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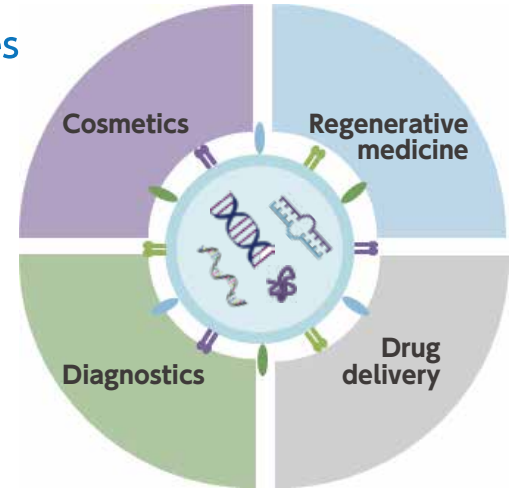
TREHALOSE SG JP, USP-NF, Ph. Eur., CP

For Exosome Production and Storage

Mesenchymal Stem Cell (MSC)-derived Exosomes

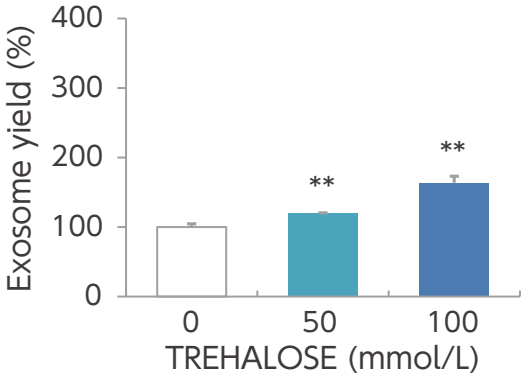
- MSC-derived exosomes are attracting attention for their application in regenerative medicine, drug delivery, diagnostics and cosmetics, and the need for a stable supply of exosomes is increasing.
- TREHALOSE SG is expected to be a powerful tool for industrial applications by increasing the exosome yield in the three processes of exosome production, purification and preservation.



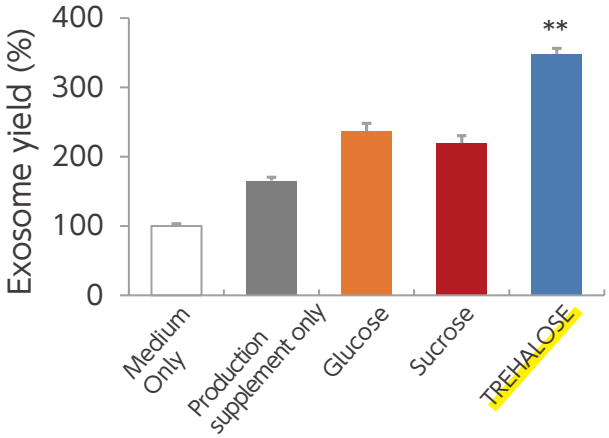
Improvement of Production Yield

- **The addition of TREHALOSE SG increases the production of MSC-derived exosomes via elevating Rab7 protein level.**
- **The combination of TREHALOSE SG with another supplement is more effective.**

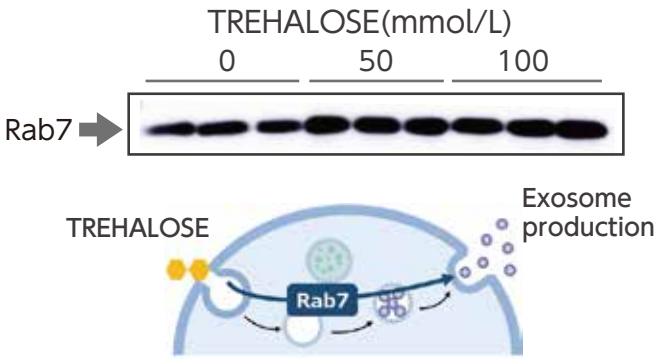
TREHALOSE alone

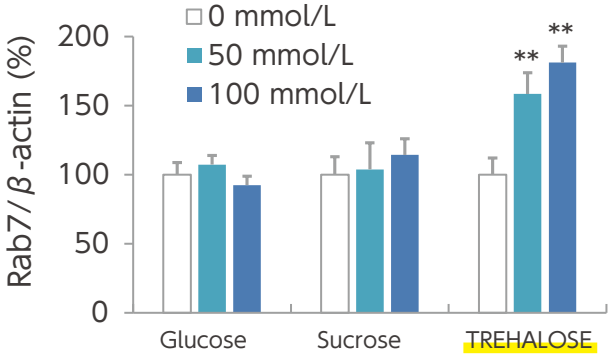


Combination Use



Mechanism





[Methods] Human adipose-derived MSCs (4×10^4 cells /2mL/well) were incubated with TREHALOSE SG in Messenchymal stem cell growth medium DXF (TAKARA) for 48 hrs, and exosome marker (CD9, CD63, CD81) positive particles in the culture supernatant were measured by flow cytometer. Effects of TREHALOSE SG were examined alone or in combination with exosome production supplements (EV-Up™, Fujifilm Wako Pure Chemical Corporation). Rab7 protein levels in MSCs were analyzed by Western blotting using anti-Rab7 antibody (Cell Signaling Technology, Inc.), and calculated relative to the β-actin signal (**$p<0.01$ vs. TREHALOSE 0 mmol/L).

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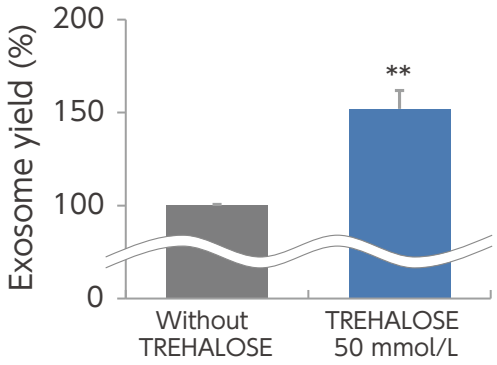
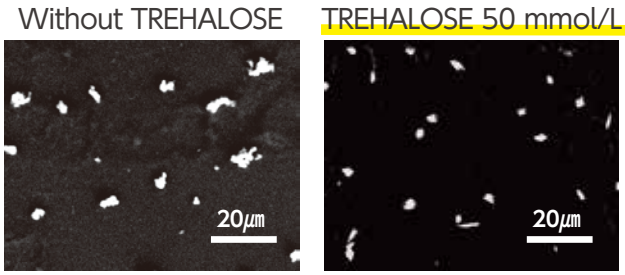
Improvement of Purification Yields

Addition of TREHALOSE SG suppresses exosome aggregation during purification.

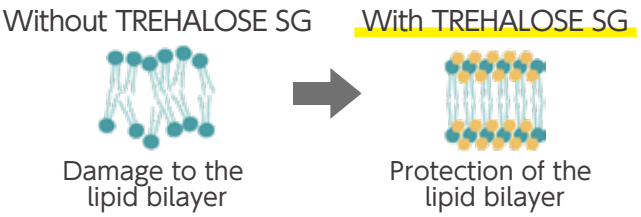




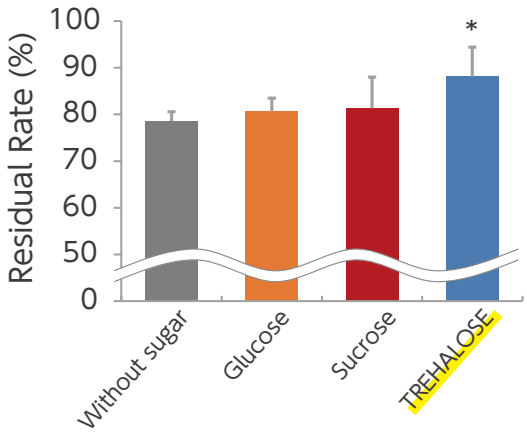
[Methods] MSC-derived culture supernatants were centrifuged using a 10-kDa ultrafiltration membrane (Amicon Ultra, Merck Millipore) at 9,000 g for 20 min at 4℃. Extracellular particles were collected and adjusted to 200 μL using 0.1 μm filtered PBS, and the number was measured using a flow cytometer. Results represent the exosome yield when the number of exosomes in the absence of TREHALOSE was settled as 100% (**p<0.01 vs. without TREHALOSE). The state of exosome was photographed using a scanning electron microscopy (× 500).

Improvement of Stability

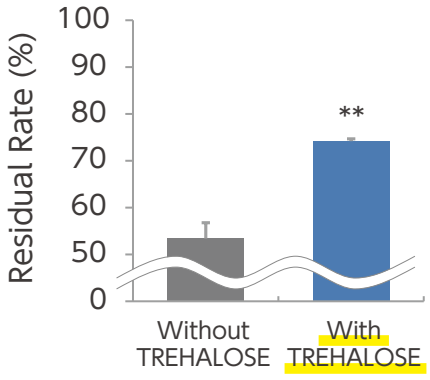
Addition of TREHALOSE SG improves the stability of exosomes during storage.



Storage at 4℃ (2 weeks)



Freeze-thawing (3 times)



[Methods] MSC-derived exosomes were suspended in 1 mL of PBS containing TREHALOSE SG or the other sugars (50 mmol/L), and the number of exosome was measured by flow cytometer after storage at 4℃ or freeze-thaw cycles (-80℃ to 4℃). Results represent residual rate of exosomes when the number of exosomes before storage was settled as 100%, and are expressed as the mean and standard deviation of three similar experiments (*p<0.05, **p<0.01 vs. without sugar or TREHALOSE).

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