Development of Probiotic Nanovesicles for Oral Delivery in Inflammatory Bowel Disease Treatment

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Introduction

Root cause of inflammatory bowel disease (IBD) remains unidentified, but it is strongly linked to loss of gut microbial diversity. Probiotics have proven therapeutics effects against GI tract infections [1]. Extracellular vesicles (EVs) of probiotics being a novel field of study and are investigated to be a safer replacement for clinical application [2].

Compared to naturally secreted EVs, engineered EVs offer higher yields and allow for more precise control over their concentration.

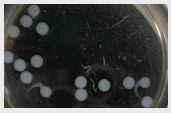
Objectives

To enhance EV survival rate in GI tract, and achieve target delivery in the colon

→ Encapsulate EVs in a pH sensitive hydrogel using 3D printing protocol. Explore different ink formulations and optimize 3D printing parameters.

To Investigate the therapeutic efficacy of EVs

→ Feed EV drug formulations via oral gavage in an in vivo IBD model



3D printed beads

In vivo oral gavage

Methodology

In vitro

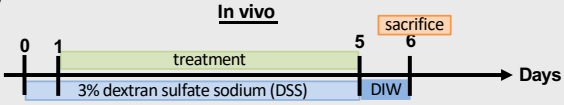
BIOX6 bioprinter with syringe pump printhead

3D printing using CELLINK BIOX6

Ink preparation:
1-5% sodium alginate (AL), 1-3% methyl cellulose (MC), crosslinking for 10min in 0.05M $CaCl_2$

Characterisation:
Rheological properties, SEM imaging, FTIR spectra, EV release profile

In vivo



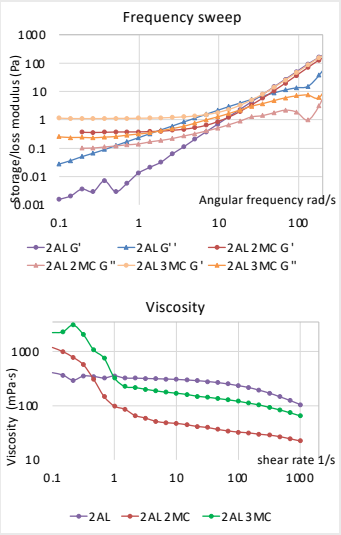
Male C57BL/6J mice, aged 8–10 weeks. 5 experimental groups (DIW, DSS, MEV, MEV beads, Alginate beads)

Daily Monitoring: Stool collection and weight measurement for Disease Activity Score (DAI).

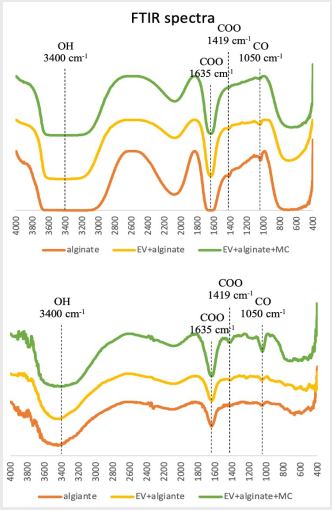
Post-Sacrifice Analysis: H&E staining and qPCR of colon tissues.

Results & Discussion

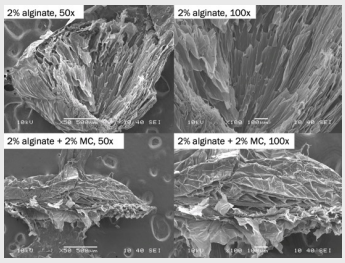
In vitro



Rheological testing shows AL and MC exhibit shear-thinning effects, with MC enhancing gel-like properties and viscosity at low shear rates for easier printing.

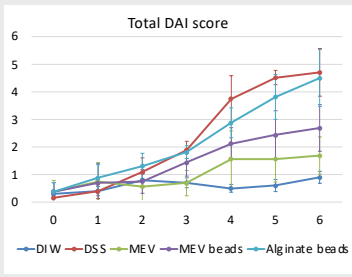


FTIR shows no new chemical bonds with AL and MC addition.



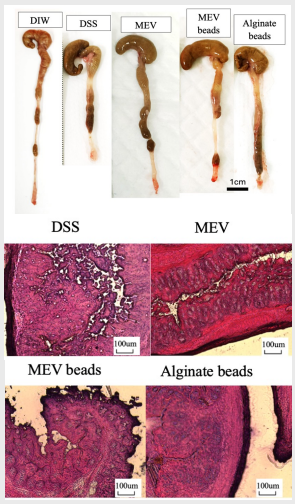
SEM shows MC results in a more porous and disordered amorphous structure.

In vivo

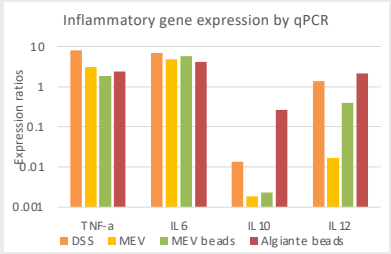


Score	Weight Loss	Stool Consistency	Blood in Stool
0	< 5%	Firm and normal	No visible blood
1	5%–10%	Slightly softer	No visible blood but detectable on screen
2	10%–15%	Very soft but maintain a shape	Slightly visible blood, obvious on screen
3	> 15%	Liquid-like stool	Obvious visible blood

DAI indicates that MEV treatment significantly suppressed colitis inflammation, while MEV beads showed lower efficacy.



MEV group displays the healthiest colon tissue with minimal fibrosis and clearly defined mucosal layers and distinct microvilli structure, consistent with the DAI results.



EV treatments significantly suppressed the expression of the proinflammatory gene IL-12, a key pathway targeted in IBD treatment [3], but did not affect the expression of other proinflammatory genes, IL-6 and TNF-α.

Conclusion

1. Methyl cellulose improved bioink printability but reduced structural strength and crosslinking efficiency.
2. EV treatment suppressed colitis symptoms, with liquid EVs showing the best efficacy, while encapsulating EVs in alginate beads were less effective due to administration challenges and partial EV release.

References
[1] A. Bezkorovainy, "Probiotics: determinants of survival and growth in the gut," Am. J. Clin. Nutr., vol. 73, no. 2 Suppl, pp. 399S-405S, Feb. 2001, doi: 10.1093/ajcn/73.2.399s.
[2] M. Morishita, M. Horita, A. Higuchi, M. Marui, H. Katsumi, and A. Yamamoto, "Characterizing Different Probiotic-Derived Extracellular Vesicles as a Novel Adjuvant for Immunotherapy," Mol. Pharm., vol. 18, no. 3, pp.
[3] Z. Tian, Q. Zhao, and X. Teng, "Anti-IL23/12 agents and JAK inhibitors for inflammatory bowel disease," Front. Immunol., vol. 15, p. 1393463, Jul. 2024, doi: 10.3389/fimmu.2024.1393463.