Sample Prep for Liquid Biopsies on a Chip: Exosomes, DNA and Beyond

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Outline

• Separation and isolation at the nanoscale
• nanoDLD on-chip separation of exosomes and nucleic acids
• Solutions & approaches to key technical challenges for chip-based sample prep:
  • Resolution limits
  • Filtration – off-chip vs. on-chip
  • Minimizing sample dilution
  • Sample extraction with higher volume chips
  • Automated exosome and dsDNA separation
IBM Research nanofabrication strategy

- State-of-the-art design, fabrication and packaging facility to rapidly prototype and integrate novel materials and structures for devices, sensors, and systems

- 40,000 sq. ft. class 100 CR, 200 mm wafer line, advanced CMOS and packaging capabilities
Exosomes and cfDNA are implicated in many health conditions

- Premetastatic niche
- Immune response
- Biomarker
- Cell apoptosis
- Disease transmission
- Diagnostic tool
- Drug vector
- Biomarker
- Inhibits immune response against fetus
- Maternal and fetal biomarker
- Promotes placental angiogenesis
- Tissue regeneration
- Protective factor
- Biomarker
- Infection spread
- Immune stimulation
- Biomarker
- Tissue regeneration

De Toro, J. et al, Frontiers in Immunology, 2015, 6, 203.
Current exosome and cfDNA isolation techniques

Exosome Isolation

Conditioned medium → UC, 10,000 x g for 1-2 hours → Cellular debris
→ UC, 100,000 x g for >4 hours → Extracellular vesicle pellet
→ Sucrose gradient for >15 hours → Contaminating EVs
→ α-CD63 beads for 2-3 hours → Isolated exosomes

Sample loss, Long run time, Liquid and protein contamination

Non-automated Batch processing
Sophisticated lab equipment
Independent detection methods

cfDNA Isolation

Blood sample → Plasma preparation for 15-30 minutes → RBCs, WBCs, platelets
→ Plasma sample
→ Spin column kit for 1-2 hours → Contaminating RNA, proteins
→ Isolated cfDNA

Sample degradation, High background, non-specific
IBM nanoDLD separation capabilities

**Nanoscale Biomarkers**
- **Exosomes** (30-150nm)
- **DNA** (2nm)
- **Viruses** (20-200nm)

**DLD Operation**

**Nanofluidics Platform**

**IBM nanoDLD On-Chip Separation**
Biomarker separation down to 20 nm with nanoscale resolution

**Applications to Biology**
- Circulating tumor cells
- Human parasites
- Blood cells


nanoDLD separation of nucleic acids

- In addition to exosomes, DNA can also be separated by size using nanoDLD
- Opportunities in sequencing prep and diagnostics

250nm gap size, 5.7° maximum angle

Array Inlet
- nanopillar array
- 100 bp dsDNA
- 1.0 kb dsDNA
- 10.0 kb dsDNA

Array Outlet
- nanopillar array
- 100 bp dsDNA
- 1.0 kb dsDNA
- 10.0 kb dsDNA

nanoDLD gap size
- 78nm
- 250nm
- 500nm
- 750nm

fraction maximum migration angle vs dsDNA basepairs (kb)
Separation resolution

- Multiple dsDNA strand separations possible with a single array – not a binary system
- Minimum bump size down to ~100bp, with a resolution limit of ~200bp difference

### dsDNA pair | $R$ | Estimated molar purity
---|---|---
0.1 – 0.25 kb | 0.0 | 50.0% - 50.0%
0.25 – 0.5 kb | 0.8 | 64.6% - 39.8%
0.1 – 1.0 kb | 1.4 | 99.9% - 95.3%
0.5 – 1.0 kb | 1.4 | 99.9% - 99.9%
1.0 – 10.0 kb | 2.4 | >99.9% - >99.9%
Physics of particle motion in nanoDLD arrays

- Using computational fluid dynamics, particle trajectories are studied to understand nanoDLD systems and develop new structures with novel functionality.

Simulation model → Model prediction and experimental verification → Heterogeneous system for high efficiency separation device

S.-C. Kim et al. (submitted)
Separation without sample dilution

- Experimentation and computation have provided a better understanding of nanoDLD fluid dynamics, enabling predictive modeling and structures with new utility.

Previous separation required dilution

Condenser structure in series w/ nanoDLD array achieves full separation w/o dilution

Sample is compressed with buffer flow to only 10-20% of array width

Full separation achieved with full-width injection

S.-C. Kim et al. (submitted)
Filtration and clog-resistant structures

Off-Chip Preload Filtration

On-Chip Filtration and Clog Mitigation

- Test is conservative estimate: run with carboxylated polystyrene beads
- Running with dsDNA or exosomes, chips survive over 24hr
Sample extraction with higher volume throughput

Prototyping
- Observational layout
- Patterned with mixed DUV/ebeam litho.
- 6-8 week prototyping short loop
- Fluidic throughput ~ 0.2µL/hr

2D Enrichment Layout
- Stackable enrichment chip
- Four large arrays in parallel
- Sample collection possible
- Fluidic throughput 50-100µL/hr per layer

3D Integrated
- Multiplexed fluidic system
- 360 arrays in parallel
- Designed for sample collection
- Fluidic throughput target: > 1mL/hr
Route to automated biomarker separation

- A practical route requires decoupling sample from system – a different approach to fluid drive is needed
Exosome size and surface marker separation and detection

Separation of Exosomes by Size

- nanoDLD enables both isolation of the total exosome population as well as subfractionation of specific exosome size ranges of interest.
- Exosomes isolated from docetaxel-resistant prostate cancer cells are smaller than exosomes from cells sensitive to the drug.
- Size-based separation allows isolation of intact exosomes (30-150 nm) from other extracellular vesicles (200-500 nm) and apoptotic bodies

Separation of Exosomes by Surface Marker

- Prostate cancer-specific exosome surface markers can be detected in nanoDLD array.
- Simultaneous two-color imaging enables detection of multiple epitopes and single exosome studies.
- Bead-coupled antibodies allows for surface marker-based separation on nanoDLD.

![Exosome size and surface marker separation diagram]
Conclusion

- Achieved separation of biocolloids down to 20nm in continuous flow
- On-chip size-based separation of exosomes and dsDNA demonstrated using nanoDLD
- Parallel architectures provide a technological path with sufficient throughput to achieve automated sample prep
- Epitope labeling and biochemical size-amplification of exosomes in nanoDLD arrays can aid diagnostics to help identify a disease state and monitor progression
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