Biological Function of Exosome-like Particles Isolated from Rose (Rosa Damascena) Stem Cell Culture Supernatant

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Abstract

Rose stem cell (RSC) extracts have been claimed to have multiple beneficial effects in skin. Many cells release extracellular vesicles, also called exosomes, that involved in cell-to-cell communication. There is however no information on whether exosomes are released in RSC cultures, and what the biological function of these may be. We hypothesized that RSC in culture can release exosome-like particles (RSCEs) and that the RSCEs may have biological function in cells relevant to skin. RSC culture supernatant was purchased and standard exosome-isolation was performed, using tangential flow filtration. The characterization of RSCEs was measured with TEM and NTA, that revealed round structures and reports the presence of particles approximate 90-200nm size. Proteins and miRNAs were isolated from the exosomes, and 206 peptides containing likely cytosolic and membrane proteins and miRNA containing Let-7 families were identified. The RSCEs were found to be non-toxic on human dermal papilla cells, whereas a high concentration of the crude supernatant induced widespread cell death. Further, the RSCEs enhanced growth of human dermal fibroblasts and increased the closure of scratch assay, whereas the crude supernatant lacked this effect. Further, the RSCEs reduce the amount of melanin in cultured melanocytes and IL-6 released by Raw264.7 cells stimulated by LPS in a dose-dependent manner. These data collectively show that RSC in culture released RSCEs that contain miRNA and proteins have multiple biological functions in skin-related assays such as fibroblast growth and melanin content in melanocytes. Combined with the anti-inflammatory function of the RSCEs, we suggest that they have appropriate features to be useful in aesthetic medicine for improving skin quality.

Introduction

Exosomes, also known as extracellular vesicles or exosome-like nanoparticles, are small membrane-enclosed structures that are released by cells into their surrounding environment[1]. They are known to transport a variety of molecules, including proteins, lipids, and nucleic acids, between cells, and they can act as vehicles for genetic information transfer[2, 3]. Plant cells also release exosomes, that have similarities to exosomes produced by animal and human cells[4, 5], but with some unique features that are specific to plants. Plant exosomes are involved in cell-to-cell communication and may play important roles in intercellular signaling, stress responses, and defense mechanisms[6].

Research on plant exosomes is still in its early stages, but there is growing interest in their potential applications in agriculture, biotechnology and even medicine[5]. For example, they could be used as delivery systems for drugs or genetic materials, or as diagnostic tools for plant diseases[7]. Vesicles extracted from different types of plants have been studied as potential drug candidates in different disease models. The plants used for this vesicle extraction include lemon, ginger, ginseng and grapes[8-11]. A major concern with these studies is, however, that they may not be studying naturally released plant EVs, as the isolation procedures can include harsh methods such as kitchen mixers, which disintegrates cell and provides membrane for possible spontaneous micro-vesiculation of these. Regardless, several of these vesicles have shown anti-inflammatory functions in cell systems and animals[12]. However, to our knowledge, no study has determined the presence of exosome-like particles in the cell supernatant of plant stem cells.

We here hypothesize that plant stem cells release exosome-like structures when cultured in vitro, and that these vesicles may harbor anti-inflammatory and potentially regenerative functions. To study this, we used rose stem cell culture supernatant to isolate any vesicles, which indeed could be isolated by standard exosome isolation procedures. The RSCEs were further characterized using electron microscopy, proteomics and transcriptomic techniques. Any anti-inflammatory or regenerative function of the RSCE was studied in fibroblasts and inflammatory cells.

Materials & Methods

Isolation and Characterization of Exosome-like structures

Rose-Stem Cell Exosomes (RSCEs) were isolated from the RSCs culture supernatant (RSC-CM), MS (Murashige and Skoog) medium with plant derived growth factors (auxin, cytokinin etc), by the tangential flow filtration (TFF)-based ExoSCRT® technology as previously described[13]. Briefly, RSC-CM was purchased from callus culture contractor and, the CM was filtrated through a 0.22μm polyethersulfone membrane filter (Merck Millipore, MA, USA) to remove non-exosomal particles such as cells, cell debris and large protein aggregates. The CM was then concentrated by tangential-flow filtration with a 100 kDa molecular weight cut-off filter membrane cartridge (GE Healthcare, Chicago, USA) and buffer exchange was performed by diafiltration with EDB1 (Dilution Buffer with 2%Trehalose in PBS). Isolated RSCEs were aliquoted into polypropylene disposable tubes and stored at -80℃ until further use. Nanoparticle tracking analysis (NTA) was performed using a NanoSight NS300 (Malvern Panalytical, Amesbury, UK) was used to measure the size distribution of isolated particles. The morphological characteristics of the RSCEs was determined using a JEOL 2100P Cryo-transmission electron microscope (JEOL, MA, USA)[14].

Cellular Uptake Assay

Purified RSCEs were labeled with the PKH67 green fluorescent linker Mini Kit (Sigma-Aldrich, Missouri, USA) according to the manufacturer's instructions. Briefly, after labeling with PKH67 the membrane of the prepared RSCEs, the reaction solution was fractionated into a MiniTrap-25 column (Sartorius, Gottingen, Germany) to remove the unbound PKH67 fluorescent dye. Thereafter, the PKH67-labeled were added to cultures with human dermal fibroblasts (HDFs; ATCC, Virginia, USA), and then observed under a fluorescence microscope to confirm that the RSCEs were taken up to cells. DAPI (Invitrogen, Massachusetts, USA) was used to stain nuclei and Cellmask (Invitrogen, Massachusetts, USA) was stained cytoplasm.

Cell Toxicity

MTT assay was performed on Human dermal papilla cells (HDPs; CefoBio, Gwangmyeong-si, Korea) after RSCEs incubation. Briefly, Human dermal papilla cells were cultured in growth medium and were seeded at 1 x 10^4 cells per well into 96-well plates and cultured for 24 h, subsequently they were treated with RSCEs and RSC-CM for 24 h. And then, cultures were incubated for 2 h with Growth medium include MTT reagent (Sigma-Aldrich, Missouri, USA) in an incubator. Absorbance was measured at 450nm using a microplate reader.

Collagen assay

Procollagen Type 1 C-peptide (PIP) was measured according to the manufacturer's instructions (Takara, Shiga, Japan). For evaluation of PIP, HDFs were seeded at 2.5 x 10^4 cells per well in a 24-well plated and incubated for 24 h and then RSCEs and RSC-CM were treated by concentration. PIP content was measured a microplate spectrophotometer at 450nm.

Scratch-Wound Assay

In order to confirm the migration of HDFs through scratch-wound assay, an Incucyte® live cell imager (Sartorius, Gottingen, Germany) capable of real-time cell observation was used. HDFs were cultured to 100% confluency on a 96-well plate and the assay was performed in accordance with the device manufacturer's manual. Briefly, with a wound maker, made by the device manufacturer, a straight scratch was created in the middle of the well, removing cells in that area. Subsequently, the wells were washed once with DPBS and the cells were treated with RSC-CM or RSCEs. Place the plate in Incucyte® was marked to ensure that images were taken in the same position and cell migration progress could be monitored continuously for 24 h.

Melanin Contents measurement

B16F10 cells were seeded at 8 x 10^3 cells per well in a 48-well plate and cultured for 24 h. After culturing, cells were treated with 1mM arbutin and RSCEs along with 100nM α-MSH for 48 h. Thereafter, the supernatant was recovered and absorbance of extra-melanin was measured at 405 nm. For intra-melanin, Cells in the plate were washed with DPBS and treated with Growth medium include CCK-8 reagent (Dojindo, Kumamoto, Japan) for 2 h. then, the supernatant harvest in a 96-well plate and measured absorbance at 450nm. Cells in the plate were washed twice with DPBS and treated 1N NaOH containing 10% DMSO at 85 °C for 30 min to dissolve. After transferring the cell lysate to a 96-well plate, absorbance was measured at 405 nm using a microplate reader. The melanin content was calculated using a standard curve, and normalized using the absorbance value of CCK-8.

Anti-inflammation assay

The Raw264.7 cells (ATCC, Virginia, USA) were seeded at 3 x 10^4 cells per well in 48-well plate and cultured at 37 °C under 5% CO_2. Cells were treated with 100 ng/ml of LPS to induce inflammation and treated with RSC-CM or RSCEs for 24 hours. Then, IL-6 was measured by ELISA (enzyme-linked immunosorbent assay) with the obtained supernatant and analyzed according to the manufacturer's protocol (R&D Systems, Minnesota, USA).

miRNA isolation & sequencing

Total RNA was isolated from RSCEs using the miRNeasy kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions and purified RNA was quantified using a spectrophotometry. Total RNA was stored at -80 °C, immediately after extraction. The miRNA sequencing analysis of the isolated samples was performed by Macrogen (Seoul, Korea). Briefly, cDNA libraries were generated using the Small RNA-Seq Kit for Illumina (Takara, Shiga, Japan). cDNA fragments are sequenced by the read length using sequence by synthesis method on the Illumina platform. After sequencing, the raw sequence reads are filtered based on quality. The adapter sequences are also trimmed off the raw sequence reads. Then, the processed reads are gathered forming a unique cluster. Unique clustered reads are aligned to miRBase v22.1 across human genome to classify known miRNAs.

Protein isolation & analysis

Protein extracts were boiled to be denatured for 10 mins at 100 ℃ by added lysis buffer final concentration SDS[15]. Protein concentration was measured by the bicinchoninic acid (BCA) method. 20 μg Proteins were separated by 12 % SDS-PAGE and in-gel digestion was conducted. Gels were fractionated into five parts according to molecular weight. in-gel digestion war performed described previously[16] gels ware divided and sliced into seven fractions according to molecular weight. sliced gels were washed with a 30% methanol and distained with 10 mM ammonium bicarbonate and 50% acetonitrile. After drying, gels were reduction with 10 mM dithiothreitol (DTT) and alkylation of cysteines with 55 mM iodoacetamide (IAA). After the gels were washed with distilled water, tryptic digestion was performed in 50 mM ammonium bicarbonate at 37°C for 12 h. Tryptic peptides was dried then extraction with extraction solution (50 mM ammonium bicarbonate and 50% acetonitrile containing 5% trifluoroacetic acid (TFA)). LC-MS/MS was conducted according to a previous procedure[17]. Tryptic digestion sample were dissolved with 0.5% TFA prior to further analysis. A 5 μL dissolved sample were onto a 100 μm × 2 cm nanoviper trap column and 15 cm × 75 μm nanoviper analysis column (Thermo Fisher Scientific) at a flow rate of 300 nL/min and were eluted with a gradient of 5%–40% acetonitrile over 95 min. All MS and MS/MS spectra captured by the Q Exactive Plus mass spectrometer (Thermo Fisher Scientific) were acquired in data-dependent top 12 mode. The MS/MS data were analyzed by using MASCOT 2.7, with a parameter corresponding to a false discovery rate (FDR) of 1%.

Data presentation and statistics

The statistical analysis was performed using Graph pas Prism® version 8.0 software (San Diego, CA, USA). Data were expressed as mean ± standard error of the mean (SEM), and comparisons of multiple samples were performed using one-way ANOVA and T-test. $P<0.05$ was considered statistically significant.

Results

Rose stem cell culture supernatant (RSC-CM) underwent exosomes isolation procedures using TFF. the supernatant was concentrated approximately 60X. In the remaining liquid, nano-tracking analysis showed the presence of particles with a reported diameter of approximately 100-200 nm (Fig 1A). Cryo-electron microscopy showed the presence of round particles with a diameter of approximately 100-200 nm (Fig 1B). The surface of the round particles appears grey, potentially because of remaining cell wall components.

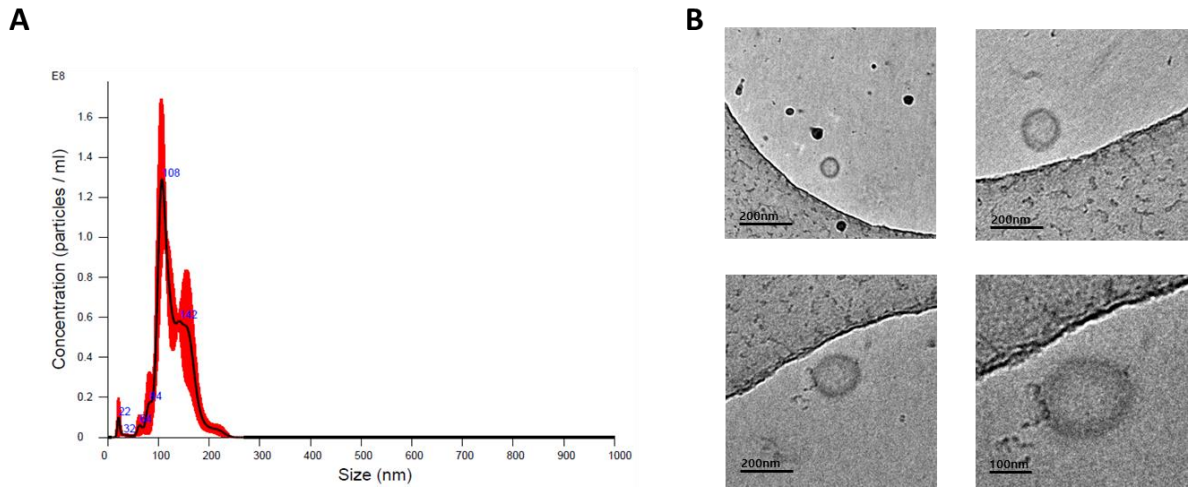


Figure 1. Characterizations of Rose-stem cell Exosomes (RSCEs). *(A) Representative histogram of particle concentrations and size distribution of RSCEs measured by nanoparticle tracking analysis (NTA). (B) Representative Cryo-TEM image of RSCEs. Scale bar: 200nm.*

Proteomics analysis of the RSCEs vs RSCs showed the presence of approximately 206 proteins in the RSCEs vs 2,098 in RSCs. The exosomes contained 35 unique proteins, and the biological process, molecular function and cellular component according to a bioinformatics analysis are shown in Figure 2. This analysis suggests the presence of several cell membrane or cell wall components, which is compatible with the exosomes-like particles being isolated indeed containing membranes. Further, several components are also related to RNA, implying the presence of RNA in the RSCEs.

The bioinformatics suggest that multiple cellular components are associated to membrane, including the presence of vacuolar membrane components, mitochondrial inner membrane proteins, as well as anchored components of membrane are present in the RSCEs. Further, the component analysis also suggests the presence of rose stem cell wall components. The biological process analysis shows the presence of “defense response to bacterium”, and “Defense response”, suggest that the RSCEs may be protective against infection or other trauma. Furthermore, the analysis of molecular function suggests enrichment of multiple enzyme pathways in the RSCEs, including peroxidase, esterase, ligase, dehydrogenase, endopeptidase, mannosidase and chitinase activity. The high significance of the term "translation" in the biological process bioinformatics, may suggest a relationship to RNA.

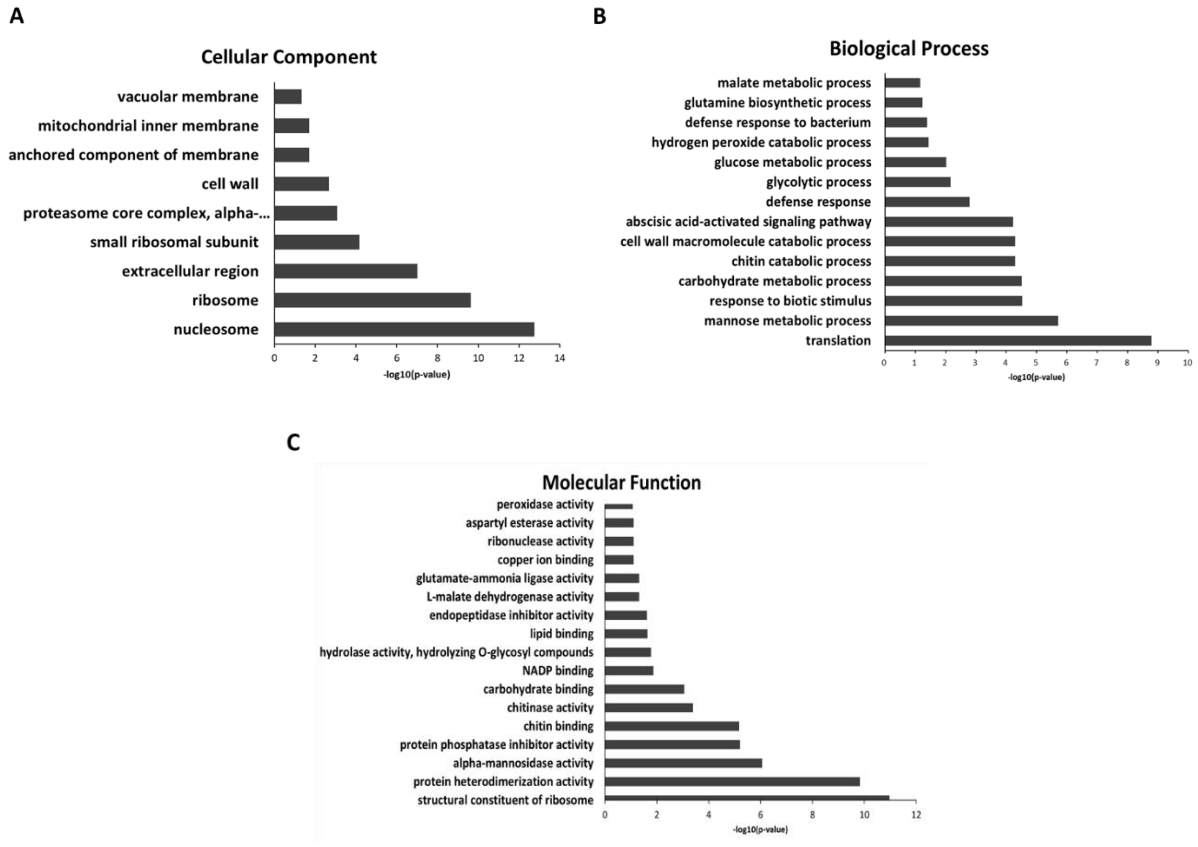


Figure 2. Proteomics analysis of Rose-stem cell Exosomes (RSCEs). *(A-C) Gene Ontology (GO) analysis of 206 identified RSCEs proteins. Enrichment of GO Cellular components, Biological process and Molecular function performed using DAVID bioinformatics are shown, with the cutoff for significance of -log10(p-value) using Benjamini-Hochberg correction. The most dominant cellular component is nucleosome, the biological process is translation and molecular function structural constituent of ribosome.*

Exosomes are known to contain and shuttle small RNA between cells[18], and therefore we isolated and sequenced RNA isolated from the RSCEs. Data show the presence of multiple microRNAs, including multiple Let7 family members (Fig 3A). In total more than 42 million bases were identified in the RSCEs. When matched to MicroRNA databases for Homo Sapiens, Zebrafish, Fruit Fly, C. Elegans and Arabidopsis, 904, 10577, 7, zero and 646 matches respectively were identified. In triplicate sequencing, the microRNAs that were consistently present were miR-8485, miR-574-5p, miR-1246, and the common functions are “regulating pathway for pluripotency of stem cells”, “Wnt signaling pathway” and “pathway in cancer” (involved in regulation of proliferation of cells) (Fig 3B).

A

No.	Mature ID	No.	Mature ID
1	hsa-let-7a-5p	15	hsa-miR-205-5p
2	hsa-let-7b-5p	16	hsa-miR-21-5p
3	hsa-let-7c-5p	17	hsa-miR-214-3p
4	hsa-let-7f-5p	18	hsa-miR-23a-3p
5	hsa-let-7g-5p	19	hsa-miR-23b-3p
6	hsa-let-7i-5p	20	hsa-miR-29b-3p
7	hsa-miR-1-3p	21	hsa-miR-3149
8	hsa-miR-10395-3p	22	hsa-miR-3942-5p
9	hsa-miR-122-5p	23	hsa-miR-4488
10	hsa-miR-1246	24	hsa-miR-4508
11	hsa-miR-1290	25	hsa-miR-574-5p
12	hsa-miR-130a-3p	26	hsa-miR-7847-3p
13	hsa-miR-184	27	hsa-miR-8485
14	hsa-miR-193b-5p		

B

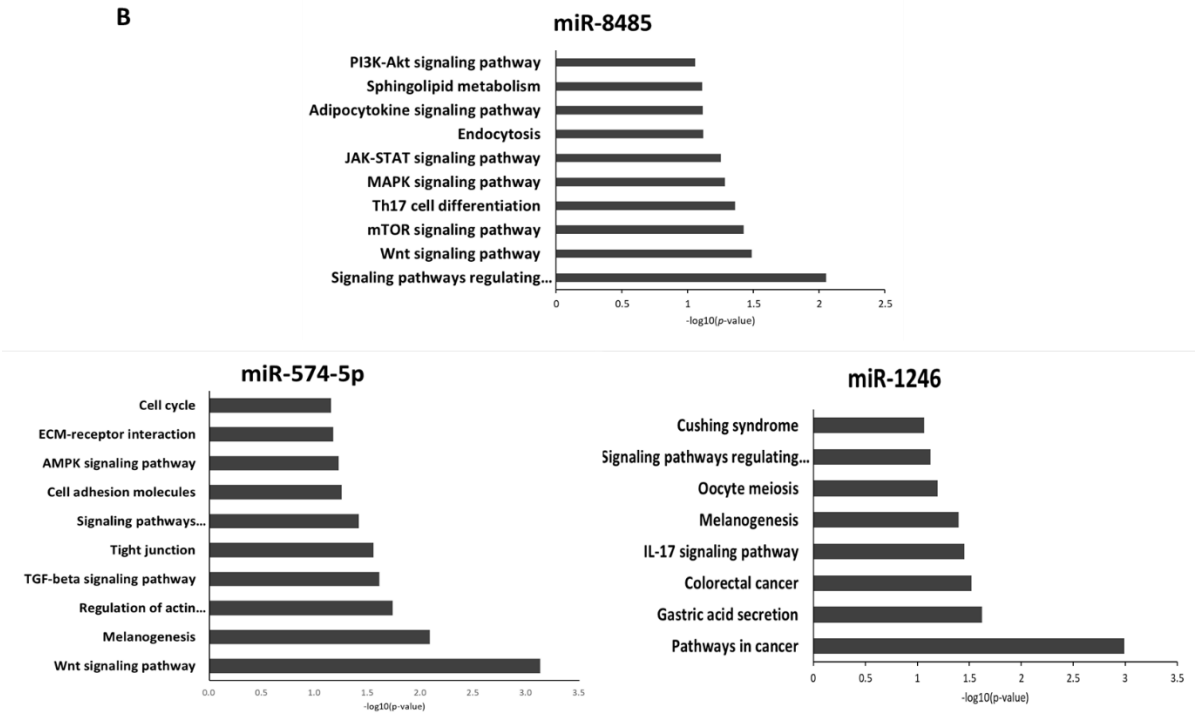


Figure 3. miRNA analysis of Rose-stem cell Exosomes (RSCEs) using small RNA sequencing using the Illumina platform. *(A) List of miRNAs that were matched against the Homo sapiens miRNA database by comparing miRNAs isolated from the RSCEs. (B) Representative miRNAs of RSCEs and their functional roles using gene ontology analysis using the miRbase platform. Multiple RSCEs miRNA were identifiable in the human database, and the bioinformatics imply that the three miRNA that were repeatedly observed in replicates, miR-8485, miR-574-5p and mir-1246 are involved in signaling pathway regulation, Wnt signaling pathway and "pathway in cancer" (most likely related to cell proliferation).*

In the next steps, we aimed at determining function of the RSCEs in human cells, primarily focusing on skin-relevant cells such as hair papilla cells, fibroblasts and melanocytes. The unseparated RSC-CM was found to reduce viability in human dermal papilla cells (HDPs). However, the RSCEs did not show any detrimental effect on viability of HDP (Fig 4).

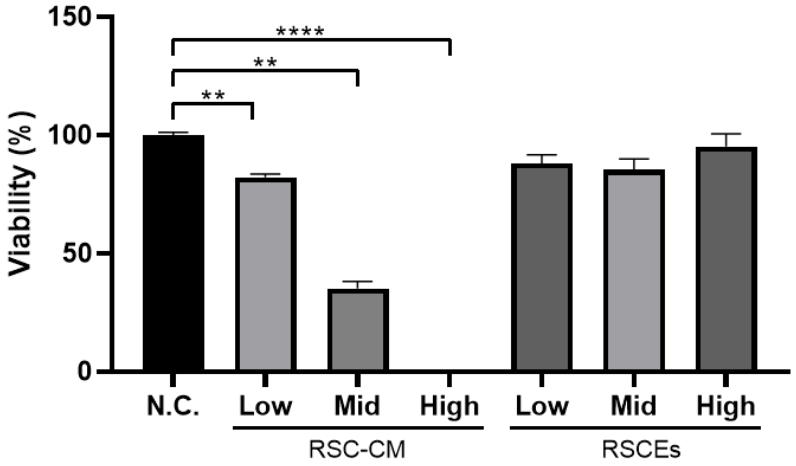
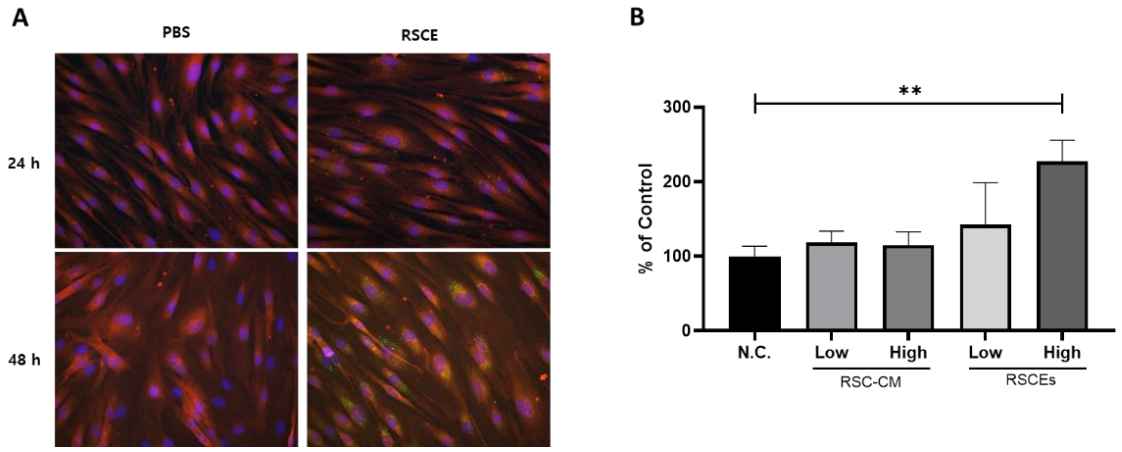


Figure 4. Cytotoxicity of Rose Stem Cell cultured supernatants (RSC-CM) and Rose-stem cell Exosomes (RSCEs) in Human Dermal Papilla cells (HDPs). *Cell metabolism was quantified by the MTT assay. After 24 h of Rose callus Cultured medium (RSC-CM) and RSCEs, the absorbance was detected at 540nm. N.C.; Normal Control, Low Conc.; 2.5E+08 particles/ml, Mid Conc.; 8.0E+08 particles/ml, High Conc.; 2.5E+09 particles/ml in Growth medium. (**;p<0.01, ****;p<0.001 vs N.C.). CM, but not RSCEs, influenced the MTT assay negatively, implying negative effects on cell viability by the CM.*

Human dermal fibroblasts (HDFs), grown in 24 well plates, were shown to take up exosomes loaded with the fluorescent dye PKH67 (green; Fig 5A). Collagen synthesis from HDFs was quantified by ELISA, and the RSCEs were shown to dose-dependently increase the pro-type collagen 1 (Fig 5B). A scratch was introduced to confluent dermal fibroblasts grown in 48 well plate wells, to mimic a wound. Applying the positive control (growth medium) resulted in close to complete culture of the scratch wound in 24 hrs. Applying RSC-CM did not result in any apparent scratch wound closure, whereas the RSCEs were almost as efficient as the positive control (Figure 5C and D).



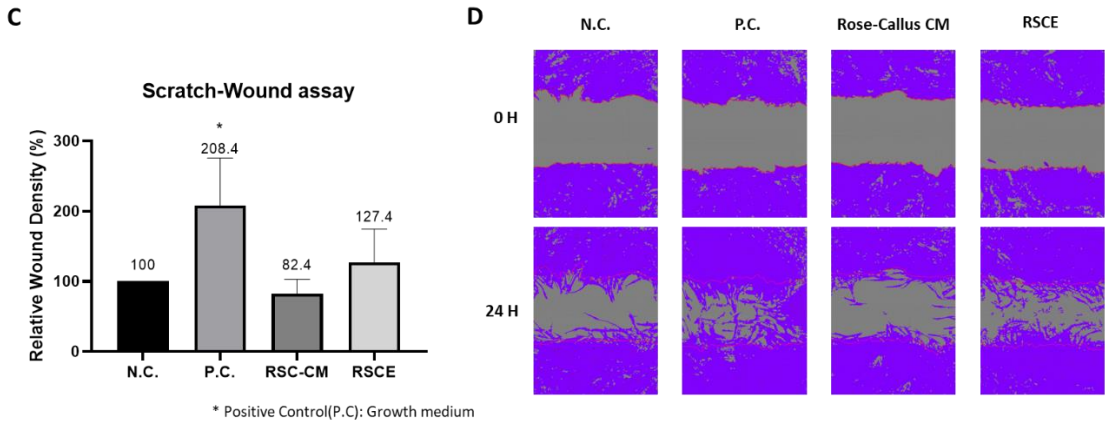


Figure 5. Uptake, Collagen synthesis and migration in Human Dermal Fibroblasts (HDFs) of Rose-stem cell Exosomes (RSCEs). *(A) RSCEs were stained with PKH67 and incubated with HDFs for 24 and 48h respectively. Blue: Nuclei were stained DAPI, Orange; Cytoplasm was stained cellmask, Green; RSCEs with PKH67. (B) RSCEs increases the collagen production in HDF cell culture supernatant in proportion to the treatment concentration more significantly for RSC- CM. (Low Conc.; 8.0E+08 particles/ml, High Conc.; 2.5E+09 particles/ml). (C, D) Representative photomicrographs of the wound edge in the scratch assay at 0 and 24h after treatment with RSC-CM and RSCEs (Conc.; 2.5E+09 particles/ml). The wound closure rate is presented as the percent scratch closure. (**; p<0.01 vs N.C.).*

The mouse melanoma cell line B16F10 was used to determine any effects of the RSC-CM or RSCEs on cell toxicity and melanin accumulation. Again, the RSC-CM reduced cell viability with approximately 40% (Figure 6A), where the RSCEs at low or high dose did not reduce cell viability. The RSCEs dose-dependently reduced melanin accumulation at 48 h time point, to a similar degree or more than the positive control (Figure 6B).

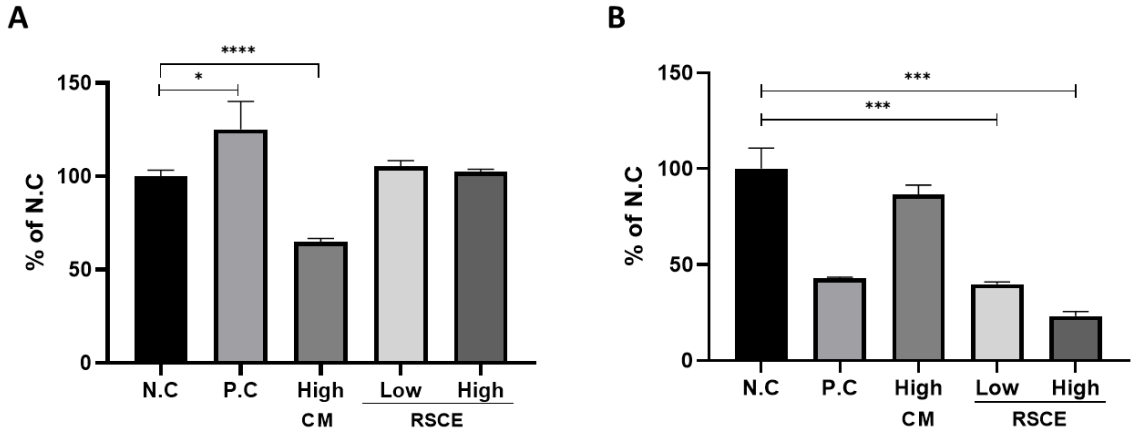


Figure 6. Cell viability and Melanin synthesis inhibit in mouse B16F10 cells of Rose-stem cell Exosomes (RSCEs). *(A) Cell viability vs of B16F10 cells (mouse melanocytes) as % of negative control (N.C.) and compared to Arbutin (1mM; P.C.), showing that RSCEs did not influence viability. (B) Melanin contents measured by absorbance (405nm) in B16F10 cell cultures, showing reduction by two concentrations of RSCEs (Low Conc.; 8.0E+08 particles/ml, High Conc.; 2.5E+09 particles/ml). (*; p<0.05, ***; p<0.005, ****; p<0.001 vs N.C.)*

RSCEs were applied to the macrophage cell line RAW264.7 that was exposed to LPS, and effects were compared to RSC-CM. In this model, neither treatment influenced cell viability in a detrimental way (Figure 7A). The RSCEs dose-dependently reduced the cytokine release from the cell line (Figure 7B).

In this experiment, a mild anti-inflammatory effect of also the high concentration of RSCEs was observed.

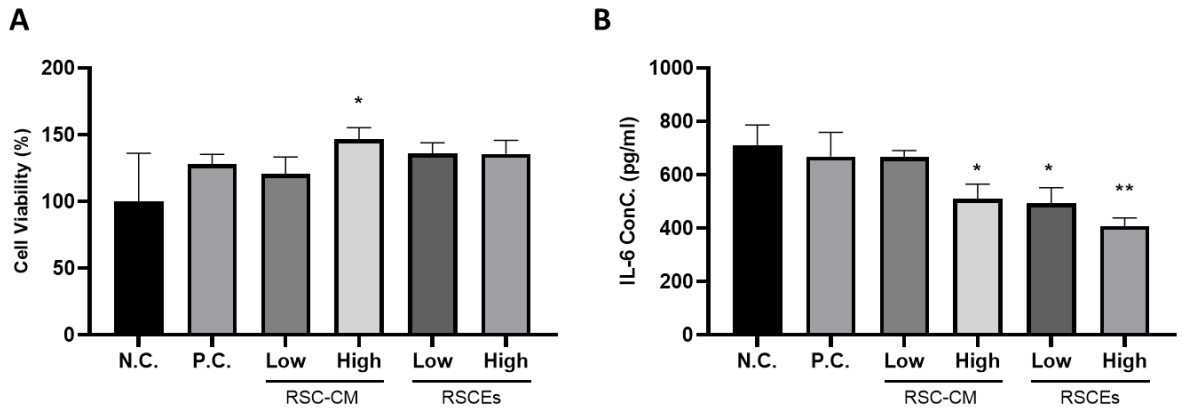


Figure 7. Cell viability and Anti-inflammation activity in mouse Raw264.7 cells of Rose-stem cell Exosomes (RSCEs). *(A) Cell viability vs negative control (N.C.) by dexamethasone (200µM; positive control; P.C.), in RAW cells exposed to LPS (100ng/mL). (B) IL-6 release by RAW cells exposed to LPS (100ng/mL) and treated with either dexamethasone (P.C.), RSC-CM (Low Conc.; 8.0E+06 particles/ml, High Conc.; 8.0E+08 particles/ml) and RSCEs (Low Conc.; 8.0E+06 particles/ml, High Conc.; 8.0E+08 particles/ml) at two different doses. (*; p<0.05, **; p<0.01 vs N.C.)*

Discussion

This is the first study to show the presence of RSCEs in the supernatant of Rose stem cell cultures (RSCs). Our compiled data suggest that the RSCEs indeed are membrane enclosed extracellular vesicles, including the presence of membrane-associated proteins and protein functions, as well as the presence of RNA. The RSCEs also stimulate skin fibroblast proliferation and collagen production, as well as in vitro wound healing. Further, the RSCEs reduce pigmentation of melanocytes, and covey anti-inflammatory function in macrophages.

Figure 1, 2 and 3, this is, to our knowledge, the first study to show the presence of RSCEs in vitro cultures. Cryo-electron microscopy visualized round structures that are similar in shape and electron density to exosomes released from eukaryotic cells[19]. To further determine that the observed particles indeed are exosomes with membranes, proteomics was performed, which showed the presence of multiple membrane associated proteins in the RSCEs, again supporting the conclusion that the structures indeed are similar to exosomes from other kingdoms including eukaryote. Importantly, the presence of RNA in the RSCEs further supports their similarity to exosomes, as they are generally known to carry functional RNA[18]. Overall, these results strongly suggests that Rose stem cells release exosomes in cell cultures.

Bioinformatics analysis of both the protein and RNA contents in the RSCEs suggests that they may have a role in cell defense, and may convey anti-inflammatory functions in cell systems. Importantly, the microRNA Let-7 family are known to be present in different types of exosomes from humans, including mesenchymal stem cell exosomes[20, 21]. This family of microRNAs are suggested to be involved in regulating inflammation, but can also attenuate cancer growth. miR-21 is considered to be abundant in many types of cells, and is a highly conserved microRNA. It has been suggested to be involved in

multiple processes in health and disease, and may participate in the stimulation of cell growth[22]. Also, miR-23 may participate in the biological functions of the RSCEs. This microRNA can regulate multiple gene and intracellular signaling, and can mediate cell- proliferation, survival, and migration, and can stimulate angiogenesis[23]. Overall, these results suggest that the cargo of the RSCEs can convey multiple biological functions in cell systems, although it is unclear exactly which cargo may be most important.

When we applied the RSCEs to different cell systems, multiple interesting phenomena were observed. Firstly, the non-exosome containing cell culture supernatant was toxic to human dermal papilla cells, whereas the exosomes did not have any such function. Further, the RSCEs were taken up by human dermal fibroblasts, and stimulate their proliferation as well as collagen production. In an in vitro scratch assay, which mimics fibroblast function in wounds, the RSCEs were shown to increase wound closure, probably by increasing both fibroblast proliferation as well as migration. In the current experiments, it is impossible to determine exactly which RSCEs cargo that mediate the functions observed in the fibroblasts, but it is likely that uptake and intracellular delivery of both protein and RNA cargo is important, as it is in other types of functional exosome-assays.

Importantly, the RSCEs are also taken up by melanocytes. Interestingly, the uptake of RSCEs was paralleled by a reduced melanin content in the cells, suggesting that the RSCEs may have a whitening function. Again, the exact molecular mechanism of this function is unclear, but is likely to involve multiple molecules delivered to the melanocytes by the RSCEs.

The RSCEs were also found to have anti-inflammatory function in a macrophage assay. Briefly, the macrophages were stimulated by LPS, which results in strong cytokine release in the macrophage supernatant, specifically IL-6. RSCEs dose-dependently reduce the IL-6 release from the macrophages. It is possible that multiple proteins and RNA-species mediate this function, also in this inflammation assay.

The collective functionality of the RSCEs on skin fibroblasts, melanocytes and inflammatory cells, suggest that they could be beneficial in treating skin, including potentially skin disease, but also to provide function in skincare products used as cosmetics or cosmeceuticals. Indeed, plant extracts are one of the main sources of materials used in the cosmetic industry[24]. A growing body of research has highlighted the potential benefits of plant-based ingredients in the cosmetics, for example including their anti-inflammatory properties. Plant ingredients are known to have a range of features, such as anti-inflammatory-, antioxidant-, antimicrobial-, and anti-aging properties, beneficial for the skin and hair[24, 25]. Additionally, in the last few years, potential anti-aging compounds isolated from plants have been reported to improve skin elasticity through various pharmacological actions[26]. Overall, this indicates that plant materials, including extracts, can be applied not only for skin care purposes but potentially also for the treatment of several skin diseases[27]. Plant extracts such as aloe vera, chamomile, green tea, rosemary, and tea tree oil have been found to improve at various skin conditions, such as acne, eczema, and psoriasis[28]. Additionally, seed oils and fruit extracts provide essential nutrients to protect and maintain the skin and hair, making them popular ingredients in beauty products. Plant-based ingredients may not only be beneficial for the skin and hair but also have environmental advantages. Plant-based products are also considered sustainable, as they are biodegradable and have a lower environmental impact than synthetic chemicals used in many cosmetics and beauty products. Therefore, investigating and further optimizing plant-based skincare products, potentially containing plant stem cell exosomes, remains important.

This study, for the first time, shows the functionality of RSCEs in cell functions relevant for the skin, including skin inflammatory diseases and potentially as cosmetics. These functions include growth of skin fibroblasts and collagen production, reduced melanin production in melanocytes, and inhibition of inflammation. Developing RSCEs for treating skin disease or as skincare products may lead to further enhanced product development.

Acknowledgement and Declaration of Interest

The authors thank professor Jan Lötvall for valuable advice in the preparation of this manuscript.

Author Contributions

Y.J.W.; Conceptualization, methodology, formal analysis, investigation, writing-original draft preparation, writing-review and editing, E.L.; methodology, formal analysis, investigation, writing-original draft preparation, S.Y.M.; investigation and writing-original draft preparation, and B.S.C.: conceptualization, and supervision. All authors have reviewed the manuscript.

Competing interests

The authors declare no competing interests.

Funding

The study was fully financed by ExoCoBio.

Data Availability

The mass spectrometry proteomics data have been deposited to the ProteomeXchange Consortium via the PRIDE partner repository (http://www.ebi.ac. uk/pride/archive) with the dataset identifier PXD043169. And miRNA raw data generated in this study was deposited in the NCBI Sequence Read Archive (SRA) under BioProject ID PRJNA980591.

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