



Purification and Functional Evaluation of Exosome-like Nanoparticles Derived from Pavlova

Moving the Heart



Rohto Pharmaceutical Co., Ltd.

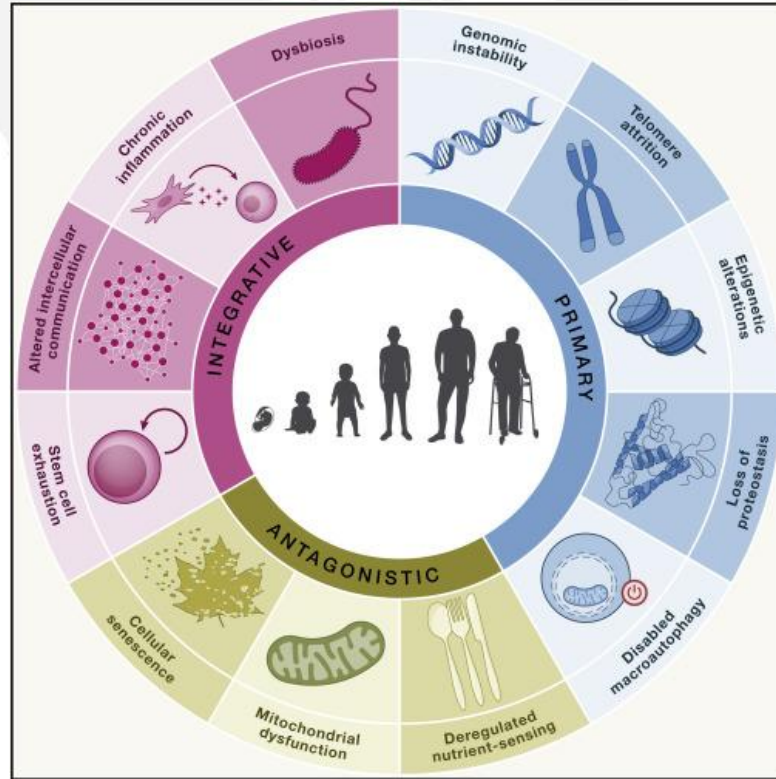
Basic Research and Development Division

Shun Kimura, Nozomi Hirose, Yuya Hayashi and Noriko Suenobu



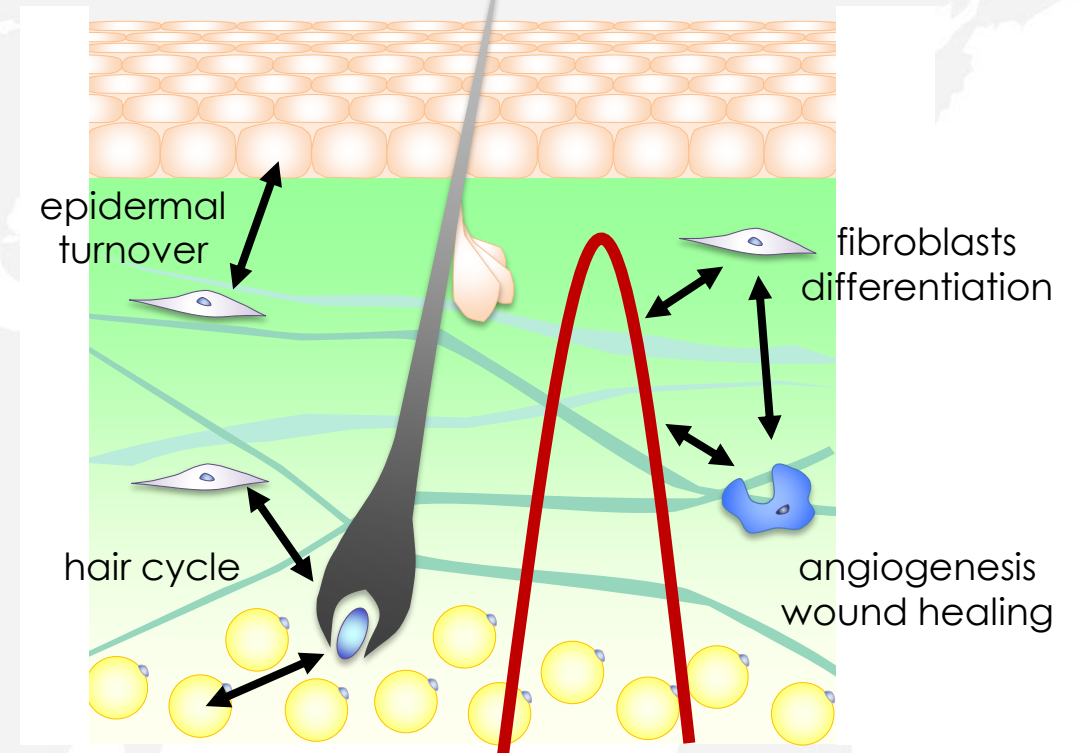
Intracellular communication is essential for homeostasis

Intercellular communication is one of the hallmarks of aging



Carlos López-Otín et al. Cell, 2023; Volume 186, Issue 2, 243-278

Intercellular Communication in the human skin

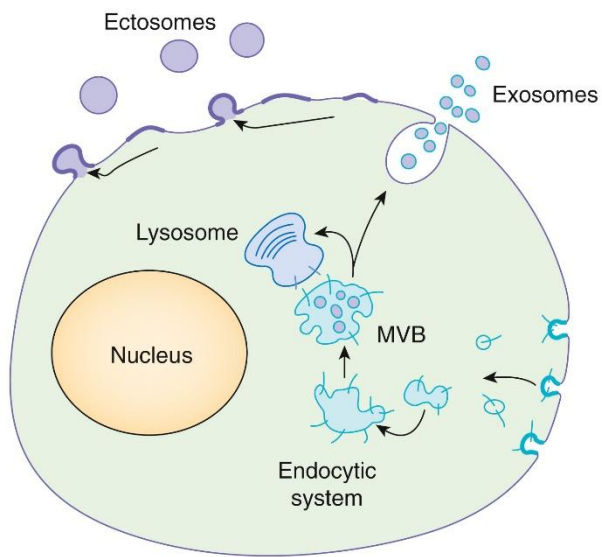


Kimura S, Tsuji T. Int J Mol Sci. 2021 May 22;22(11):5474.
Kimura S et al. EMBO Rep. 2026 May;27(9):2319-2344.

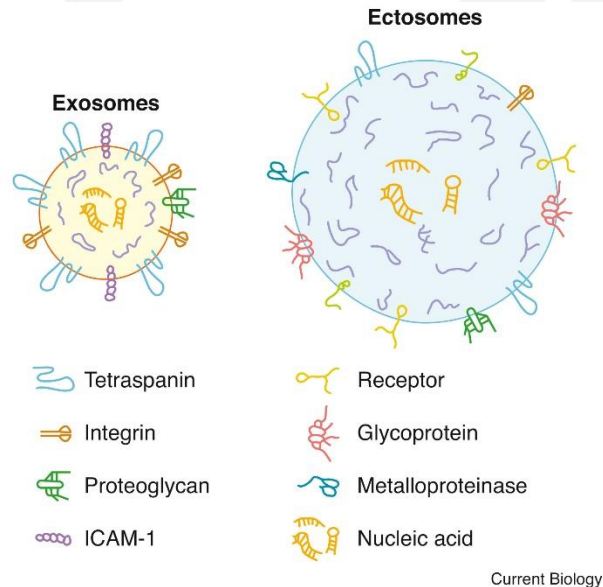
Important target mechanism for developing advanced skincare aimed at caring for skin morphology and function

Extracellular Vesicles in Intercellular Communication

Extracellular vesicles



Current Biology

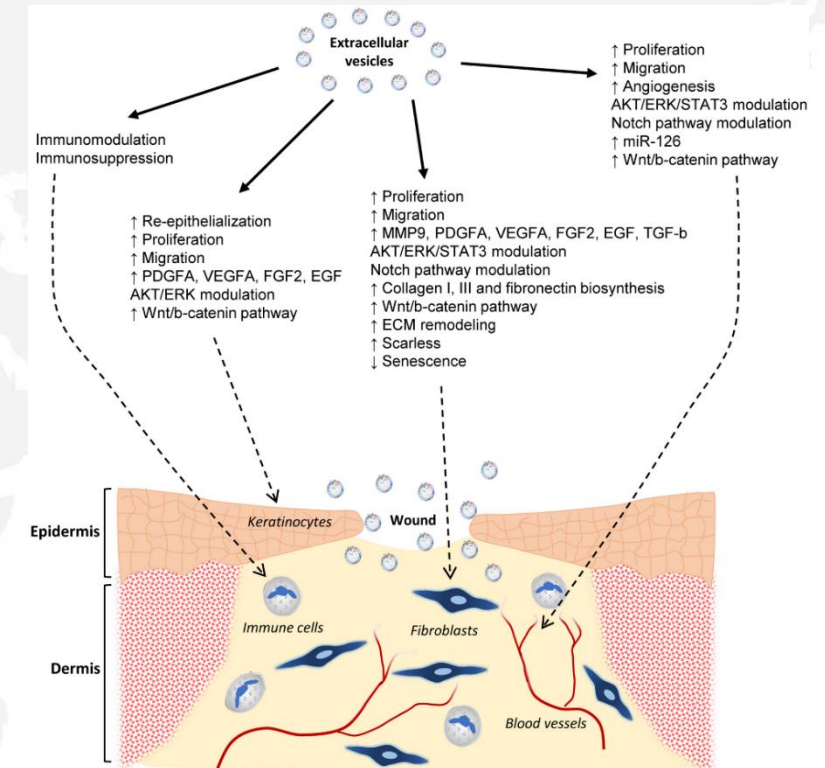


Current Biology

Definition of exosome
endosome-derived extracellular vesicles
typically less than 200 nm in diameter.

Ref. Meldolesi J et al. Current Biology, 28, R435-R444

Effects of human MSC-derived exosomes on skin wound healing



Ref. Casado-Díaz A et al. Front. Bioeng. Biotechnol. 8:146. 2020

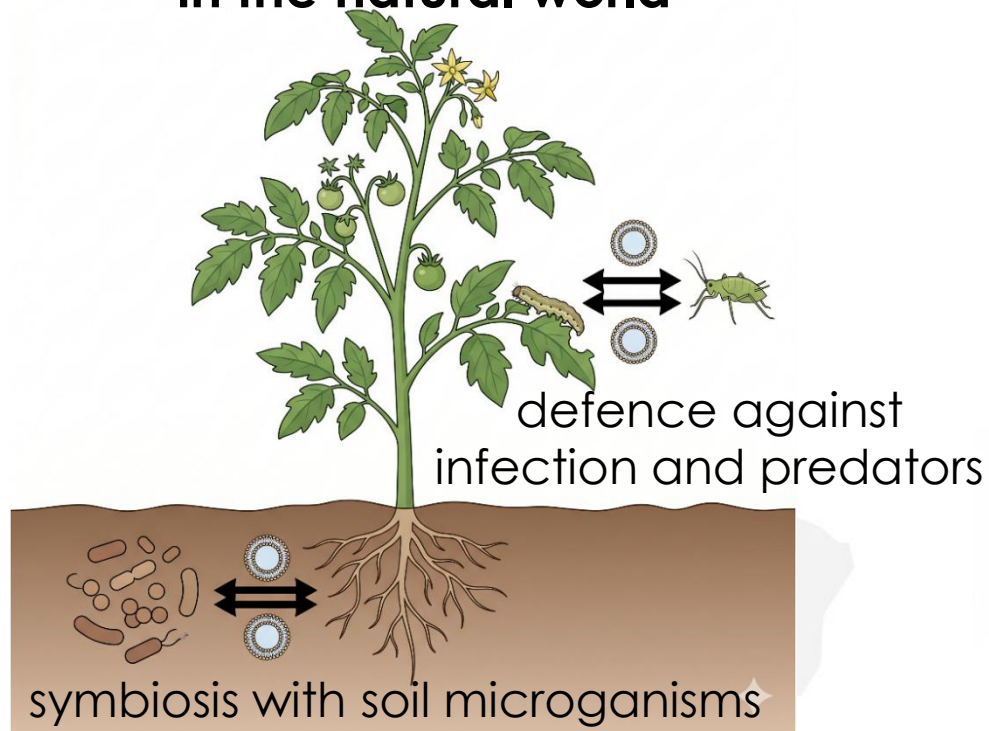
While human-derived exosomes offer high functional potential, ensuring their safety and quality requires extraordinarily rigorous evaluation.

Cross-Kingdom communications mediated by Phyto-exosomes

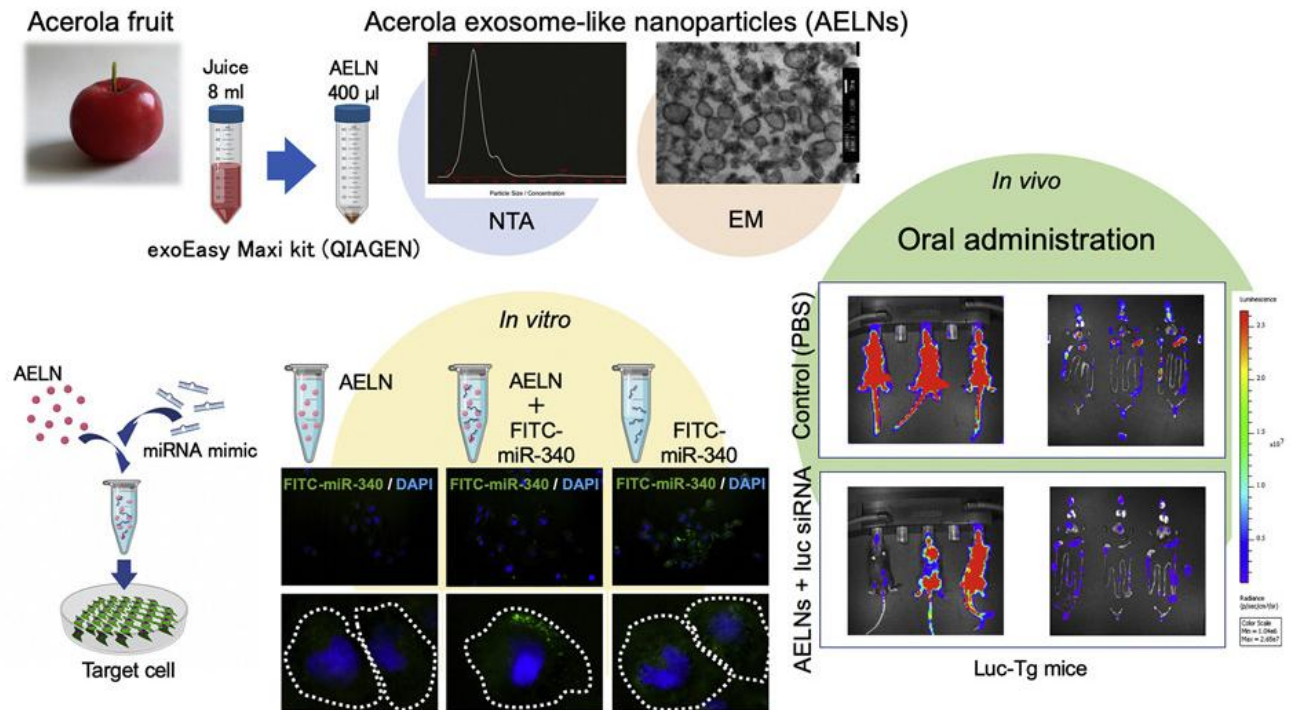
“Phyto-exosomes”

lipid bilayer exosome like nano-vesicles (ELNs) of non-animal natural origin

Cross-Kingdom communication
in the natural world



DDS potential of fruit juice derived ELNs



Umezumi T et al. Mol Ther Methods Clin Dev. 2021 Mar 10;21:199-208.

Phyto-exosomes represent a treasure trove of bioactive molecules and, can be exploited as natural drug delivery systems (DDS).

Which biological species should we source phyto-exosomes?



We turned our attention to Japan's rich biodiversity, supported by its unique climate, and to fermentation technologies as key assets.

Selection of *Pavlova* from the Okinawan Microalgae Library

A library of approximately 1000 species of algae native to Okinawa.



Comparison of nutrient content

	Pavlova	Spirulina	Chlorella	Euglena
	> 61	> 50	> 59	> 59
Protein (g/100g)	46.9	65.8	58	50.1
Lipids(g/100g)	23.8	7.5	8.9	21.3
DHA(mg/100g)	460	—	—	100
EPA(mg/100g)	3760	—	—	300

Pavlova sp...

- inhabiting the mangrove of northern Okinawa.
- Strong resilience to environmental stress
Dryness, UV, heat, osmotic pressure
- contains abundant functional molecules
(omega-3 fatty acids and fucoxanthin)

We selected *Pavlova* as our candidate organism, expecting it to yield phyto-exosomes with exceptional stress-resilience properties.

Manufacturing of Pav-ELNs

7-ton-scale cultivation plant
utilizing deep ocean water in Okinawa



harvest
culture medium

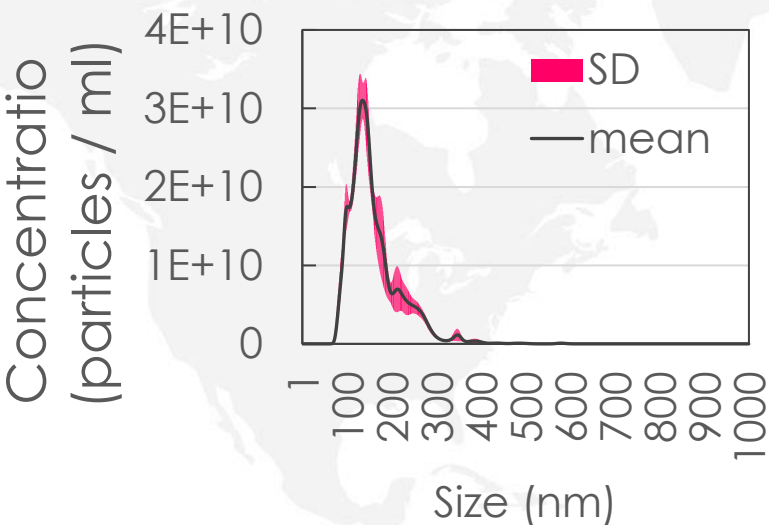
Purification process

1. Thermal treatment
105°C, 4 min
2. Filtration
0.2 μ m membrane
3. Concentration
300 kDa hollow fiber
membrane
4. Filtration
0.22 μ m membrane

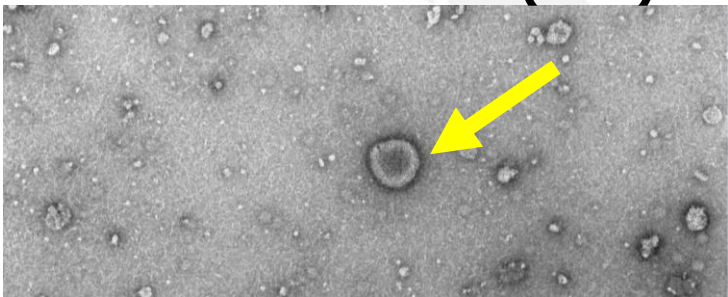
We established a stable, industrial-scale manufacturing process.

Characterization of Pav-ELNs

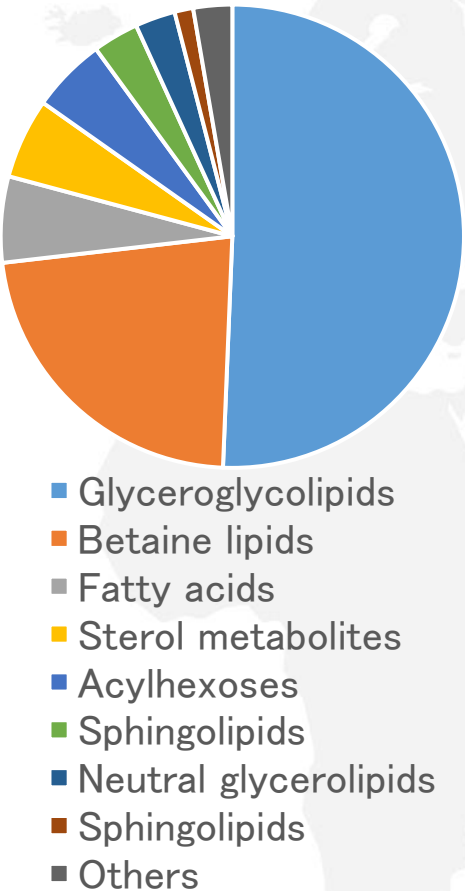
Particle size & concentration



Particle structure (TEM)



Lipidome of Pav-ELNs



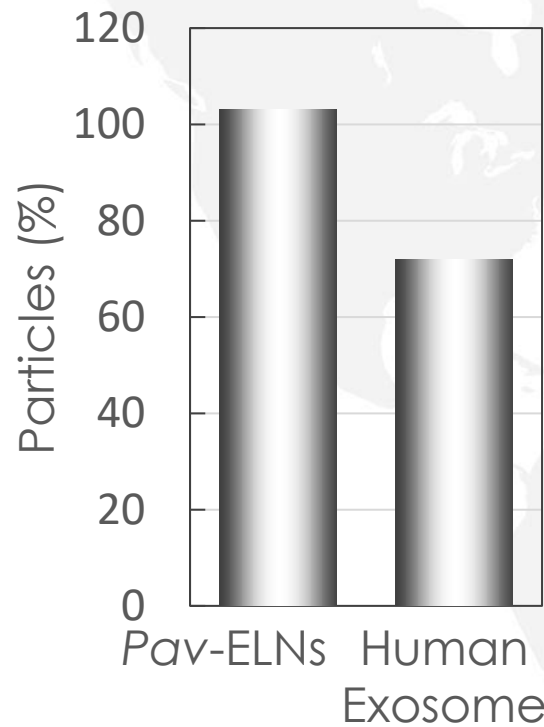
Size distribution of RNA within vesicles



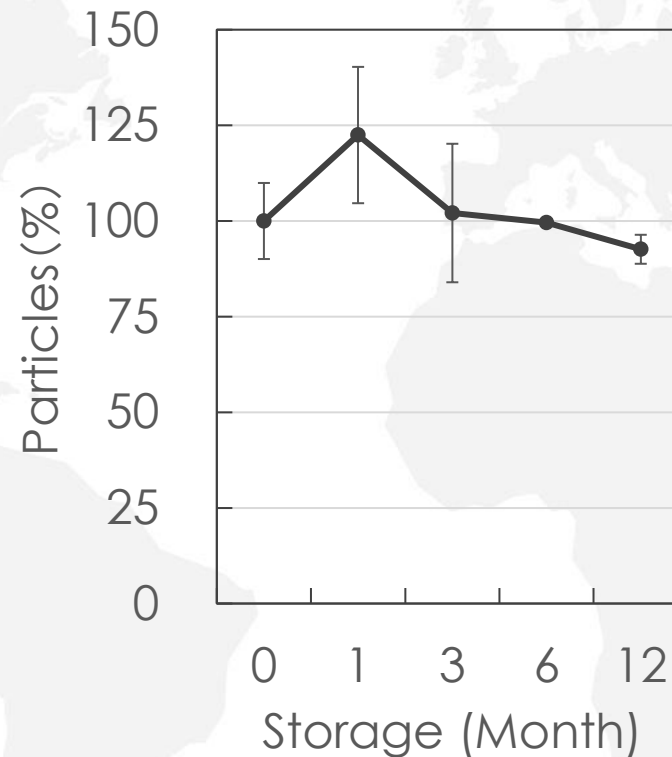
Pav-ELNs are lipid bilayer nanoparticles containing functional lipids and RNA.

Stability and DDS function of Pav-ELNs

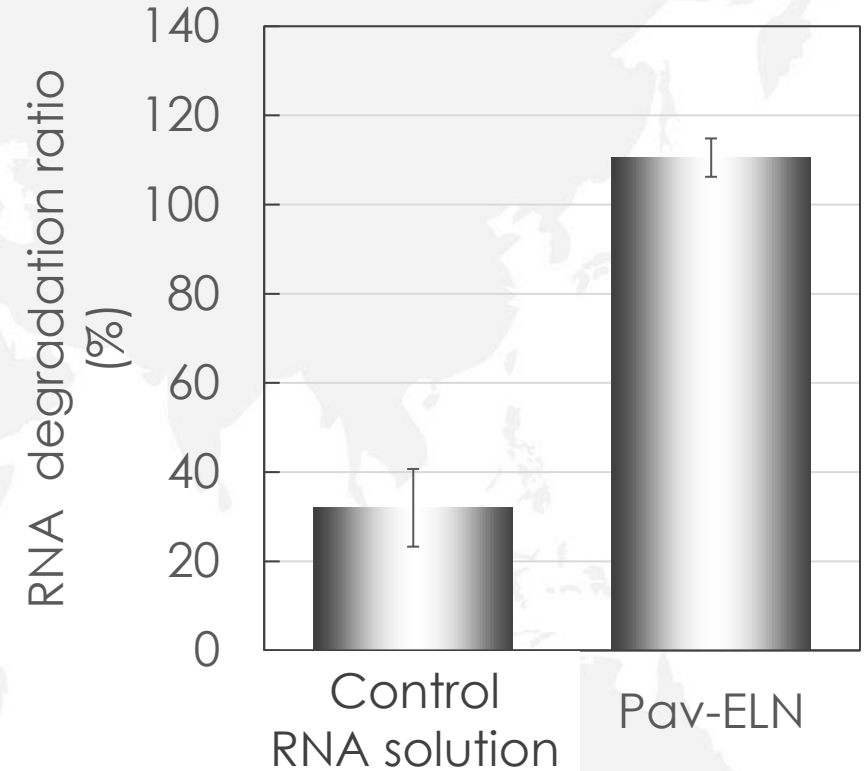
**Stability test at 40°C
for three months**



**Stability test at 4°C
in Pav-ELNs/30% BG
formulation**



**ELNs-RNA stability
after RNase I treatment**



Pav-ELNs are stable over extended periods in polyol solutions and are capable of protecting their encapsulated cargo from degradation.

Cellular Uptake of *Pav*-ELNs

Method: PKH26-labeled *Pav*-ELNs were added to skin cells and incubated for 24hr.

Epidermal Keratinocytes

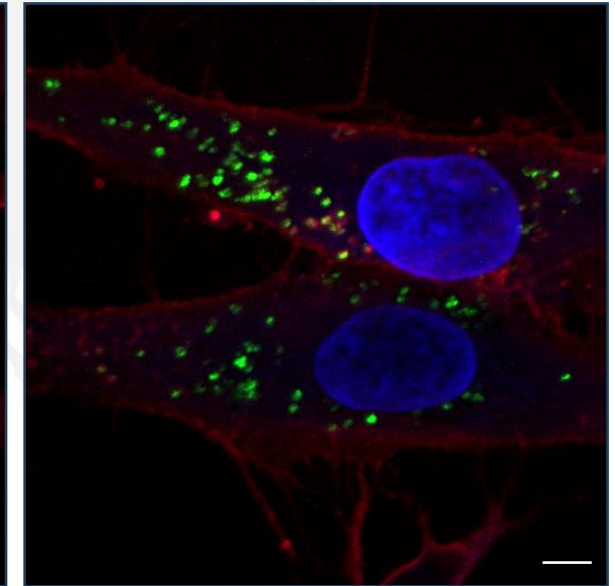
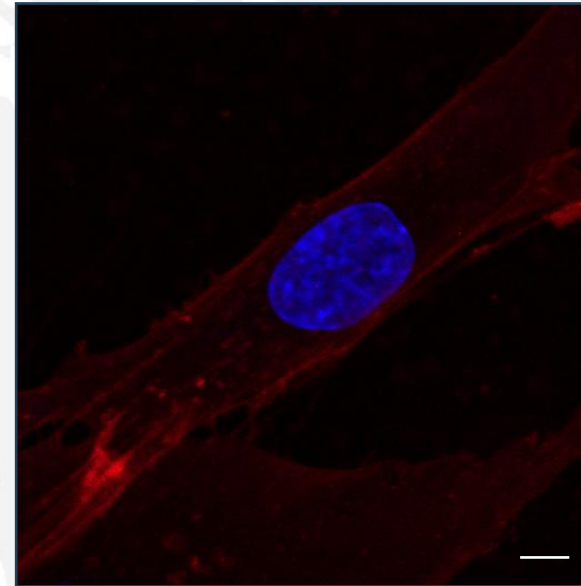
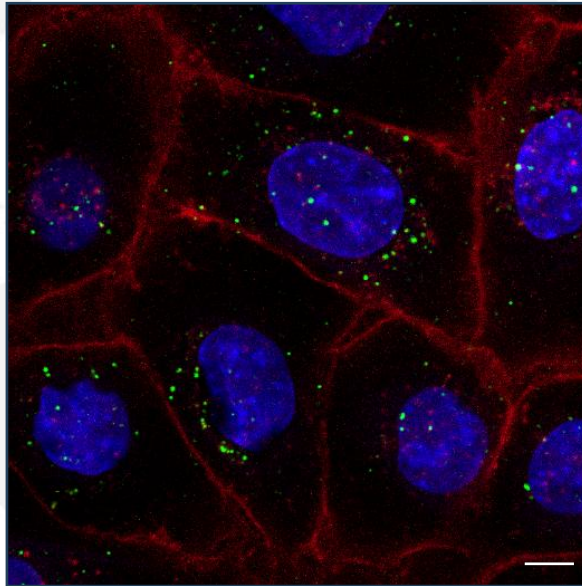
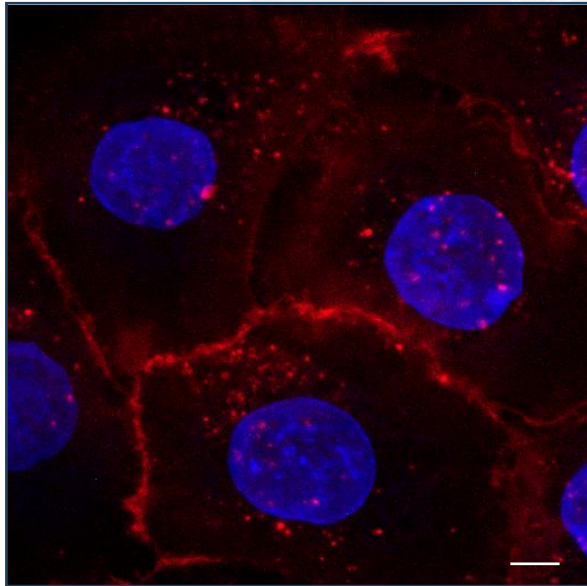
Dermal Fibroblasts

negative control
WGA/Hoechst33342

PKH26 labelled *Pav*-ELNs
WGA/Hoechst33342

negative control
WGA/Hoechst33342

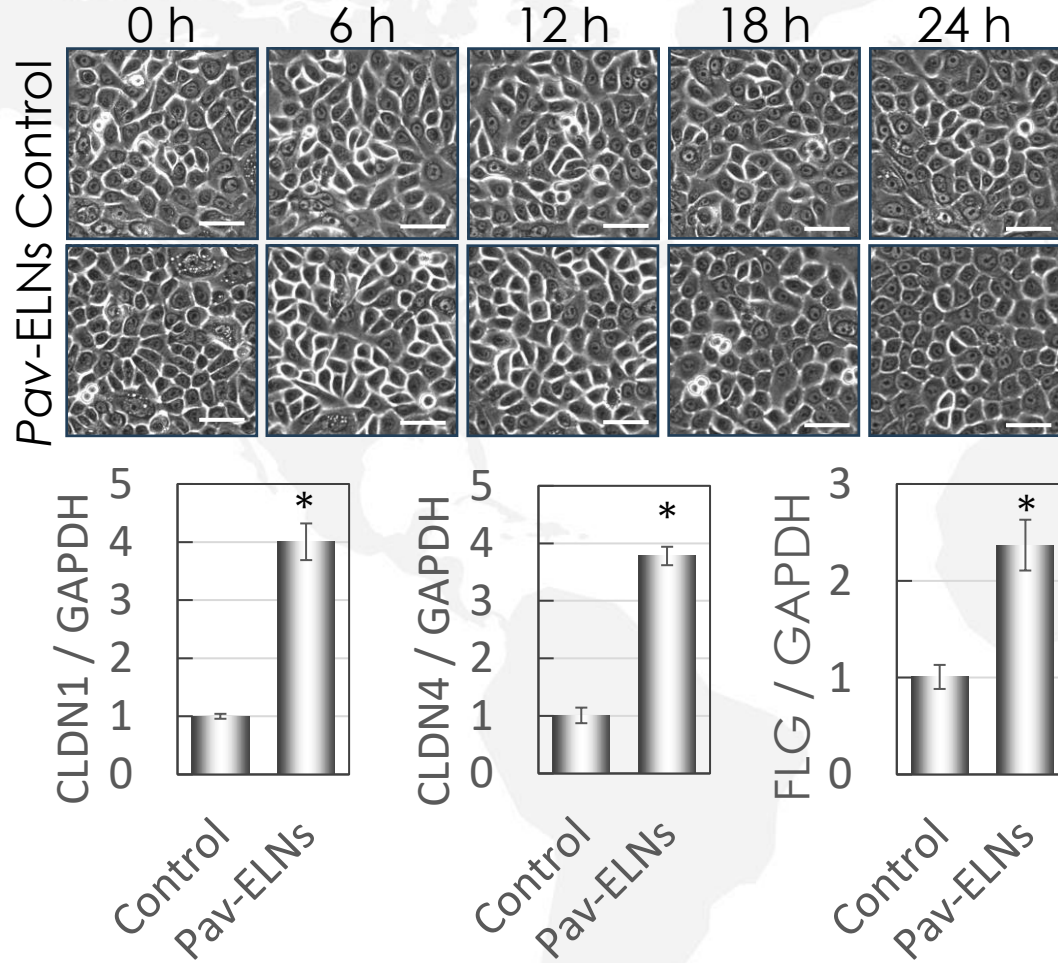
PKH26 labelled *Pav*-ELNs
WGA/Hoechst33342



Pav-ELNs are internalized and accumulated within both skin cells.

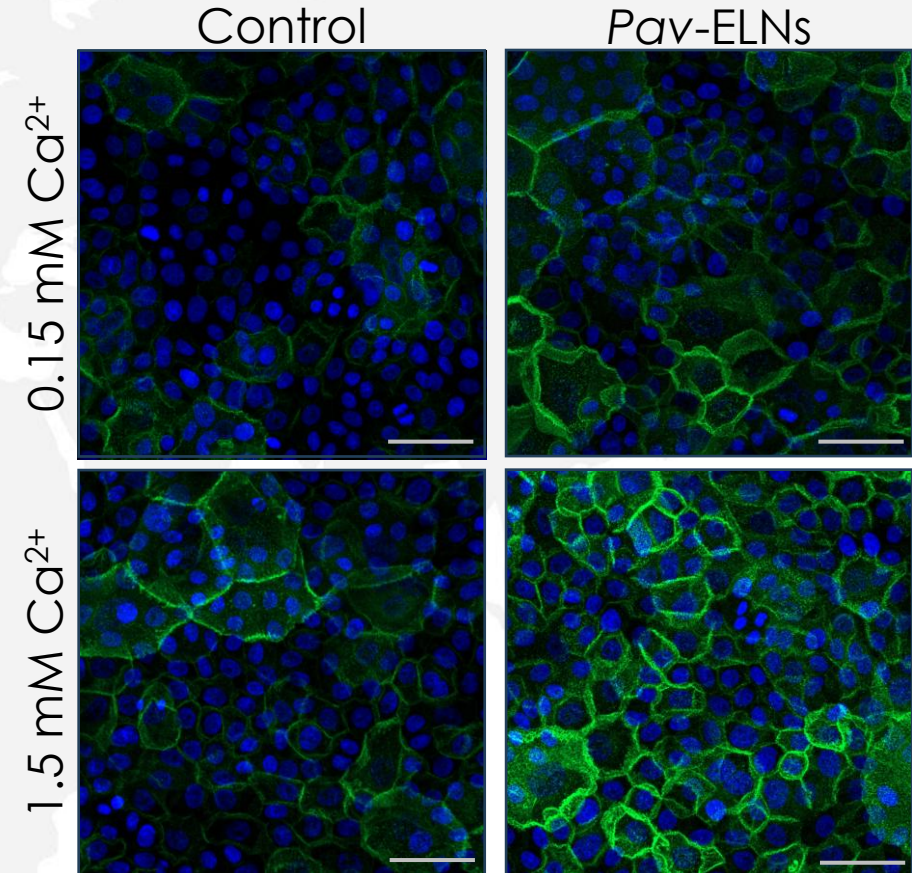
Effects of *Pav*-ELNs on Epidermal Keratinocytes

Epidermal cell differentiation induction



* $P < 0.001$, as assessed by student's t-test

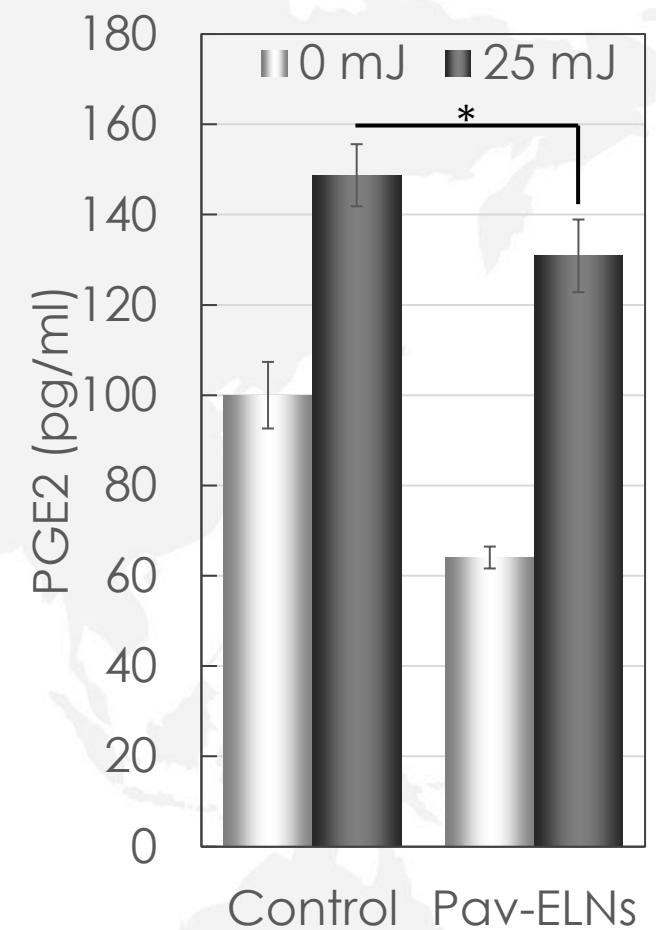
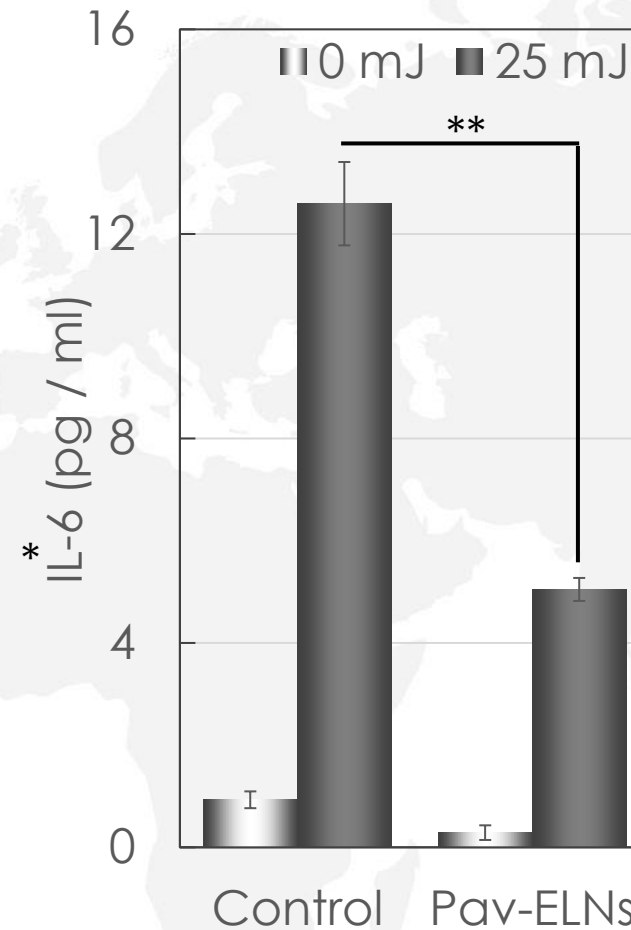
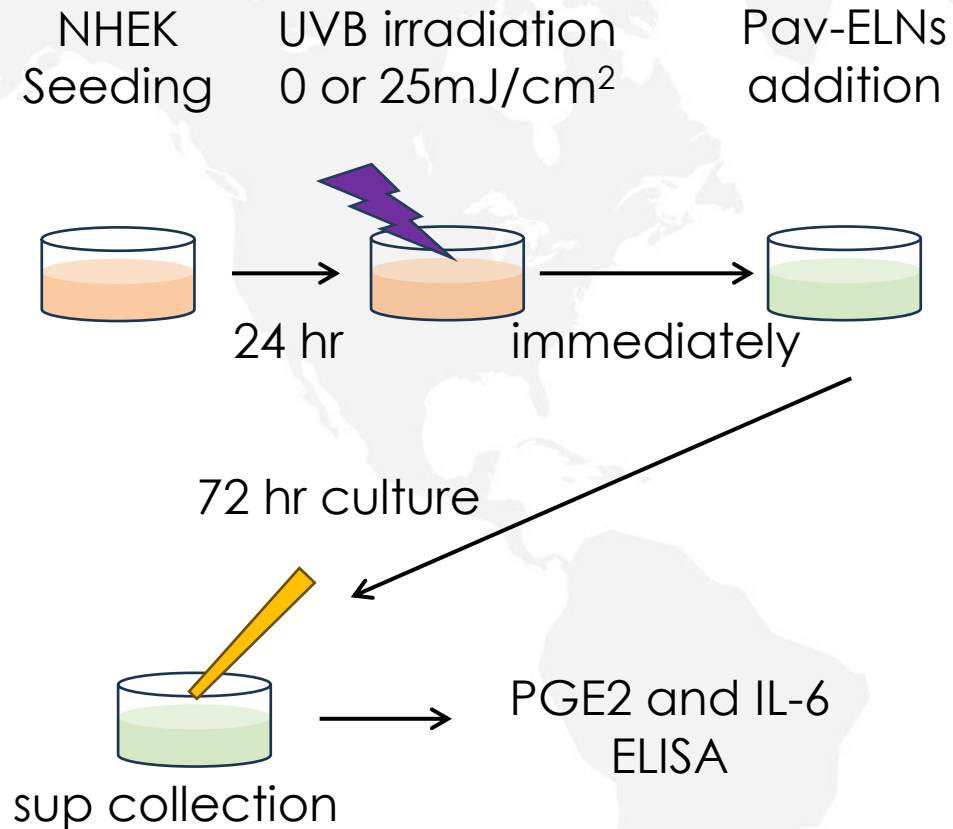
Tight Junction Formation



Pav-ELNs possess the ability to induce epidermal barrier formation.

Anti-inflammation effects of Pav-ELNs

Method

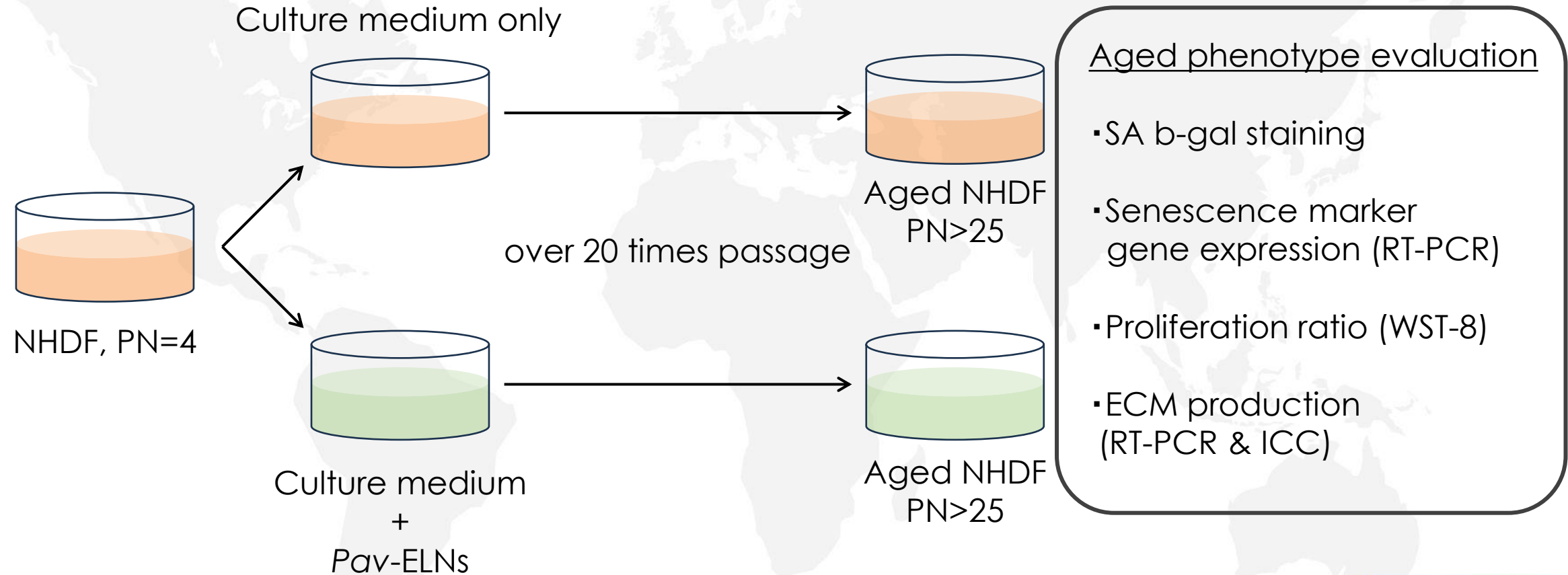


*P<0.05, **P < 0.001, as assessed by student's t-test

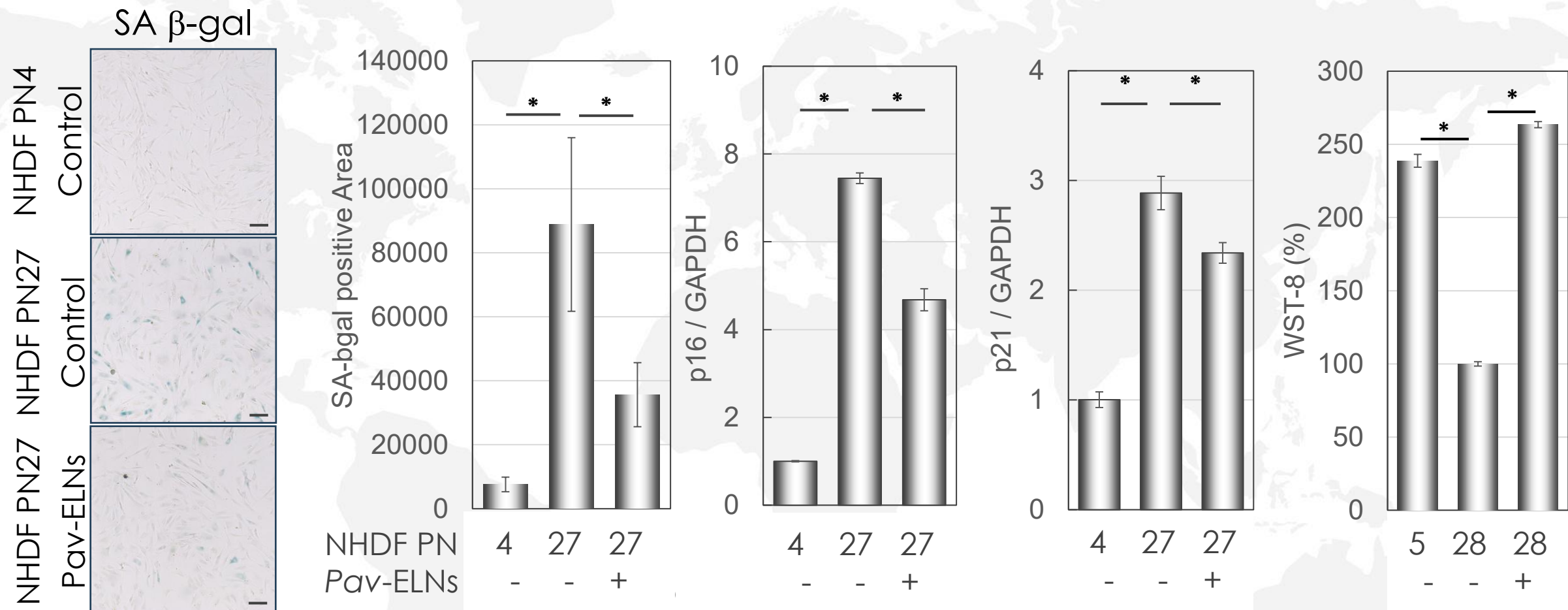
This finding suggests that Pav-ELNs are efficacious against UV-induced skin roughness and hyperpigmentation.

Anti-cellular senescence effects of Pav-ELNs: Experimental design

Induction of replicative senescence in normal human dermal fibroblasts (NHDF)



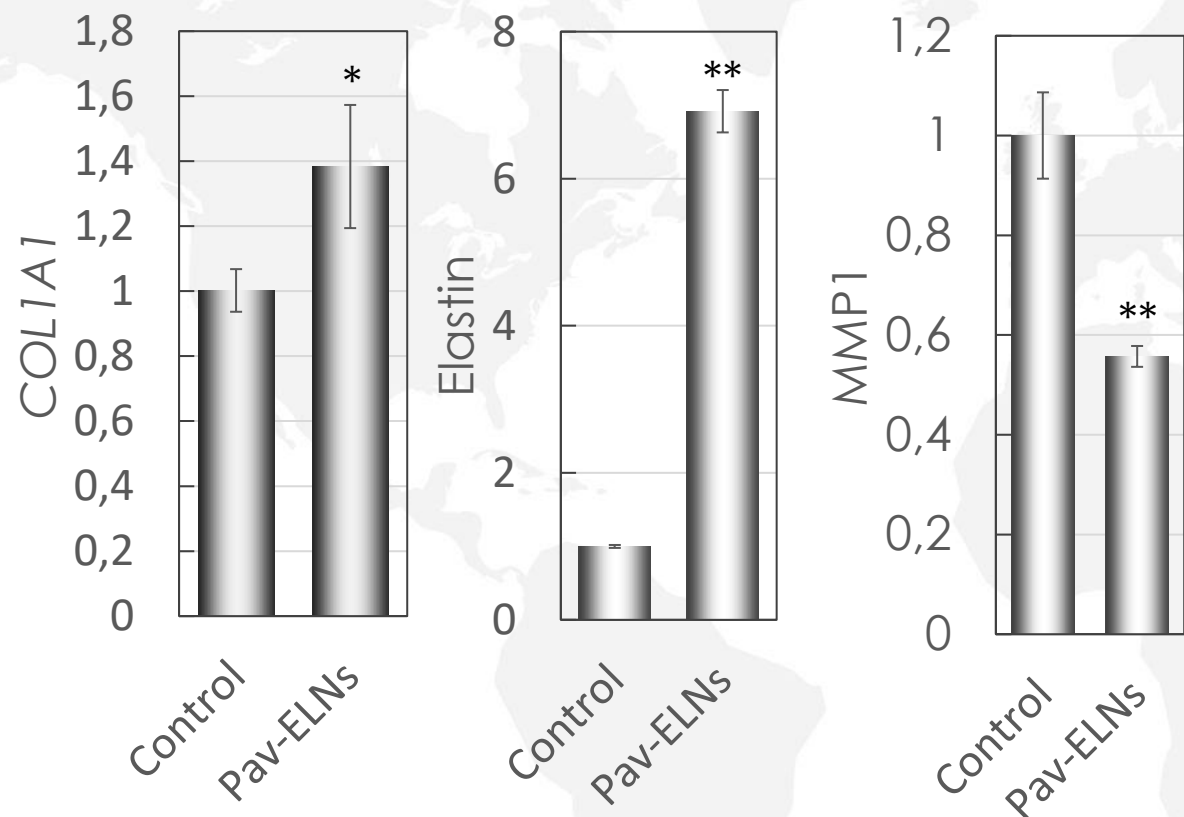
Anti- cellular senescence effects of Pav-ELNs



Pav-ELNs possess the capacity to prevent cellular senescence.

Effects of Pav-ELNs on ECM Synthesis

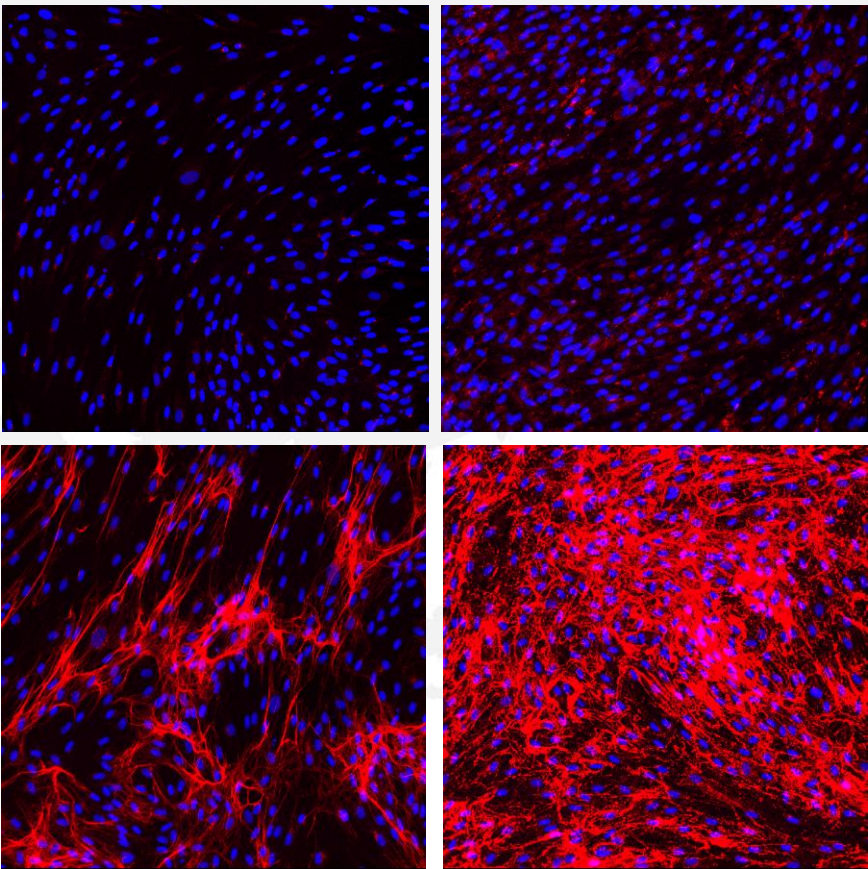
ECM related gene expression (RT-PCR)



*P<0.05, **P < 0.001, as assessed by student's t-test

Collagen fiber deposition (ICC)

Control Pav-ELNs



Control
Magnesium
ascorbyl
phosphate

Pav-ELNs hold potential for
addressing age-associated wrinkles and sagging

Summary of Pav-ELNs Properties

- **Stability**

Pav-ELNs exhibit greater stability than human-derived exosomes and remain stable for extended periods in polyol solutions.

- **Cargo protection**

Pav-ELNs encapsulate antioxidant lipids and RNA, protecting cargo from enzymatic degradation (e.g., RNase).

- **Cellular uptake**

Pav-ELNs are internalized and accumulate within both NHEK and NHDF in vitro.

- **Effects on keratinocytes (NHEK)**

Pav-ELNs induce epidermal differentiation, promote barrier formation (tight junction), and exert anti-inflammatory effects against UVB.

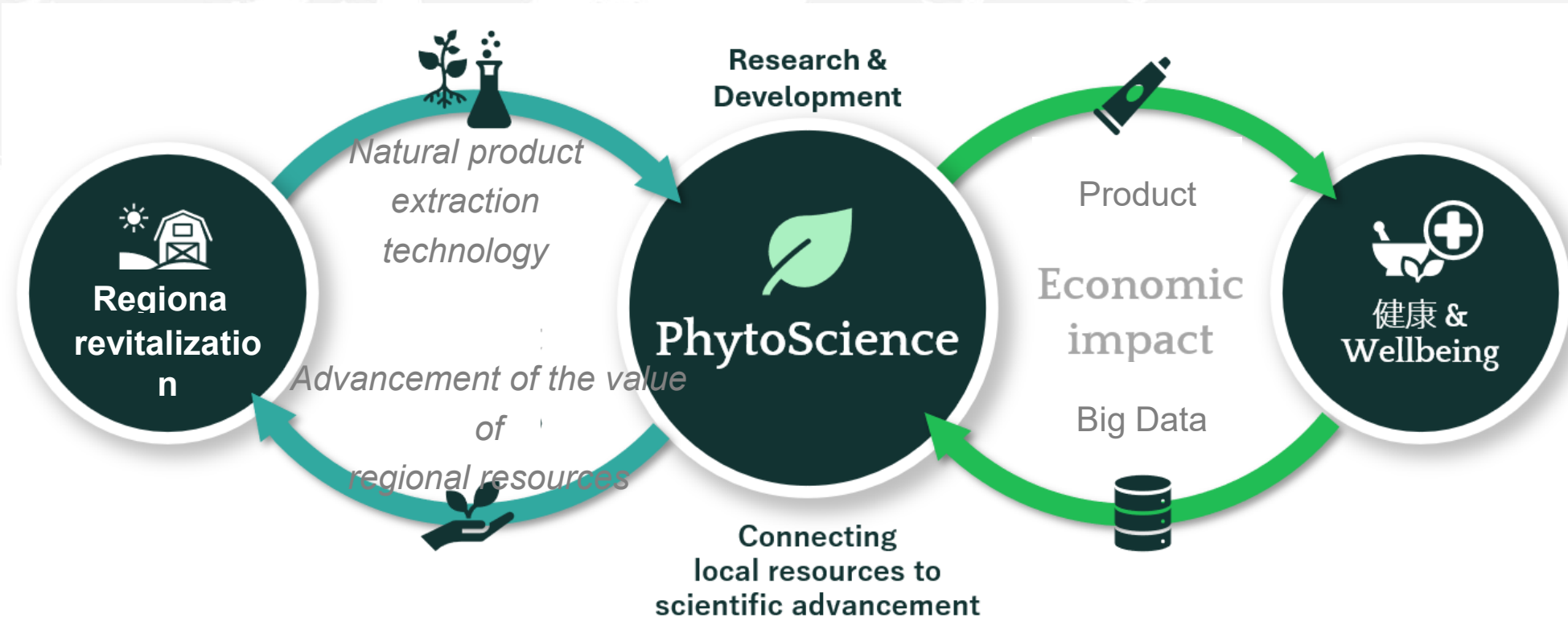
- **Effects on fibroblasts (NHDF)**

Pav-ELNs suppress replicative cellular senescence and stimulate ECM synthesis (upregulation of COL1A1, ELN; suppression of MMP1, MMP3).

We have manufactured Pav-ELNs, which have functional potential for nucleic acid DDS and anti-aging, on an industrial scale.

Our Goal: 'Phytoscience Initiative'

Accelerating Value Creation by Scientifically Utilizing the Power of Nature



We believe that by advancing the research, development, and product application of regional materials through phytoscience, we can achieve both **Longevity** and **Regional Revitalization**.