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Comments on "Tracking adipose-derived stem cell exosomes applied in a mouse crush injury model: insights from fluorescent labeling and spatial transcriptomics – an experimental study"

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Dear Editor,

We read with great interest the experimental study by Rau *et al*, which employed fluorescent labeling and spatial transcriptomics to investigate the biodistribution and transcriptional effects of adipose-derived stem cell (ADSC) exosomes in a murine sciatic nerve crush injury model[1]. The authors elegantly demonstrate that exosome labeling enables *in situ* tracking, and their spatial transcriptomics approach reveals injury-site-specific gene expression changes following exosome application. While these findings advance our understanding of exosome biodistribution and potential molecular effects in peripheral nerve regeneration, several methodological and translational considerations warrant further discussion before such strategies can be robustly translated into clinical settings.

Exosome labeling and tracking limitations

The study relies on PKH26 fluorescent dye for exosome labeling, a widely used but nonspecific lipophilic tracer that can persist after exosome degradation and potentially transfer to host cell membranes[2]. This raises the possibility of overestimating retention time and tissue localization. Complementary approaches such as genetically engineered reporter constructs (e.g., CD63-GFP fusion), nanoparticle tracking analysis combined with isotope labeling, or *in vivo* imaging modalities could improve tracking specificity and quantify degradation kinetics[3].

Model-specific considerations for biodistribution

The biodistribution patterns reported are specific to the mouse sciatic nerve crush model and may not extrapolate directly to other tissues or injury types. The local tissue architecture, vascular permeability, and immune microenvironment can substantially influence exosome retention and uptake[4]. Comparative biodistribution studies across multiple injury models, including systemic injury or inflammation, would clarify whether the observed localization is a generalizable property of ADSC exosomes or model-specific.

Spatial transcriptomics interpretation and mechanistic inference

The use of spatial transcriptomics is a notable strength, allowing spatially resolved mapping of gene expression in injured and treated tissues. However, transcriptional changes alone cannot establish causality or distinguish between direct exosome-mediated signaling and secondary effects from altered immune or glial cell activity. Functional validation, such as pathway inhibition, targeted gene knockdown, or proteomic correlation, would be necessary to confirm that the identified molecular pathways are indeed central to the regenerative effects[5].

Exosome characterization and reproducibility

The study briefly reports nanoparticle size distribution and surface marker expression, but lacks comprehensive exosome profiling according to the latest MISEV (Minimal Information for Studies of Extracellular Vesicles) 2023 guidelines[6]. Detailed characterization, including RNA cargo profiling, protein composition, particle concentration, and purity metrics, is essential for reproducibility and for linking specific exosome components to observed transcriptional effects.

Dosing, administration route, and pharmacokinetics

Only a single exosome dose and the local administration route were tested. Dose–response relationships, repeated dosing schedules, and alternative delivery routes (e.g., systemic vs intraneural) remain unexplored. Moreover, pharmacokinetic parameters such as clearance rate, half-life, and tissue-specific uptake were not quantified, which are critical for designing therapeutic protocols in humans[7].

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Immunologic and off-target effects

While exosomes are generally considered low-immunogenic, immune responses can be influenced by donor–recipient mismatch, injury context, and exosome surface proteins. No immune profiling (e.g., cytokine panels, immune cell infiltration) was reported. Additionally, biodistribution to nontarget organs (e.g., liver, spleen, lung) may have functional consequences that require systematic evaluation, especially in chronic dosing scenarios[8].

Translational considerations for clinical application

Exosome therapies are classified as biologics or advanced therapy medicinal products in most jurisdictions, requiring stringent manufacturing controls. Large-scale GMP-compliant production, stability testing, batch consistency, and validated potency assays remain necessary steps before clinical trials. Furthermore, scaling spatial transcriptomics-based mechanistic monitoring to human patients presents cost and feasibility challenges; surrogate biomarkers of treatment response will need to be developed[9].

In conclusion, Rau *et al* provide valuable insights into the *in situ* tracking and molecular mapping of ADSC exosomes in peripheral nerve injury, demonstrating the potential of integrating imaging and transcriptomic profiling for regenerative medicine research. To accelerate clinical translation, future studies should incorporate more specific and quantitative tracking methods, expanded injury models, comprehensive exosome characterization, functional pathway validation, immune safety profiling, and pharmacokinetic modeling. Addressing these aspects will be critical for establishing ADSC exosomes as a reproducible, safe, and mechanistically understood therapeutic modality in nerve repair and beyond. This discussion was framed within the context of the TITAN guideline, which underscores the importance of openly disclosing the role of artificial intelligence in research reporting[10].

Ethical approval

Not applicable.

Consent

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Author contributions

E.R.: Writing and editing the draft. J.R.S.: Writing and editing the draft. M.F.-R.: Study design, data collection, and writing and editing the draft.

Conflicts of interest disclosure

There are no conflicts of interest.

Guarantor

All of the authors (Esmaeil Ranjbar, Javad Raouf Sarshoori, and Mahdi Fasihi-Ramandi) are accepting full responsibility for the work and the conduct of the study, had access to the data, and controlled the decision to publish.

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