Supplementary Material

Extracellular vesicles produced by 3D cultured MSCs promote wound healing by regulating macrophage activation through ANXA1

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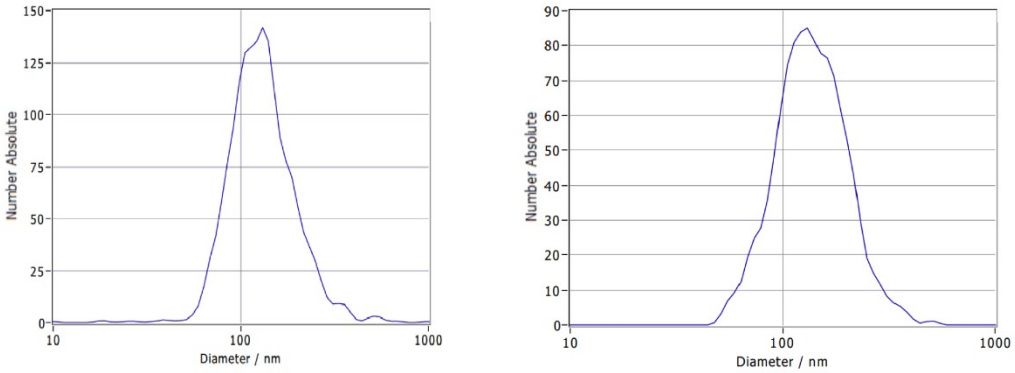
Supplement Data

A



2D-EVs NTA　　3D-EVs NTA

Figure S1 (A) Size distribution of 2D-Exo and 3D-Exo detected by NTA.

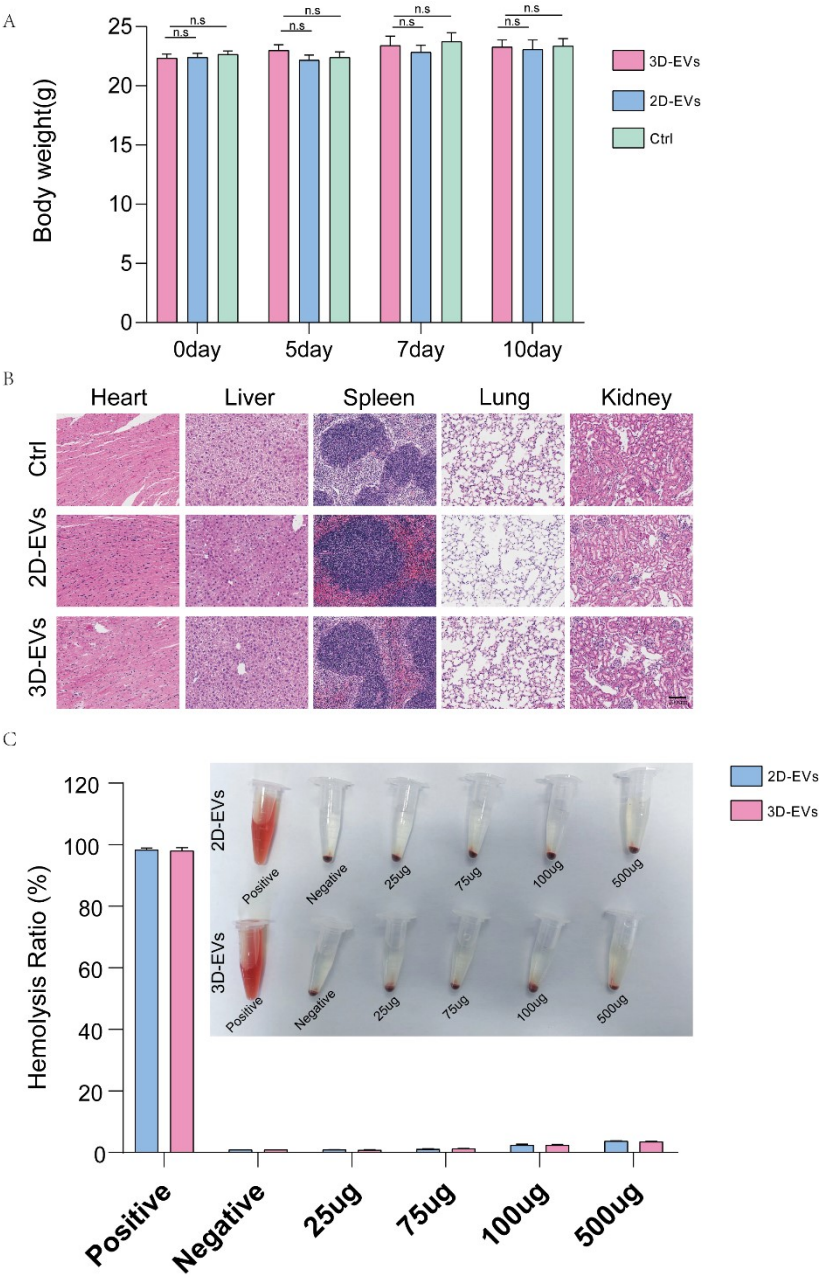


Figure S2. Preliminary toxicity study in vivo. A. Statistical analysis of the mice body weight change in the Ctrl group, 2D-EVs group, and 3D-EVs group. B. Histological toxicological observation of H&E staining of the heart, liver, spleen, lung, and kidney with different treatments, bar=200 μm. C. Pictures of erythrocytes and exosomes after culture. H_2O was used as a control. D. Hemolysis ratio in corresponding groups.

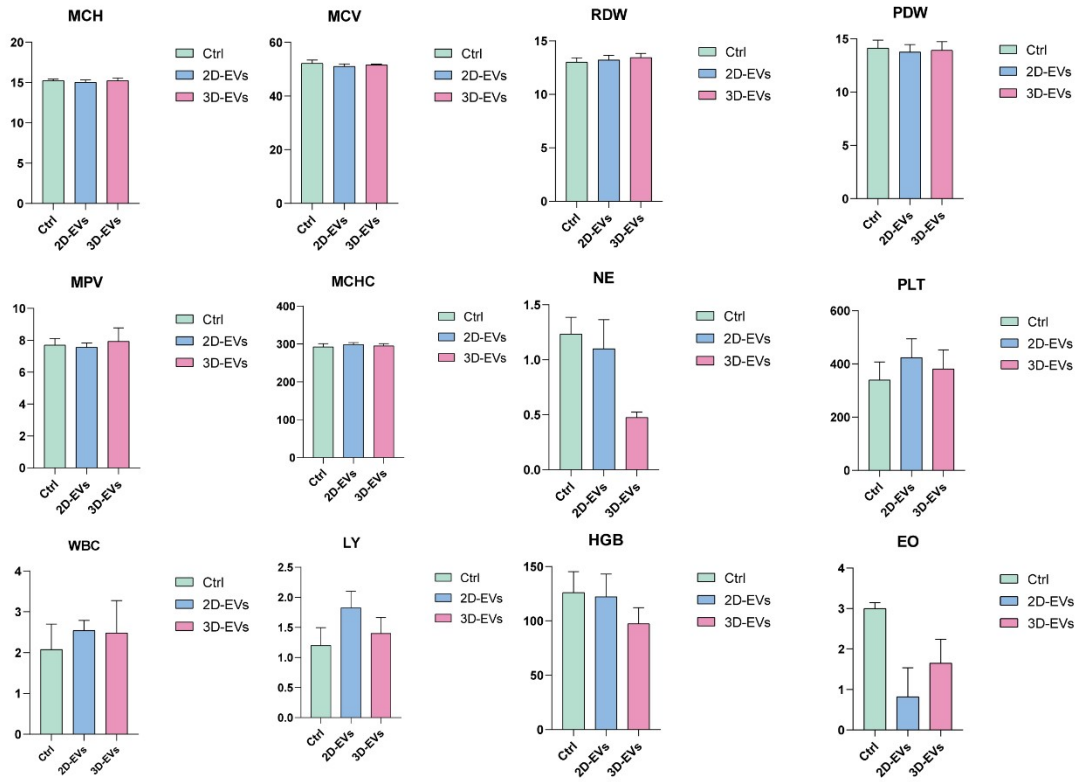


Figure S3. Mice blood routine. A. Statistical analysis of the mice blood routine change in the Ctrl group, 2D-EVs group, and 3D-EVs group.

Preliminary toxicity study in vivo

The outstanding properties and potential of exosomes secreted by 3D cultured MSCs for wound repair were identified in the main body of data. At the same time, we also evaluated the biocompatibility of exosomes *in vivo*, which is crucial for their practical application. Due to the direct contact of exosomes with the wound, histological examination and blood biochemical analysis were used to evaluate the biocompatibility of the *in vivo* treatment.

Data on body weight changes in mice at different time points of 0, 5, 7, and 10 days showed that 2D-EVs and 3D-EVs did not affect body weight changes in mice. We performed HE staining of the heart, liver, spleen, lung, and kidney of mice and found no difference in histological staining of the heart, liver, spleen, lung, and kidney of mice after treatment with 2D-EVs and 3D-EVs.

Biochemical analyses were performed on day 10 after injury in mice to assess the

hemolytic properties of exosomes at the cellular level by measuring the hemolytic properties of erythrocytes. We found no significant hemolytic effect with exosomes at different protein concentrations. The hemolysis of erythrocytes was significantly reduced after exosomes were used. These results provide strong evidence that exosomes can be used as a safe and effective therapeutic strategy to accelerate wound healing.