Clinical, Cosmetic and Investigational Dermatology

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ORIGINAL RESEARCH

Lactobacillus brevis-Derived Exosomes Enhance Skin Barrier Integrity by Upregulating Key Barrier-Related Proteins

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Introduction: The human skin, comprising the epidermis, dermis, and subcutaneous fat layers, serves as a critical barrier against external stimuli. The integrity of this barrier function is essential for preventing skin damage and diseases. When compromised, it can lead to various dermatological issues.

Methods: This study investigated the efficacy of *Lactobacillus brevis* J2K55-derived exosomes (LBDEs) on enhancing skin barrier function. High-purity LBDEs were produced and characterized using nanoparticle tracking analysis and Cryo-TEM, concentrated to 1.52×10^8 particles/mL with sizes ranging from 50 to 200 nm. The LBDEs were then applied to human keratinocytes, HaCaT cells, and a live human skin model to analyze the expression of genes significant to skin barrier function.

Results: In vitro experiments demonstrated that 2.5% LBDEs increased Filaggrin mRNA expression by 301.80% compared to the control. In an ex vivo skin damage model induced by physical stimulation and UVB (Ultraviolet B) irradiation, 1% LBDEs treatment significantly upregulated the expression of key barrier-related proteins, including Aquaporin-3 (180.8%), Claudin-1 (205.4%), Filaggrin (309.9%), Loricrin (365.2%), and Serine palmitoyltransferase (191.3%), in comparison to the friction and UVB-induced control group.

Conclusion: These results suggest that LBDEs have potential in enhancing skin barrier function, as evidenced by increased expression of crucial barrier-related proteins in both in vitro and ex vivo models.

Keywords: exosome, skin barrier, lactobacillus brevis, nanoparticle tracking analysis, Cryo-TEM

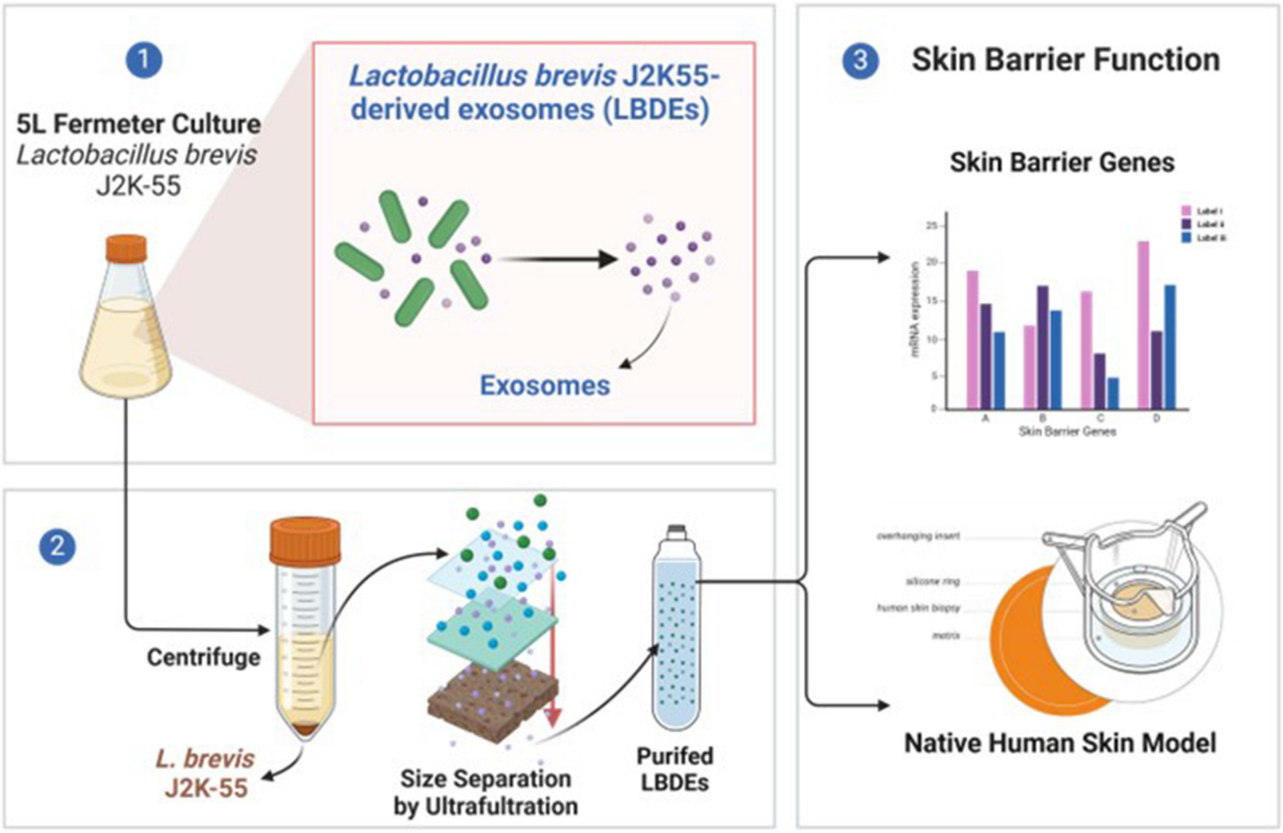
Introduction

As interest in skin health and beauty continues to grow, there is a corresponding increase in interest in materials that protect the health of the skin. The skin, which serves to separate the body's internal and external environments, acts as a chemical and physical barrier, preventing the loss of moisture and protecting the body from damage. Furthermore, the skin plays a significant role in thermoregulation and immune defense. However, the skin barrier can be compromised by a number of environmental factors, including ultraviolet radiation and air pollution, as well as by irregular lifestyle habits and alcohol and tobacco use.[1]

The regulation of the skin barrier is a complex process influenced by molecular, immunological, microbiome-related, and environmental factors. Key regulators include interleukins, NF-κB, and sirtuin 3, which maintain skin acidity, ceramide levels, and immune homeostasis, while transcription factors like GRHL1 and GRHL3 play essential roles in epidermal formation and repair.[2,3] The microbiome plays a critical role in epidermal differentiation and repair, with commensal bacteria signaling through the aryl hydrocarbon receptor (AHR) to enhance barrier function,[4] and gut-derived microbial exosomes modulating immune responses to reduce inflammation.[5] Additionally, hair follicles contribute to barrier regulation by modulating desquamation and inflammatory responses, particularly via IL-17a signaling,[6] while

Received: 8 January 2025
Accepted: 18 April 2025
Published: 8 May 2025

Graphical Abstract



C10orf99/GPR15L influences keratinocyte function and inflammation in conditions like psoriasis.[7] Future research is expected to explore personalized treatments that integrate genetics, microbiome analysis, and immune modulation for more effective interventions in skin barrier-related diseases.

Recent research on skin barrier regulation and drug delivery highlights the development of advanced formulations such as microemulsion-based gels (μEGs) and silica nanoparticle-embedded smart gels for enhanced transdermal drug delivery. μEGs provide a superior alternative to traditional topical formulations by offering high stability, structural flexibility, and controlled drug release, as demonstrated in studies with celecoxib (CXB), diclofenac sodium (DF-Na), and naproxen sodium (NP-Na), which showed significantly improved permeation through the skin barrier with sustained drug release over 8–10 hours.[8,9] Similarly, silica nanoparticle-embedded smart gels, such as those formulated with minoxidil (MXD), exhibit high porosity and strong drug encapsulation properties, allowing for a prolonged release over 12 hours, making them effective for cutaneous drug administration.[10] Additionally, while advanced drug delivery systems optimize transdermal absorption, diagnostic techniques such as fine-needle aspiration cytology (FNAC) are emerging as valuable tools for identifying skin adnexal tumors, complementing histopathology in the diagnosis and management of skin-related conditions.[11] These findings collectively underscore the growing potential of nanotechnology-based formulations and minimally invasive diagnostic approaches in enhancing skin barrier function, improving drug delivery, and advancing dermatological diagnostics.

Extracellular vesicles (EVs) are double-lipid membrane structures secreted by cells. They can be classified into microvesicles, exosomes, and apoptotic bodies, and are differentiated according to their production and release pathways, size, contents, and function. Despite their variation in size and secretion mechanism, they are collectively referred to as

vesicles. These are secreted into the extracellular space and are composed of a variety of substances with biological activity, including proteins, lipids, nucleic acids, and metabolites. Given these characteristics, EVs are regarded as proxies of cells, encapsulating a range of cellular components, including DNAs, RNAs, lipids, metabolites, cytoplasmic and cell surface proteins, contingent on the cell of origin. Their potency may vary, reflecting the characteristics and status of the parent cell from which they are secreted.[12]

Currently, with the advancement of EVs isolation technology, various functional studies of exosomes are being conducted. These studies have revealed diverse effects of exosomes, including angiogenesis promotion, collagen synthesis enhancement, and inflammation regulation.[13,14] EVs are obtained from a variety of sources, with the three main types being human stem cell-derived exosomes (particularly those isolated from adipose-derived stem cells), plant-derived exosomes, and microbiome-derived exosomes.[15,16]

Exosomes play an important role in skin barrier regulation and regeneration, and stem cell- and microbial-derived exosomes in particular have excellent skin barrier repair and anti-inflammatory effects.[5,17–19] Alternative sources, such as milk-derived exosomes, are opening up new possibilities in skincare and therapeutics, and gut microbial-derived exosomes are also gaining traction in skin health research.[20] In the future, clinical applications of exosomes are expected to expand with advances in mass production and purification technologies.[14]

Lactobacillus-derived EVs have been successfully isolated from culture supernatants, and their characteristics and functions have been elucidated. Various Lactobacillus species have demonstrated specific therapeutic effects: *Lactobacillus paracasei* has shown efficacy in treating skin inflammatory diseases, *Lactobacillus plantarum* has exhibited benefits in addressing skin aging, and *Lactobacillus rhamnosus* has demonstrated effectiveness in treating colitis.[21–23] However, studies on EVs derived from other probiotic species remain limited.

Exosomes are exceptionally small (50–200 nm in diameter), approximately 1/250 to 1/600 the size of human skin pores. Their cell-like composition confers advantages such as low irritation potential and ease of skin absorption and penetration.[24] *Lactobacillus* species have been extensively studied for their probiotic properties and skin health benefits, including their ability to modulate inflammation and promote barrier repair.[16] However, despite the growing interest in *Lactobacillus*-derived exosomes, there is currently limited research on *L. brevis* exosomes and their specific impact on skin barrier integrity. In this study, exosomes were isolated and purified from a *Lactobacillus brevis* strain using an ultrafiltration method. *Lactobacillus brevis* was selected for this study based on its well-documented probiotic properties and potential for skin health applications. This strain has been widely studied for its anti-inflammatory, antioxidant, and immunomodulatory effects,[25] which are critical for maintaining skin homeostasis and barrier integrity. Additionally, *L. brevis* has been reported to enhance keratinocyte function and promote lipid metabolism, both of which contribute to a stronger epidermal barrier.[26,27] This study aims to fill the knowledge gap by investigating the functional role of *L. brevis*-derived exosomes (LBDEs) in skin barrier enhancement. By analyzing their effects on barrier-related protein expression, we provide new insights into the potential therapeutic applications of *L. brevis* exosomes in skincare and dermatological treatments.

The presence and characteristics of these exosomes were confirmed through Nanoparticle Tracking Analysis (NTA) and Cryogenic Transmission Electron Microscopy (Cryo-TEM). Furthermore, in vitro and ex vivo experiments were conducted to evaluate the efficacy of these exosomes in improving the skin barrier function. The results of this study confirm the potential of probiotic-derived exosomes as cosmetic ingredients that can contribute to skin health.

Materials And Methods

Materials and Equipment

Bacterial culture was done using MRS agar and broth. Mammalian cells were cultured in DMEM (Dulbecco's Modified Eagle Medium) from Sigma (St. Louis, MO, USA). Thiazolyl blue tetrazolium bromide, DMSO (98% and 99.9% each, Sigma, St. Louis, MO, USA), FBS, and antibiotic-antimycotic solution (Corning, Corning, NY, USA) were used. NucleoZOL was from Macherey-Nagel (Düren, Germany), and the HiSenScript RH(-) RT PreMix Kit from Intron Biotechnology (Seongnam, Korea). Biofact 2X Real-Time PCR Master Mix was from Biofact (Daejeon, Korea). Other reagents were of analytical grade. Equipment included a 5L KoBioTech fermentor (Incheon, Korea), Supra R17

centrifuge (HANIL SCIENCE, Daejeon, Korea), FMX-B ultrafiltration system (BKT CO, Anyang, Korea), Spectramax ABS Plus microplate reader (Molecular Devices, San Jose, CA, USA), and QuantStudio 3 PCR System (Applied Biosystems, Waltham, MA, USA).

Cell Culture and Purification of Exosome

Lactobacillus brevis J2K-55, isolated from Jeju dongchimi, was grown in MRS broth. After initial aerobic incubation at 150 rpm and 35°C for 24 hours, the culture was transferred to a 5L fermenter and incubated at 35°C for 48 hours at 200 rpm and 1 vvm. The culture was centrifuged at 10,000 rpm for 10 minutes, followed by ultrafiltration to remove medium components. Exosomes (LBDEs) were obtained by filtering out particles larger than 200 nm using a 0.1 μm PVDF membrane.

Analysis of Lactobacillus Brevis-Derived Exosomes (LBDEs)

NTA was used to measure LBDE size, distribution, and concentration with a NanoSight NS300 (Malvern Panalytical, Malvern, UK). Cryo-TEM validated the lipid bilayer structure of exosomes using samples rapidly frozen in liquid nitrogen and observed at 150,000x magnification using a Tecnai G2 Spirit TWIN (FEI, Hillsboro, OR, USA).

Cell Culture

HaCaT cells were kindly provided by Professor Sang-Bae Han (College of Pharmacy and Medical Research Center, Chungbuk National University, South Korea) and maintained according to the supplier's recommended culture conditions. Cells were cultured in DMEM with 10% FBS and 1% antibiotic-antimycotic solution, maintained in a 37°C incubator with 5% CO_2. Subculturing was done every 48 hours.

Cell Viability Assay

HaCaT cells were seeded at a density of 1.0×10^4 cells/well in 180 μL of medium and incubated for 24 hours at 37°C with 5% CO_2. LBDEs were diluted, and 20 μL of each concentration was added to the wells. After 24 hours, 20 μL of MTT solution (5 mg/mL) was added and incubated for two hours. Formazan crystals were dissolved in 100 μL DMSO, and absorbance was measured at 590 nm using a microplate reader.

Real-Time Polymerase Chain Reaction

HaCaT cells were seeded at a density of 1.5×10^5 cells/well in a 6-well plate and incubated for 18 hours. The medium was replaced with serum-free DMEM, and LBDE samples were added. After 24 hours, RNA was isolated using NucleoZOL (Macherey-Nagel, Düren, Germany), and cDNA synthesis was performed using the HiSenScript RH(-) RT PreMix Kit (Intron Biotechnology, Seongnam, Korea). PCR was conducted using Biofact 2X Real-Time PCR Master Mix (Biofact, Daejeon, Korea), and quantification was performed with the QuantStudio 3 system (Applied Biosystems, Waltham, MA, USA). Primer conditions are listed in Table 1.

Table 1 Forward and Reverse Primer PCR Sequence for qRT-PCR

Gene	Primer	Primer sequence
LOR	Forward Reverse	AAGAAGCCATTGAGCTCTCCG TTATTGACTGAGGCACTGGGG
FLG	Forward Reverse	GCTGAAGGAACTTCTGGAAAAGG GTTGTGGTCTATATCCAAGTGATC
GAPDH	Forward Reverse	5'-GTCTCCTCTGACTTCAACAGCG-3' 5'-ACCACCCTGTTGCTGTAGCCAA-3'

H&E and Immunofluorescence

Skin damage was induced by physical stimulation and UVB irradiation, followed by treatment with 1% LBDEs. The NativeSkin (Genoskin, Salem, MA, USA) live human skin model was utilized. Histological observation was done using H&E staining.[28] Immunofluorescence was used to analyze protein expression (Claudin-1, Filaggrin, Loricrin, SPT). The following primary antibodies were used for staining: Claudin-1 (Santa Cruz, sc-166338), Filaggrin (Abcam, ab81468), Loricrin (Abcam, ab176322), SPT (Abcam, ab236900), and Aquaporin-3 (Abcam, ab125219). Protein expression levels were quantified with Image J software, and statistical analysis was done using Prism 7 software with Tukey's multiple comparison test.

Ex vivo Model Description

The NativeSkin human skin explant model (GenoSkin SAS, France, Lot No. 20230904.03) was used to assess the effects of *Lactobacillus brevis*-derived exosomes (LBDEs) on skin barrier integrity. The skin explants were obtained from a 33-year-old female donor (abdominal region) with no known skin diseases or allergies. To induce skin barrier disruption, a frictional stress model was applied by scratching the skin surface with a sterilized cotton swab for 60 minutes, followed by 50 mJ/cm² UVB irradiation. Then, 20 µL of LBDEs (1% in water) was topically applied, and the explants were incubated at 37°C in a 5% CO_2 incubator. Samples and media were replaced every 24 hours for 72 hours, after which tissues were harvested for histological and molecular analysis.

Statistical Analysis

Experiments were conducted in triplicate. Data are shown as mean ± SD. Statistical analysis was performed using Excel, with significance set at $p < 0.05$ using the *t*-test. Homogeneity of variance was confirmed using the F-test.

Results

Characterization of Lactobacillus Brevis-Derived Exosomes (LBDEs)

Lactobacillus brevis-derived exosomes (LBDEs) were characterized using NTA. The average particle size was measured at 133.3 nm, with a mode size of 105.3 nm and a standard deviation of 59.7 nm, indicating a distribution within this range. The particle concentration was quantified at 1.52×10^8 particles/mL (Figure 1A, Table 2). Cryo-transmission electron microscopy (Cryo-TEM) was employed to further assess the particle morphology. The exosomes were observed to have sizes ranging from 50 to 200 nm, consistent with NTA results. In addition, the presence of a lipid bilayer structure was observed, which is characteristic of exosomal particles (Figure 1B). The combination of NTA and Cryo-TEM data provides a detailed characterization of LBDEs, verifying their size distribution, concentration, and structural features, showing their exosomal nature.

Effect of Lactobacillus Brevis-Derived Exosomes (LBDEs) on Skin Barrier

Before evaluating the barrier-enhancing effects of LBDEs on HaCaT cells, we first assessed cell viability to ensure that the LBDE concentrations used were not cytotoxic. HaCaT cells were treated with varying concentrations of LBDE (0.5%, 1%, and 2.5%) for 24 hours (Figure 2A). Cell viability was measured using the MTT assay, a common colorimetric assay for assessing cell metabolic activity as an indicator of cell viability. The results indicated that LBDE concentrations up to 2.5% did not significantly affect cell viability, with viability rates remaining above 90%.

To evaluate the barrier-enhancing effects of LBDEs on HaCaT cells, the mRNA expression levels of the key skin barrier proteins, Loricrin and Filaggrin, were quantitatively analyzed using real-time PCR. The results demonstrated a significant upregulation in the expression of both genes following LBDE treatment. Specifically, treatment with 2.5% LBDEs led to a 2-fold increase in Loricrin mRNA (*LOR*) expression compared to the untreated control group, suggesting a strong induction of this protein, which plays a critical role in maintaining the integrity of the skin barrier. Additionally, treatment with 2.5% LBDEs resulted in a 3-fold increase in Filaggrin mRNA (*FLG*)expression relative to the control group. Filaggrin is essential for epidermal differentiation and hydration,[29] and the observed increase indicates a potential role of LBDEs in enhancing skin barrier function (Figure 2B and C).

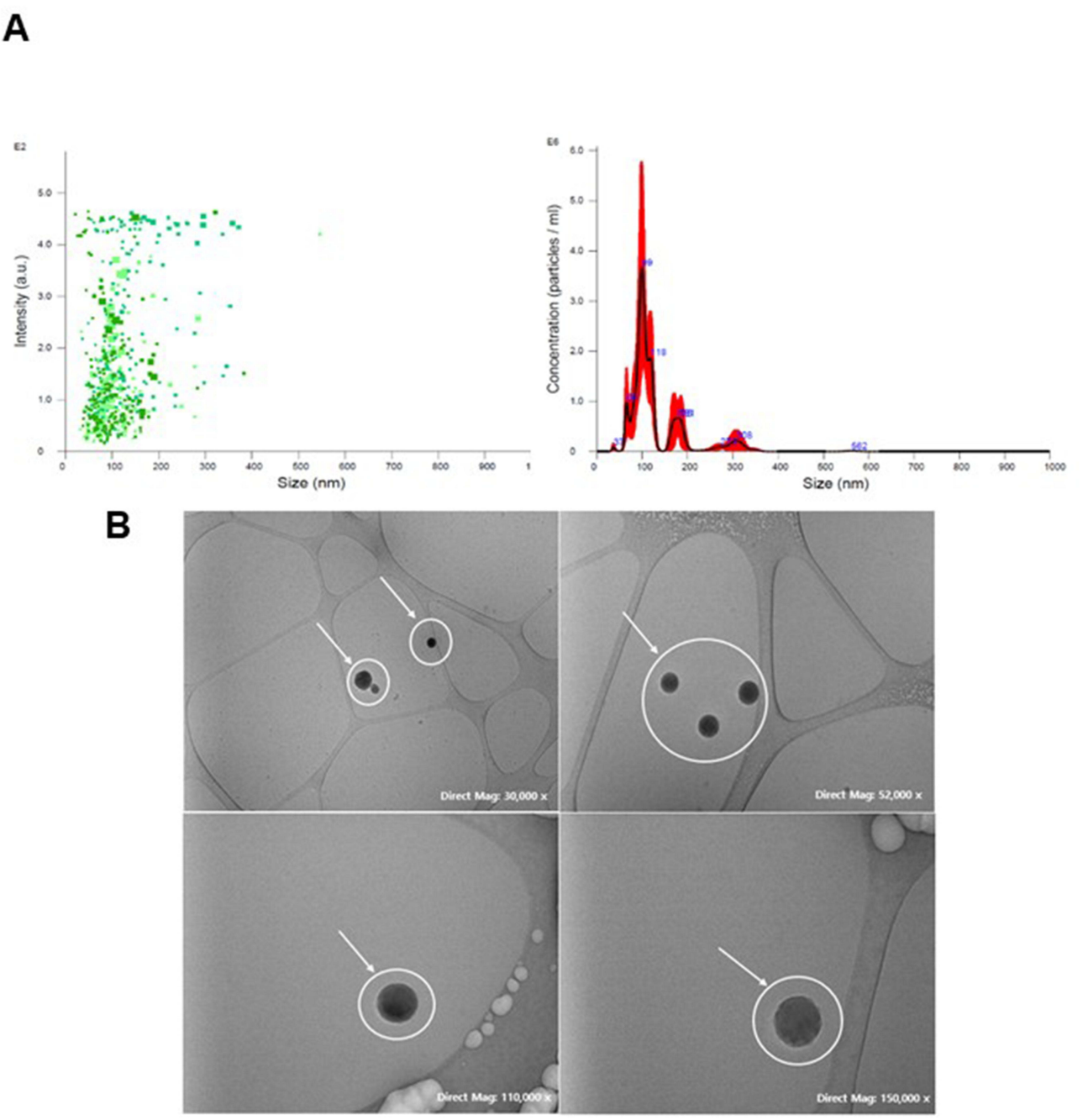


Figure 1 (A) Nanoparticle tracking analysis of exosomes isolated from *Lactobacillus brevis* J2K-55. (**A**) The scattering distributions are presented from three consecutive 30s runs for nanoparticles. Representative graph also shows particle concentration and their size measurements. (**B**) Cryo-TEM image of exosomes derived from *Lactobacillus brevis* J2K-55 at different magnifications.

These findings suggest that LBDEs promote the expression of critical structural proteins involved in skin barrier maintenance in a dose-dependent manner, highlighting their potential utility in therapeutic applications aimed at improving skin barrier function. The dose-specific responses of Loricrin and Filaggrin further support the efficacy of LBDEs in modulating skin barrier-related genes.

Table 2 Results of Nanoparticle Tracking Analysis

Mean (nm)	Mode (nm)	Standard Deviation (nm)	D10 (nm)	D50 (nm)	D90 (nm)	Concentration (Particles/mL)
133.3 ±21.0	105.3 ±5.9	59.7 ±8.1	84.9 ±11.1	109.6 ±10.3	202.0 ±54.9	1.52×10^8 $\pm 2.96 \times 10^7$

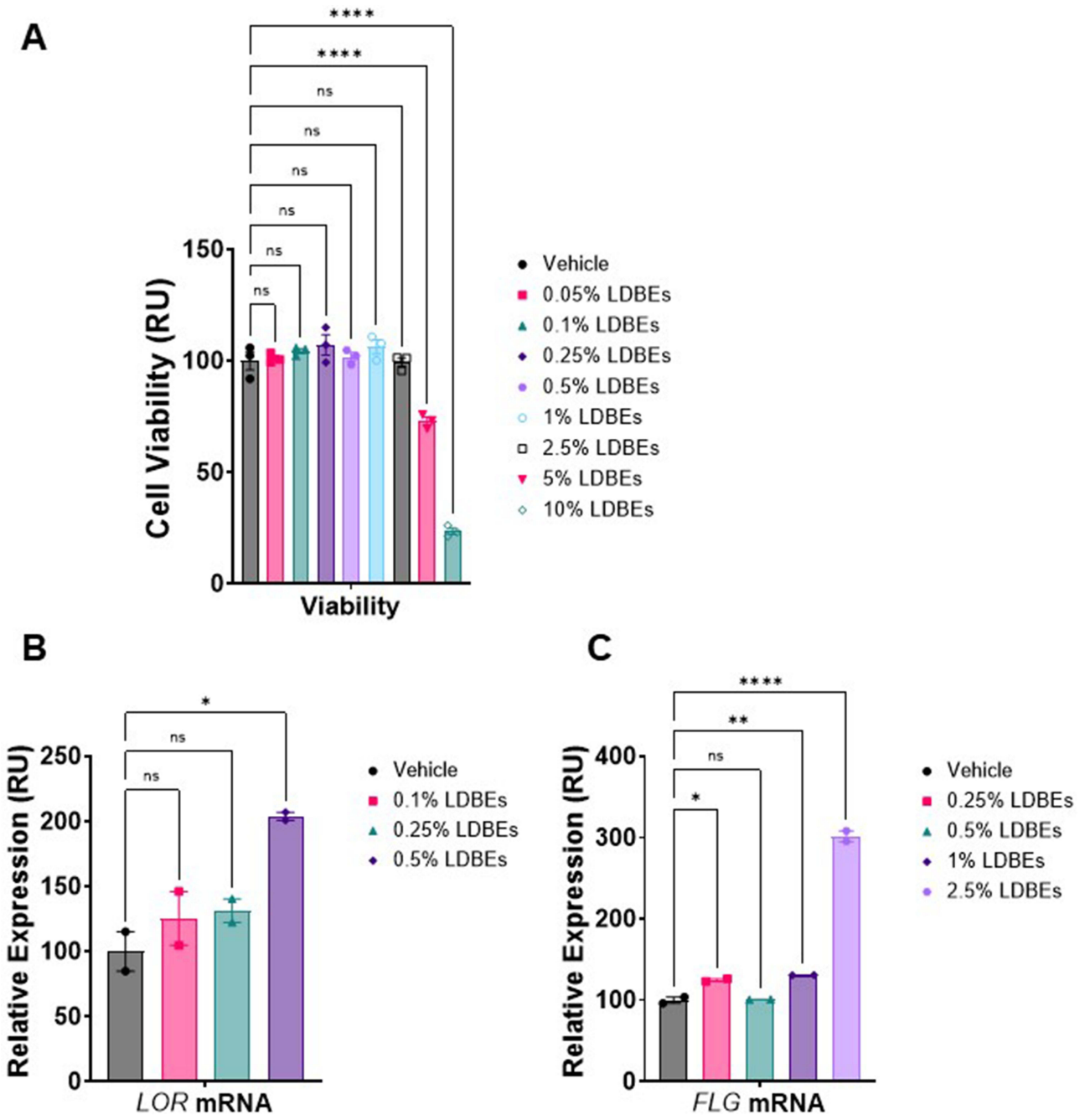


Figure 2 (**A**) Evaluation of cell viability according to concentration of LBDEs in RAW 264.7 cells. The mRNA expression levels of (**B**) Loricrin, (**C**) Filaggrin in the keratinocytes as determined by quantitative real-time PCR analysis. One-way ANOVA revealed statistical significance with *p<0.05, **p<0.01, ****p<0.001, ns: non-significant.

Effect of LBDEs on Skin Barrier

To assess the histological changes and protein expression levels by treatment of LBDEs in the NativeSkin live human skin model, hematoxylin and eosin (H&E) staining and immunofluorescence (IF) experiments were conducted (Figure 3A). Skin barrier damage was induced through mechanical friction using a cotton swab, followed by UVB irradiation at 50 mJ to simulate environmental stress. The H&E staining results revealed significant histological alterations in the skin tissue of the friction and UVB-induced damage model, including epidermal keratin loss, spongiosis, and atypical keratinization. In contrast, treatment with 1% LBDEs markedly reduced the extent of these histological damages, indicating a protective effect on the skin structure. These protective effects were previously

https://doi.org/10.2147/CCID.S512793

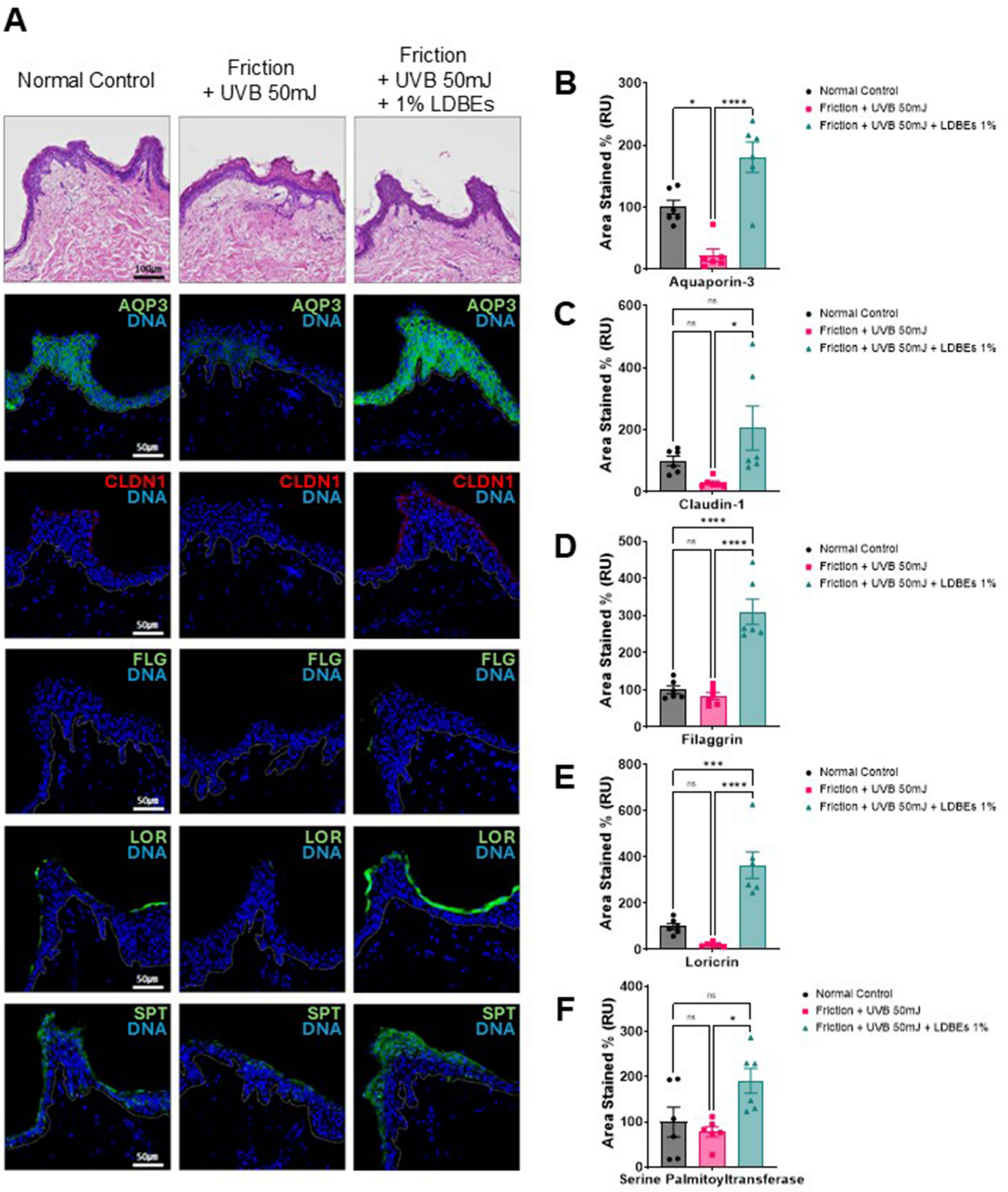


Figure 3 (**A**) Histological results of Hematoxylin and Eosin (H&E) staining. Quantification of the percentage of stained area are assessed from immunofluorescent staining of, (**B**) Aquaporin-3, (**C**) Claudin-1, (**D**) Filaggrin, (**E**) Loricrin and (**F**) Serine palmitoyltransferase. One-way ANOVA revealed statistical significance with *p<0.05, ***p<0.005, ****p<0.001, ns: non-significant.

suggested by gene expression analysis, which demonstrated that Loricrin (*LOR*) and Filaggrin (*FLG*), key barrier-related proteins, were significantly upregulated following LBDE treatment. Notably, *FLG* expression was significantly increased from 1% of LBDEs, aligning with the concentration selected for ex vivo studies based on preliminary in vitro findings (Figure 1). These results suggest that LBDEs not only mitigate structural damage but also actively enhance skin barrier integrity at the molecular level, reinforcing their potential as a therapeutic agent for barrier restoration.

Immunofluorescence analysis revealed statistically significant reductions in the expression levels of key skin barrier proteins in the friction and UVB-induced damage group compared to the normal control group. Specifically, Aquaporin-3, Claudin-1, Filaggrin, Loricrin, and Serine palmitoyltransferase (SPT) expression levels were decreased by 22.6%, 23.2%, 83.7%, 21.0%, and 78.3%, respectively. These reductions reflect the compromised skin barrier function caused by friction and UVB exposure. In contrast, treatment with 1% LBDEs significantly restored the expression of these proteins in the friction + UVB 50 mJ + 1% LBDEs group, with increases in Aquaporin-3, Claudin-1, Filaggrin, Loricrin, and SPT expression levels by 180.8%, 205.4%, 309.9%, 365.2%, and 191.3%, respectively, compared to the Friction + UVB 50 mJ group. These findings suggest that LBDE treatment not only mitigated the damage but also enhanced the expression of proteins critical for skin barrier integrity (Figure 3B–F).

Overall, the results demonstrate that skin barrier function, as indicated by protein expression levels, was significantly impaired following physical and UVB-induced damage. However, treatment with 1% LBDEs was able to significantly improve skin barrier integrity, as evidenced by the restoration of key protein levels.

Discussion

This research highlights the beneficial effects of *Lactobacillus brevis*-derived exosomes (LBDEs) on improving skin barrier function. Characterization of LBDEs using NTA and Cryo-Transmission Electron Microscopy (Cryo-TEM) confirmed their typical size and lipid bilayer structure, hallmark features of exosomes. In HaCaT cell, LBDEs demonstrated non-cytotoxic behavior up to a concentration of 2.5%, with cell viability maintained above 90%. Treatment with LBDEs significantly increased the expression of essential skin barrier proteins, Loricrin and Filaggrin, in a dose-dependent manner. These results suggest that LBDEs enhance the synthesis of structural proteins vital for skin integrity and hydration, supporting their potential use in promoting skin health, especially in conditions with compromised barrier function.

The barrier-protective effects of LBDEs were further validated in an ex vivo NativeSkin human skin model, where skin damage was induced through mechanical friction and UVB radiation. Histological analysis showed that LBDE treatment effectively mitigated damage, preserving the structural integrity of the skin. Immunofluorescence staining indicated significant restoration of key skin barrier proteins, including Claudin-1, Filaggrin, Loricrin, and Serine palmitoyltransferase (SPT), all of which were reduced by the induced damage. These findings reveal that LBDEs not only reduce damage but also actively promote the expression of critical barrier proteins, underscoring their potential therapeutic value in managing skin conditions characterized by barrier dysfunction, such as atopic dermatitis or psoriasis.

Despite these promising results, several limitations of the study should be considered. First, the reliance on in vitro and ex vivo models, while insightful, does not fully capture the complexity of the human skin environment, including the roles of immune responses and vascularization. To address this, it is essential to acknowledge the complementary role of in vivo studies in validating findings obtained from in vitro and ex vivo models.[30,31] While in vitro models provide controlled environments for studying skin barrier function and drug permeability, they lack the dynamic interactions present in living systems. Similarly, ex vivo models, though preserving structural integrity, do not fully replicate the physiological responses of intact human skin. Studies have highlighted the importance of integrating in vitro, ex vivo, and in vivo approaches to obtain a more comprehensive understanding of skin barrier mechanisms.[32,33] Furthermore, the development of advanced in vitro skin equivalents and organ-on-a-chip technologies has improved the physiological relevance of these models, bridging the gap between laboratory-based studies and clinical applications.[34] Future studies should focus on integrating multi-layered, vascularized skin models to better mimic immune responses, wound healing, and transdermal drug delivery in a physiologically relevant manner.

Additionally, the study primarily focused on the short-term effects of LBDE treatment, leaving the long-term impacts unexamined. Prolonged use of LBDEs might provide additional insights into sustained benefits or potential side effects.

While no cytotoxicity was observed in vitro at the tested concentrations, extended exposure studies are needed to assess potential immunogenic responses, microbiome interactions, and systemic absorption risks. Future research should focus on in vivo validation, large-scale safety assessments, and formulation stability to ensure clinical and commercial feasibility. Moreover, the investigation was limited to a few key skin barrier proteins, and the broader molecular mechanisms by which LBDEs enhance skin health remain to be elucidated. Previous studies suggest that probiotic-derived exosomes may regulate keratinocyte differentiation, lipid metabolism, and tight junction integrity, potentially through pathways involving Filaggrin (FLG), Loricrin (LOR), and Claudin-1.[35,36] Further research, including RNA sequencing and proteomic analysis, will be necessary to elucidate the exact pathways involved.

Furthermore, a suitable positive control for LOR and FLG upregulation was difficult to establish, as most skin barrier-related treatments primarily focus on anti-inflammatory effects or ceramide-based physical reinforcement rather than directly enhancing structural protein expression. To address this limitation, the ex vivo experiments demonstrated a simultaneous upregulation of multiple structural proteins, including Filaggrin (FLG), Loricrin (LOR), Claudin-1, and Aquaporin-3, providing strong evidence of LBDEs' role in barrier enhancement. This approach helped compensate for the lack of a direct positive control, allowing a more comprehensive evaluation of LBDEs' effects on skin barrier integrity. Future studies should explore additional comparative agents or well-characterized modulators of structural protein expression to further validate these findings.

To address these limitations, future studies should incorporate in vivo clinical trials to confirm the safety and efficacy of LBDEs in humans, particularly under long-term use conditions. Further research should also focus on understanding the molecular pathways through which LBDEs exert their effects, with an expanded focus on inflammatory processes and lipid metabolism. Comprehensive protein and lipid profiling using advanced techniques such as proteomics and lipidomics could provide deeper insights into the full scope of LBDE-mediated skin barrier enhancement. Additionally, optimizing delivery methods and determining the appropriate dosing regimen will be crucial for translating these findings into practical, clinically applicable treatments.

The findings from this study highlight the potential of *Lactobacillus brevis* J2K55-derived exosomes (LBDEs) in enhancing skin barrier function through the upregulation of key barrier-related proteins. The significant increase in Filaggrin, Claudin-1, Loricrin, Aquaporin-3, and Serine palmitoyltransferase expression suggests that LBDEs contribute to reinforcing the structural integrity of the skin barrier. These proteins play essential roles in maintaining epidermal hydration, lipid organization, and overall barrier stability, which are crucial for preventing external stress-induced skin damage. Additionally, the protective effects observed in the friction and UVB-induced skin damage model indicate that LBDEs may be effective in counteracting environmental stressors that compromise barrier function. These results suggest the potential application of microbial-derived exosomes in skincare formulations or therapeutic interventions aimed at strengthening the skin barrier and preventing dermatological conditions associated with barrier dysfunction.

This study suggests the potential of *Lactobacillus brevis* to produce exosomes and demonstrated their efficacy in strengthening the skin barrier through both in vitro and ex vivo experiments. Their ability to upregulate key structural proteins suggests potential use in skincare formulations targeting sensitive, dry, or damaged skin. Additionally, their natural origin and exosome-based delivery system provide advantages over traditional skincare ingredients, making them suitable for cosmeceuticals and therapeutic interventions. Further studies are needed to explore additional effects of these exosomes, such as anti-inflammatory, regenerative, whitening, anti-wrinkle, antioxidant, and moisturizing benefits, beyond skin barrier enhancement.

Author Contributions

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

Funding

This study was supported by the National Research Foundation of Korea (NRF) grant funded by the Korean government (MSIT) (2022R1C1C1007334). This work was supported by the Glocal University 30 Project Fund of Gyeongsang National University in 2025.

Disclosure

Yong-Han Cho, Ji-Woo Kim, Nari Kim, Hee-Sik Kim, Jun-Hwan Jang, and Jun-Tae Bae are employees of J2KBIO Corp., a company engaged in the commercial production of cosmetic raw materials. The company has a vested interest in the microorganisms discussed in this study. The remaining authors certify that they have no conflicts of interest.

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