Shaping extracellular vesicle fate through functional protein corona formation for liver fibrosis treatment

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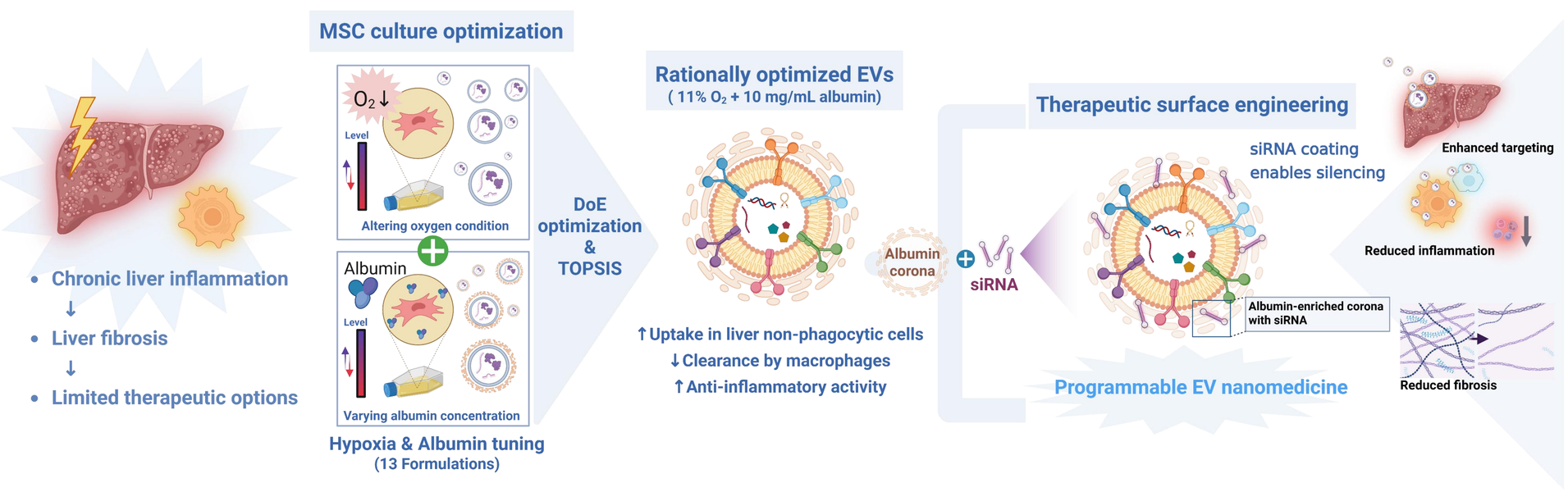


Engineering EV Fate Through Protein Corona Design

INTRODUCTION

Liver fibrosis problem

- Progressive inflammatory disease
- No approved anti-fibrotic therapy.
- MSC-EVs are promising but rapidly cleared.

Previous finding

Albumin corona improved liver targeting and retention (1).

Current hypothesis

Optimized corona engineering can enhance EV functionality and enable therapeutic surface loading.

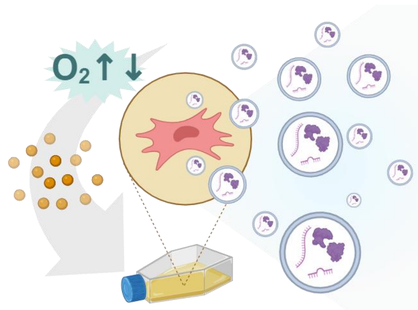
Methods

STEP 1: Generation of corona-functionalized EV libraries

MSC-EVs were generated under varying:

- Oxygen tensions
- Albumin types (BSA VS lipid-rich BSA)
- Albumin concentrations

26 EV formulations generated using DoE



STEP 2: Multi-parametric EV evaluation

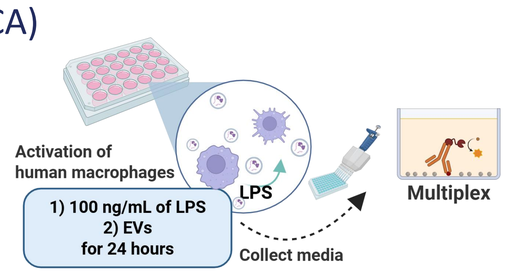
A. Physicochemical characterization

- Size & concentration > Nanoparticle tracking analysis (NTA) > Yield
- Protein quantification > MicroBCA
- Protein per particle (NTA + MicroBCA)

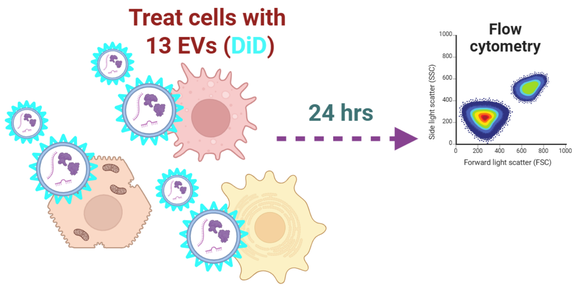
B. Anti-inflammatory assessment

- TNF-α
- IL-6
- IL-1β

Key drivers of liver fibrosis

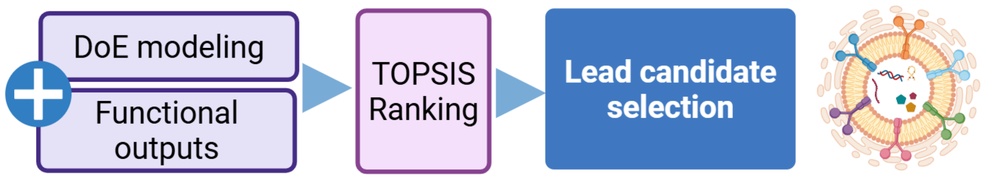


C. Cellular uptake profiling



- Macrophages (differentiated THP-1)
- Hepatocytes (HEPG2)
- Liver stellate cells (LX-2)

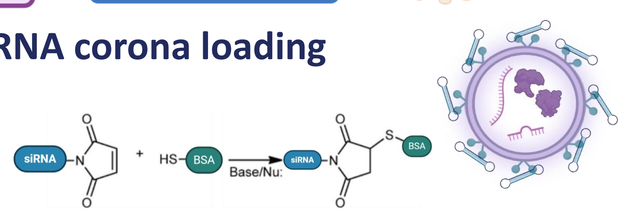
STEP 3: Multi-objective optimization and candidate selection

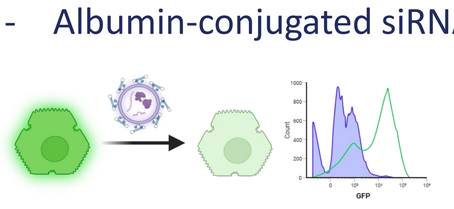


STEP 4: Modular therapeutic siRNA corona loading

A. siRNA loading

- Thiol–maleimide chemistry
- Albumin-conjugated siRNA





B. Validation

GFP silencing assay (HEPG2 EGFP) compared with LNPs and non-optimized EVs

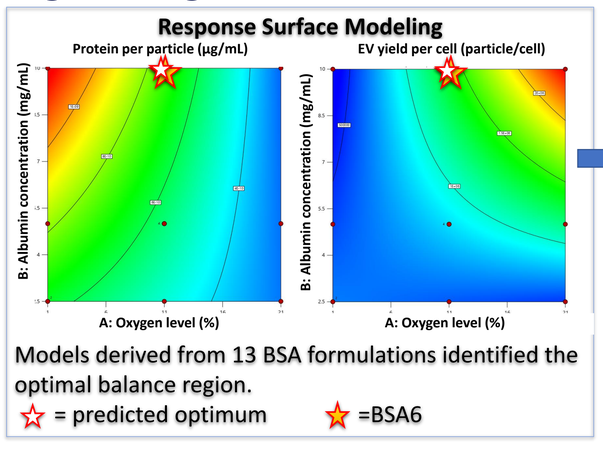
RESULTS

1) Design space engineering

Inputs
1)O_2 levels
2)Albumin type (BSA & Lipid-rich BSA)
3)Albumin concentration

26 Formulations generated (13 Formulations for each BSA type)

Only the **BSA subgroup** produced robust predictive models.



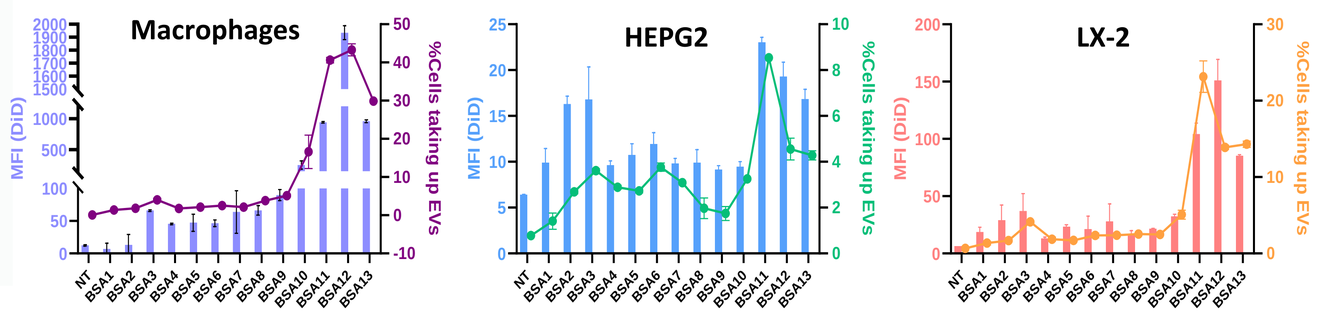
Models derived from 13 BSA formulations identified the optimal balance region.
☆ = predicted optimum ★ =BSA6

Outputs
☆ Predicted optimum (~10.6% O_2, 10 mg/mL albumin)
★ Selected lead formulation (BSA6) (~11% O2, 10 mg/mL albumin)

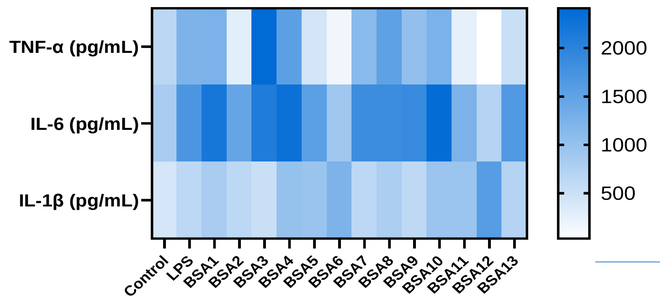
Key 1: A model-predicted optimum near 10.6% O_2 guided selection of BSA6 (11% O_2) as a practical lead formulation balancing EV productivity and corona enrichment.

2) Functional evaluation

A. Cellular uptake profile



B. Inflammatory responses mediated by EVs in activated macrophage



Key 2: EV formulations exhibited distinct uptake behaviors and inflammatory responses, supporting the need for integrated multicriteria selection.

3) Integrated candidate selection

Key 3: Integrated multicriteria analysis identified BSA6 as the lead formulation balancing EV physicochemical properties and biological functionality.

Criteria
EV yield
Albumin corona
TNF-α reduction
IL-6 reduction
IL-1β reduction
↑HEPG2 uptake
↑LX-2 uptake
↓Macrophage uptake

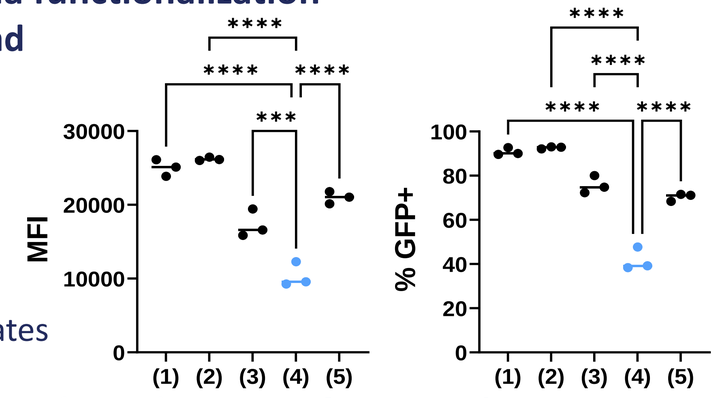
→ TOPSIS → Multi-criteria weighting → Distance-to-ideal analysis → Candidate ranking

Ranking
★ BSA6
BSA13
BSA11
BSA12
BSA3
BSA2

4) Programmable therapeutic corona functionalization

A. Functional siRNA delivery using lead EV formulation

1) Non-treated
2) Free-conjugates (BSA-siGFP)
3) LNPs encapsulated with conjugates
4) BSA6 coated with conjugates
5) Non-optimized EVs coated with conjugates



Key 4: Distinct EV corona environment (BSA6) support functional siRNA surface loading and gene silencing.

CONCLUSION & FUTURE WORK

These findings establish protein corona engineering as a rational strategy for developing tunable EV-based therapeutics for liver fibrosis. Ongoing studies aim to validate the in vivo therapeutic performance and translational potential of optimized and modularly functionalized EV systems.

ACKNOWLEDGEMENT

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REFERENCE

(1) Liam-Or, Revadee et al. "Cellular uptake and in vivo distribution of mesenchymal-stem-cell-derived extracellular vesicles are protein corona dependent." Nature nanotechnology vol. 19,6 (2024): 846-855.