How did you get in here?

A closer look at the interaction between adipose stem cell derived extracellular vesicles and Schwann cells.

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Objective

Small extracellular vesicles (**EVs**) are involved in a plethora of physiological and pathophysiological processes, however, many known unknowns regarding the biology of EVs remain (1, 2). In this study, we investigate how EVs isolated from adipose tissue derived stem cells (**ASCs**) interact with Schwann cells (**SCs**), which have been ascribed an essential role in nerve repair. ASC-EVs have been shown to increase proliferation of SCs (3), making them a promising candidate for autologous therapy following injury.

Material and Methods

SCs and ASCs were isolated from young adult male Lewis rats according to well established protocols. ASC-EVs were obtained following a 24-hour serum free conditioning period by differential ultracentrifugation.
SCs and ASCs were immunophenotyped with confocal laser scanning microscopy, EVs were immunophenotyped with imaging flow cytometry (IFC), and analyzed regarding their morphology by nanoparticle tracking analysis (NTA), atomic force microscopy (AFM) and both scanning- and transmission electron microscopy (SEM, Cryo-TEM). Interactions between SCs and EVs were recorded by live cell imaging (LCI), and further analyzed by 3D reconstructions of confocal micrographs, as well as TEM.
After we established the internalization of ASC-EVs by SCs, the membrane transit was characterized by specific biochemical inhibition of the two most commonly described modes of entry for EVs, clathrin-mediated endocytosis (CME) and macropinocytosis (MP). To quantify the internalization, IFC was used following biochemical inhibition, and an automated analysis pipeline was established to determine internalization quality (*internalization score*) and quantity (*spot count*). In **figure 1** a representative gallery of an SC following incubation with fluorescently labeled EVs is presented.

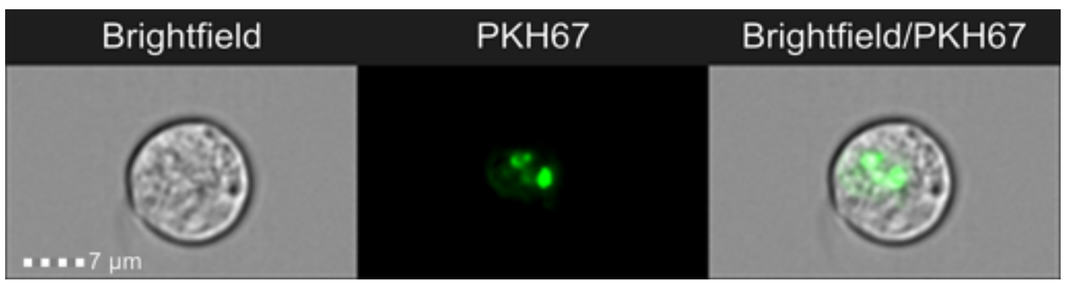

Figure 1: Imaging flow cytometry gallery. Brightfield image of a SCs (left), fluorescence image of PKH67-labeled ASC-EVs (middle), merge of brightfield and fluorescence channels (right).

Results

Upon initial contact with SCs, ASC-EVs were moved along the membrane until they were internalized and subsequently transported towards the cell's nucleus, where they were accumulated. The inhibition of specific endocytosis pathways revealed that in SCs, the internalization of ASC-EVs is mainly mediated by clathrin, though alternative modes of membrane transit are likely involved, as no complete block of ASC-EV-internalization could be achieved. Upon internalization of ASC-EVs, we observed an increase in SC proliferation in a time- and dose dependent manner, up to 2.5-fold compared to untreated SCs within 72h.

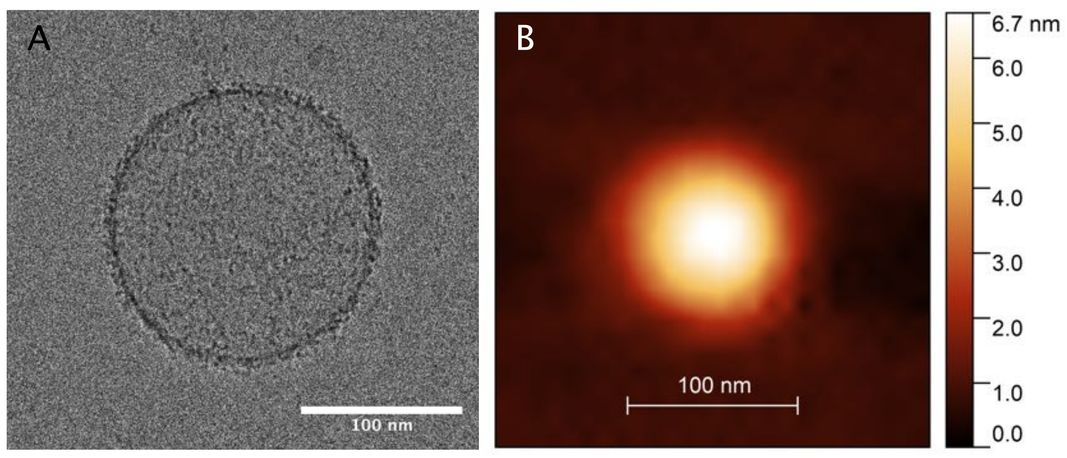

Figure 2: Characterization of ASC-EVs. Cryo-TEM micrograph (A) and AFM topography (B) of a representative ASC-EV

Figure 3: Transparent surface rendering of a 3D reconstructed confocal laser scanning micrograph. After transit through the SC-membrane (white), ASC-EVs (red) accumulate around the nucleus (blue) following internalization.

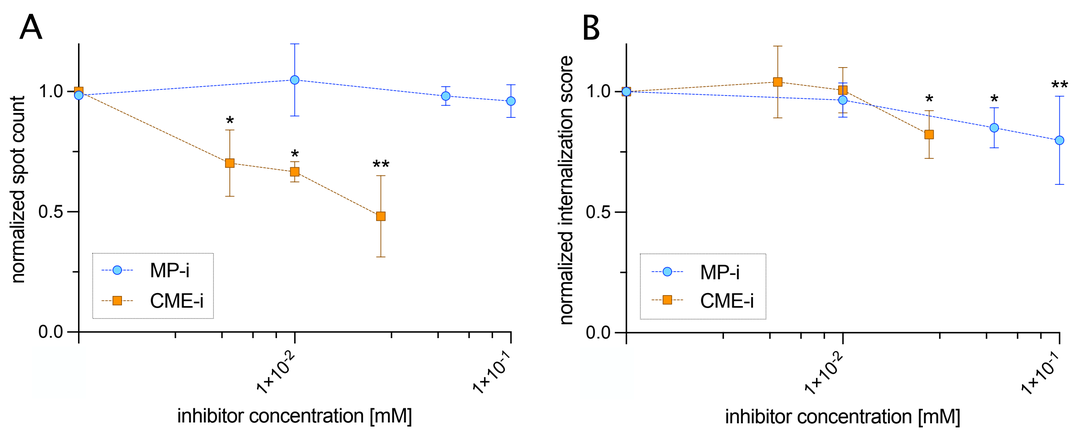

Figure 4: Internalization quantity and quality of ASC-EVs by SCs is affected by specific inhibition of endocytic pathways. (A) The internalization quantity (*spot count*) is significantly reduced upon inhibition of CME, however, remains unaffected by inhibiting MP. (B) The inhibition of both MP and CME significantly affected internalization quality (*internalization score*).

Conclusion

We established that ASC-EVs can enhance proliferation in SCs, crucial for peripheral nerve regeneration. This response is activated upon internalization of ASC-EVs. We identified the major mode of internalization, however, alternative modes of internalization likely involved. The potential therapeutic application of EVs necessitates understanding the underlying processes, especially the interaction with target cells. Our investigations provide a deeper understanding of the cellular signal transduction during peripheral nerve regeneration upon stimulation with ASC-EVs and adds to the knowledge needed to harness the full potential of EVs for therapeutic purposes.

References

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