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Three-dimensional mechanical microenvironment enhanced osteogenic activity of mesenchymal stem cells-derived exosomes

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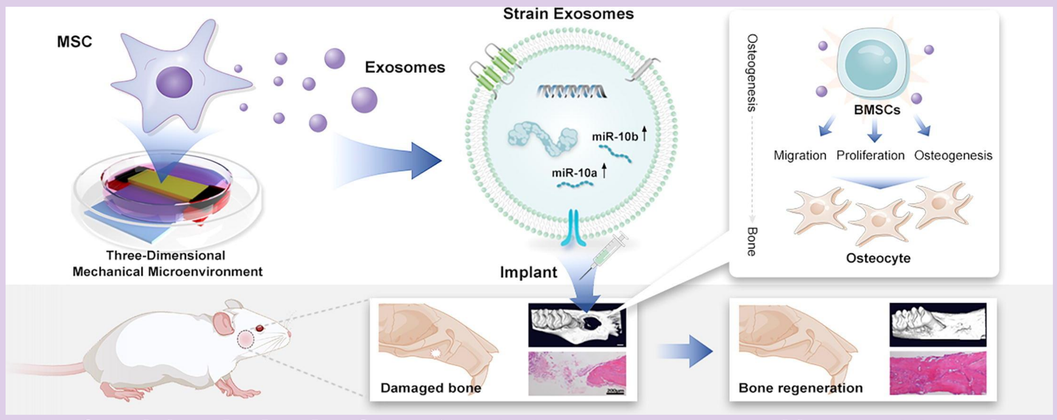
HIGHLIGHTS

- 3D strain microenvironment fabricated by magnetically stretched collagen hydrogels.
- The 3D mechanical stimulation dramatically alters exosomal miRNA content.
- The 3D strain microenvironment favors the highly effective osteogenesis of MSCs derived exosomes.
- 3D microenvironment could enhance exosomes-based cell-free regenerative therapy.

ABSTRACT

Exosomes derived from **mesenchymal stem cells (MSCs)** through the paracrine pathway have shown great potentials in **cell-free therapy for regenerative medicine**. Although MSCs have been recently known to respond to the **mechanical microenvironment** and thus orchestrate their behaviors and functions, the effects of such a biophysical cue on the paracrine pathway of MSCs, especially on the regulation of characteristics and bioactivity of MSC-derived exosomes have rarely been reported.

In this study, we used periodontal ligament stem cells (PDLSCs), which were cultured in the **three-dimensional(3D) microscale magnetically stretched collagen hydrogels**, as the model MSCs to explore the **changes in characteristics** (e.g., morphology, size distribution, typical surface protein expression, miRNA content, and internalization) and **bioactivity** of MSC-derived exosomes in response to matrix strain.



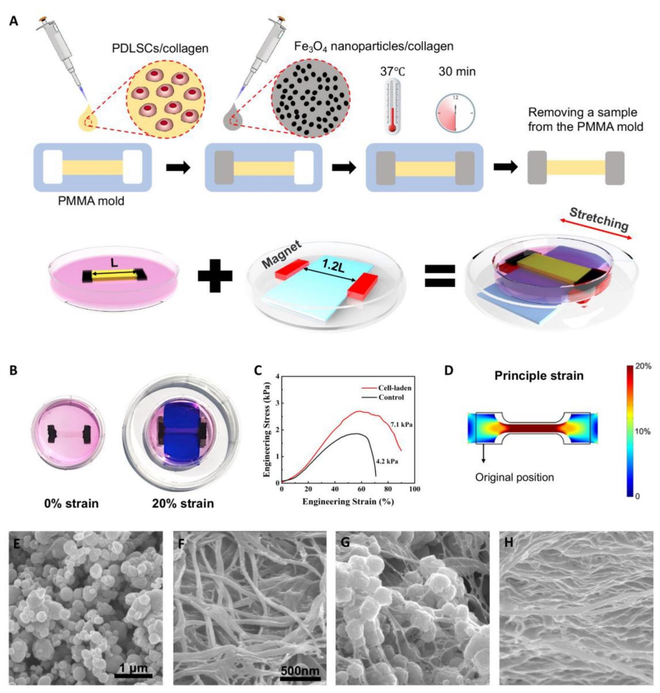
We found that **the levels of 25 miRNAs** in exosomes secreted by PDLSCs in the 3D strain microenvironment (SM-Exo) were dramatically different from those obtained from the 3D culture microenvironment (Exo). Next, the **bioactivity of SM-Exo** was significantly enhanced as reflected by **the improved proliferation, migration, and osteogenic differentiation** of target cells (e.g., BMSCs). This was further confirmed by in vivo studies, in which PDLSC-derived exosomes obtained from the 3D strain microenvironment showed **stronger bioactivity to repair alveolar bone defects** in SD rats.

This study proposed a novel strategy to promote **alveolar bone regeneration** via altered **PDLSC-derived exosomes** in the **3D strain** microenvironment, and such an engineered mechanical microenvironment would be an effective tool in other regenerative studies and clinical applications.

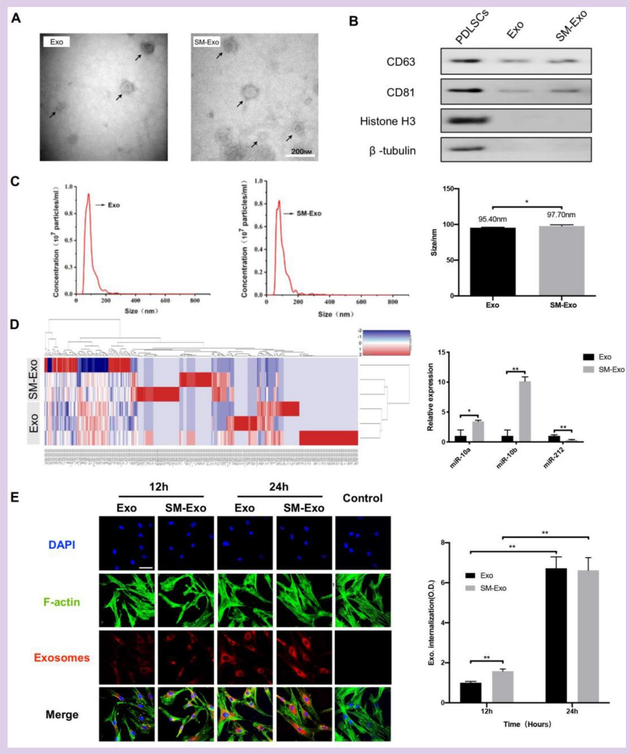
METHODS

Engineering of the 3D cell mechanical microenvironment based on the microscale magnetically stretched cell-laden collagen hydrogels.

(A) Schematic figure of engineering the 3D cell mechanical microenvironment with 20% strain
(B) Photos of the engineered 3D cell mechanical microenvironment with or without 20% strain
(C) Stress–strain curves of the PDLSC-laden collagen hydrogels
(D) Simulated strain distribution in the middle section of collagen hydrogel under magnetic stretching showed a highly uniform mechanical field
(E) SEM micrographs of the Fe3O4 nanoparticle at low magnification. Scale bar: 1 μm
(F,G,H) SEM micrographs at a high magnification of Collagen hydrogel, Fe3O4 blended collagen hydrogel, and stretched collagen hydrogel. Scale bar: 500 nm.



RESULTS

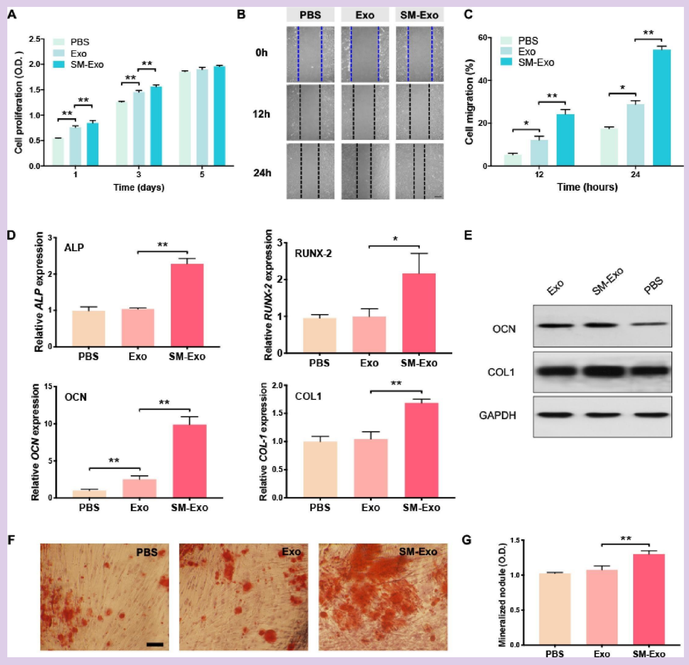


Characteristics of Exosomes and exosomal content.

(A) Representative TEM images showing the morphologies of Exo and SM-Exo
(B) Typical surface protein expressions of PDLSCs, Exo, and SM-Exo
(C) Size distribution and average particle diameter of Exo and SM-Exo
(D) Heatmap diagram of differential miRNA expression between Exo and SM-Exo and quantitative real-time PCR validated the change of miRNA in SM-Exo
(E) Representative confocal microscopy images showing the internalization process of Exo and SM-Exo
(F) Quantitative analysis of internalization of Exo and SM-Exo

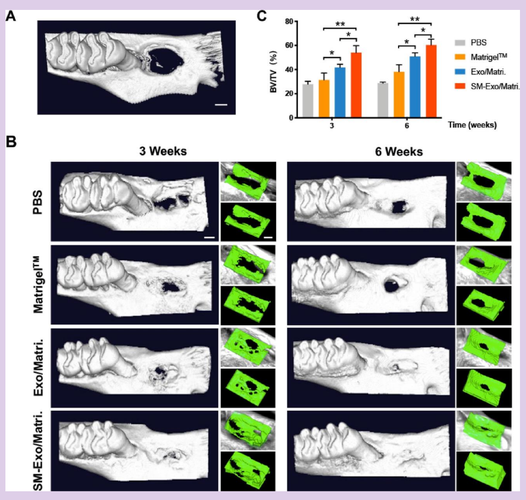
3D strain microenvironment affected bioactivity of PDLSC-derived exosomes in vitro.

(A) BMSC proliferation on day 1, 3, and 5 were assessed via CCK-8 assay
(B) BMSC migration was observed by the microscope
(C) Quantitative analysis of cell migration
(D) Real-time PCR results showed the relative mRNA expression of osteogenesis-related genes including ALP, RUNX 2, OCN, and COL 1, respectively
(E) Protein expressions of OCN and COL 1 were investigated by the western-blotting method
(F) Alizarin red staining was used to characterize the mineralized nodules produced from BMSCs
(G) Quantitative analysis of mineralized nodule formation



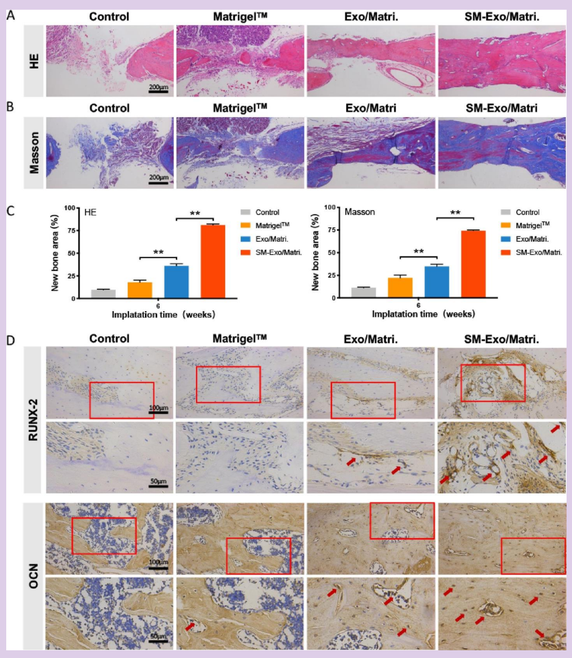
Micro-CT analysis of new bone formation in rat alveolar bone defects treated with PDLSC-derived exosomes at 3 and 6 weeks of healing.

(A) Representative 3D reconstructed micro-CT image of rat alveolar bone defect model after surgery
(B) Representative 3D reconstructed micro-CT images showing the newly regenerated bone tissue in the defects treated with the PBS, Matrigel™, Exo/Matri. and SM-Exo/Matri., respectively at 3 weeks and 6 weeks of healing
(C) Quantitative analysis of the regenerated bone volume fraction within the original defects (BV/TV, %) in different groups at 3 and 6 weeks of healing



3D strain microenvironment promoted the osteogenesis efficiency of PDLSC - derived exosomes in vivo.

(A) HE staining and
(B) Masson's trichrome staining of the rat alveolar bone defects showed the new bone formation in various alveolar bone defects treated with the PBS, Matrigel™, Exo/Matri. and SM-Exo/Matri., respectively
(C) Quantitative analysis of the new bone area in HE staining images and Masson staining images were carried out using the Image-Pro Plus software
(D) Immumohistochemical staining of osteogenic proteins including RUNX 2 and OCN. Red arrows indicate positive brown stained.



CONCLUSION

In summary, we used **natural collagen** to engineer a **3D high biocompatible hydrogel** that is effective for applying the mechanical strain via microscale magnetically stretching the collagen hydrogels. The results revealed strong influences of the 3D strain microenvironment on the characteristics and functions of PDLSC-derived exosomes. Although the matrix strain did not affect morphology, size distribution, and internalization of PDLSC-derived exosomes, the SM-Exo miRNA expression profile was significantly different from Exo. **In vitro studies** demonstrated that the mechanical stretching dramatically enhanced the bioactivity of PDLSC-derived exosomes, as reflected by the improved **proliferation, migration, and osteogenic differentiation** of target cells (e.g., BMSCs). Moreover, **in vivo studies** also revealed that the matrix strain significantly promoted bioactivity of PDLSC-derived exosomes that facilitated new bone formation and repair of alveolar bone defects by **upregulating the protein expression levels of RUNX 2 and OCN**.

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RESEARCH INTEREST

- Exosomes
- Mesenchymal stem cells
- Tissue engineering
- Regenerative medicine

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