# Therapeutic potential of biofilm-derived bacterial extracellular vesicles

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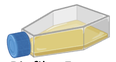
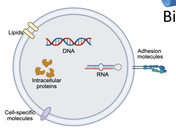
## Background

- Inflammatory bowel disease (IBD) affects millions of people in the U.S.
- Symptoms of IBD are caused by the immune system in response to dysbiosis in the gut, genetic predisposition, and/or environmental factors
- Live probiotic strains have shown promise as a treatment in reducing inflammation, but suffer from poor recolonization efficiency and viability in the gut
- Bacteria-derived extracellular vesicles (bEVs) are natural lipid bound nanoparticles containing a variety of cargo including proteins, lipids, and nucleic acids, and do not possess the same limitations as their respective host bacteria
- The application of live probiotics has demonstrated higher clinical potency when grown as a biofilm as opposed to a typical planktonic state


Planktonic Form


Biofilm Form



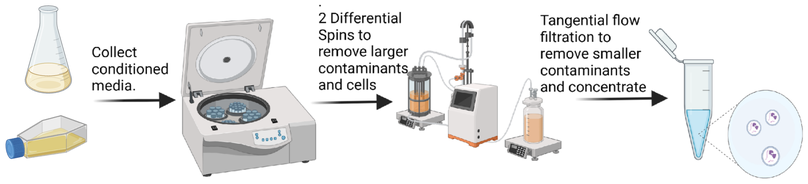
Bacteria-derived Extracellular Vesicle (**bEV**)

Despite their benefits, bEVs struggle in terms of offering the same anti-inflammatory potency as their live probiotic counterparts

To combat this, we aim to explore the potential of bEVs derived from biofilm-grown probiotic strains as a novel treatment for the symptoms of IBD

## Methods

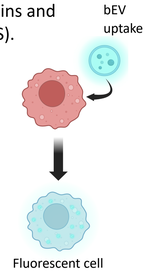
### Probiotic Culture and bEV Isolation




*Limosilactobacillus reuteri* and *Lactiplantibacillus planterum*, probiotic bacteria endogenous to the gut, were cultured in planktonic and biofilm form, under aerobic and anaerobic conditions. Vesicles were isolated using a series of differential spins to remove cell debris and other unwanted particles, followed by tangential flow filtration to separate bEVs from free proteins and other unwanted secretions, as well as to transfer bEVs into phosphate-buffered saline (PBS).

### Validation of bEV Uptake by Mammalian Cells

Planktonic and biofilm-derived bEVs were dyed with SYTO 41 nucleic acid dye and placed in solution with RAW264.7, HT29-MTX, and Caco2 cell lines. Each cell line was exposed to $5 \times 10^9$ bEVs/mL for 24 hours. Flow cytometry was then used to measure cell fluorescence in order to confirm successful uptake.

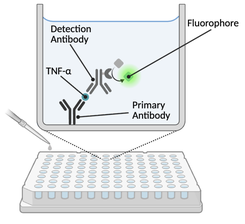



### Inducing Macrophage Inflammatory Response

RAW264.7 cells were grown in a 48-well plate and were treated with $5 \times 10^9$ planktonic and biofilm-derived bEVs/mL for 18-24 hours. Macrophages were then exposed to lipopolysaccharide for 4 hours, and supernatants were collected.

### Anti-inflammatory Quantificiation

Macrophage supernatant samples were placed in a 96-well plate and analyzed using ELISA. TNF-α levels were read at a wavelength of 450nm, as well as 570nm for normalization.



96-well plate setup used in ELISA

## Results

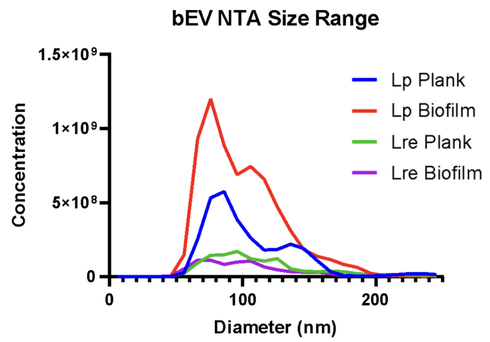



**Figure 1. Sample of NTA results from isolated bEVs.** NTA was utilized to image vesicles acquired from both *L. reuteri* and *L. planterum*. The size profiles of these particles were consistent between the two species, as well as between planktonic and biofilm conditions. Concentration values were later used to treat macrophages with equal amounts of particles from each experimental group.

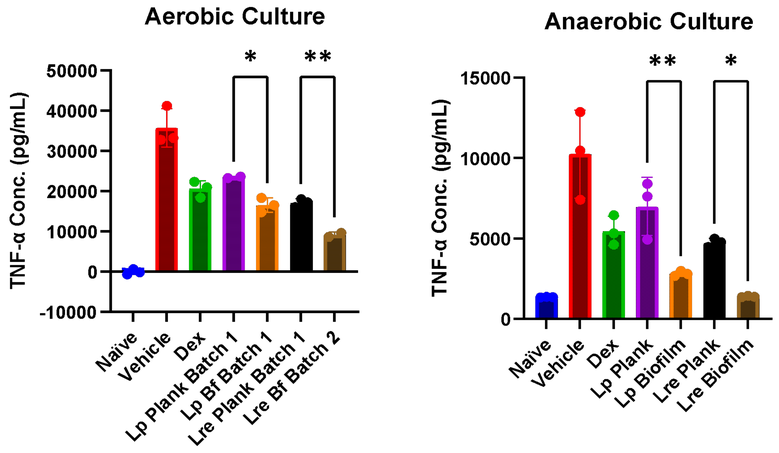



**Figure 3. Biofilm-derived bEVs significantly reduce TNF-α levels.** Concentrations of TNF-α were measured for macrophages exposed to various treatments. Groups include negative control (Naive), vehicle control treated with PBS (Vehicle), positive control treated with 1.0 µg/mL Dexamethasone (Dex), and experimental groups treated with $5 \times 10^9$ particles of their respective vesicle type, under both aerobic and anaerobic conditions. Macrophages treated with vesicles derived from both biofilm-grown *L. reuteri and L. planterum* produced fewer concentrations of inflammatory cytokine TNF-α, compared to their traditionally planktonic-grown counterparts. These results were found to be significant in both groups using one-way ANOVA. Data are expressed as mean +/- standard deviation. (* $p < 0.05$; ** $p < 0.01$)

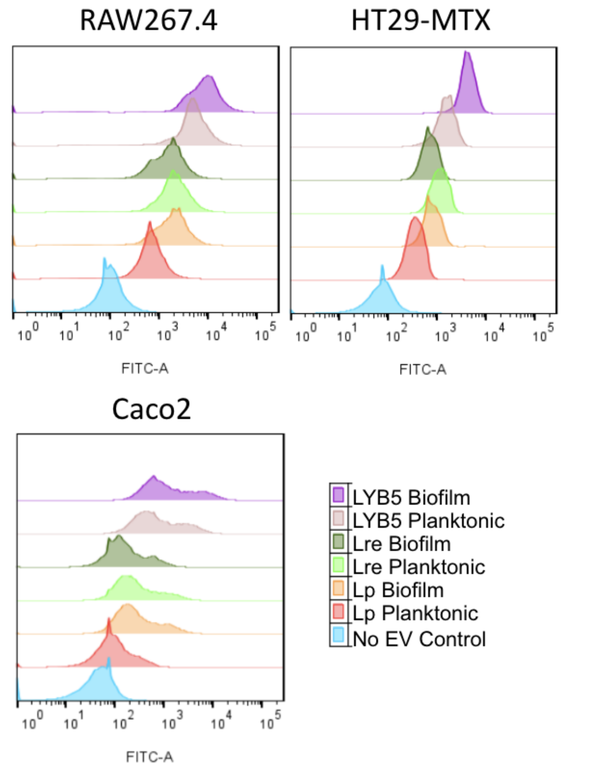


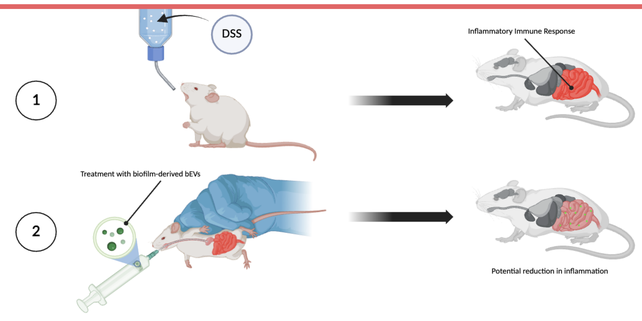

**Figure 2. Validation of bEV uptake by mammalian cell lines.** RAW264.7, HT29-MTX, and Caco2 cell lines treated with SYTO 41-dyed bEVs were analyzed by flow cytometry. Cells demonstrated higher levels of fluorescence when exposed to both planktonic and biofilm-derived bEVs, indicating successful uptake and delivery of target molecules.

## Conclusions

- BEVs produced by *L. reuteri* and *L. planterum* have comparable size profiles
- Mammalian cells successfully take up bEVs and demonstrate presence of contents
- Macrophages treated with biofilm-derived bEVs demonstrated significantly lower inflammatory cytokine concentrations compared to macrophages treated with planktonic bEVs
- Biofilm *L. reuteri*-derived bEVs consistently demonstrated higher anti-inflammatory potency in contrast to biofilm *L. planterum*-derived bEVs and even the 1.0 µg/mL Dexamethasone positive control
- Biofilm-derived bEVs acquired from anaerobic cultures demonstrated lower inflammatory response in macrophages as opposed to those acquired from aerobic cultures

## Future Directions

- Comparing efficacy of biofilm-derived bEVs to other popular treaments for IBD
- Increasing the efficiency and cost-effectiveness of biofilm-derived bEV production
- Genetic engineering of host bacteria to produce more vesicles and/or more potent bEVs
- Animal testing of biofilm-derived bEVs in mouse models with dextran sulfate sodium (DSS)-induced colitis with analysis of body weight, colon length, and gut microbiome




## Acknowledgements

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