Biocalorimetry to predict extracellular vesicle production and improve biomanufacturing

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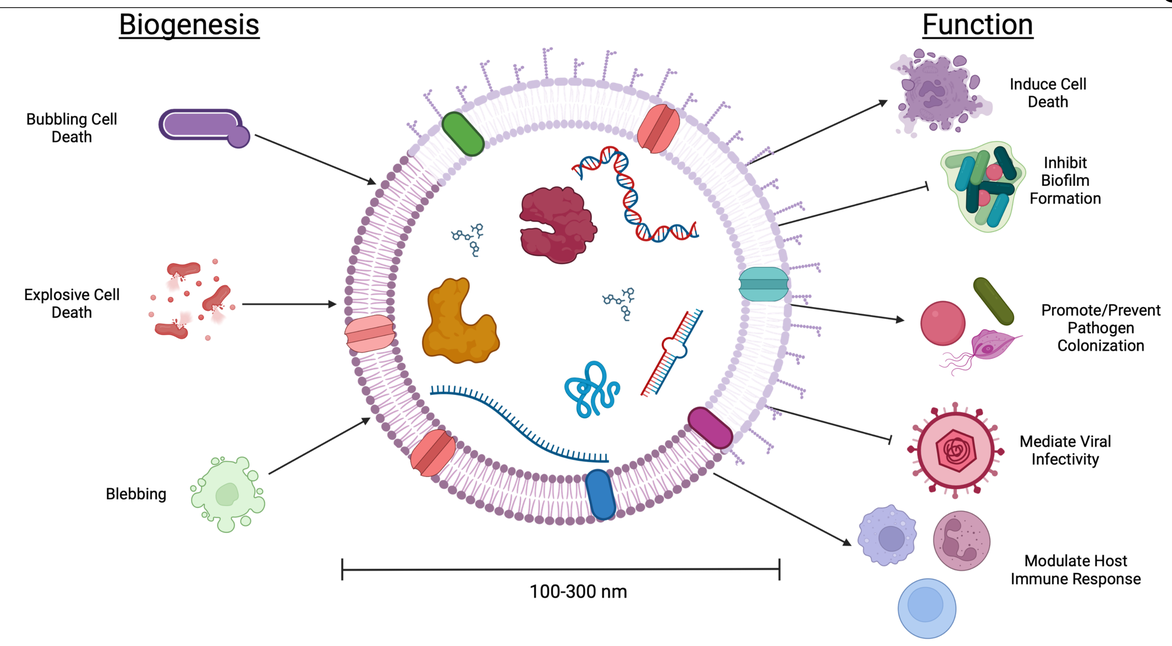
Background



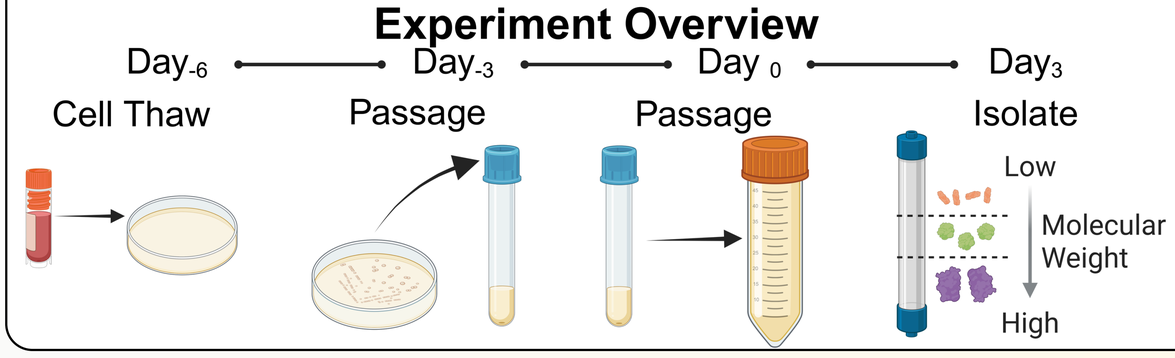
- Bacteria produce bacterial extracellular vesicles (bEVs)
- bEVs carry different cargos and enable cellular communication
- bEVs are ideal drug delivery nanocarriers
- Trillions of bEVs are needed to support clinical applications
- Bioprocesses are needed to manufacture bEVs
- Shift in bEV biogenesis based on metabolic activity
- Biocalorimetry can be used to predict cell growth & bEV biogenesis

Hypothesis

We hypothesize that bEV characteristics can be optimized or drug delivery by controlling culture parameters including carbon source, oxygen saturation, pH, and temperature.

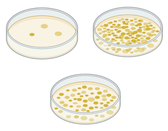
Methods

Experiment Overview

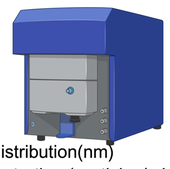


Analysis

Colony Forming Unit (CFU) Analysis



Nanoparticle Tracking Analysis (NTA)



Evaluate:
- Size distribution(nm)
- Concentration (particles/mL)
- Zeta Potential (mV)

Condition	Parameter
Temperature	30°C, 37°C
pH level	4.5, 5.5, 6.5, 7.5
Carbon Source	Galactose, Glucose, Fructose, Lactose, Maltose, Sucrose

Results

Biocalorimetry predicts cell growth & bEV production

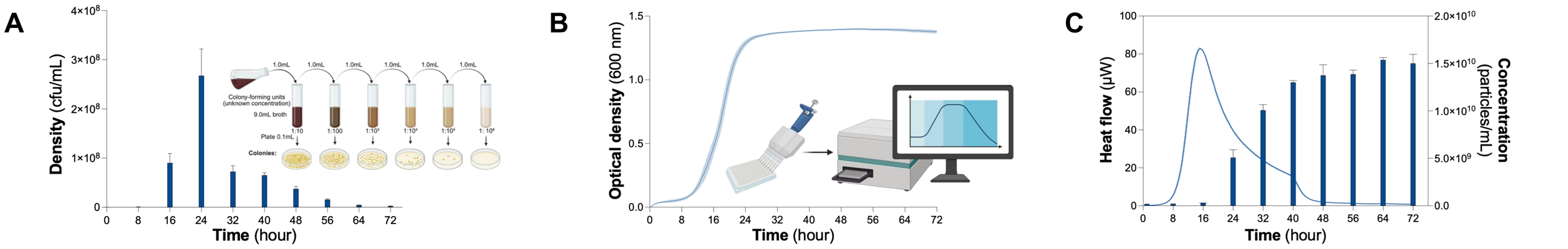


Figure 1. Cell growth profiles modeled via (**A**) colony forming unit analysis (CFU), (**B**) optical density measurements (OD_{600}), and (**C**) biocalorimetry**.** (n = 4)

Culture parameters affect bEV characteristics

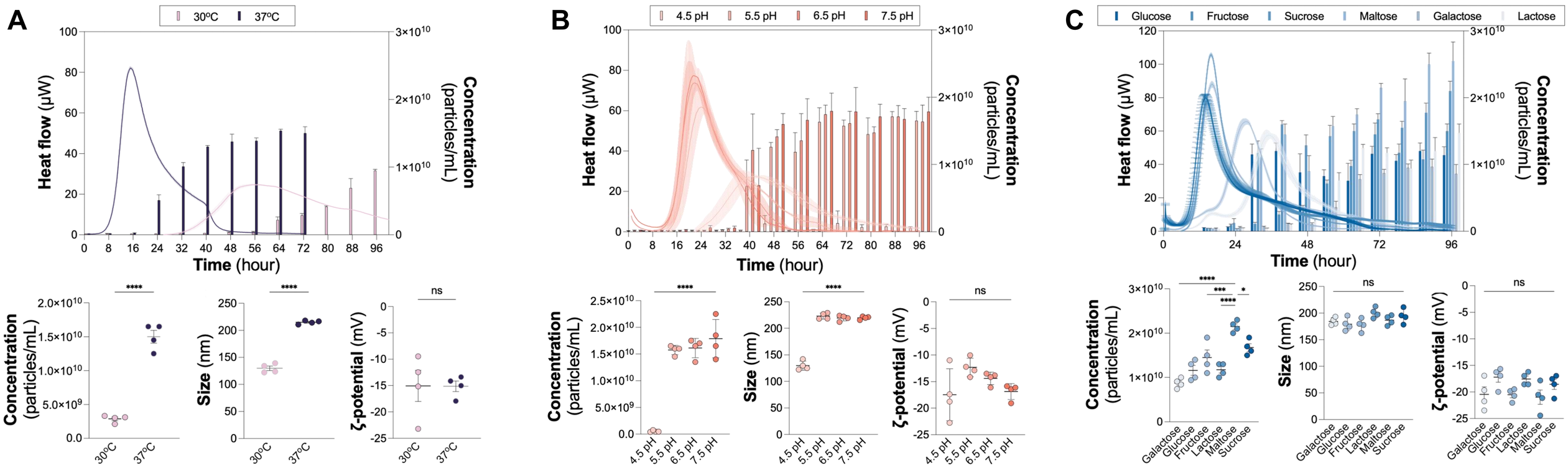


Figure 2. Heat flow profiles and characterization of bEVs isolated from *L. crispatus* different (**A**) temperatures, (**B**) pH levels, and (**C**) carbon sources**.** (n = 4)

Summary

✓Bioprocesses are required to manufacture bEVs for therapeutic applications & clinical translation.

✓We demonstrate biocalorimetry as a tool to predict both cell growth & bEV production.

✓We examine the effects of various culture parameters on bEV physical characteristics.

➔Future work will utilize biocalorimetry to identify optimal culture parameters for bEV production.

References
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2. Moore, Petersen, Zierden. *Nanoscale*. 2024
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4. Steinman, Kirian, Zierden, accepted *Methods in Mol Biol*. 2024
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