA using EV concentrations ranging from 5×10^6 to 1×10^9 particles/mL. In the conventional format, optical density (OD) values increased from 0.401 at 5×10^6 particles/mL to 0.543 at 1×10^9 particles/mL, yielding a moderate linear correlation ($R^2 = 0.8388$) (Fig. 4F). The DMF-based ELISA produced comparable concentration-dependent OD values, ranging from 0.273 to 0.351, but exhibited a stronger linear relationship ($R^2 = 0.8935$) (Fig. 4G). Although the absolute signal intensity on the DMF platform was approximately 30–35% lower—primarily due to the reduced assay volume and droplet scale—the platform showed better proportionality and reproducibility across the tested range. This enhanced correlation arises from the inherently uniform droplet sizes, efficient on-chip mixing, and minimal reagent loss characteristic of digital microfluidics.

Overall, these findings demonstrate that the DMF-based ELISA can reliably and consistently detect EVs with performance comparable to conventional assays, while offering significant advantages in assay time (30 min) and reagent consumption (30 μ L). The results further support the potential of DMF technology as a foundation for next-generation, low-volume, immunoassay workflows in the future.

4. Conclusions

The present work was intentionally designed as a technological “proof-of-concept” and “engineering validation” of a commercially available, unmodified DMF platform. Within this framework, we demonstrated that essential bioanalytical operations required for EV analysis—including droplet manipulation, immunomagnetic EV capture, on-chip washing, and colorimetric immunodetection—can be successfully implemented on the OpenDrop V4 system without any hardware modifications. Importantly, the on-chip washing experiments

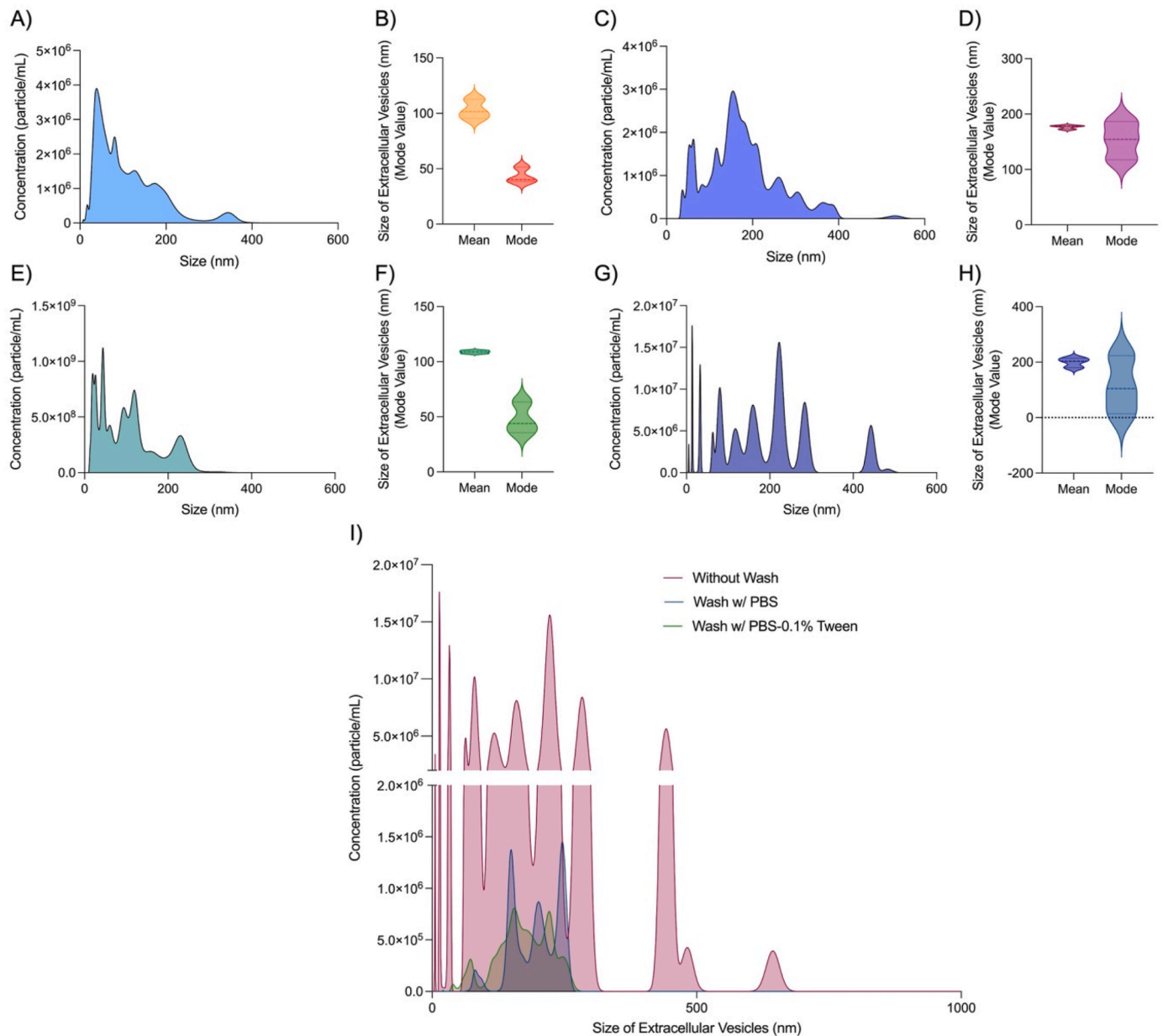


Fig. 2. Comparison of EV isolation methods and their corresponding nanoparticle tracking analysis (NTA) results, including particle concentration as well as mean and mode size distributions. A–B) Ultracentrifugation, C–D) ultrafiltration, E–F) magnetic bead-based isolation, and G–H) DMF platform are presented. I) NTA size distribution of EV samples without washing, washed with PBS, and washed with PBS supplemented with 0.1% Tween for assessing the washing strategies for DMF-based workflows.

provided direct experimental evidence that magnetically retained EV–bead complexes remain stable during droplet-based washing while substantially reducing non-specifically associated particles by more than one order of magnitude. Although further developments will be required to achieve a fully integrated and autonomous diagnostic workflow, these results establish the technical feasibility of performing sequential EV processing and detection steps on an accessible, off-the-shelf DMF platform. Collectively, this study provides a practical foundation for future efforts aimed at integrating sample preparation, washing, and detection into fully automated POC EV analysis systems.

Overall, this study represents a validation of a commercially available DMF platform for EV isolation and detection. This study denotes that magnetic bead-based EV isolation and on-chip ELISA detection can be reliably performed on an off-the-shelf DMF platform without requiring any hardware modifications. To our knowledge, this represents the first demonstration of fully integrated EV isolation and on-chip

immunodetection on the OpenDrop platform, establishing a complete analytical workflow on a single unmodified commercial device and representing a meaningful step toward democratizing EV-based diagnostics by removing the fabrication barrier that currently limits translation of DMF-based EV workflows beyond specialized research environments. Across all comparative evaluations, the DMF system reproduced the analytical trends observed with ultracentrifugation, ultrafiltration, and conventional benchtop immunoassays, while operating with significantly reduced reagent consumption and shorter assay times. The EVs isolated using the DMF-assisted magnetic bead strategy showed preserved morphology, narrow size distributions, and tetraspanins expression, confirming that the platform provides a gentle, affinity-driven approach that maintains vesicle integrity. Moreover, the linearity of the DMF-based ELISA ($R^2 = 0.8935$) underscores its ability to deliver quantitative and reproducible immunodetection at microvolume scales.

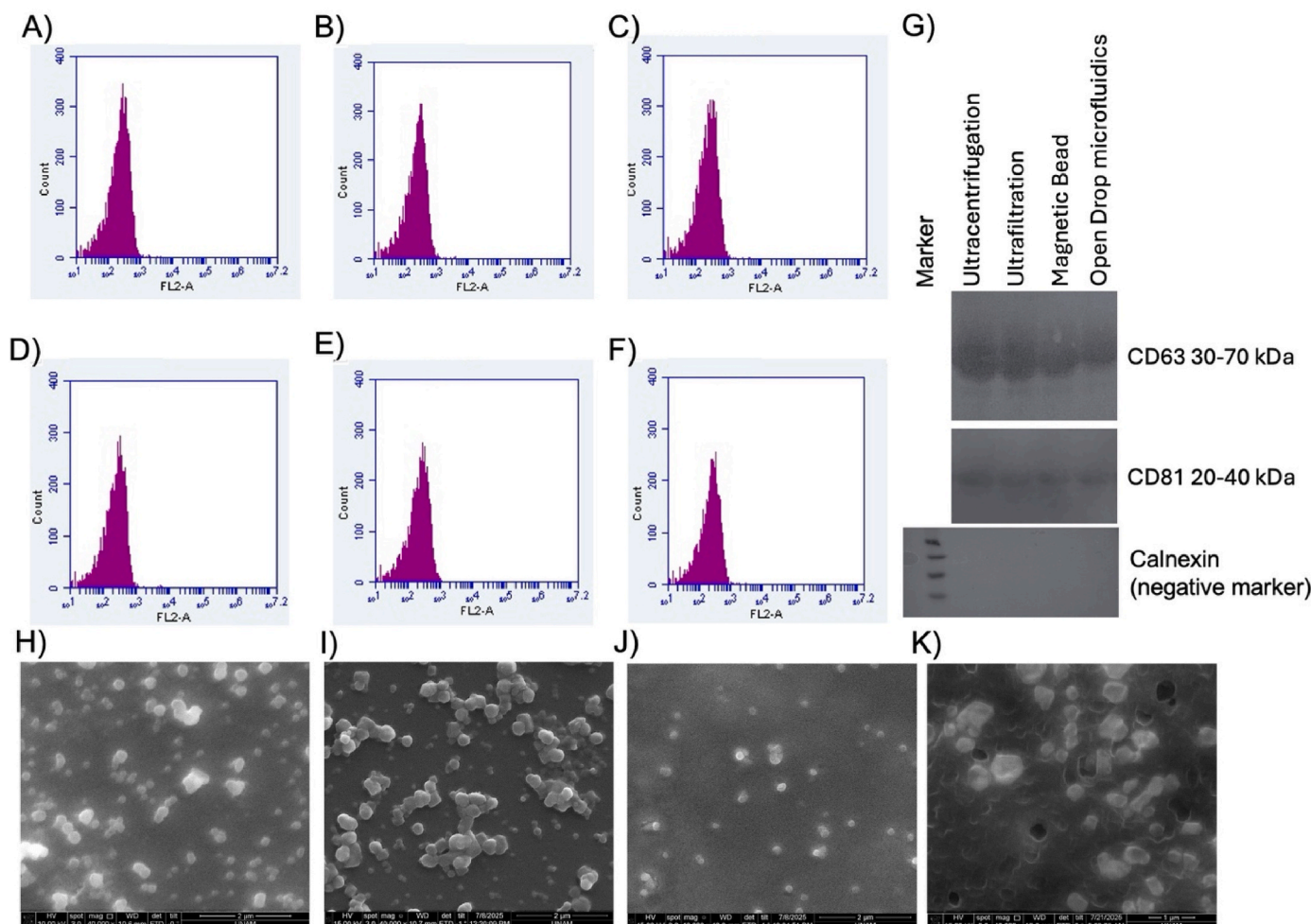


Fig. 3. Characterization of EVs isolated using different methods. A–F) Flow cytometry histograms of EVs captured via magnetic bead-based isolation at varying concentrations: A) 1×10^9 , B) 5×10^8 , C) 1×10^8 , D) 5×10^7 , E) 1×10^7 , and F) 5×10^6 particles/mL, showing particle counts across fluorescence intensity (FL2-A) and demonstrating concentration-dependent detection. G) Western blot analysis of canonical tetraspanin markers, i.e., CD63, CD81 and negative marker Calnexin, on the membrane of EVs isolated by ultracentrifugation, ultrafiltration, magnetic bead-based capture, and DMF platform, indicating preservation of membrane protein integrity. Scanning electron microscopy (SEM) images comparing EV morphology, surface topography, and size distribution across isolation methods. H) ultracentrifugation, I) ultrafiltration, J) magnetic bead-based isolation, and K) DMF platform, highlighting differences in uniformity and vesicle integrity.

Herein, EVs derived from conditioned cell culture media were evaluated as a proof-of-concept study; however, extending DMF-based workflows to more complex biological fluids such as plasma or serum represents a pivotal next step. While the present approach demonstrated robust performance in conditioned cell culture media, which served as a controlled model system for platform validation, biological fluids such as plasma, serum, or saliva present substantially greater compositional complexity and may introduce additional biofouling challenges. Addressing these challenges will likely require the incorporation of advanced anti-fouling surface chemistries and optimized sample-processing strategies. In the present study, owing to its hydrophobic feature, silicone oil pre-coating of the device surface was employed as a standard anti-fouling measure, which reduces biomolecule adsorption and facilitates droplet actuation under controlled conditions. However, more robust and controllable surface engineering will be required to achieve clinical sample compatibility. Promising strategies identified in the literature include Pluronic F-127 or PEG-based surface coatings, which form steric barriers against protein adsorption without compromising droplet actuation performance, as well as optimized fluorocarbon dielectric layers and BSA blocking protocols (Ho et al., 2023; Latip et al., 2017; Luk et al., 2008; Rashid et al., 2021; Sista et al., 2008). On-chip sample pre-processing steps — such as programmatic dilution or pre-filtration droplets routed prior to the immunocapture zone —

offer additional means of reducing the fouling burden without requiring external instrumentation (Mei et al., 2014). Implementing and systematically evaluating these strategies within the context of plasma- or serum-derived EV workflows represents a well-defined and tractable next step for translating the present platform toward clinical diagnostic applications. The integration and systematic evaluation of such anti-fouling approaches in clinically relevant biofluids represents an important next step toward the translation of this platform and will be the subject of future investigations. Notably, magnetic bead-based immunoaffinity capture has already demonstrated strong performance in complex biofluids, suggesting that, with appropriate modifications, the DMF workflow described here can be adapted for clinical applications. Finally, the slightly larger mean EV diameter observed on the DMF platform is consistent with the preferential enrichment of tetraspanin-high EV subpopulations through immunoaffinity capture, a phenomenon well documented in the literature (Kowal et al., 2016; Théry et al., 2018), rather than an inherent limitation of the system. This effect can be mitigated by optimizing bead size, surface functionalization, and droplet actuation parameters to achieve more uniform EV capture across diverse size populations. Overall, despite these manageable challenges, the DMF platform offers a compelling, programmable, and reagent-efficient framework for EV isolation and quantitative immunodetection. With improvements in on-chip washing, anti-fouling

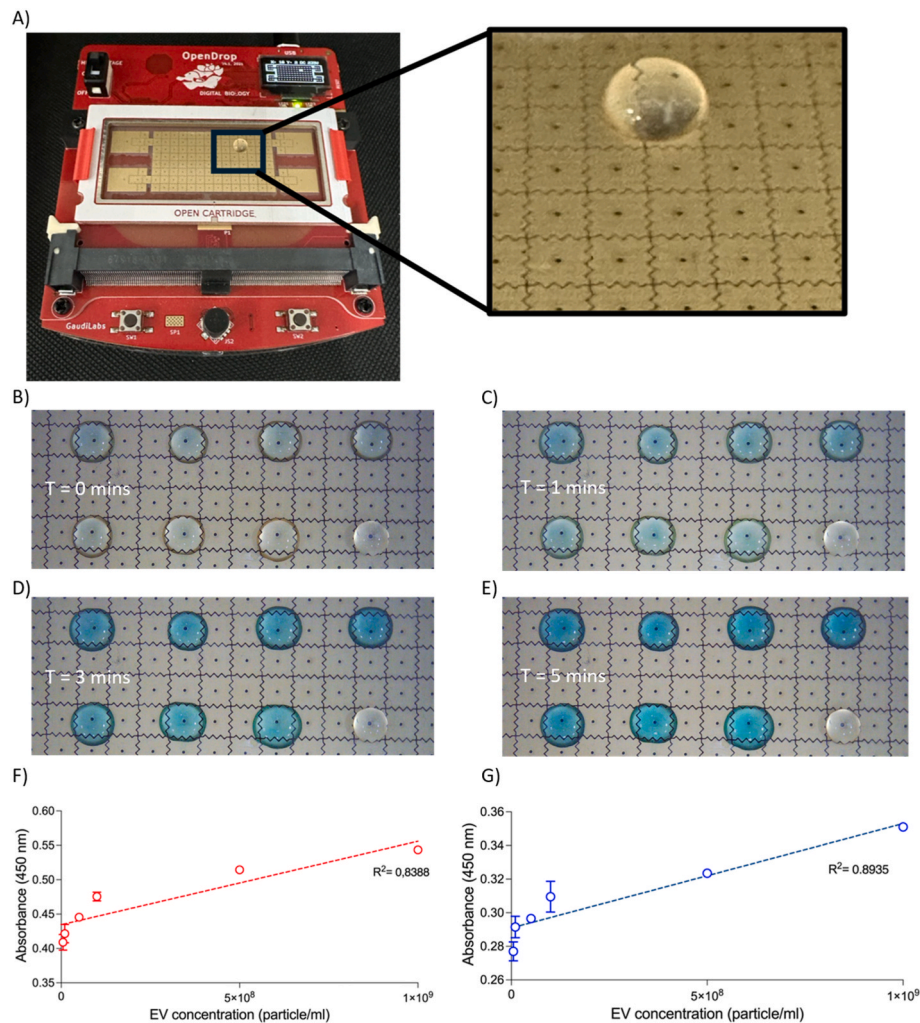


Fig. 4. DMF-based handling of magnetic bead-tagged EVs is demonstrated. A) A droplet containing magnetic bead-bound EVs positioned over a magnetic pad beneath the cartridge. B) Eight droplets containing ELISA reagents with varying EV concentrations (1×10^9 , 5×10^8 , 1×10^8 , 5×10^7 , 1×10^7 , 5×10^6 particles/mL) distributed on the DMF platform. C–E) Represented images of the droplets at 1, 3, and 5 min, illustrating droplet stability, mixing, and EV–bead interactions over time. ELISA-based quantification of EVs across different concentrations are further assessed quantitatively. F) Conventional ELISA results showing absorbance at 450 nm as a function of EV concentration (particles/mL). G) DMF platform-based ELISA results demonstrating absorbance signals across the same concentration range.

chemistry, optical signal enhancement, and multiplexed operation, DMF technology has the potential to evolve into a fully integrated, automated system for portable EV isolation and detection — serving as a stepping stone toward point-of-care diagnostic applications.

CRediT authorship contribution statement

Murat Alp Güngen: Conceptualization, Formal analysis, Investigation, Methodology, Validation, Visualization, Writing – original draft.
Eylul Gulsen Yilmaz: Conceptualization, Formal analysis, Investigation, Methodology, Validation, Visualization, Writing – original draft.
Fatih Inci: Conceptualization, Formal analysis, Investigation, Methodology, Project administration, Supervision, Validation, Visualization, Writing – original draft.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

Fatih Inci gratefully acknowledges support from the Scientific and Technological Research Council of Turkey (TÜBİTAK) 2232 International Fellowship for Outstanding Researchers (Project No: 118C254). However, the entire responsibility of the publication/article belongs to the owner of the publication/article. The financial support received from TÜBİTAK does not mean that the content of the publication is approved in a scientific sense by TÜBİTAK. Fatih Inci gratefully acknowledges the support from TÜBİTAK Incentive Award, Turkish Academy of Sciences - Outstanding Young Scientists Award Program (TÜBA-GEİP), Young Scientist Awards Program (BAGEP) from Science Academy, TÜSEB Aziz Sancar Incentive Award, Istanbul Technical University (ITU) Science Award, Dr. Nejat Eczacıbaşı Medical Incentive Award, and Science Award from Izmir Biomedicine and Genome Center (IBG).

Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.biosx.2026.100816>.

Data availability

Data will be made available upon reasonable request.

References

- Abreu, C.M., Costa-Silva, B., Reis, R.L., Kundu, S.C., Caballero, D., 2022. *Lab Chip* 22, 1093–1125.
- Akceoglu, G.A., Saylan, Y., Inci, F., 2021. *Adv. Mater. Technol.* 6, 2100049. <https://doi.org/10.1002/ADMT.202100049>.
- Albin, D., Buecherl, L., Kochavi, E., Niehaus, E., Novack, S., Urugoda, S., Myers, C.J., Alistar, M., 2024. *IEEE Trans. Biomed. Eng.* 71, 217–226. <https://doi.org/10.1109/TBME.2023.3295418>.
- Alistar, M., Gaudenz, U., 2017. *Bioengineering* 4, 45.
- Allelein, S., Medina-Perez, P., Lopes, A.L.H., Rau, S., Hause, G., Kölsch, A., Kuhlmeier, D., 2021. *Sci. Rep.* 11, 1–12. <https://doi.org/10.1038/s41598-021-91129-y>, 2021 111.
- Amin, S., Massoumi, H., Tewari, D., Roy, A., Chaudhuri, M., Jazayerli, C., Krishan, A., Singh, M., Soleimani, M., Karaca, E.E., 2024. *Int. J. Mol. Sci.* 25, 2730.
- Andreu, Z., Yáñez-Mó, M., 2014. *Front. Immunol.* 5, 442. <https://doi.org/10.3389/fimmu.2014.00442>.
- Arjmand, E.M., Grether, G., Bustos-Quevedo, G., Atanga, J., Sánchez-Martín, P., Van Deun, J., Hutzenlaub, T., Nazarenko, I., Paust, N., Lueddecke, J., 2025. *bioRxiv*, 674190. <https://doi.org/10.1101/2025.09.04.674190>, 2025.09.04.
- Atabay, M., Inci, F., Saylan, Y., 2025. *Biosens. Bioelectron.* 277. <https://doi.org/10.1016/j.bios.2025.117275>.
- Azad, B.K.D., Roozbahani, A., Tabatabaei, S.M., Valinejad, A., Fazelian, A., Shahmoradi, K., Khatami, F., Aghamir, S.M.K., Kolahdouz, M., 2025. *Sci. Rep.* 15, 4578. <https://doi.org/10.1038/s41598-025-89343-z>.
- Bajo-Santos, C., Priedols, M., Kaukis, P., Paidere, G., Gerulis-Bergmanis, R., Mozolevskis, G., Abols, A., Rimsa, R., 2023. *Int. J. Mol. Sci.* 24, 7971.
- Chen, J., Zheng, M., Xiao, Q., Wang, H., Chi, C., Lin, T., Wang, Y., Yi, X., Zhu, L., 2024. *Micromachines* 15, 630.
- Convery, N., Gadegaard, N., 2019. *Micro Nano Eng.* 2, 76–91. <https://doi.org/10.1016/j.mne.2019.01.003>.
- Dai, Y., Cui, Y., Li, J., Li, P., Huang, X., 2025. *APL Bioeng.* 9, 31502. <https://doi.org/10.1063/5.0273892>.
- Derin, E., Yilmaz, E.G., Erdem, O., Aslan, Y., Kafaden, A., Çelik, S., Çelik, U., Inci, F., 2025. *ACS Appl. Mater. Interfaces* 17 (47), 64048–64064.
- Derin, E., Inci, F., 2022. *ACS Sens.* 7, 358–385. <https://doi.org/10.1021/acssensors.1c01781>.
- Dong, L., Zieren, R.C., Horie, K., Kim, C.-J., Mallick, E., Jing, Y., Feng, M., Kuczler, M.D., Green, J., Amend, S.R., Witwer, K.W., de Reijke, T.M., Cho, Y.-K., Pienta, K.J., Xue, W., 2020. *J. Extracell. Vesicles* 10, e12044. <https://doi.org/10.1002/jev2.12044>.
- Gholizadeh, S., Draz, M.S., Zarghooni, M., Sanati-Nezhad, A., Ghavami, S., Shafiee, H., Akbari, M., 2017. *Biosens. Bioelectron.* 91, 588–605.
- Guo, S.-C., Tao, S.-C., Dawn, H., 2018. *J. Extracell. Vesicles* 7, 1508271.
- György, B., Szabó, T.G., Pászto, M., Pál, Z., Misják, P., Aradi, B., László, V., Pállinger, É., Pap, E., Kittel, Á., Nagy, G., Falus, A., Buzás, E.L., 2011. *Cell. Mol. Life Sci.* 68, 2667–2688. <https://doi.org/10.1007/S00018-011-0689-3>, 2011 6816.
- Hassanzadeh-Barforoushi, A., Sango, X., Johnston, E.L., Haylock, D., Wang, Y., 2025. *J. Extracell. Vesicles* 14, e70132. <https://doi.org/10.1002/jev2.70132>.
- Havers, M., Broman, A., Lenshof, A., Laurell, T., 2023. *Anal. Bioanal. Chem.* 415, 1265–1285.
- Ho, M., Au, A., Flick, R., Vuong, T.V., Sklavounos, A.A., Swyer, I., Yip, C.M., Wheeler, A. R., 2023. *ACS Appl. Mater. Interfaces* 15, 6326–6337. <https://doi.org/10.1021/acsami.2c17317>.
- Inci, F., 2022. *Langmuir* 38, 1897–1909. <https://doi.org/10.1021/ACS.LANGMUIR.1C03119/ASSET/IMAGES/MEDIUM/LA1C03119.0010.GIF>.
- Kalyani, N., Pastor, M.C., Pezzarossa, L., Moller-Hansen, I., Tanev, G., Dimaki, M., Borodina, I., Madsen, J., Svendsen, W.E., 2025. *Talanta* 282, 127001. <https://doi.org/10.1016/j.talanta.2024.127001>.
- Kim, Y., Jeon, S., Lee, K., Shin, S., 2025. *bioRxiv*, 645827. <https://doi.org/10.1101/2025.03.27.645827>, 2025.03.27.
- Küçük, B.N., Yilmaz, E.G., Aslan, Y., Erdem, Ö., Inci, F., 2024. *ACS Appl. Bio Mater.* 7, 5841–5860. <https://doi.org/10.1021/acsbm.4c00782>.
- Kowal, J., Arras, G., Colombo, M., Jouve, M., Morath, J.P., Primdal-Bengtson, B., Dingli, F., Loew, D., Tkach, M., Théry, C., 2016. *Proc. Natl. Acad. Sci.* 113, E968–E977.
- Latip, E.N.A., Coudron, L., McDonnell, M.B., Johnston, I.D., McCluskey, D.K., Day, R., Tracey, M.C., 2017. *RSC Adv.* 7, 49633–49648. <https://doi.org/10.1039/C7RA10920B>.
- Li, B., Wu, W., Xu, W., Qian, H., Ji, C., 2025. *J. Nanobiotechnol.* 23, 678. <https://doi.org/10.1186/s12951-025-03768-2>.
- Liang, L.-G., Sheng, Y.-F., Zhou, S., Inci, F., Li, L., Demirci, U., Wang, S., 2017. In: *Extracellular Vesicles: Methods and Protocols*. Springer, pp. 355–364.
- Lin, Y., Zhang, C., Xiang, P., Shen, J., Sun, W., Yu, H., 2020. *J. Extracell. Vesicles* 9, 1722385. <https://doi.org/10.1080/20013078.2020.1722385>.
- Liu, F., Kc, P., Zhang, G., Zhe, J., 2016. *Anal. Chem.* 88, 711–717. https://doi.org/10.1021/ACS.ANALCHEM.5B02716/ASSET/IMAGES/LARGE/AC-2015-02716A_0008.JPG.
- Lo, T.-W., Liu, J., Zhang, Y., Kim, Y., Yao, J., Dong, H., Lucien, F., Liu, Y., 2025. *Small* 21, e04335. <https://doi.org/10.1002/sml.202504335>.
- Luk, V.N., Mo, G.C.H., Wheeler, A.R., 2008. *Langmuir* 24, 6382–6389. <https://doi.org/10.1021/la7039509>.
- Meggiolaro, A., Moccia, V., Brun, P., Pierno, M., Mistura, G., Zappulli, V., Ferraro, D., 2022. *Biosensors* 13, 50.
- Meggiolaro, A., Moccia, V., Brun, P., Pierno, M., Mistura, G., Zappulli, V., Ferraro, D., 2023. *Biosensors*. <https://doi.org/10.3390/bios13010050>.
- Mei, N., Seale, B., Ng, A.H.C., Wheeler, A.R., Oleschuk, R., 2014. *Anal. Chem.* 86, 8466–8472. <https://doi.org/10.1021/ac5022198>.
- Oksvold, M.P., Neurauder, A., Pedersen, K.W., 2015. *Methods Mol. Biol.* 1218, 465–481. https://doi.org/10.1007/978-1-4939-1538-5_27.
- Parlatan, U., Ozen, M.O., Kecoglu, I., Koyuncu, B., Torun, H., Khalafkhan, D., Loc, I., Ogut, M.G., Inci, F., Akin, D., 2023. *Small* 19, 2205519.
- Peng, C., Zhang, Z., Kim, C.-J., Ju, Y.S., 2014. *Lab Chip*. <https://doi.org/10.1039/c3lc51223a>.
- Rajagopal, C., Harikumar, K.B., 2018. *Front. Oncol.* 8, 66. <https://doi.org/10.3389/FONC.2018.00066>.
- Rashid, S., Ward-Bond, J., Krupin, O., Berini, P., 2021. *Colloids Surf. B Biointerfaces* 208, 112138. <https://doi.org/10.1016/j.colsurfb.2021.112138>.
- Samiei, E., Tabrizian, M., Hoorfar, M., 2016. *Lab Chip* 16, 2376–2396. <https://doi.org/10.1039/C6LC00387G>.
- Saylan, Y., Altıntaş, Ö., Yilmaz, E.G., Akceoglu, G.A., Inci, F., Yilmaz, F., Denizli, A., 2025. *Microchem. J.* 116029.
- Shirejini, S.Z., Inci, F., 2022. *Biotechnol. Adv.* 54, 107814. <https://doi.org/10.1016/j.biotechadv.2021.107814>.
- Sista, R., Hua, Z., Thwar, P., Sudarsan, A., Srinivasan, V., Eckhardt, A., Pollack, M., Pamula, V., 2008. *Lab Chip* 8, 2091–2104. <https://doi.org/10.1039/B814922D>.
- Sun, J., Li, Z., Chen, Y., Chang, Y., Yang, M., Zhong, W., 2025. *Anal. Chem.* 97, 6922–6937.
- Tauro, B.J., Greening, D.W., Mathias, R.A., Ji, H., Mathivanan, S., Scott, A.M., Simpson, R.J., 2012. *Methods* 56, 293–304. <https://doi.org/10.1016/J.YMETH.2012.01.002>.
- Théry, C., Witwer, K.W., Aikawa, E., Alcaraz, M.J., Anderson, J.D., Andriantsitohaina, R., Antoniou, A., Arab, T., Archer, F., Atkin-Smith, G.K., 2018. *J. Extracell. Vesicles* 7, 1535750.
- Tian, F., Liu, C., Deng, J., Sun, J., 2022. *Accounts mater. Res.* 3, 498–510.
- Tong, Z., Xu, X., Shen, C., Yang, D., Li, Y., Li, Q., Yang, W., Xu, F., Wu, Z., Zhou, L., 2025. *Biosens. Bioelectron.* 270, 116976.
- Tong, Z., Yang, D., Shen, C., Li, C., Xu, X., Li, Q., Wu, Z., Ma, H., Chen, F., Mao, H., 2024. *Anal. Chim. Acta* 1296, 342337.
- Welsh, J.A., Goberdhan, D.C.I., O'Driscoll, L., Buzas, E.I., Blenkiron, C., Bussolati, B., Cai, H., Di Vizio, D., Driedonks, T.A.P., Erdbrügger, U., Falcon-Perez, J.M., Fu, Q.-L., Hill, A.F., Lenassi, M., Lim, S.K., Mahoney, M.G., Mohanty, S., Möller, A., Nieuwland, R., Ochiya, T., Sahoo, S., Torrecillas, A.C., Zheng, L., Zijlstra, A., Abuelreich, S., Bagabas, R., Bergese, P., Bridges, E.M., Bruciale, M., Burger, D., Carney, R.P., Cocucci, E., Crescitelli, R., Hanser, E., Harris, A.L., Haughey, N.J., Hendrix, A., Ivanov, A.R., Jovanovic-Talisman, T., Kruh-Garcia, N.A., Ku'ulei-Lyn Faustino, V., Kyburz, D., Lässer, C., Lennon, K.M., Lötvall, J., Maddox, A.L., Martens-Uzunova, E.S., Mizenko, R.R., Newman, L.A., Ridolfi, A., Rohde, E., Rojalin, T., Rowland, A., Saftics, A., Sandau, U.S., Saugstad, J.A., Shekari, F., Swift, S., Ter-Ovanesyan, D., Tosar, J.P., Useckaite, Z., Valle, F., Varga, Z., van der Pol, E., van Herwijnen, M.J.C., Wauben, M.H.M., Wehman, A.M., Williams, S., Zendriani, A., Zimmermann, A.J., Consortium, M., Théry, C., Witwer, K.W., 2024. *J. Extracell. Vesicles* 13, e12404. <https://doi.org/10.1002/jev2.12404>.
- Xu, X., Cai, L., Liang, S., Zhang, Q., Lin, S., Li, M., Yang, Q., Li, C., Han, Z., Yang, C., 2023. *Lab Chip* 23, 1169–1191. <https://doi.org/10.1039/D2LC00756H>.
- Zhang, S., Deng, J., Li, J., Tian, F., Liu, C., Fang, L., Sun, J., 2022. *TrAC Trends Anal. Chem. (Reference Ed.)* 157, 116817.