ECMC-P 2025 Conference

The 1st International Electronic Conference on Medicinal Chemistry and Pharmaceutics

01–30 November 2025 | Online

MDPI

Artificial cell derived vesicles from Ginsenoside Rg1-primed mesenchymal stromal cells mitigate oxidative stress and DNA damage in myocardial ischemic/reperfusion injury

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INTRODUCTION & AIM

Myocardial ischemia/reperfusion injury (MI/RI) remains a major challenge in the treatment of acute myocardial infarction due to the lack of effective therapeutic options. Despite advancements in interventional techniques like percutaneous coronary intervention, MI/RI-induced oxidative stress, inflammatory responses, and cardiomyocyte apoptosis still lead to poor long-term prognosis for patients. While mesenchymal stromal cells (MSCs) and their derivates show promising potential for MI/RI therapy, their clinical application is hindered by low transplantation efficiency—often resulting from poor cell retention in the ischemic myocardium—and insufficient yield for large-scale clinical use. In this study, we engineered nanoscale artificial cell-derived vesicles (ACDVs) by extruding Ginsenoside Rg1-primed MSCs (Rg1-MSCs), resulting in Rg1-ACDVs. Rg1-ACDVs displayed superior therapeutic efficacy compared to non-primed ACDVs and extracellular vesicles derived from Rg1-MSCs (Rg1-EVs), as evidenced by reduced myocardial infarct size in rat MI/RI models. Multi-omics analysis revealed that Rg1-ACDVs possess distinct molecular signatures associated with promoting cell cycle progression and reducing DNA damage, including upregulated expression of DNA repair-related proteins and cell cycle regulators. These findings were further validated experimentally, demonstrating that Rg1-ACDVs effectively reduce reactive oxygen species (ROS) accumulation—an important driver of MI/RI—and mitigate DNA damage both in vitro (in cultured cardiomyocytes) and in vivo (in rat MI/RI models). This study highlights the synergistic benefits of combining Ginsenoside Rg1 priming (which modulates MSC paracrine function) with nanoscale engineering (which optimizes vesicle delivery), and introduces Rg1-ACDVs as a scalable and innovative strategy, offering a promising approach for improving clinical outcomes in MI/RI therapy.

METHOD

1 Generation and Characterization of ACDVs

Porous microcarriers were soaked in MSC culture medium at 4 °C for 12 h. Thereafter, the microcarriers and MSCs were transferred to a spinner flask, which was set to a cyclic program (40 rpm for 5 min, followed by 0 rpm for 25 min). After 24 h of culture, MSCs were further cultured for 4 days with or without 125 μM ginsenoside Rg1, and the spinner flask speed was adjusted to a constant 40 rpm. On days 3 and 5 of culture, the number of MSCs and their viability were determined via Trypan blue staining and Cal-AM/PI staining, respectively.

Subsequently, MSC-conditioned medium was collected for extracellular vesicle (EV) isolation, while MSCs were harvested on day 5 for ACDV preparation. ACDVs were generated by subjecting MSCs to serial extrusion through filters with progressively reduced pore sizes (10 μm, 5 μm, and 1 μm). For purification, the crude ACDV mixture was first centrifuged at 300×g and 2000×g for 10 min each, followed by ultracentrifugation at 100,000×g for 70 min. The resulting pellet was resuspended in phosphate-buffered saline (PBS) and subjected to another round of ultracentrifugation under the same conditions (100,000×g for 70 min). The purified ACDVs were resuspended in PBS and stored at −80 °C for subsequent experiments.

The characterization of ACDVs (including morphology, size, protein composition, and zeta potential) was performed using transmission electron microscopy (TEM), nanoparticle tracking analysis (NTA), and Western blot assay, as described previously [19].

2 Flow Cytometry Assay

Flow cytometry was used to verify the stemness of MSCs via immunostaining. The antibodies employed were as follows: anti-CD73-PE, anti-CD90-FITC, anti-CD105-PE, anti-CD14-FITC, anti-CD34-FITC, anti-CD19-PE, anti-CD45-APC, and anti-HLA-DR-PE (all purchased from Biolegend, San Diego, CA, USA).

3 Immunofluorescence Staining

To assess DNA damage, an H_2O_2-induced injury model was established in H9c2 and AC16 cells. After treatment with nanovesicles, cells were washed three times with PBS, fixed with 4% paraformaldehyde, permeabilized with 0.3% Triton X-100, and blocked with 5% bovine serum albumin (BSA) to reduce non-specific antibody binding.

Primary antibodies—including anti-Histone H2A.X (Abcam, UK) and anti-8-OHdG (E-8, USA)—were added and incubated overnight at 4 °C. After washing, cells were incubated with fluorophore-conjugated secondary antibodies (EarthOx, diluted 1:200) and counterstained with 4',6-diamidino-2-phenylindole (DAPI) to visualize cell nuclei. Fluorescence images were captured using a confocal microscope (Leica, Germany).

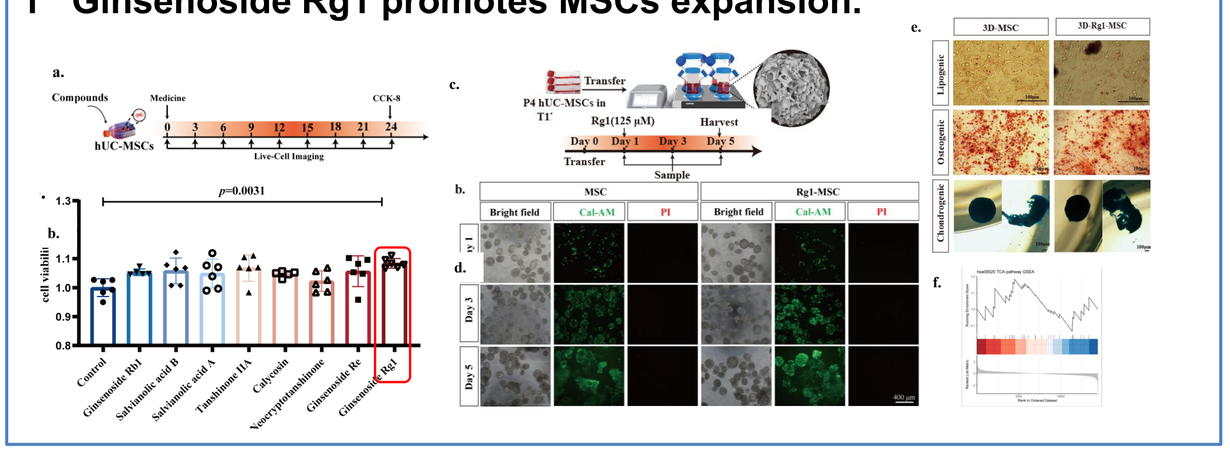
4 Evaluation of Oxidative Stress

For frozen tissue sections, ROS staining solution was applied, and sections were incubated at 37 °C for 60 min in a light-proof thermostat. ROS signals were observed and imaged under a fluorescence microscope.

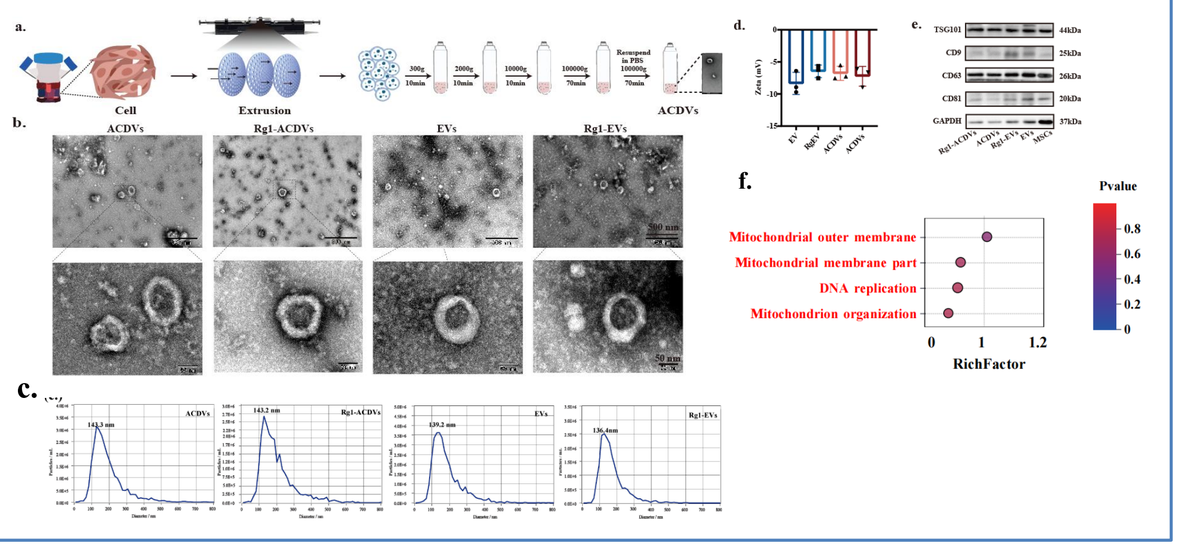
To evaluate mitochondrial superoxide ion levels, cells were stained with MitoSOX (Beyotime, China) according to the manufacturer's instructions.

RESULTS & DISCUSSION

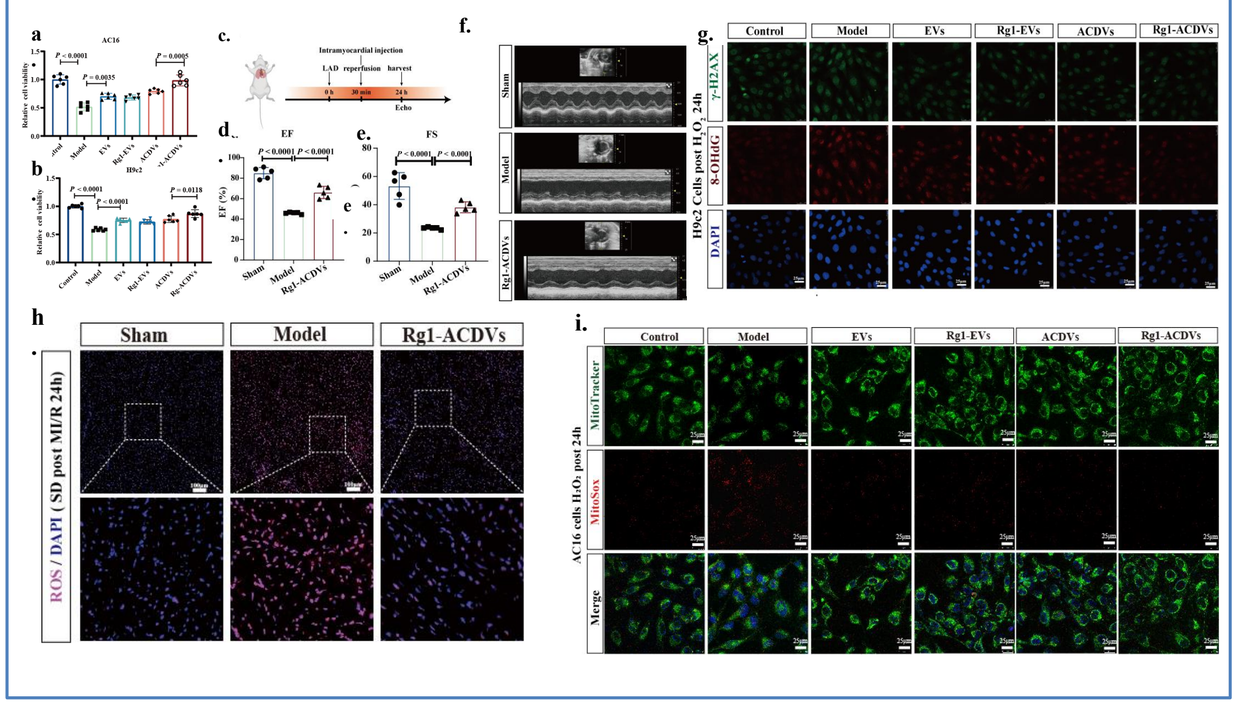
1 Ginsenoside Rg1 promotes MSCs expansion.



2 Rg1-ACDVs displayed distinguished characteristics compared to other MSC derivates.



3 Rg1-ACDVs demonstrate the therapeutic efficiency .



CONCLUSION

This study demonstrates that Ginsenoside Rg1-primed ACDVs(Rg1-ACDVs) synergistically combine nanoengineering technology with a 10-fold increase in yield, establishing a synthetic nanovesicle platform that addresses oxidative stress and DNA damage while remaining biocompatible, offering a promising approach to improve clinical outcomes in MI/RI therapy.

FUTURE WORK / REFERENCES

[1] Zhao, S., et al., Artificial cell derived vesicles from Ginsenoside Rg1-primed mesenchymal stromal cells mitigate oxidative stress and DNA damage in myocardial ischemic/reperfusion injury. Nano Research, 2025. 18(6): p. 94907535.