IFSCC 2025 full paper (IFSCC2025-1044)

"The role of miRNA167 in skin improvement : Insight from extracellular vesicles derived from Rock Samphire (Crithmum maritimum)"

Soll Jin *[1], ChangHoe Ku[2], Hye Jin Kim [1], Jae-Goo Kim [1], Hee Cheol Kang [1], Jae Sung Hwang[2] and Mi Jung Kim [1]

[1]Human & Microbiome Communicating Laboratory, GFC Life Sciences Co., Ltd, Hwaseong 18471, Republic of Korea; [2]Department of Genetics & Biotechnology, Graduated School of Biotechnology, College of Life Sciences, Kyung Hee University, Yongin 17104, Republic of Korea

1. Introduction

Halophytes like Rock Samphires (*Critimum matrimum*) thrives in saline environments and are rich in bioactive compounds beneficial for skin health [1,2]. Recently, extracellular vesicles (EVs) derived from plants have emerged as carriers of bioactive molecules, including microRNAs (miRNAs), with therapeutic potential [3,5]. Among these, miR167 is notable for its role in regulating plant development and stress responses [6-10]. This study explores miR167 from C. *maritimum*-derived EVs (Cm-callus EVs) and its effects on skin regeneration and wound healing in human skin fibroblasts.

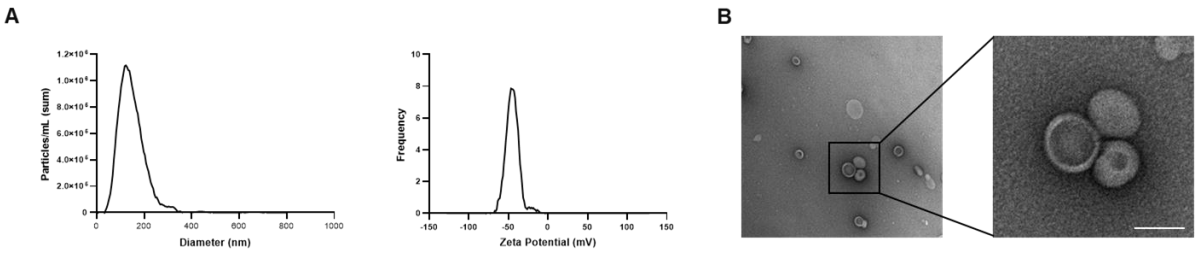
2. Materials and Methods

C. *maritimum* callus was induced and cultured in a bioreactor for EV isolation. Cm-callus EVs were isolated through filtering and ultracentrifugation, and characterized via nanoparticle tracking analysis (NTA), zeta potential, and transmission electron microscopy (TEM). Their effects on human foreskin fibroblasts (HFF) were evaluated using cell viability, wound healing assays, qRT-PCR, and immunofluorescence (IF). Small RNA sequencing identified miRNA content, and miR167 was further analyzed using mimic transfection and bioinformatics tools to predict target genes.

3. Results

3.1. Characterization of Extracellular Vesicles (EVs) from Callus of C. maritimum

Cm-callus EVs exhibited a size of 136.6 nm and a zeta potential of -44.98 mV, indicating stability and suitability for transdermal delivery (Figure 1).

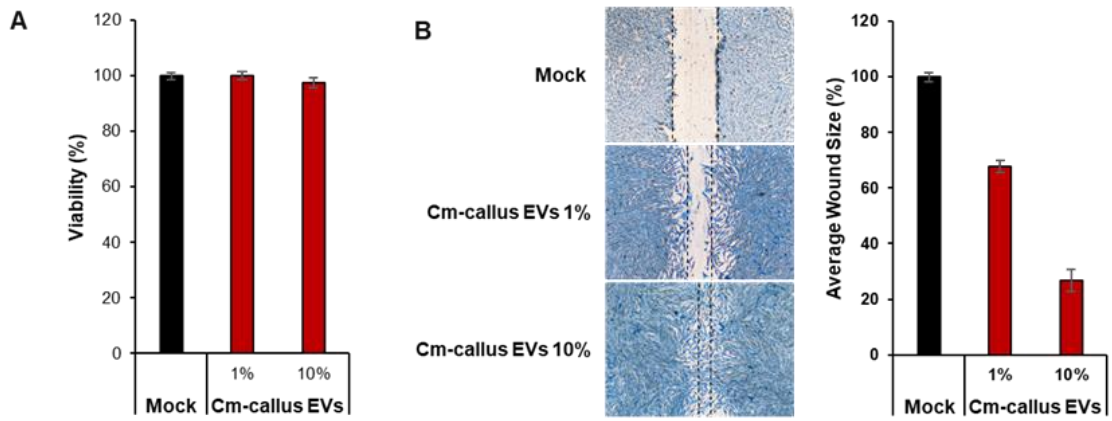


Sample	Concentration (Particles/mL)	Particle size (nm)	Zeta Potential (mV)
Cm-callus EVs	2.6 x 10^{10}	136.6	-44.98

Figure 1. Characterization of Cm-callus EVs. (A) NTA and zeta potential of Cm-callus EVs, result as 2.6 x 10^{10} particles/mL of concentration, 136.6 nm of mean size, and -44.98 mV of zeta potential. (B) TEM images of Cm-callus EVs. (Scale Bar; 100 nm)

3.2. Effect of Cm-callus EVs on Wound Healing and Skin Regeneration in Human Foreskin Fibroblast

Treatment with Cm-callus EVs enhanced wound healing in HFF cells, reducing wound size by over 70% (Figure. 2B) without affecting cell viability (Figure. 2A), and modulated skin-regeneration-related genes, increasing COL1A1 and VEGFA while decreasing MMP1 expression (Figure. 2C). Especially, COL1A1 expression, which was reduced by UV irradiation, was restored by more than 90% after treatment with Cm-callus EVs (Figure. 2D, E).



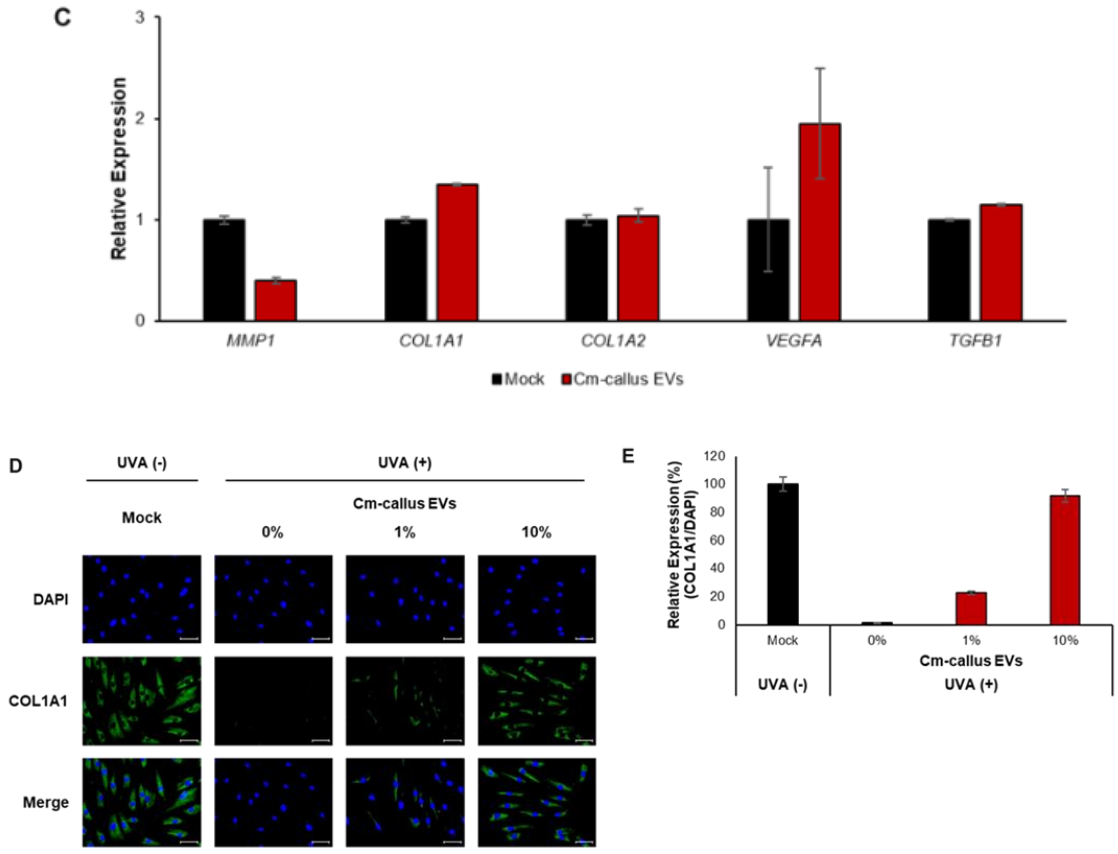


Figure 2. *In vitro* functional characterization of Cm-callus EVs. (A) No significant changes in cell viability of HFF cells after treatment of Cm-callus EVs. (B) Decreased average wound size in wounded HFF cells dose-dependently after treatment of Cm-callus EVs (left) and evaluation of average wound size in [%] (right). (C) Changes in mRNA expression of MMP1, COL1A1, COL1A2, VEGFA, TGFB1 genes in HFF cells following treatment of Cm-callus EVs. (D, E) Changes and recovery in protein expression of COL1A1 in HFF cells by treatment of Cm-callus EVs after UV irradiation (D) and evaluation of relative expression of COL1A1 normalized with DAPI (E). (green: COL1A1 labeled by Alexa Fluor 488; blue: nucleus labeled by DAPI, Scale bar; 50 μm)

3.3. Enrichment of tae-miR167c-5p in Cm-callus EVs and effect of miR167 from Cm-callus EVs on Wound Healing and Skin Regeneration in Human Foreskin Fibroblast

Small RNA sequencing confirmed miR167 in Cm-callus EVs (Figure 3), and mimic transfection reproduced their regenerative effects (Figure 4)

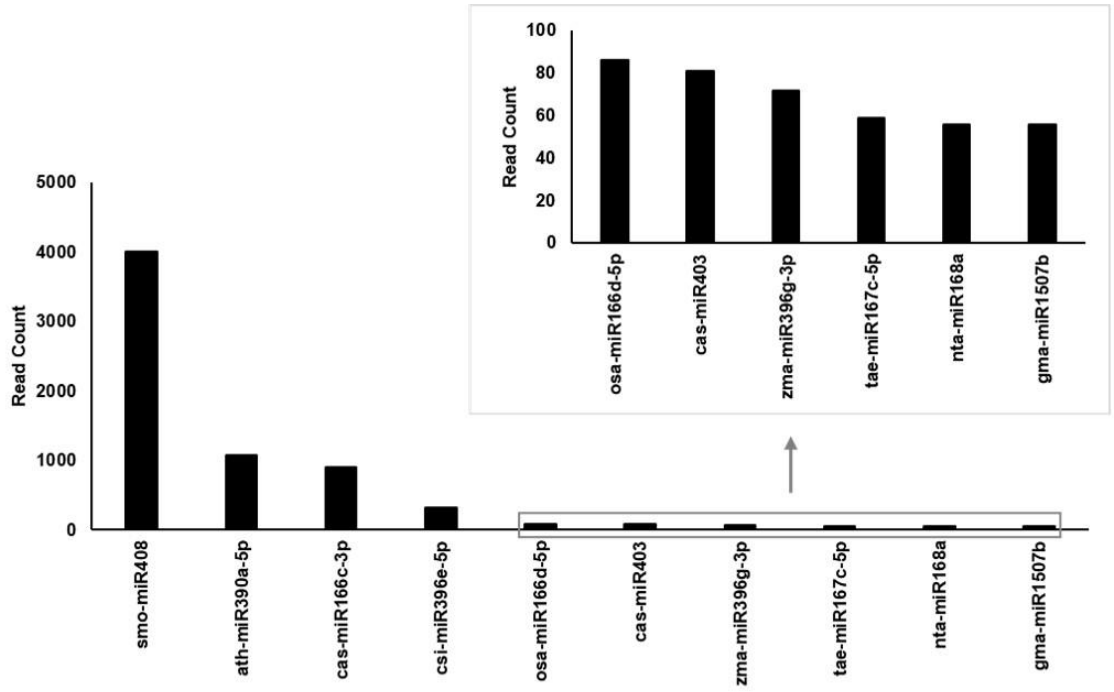
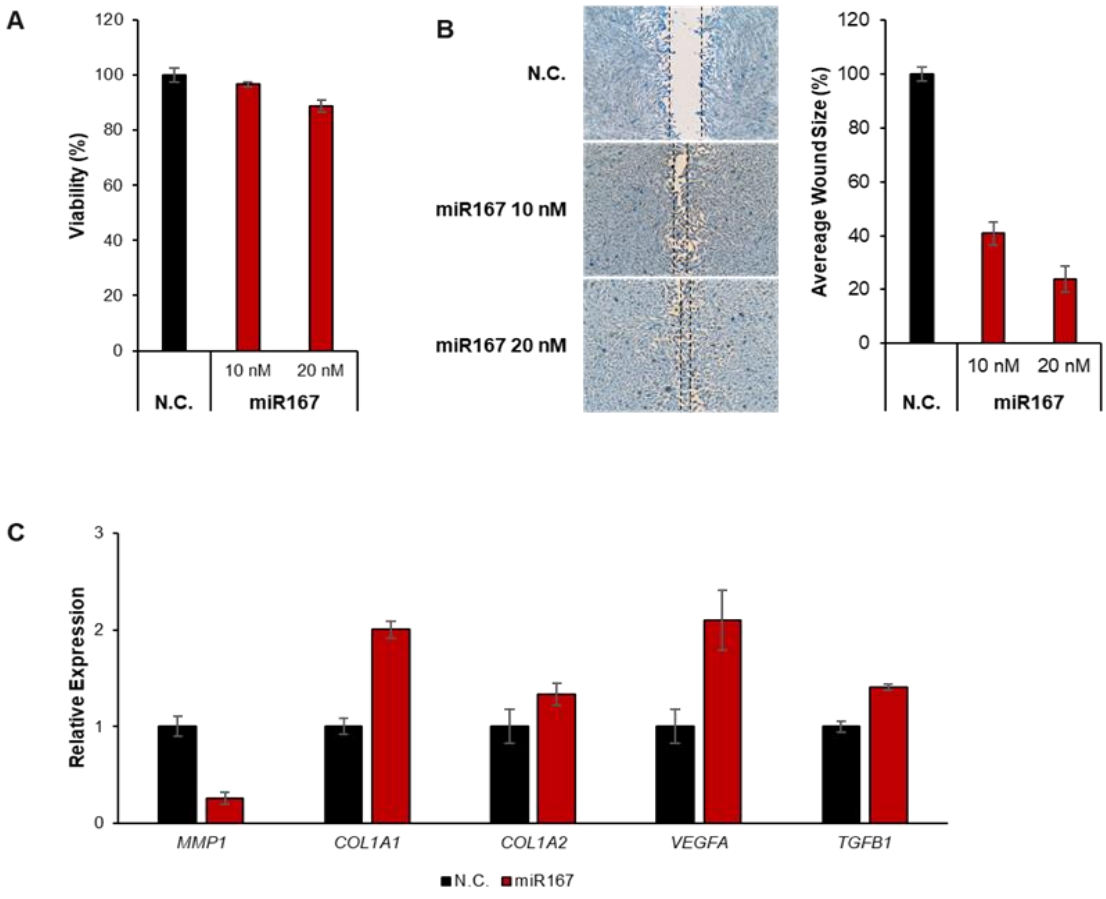


Figure 3. List of miRNAs present in Cm-callus EVs. Top 10 list of miRNAs with highest number of read count in Cm-callus EVs including miR167(tae-miR167c-5p), those with read count of less than 100 are enlarged.



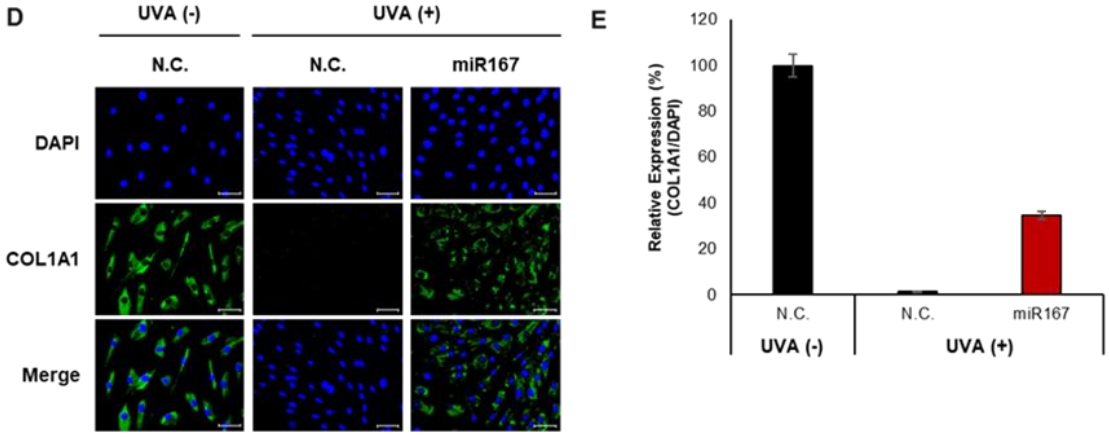


Figure 4. *In vitro* functional characterization of miR167. (A) No significant changes in cell viability of HFF cells after transfection of miR167 mimic. (B) Decreased average wound size in wounded HFF cells dose-dependently following transfection of miR167 mimic (left) and evaluation of average wound size in [%] (right). (C) Changes in mRNA expression of MMP1, COL1A1, COL1A2, VEGFA, TGFB1 genes in HFF cells after transfection of miR167 mimic. (D, E) Changes and recovery in protein expression of COL1A1 in HFF cells by transfection of miR167 mimic after UV irradiation (D) and evaluation of relative expression of COL1A1 normalized with DAPI (E). (green: COL1A1 labeled by Alexa Fluor 488; blue: nucleus labeled by DAPI, Scale bar; 50 μm)

3.6. PPP3R2 could be a Target of miR167

Bioinformatics suggested PPP3R2 (Table 1), linked to MAPK and NFAT signaling pathways, as a potential miR167 target involved in skin regeneration.

Table 1. Predicted target genes of miR167 (Top 10)

Putative Human Target Genes of tae-miR167c-5p (from. psRNATarget V2)			
Rank	**Target Accession**	**mRNA Target Aligned Fragment (5'-3')**	**Inhibition**
1	NM_147180|PPP3R2	UUAUGUCAUGUUGGUAGCUUUA	Cleavage
2	NM_001122853|MYOZ3	GAUGAUGAUGAUGGCAGCUUUA	Cleavage
3	NM_006830|UQCR11	GUUGAUCAUGCUGGUGGCUUGG	Cleavage
4	NM_012479|YWHAG	AUGGAUCGUGUUGGUAUUUUCA	Cleavage
5	NM_015026|MON2	UAUGAUCAUGCAGUUAGCUUCA	Translation
6	NM_080391|PTP4A2	UCAGAGAAUGCUGGUAGCUUAA	Cleavage
7	NM_022340|ZFYVE20	CAGGAUCGUGCUGGUAGCACCA	Cleavage
8	NM_003672|CDC14A	GGGAAUCAUGUUGACAGUUUUA	Cleavage

9	NM_006004|UQCRH	UUGGCUUAGGCUGGUAGCUUCU	Cleavage
10	NM_001089591|UQCRHL	UUGGCUUAGGCUGGUAGCUUCU	Cleavage

4. Discussion

PDEVs are nanosized (30-150 nm) membrane vesicles that carry biomolecules that influence plants development and protect plants against pathogens [4,12]. In addition, PDEVs have been shown to exchange information with mammals across kingdoms [12]. Recently, PDEVs have gained attention as novel biologically active materials for skin care [3]. The zeta potential and size of EVs are critical factors in evaluating their stability and effectiveness as drug delivery systems [13]. Cm-callus EVs exhibit a zeta potential of -44.98 mV and an average size of 136.6 nm (Fig. 1A), characteristics that are particularly advantageous for their application as drug delivery systems. The zeta potential, indicative of surface charge, reflects a strong negative charge in Cm-callus EVs, which promotes electrostatic repulsion between vesicles, ensuring excellent colloidal stability and preventing aggregation [14]. Additionally, a negative charge may improve cellular uptake by facilitating interactions with positively charged components of the cell membrane, which is a key factor for effective drug delivery [15]. The average size of the Cm-callus EVs, 136.6 nm, fell within the optimal rage (100-200 nm) for drug delivery systems. Nanoparticles of this size are efficiently internalized by cells through endocytic pathways such as clathrin-mediated endocytosis, enabling targeted therapeutic delivery [16,17]. Their size also contributes to favorable biodistribution and prolonged retention in the bloodstream [18]. Moreover, nanoparticles under 150 nm can exploit the enhanced permeability and retention (EPR) effect, allowing passive accumulation in tissues with leaky vasculature, such as inflamed or tumor regions, further enhancing their targeting capabilities [19]. Taken together, the combination of a highly negative zeta potential and an optimal size of Cm-callus EVs positions them as a promising platform for drug delivery. These properties not only ensure high stability and efficient cellular uptake but also provide the potential to encapsulate and protect bioactive molecules, target specific tissues, and minimize systemic toxicity. These characteristics underline the significant potential of Cm-callus EVs for advancing therapeutic applications and warrant further exploration of their functionalization and clinical use, making them highly suitable for drug delivery applications. In this study, we demonstrated the role of Cm-callus EVs in promoting skin regeneration. The regenerative capability of Cm-callus EVs was validated through wound healing assay. The treatment of fibroblasts with Cm-callus EVs reduced wound size by more than 70% (Fig. 2B). As wound healing and skin regeneration progressed, the expression of MMP1 and MMP3 was downregulated along with an upregulation in COL1A1, COL1A2 and VEGFA expression [20,21]. These trends were consistent with the observed downregulation of MMP1 mRNA

and the upregulation of COL1A1 and VEGFA mRNA in fibroblast treated with Cm-callus EVs (Fig. 2C). In particular, the UV-induced reduction in COL1A1 protein expression was reversed following treatment with Cm-callus EVs (Fig. 2D, E). These findings strongly suggest that Cm-callus EVs promote skin regeneration. To further elucidate the specific components of EVs responsible for their regenerative effects, we focused on the miRNA, which are key biomolecules in EVs. miR167, well-studied miRNA known to confer salt-tolerance to halophytes, is of particular interest [9,10]. Small RNA-sequencing (sRNA-seq) revealed that Cm-callus EVs contained more than 100 miRNAs, including miR167 (Fig 3). Functional analyses confirmed that miR167 possessed skin-regenerative properties comparable to or even exceeding those of Cm-callus EVs. Transfection of fibroblasts with the miR167 mimic reduced the wound size by approximately 80% (Fig. 4B). Additionally, the changes in gene expression related to skin regeneration observed after miR167 transfection were consistent with those observed after Cm-callus EV treatment (Fig. 4C-E). These results establish miR167 as a key contributor to the skin regenerative effects of Cm-callus EVs. miRNAs regulate target mRNAs through mechanism such as mRNA degradation and translation inhibition. In this study, we investigated the potential targets of miR167, a miRNA enriched in Cm-callus EVs, using the small RNA target analysis server, psRNATarget [11]. Our results identified approximately 100 candidate target genes, with PPP3R2 as the top candidate. Notably, PPP3R2 was highly ranked in both versions of the prediction tool, indicating strong potential for miR167 to regulate its expression. PPP3R2 is related to both the MAPK and NFAT signaling pathways, which are critical for various cellular processes, including skin regeneration and repair. The MAPK pathway plays an essential role in regulating cell proliferation, differentiation, and survival, processes that are crucial for wound healing and tissue regeneration [22,23]. The NFAT pathway, on the other hand, is involved in immune response regulation and has been shown to influence collagen production, a key factor in skin elasticity and structure [24,25]. Both pathways contribute to the maintenance of skin integrity by modulating fibroblast function, collagen synthesis, and inflammatory responses, which are essential for combating signs of aging. The prediction that miR167 targets PPP3R2, a gene involved in these pathways, opens up an intriguing possibility for its anti-aging role. By modulating PPP3R2 expression, miR167 could influence the MAPK and NFAT pathways, potentially enhancing skin regeneration and slowing down the aging process. This aligns with the regenerative effects observed with Cm-callus EVs treatment in fibroblast wound healing assay, where key genes involved in collagen synthesis (such as COL1A1 and COL1A2) were upregulated, and markers of tissue degradation, such as MMP1, were downregulated. Thus, the potential targeting of PPP3R2 by miR167 may represent a mechanism through which Cm-callus EVs promote skin regeneration and exhibit anti-aging effects. Although our findings

provide strong computational evidence linking miR167 with PPP3R2 and the regulation of important signaling pathways in skin regeneration, further experimental studies are required to validate these predictions. Specifically, the functional confirmation of PPP3R2 as a target of miR167 and its involvement in skin regeneration processes are key to fully understanding the therapeutic potential of miRNA-based treatments in anti-aging skincare.

5. Conclusion

In conclusion, this study provides evidence that Cm-callus EVs, particularly their content of miR167, promote skin regeneration by modulating the key genes involved in tissue repair and collagen synthesis. Although the exact molecular mechanisms remain to be elucidated, our findings support the potential use of plant-derived EVs as a novel approach for enhancing skin regeneration, with miR167 playing a crucial role. Further studies are needed to validate the identified targets of miR167 and to explore the therapeutic potential of Cm-callus EVs in skin regeneration.

6. References

[1] Radman, S., et al., Sea Fenel (*Crithmum maritimum* L.) Flowers as an Emerging Source of Bioactive Compounds, Pol. J. Food Nutr. Sci. 74 (2024), 221-231.

[2] Generalić Mekinić, I., et al., Sea fennel (*Crithmum maritimum* L.): phytochemical profile, antioxidative, cholinesterase inhibitory and vasodilatory activity, J Food Sci Technol. 53 (2016), 3104-3112.

[3] Subha, D., et al., Plant derived exosome-like Nanovesicles: an updated overview, Plant Nano Biology 3 (2023), 100022.

[4] Alzahrani, F. A., et al., Plant-Derived Extracellular Vesicles and Their Exciting Potential as the Future of Next-Generation Drug Delivery, Biomolecules 13 (2023), 839.

[5] Saiyed, A. N., Vasavada, A. R. & Kaid Johar, S. R., Recent trends in miRNA therapeutics and the application of plant miRNA for prevention and treatment of human diseases. Futur J Pharm Sci. 8 (2022), 24.

[6] Liu, X., Huang, S. & Xie, H., Advances in the regulation of plant development and stress response by miR167, Front Biosci (Landmark Ed). 26 (2021), 655-665.

[7] Díez-Sainz, E., et al., MicroRNAs from edible plants reach the human gastrointestinal tract and may act as potential regulators of gene expression, J Physiol Biochem. 80 (2024), 655-670.

[8] Philip, A., Ferro, V. A. & Tate, R. J., Determination of the potential bioavailability of plant microRNAs using a simulated human digestion process, Mol Nutr Food Res. 59 (2015), 1962-72.

[9] Islam, W., Waheed, A., Naveed, H. & Zeng, F., MicroRNAs Mediated Plant Responses to Salt Stress, Cells 11 (2022), 2806.

[10] Gharat, S. A. & Shaw, B. P., Novel and conserved miRNAs in the halophyte *Suaeda maritima* identified by deep sequencing and computational predictions using the ESTs of two mangrove plants, BMC Plant Biol. 15 (2015), 301.

[11] Dai, X., Zhuang, Z. & Zhao, P. X., psRNATarget: a plant small RNA target analysis server (2017 release), Nucleic Acids Res. 46 (2018), W49-W54.

[12] Cho, J. H., et al., Confrmation of plant-derived exosomes as bioactive substances for skin application through comparative analysis of keratinocyte transcriptome, Appl Biol Chem. 65 (2022), 8.

[13] Midekessa, G., et al., Zeta Potential of Extracellular Vesicles: Toward Understanding the Attributes that Determine Colloidal Stability, ACS Omega 5 (2020), 16701-16710.

[14] Pochapski, D. J., et al., Zeta Potential and Colloidal Stability Predictions for Inorganic Nanoparticle Dispersions: Effects of Experimental Conditions and Electrokinetic Models on the Interpretation of Results, Langmuir 37 (2021), 13379-13389.

[15] Öztürk, K., Kaplan, M. & Çalış, S., Effects of nanoparticle size, shape, and zeta potential on drug delivery, Int J Pharm. 666 (2024), 124799.

[16] Tang, X. R., et al., How big nanoparticles carry small ones into cells: Actions captured by transmission electron microscopy., Colloids Surf B Biointerfaces 245 (2025), 114272.

[17] Rennick, J. J., Johnston, A. P. R. & Parton, R. G., Key principles and methods for studying the endocytosis of biological and nanoparticle therapeutics, Nat Nanotechnol. 16 (2021), 266-276.

[18] Hoshyar, N., Gray, S., Han, H. & Bao, G., The effect of nanoparticle size on in vivo pharmacokinetics and cellular interaction, Nanomedicine (Lond) 11 (2016), 673-92.

[19] Prabhakar, U., et al., Challenges and Key Considerations of the Enhanced Permeability and Retention Effect for Nanomedicine Drug Delivery in Oncology, Cancer Res. 73 (2013), 2412-7.

[20] Kunhorm, P., Chaicharoenaudomrung, N. & Noisa, P., Cordycepin-induced Keratinocyte Secretome Promotes Skin Cell Regeneration, In Vivo 37 (2023), 574-590.

[21] Lee, Y. I., et al., Exploring the Safety and Efficacy of Organic Light-Emitting Diode in Skin Rejuvenation and Wound Healing, Yonsei Med J. 65 (2024), 98-107.

[22] Leyane, T. S., Jere, S. W. & Houreld, N. N., Cellular Signalling and Photobiomodulation in Chronic Wound Repair, Int J Mol Sci. 22 (2021), 11223.

[23] Cargnello, M. & Roux, P. P., Activation and Function of the MAPKs and Their Substrates, the MAPK-Activated Protein Kinases, Microbiol Mol Biol Rev. 75 (2011), 50-83.

[24] Lin, Y., et al., NFAT signaling dysregulation in cancer: Emerging roles in cancer stem cells, Biomed Pharmacother. 165 (2023), 115167.

[25] Manabe, T., Park, H. & Minami, T., Calcineurin-nuclear factor for activated T cells (NFAT) signaling in pathophysiology of wound healing, Inflamm Regen. 41 (2021), 26.