Delivery of Receptor Antagonist of IL-1 using Cationic Exosomes for Cartilage Targeting and Repair

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INTRODUCTION: Osteoarthritis (OA) is a degenerative joint disease that affects tissues like cartilage. Cartilage has a high negative fixed charge density (FCD) due to negatively charged glycosaminoglycan chains (GAGs)[1]. This high negative charge along with rapid clearance from the intra-articular joint space makes drug delivery to cartilage extremely challenging. Interleukin-1 (IL-1), a proinflammatory cytokine, is elevated in OA and promotes catabolic processes by activating several signaling cascades[2]. IL-1RA is the receptor antagonist of IL-1 and blocks the IL-1 activity by competitively binding with the receptor. Recently, it has been seen that MSC-derived exosomes can enable cartilage repair in injury- stimulated OA animal models[3]. However, the negative charge of the exosomes lipid bilayer hinders their penetration in the anionic cartilage matrix. In our previous work, we designed cartilage targeting arginine rich cationic peptide carrier with a net charge of +14 (CPC+14) that showed full depth penetration in cartilage and long retention time[4]. We hypothesize that by modifying the surface of exosomes to make them positively charged for delivering IL-1RA into the cartilage, sustained administration will be possible with a single dose via depot delivery. We have synthesized **cationic exosomes** by conjugating CPC+14 and a cationic glycoprotein Avidin to their lipid bilayer for effective targeting in the cartilage along with IL-1RA anchored for therapeutic effects (**Fig. 1A**).

METHODS: The hydrophobic part of DSPE-PEG (2000)-Azide (DPA) was inserted in the lipid bilayer of exosomes. A cross linker, DBCO-NHS ester was introduced to conjugate CPC+14 [RRRR(NNRRR)$_3$R] to DPA via click chemistry. DSPE-PEG-Biotin was used to conjugate Avidin on the surface of exosomes. For examining the transport properties of cationic Exos (Exo-Avidin and Exo-CPC+14), 3mm×1mm IL-1α treated GAG depleted cartilage explants were treated with labelled cationic Exos for 48 h. Following the treatment, the explants were fixed, cryo-sectioned and stained with DAPI and WGA to visualize the nucleus and chondrocyte membrane respectively. Primary chondrocytes were incubated with labelled cationic Exos for 2.5 h to measure their uptake using a flow cytometer. Exo-CPC+14-IL-1RA and Exo-Av-IL-1RA were synthesized by optimizing the loading of labeled CPC+14 (Cy5), Avidin (Texas red) and IL-1RA (FITC) to achieve maximal reduction of negative charge on Exos. Size, zeta potential, polydispersity index (PDI), and loading on Exos were measured. The biological effectiveness of Exo-CPC+14-IL-1RA and Exo-Av-IL-1RA was investigated in IL-1α challenged cartilage discs. Following two doses on Day 0 and Day 4, the cumulative GAG loss was calculated using the DMMB assay. Finally, cytotoxicity of the surface modified Exos was assessed in primary bovine chondrocytes using the MTT assay. One-way ANOVA with post-hoc Tukey's Honestly Significant Difference (HSD) test was used for comparisons between different treatment conditions. Data are presented as Mean ± SD. $P < 0.05$ were considered statistically significant.

RESULTS: Exo-CPC+14 and Exo-Av showed full depth penetration in the IL-1α treated cartilage and were observed to be uptaken by chondrocytes residing in the deep zone of the cartilage tissue, confirmed by the overlapping fluorescence of cationic Exos (green), nucleus (blue) and chondrocyte membrane (red) (**Fig. 1B**). Cellular chondrocyte uptake study showed that Exo-Av resulted in about twice the cellular uptake compared to native Exos (**Fig. 1C**). We successfully synthesized cationic Exos by anchoring cationic motifs like CPC+14 and Avidin on the surface of Exos along with IL-1RA and reversed the zeta potential from -25.4 ± 1.3 to -1.6 ± 1.1 and -2.3 ± 0.85 respectively (**Fig. 1D**). From the *in vitro* experiment, it can be seen that Exo-CPC+14-IL-1RA and Exo-Av-IL-1RA significantly reduced the IL-1α-induced GAG loss while IL-1RA alone could not (**Fig. 1E**). Exo-CPC+14-IL-1RA and Exo-Av-IL-1RA exhibited over 90% cell viability confirming that modified exosomes were not cytotoxic and safe to use (**Fig. 1F**).

DISCUSSION: Here, we engineered charge-reversed exosomes anchored with IL-1RA with a zeta potential close to neutral and demonstrated full-thickness penetration in bovine cartilage *in vitro* implying their potential as effective targeted delivery systems. Moreover, the cationic exosomes exhibited superior chondrocyte uptake which further confirms their targeting potential. The IL-1RA anchored cationic exosomes significantly suppressed the IL-1α-induced GAG loss in cartilage explant models over 8 days while free IL-1RA did not. Ongoing work includes optimization designs to increase drug anchoring capacity on cationic Exos such that a sustained therapeutic effect can be achieved with only a single dose in *in vivo* models.

SIGNIFICANCE/CLINICAL RELEVANCE: We, for the first time, synthesize cationic exosomes that can effectively target, penetrate, and be retained in cartilage for effective delivery of a 17kDa receptor antagonist of IL-1 for the treatment of OA. This Exo-based delivery system has the potential to generate intra-cartilage drug depots following its intra-articular administration and facilitate delivery of any anchored drug to its cell targets.

REFERENCES: [1] Bajpayee+, Nature Rheum 2017; [2] Mehta+, Arthritis Res Ther 2019; [3] Chen+, Membranes (Basel); [4] Vedadghavami+, Acta Biomater 2019.

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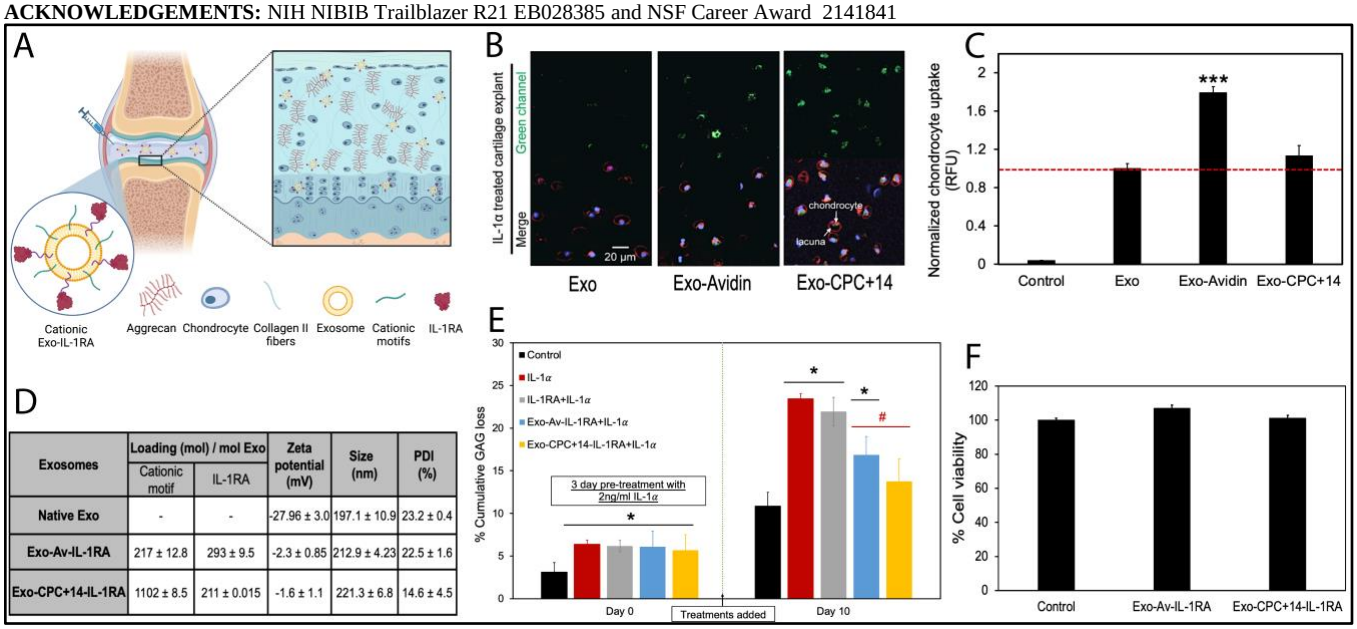

Exosomes	Loading (mol) / mol Exo		Zeta potential (mV)	Size (nm)	PDI (%)
	Cationic motif	IL-1RA			
Native Exo	-	-	-27.96 ± 3.0	197.1 ± 10.9	23.2 ± 0.4
Exo-Av-IL-1RA	217 ± 12.8	293 ± 9.5	-2.3 ± 0.85	212.9 ± 4.23	22.5 ± 1.6
Exo-CPC+14-IL-1RA	1102 ± 8.5	211 ± 0.015	-1.6 ± 1.1	221.3 ± 6.8	14.6 ± 4.5

Fig.1A. Schematic of cationic Exos modified with CPC+14 and Avidin along with IL-1RA. **B.** Transport of Exo-CPC+14 and Exo-Avidin IL-1α treated bovine cartilage explants (Exos- green, nucleus- blue, chondrocyte membrane- red). **C.** Chondrocyte uptake of Exo-CPC+14 and Exo-Av (*** vs native Exos; $p<0.001$). **D.** Loading of cationic motifs, and IL-1RA on Exos with size, zeta potential, and PDI. **E.** % Cumulative GAG loss in IL-1α treated cartilage explants; IL-1 (2ng/ml), IL-1RA (5000ng/ml) (* vs control, # vs IL-1; $p<0.05$). **F.** Cytotoxicity of IL-1RA anchored cationic Exos using MTT assay.