OP-026 [Apitherapy]
Exploring the Skin Rejuvenating Properties of Royal Jelly through Stem Cell Activation

Nobuaki Okumura[1], Hideto Okamoto[1], Takashi Ito[2], Mariko Moriyama[3], Hiroyuki Moriyama[3], Tomohiro Itoh[4], [1]Yamada Bee Company Group Institute for Beauty Science, Yamada Bee Company, Inc.
[2]Institute for Bee Products and Health Science, Yamada Bee Company, Inc
[3]Pharmaceutical Research and Technology Institute, Kindai University.
[4]Department of Life Sciences Graduate School of Bioresources, Mie University

INTRODUCTION: Royal jelly has been historically utilized as a beauty ingredient and is still widely used as a cosmetic material today. However, the precise mechanisms by which royal jelly benefits skin health are not yet fully understood. It is proposed that the epidermis, dermis, and fat layers that make up the skin each contain distinct stem cells. Epidermal stem cells, located in the basal membrane of the skin, contribute to epidermal turnover and wound healing. On the other hand, mesenchymal stem cells are mainly found in the fat layer. They can differentiate into dermal fibroblasts and also secrete extracellular vesicles (EVs), which induce collagen production by dermal fibroblasts. In this study, we evaluated the effects of royal jelly on skin regeneration and rejuvenation through these stem cells.

METHODS: An epidermal three-dimensional skin model was used to assess epidermal stem cell activation. We added royal jelly during the construction of the 3D skin model and then histologically analyzed the thickness of the formed epidermis, as well as the expression of stem cell markers. To evaluate the impact on mesenchymal stem cells, we treated human adipose-derived mesenchymal stem cells with royal jelly and then investigated the number of EVs secreted into the culture supernatant. We also evaluated the collagen production activity of these vesicles when applied to dermal fibroblasts.

RESULTS: The royal jelly treatment resulted in increased thickness of the 3D skin model and enhanced expression of the epidermal stem cell marker p63 and the proliferation marker Ki67. These findings suggest that royal jelly promotes turnover by activating epidermal stem cells. Additionally, treatment of mesenchymal stem cells with royal jelly led to an increased release of EVs into the culture supernatant. When these vesicles were applied to fibroblasts, those derived from royal jelly-treated mesenchymal stem cells induced higher collagen production.

CONCLUSION: This study demonstrates the potential of royal jelly that activates skin-resident stem cells and likely contributing to anti-aging effects on the skin. Royal jelly is a promising ingredient that contributes to skin health and rejuvenation.

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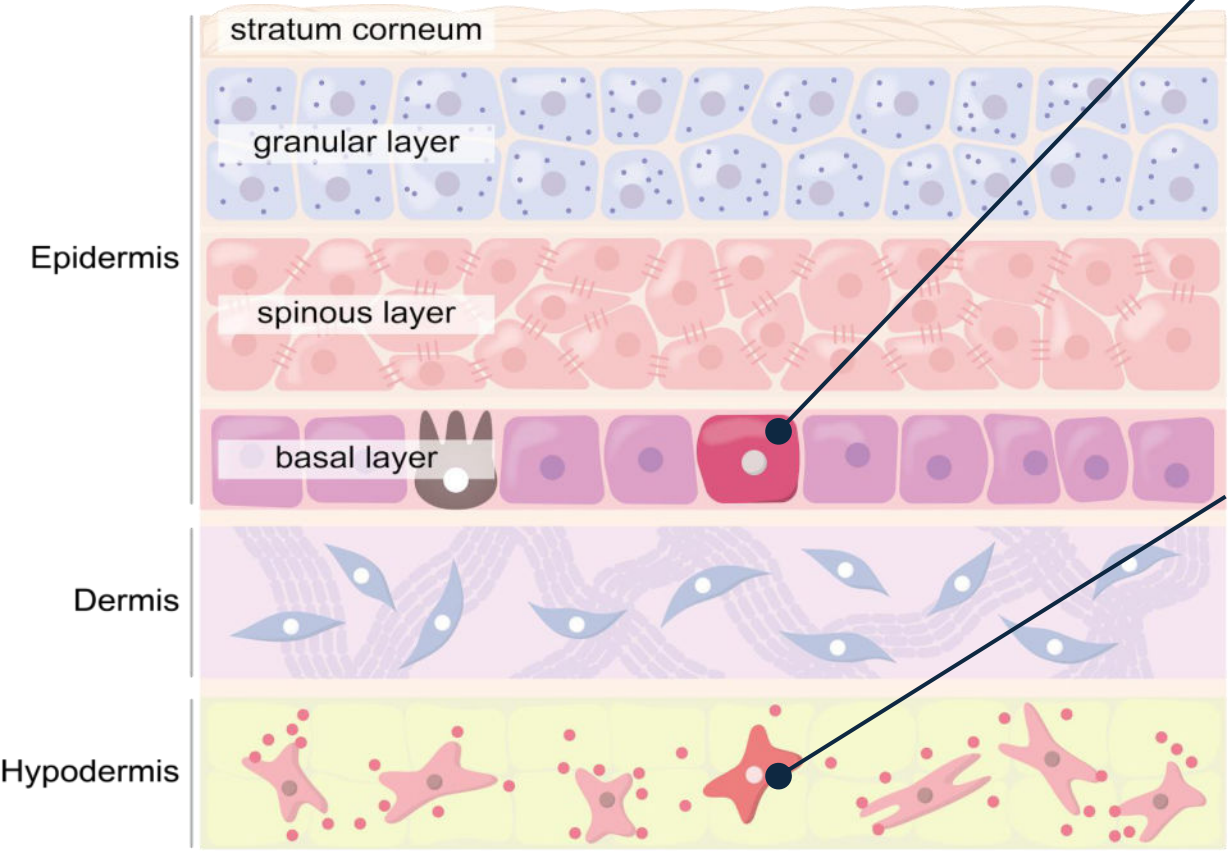
*Yamada Bee Company, Inc., Japan

Stem Cells: The Engine of Skin Rejuvenation



■ Epidermal Stem Cells (ESCs)

Location:
Basal layer, Hari follicle bulge
Functions:
Maintain the barrier function by differentiating into new epidermal cells/
Aging:
A decrease in number and slower renewal lead to thinner skin.

■ Adipose derived Stem Cells (ADSCs)

Location:
Hypodermis
Functions:
Maintain dermal firmness and secrete “message capsules (Extracellular vehicles; EVs)” that boost collagen production.
Aging:
A decrease in number and function lead to wrinkles.

Maintaining these skin stem cells is the key to skin rejuvenation

Royal Jelly: Benefit for Skin Function

- Yellowish-white creamy substance secreted by honeybees (Apis mellifera) for an essential nutrient for the maturation of bee larvae into queen bees.
- Contains multi nutrients such as proteins, carbohydrates, lipids and minor components like phenolic compounds.
- Traditionally used as apitherapy for maintaining heath and beauty.
- Use for the cosmetic ingredient all over the world.

The effect of RJ on the skin stem cells have not been understood

Testing RJ on Epidermal Stem Cells (EPCs)

1. Growth & Renewal Ability
Does RJ help stem cells multiply?

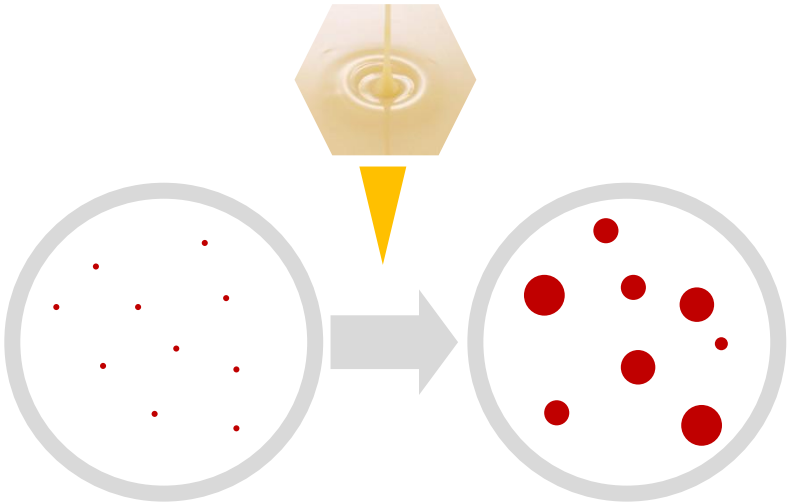
Measurement
- The number of cell colonies

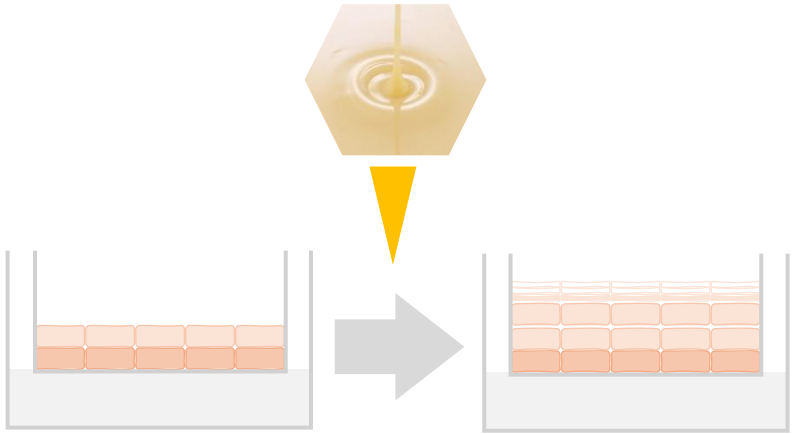
2. Building a Healthy Epidermis
Dose RJ help build a proper skin layer?

Measurement
- Epidermis thickness
- EPCs markers; Ki67, p63 and K14

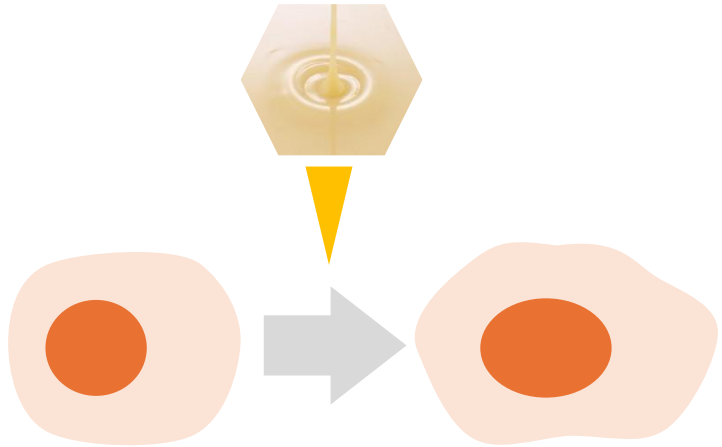
3. Fighting Cellular Aging
Dose RJ protect stem cells from aging?

Measurement
- Population doubling level (PDL)
- Aging marker; SA-β-Gal





3D skin model (Epidermis model)

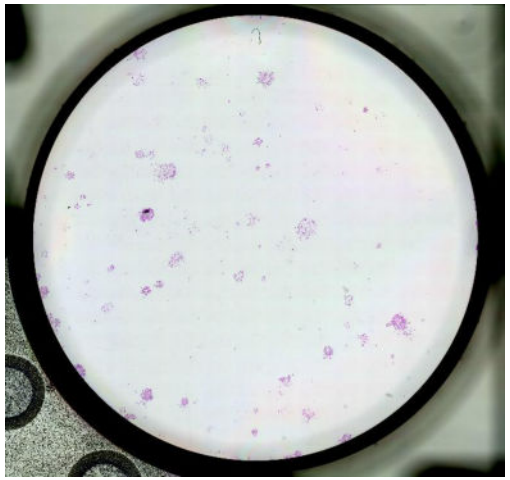


Normal cell

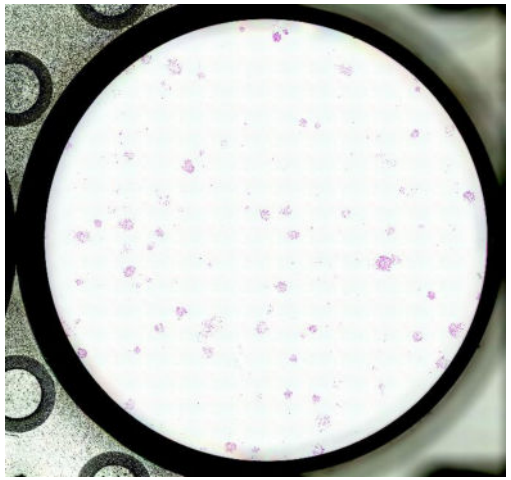
Aged cell

RJ increased colony formation

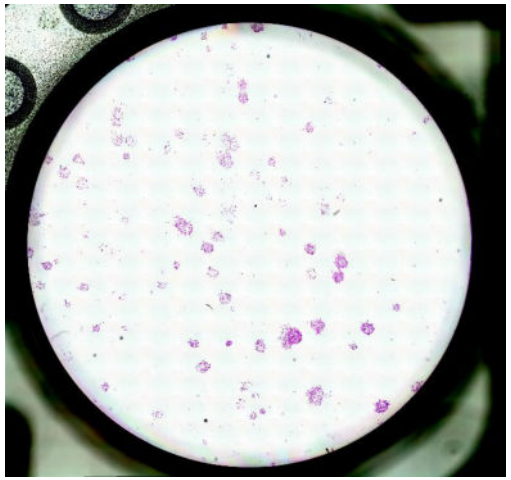
Control

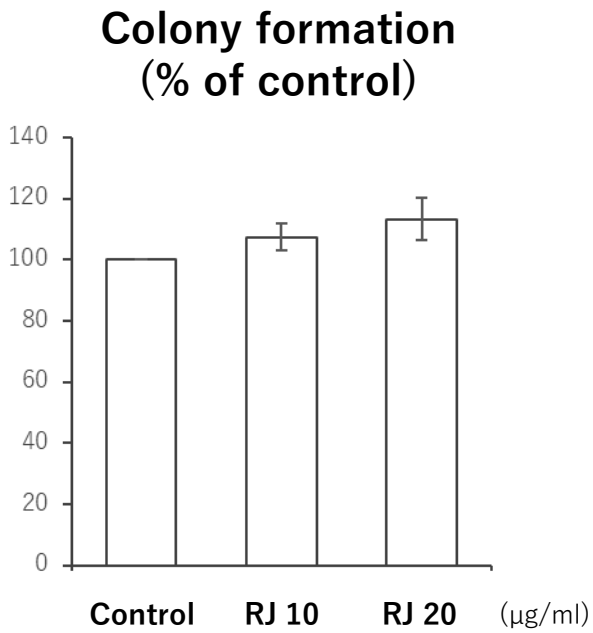


RJ 10 μg/ml



RJ 20 μg/ml



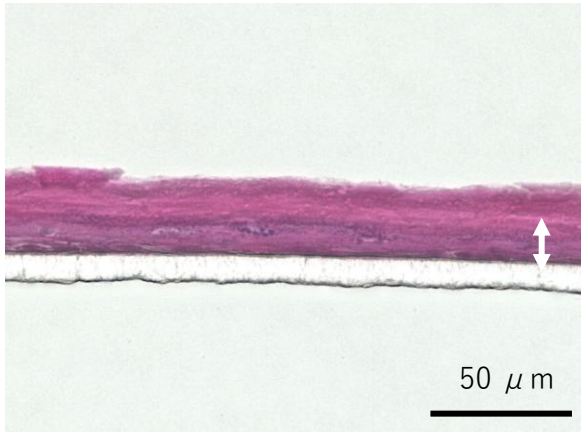


Graph represents the mean ± SD
n = 3-4 by Student's t-test.
**$P < 0.01$.

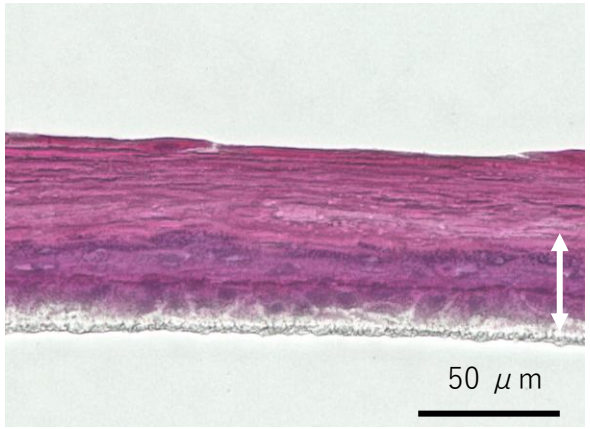
Okumura et al.,
Unpublished data

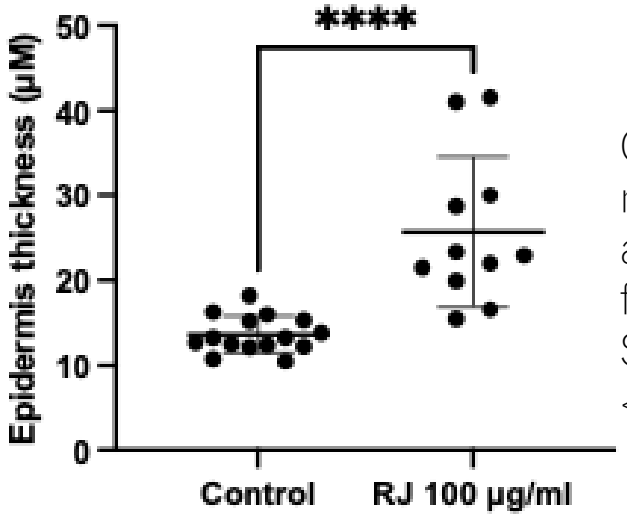
RJ induced healthier epidermal development in 3D skin model

Control



RJ 100 μg/ml

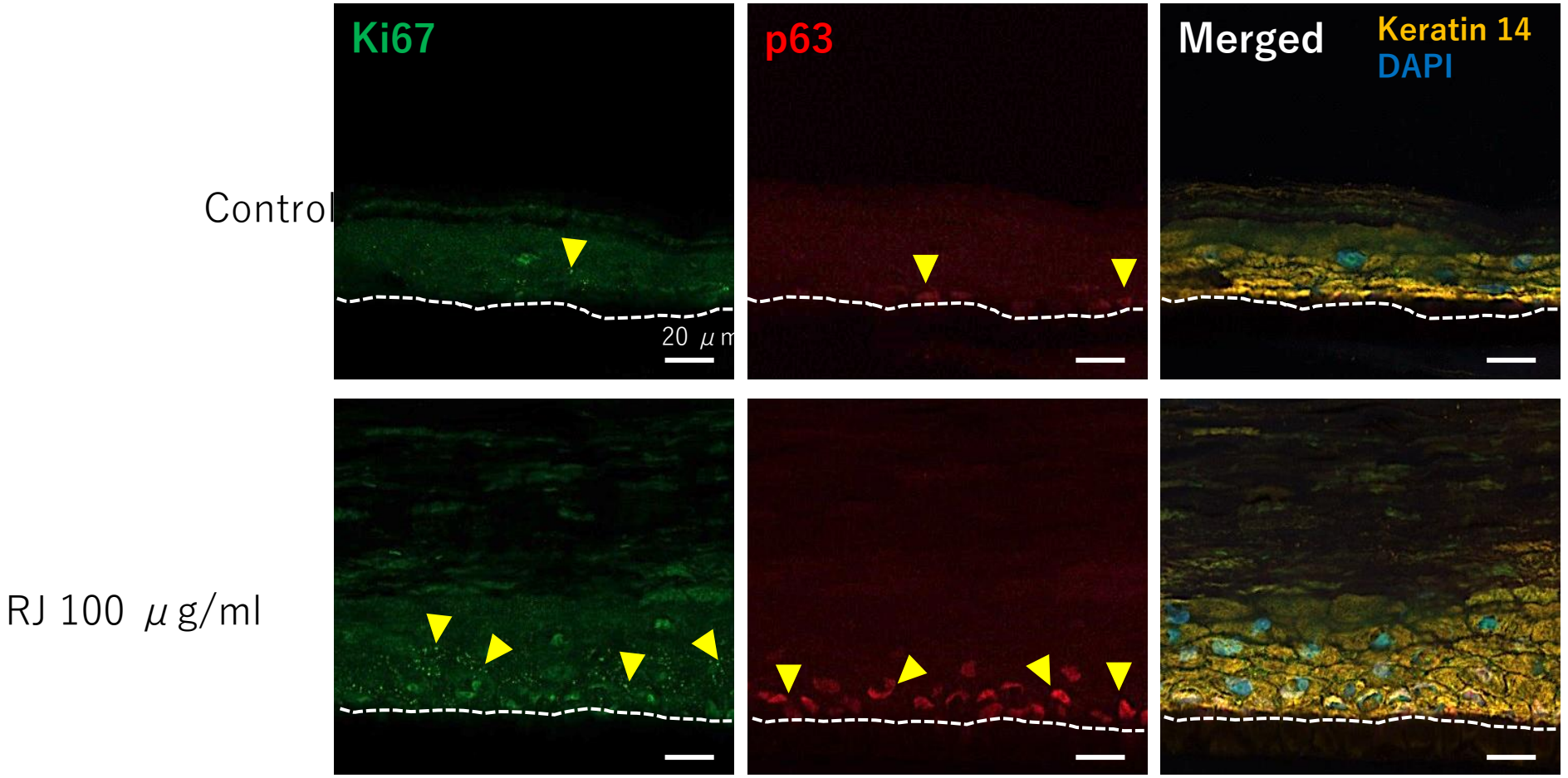




Graph represents the mean ± SD ($n = 3$; averaged over 12 - 15 fields of view) by Student's *t*-test. ****$P < 0.0001$.

Moriyama et al., *Biol Pharm Bull*. 2024
https://doi.org/10.1248/bpb.b24-00607

RJ upregulated ESC makers in 3D skin model



Moriyama et al., *Biol Pharm Bull*. 2024
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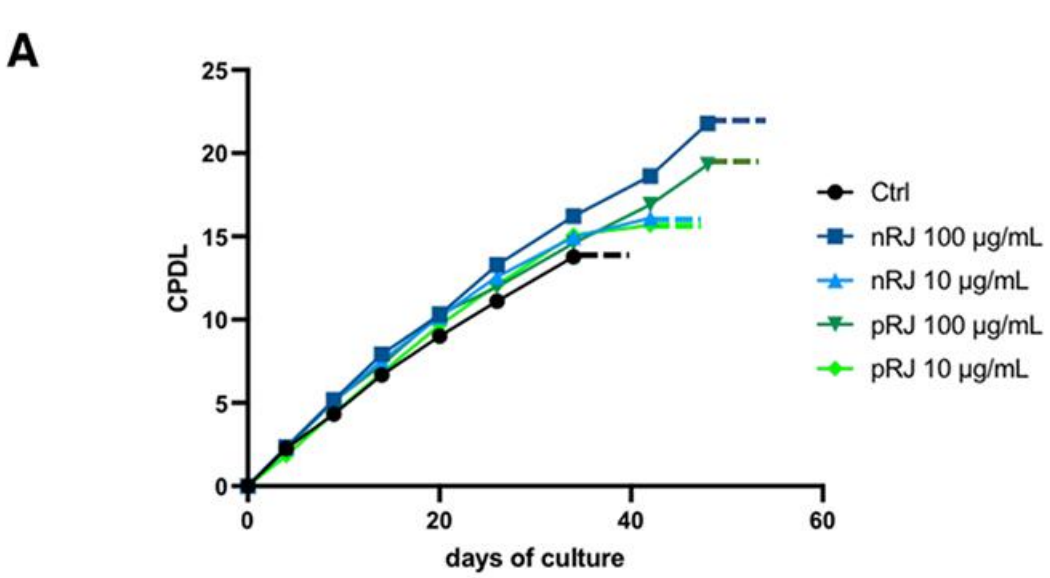
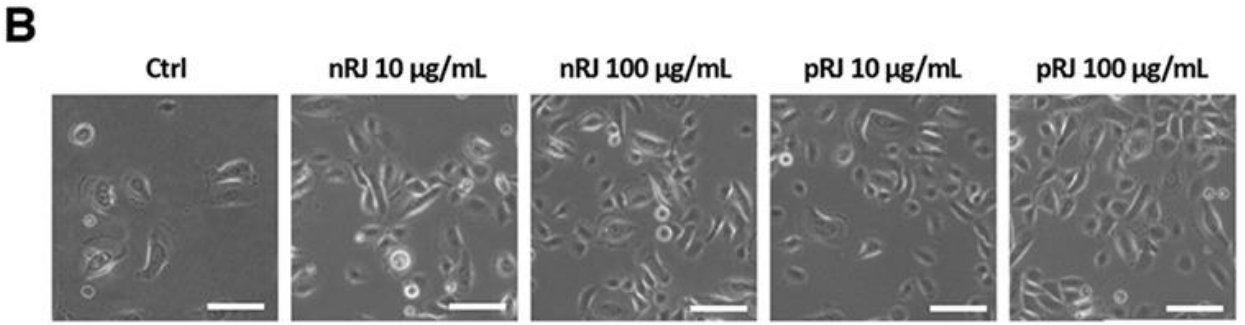
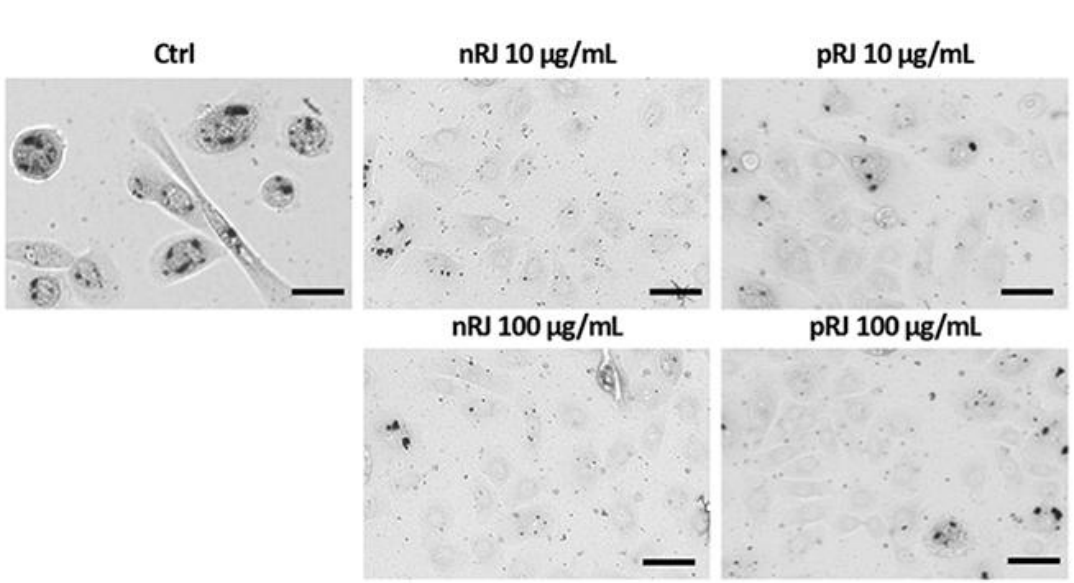
RJ impaired epidermal cell senescence







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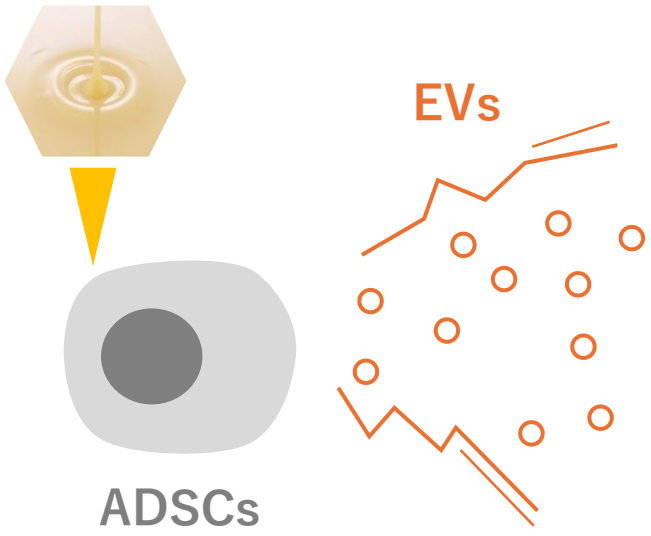
Testing RJ on Adipose derived Stem Cells (ADSCs)

1. EVs secretion ability

Dose RJ stimulate **“Extracellular Vesicles (EVs)”** secretion from stem cells?

Measurement

- The number and size of EVs

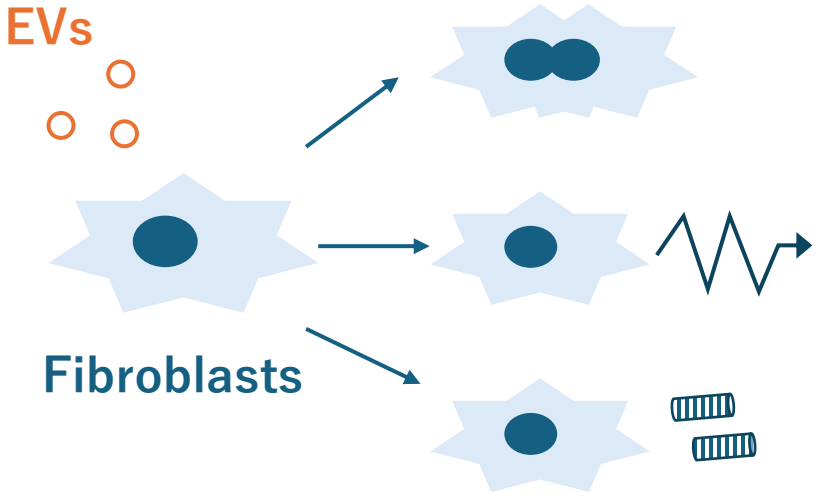


2. Function of EVs on fibroblasts

Do EVs from different sources affect dermal fibroblast function?

Measurement

- Fibroblast growth
- Migration (Wound healing)
- Collagen production

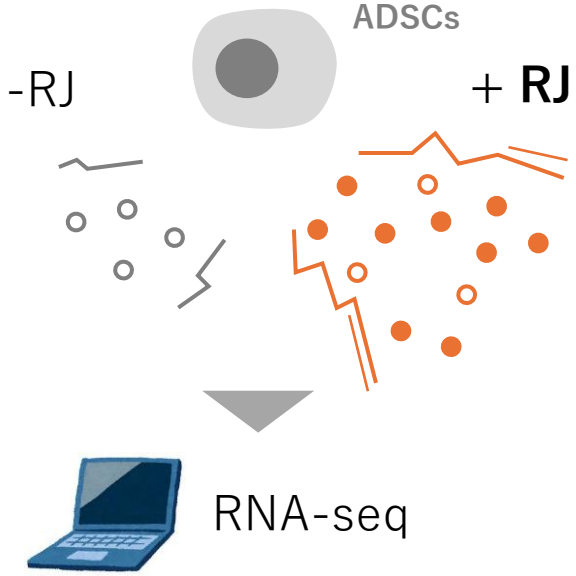


3. Mechanism of EVs' action

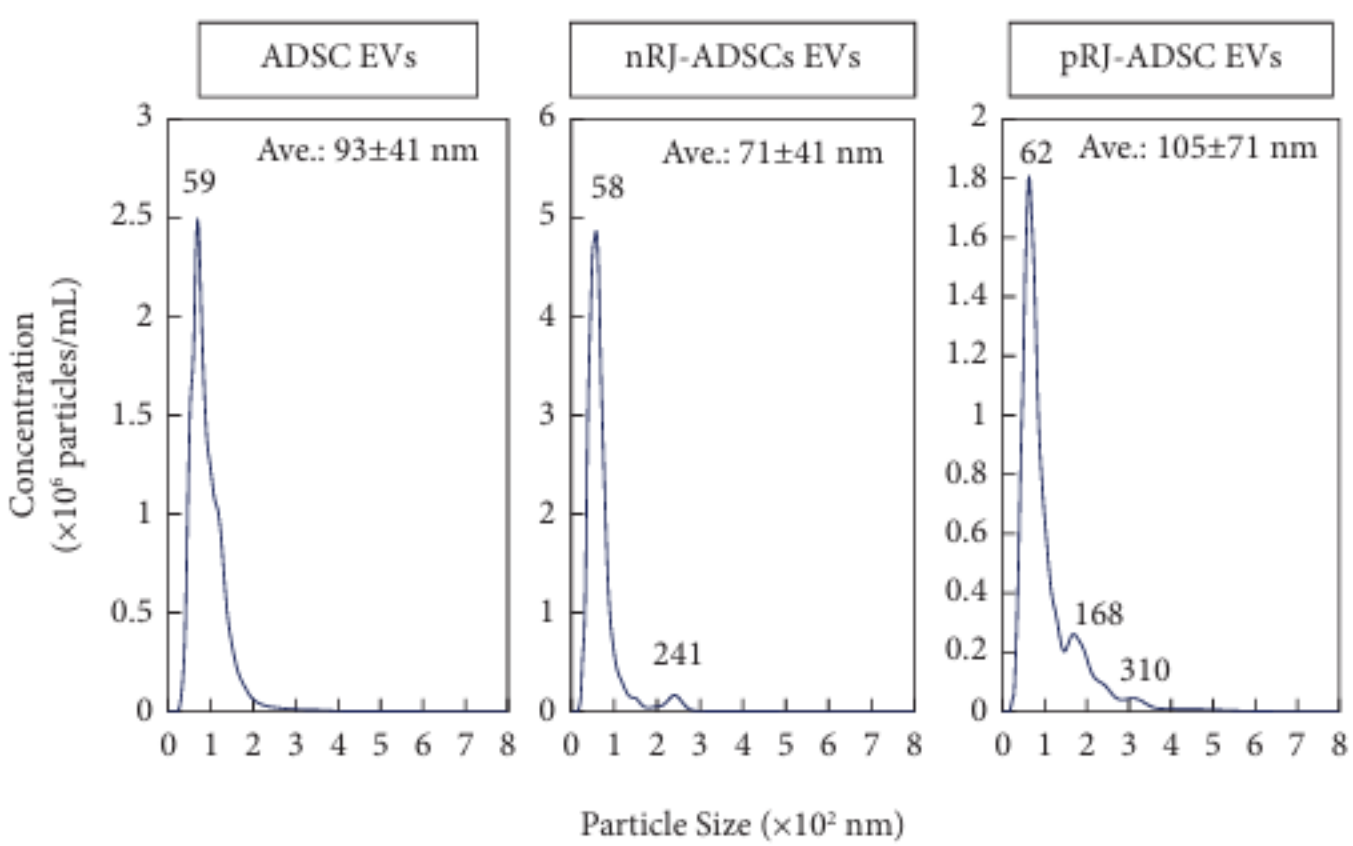
What causes the functional difference in EVs?

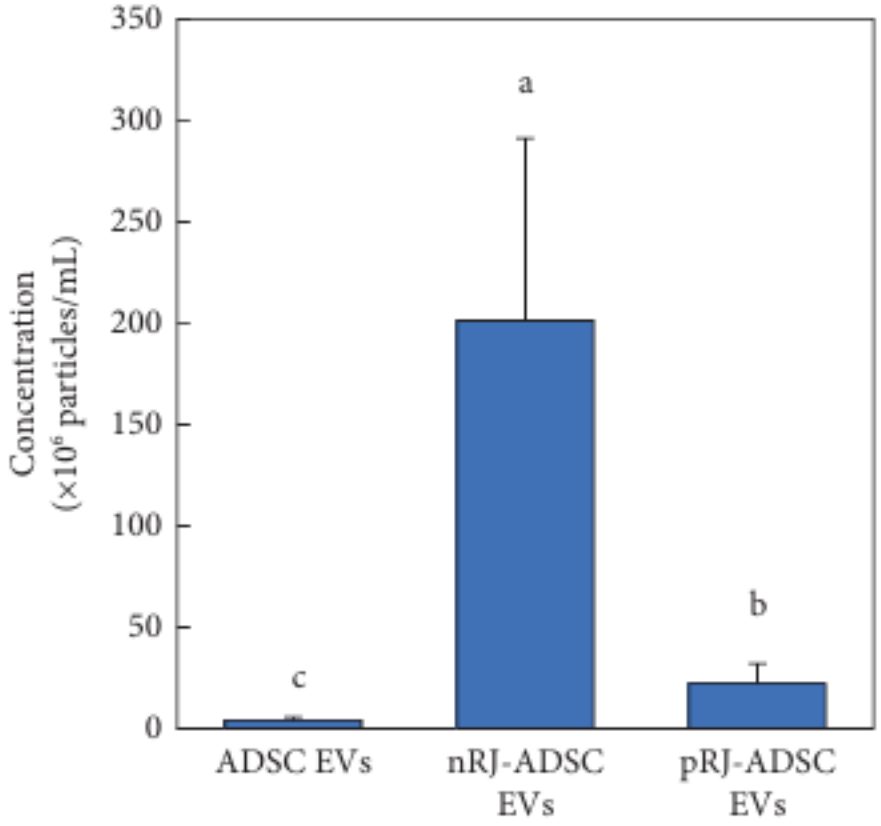
Measurement

- Matrix-related mRNAs
- Micro RNAs



RJ increased the number of EV secretion.

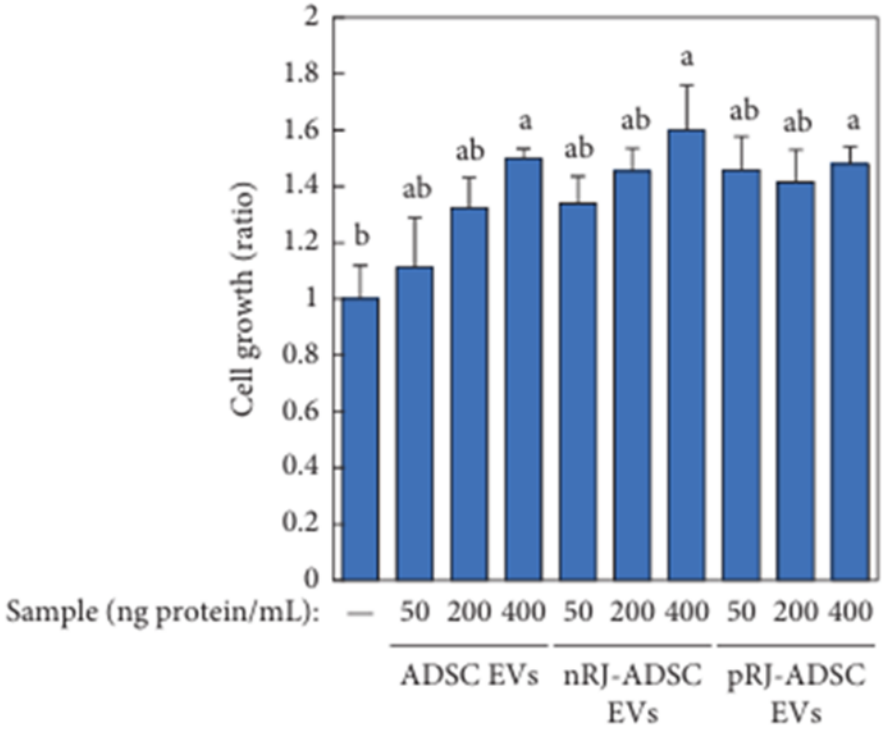




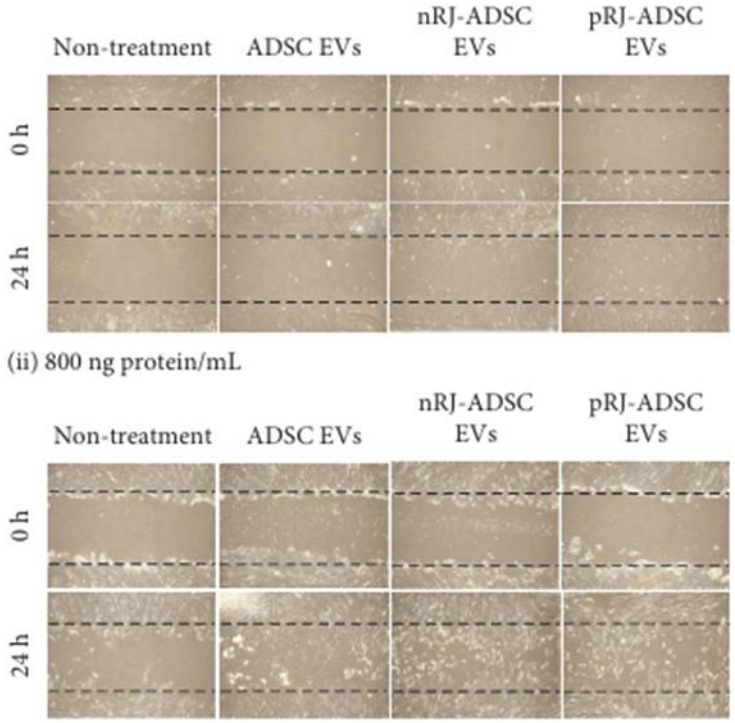
Itoh et al., *Dermatol Ther*, 2023
https://doi.org/10.1155/2023/7950026

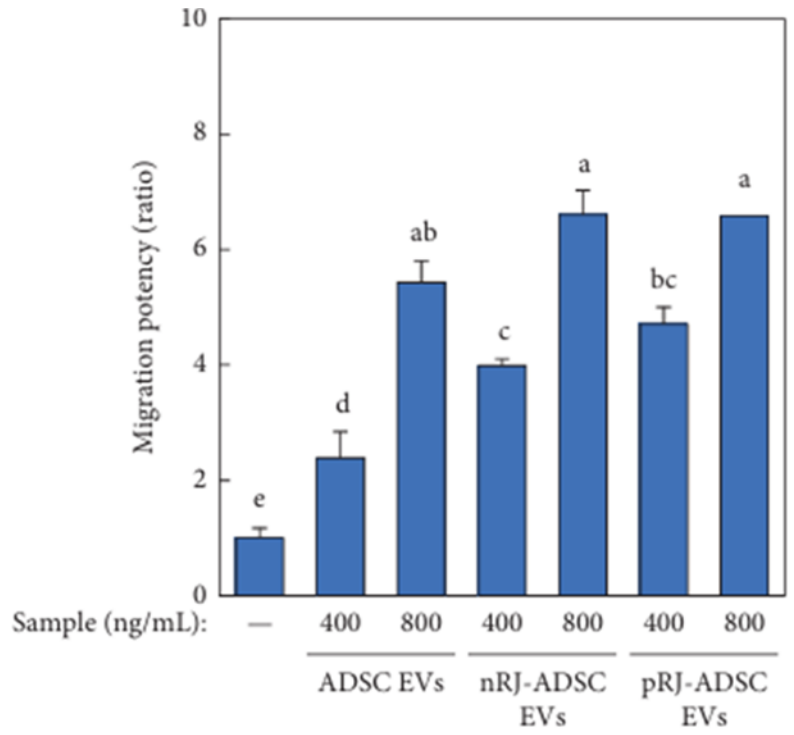
RJ-stimulated ADSC secreted EV activated dermal fibroblast functions

a) Growth assay



b) Migration assay





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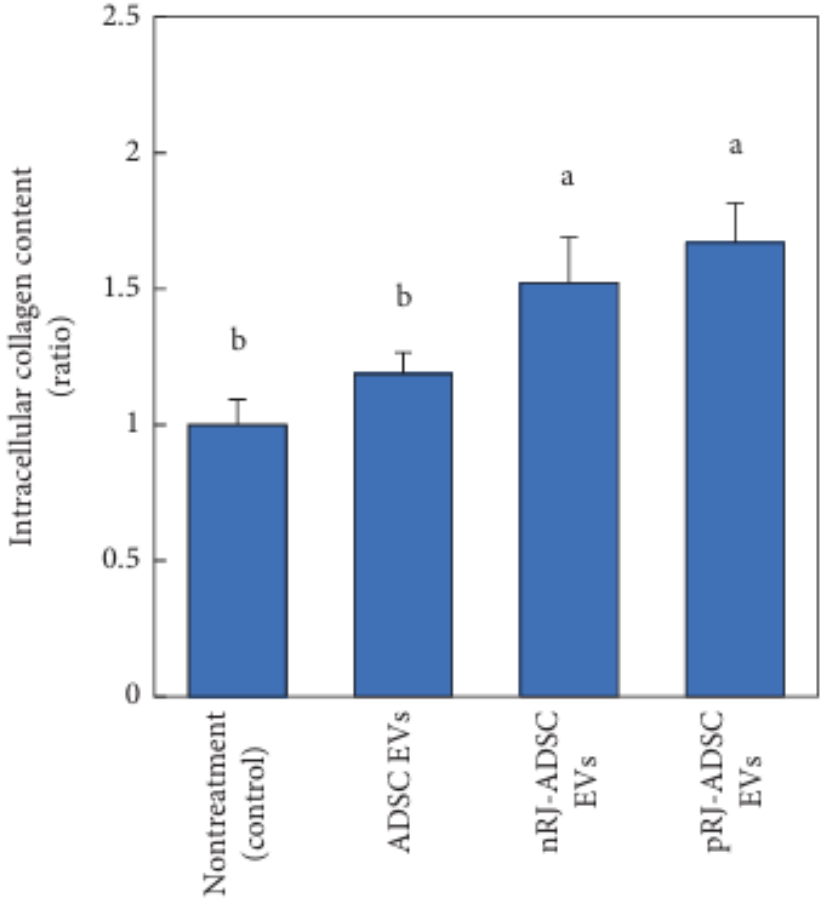
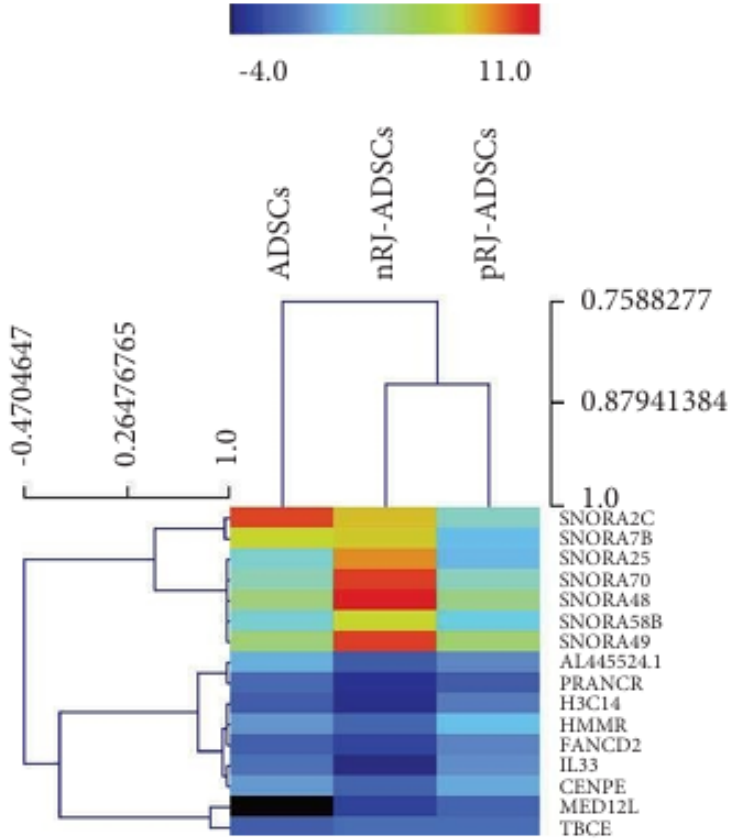
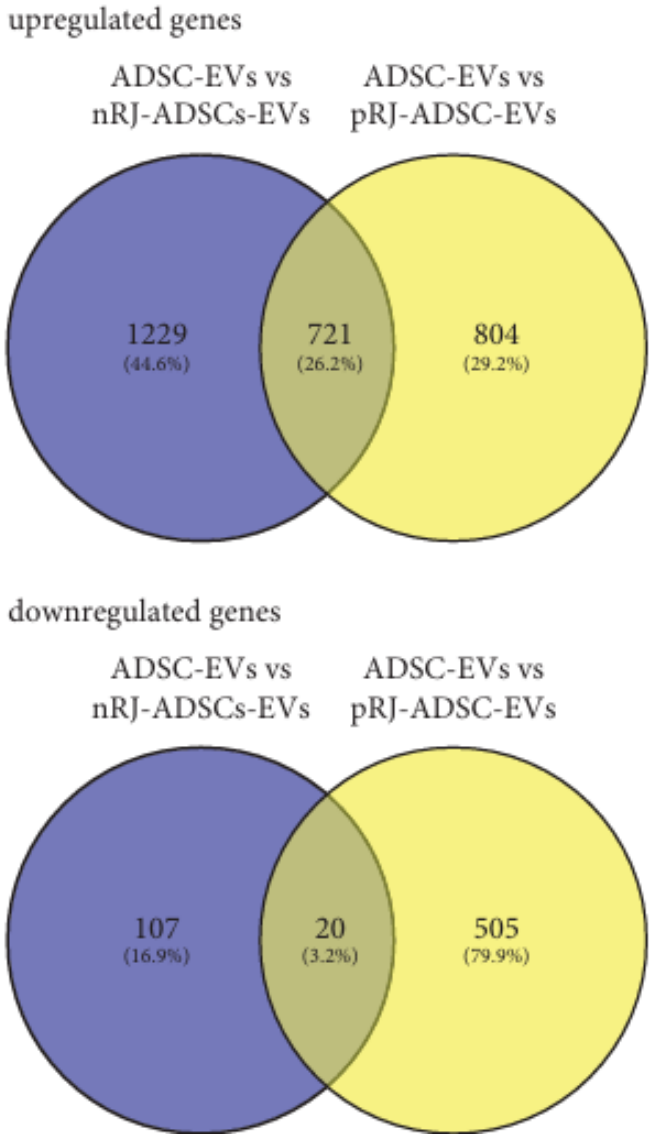
RJ-stimulated ADSC secreted EV stimulated collagen production in dermal fibroblast

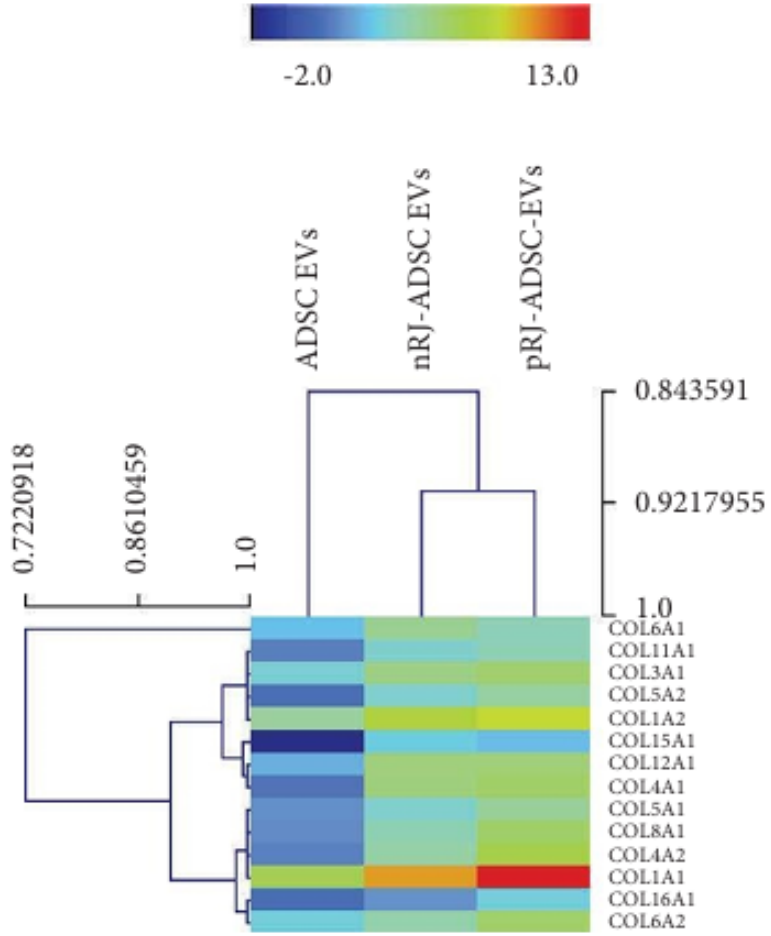


Itoh et al., *Dermatol Ther*, 2023
https://doi.org/10.1155/2023/7950026

RJ-stimulated ADSC secreted EV contains collagen genes transcripts







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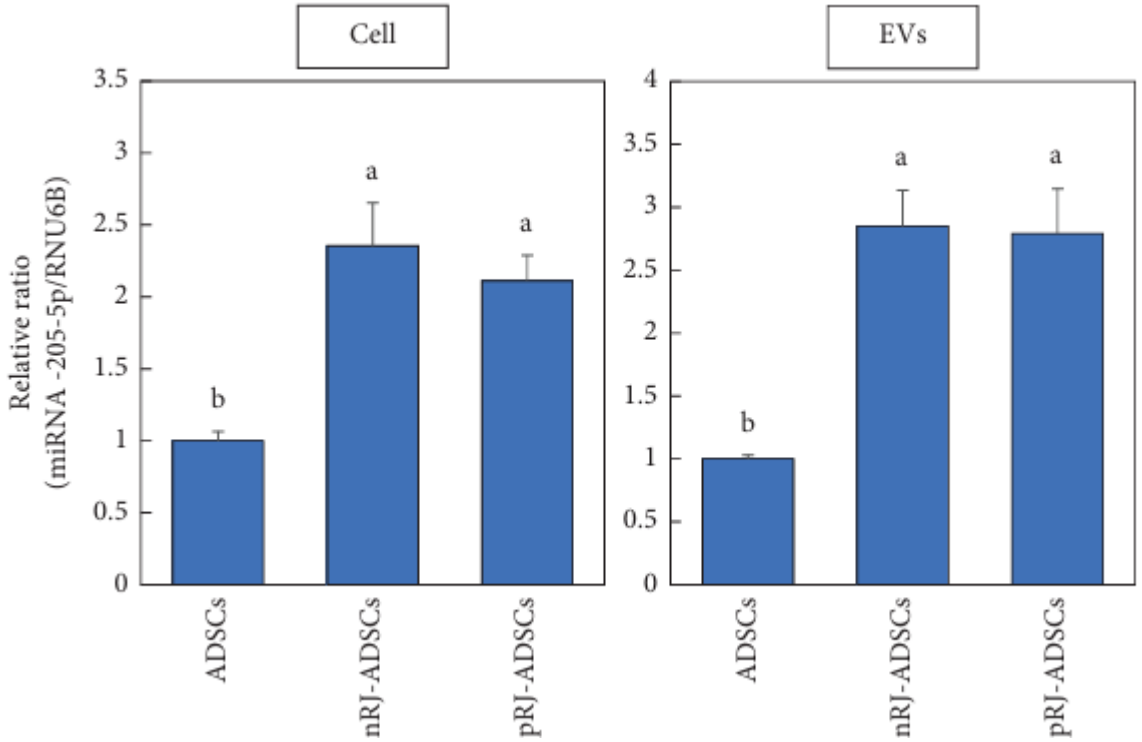
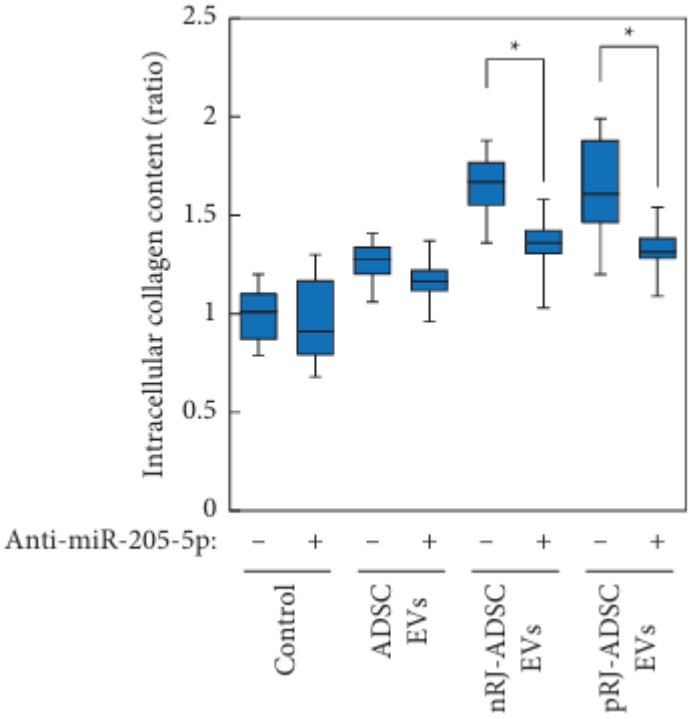
RJ-stimulated ADSC secreted EV contains miR-205-5p that regulated collagen contents.





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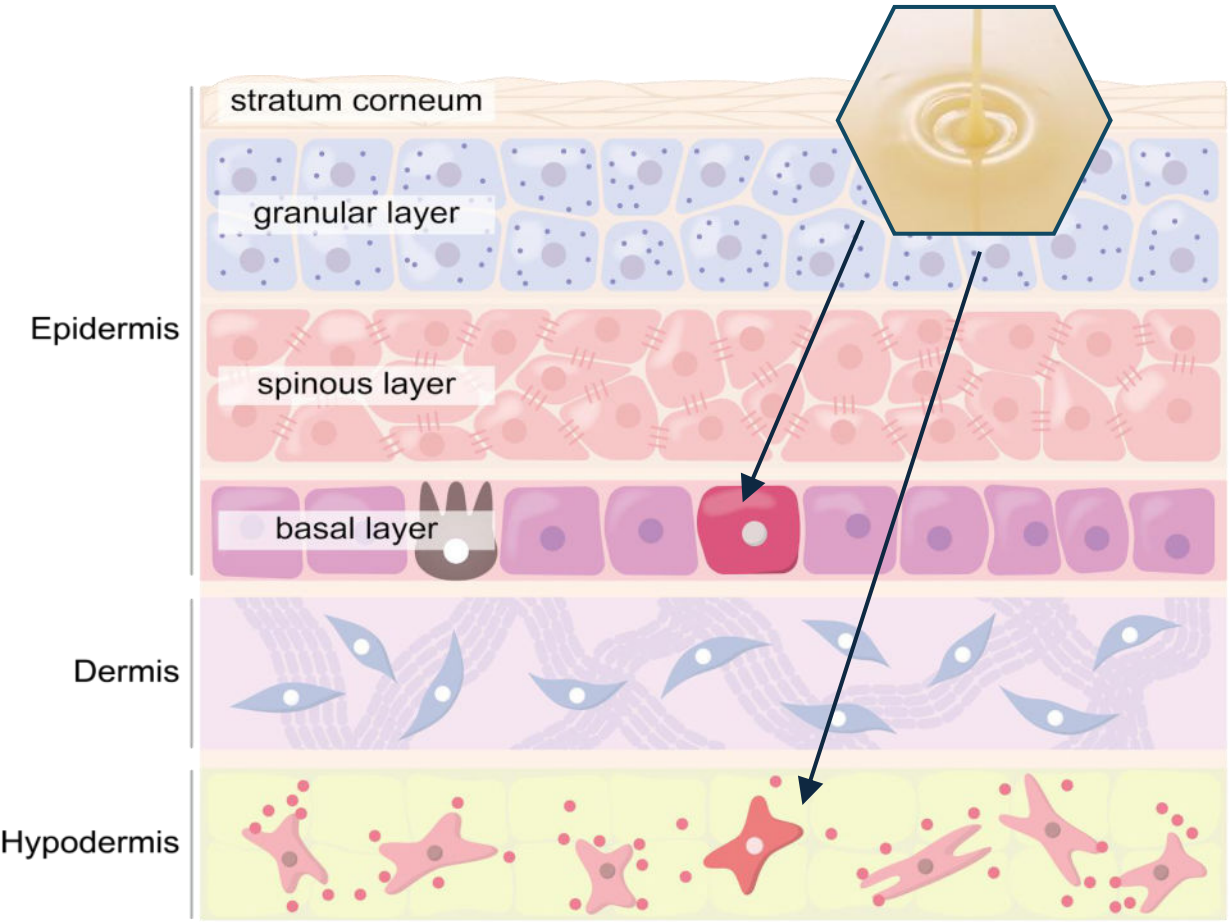
Conclusion: The effect of RJ on Skin Stem Cells



■ Epidermal Stem Cells (ESCs)

- ✓ Activate EPCs function
- ✓ Inhibit cellular senescence

RJ activates EPCs and have regenerative capacity of epidermal structures.

■ Adipose derived Stem Cells (ADSCs)

- ✓ Stimulated EV production in ADSCs
- ✓ Changed EV qualitied to ECM productions

RJ stimulated dermal functions through ADSCs function.

RJ is a promising ingredient that contributes to skin rejuvenation.

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Mariko Moriyama
(EPCs studies)

MIE UNIVERSITY

Tomohiro Itoh
(ADSCs studies)

アピセラピーを追究する。
山田養蜂場
YAMADA BEE FARM

Takashi Ito
Hideto Okamoto
(Stem Cell Research project)

Koshiro Yoshida
(Graphical images)