Human Adipose Stem Cells-derived Vesicles Improve Pain and Reduce Cartilage Destruction in an Osteoarthritis Rat Model

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Abstract

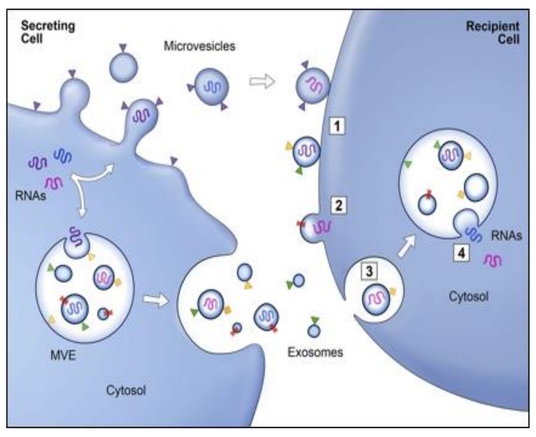
Extracellular vesicles (EVs) released from human mesenchymal stromal cells (hMSCs) have provided significant benefits in various disease models. However, most mammalian cells secrete a small amount of EVs, limiting the clinical applications. The next-generation EV-mimetic vesicles (or cell-derived vesicles, CDVs) that we recently developed by serial extrusion method enabled the production of a larger quantity of vesicles, thereby providing a more powerful venue for therapeutics development. In this study, we aimed to test the efficacy of the CDVs derived from human adipose-derived stem cells (hASCs) in a rat osteoarthritis (OA) model.

The hASC-derived CDVs were produced by serial extrusion of cells through filters with pore sizes of 10, 5, and 1 μm. The physical and biochemical properties of CDVs were analyzed by transmission electron microscopy (TEM), nanoparticle analysis system (NTA), western blot, and flow cytometry. CDVs were injected into the joints of a MIA-induced OA rat model. Improvement in pain was assessed after CDV injections by paw withdrawal latency and weight bearing ratio on hind limbs, whereas the joint regeneration was evaluated by histological methods. We also estimated the effects of CDVs on the proliferation and migration of OA patient-derived chondrocytes in vitro by cell counting and scratch assays.

The hASC-CDVs were 50-150 nm in diameter and expressed multiple EV-associated tetraspanin markers (CD9, CD63, and CD81). CDV-treated OA mice exhibited significant improvements in both paw withdrawal and weight bearing tests 17 days after treatment compared to vehicle (PBS) control. Further, histological cartilage repair significantly improved in the samples treated with CDV at 24 days. Similarly, the migration and proliferation of chondrocytes were enhanced by CDVs in a dose-dependent manner.

This study demonstrates for the first time the efficacy of hASC-CDVs in OA model. Notably, we showed that hASC-CDVs could improve pain and promote cartilage regeneration. These results support the potential application of hASC-CDVs in OA, via local administration into affected joints.

Introduction: hMSC-CDVs and opportunities

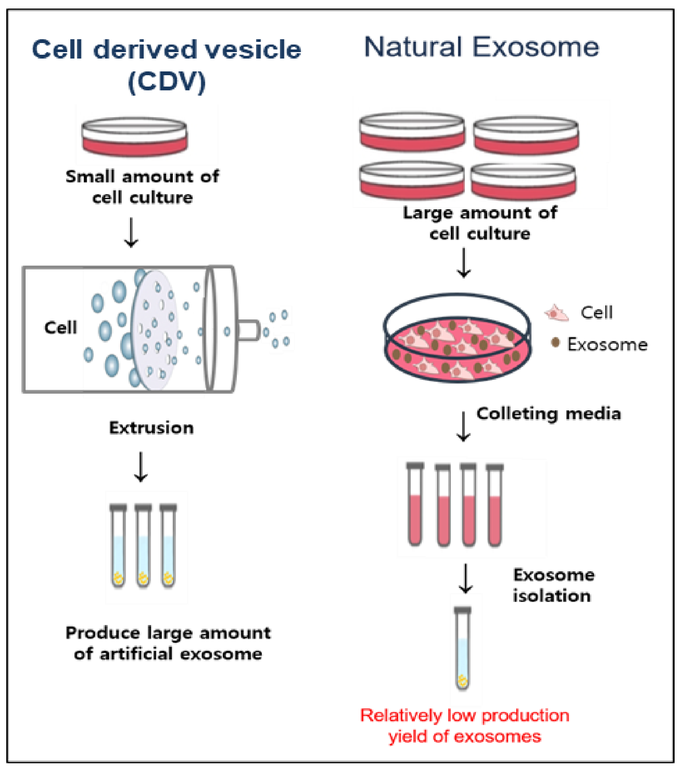


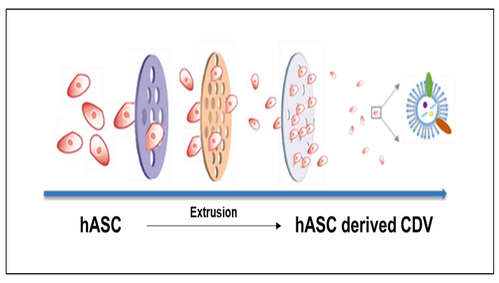
➢ **Exosomes**

-Nano-sized (80-150nm) signaling molecules secreted by cells

-Secreted in small quantities

Ref) Extracellular vesicles: Exosomes, microvesicles, and friends, JCB, vol. 200 no. 4 373-383, 2013





➢ **Cell Derived Vesicles (CDVs)**

-Cost-Effective

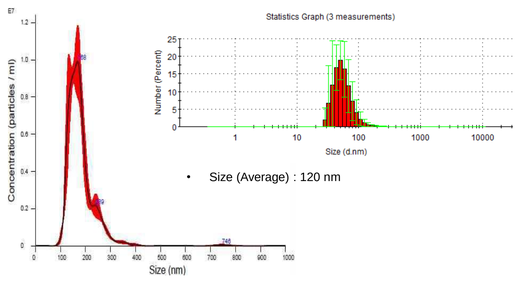
: Higher yield of production than natural exosomes

-Platform Technology

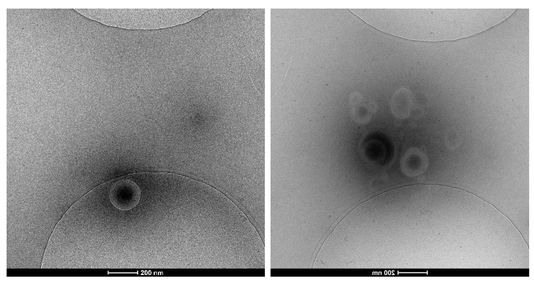
: Applicable to all human cells

hASC-CDV Characterization

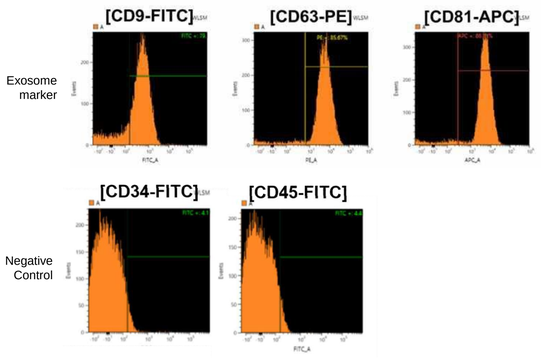
A) Size distribution



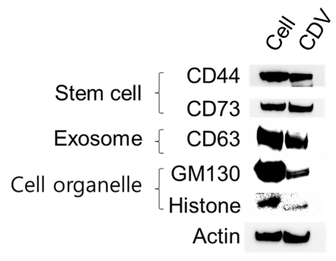
B) Cryo-TEM Imaging



C) FACS: Tetraspanin makers



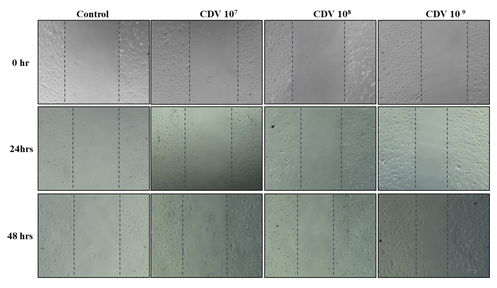
D) Western blotting



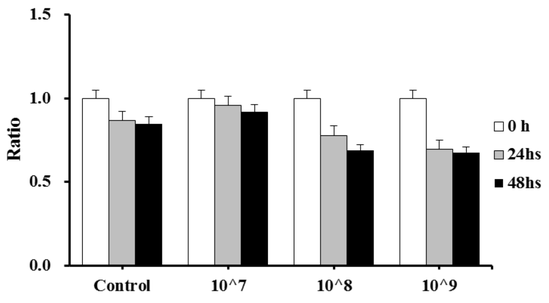
Characterization of hASC-CDV. (A) Size distribution of CDVs. (B) The morphology of CDV was imaged using electron microscopy. (C) Flow cytometry analysis of CDVs for tetraspanin markers, CD9, CD81 or CD63. (D) Western blotting for stem cell, exosomes and cell organelle markers.

In vitro assay using OA patient chondrocytes

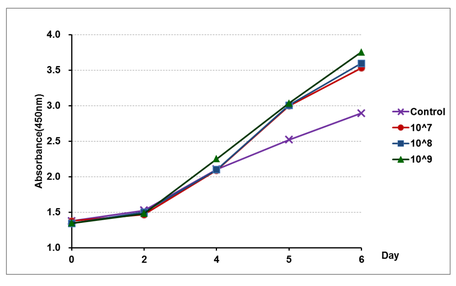
A) Scratch wound assays on OA patient-chondrocyte



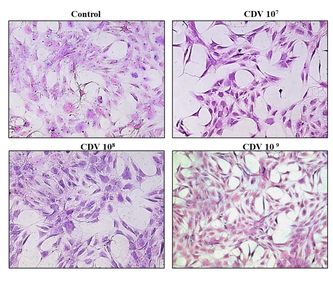
B) Scratch wound assays on OA patient-chondrocyte



C) Effect of MSC-CDV on proliferation In OA patient-chondrocytes



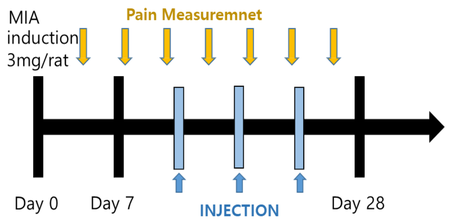
D) Safranin-O staining on OA patient-chondrocyte



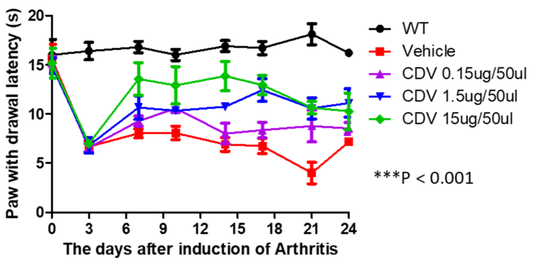
In vitro assay using OA patient-derived chondrocytes (A),(B) hASC-CDVs enhance the migration of OA-derived patient chondrocytes. (C) Effect of CDV on the proliferation of OA-derived patient chondrocytes; OA-derived patient chondrocytes were treated with various concentrations of CDVs (1E+7, 1E+8, 1E+9 particles/ml) for 2,4,5 and 6day. (D) Histological analysis of cartilage repair by safranin-O.

Effect of hASC-CDV in OA rat model

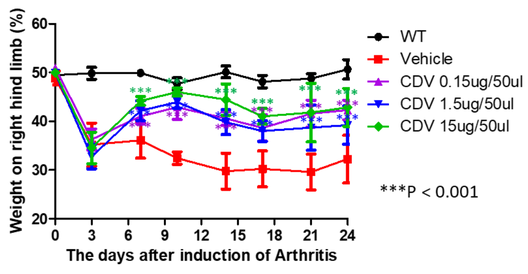
A) Scheme



B) Pain measurement

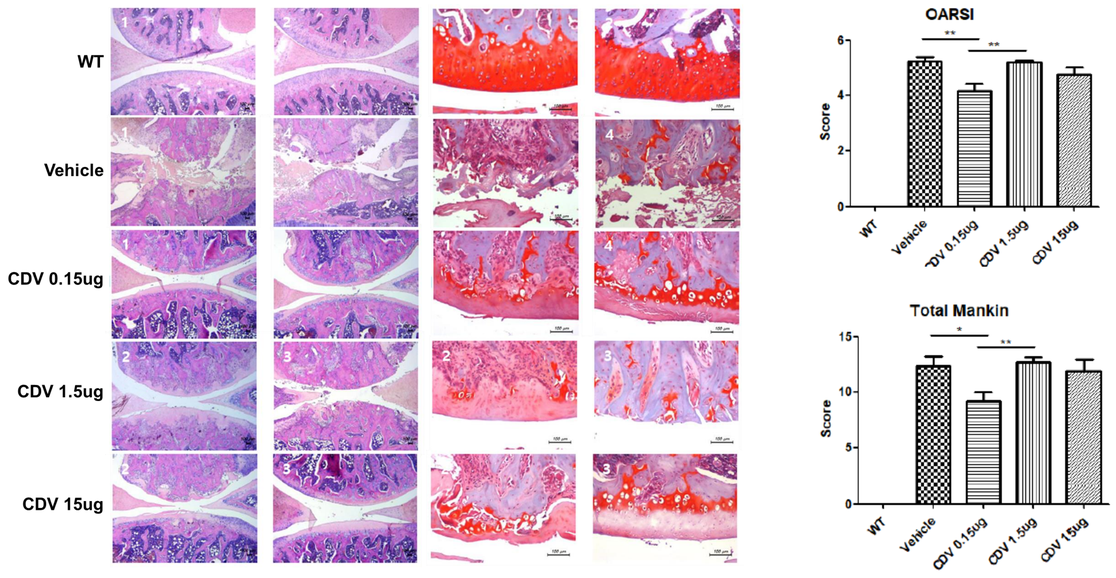


C) Weight bearing



Effects of hACS-CDVs on pain behaviors in MIA-induced OA rats. Experimental scheme (A), paw withdrawl responses to mechanical stimli (B) and weight bearing on the affected hind limb (C) were assessed. Data are expressed as mean±SEM.

Histological analysis



Histological evaluation of joints after treatment of hASC-CDVs in MIA-induced OA rats. Knee joints of OA rats treated with vehicle or hASC-CDVs were stained with hematoxylin and eosin and Safranin-O (A),(B), The OARSI index and the total mankin level were assessed (B).

Conclusion

- **Notably, hASC-CDVs were shown to improve pain and promote cartilage regeneration.**
- **These results support the potential application of hASC-CDVs in OA, via local administration into affected joints.**