Enhancing the storage stability and delivery of extracellular vesicles by using microneedles

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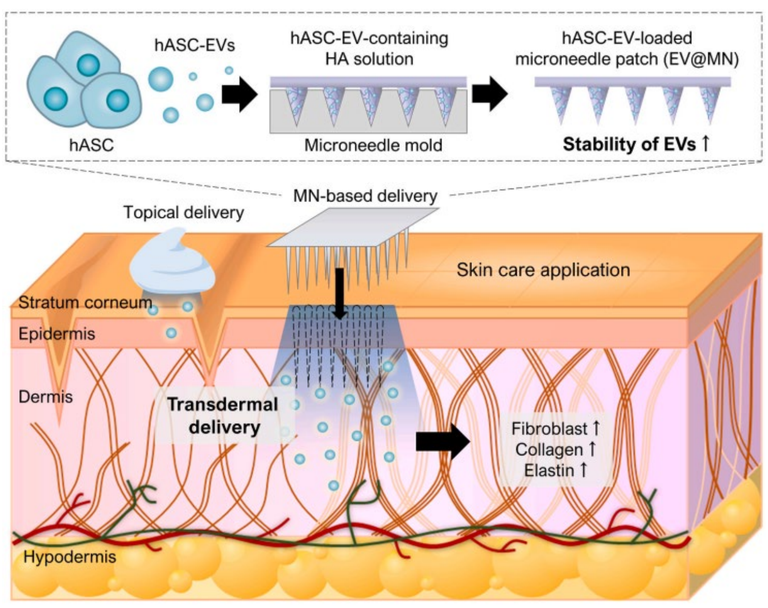
Abstract

Stem cell-derived extracellular vesicles (SC-EVs) are nano-sized vesicles that possess biological regenerative effects due to their internal contents, including protein and mRNA, originating from those found in their parent cells. While SC-EVs have extensive potential for skin care, their applications are limited because of poor long-term storage stability and insufficient delivery to the dermis layers. In this study, we have developed SC-EVs-loaded dissolving microneedles (EV_MNs) to overcome these limitations. EV_MNs maintained the stability of EVs for over six months at -20˚C and even at 4˚C, which was confirmed by a maintained number of SC-EVs, biological activity on fibroblasts, and acetylcholinesterase function. EV_MNs demonstrated prolonged EV retention at the administration site of the mouse for up to seven days, which is significantly longer than conventional intradermal injections. EV_MN-treated skin showed increased dermal thickness, elevated collagen and elastin content, and enhanced fibroblast proliferation. This approach significantly improved the effect on fibroblast function related to anti-aging applications. This study provides a promising strategy for clinical EV applications, addressing current challenges in storage and transdermal delivery.

Introduction



Scheme 1. The fabrication of EV@MN and skincare applications

Results

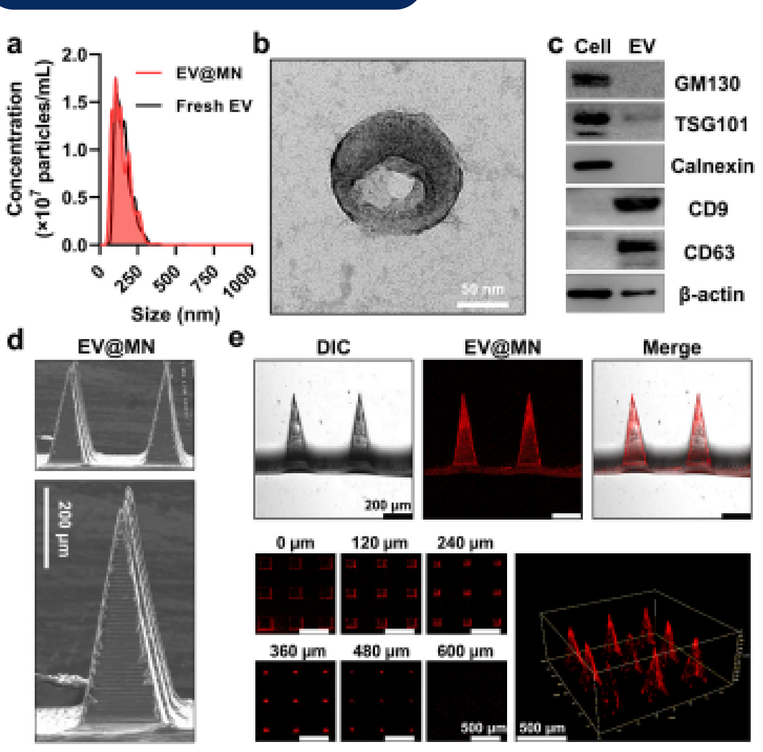


Fig. 1. Characterization of EV-loaded MN.
(a) Size distribution of fresh hASC-EVs and solution of EV@MN by NTA. (b) TEM image of hASC- EVs. (c) Biomarkers of hASC- EVs. (d) SEM images of EV@MN. (e) CLSM images of MN loaded with fluorescent hASC- EVs.

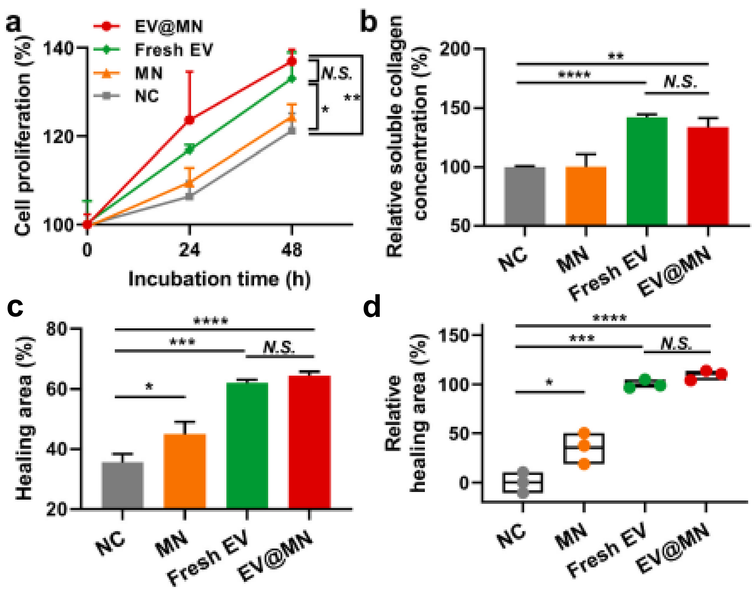


Fig. 2. Biological functions of EV@MN.
(a) Proliferation of HDFs. (b) Soluble collagen generation of HDFs at 24 h. (c) absolute or (d) relative healing area calculated from the scratch assay.

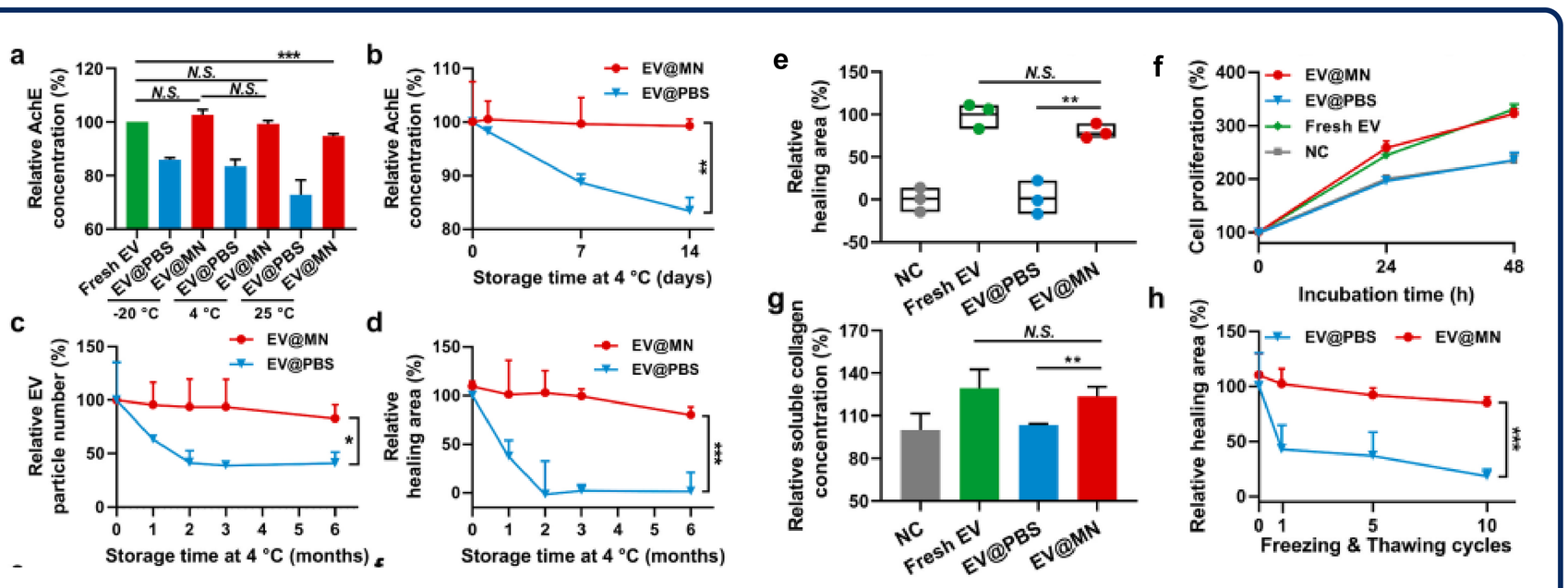


Fig. 3. Stability of EVs in EV@MN.
Acetylcholine esterase (AchE) concentration of hASC-EVs at different (a) storage conditions and (b) time points. (c) Relative hASC-EV numbers after various storage periods. (d) Relative healing area of HDFs in a scratch assay when treated with EV@MN after various storage periods. (e) Relative healing area, (f) proliferation, and (g) soluble collagen generation of HDFs treated different formulations after six months of storage. (h) Relative healing area of HDFs treated with samples which underwent several freeze-thawing cycles.

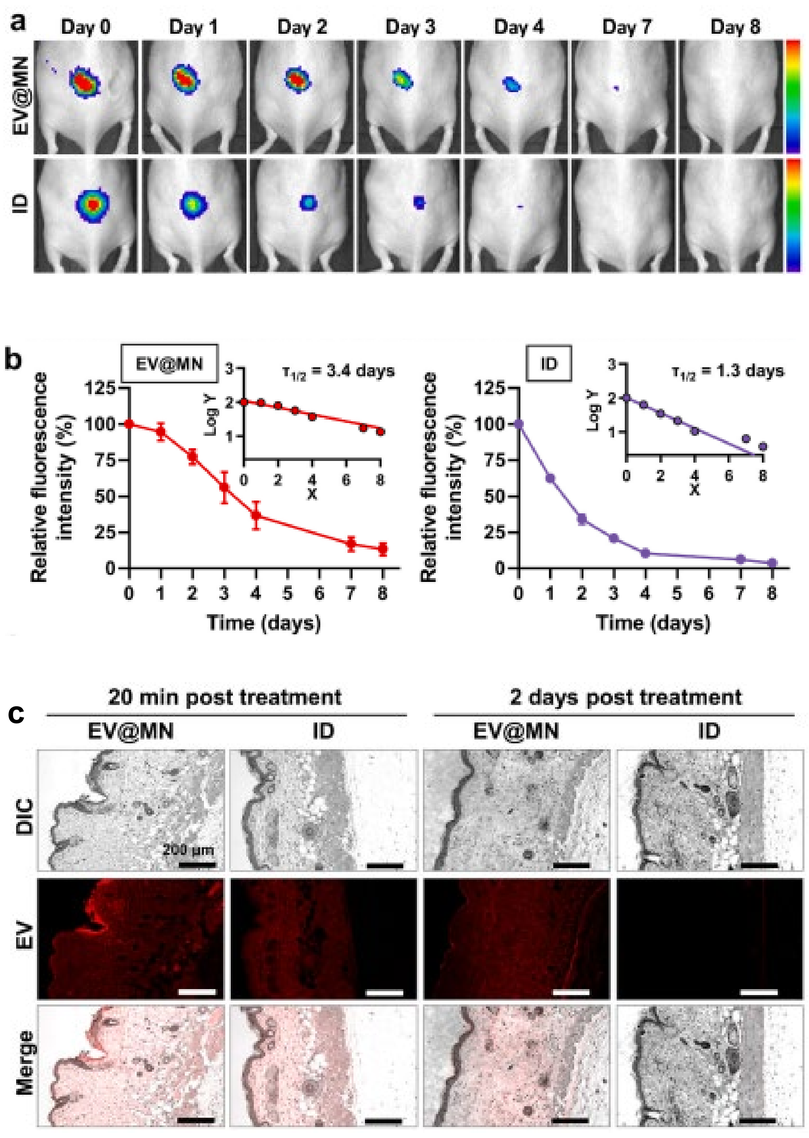


Fig. 4. *In vivo* biodistribution of hASC-EVs after EV@MN transdermal application.
(a) Time-dependent fluorescence images of SKH1 mice after different treatments. (b) Relative fluorescence intensity at the region of interest. Error bars represent the standard deviation. (c) CLSM images of skin tissue sections after different treatments. NT, non-treated; ID, intradermal injection; Topi, topical administration.

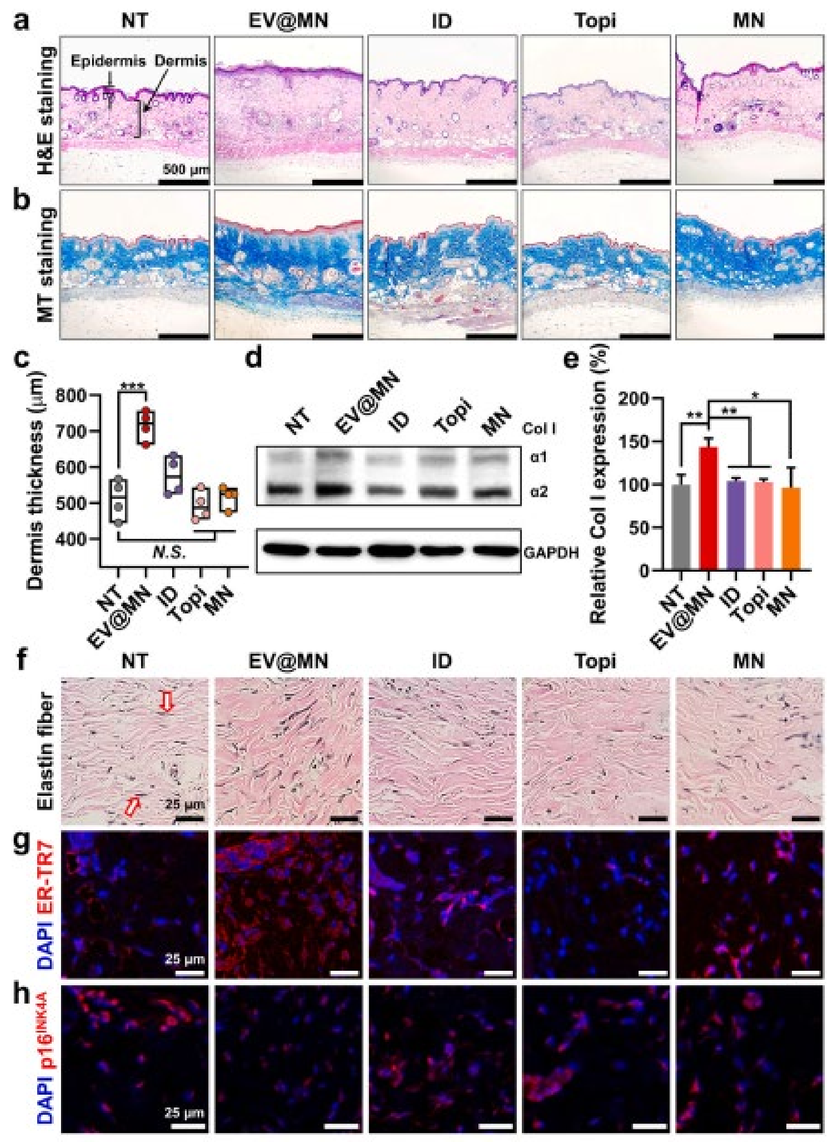


Fig. 5. BioTopical application of EV@MN.
(a) H&E and (b) Masson's trichrome staining. (c) Dermal thickness analysis of (a). (d) Western blot image and (e) quantified analysis of collagen type 1 in skin tissues. Representative images of (f) elastin fiber (arrows), (g) ER-TR7 (HDF biomarkers), and (h) $p16^{INK4A}$ (senescent biomarkers) expression in skin.

Conclusion

This study successfully made hASC-EVs loaded dissolving microneedle. The hASC-EVs' biological functions remained intact for six months at 4 ºC in microneedle. EV@MN allowed for intradermal administration, resulting in the release of hASC-EVs into the dermis. The approach significantly improved the effects of hASC-EVs, offering a solution for clinical applications while addressing storage and delivery issues. EV@MN has a lot of potential as a cell-free treatment for delivering hASC-EVs.