Supporting information

Hybrid lipid nanoparticles derived from human mesenchymal stem cell extracellular vesicles by microfluidic-sonication for collagen I mRNA delivery to human tendon progenitor stem cells

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Materials and methods

TSPCs isolation

Briefly, tendon samples were obtained from different 43 and 51-years-old patients during surgery for the reconstruction of injured Achilles tendon. Samples were collected following the guidelines of the Declaration of Helsinki approved by the Institutional Review of San Giovanni di Dio e Ruggi d'Aragona Hospital (Salerno, Italy).

The isolation of primary cells was performed following enzymatic digestion of the sample. Firstly, tendon samples were washed with sterile phosphate buffer saline (PBS) 1x (Corning, Manassas, VA, United States) with 1% Penicillin/Streptomycin (Corning, Manassas, VA, United States) and 1% Amphotericin B (Corning, Manassas, VA, United States), and subsequently minced in small fragments to be digested with Trypsin-EDTA (Corning, Manassas, VA, United States) for 30 min at 37 ºC. Afterwards, α-MEM (Corning, Manassas, VA, United States) supplemented with 1% Glutagro™ (Corning, Manassas, VA, United States), 20% fetal bovine serum (FBS) (Gibco™, Walthan, MA, USA), 1% Penicillin/Streptomycin and 1% Amphotericin were added for trypsin neutralization, filtered with a 70 μm cell strainer and centrifuged at 1400 RPM for 10 min. The isolated cells were resuspended in growth medium and left to adhere at 37 ºC with 5% CO_2 and air in α-MEM medium with the previously described supplementation. The cell medium was changed every 2-3 days.

Results and discussion

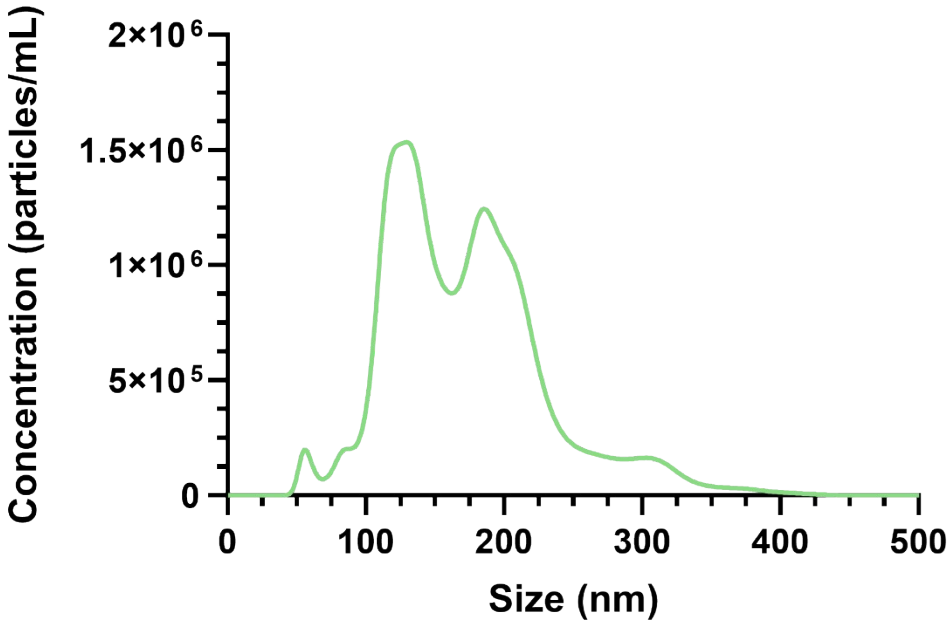


Figure S1. Size and particle concentration of MSC-derived EVs determined by NTA.

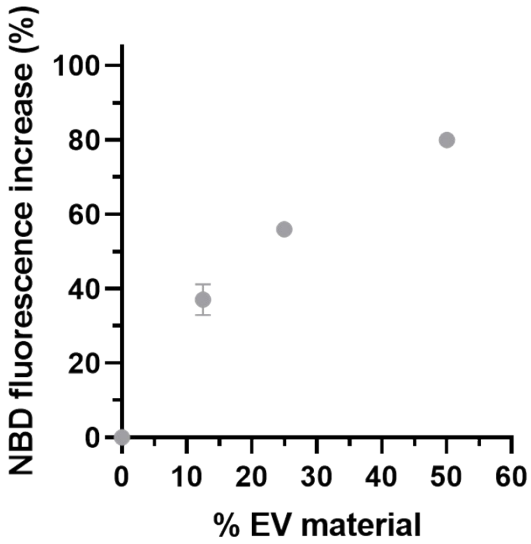
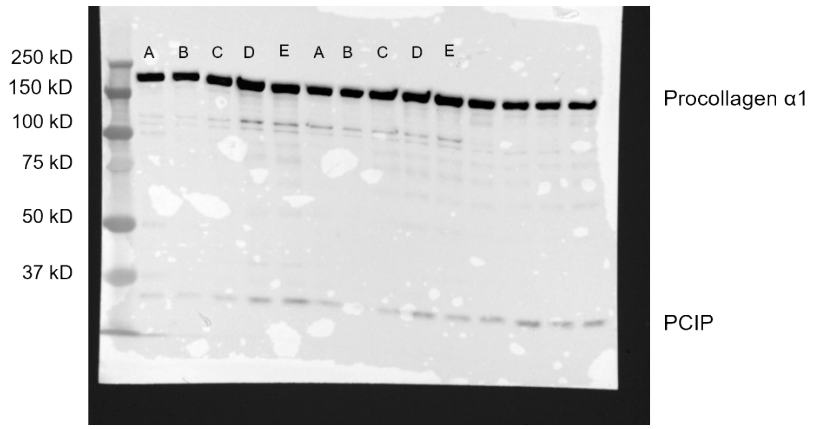


Figure S2. Calibration curve between the percentage of maximal NBD fluorescence and the percentage of addition of external material into the lipid structure. Different formulations different amounts of both conjugated lipids were prepared, mimicking an increase in the distance between both fluorochromes. Formulations were prepared with fluorescent lipids ratios of 1.5, 1.3125, 1.125 and 0.75 of donor and acceptor lipids. NBD fluorescence was quantified using the same amount of fluorescent lipids.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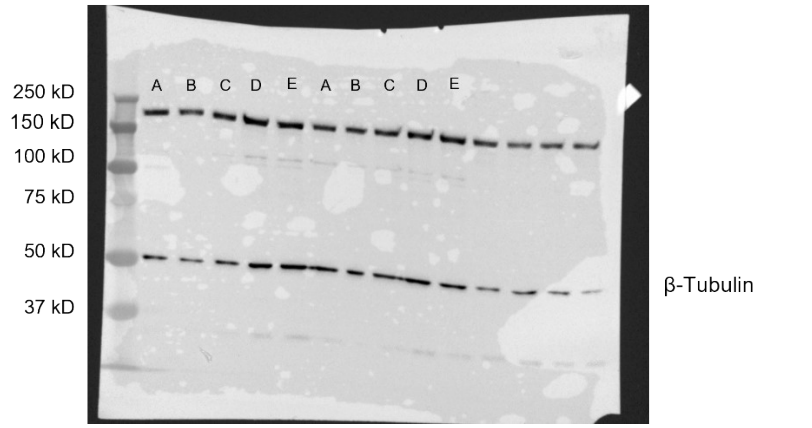
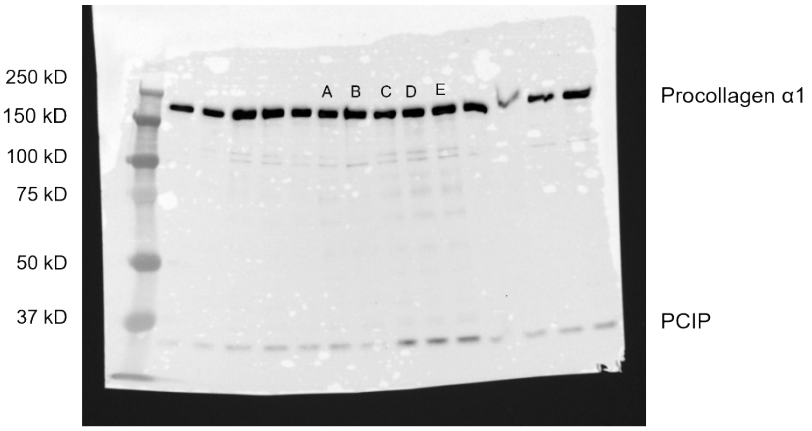
150 kD
100 kD
75 kD
50 kD
37 kD
Procollagen α1
PCIP


A B C D E A B C D E
250 kD
150 kD
100 kD
75 kD
50 kD
37 kD
β-Tubulin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Procollagen α1
PCIP

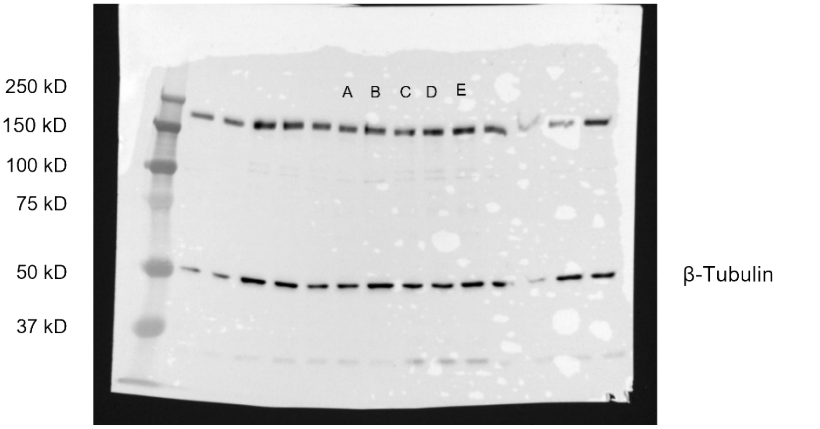
A B C D E
250 kD
150 kD
100 kD
75 kD
50 kD
37 kD
β-Tubulin

Figure S3. Raw images of Western-Blot protein quantification. The biological replicates were run simultaneously in two gels and the labelled columns represent (A) Negative control, (B) Empty LNPs, (C) Empty MSC-Hyb, (D) mRNA-loaded LNPs, (E) mRNA-loaded MSC-Hyb. The analysed protein bands were highlighted around 180 kD for Procollagen α1, 35 kD for PCIP and 50 kD for β-Tubulin.