Detection of extracellular vesicles: size does matter

Edwin van der Pol

November 16th, 2018
Outline

- PhD research
  - Extracellular vesicles
  - Size does matter
  - Flow cytometry

- Outlook
  - Standardisation
  - Clinical studies
  - Small *and* fast
Extracellular vesicles
Extracellular vesicles

- Cells release EVs: biological nanoparticles with receptors, DNA, RNA
- Specialized functions
- Clinically relevant

van der Pol et al. *Pharmacol Rev* 2012
Vesicle-based “liquid biopsy”

<table>
<thead>
<tr>
<th>Hematology parameter</th>
<th>Concentration (vesicles mL⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Platelet vesicle count</td>
<td>2.3 – 6.2 \cdot 10^9</td>
</tr>
<tr>
<td>Erythrocyte vesicle count</td>
<td>7.0 – 8.6 \cdot 10^{10}</td>
</tr>
<tr>
<td>Reticulocyte vesicle count</td>
<td>3.9 – 15.6 \cdot 10^8</td>
</tr>
<tr>
<td>Leukocyte vesicle count</td>
<td>6.2 – 16.4 \cdot 10^7</td>
</tr>
<tr>
<td>Total vesicle count</td>
<td>7.3 – 9.4 \cdot 10^{10}</td>
</tr>
</tbody>
</table>

rare EVs

all EVs
Problem: counting vesicles is difficult

- Reported concentrations of blood vesicles differ >$10^6$-fold
- Clinical data cannot be compared

“Gąsecka’s law”
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Size distribution of extracellular vesicles?

Extracellular vesicles

Reference particles
Size distribution of extracellular vesicles
Size distribution of extracellular vesicles
Size distribution of extracellular vesicles

![Graph showing size distribution and concentration of extracellular vesicles using different techniques.](image-url)
Size distribution of extracellular vesicles

Size distribution of extracellular vesicles

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Problem: count the black grains
Solution: flow cytometry

- Laser
- Forward scatter detector
- Side scatter detector
- Fluorescence channels
- Electronics and computer

image: semrock.com
Problem: which size of vesicles do I measure?

Flow cytometry:

![Flow cytometry graph](image)

[Image of TEM showing vesicles with scale bar of 500 nm]
Flow cytometry calibration before 2012

Flow cytometry:

- 900 nm polystyrene particles
- 500 nm polystyrene particles
Relate scatter to diameter of beads
Relate scatter to diameter of beads

![Graph showing side scatter vs. diameter for polystyrene beads. The x-axis represents diameter in nanometers, and the y-axis represents side scatter in arbitrary units (a.u.). The graph includes a line and data points indicating the scatter relationship.]
Relate scatter to diameter of beads

![Graph showing the relationship between side scatter (a.u.) and diameter (nm) for polystyrene and silica beads with corresponding theory curves and data points.]

- Data polystyrene beads
- Data silica beads
- Theory polystyrene spheres ($n_{\text{polystyrene}} = 1.605$)
- Theory silica spheres ($n_{\text{silica}} = 1.445$)
Relate scatter to diameter of vesicles

![Graph showing the relationship between side scatter (a.u.) and diameter (nm) for data polystyrene beads, data silica beads, theory polystyrene spheres ($n_{\text{polystyrene}} = 1.605$), theory silica spheres ($n_{\text{silica}} = 1.445$), and theory vesicles ($n_{\text{core}} = 1.38 \pm 0.02$, $n_{\text{shell}} = 1.48$).]
Particles that are too small to be detected generate a signal!

89 nm silica beads

urine EVs <220 nm
Flow cytometry

- Laser
- Fluorescence channels
- Electronics and computer
- Side scatter detector
- Forward scatter detector
At a concentration of $10^{10}$ vesicles ml$^{-1}$, >800 vesicles are simultaneously present in the beam.
Invisible vesicles swarm within the iceberg

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How to calibrate flow cytometers?

flow rate (µL/min) ?

fluorescence (MESF) ?

side scatter (nm²) ?

forward scatter (nm²) ?

laser
Solution

- One reference material to calibrate them all
- Properties similar to extracellular vesicles
  - scatter (size + refractive index)
  - fluorescence
  - concentration

Tolkien (1954)
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Clinical studies on extracellular vesicles

- Myocardial infarction
  - Therapy monitoring
- Stroke
  - Differentiate between ischemic stroke and hemorrhage stroke in acute phase
- Prostate cancer
  - Diagnoses
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Problem: small *or* fast

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<tr>
<th>Vesicles Diameter (nm)</th>
<th>Measurement Time</th>
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<td>&gt;150</td>
<td>15 minutes</td>
</tr>
<tr>
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![Graph showing concentration versus diameter](image)
**Solution: EV-Radar, small and fast**

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![Graph showing vesicle concentration vs. diameter with a power-law fit](image)

Data:
- Concentration (vesicles ml⁻¹)
- Diameter (nm)

Power-law fit:
- Black line: Data
- Red line: Power-law fit
Conclusion

- Body fluids contain extracellular vesicles with clinical information
- The size of extracellular vesicle matters!
Acknowledgements

- My parents
- My wife

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  Amsterdam University Medical Centers
    ➢ Ton van Leeuwen
    ➢ Rienk Nieuwland
    ➢ Guus Sturk
    ➢ Frank Coumans

- More info about extracellular vesicles: edwinvanderpol.com