Tiny Packages, Big Impact: Functional Evaluation of Lyophilized Platelet and MSC-EVs

Beklem Bostancioglu, PhD, Cellcolabs, Solna, Sweden
Şirin Korulu Koç, Tayfun Tatar, Paolo Guazzi, HansaBioMed Life Sciences, Tallinn, Estonia

Introduction

Extracellular vesicles from different sources are attracting interest for novel modalities in regenerative medicine. With their therapeutic potential, mesenchymal stem cells (MSCs) and platelets are among the most commonly studied sources of EVs for their regenerative use. Yet, among many other challenges regarding the scalability and biosafety of such approaches, the preservation of EVs is a major concern to establish standardized approaches.

In this study, Cellcolabs evaluated HansaBioMed's lyophilized EV reference materials alongside internally produced adipose MSC-derived EV preparations to assess biological activity across multiple regenerative in-vitro assays. These reference EV materials are all lyophilized by HansaBioMed for extended preservation in stable form. Across all evaluated assays, the EV preparations demonstrated measurable biological responses, including cellular uptake, wound healing and angiogenic activity.

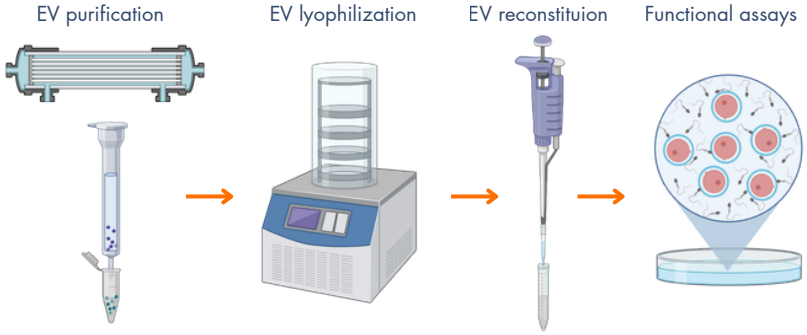

Figure 1: Representative images of the study workflow

Materials and Methods

In this study, HansaBioMed's lyophilized platelet EVs (HBM-PET-100) and adipose tissue MSC EVs (HBM-MSC-100) were utilized for functional assessment in comparison with EV samples purified and lyophilized by HansaBioMed from adipose tissue MSC expansion medium conditioned with human platelet lysate, provided by Cellcolabs. Platelet EVs were prepared using ion exchange chromatography and size exclusion chromatography (SEC) (HBM-PEV). Both MSC-EVs samples were purified with a combination of tangential flow filtration (TFF) (HBM-TFF-MV&HBM-TFF-EVs-S) and SEC.

For in-vitro functional assessment, human fibroblast cell cultures were used by Cellcolabs. Cellular uptake was performed using the Incucyte® Exofluor Green EV Labelling Kit (Sartorius) according to the manufacturer's instructions. Wound healing assays were utilized for assessing EV-mediated cell migration and wound closure. Finally, angiogenesis assays were used to evaluate endothelial tube formation as an indicator of pro-angiogenic activity.

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HansaBioMed Life Sciences
Mäealause 2/1, 12618 Tallinn, Estonia
info@hansabiomed.eu
www.hansabiomed.eu

Results

Cellular Uptake

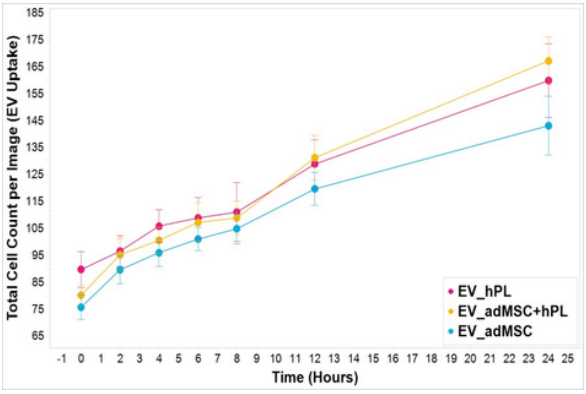

Figure 2: Comparison of cell count per image for HBM platelet EVs (EV_hPL) and MSC-EV (EV_adMSC) reference materials with EVs isolated from human platelet lysate-conditioned MSC media (EV_adMSC+hPL)

All EV preparations demonstrated successful cellular uptake, indicating active interaction with recipient cells. Observed outcomes:

- Increased intracellular fluorescence signal
- Time-dependent EV internalization
- Consistent uptake across evaluated EV preparations

Wound Healing Activity

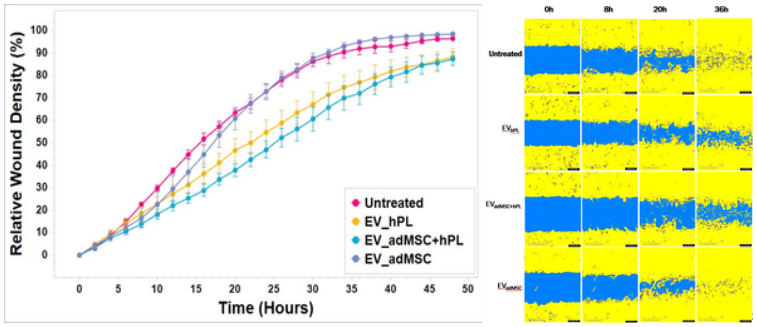

Figure 3: Comparison of relative wound densities and wound closures for HBM platelet EVs (EV_hPL) and MSC-EV (EV_adMSC) reference materials with EVs isolated from human platelet lysate-conditioned MSC media (EV_adMSC+hPL)

EV-treated groups demonstrated improved wound closure compared with untreated controls. Observed outcomes:

- Accelerated cell migration
- Improved wound closure dynamics
- Regenerative-associated cellular responses

Pro-Angiogenic Activity

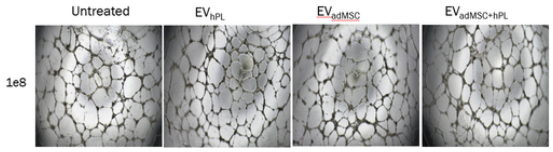

Figure 4: Microscope images showing network formation for HBM platelet EVs (EV_hPL) and MSC-EV (EV_adMSC) reference materials with EVs isolated from human platelet lysate-conditioned MSC media (EV_adMSC+hPL)

EV-treated groups promoted endothelial tube formation and vascular network development. Observed outcomes:

- Increased branching structures
- Enhanced mesh formation
- Improved organization of endothelial networks

Conclusion

Across all evaluated assays, the EV preparations demonstrated measurable biological activity, supported by efficient cellular uptake, positive effects on wound healing-associated responses, promotion of angiogenic activity, and compatibility with multiple in-vitro assay systems.

The study reveals that HBM reference EV materials are biologically active and suitable for use in functional EV research workflows for EV assay benchmarking, method validation, or cross-platform comparisons. It further underlines that HBM EV preparation workflow consisting of TFF+SEC-based EV purification followed by lyophilization preserves the functionalities of EVs.

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