

1 **Biological Function of Exosome-like Particles Isolated from Rose** 2 **(*Rosa Damascena*) Stem Cell Culture Supernatant**

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8 **Abstract**

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

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38 Introduction

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

46 Research on plant exosomes is still in its early stages, but there is growing interest in their potential
47 applications in agriculture, biotechnology and even medicine[5]. For example, they could be used as
48 delivery systems for drugs or genetic materials, or as diagnostic tools for plant diseases[7]. Vesicles
49 extracted from different types of plants have been studied as potential drug candidates in different
50 disease models. The plants used for this vesicle extraction include lemon, ginger, ginseng and grapes[8-
51 11]. A major concern with these studies is, however, that they may not be studying naturally released
52 plant EVs, as the isolation procedures can include harsh methods such as kitchen mixers, which
53 disintegrates cell and provides membrane for possible spontaneous micro-vesiculation of these.
54 Regardless, several of these vesicles have shown anti-inflammatory functions in cell systems and
55 animals[12]. However, to our knowledge, no study has determined the presence of exosome-like
56 particles in the cell supernatant of plant stem cells.

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

63

64 Materials & Methods

65 Isolation and Characterization of Exosome-like structures

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

79 **Cellular Uptake Assay**

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

88 **Cell Toxicity**

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

95 **Collagen assay**

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

100 **Scratch-Wound Assay**

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

109 **Melanin Contents measurement**

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

119 **Anti-inflammation assay**

120 The Raw264.7 cells (ATCC, Virginia, USA) were seeded at 3×10^4 cells per well in 48-well plate and
121 cultured at 37 °C under 5% CO₂. Cells were treated with 100 ng/ml of LPS to induce inflammation and
122 treated with RSC-CM or RSCEs for 24 hours. Then, IL-6 was measured by ELISA (enzyme-linked
123 immunosorbent assay) with the obtained supernatant and analyzed according to the manufacturer's
124 protocol (R&D Systems, Minnesota, USA).

125 **miRNA isolation & sequencing**

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

135 **Protein isolation & analysis**

136 Protein extracts were boiled to be denatured for 10 mins at 100 °C by added lysis buffer final
137 concentration SDS[15]. Protein concentration was measured by the bicinchoninic acid (BCA) method.
138 20 µg Proteins were separated by 12 % SDS-PAGE and in-gel digestion was conducted. Gels were
139 fractionated into five parts according to molecular weight. in-gel digestion was performed described
140 previously[16] gels were divided and sliced into seven fractions according to molecular weight. sliced
141 gels were washed with a 30% methanol and destained with 10 mM ammonium bicarbonate and 50%
142 acetonitrile. After drying, gels were reduction with 10 mM dithiothreitol (DTT) and alkylation of
143 cysteines with 55 mM iodoacetamide (IAA). After the gels were washed with distilled water, tryptic
144 digestion was performed in 50 mM ammonium bicarbonate at 37°C for 12 h. Tryptic peptides were dried
145 then extraction with extraction solution (50 mM ammonium bicarbonate and 50% acetonitrile
146 containing 5% trifluoroacetic acid (TFA)). LC-MS/MS was conducted according to a previous
147 procedure[17]. Tryptic digestion sample were dissolved with 0.5% TFA prior to further analysis. A 5 µL
148 dissolved sample were onto a 100 µm × 2 cm nanoviper trap column and 15 cm × 75 µm nanoviper
149 analysis column (Thermo Fisher Scientific) at a flow rate of 300 nL/min and were eluted with a gradient
150 of 5%–40% acetonitrile over 95 min. All MS and MS/MS spectra captured by the Q Exactive Plus mass
151 spectrometer (Thermo Fisher Scientific) were acquired in data-dependent top 12 mode. The MS/MS
152 data were analyzed by using MASCOT 2.7, with a parameter corresponding to a false discovery rate
153 (FDR) of 1%.

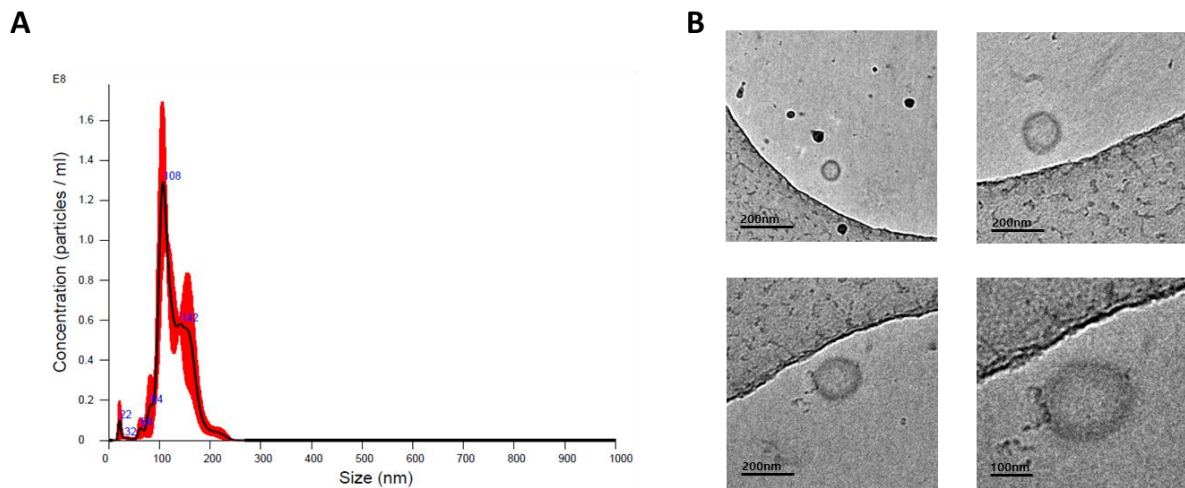
154 **Data presentation and statistics**

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

159

160 Results

161 Rose stem cell culture supernatant (RSC-CM) underwent exosomes isolation procedures using TFF. the
162 supernatant was concentrated approximately 60X. In the remaining liquid, nano-tracking analysis
163 showed the presence of particles with a reported diameter of approximately 100-200 nm (Fig 1A).
164 Cryo-electron microscopy showed the presence of round particles with a diameter of approximately
165 100-200 nm (Fig 1B). The surface of the round particles appears grey, potentially because of remaining
166 cell wall components.



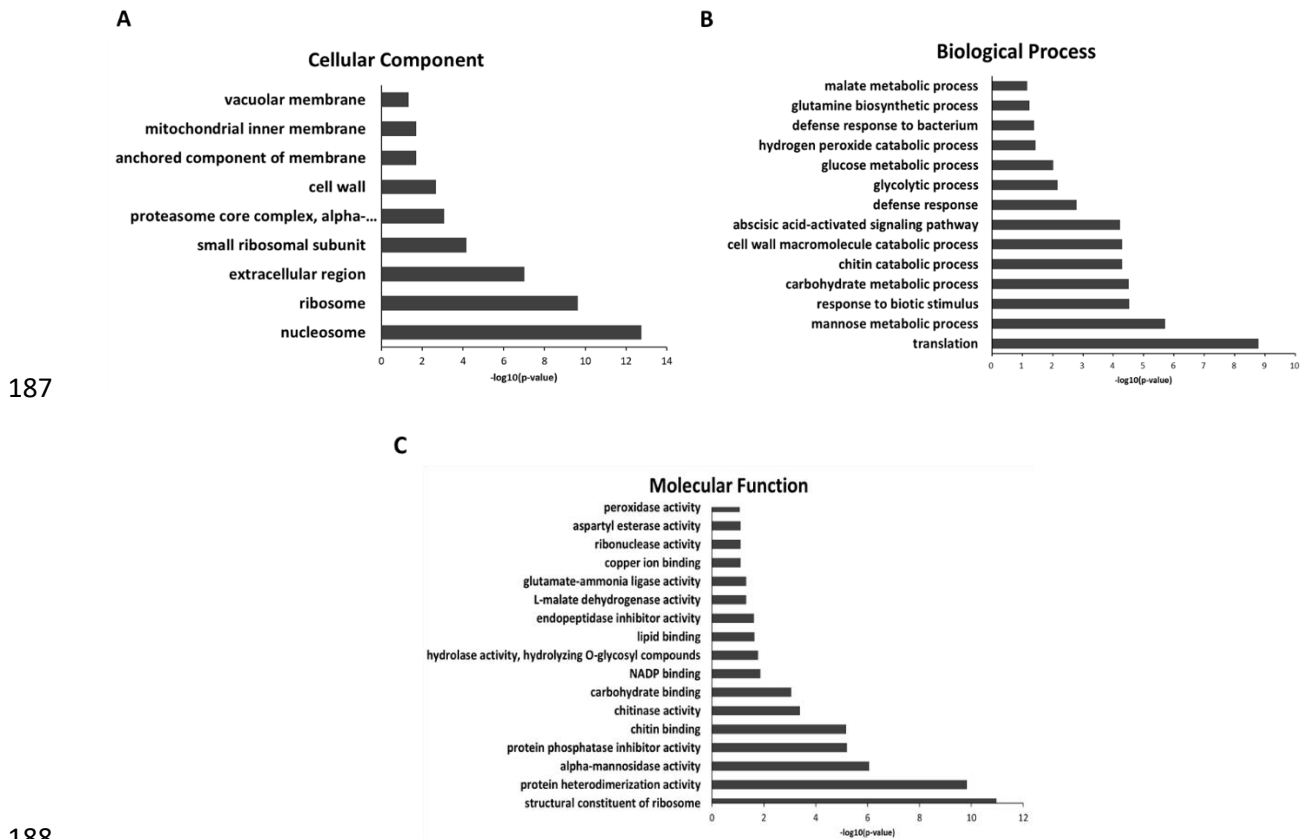
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168 **Figure 1. Characterizations of Rose-stem cell Exosomes (RSCs).** (A) Representative histogram of particle
169 concentrations and size distribution of RSCs measured by nanoparticle tracking analysis (NTA). (B)
170 Representative Cryo-TEM image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

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

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

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189 **Figure 2. Proteomics analysis of Rose-stem cell Exosomes (RSCes).** (A-C) Gene Ontology (GO) analysis of 206
 190 identified RSCes proteins. Enrichment of GO Cellular components, Biological process and Molecular function
 191 performed using DAVID bioinformatics are shown, with the cutoff for significance of $-\log_{10}(p\text{-value})$ using
 192 Benjamini-Hochberg correction. The most dominant cellular component is nucleosome, the biological process is
 193 translation and molecular function structural constituent of ribosome.

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

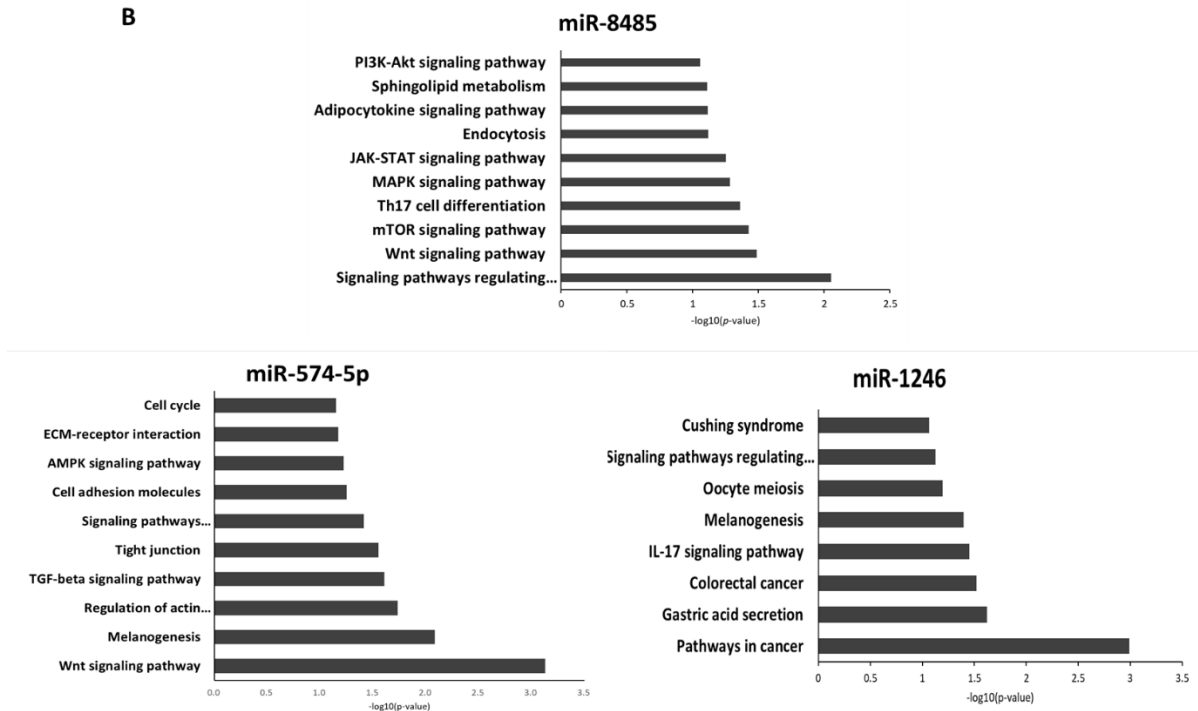
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A

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



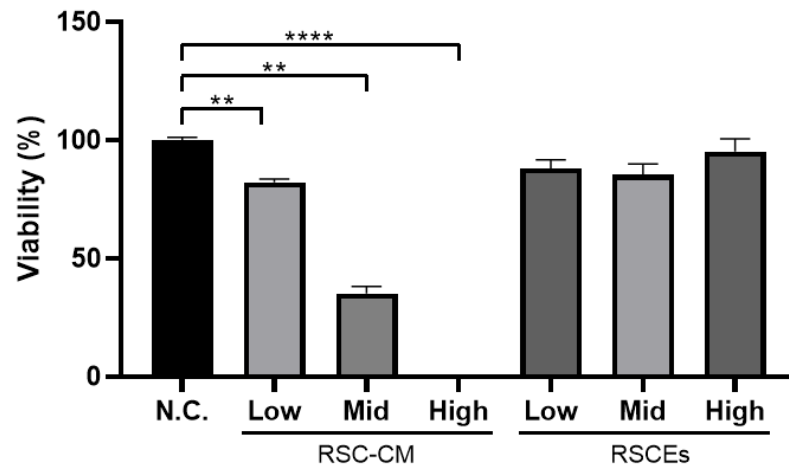
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205 **Figure 3. miRNA analysis of Rose-stem cell Exosomes (RSCs) using small RNA sequencing using the Illumina**
 206 **platform.** (A) List of miRNAs that were matched against the Homo sapiens miRNA database by comparing miRNAs
 207 isolated from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

212

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

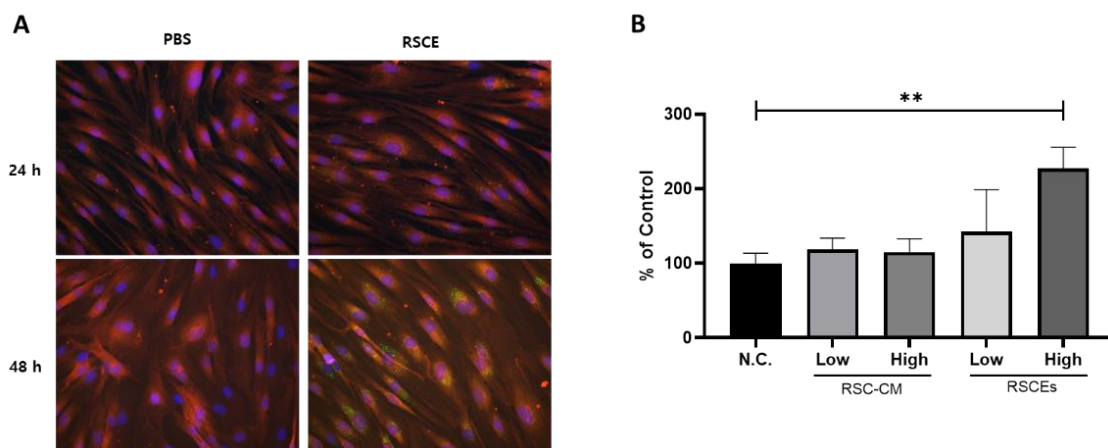
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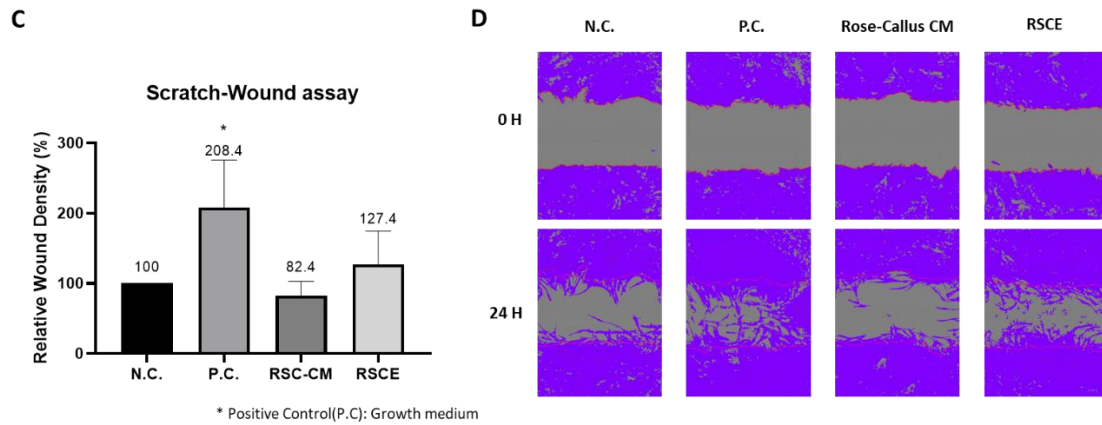
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219 **Figure 4. Cytotoxicity of Rose Stem Cell cultured supernatants (RSC-CM) and Rose-stem cell Exosomes (RSCEs)**
220 **in Human Dermal Papilla cells (HDPs).** Cell metabolism was quantified by the MTT assay. After 24 h of Rose callus
221 Cultured medium (RSC-CM) and RSCEs, the absorbance was detected at 540nm. N.C.; Normal Control, Low Conc.;
222 $2.5E+08$ particles/ml, Mid Conc.; $8.0E+08$ particles/ml, High Conc.; $2.5E+09$ particles/ml in Growth medium.
223 (**; $p < 0.01$, ****; $p < 0.001$ vs N.C.). CM, but not RSCEs, influenced the MTT assay negatively, implying negative
224 effects on cell viability by the CM.

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



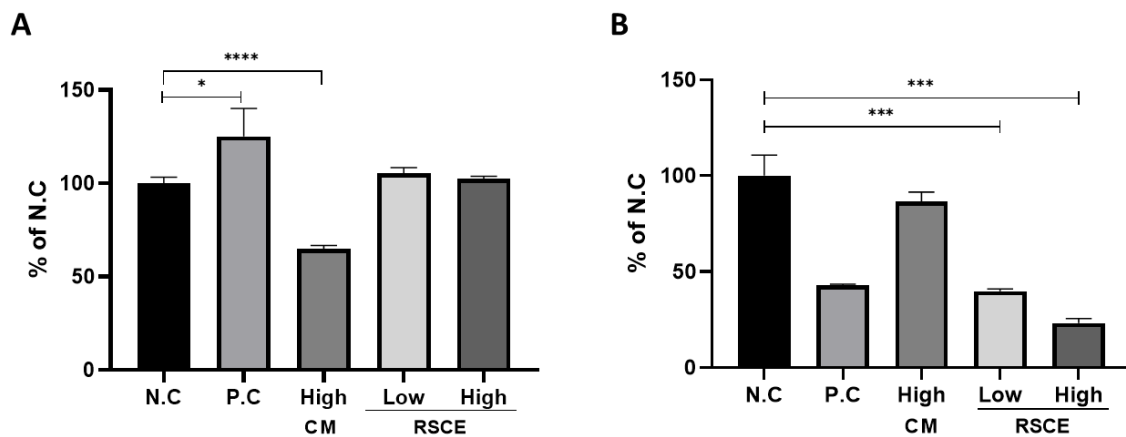
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233

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

242 The mouse melanoma cell line B16F10 was used to determine any effects of the RSC-CM or RSCs on
 243 cell toxicity and melanin accumulation. Again, the RSC-CM reduced cell viability with approximately
 244 40% (Figure 6A), where the RSCs at low or high dose did not reduce cell viability. The RSCs dose-
 245 dependently reduced melanin accumulation at 48 h time point, to a similar degree or more than the
 246 positive control (Figure 6B).

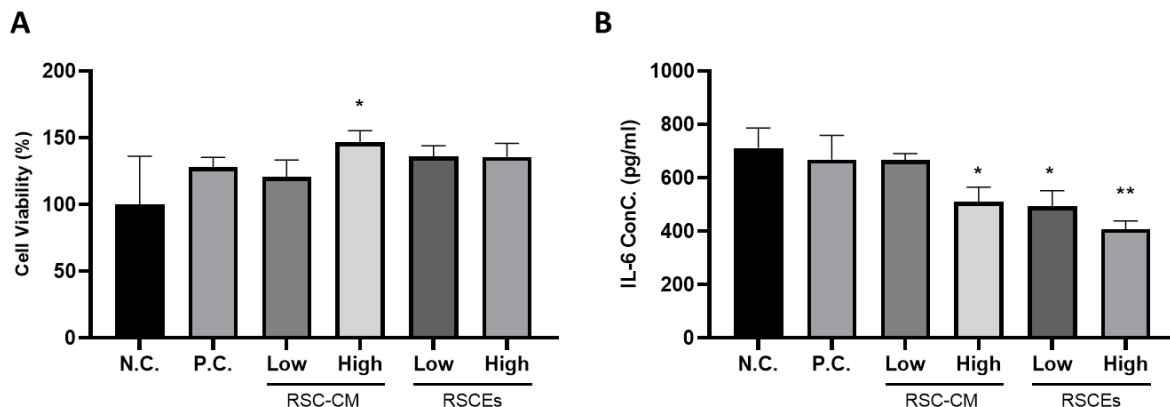


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248 **Figure 6. Cell viability and Melanin synthesis inhibit in mouse B16F10 cells of Rose-stem cell Exosomes (RSCs).**
 249 (A) Cell viability vs of B16F10 cells (mouse melanocytes) as % of negative control (N.C.) and compared to Arbutin
 250 (1mM; P.C.), showing that RSCs did not influence viability. (B) Melanin contents measured by absorbance
 251 (405nm) in B16F10 cell cultures, showing reduction by two concentrations of RSCs (Low Conc.; $8.0E+08$
 252 particles/ml, High Conc.; $2.5E+09$ particles/ml). (*; $p < 0.05$, ***, $p < 0.005$, ****, $p < 0.001$ vs N.C.)

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

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



258

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

265

266 Discussion

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

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

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

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

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

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

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

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

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

336

337 **Acknowledgement and Declaration of Interest**

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339 **Author Contributions**

340 Y.J.W.; Conceptualization, methodology, formal analysis, investigation, writing-original draft
341 preparation, writing-review and editing, E.L.; methodology, formal analysis, investigation, writing-
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344

345 **Competing interests**

346 The authors declare no competing interests.

347

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350

351 **Data Availability**

352 The mass spectrometry proteomics data have been deposited to the ProteomeXchange Consortium
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354 PXD043169. And miRNA raw data generated in this study was deposited in the NCBI Sequence Read
355 Archive (SRA) under BioProject ID PRJNA980591.

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