Effects of Extracellular Vesicles on Skin Aging Biomarkers in a 3D Reconstructed Skin Model

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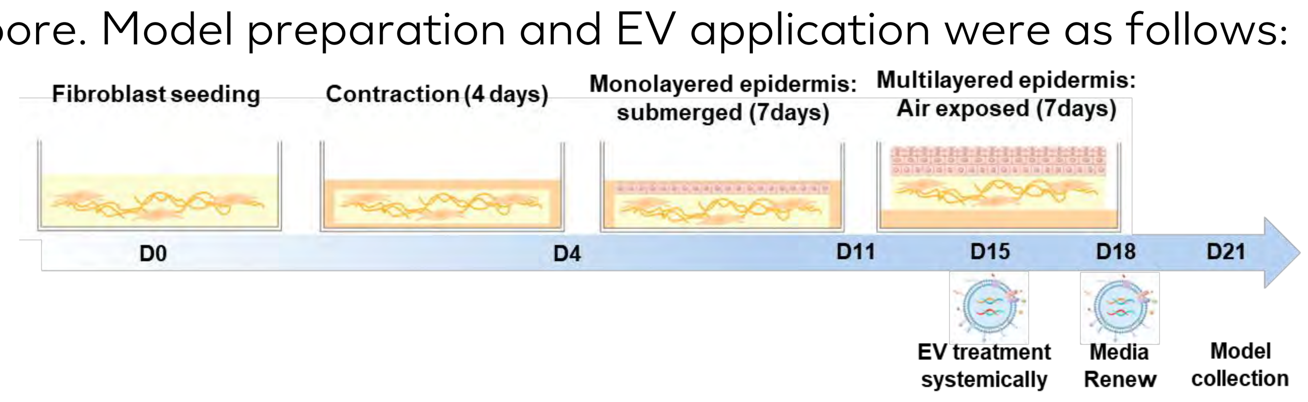
1 INTRODUCTION

Extracellular vesicles (EVs) are released by most cells and carry diverse components including proteins, nucleic acids, and lipids [1,2]. While the regenerative potential of EVs is well-documented, much of the published data demonstrating EV effectiveness are from 2D *in vitro* and animal models [3]. Reconstructed human skin models offer a more physiologically relevant 3D microenvironment compared to 2D culture, while also supporting assessment of biomarkers linked to skin aging [4,5]. In this study, 3D organotypic skin models comprising an epidermis and dermis were used to understand the potential of EVs to impact biomarkers associated with skin aging.

2 MATERIALS & METHODS

EV Materials & Characterization: Adipose-derived stem cells (ADSC) and umbilical cord mesenchymal stem cells (UC-MSC) EVs were isolated from conditioned media, then characterized for size/count by Nanoparticle Tracking Analysis (NTA, Zeta View PMX 110), and morphology by Cryo-TEM (Talos F200C G2). EV biomarkers CD63, HSP70, TSG101 and calnexin (negative control) were detected via Western blot.

Reconstructed Human Skin Models: The reconstructed skin models were produced using primary cells isolated from surgical waste with patient consent and ethical approval from the review board of the Skin Research Institute of Singapore. Model preparation and EV application were as follows:



Histology: 10mm biopsy punches were used to obtain sections for hematoxylin and eosin (H&E) and immunofluorescence (IF) staining. Briefly, H&E samples were formaldehyde-fixed and paraffin-embedded, while IF samples were O.C.T-embedded, cryo-sectioned and stained with primary antibodies (Ki67 and Collagen IV), secondary Alexa Fluor 488, and DAPI.

Transcriptomics: RNA-seq involved total RNA extraction from 5-6 replicates per condition, Illumina sequencing, EdgeR-based differentially expressed gene (DEG) identification (|fold change|>1.5, p<0.05), and pathway enrichment by ClusterProfiler.

3 RESULTS

ADSC and UC-MSC EVs possess EV characteristics and are internalized by fibroblasts

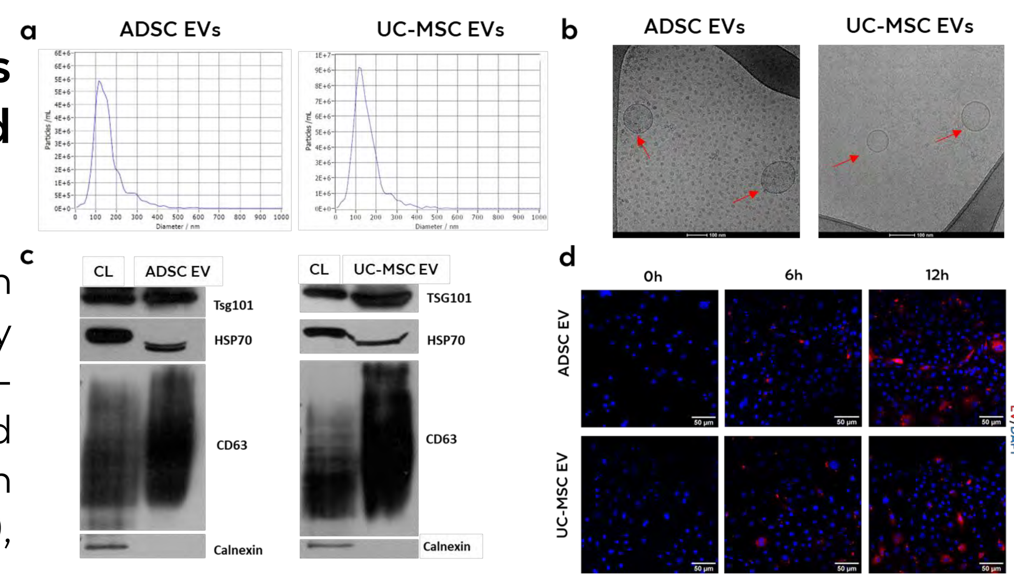


Figure 1. (a) Size and concentration measured by NTA; (b) EV morphology visualization (red arrows) with Cryo-TEM. Scale bar, 100 nm; (c) EV related biomarkers confirmed with Western blot and compared to cell lysate (CL), (d) EV uptake by human fibroblasts.

EVs impact epidermal thickening, keratinocyte proliferation, and the dermal-epidermal junction ECM in 3D reconstructed skin models

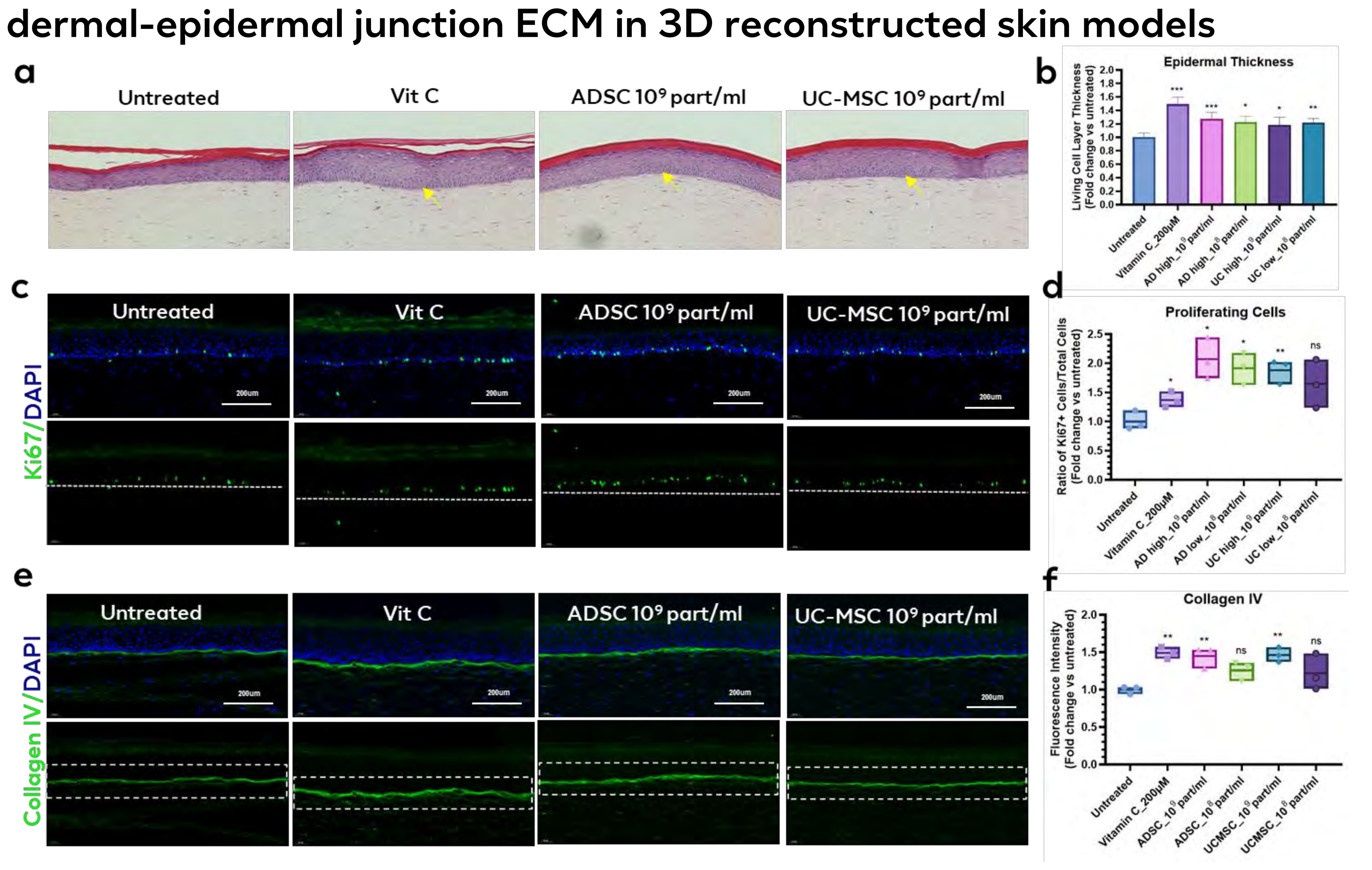


Figure 2. (a-b) H&E staining of the skin model section and the quantitative results of the thickness of the living cell layers. (c-d) IF images of Ki67-positive cells and associated image analysis. (e-f) IF images for Collagen IV in skin model section and image analysis. One-way ANOVA, *p<0.05, **p<0.01, ***p<0.001 vs Untreated.

Bulk transcriptomics of EV-treated reconstructed skin models reveal unique regulation on gene expression pathways

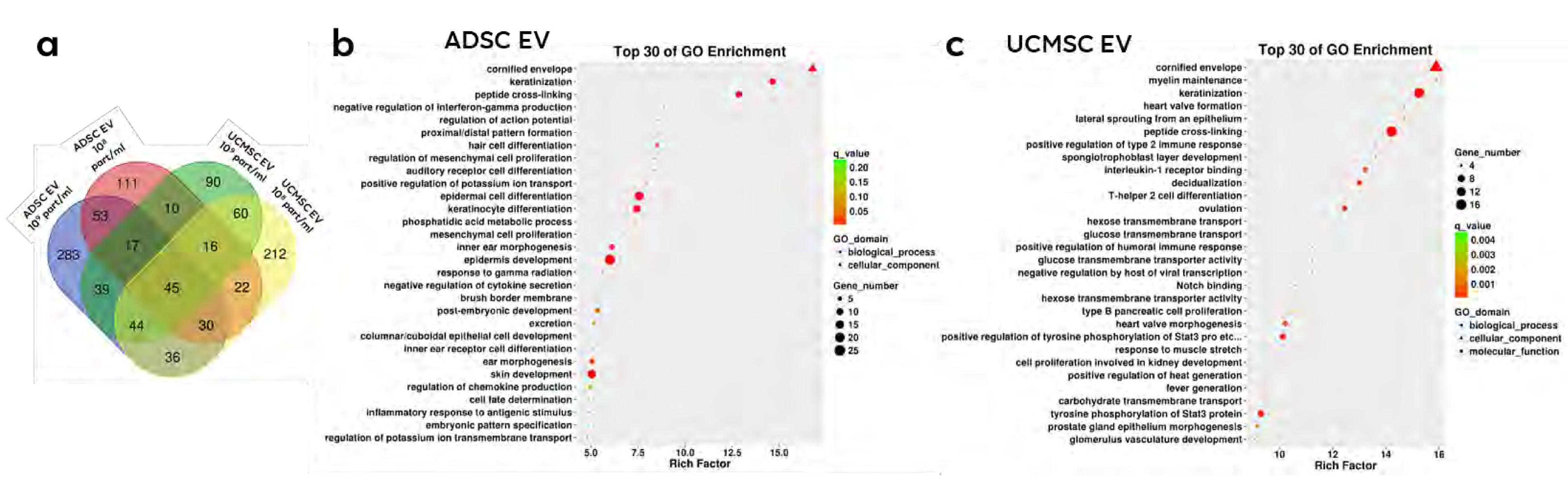


Figure 3. (a) Venn plot of the DEGs identified with application of ADSC EVs and UC-MSC EVs. (b-c) DEGs regulated by ADSC EVs and UC-MSC EVs were enriched in GO database.

4 CONCLUSION

- EVs linked to regenerative behaviors impact skin aging biomarkers in reconstructed skin models. However, gene expression analysis identifies potential differences in the signature of EVs from different sources.
- **3D reconstructed human skin models are relevant and reproducible tools to assess aging-associated biomarkers impacted by EVs.**
- EV performance is highly-dependent on many factors, including cell source, manufacturing process, and stability. Properly-controlled clinical studies are needed to confirm benefits of EVs on skin aging and rejuvenation.

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