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Research on the Synergistic Mechanisms of Dual-Ball PDRN and Dual-Vesicle in Repairing Mitochondrial Dysfunction

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Abstract: *Objective:* Mitochondrial dysfunction[1-2] is not only associated with various systemic diseases but also closely related to skin aging and skin conditions (such as melasma, acne, psoriasis, etc.). Mitochondrial dysfunction can lead to decreased energy metabolism, weakened antioxidant capacity, and accelerated apoptosis in skin cells, thereby causing problems such as skin sagging, increased wrinkles, and pigmentation. In contrast, through the synergistic action of multiple active ingredients, it is possible to comprehensively improve the energy metabolism of skin cell mitochondria, ATP production, NAD^+ levels per unit protein, mitochondrial morphology, and mitochondrial autophagy[3-5], thereby effectively repairing mitochondrial dysfunction: 1% Polyglycan® Probiotic PDRN (component content includes: hydrolyzed DNA 0.01-0.06%), 1% Polyglycan® Microspherical PDRN (component content: mannitol 55%, trehalose 30%, sodium DNA 10%, sodium hyaluronate 5%), 1% Polyglycan® Ginseng Root Extract (component content includes: ginseng root extract 95-96.8%), 0.35% Polyglycan® Red Ginseng Fermentation Vesicles (lactobacillus fermentation lysate 59.8-61.8%, lactobacillus/ginseng root fermentation filtrate 35-37%). This combination system, as a topical formulation, achieves the effect of repairing mitochondrial dysfunction. This technology stands out with its natural, convenient, non-invasive, and long-lasting effects, fundamentally solving skin aging problems and providing consumers with a safer, more comfortable, and comprehensive anti-aging experience. *Methods:* The raw materials of dual-ball PDRN (Polydeoxyribonucleotide) and dual-vesicles were dissolved and uniformly dispersed in a pre-designed matrix system separately to prepare a stable combination system for later use. Human dermal fibroblasts were exposed to UVA (ultraviolet A) irradiation to induce cellular damage and simulate a state of cellular stress. On this basis, the concentration of NAD^+ (nicotinamide adenine dinucleotide) per unit protein, the content of ATP (adenosine triphosphate), and the changes in mitochondrial morphology were detected to systematically evaluate the regulatory effects of the combination system on mitochondrial function. The regulatory effects of the combination system on autophagy were assessed by detecting the changes in LC3b (Microtubule-Associated Protein 1 Light Chain 3B) content. Through the above methods, the biological effects of the dual-ball PDRN and dual-vesicle combination system at the cellular level were comprehensively explored. Results:Based on UVA-irradiated fibroblasts, the concentration of NAD^+ per unit protein significantly increased by 29.52% and the content of ATP significantly increased by 105.88% after 24 hours of treatment with the combination. Mitochondrial morphology within the cells appeared as elongated rods, with normal fusion and fission occurring. After 48 hours of treatment, the concentration of NAD^+ per unit protein significantly increased by 34.86% and the content of ATP significantly increased by 141.03%. Based on fibroblasts, the content of human

microtubule-associated protein 1 light chain 3b (LC3b) significantly increased by 102.00% after 24 hours of treatment with the combination. *Conclusion:* In this study, the effects of the dual-ball PDRN and dual-vesicle combination system on mitochondrial function in fibroblasts were investigated. The results demonstrated that the combination system could improve mitochondrial morphology, increase the concentration of NAD^+ per unit protein and the content of ATP, indicating its regulatory role on mitochondrial function. Additionally, it could enhance the content of human microtubule-associated protein 1 light chain 3b (LC3b), showing its regulatory effect on autophagy.
Keywords: Spherical PDRN; Probiotic Spherical PDRN; Dual Vesicles; Mitochondrial Dysfunction; Anti-aging; Sensitive Skin

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1. Introduction

With the increasing demand for skin anti-aging solutions, a wide variety of anti-aging products and treatment options have emerged on the market. However, current solutions have many limitations, mainly in the following aspects: Limitations of existing technologies: The mechanisms of action are not comprehensive. Many existing anti-aging products mainly focus on improving surface symptoms, such as reducing wrinkles and brightening the skin tone, without addressing the root cause of cellular organelle dysfunction. Dysfunction of cellular organelles is one of the core mechanisms of skin aging, including the decline of mitochondrial function and the decrease of cellular energy metabolism. These factors can lead to the accumulation of cellular damage and accelerated aging. Lack of soothing and repair functions: Some anti-aging products may cause skin irritation during use, leading to discomfort. Beauty enthusiasts not only need anti-aging effects but also expect products to have soothing and repair properties to reduce skin irritation and promote the skin's self-repair ability. Insufficient long-term effects and stability: Some traditional anti-aging products may show certain effects in the short term, but their effects are often unstable with long-term use, and may even rebound or worsen skin problems. This is mainly because these products do not fundamentally solve the root causes of skin aging, such as the accumulation of cellular damage and organelle dysfunction. Given the limitations of existing anti-aging products, there is an urgent need in the market for a solution that can address the root causes of skin aging. The ideal anti-aging product should have the following characteristics: Comprehensive mechanism of action: It should target multiple aging mechanisms, including organelle dysfunction and the accumulation of cellular damage, to fundamentally improve skin health. Soothing and repair functions: While providing anti-aging benefits, it should also be able to soothe the skin, reduce irritation, and promote skin repair, enhancing the user experience. Long-term effects and stability: It should not only be effective in the short term but also maintain stable effects with long-term use, avoiding rebound or worsening of skin problems. Based on the above market demands and technological gaps, this invention proposes an innovative anti-aging cosmetic composition and its preparation method. This composition, through the synergistic action of multiple active ingredients, can comprehensively improve organelle dysfunction and reduce the accumulation of cellular damage, thereby fundamentally solving the problem of skin aging.

2. Experimental Section

2.1. Main Instruments and Reagents

DMEM culture medium (Gibco), newborn bovine serum (NBS, Lanzhou Rongyue), MTT (Sigma), DMSO (Sigma), PBS (Solarbio), quercetin (Aladdin), rapamycin (Sigma), paraformaldehyde (Biosharp), Cell Meter Live Cell ATP Detection Kit (Xi'an Beyotime), Mitochondrial Morphology Kit (Thermo Fisher), NAD^+/NADH Detection Kit (Beyotime), anti-human microtubule-associated protein 1 light chain 3b (LC3b) antibody (Abcam). CO_2 incubator (Thermo, 150I), laminar flow cabinet (Sujing Antai, SW-CJ-2F), microplate reader (BioTek, Epoch), inverted microscope (Olympus, CKX53),

fluorescence microscope (Olympus, BX43), super-resolution microscope (NanoInsights-Tech Co., Multi-SIM), UVA irradiation device (Philips), stirrer (EUROSTAR 60), Polyglycan® Spherical PDRN samples (Shaanxi Bohong Synthetic Biotechnology Co., Ltd., batch no. 241002), Polyglycan® Probiotic Spherical PDRN (Shaanxi Bohong Synthetic Biotechnology Co., Ltd., batch no. 231005), Polyglycan® Ginseng Root Extract (Shaanxi Microbelle Biotechnology Co., Ltd., batch no. 241113), Polyglycan® Red Ginseng Fermentation Vesicles (Shaanxi Microbelle Biotechnology Co., Ltd., batch no. 2410910), butylene glycol (OXEA), glycerin (Hangzhou Zanu Oil & Fat Technology Co., Ltd.), xanthan gum, preservatives.

2.2. Testing Methods

2.2.1. Dual-Ball PDRN and Dual-Vesicle Combination System

Polyglycan® Super-Assembled PDRN: Polyglycan super-assembly technology is a process that uses polysaccharides or derivative targeting polysaccharides as precursors. By leveraging or artificially designing weak interactions between molecules in each layer, it assembles peptides, proteins, nucleic acid active molecules, or other active small molecules. This spontaneous association forms structurally complete, stable, and functional molecular aggregates or supramolecular structures. Polydeoxyribonucleotide (PDRN) is a polynucleotide active substance with a molecular weight ranging from 50 to 1500 kDa, containing 50% double-stranded deoxyribonucleotides and 90% single-stranded deoxyribonucleotides. Polyglycan™ super-assembled PDRN has superior transdermal absorption and target recognition capabilities, which can repair damaged skin cells and maintain long-term skin stability. Polyglycan® Probiotic Spherical PDRN: Polyglycan® utilizes a layered microspherization controlled-release technology platform. By leveraging or designing weak interactions between molecules in each layer, it allows peptides, proteins, nucleic acids, polysaccharides, and other active macromolecules to interact with other active molecules, spontaneously forming structurally complete, stable, and functional molecular aggregates or particles. This technology uses probiotics as raw materials to extract PDRN, balancing the extracellular matrix and exerting corresponding effects. Polyglycan® Red Ginseng Fermentation Vesicles: Using red ginseng as the raw material, this technology employs synthetic biology principles for precise fermentation to address the issue of low active content of saponins. Utilizing probiotic extracellular vesicle technology, cells are restructured to encapsulate saponins, enhancing the permeability of active substances. Polyglycan® Ginseng Root Extract: Based on the application research of extracellular vesicles derived from natural animals, plants, and probiotics, this technology uses bioengineering to modify and enhance the efficacy of these vesicles. Ginseng extract is modified using this technology. Extracellular vesicles have a phospholipid bilayer structure similar to cell membranes, making them easily absorbed by cells and providing inherent permeability advantages. Technical adjustments can be made to enhance the drug delivery capabilities, stability, solubility, and targeting of these vesicles. The ginseng extract is enriched with bioactive components such as ginsenosides, which have strong antioxidant capabilities. They can neutralize free radicals and reduce oxidative stress damage to skin cells. They also promote skin microcirculation, increasing blood supply to the skin and thereby enhancing skin metabolism, which helps reduce the appearance of wrinkles and fine lines. Additionally, ginseng extract can stimulate collagen synthesis while inhibiting collagen degradation, thus enhancing skin elasticity and firmness.

Xanthan gum and humectants were mixed, and purified water was added. The mixture was stirred uniformly at 80-85°C to obtain phase A. After adding the skin feel regulator to phase A and cooling it down to 40-45°C, the following four components were added in proportion: 1% Polyglycan® Probiotic Spherical PDRN (containing 0.01-0.06% hydrolyzed DNA), 1% Polyglycan® Microspherical PDRN (containing 55% mannitol, 30% trehalose, 10% sodium DNA, and 5% sodium hyaluronate), 1% Polyglycan® Ginseng Root Extract (containing 95-96.8% ginseng root extract), and 0.35% Polyglycan® Red Ginseng Fermentation Vesicles (containing 59.8-61.8% lactobacillus fermentation lysate and 35-37% lactobacillus/ginseng root fermentation filtrate). Preservatives were also added and the mixture was kept at a constant temperature for 30 minutes to obtain the final composition.

2.2.2. Cell Viability Assay Method

Cell Seeding:After thawing the cells, when the confluence reaches approximately 60%, seed the cells into a 96-well plate and incubate overnight in a CO_2 incubator (37°C, 5% CO_2). Experimental Grouping: The experiment includes a zero-adjustment group, a solvent control group, a positive control group, and a sample group. In the sample group, each sample is tested at eight different concentrations, with three replicate wells for each concentration. When the confluence in the 96-well plate reaches 50% to 60%, the cells are treated with the respective agents. The solvent control group receives 200 μL of culture medium per well, the positive control group receives 200 μL of culture medium containing 10% DMSO per well, and the sample group receives 200 μL of culture medium containing the respective concentration of the test sample per well. The zero-adjustment group has no cell seeding and only receives 200 μL of cell culture medium. After treatment, the 96-well plate is placed back into the CO_2 incubator (37°C, 5% CO_2) for 24 hours of cultivation. After 24 hours of incubation, discard the supernatant and add MTT working solution (0.5 mg/mL), then incubate at 37°C in the dark for 4 hours. After incubation, discard the supernatant again and add 150 μL of DMSO to each well, then read the OD value at 490 nm. Calculation of Relative Cell Viability:Relative cell viability (%) = (Sample well OD - Zero-adjustment well OD) / (Solvent control well OD - Zero-adjustment well OD) × 100%.

2.2.3. Cell Morphology Assay Method

After thawing the cells, when the confluence reaches approximately 60%, seed the cells into a 24-well plate and incubate overnight in a CO_2 incubator (37°C, 5% CO_2). The experiment includes a solvent control group and a sample group. In the sample group, each sample is tested at five different concentrations. When the confluence in the 24-well plate reaches 50% to 60%, the cells are treated with the respective agents. The solvent control group receives 1 mL of culture medium per well, and the sample group receives 1 mL of culture medium containing the respective concentration of the test sample per well. After treatment, the 24-well plate is placed back into the CO_2 incubator (37°C, 5% CO_2) for 24 hours of cultivation. After incubation, discard the supernatant and take photographs under an inverted microscope.

2.2.4. Testing on Fibroblasts Irradiated with UVA

After thawing the cells, when the confluence reaches approximately 60%, seed the cells into a 6-well plate and incubate overnight in a CO_2 incubator (37°C, 5% CO_2). Prepare the working solutions of the test substances according to the test groups, and conduct tests using the WST-8 colorimetric method and fluorescence probe method. Dosage Administration:According to the test groups, when the confluence in the 6-well plate reaches 30% to 50%, administer the substances to the cells. For the blank control group and negative control group, add 2 mL of culture medium per well. For the positive control group, add 2 mL of culture medium containing quercetin per well. For the sample group, add 2 mL of culture medium containing the respective concentration of the test sample per well. After administration, place the 6-well plate back into the CO_2 incubator (37°C, 5% CO_2) for 24 hours of incubation. UVA Irradiation: According to the test groups, except for the blank control group, all other groups are subjected to UVA irradiation at a dose of 30 J/cm². After irradiation, continue to culture in the CO_2 incubator (37°C, 5% CO_2) for an additional 24 or 48 hours. NAD^+ Concentration per Unit Protein Detection:After incubation, collect the cells and perform the detection according to the instructions of the NAD^+/NADH Detection Kit. ATP Content Test: Conduct ATP content detection using the Cell Meter Live Cell ATP Detection Kit. Observe the staining results under a fluorescence microscope, take photos, and collect images for analysis using Image-Pro® Plus software.Calculation of Enhancement Rate: Enhancement rate (%) = (Sample group - Negative control group) / Negative control group × 100%.

2.2.5. Testing Based on Fibroblasts

Cell Seeding: After thawing the cells, when the confluence reaches approximately 60%, seed the cells into a 24-well plate and incubate overnight in a CO_2 incubator (37°C, 5% CO_2). Preparation of Solutions: Prepare the working solutions of the test substances according to the test groups. Dosage Administration: According to the test groups, when the confluence

in the 24-well plate reaches 40% to 60%, administer the substances to the cells. For the blank control group, add 1 mL of culture medium per well. For the positive control group, add 1 mL of culture medium containing rapamycin per well. For the sample group, add 1 mL of culture medium containing the respective concentration of the test sample per well. After administration, place the 24-well plate back into the CO_2 incubator (37°C, 5% CO_2) for 24 hours of incubation. LC3b Content Test: Fix the cells with 4% paraformaldehyde for 30 minutes, followed by immunofluorescence detection. Observe the staining results under a fluorescence microscope, take photos, and collect images for analysis using Image-Pro® Plus software. Calculation of Enhancement Rate:Enhancement rate (%) = (Sample group - Negative control group) / Negative control group × 100%.

2.3. Statistical Analysis

Data Analysis:GraphPad Prism was used for data plotting, with results expressed as Mean ± SD. Comparisons between groups were conducted using the t-test for statistical analysis. All statistical results were two-tailed. A P-value < 0.05 was considered to indicate a significant difference, a P-value < 0.01 was considered to indicate a highly significant difference, and a P-value < 0.001 was considered to indicate an extremely significant difference.

3. Test Results

3.1. Cell Viability Assay

Based on the results of the MTT assay and morphological observations, it was concluded that the samples did not exhibit significant cytotoxicity at a concentration of 2.5% in fibroblast (**Figure 1).**

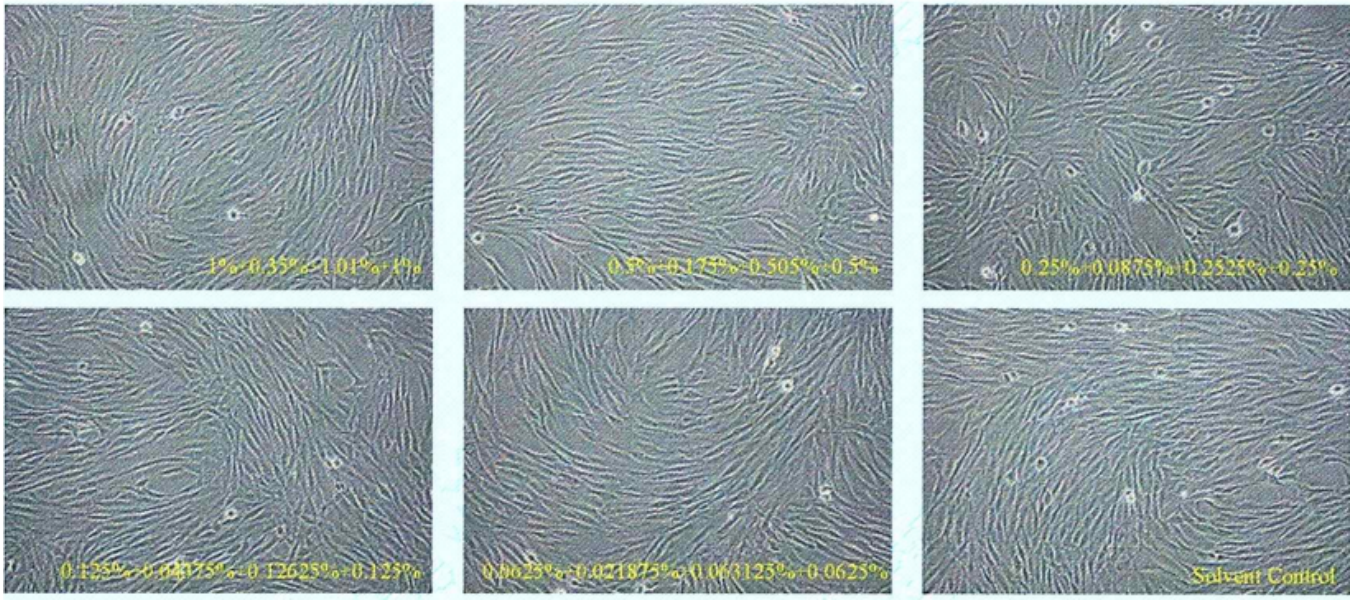

Figure 1. Cell Viability and Morphology Diagram

3.2. Test Results Based on UVA-Irradiated Fibroblasts

As shown in **Table 2**, compared with the BC group, the concentration of NAD^+ per unit protein in the NC group significantly decreased, indicating that the stimulation conditions used in this test were effective. Compared with the NC group, the concentration of NAD^+ per unit protein in the PC group significantly increased, indicating that the positive control used in this test was effective. Compared with the NC group, the concentration of NAD^+ per unit protein in the sample group significantly increased at a certain concentration, with an increase rate of 29.52% after 24 hours and 34.86% after 48 hours (**Table 1-2**).

Table 1. Summary of NAD^+ Concentration per Unit Protein - 24h Detection Results

Sample Name	Relative Average IOD	SD	*P*-value	Enhancement Rate(*vs*NC)
BC(Blank)	205.29	13.61	/	/
NC(Negative)	77.60	1.14	0.000###	/
PC(Quercetin 300nM)	101.40	3.04	0.000***	/
Sample Group	100.51	2.52	0.000**	29.52%

Table 2. Summary of NAD^+ Concentration per Unit Protein - 48h Detection Results

Sample Name	Relative Average IOD	SD	*P*-value	Enhancement Rate(*vs*NC)
BC(Blank)	307.64	2.63	/	/
NC(Negative)	133.84	1.51	0.000###	/
PC(Quercetin 300nM)	191.40	2.25	0.000***	/
Sample Group	180.49	1.43	0.000**	34.86%

Note 1:When performing statistical analysis using the 1test method, significance compared to the BC group is indicated by #, with P-value < 0.05 represented as #, P-value < 0.01 represented as ##, and P-value < 0.001 represented as ###; significance compared to the NC group is indicated by *, with P-value < 0.05 represented as *, P-value < 0.01 represented as **, and P-value < 0.001 represented as ***.

Note 2: P-value < 0.05 is indicated by #, indicating a significant difference; P-value < 0.01 is indicated by ##, indicating a highly significant difference; P-value < 0.001 is indicated by ###, indicating an extremely significant difference. P-value < 0.05 is also indicated by *, indicating a significant difference; P-value < 0.01 is indicated by **, indicating a highly significant difference; P-value < 0.001 is indicated by ***, indicating an extremely significant difference.

3.3. ATP Content Test Results

As shown in **Table 3**, compared with the BC group, the ATP content in the NC group significantly decreased, indicating that the stimulation conditions used in this test were effective. Compared with the NC group, the ATP content in the PC group significantly increased, indicating that the positive control used in this test was effective. Compared with the NC group, the ATP content in the sample group significantly increased at a certain concentration, with an increase rate of 105.88% after 24 hours and 141.03% after 48 hours(**Table 3-4**).

Table 3. Summary of ATP Detection Results - 24h

Sample Name	Relative Average IOD	SD	*P*-value	Enhancement Rate(*vs*NC)
BC(Blank)	1.00	0.06	/	/
NC(Negative)	0.51	0.07	0.001##	/
PC(Quercetin 300nM)	0.97	0.06	0.001**	/
Sample Group	1.05	0.01	0.000***	105.88%

Table 4. Summary of ATP Detection Results - 24h

Sample Name	Relative Average IOD	SD	*P*-value	Enhancement Rate(*vs*NC)
BC(Blank)	1.00	0.13	/	/
NC(Negative)	0.39	0.08	0.002##	/
PC(Quercetin 300nM)	0.89	0.04	0.001**	/
Sample Group	0.94	0.05	0.001***	141.03%

3.4. Mitochondrial Morphology Results

As shown in **Figure 2**, compared with the BC group, the mitochondria in the NC group exhibited fusion impairment, indicating that the stimulation conditions used in this test were effective. Compared with the NC group, the mitochondria in the PC group appeared as elongated rods with normal fusion and fission, indicating that the positive control used in this test was effective. Compared with the NC group, the mitochondria in the sample group appeared as elongated rods with normal fusion and fission at a certain concentration, indicating that the sample could improve the mitochondrial morphology in fibroblasts at this concentration(**Figure 2**).

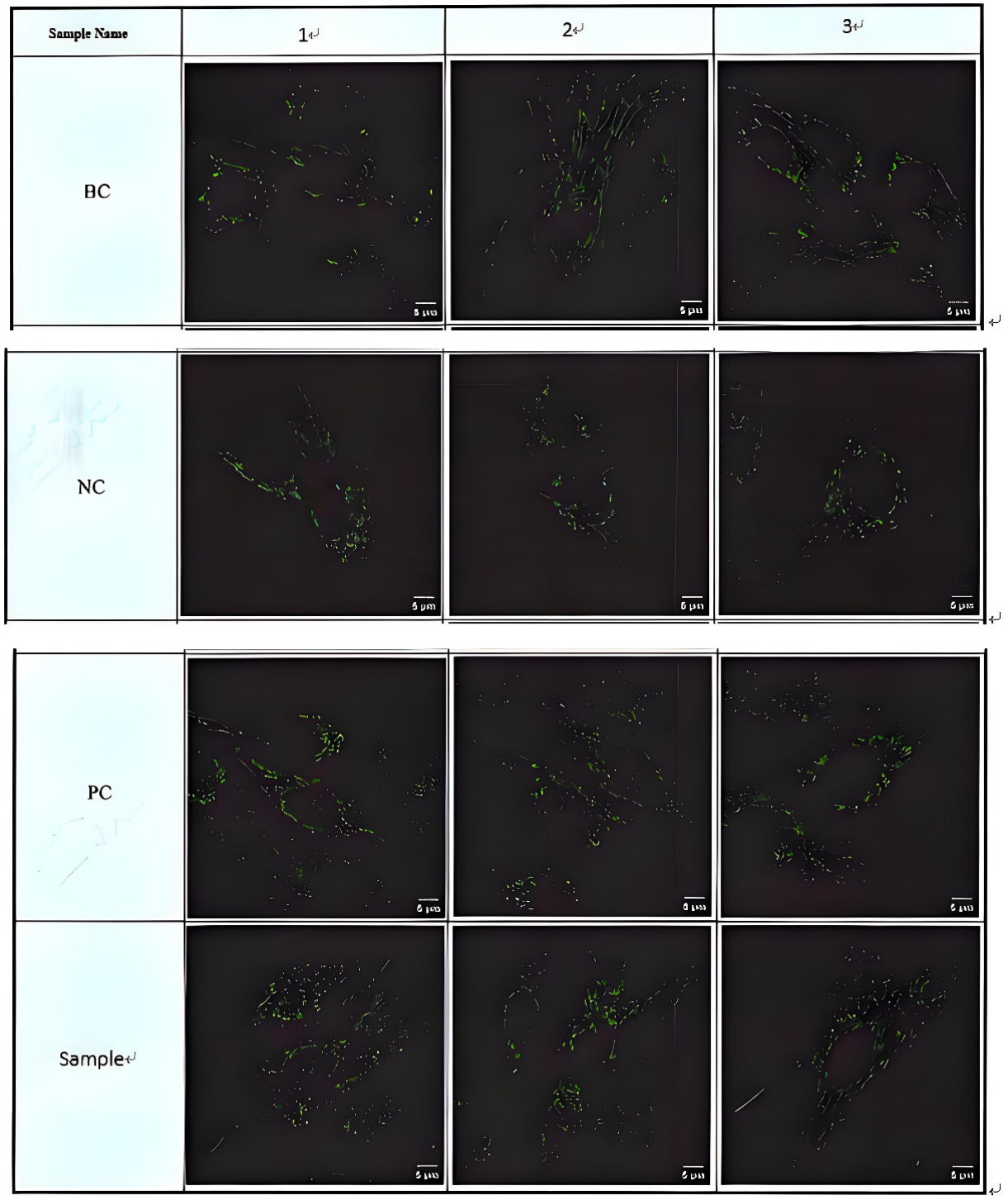

Figure 2. Mitochondrial Morphology Diagram

3.5. Test Results Based on Fibroblasts

As shown in **Table 4**, compared with the BC group, the LC3b content in the PC group significantly increased, indicating that the positive control used in this test was effective. Compared with the BC group, the LC3b content in the sample group significantly increased at a certain concentration, with an increase rate of 102.00%(**Table 5**).

Table 5. Summary of LC3b Content Results

Sample Name	Relative Average IOD	SD	*P*-value	Enhancement Rate(*vs*NC)
BC(Blank)	1.00	0.16	/	/
PC(Rapamycin 50 nM)	2.29	0.32	0.003**	/
Sample Group	2.02	0.04	0.000***	102.00%

4. Conclusion

In this study, four combinations of substances were used: 1% Polyglycan® Probiotic Spherical PDRN (containing 0.01-0.06% hydrolyzed DNA), 1% Polyglycan® Microspherical PDRN (containing 55% mannitol, 30% trehalose, 10% sodium DNA, and 5% sodium hyaluronate), 1% Polyglycan® Ginseng Root Extract (containing 95-96.8% ginseng root extract), and 0.35% Polyglycan® Red Ginseng Fermentation Vesicles (containing 59.8-61.8% lactobacillus fermentation lysate and 35-37% lactobacillus/ginseng root fermentation filtrate). These were applied to fibroblasts using a topical application technique.Based on UVA-irradiated fibroblasts, at a certain concentration, the combination significantly increased the concentration of NAD^+ per unit protein by 29.52% and the content of ATP by 105.88% after 24 hours of treatment. The mitochondrial morphology within the cells appeared as elongated rods with normal fusion and fission. After 48 hours of treatment, the concentration of NAD^+ per unit protein significantly increased by 34.86%, and the content of ATP significantly increased by 141.03%. These results indicate that the combination can improve mitochondrial morphology and increase the concentration of NAD^+ per unit protein and ATP content, demonstrating its regulatory effect on mitochondrial function.Based on fibroblasts, at a certain concentration, the combination significantly increased the content of human microtubule-associated protein 1 light chain 3b (LC3b) by 102.00% after 24 hours of treatment. This indicates that the combination can enhance the content of LC3b, showing its regulatory effect on autophagy.

5. Discussion

This study thoroughly investigated the potential applications of four combinations of substances (1% Polyglycan® Probiotic Spherical PDRN, 1% Polyglycan® Microspherical PDRN, 1% Polyglycan® Ginseng Root Extract, and 0.35% Polyglycan® Red Ginseng Fermentation Vesicles) in the field of anti-aging. A series of experiments confirmed that this combination system can significantly increase the cellular content of ATP and NAD^+, improve mitochondrial morphology, and promote autophagy at specific concentrations. These findings provide strong scientific evidence for the application of topical techniques in the field of anti-aging. ① Improvement of Mitochondrial Function: Experimental results showed that, in the fibroblast model irradiated with UVA, after 24 hours of treatment with the combination at a certain concentration, the concentration of NAD^+ per unit protein significantly increased by 29.52%, and the content of ATP significantly increased by 105.88%. The mitochondrial morphology within the cells appeared as elongated rods with normal fusion and fission. After 48 hours of treatment, the concentration of NAD^+ per unit protein significantly increased by 34.86%, and the content of ATP significantly increased by 141.03%. These data indicate that the combination can effectively improve mitochondrial morphology and increase the concentration of NAD^+ and ATP, thereby regulating mitochondrial function. As the powerhouses of the cell, the improved function of mitochondria is of great significance for maintaining cellular vitality and delaying aging. ② Regulation of Autophagy:Further experiments showed that, in the fibroblast model, after 24 hours of treatment with the combination at a certain concentration, the content of human

microtubule-associated protein 1 light chain 3b (LC3b) significantly increased by 102.00%. LC3b is a key protein in the process of autophagy [6-7], and its significant increase indicates that the combination can effectively regulate autophagy. Autophagy is an important mechanism for cells to clear damaged and aged organelles. By promoting autophagy, the combination helps maintain the healthy state of cells and delay cellular aging. ③ Advantages of the Combination System:The combination system used in this study has unique advantages. On the one hand, the dual-sphere PDRN (Polyglycan® Probiotic Spherical PDRN and Polyglycan® Microspherical PDRN) can provide a variety of bioactive substances to promote cellular metabolism and repair. On the other hand, the dual-vesicle (Polyglycan® Ginseng Root Extract and Polyglycan® Red Ginseng Fermentation Vesicles) can effectively protect and deliver active ingredients, enhancing their permeability and bioavailability in the skin. This combination of dual-sphere and dual-vesicle not only enhances the synergistic effect of each component but also improves the stability and effectiveness of the product. ④ Clinical Application Prospects:The findings of this study provide strong scientific evidence for the application of topical techniques in the field of anti-aging. By improving mitochondrial function and regulating autophagy, this combination system is expected to become an effective anti-aging treatment. In clinical applications, this topical product can be conveniently applied to the skin surface, providing consumers with a simple and effective anti-aging option. Future research will further explore the application effects of this combination system in different skin types and stages of aging, as well as its long-term safety and effectiveness.

In conclusion, this study experimentally verified the significant effects of the four combinations of substances in improving mitochondrial function and regulating autophagy, providing strong support for the application of topical techniques in the field of anti-aging. With further research and development, this combination system is expected to become an innovative anti-aging solution. In summary, the combination system of dual-sphere PDRN and dual-vesicle has shown significant anti-aging effects by repairing mitochondrial dysfunction. Its topical application form is not only convenient to use but also provides long-term and stable protection for the skin, with broad application prospects.

Disclosure statement

The author declares no conflict of interest.

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