

A Flight-Ready Experimental Framework to Quantify Regenerative Resilience in Wharton's Jelly Mesenchymal Stromal Cells and Exosome-Qualified Extracellular Vesicles

Adriana Paulina Gudiño Reyes

drgudino@merrootlab.com.mx

MER Root Lab

Systematic Review

Keywords: regenerative resilience, human spaceflight, Wharton's jelly, mesenchymal stromal cells, exosomes, small extracellular vesicles, cellular senescence, space radiation, microgravity, mitochondrial stress, recovery biology

Posted Date: October 14th, 2026

DOI: <https://doi.org/10.21203/rs.3.rs-11209186/v1>

License:  This work is licensed under a Creative Commons Attribution 4.0 International License.

[Read Full License](#)

Additional Declarations: No competing interests reported.

A Flight-Ready Experimental Framework to Quantify Regenerative Resilience in Wharton's Jelly Mesenchymal Stromal Cells and Exosome-Qualified Extracellular Vesicles

Adriana Paulina Gudiño Reyes, MD

MER Root Lab — Regenerative Medicine and Space Health, Cancún, Quintana Roo, Mexico

ORCID: <https://orcid.org/0009-0001-0418-8499> | Correspondence: drgudino@merrootlab.com.mx

Article type: Review | Status: Pre-experimental; no flight or outcome data are claimed.

Abstract

RAD-SEN-REGEN introduced regenerative resilience as the capacity of cells and tissues to preserve identity, control damage-associated signaling and recover homeostasis across the human-spaceflight exposure continuum. The present manuscript converts that concept into a testable, flight-ready experimental architecture. Low-passage Wharton's jelly-derived mesenchymal stromal cells (WJ-MSCs) and their exosome-qualified small extracellular-vesicle preparations are positioned as paired human-relevant platforms to resolve three questions: how altered gravity and space-relevant radiation reshape regenerative reserve; whether cell-free signaling modifies the trajectory of injury and recovery; and which manufacturing, potency and safety attributes predict resilience across terrestrial, analogue and orbital environments. The design separates microgravity, radiation and combined-exposure effects; includes time-matched 1-g controls, hardware controls and recovery intervals; and integrates DNA-damage, senescence, mitochondrial, redox, inflammatory, identity and functional-potency endpoints. It also defines nomenclature and certificate-of-analysis requirements for exosome designation, prespecified go/no-go thresholds, sample-retention strategy and a staged path from ground qualification to low-Earth-orbit validation. This framework does not claim therapeutic efficacy or advocate astronaut administration. It establishes the experimental evidence required before regenerative countermeasures can be considered for regulated human investigation.

Keywords: regenerative resilience; human spaceflight; Wharton's jelly; mesenchymal stromal cells; exosomes; small extracellular vesicles; cellular senescence; space radiation; microgravity; mitochondrial stress; recovery biology

1. Introduction: from a concept to a measurable system

Human spaceflight perturbs mitochondrial regulation, redox balance, immune signaling, vascular biology, genome stability and tissue homeostasis. Many changes partially normalize after return, yet functional recovery does not establish molecular neutrality. A subset of inflammatory, genomic,

vascular, ocular and cognitive changes may persist beyond landing, supporting the need to distinguish performance recovery from biological restoration [1–3].

RAD-SEN-REGEN therefore defines regenerative resilience as a dynamic property rather than a single biomarker: the capacity to resist injury, constrain maladaptive signaling and regain a qualified biological state after exposure. The first RAD-SEN-REGEN Perspective organized readiness, surveillance, protection and recovery across the mission continuum. This second paper operationalizes the framework as a sequence of falsifiable experiments using traceable WJ-MSC and cell-free products.

The model deliberately avoids assuming that MSCs or exosomes are universally beneficial. Microgravity can alter cytoskeletal organization, YAP/TAZ signaling, proliferation, lineage commitment, mitochondrial function and senescence, while high-charge and high-energy radiation can produce clustered damage and radiation-specific senescent phenotypes [4–8]. The biological state of the source cells may also change extracellular-vesicle composition and potency. Product identity and manufacturing history must therefore be treated as causal experimental variables, not administrative metadata.

2. Study objectives and hypotheses

2.1 Primary objective

To quantify regenerative resilience as the trajectory of injury, adaptation and recovery in qualified WJ-MSCs exposed to altered gravity, space-relevant radiation and their combination, with and without an exosome-qualified WJ-MSC-derived extracellular-vesicle intervention.

2.2 Prespecified hypotheses

- H1 — Exposure effect: microgravity and space-relevant radiation will produce distinct, non-additive changes in DNA-damage signaling, senescence, mitochondrial function, redox state and MSC potency.
- H2 — Recovery effect: normalization of viability or morphology will not necessarily coincide with restoration of molecular and functional endpoints after return to 1 g.
- H3 — Product-state effect: passage, population doubling, cryopreservation history and baseline potency will explain part of the inter-lot variation in postexposure recovery.
- H4 — Cell-free modulation: a prespecified exosome-qualified preparation may alter the slope or extent of recovery, but benefit will be endpoint- and context-dependent rather than assumed.
- H5 — Safety boundary: a reduction in inflammatory markers without preserved genomic stability, identity and growth control will not qualify as regenerative recovery.

3. Operational definition of regenerative resilience

Regenerative resilience will be evaluated across three linked dimensions. No single endpoint will be sufficient.

Table 1. Operational dimensions and core measures of regenerative resilience.

Dimension	Operational question	Core measures
Resistance	How much injury occurs during exposure?	Viability; apoptosis; γ H2AX/53BP1; ROS; mitochondrial membrane potential; ATP
Control	Is damage-associated signaling contained?	p16INK4a; p21; SA- β -gal; NF- κ B; IL-6; IL-8; TNF- α ; SASP profile
Recovery	Does the system regain identity and function?	CD73/CD90/CD105; negative markers; clonogenicity; differentiation; immunomodulatory or angiogenic potency; recovery kinetics
Safety	Is apparent recovery biologically acceptable?	Karyotype/genomic stability; abnormal growth; oncogenicity-relevant panel; sterility; mycoplasma; endotoxin

A composite Regenerative Resilience Index may be explored only after individual endpoints are reported transparently. Directionality, weighting and clinically meaningful thresholds must be prespecified in a statistical analysis plan and validated independently; a post hoc score must not replace the underlying data.

4. Biological materials and product qualification

4.1 WJ-MSC qualification

WJ-MSCs will be obtained from a licensed, traceable manufacturer without identifying the commercial source in the scientific hypothesis. Each lot will retain donor eligibility documentation, tissue origin, isolation and expansion history, passage, cumulative population doublings, culture medium, cryopreservation conditions, viability and chain of custody. Identity will include adherence and an ISCT-aligned phenotype (CD73, CD90 and CD105 positive; CD45, CD34, CD14 or CD11b, CD19 or CD79 α , and HLA-DR negative), supplemented by assays tied to the intended mechanism of action [9,10]. Sterility, mycoplasma, endotoxin, genomic stability and lot-consistency data will be required before flight allocation.

4.2 Explicit exosome/sEV nomenclature and certificate requirements

The paired cell-free product will be described as WJ-MSC-derived small extracellular vesicles (WJ-MSC-sEVs) unless the manufacturing record supports an exosomal biogenesis designation. Regulatory authorization, vendor licensing and lot traceability establish legitimacy and manufacturing accountability; they do not alone establish vesicle biogenesis. The term exosome-qualified will be used when the certificate and supporting methods document the source-cell state, conditioned-medium collection, separation process, particle concentration and size distribution, morphology or orthogonal structural evidence, positive EV-associated proteins, relevant negative or contaminant markers, sterility, endotoxin, mycoplasma, storage stability and a functional potency assay consistent with MISEV2023 [11,12].

Table 2. Minimum certificate-of-analysis information and terminology for WJ-MSC-derived extracellular-vesicle preparations.

COA domain	Minimum information before flight	Manuscript terminology if absent
Identity	Particle number; size distribution; ≥3 complementary markers/assays; source-cell identity	MSC-derived EV preparation
Purity	Protein-to-particle context; non-EV/contaminant assessment; process controls	EV-enriched preparation
Biogenesis	Methods and evidence supporting endosomal origin	Do not claim “exosome”
Safety	Sterility; endotoxin; mycoplasma; adventitious-agent controls	Not flight qualified
Potency	Dose-response assay linked to the proposed mechanism	Exploratory material only
Stability	Container closure; temperature; freeze–thaw; duration; post-thaw recovery	No operational shelf-life claim

For the final submission, exact COA values will replace controlled placeholders for lot number, particle concentration, modal/median size, marker panel, endotoxin, sterility, storage condition and potency. Product branding will remain omitted unless disclosure is scientifically or legally required.

5. Experimental architecture

5.1 Factorial core design

Table 3. Factorial core experimental design for RAD-SEN-REGEN II.

Factor	Levels	Purpose
Gravity	1 g hardware control; simulated microgravity; real microgravity/LEO	Separate platform artifacts from gravity-dependent biology
Radiation	Sham; terrestrial reference radiation; space-relevant mixed or HZE exposure	Resolve dose/quality-specific injury
Biological condition	WJ-MSC alone; WJ-MSC + vehicle; WJ-MSC + prespecified sEV/exosome dose	Test cell-free modulation without assuming benefit
Time	Baseline; early exposure; late exposure; early recovery; delayed recovery	Measure trajectories rather than terminal snapshots
Material	≥3 independently qualified cell lots; matched EV lots where feasible	Estimate biological and manufacturing variability

The first powered experiment should avoid unnecessary arms. A staged design is recommended: Stage A qualifies materials and dose range under terrestrial stress; Stage B separates simulated microgravity and radiation; Stage C tests combined exposure; Stage D transfers the locked protocol

to real microgravity. Randomization, blinded image analysis, predefined exclusion criteria and time-matched controls will be retained across stages.

5.2 Exposure and recovery sequence

- T-30 to T-1 days — lot qualification, stability testing, baseline imaging and hardware compatibility.
- T0 — standardized thaw/recovery and allocation to randomized experimental units.
- Exposure — continuous environmental logging with scheduled imaging and minimal crew intervention.
- Return/recovery — transfer to a defined 1-g recovery condition without changing unplanned variables.
- Postrecovery — destructive molecular assays, retained samples and confirmatory functional testing.

5.3 Exosome intervention logic

Dose will be defined by particle number and, secondarily, protein content rather than an unqualified vial count. A terrestrial dose–response study will identify a non-cytotoxic, mechanistically active range. Pre-exposure, during-exposure and recovery-phase administration answer different questions and must not be pooled. The initial flight experiment should select one timing supported by ground data; recovery-phase dosing is the most direct test of the RAD-SEN-REGEN concept, whereas prophylactic dosing evaluates protection rather than regeneration.

6. Measurement framework

Table 4. Measurement framework and primary biological readouts.

Domain	Primary readouts	Interpretive role
DNA damage	γ H2AX and 53BP1 foci; comet assay; micronuclei where feasible	Damage burden and persistence
Senescence	p16INK4a; p21; SA- β -gal; cell-cycle profile; SASP	Arrest, phenotype and signaling
Mitochondria/redox	ROS; GSH/GSSG; NAD ⁺ /NADH; membrane potential; ATP; respiration	Energetic reserve and oxidative balance
Inflammation	IL-6; IL-8; TNF- α ; NF- κ B-linked signaling; multiplex secretome	Contextual inflammatory control
MSC identity/potency	Phenotype; clonogenicity; differentiation; selected MoA-linked potency assay	Recovery of qualified function
EV product	Particle recovery; size; markers; cargo stability; potency after exposure	Operational and biological stability
Morphodynamics	Live-cell morphology; confluence; migration; apoptosis reporters	Temporal resolution and anomaly detection

Live-cell imaging should be treated as a measurement layer, not the sole biological endpoint. An Etaluma-class compact fluorescence microscope or equivalent may enable repeated imaging, but optical configuration, field selection, focus strategy, phototoxicity, data volume, calibration and compatibility with the selected culture hardware require independent verification before naming a flight provider.

7. Hardware, environmental metadata and sample recovery

Flight hardware must maintain containment, gas exchange, temperature and media conditions while distinguishing hardware-induced shear, hypoxia, nutrient limitation and launch vibration from microgravity effects. Environmental data—including temperature, CO₂, humidity where relevant, acceleration and radiation dosimetry—will be synchronized with biological timestamps. A parallel ground unit should reproduce the flight temperature and operational timeline as closely as practical.

Whenever return logistics permit, the design will recover both viable cells and cell-free fractions. Samples will be partitioned for immediate viability and potency, fixed imaging, nucleic-acid analysis, protein/secretome analysis and biobanking. Predefined stabilization windows are essential because uncontrolled return and processing delays can erase or mimic recovery biology.

8. Statistics, reproducibility and go/no-go criteria

The experimental unit is the independent culture chamber or bioreactor, not each image or each cell. Technical fields are nested observations. Power calculations will use terrestrial pilot variance and a prespecified primary endpoint; mixed-effects models will account for exposure, time, treatment and lot, with chamber and lot as random effects where appropriate. Multiplicity control and missing-data rules will be defined before unblinding.

Table 5. Prespecified go/no-go decision criteria for experimental progression.

Decision gate	Proceed when	Stop or redesign when
Material qualification	Identity, sterility, stability and potency meet locked specifications	COA gaps, lot drift, abnormal growth or failed safety release
Hardware qualification	Viability and primary endpoints remain measurable in 1-g hardware controls	Hardware effect overwhelms biological contrast
Ground efficacy signal	Directionally consistent effect across independent lots without safety penalty	Single-lot effect, cytotoxicity or discordant genomic safety
Flight readiness	Protocol is executable with locked timing, imaging and recovery chain	Unresolved containment, temperature, imaging or sample-return risk
Human translation	Replicated mechanistic benefit plus toxicology and regulatory pathway	Biomarker improvement without functional recovery or safety

9. Oncology, ethics and regulatory boundary

Anti-inflammatory or trophic effects are not intrinsically beneficial. A preparation that suppresses cytokines while increasing survival or proliferation of genomically abnormal cells would fail the framework. Oncologic vigilance includes source-material qualification, genomic-stability assessment, abnormal-growth surveillance, mechanism-specific potency, context-appropriate tumor-support assays and long-term follow-up before any human application.

The experiments proposed here use human-derived manufactured cellular materials and do not themselves constitute astronaut treatment. Ethics and regulatory requirements will depend on donor consent and provenance, jurisdiction, material transfer, biosafety, launch-provider requirements and whether future work involves identifiable human data or administration. Any analogue-mission monitoring of participants will be submitted as a separate human-subject protocol; it will not be represented as evidence of cellular microgravity or radiation effects.

10. Translational sequence and publication program

Table 6. Translational sequence and publication program for RAD-SEN-REGEN.

Paper / phase	Scientific contribution	Evidence status
RAD-SEN-REGEN I	Defines regenerative resilience and the exposure continuum	Published/submitted Perspective
RAD-SEN-REGEN II	Operationalizes hypotheses, materials, endpoints, controls and flight gates	Present study-design manuscript
RAD-SEN-REGEN III	Final mission protocol, hardware validation and preregistered analysis plan	Before launch
RAD-SEN-REGEN IV	Primary microgravity/radiation and recovery results	After data lock
RAD-SEN-REGEN V	Recovered-cell, EV cargo, potency and multi-omic analysis	Postflight mechanistic analysis
RAD-SEN-REGEN VI	Cross-platform comparison: ISS versus another LEO laboratory/platform	Only after harmonized exposure and hardware controls
RAD-SEN-REGEN VII+	Replication, countermeasure optimization and regulated translation	Evidence dependent

This sequence prevents a launch announcement from becoming a substitute for science. Each paper should answer a distinct question, retain negative findings and preserve the distinction between platform feasibility, biological mechanism, countermeasure activity and human therapeutic translation.

11. Discussion

RAD-SEN-REGEN II advances the original framework by converting regenerative resilience into a temporal and falsifiable construct. The design does not ask only whether exposure increases ROS, p21 or γ H2AX. It asks whether damage resolves, whether identity and potency return, whether recovery differs by material quality, and whether a cell-free product changes that trajectory without creating a genomic or oncologic trade-off.

The paired use of WJ-MSCs and exosome-qualified EVs provides a mechanistically connected system: the producer cell can be characterized before and after exposure, while the secreted product can be evaluated for identity, stability, cargo and function. This pairing is stronger than treating cells and exosomes as interchangeable therapies. It can reveal whether microgravity or radiation changes the product itself, whether preflight manufacturing state predicts orbital behavior, and whether a recovery signal remains reproducible across lots.

The framework also creates a disciplined boundary between RadSenRegen and complementary digital systems. Cognitive-AI integration, wearable physiological monitoring and real-time decision support may eventually connect cellular, environmental and human data, but those capabilities require a separate validation architecture. They should enter the program only when their inputs, outputs, responsibility and regulatory status are defined.

Limitations include the imperfect correspondence between simulated and real microgravity, the challenge of reproducing the mixed radiation field, small orbital sample sizes, platform-specific hardware effects, lot variability and the possibility that in-vitro cellular recovery will not predict organ-level or human recovery. These limitations are reasons for staged comparison and replication, not for collapsing all exposures into a single resilience claim.

12. Conclusion

Regenerative resilience can become a measurable objective for space biology only when recovery is evaluated with the same rigor as injury. RAD-SEN-REGEN II establishes a flight-ready architecture in which qualified WJ-MSCs and exosome-qualified extracellular vesicles are studied through exposure, adaptation and recovery, with material quality, potency and safety integrated into interpretation. The framework is deliberately conditional: no anti-inflammatory, morphological or performance improvement is accepted as regeneration unless identity, function and genomic safety are preserved. This structure provides a defensible path from terrestrial qualification to orbital experimentation and, only if justified by replicated evidence, to future regulated human investigation.

Declarations

Ethics approval and consent to participate: Not applicable to this study-design manuscript. No new experiment involving human participants or animals is reported.

Competing interests: The author is affiliated with MER Root Lab, which operates in regenerative medicine. Commercial manufacturers and flight providers are not identified in this pre-experimental framework. Any later material or service support will be disclosed in the relevant protocol and results manuscripts.

Funding: No external funding is declared for preparation of this manuscript draft.

Author contributions: APGR conceived the RAD-SEN-REGEN framework and the translational research program.

Generative AI statement: OpenAI ChatGPT was used to support language editing, structural organization and document preparation. The author must verify all scientific content, source references and final journal requirements before submission.

References

1. Garrett-Bakelman FE, Darshi M, Green SJ, et al. The NASA Twins Study: A multidimensional analysis of a year-long human spaceflight. *Science*. 2019;364:eaau8650. doi:10.1126/science.aau8650.
2. da Silveira WA, Fazelinia H, Rosenthal SB, et al. Comprehensive multi-omics analysis reveals mitochondrial stress as a central biological hub for spaceflight impact. *Cell*. 2020;183:1185–1201.e20. doi:10.1016/j.cell.2020.11.002.
3. Afshinnekoo E, Scott RT, MacKay MJ, et al. Fundamental biological features of spaceflight: advancing the field to enable deep-space exploration. *Cell*. 2020;183:1162–1184. doi:10.1016/j.cell.2020.10.050.
4. Pala R, et al. Mesenchymal stem cell behavior under microgravity. *Int J Mol Sci*. 2023;24:8333. doi:10.3390/ijms24098333.
5. Lv W, et al. YAP inhibition alleviates simulated microgravity-induced mesenchymal stem cell senescence and mitochondrial dysfunction. *Antioxidants*. 2023;12:990.
6. Pitcher LE, Mukherjee B, Saathoff AM, et al. Space radiation induces distinct senescent phenotypes: implications for space travel. *npj Aging*. 2025;11:95. doi:10.1038/s41514-025-00272-7.
7. Pham J, Isquith J, Balaian L, et al. Nanobioreactor detection of space-associated hematopoietic stem and progenitor cell aging. *Cell Stem Cell*. 2025;32:1403–1420.e8. doi:10.1016/j.stem.2025.07.013.
8. Chen Z, Luo Q, Lin C, et al. Simulated microgravity inhibits osteogenic differentiation of mesenchymal stem cells via depolymerizing F-actin to impede TAZ nuclear translocation. *Sci Rep*. 2016;6:30322. doi:10.1038/srep30322.
9. Dominici M, Le Blanc K, Mueller I, et al. Minimal criteria for defining multipotent mesenchymal stromal cells. *Cytotherapy*. 2006;8:315–317. doi:10.1080/14653240600855905.
10. Viswanathan S, Shi Y, Galipeau J, et al. Mesenchymal stem versus stromal cells: ISCT position statement on nomenclature. *Cytotherapy*. 2019;21:1019–1024. doi:10.1016/j.jcyt.2019.08.002.
11. Théry C, Witwer KW, Aikawa E, et al. Minimal information for studies of extracellular vesicles 2018 (MISEV2018). *J Extracell Vesicles*. 2018;7:1535750. doi:10.1080/20013078.2018.1535750.
12. Welsh JA, Goberdhan DCI, O'Driscoll L, et al. Minimal information for studies of extracellular vesicles (MISEV2023): from basic to advanced approaches. *J Extracell Vesicles*. 2024;13:e12404. doi:10.1002/jev2.12404.
13. Wagner W, Horn P, Castoldi M, et al. Replicative senescence of mesenchymal stem cells: a continuous and organized process. *PLoS One*. 2008;3:e2213. doi:10.1371/journal.pone.0002213.
14. Grimm D, Egli M, Krüger M, et al. The effects of microgravity on differentiation and cell growth in stem cells and cancer stem cells. *Stem Cells Transl Med*. 2020;9:882–894. doi:10.1002/sctm.20-0084.
15. van den Nieuwenhof DWA, Moroni L, Chou J, et al. Cellular response in three-dimensional spheroids and tissues exposed to real and simulated microgravity: a narrative review. *npj Microgravity*. 2024;10:102. doi:10.1038/s41526-024-00442-z.
16. Capri M, Conte M, Ciorca E, et al. Long-term human spaceflight and inflammaging: Does it promote aging? *Ageing Res Rev*. 2023;87:101909. doi:10.1016/j.arr.2023.101909.