A Scalable Platform for Producing and Surface-Engineering Extracellular Vesicles with Enhanced Reparative Properties

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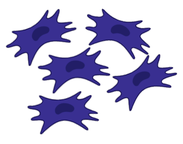
Introduction

- Extracellular vesicles (EVs) are cell-derived nanoparticles that facilitate intercellular communication through the transfer of bioactive cargo.
- Mesenchymal stromal cell (MSC)-derived EVs are widely studied for their immunomodulatory and tissue-supportive biological properties.
- Inflammation and tissue injury are common features of many conditions relevant to military health.
- Broader adoption of EV technologies is limited by challenges in scalable manufacturing, product consistency, and effective delivery to target cells.
- This study combined scalable EV production with CD47 surface engineering, a "don't eat me" signal that may enhance EV persistence and cellular uptake.

Methods

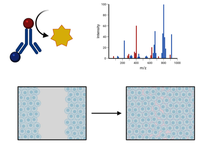
1. Cell source
2. 3-D production
3. EV isolation
4. Quality testing
5. CD47 engineering
6. In vitro repair



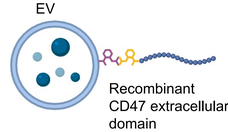
hTERT-immortalized MSCs (ATCC® SCRC-4000™)
- Extended proliferative capacity
- Lower variability

Fixed-bed bioreactor
- High density cell growth
- Real-time process monitoring

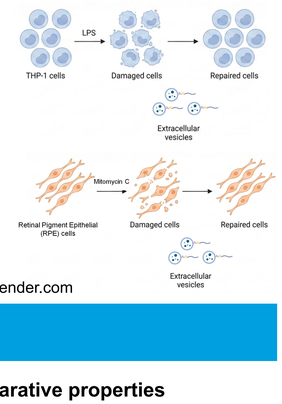
Tangential flow filtration (TFF) + ultrafiltration
- Buffer exchange & concentration
- Enrichment of EV populations



Biochemical & functional assays
- Size & yield
- Identity & sterility
- Reparative properties



Click chemistry
- Improves EV uptake and persistence
- Enhanced anti-inflammatory and reparative properties



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Results

Increased EV yield from fixed-bed bioreactor

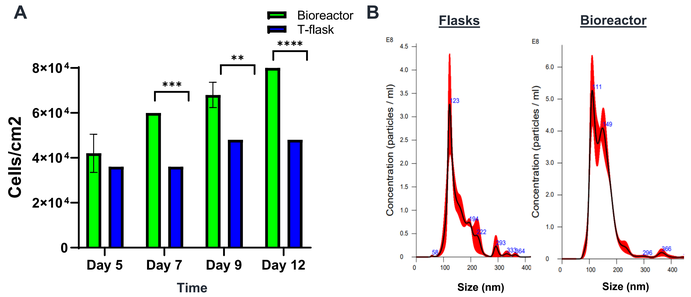

Figure 1: Enhanced yield from fixed-bed bioreactor. EVs produced from hTERT-MSCs cultured in a fixed-bed bioreactor were compared with EVs generated from a comparable static flask culture production run. (A) Cell counts were performed throughout each production to obtain cell yield over time. *n* = 2. (B) Nanoparticle tracking analysis (NTA) was performed to assess the concentration and size profiles of isolated EVs. (C) The average particles/mL and (d) average diameter between EV samples are shown. Each sample was analyzed in duplicate with three readings per replicate (*n* = 6). * = p < 0.05; ** = p < 0.01. Adapted from Cuba et al. 2026.

Enrichment of EV protein cargo

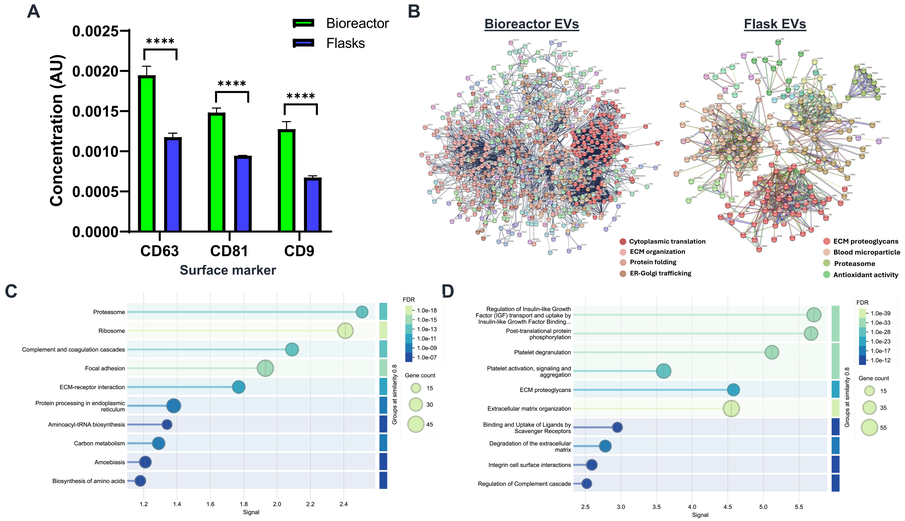

Figure 2: Characterization of EV-associated proteins in hTERT MSC EVs. (A) Multiplex immunoassay profiling of EV-associated tetraspanins in bioreactor- and flask-derived EVs. Relative EV concentrations were determined using recombinant EV calibrators and are reported as arbitrary units (AU). *n* = 3. **** = p < 0.0001. (B) STRING network analysis identified distinct protein interaction networks in bioreactor- and flask-derived EVs. (C-D) Reactome pathway enrichment analysis highlighted biological pathways associated with proteins detected in (C) bioreactor- and (D) flask-derived EVs. Bubble size indicates protein count, and color indicates FDR-adjusted significance. Adapted from Cuba et al., 2026.

CD47 surface engineering increases reparative properties

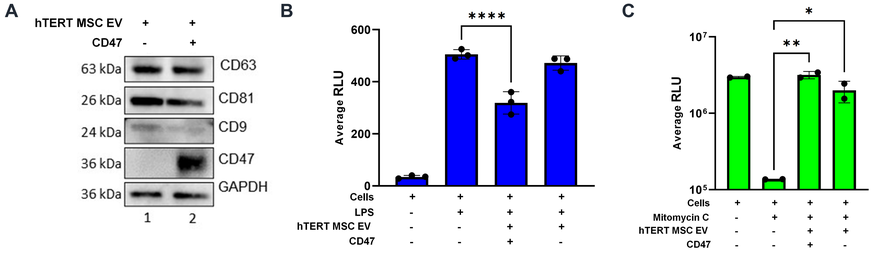

Figure 3: CD47 surface engineering of hTERT MSC EVs. hTERT-MSC EVs were modified with a recombinant human CD47 protein fragment (aa 19-139) using click chemistry and subsequently purified by size-exclusion chromatography. (A) western blot analysis confirmed expression of EV-associated tetraspanins and successful incorporation of CD47. (B) ThawReady™ THP-1 NF-kB-Luc2 monocytes (ATCC® TIB-202-NFkB-LUC2-AR™) were challenged with LPS for 3 hours followed by treatment with EVs. After 24 hours, NF-κB activation was quantified by luciferase reporter assay. *n* = 3. **** = p < 0.0001. (C) Human RPE cells (ATCC® CRL-2302™) were treated with Mitomycin C (0.5 µg/ml) for 12 hours followed by treatment with EVs. After 48 hours, cell viability was quantified by CellTiter-Glo assay (Promega). *n* = 2. * = p < 0.05; ** = p < 0.01.

Conclusions

- **Scalable production:** Fixed-bed bioreactor culture substantially increased EV yield compared to conventional flask culture.
- **Molecular integrity:** Bioreactor-derived EVs retained characteristic EV features and complex protein cargo.
- **Biological relevance:** Functional activity was maintained following scale-up and further enhanced through CD47 surface engineering.

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