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LETTER TO THE EDITOR

Potential effects of HEK293 cell-derived exosomes for dermal application

Dear editor,

Exosomes are a part of endosome-originated extracellular vesicles (EVs), which have a size ranging from 40 to 160 nm and function in cell-to-cell and tissue-to-tissue signaling.[1] Although the isolation of pure exosomes has been a challenging issue until now, exosomes have been widely studied in biomedical purposes. In this context, the choice of parent cells is crucial in acquiring the intended properties of exosomes and facilitating the preparation of them. For instance, exosomes derived from mesenchymal stem cells (MSCs) are preferred due to their apparent functions, such as anti-inflammation, tissue regeneration, and antioxidant activity.[2] However, MSCs are primary cells that require specific culture conditions, and have limited proliferative capacity and functional variability between donors.[3]

HEK293 cells are immortalized human embryonic kidney cells, widely used in research due to their robust and fast-growing properties. Consequently, researchers are studying HEK293 cell-derived exosomes (H-exo) as potential drug delivery vehicles through the circulatory system.[4] However, the activity of H-exo itself has received limited attention. In this letter, we present preliminary results of H-exo activity on human dermal fibroblast (HDF) cells, aiming to provide insights for researchers considering exosome-based dermatological treatments.

We cultured HEK293 cells in 10 L with 1% FBS, and exosomes were isolated using a two-step microfiltration (MW 10 K ~ 100 K) and ultracentrifugation (100 000 × *g*, 1 h). Figure S1 provides details on the basic properties of the exosomes. To determine the particle size distribution and concentration of the final isolate, we performed nanoparticle tracking analysis (NTA). The average size and concentration were found to be 165.1 ± 66.8 nm and $5.48 \times 109 \pm 5.4^{9} \times 10^{8}$/mL, respectively (Figure S1A). Transmission electron microscopy (TEM) was used to observe the morphology of the exosomes. The TEM image supported the particle size determined by NTA analysis (Figure S1B). Furthermore, Western blot analysis targeting exosome-specific biomarkers such as Alix, CD63, CD9, and TSG101 confirmed the enrichment of exosomes in the final isolates (Figure S1C).

The isolated H-exo were applied to HDF cells to evaluate their effect on cell proliferation, irritation protection, anti-inflammation, and aging-cell specific uptake efficiency. For comparison, we also tested the effects of soybean extract, which has been reported to have antiaging, skin hydration, and anti-inflammation properties.[5] In this experiment, SeleMix-AT™ (Genomine, Co., Pohang, Republic of Korea) was used as a material for soybean extract sample. SeleMix-AT™ is a commercially produced cosmetic active ingredient in the company (http://genomine.com/en/products_en/cosmeticmaterials_en/selemixat_en.html).

Figure 1A demonstrates that HDF cell proliferation significantly increased to 127% of control cells when exposed to 1% H-exo. The control cells were not treated either 1% H-exo or SeleMix-AT™. In comparison, HDF cells exposed to 1% SeleMix-AT™ exhibited a proliferation of 112% of control cells. These results indicate that H-exo treatment effectively induced dermal cell proliferation comparable to the soybean extracts.

In Figure 1B, the cell protective impact of H-exo was evaluated following treatment with 0.01% SDS to HDF cells. Upon exposure to 0.01% SDS, the application of 1% H-exo resulted in a significant increase of cell proliferation compared to the condition without 1% H-exo (control cells), reaching up to 122% of the viability observed in control cells. Conversely, 1% SeleMix-AT™ did not demonstrate significant protection activity.

To assess the anti-inflammatory effect of H-exo, we analyzed the level of prostaglandin E2 (PGE2) released from HDF cells following H-exo treatment, as PGE2 serves an indicator of inflammatory response.[6] Soybean extracts were known to possess anti-inflammatory effects, inhibiting PGE2 production in Raw 264.7 cells.[7] As depicted in Figure 1C, treatment with 1% H-exo significantly reduced the PGE2 level in culture media of HDF cells, similar to the effect observed with 1% SeleMix-AT™. This result suggests that H-exo may exhibit anti-inflammatory activity in dermal treatments, akin to soybean extract.

Furthermore, we conducted a comparison of H-exo uptake efficiency between young and senescent cells (passage 14 vs. 54). As illustrated in Figure 1D, the fluorescence intensity from DiR-labeled H-exo was notably enhanced in senescent cells, and this difference was statistically significant.

In conclusion, H-exo demonstrated effective activities for dermatological treatment, including cell proliferation, protection from skin irritation, and anti-inflammation. Moreover, the enhanced uptake of H-exo into senescent cells suggests its potential as an

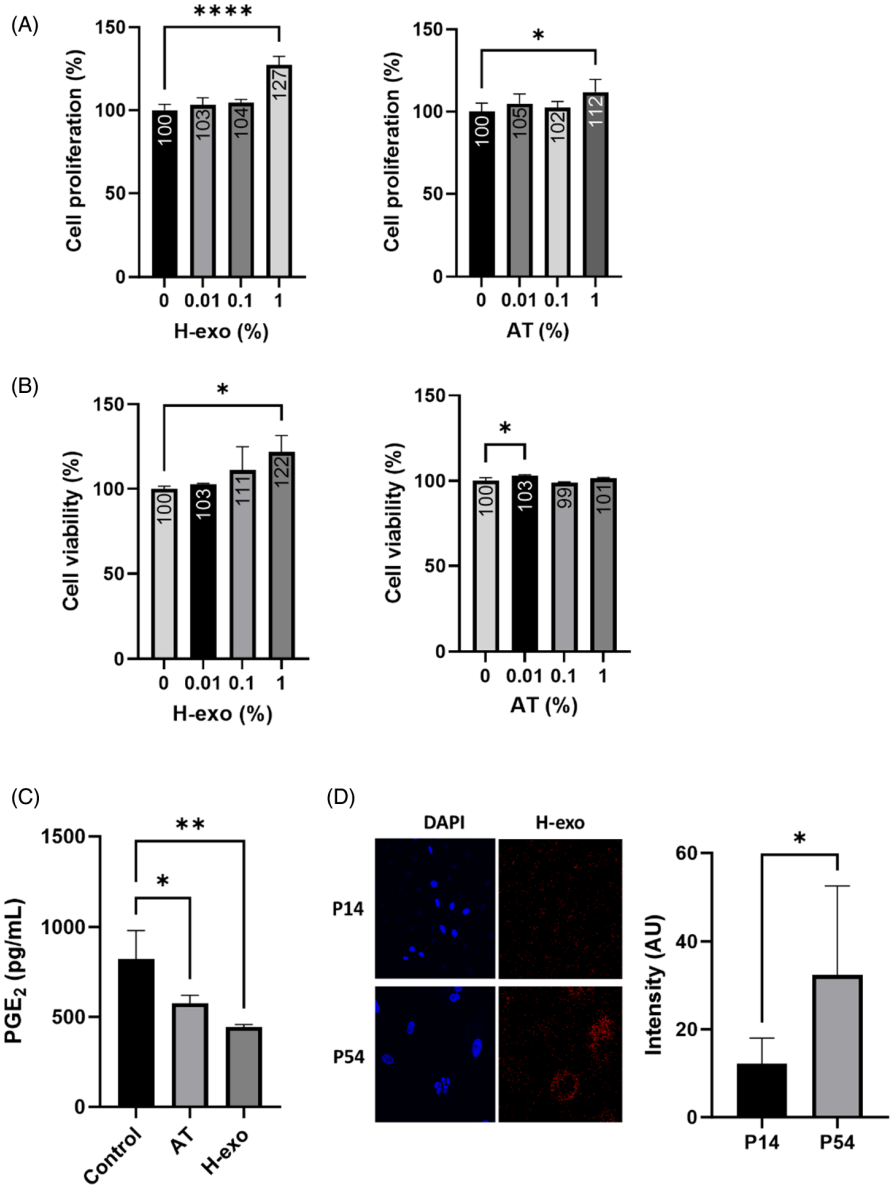

FIGURE 1 The activity of HEK293-derived exosomes (H-exo) compared to commercialized soybean extract used for a cosmetic ingredient, SeleMix-AT™. (A) Effect of H-exo and AT (SeleMix-AT™) on human dermal fibroblast (HDF) cell proliferation. (B) Protection effect of H-exo and AT on irritated HDF cells by 0.01% SDS treatment. Changes in cell viability were measured using CCK-8 assay. (C) Prostaglandin E2 (PGE2) expression level in the culture media was measured by ELISA, following the treatment of 1% H-exo or 1% AT to HDF cells. (D) Uptake efficiency of DiR-labeled H-exo into young (passage 14) and senescent (passage 54) HDF cells were measured by confocal microscope (FV1200, Shinjuku, Japan). The intensity of DiR fluorescence was quantified using ImageJ software (http://imagej.nih.gov/ij, Java 1.8.0_345). Statistical significance was determined by one-way ANOVA using Prism (ver. 10.0.0, GraphPad Software). $^{*}p<0.05$; $^{**}p<0.01$; $^{****}p<0.0001$.

efficient delivery system for antiaging active ingredients following encapsulation. The affordability and well-regulated culture techniques associated with HEK293 cells enhance the advantages of utilizing H-exo in dermal application, particularly in comparison to alternative parent cells like primary cells (e.g., MSCs).

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CONFLICT OF INTEREST STATEMENT

The authors have no conflict of interest to declare.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

ETHICS STATEMENT

The authors confirm that the ethical policies of the journal, as noted on the journal's author guidelines page, have been adhered to. No ethical approval was required as human-or animal-derived samples are not used in this study.

Jung-Hee Kim PhD
Eun-Sook Choi PhD
Eunjoo Kim PhD

Division of Electronics and Information System Research, Daegu Gyeongbuk Institute of Science and Technology (DGIST), Daegu, Korea

Correspondence
Eunjoo Kim, Division of Electronics and Information System Research, Daegu Gyeongbuk Institute of Science and Technology (DGIST), Techno-jungangdaero 333, Dalsung-gun, Daegu 42988, Korea.
Email: ejkim@dgist.ac.kr

ORCID

Eunjoo Kim https://orcid.org/0000-0001-5328-1182

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