# Janus Base Nanopieces Delivered mRNA Therapeutics against Cartilage Degradation and Joint Pain for Osteoarthritis Treatment

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INTRODUCTION: Over 32.5 million adults in the U.S. suffer from osteoarthritis (OA), often characterized by pain and discomfort arising from the deterioration of articular cartilage. While protein-based drugs like the interleukin-1 receptor antagonist peptide (Anakinra) have been developed, they have not proven effective in clinical trials. Recently, the rise of mRNA therapeutics offers hope for a more effective treatment than traditional protein drugs. However, for mRNA therapies to work for OA, they must be delivered into thick, difficult-to-penetrate tissues and cell cytoplasm. As such, major challenges of mRNA delivery include the dense extracellular matrix (ECM) and endosomal entrapment of the delivered mRNA. In this study, we have specially designed and synthesized a new generation of Janus base nanotubes (self-assembled nanotubes inspired by DNA base pairs) to combine with mRNA to form rod-shaped delivery vehicles called Janus base nanopieces (JBNps). Unlike conventional spherical delivery vehicles such as lipid nanoparticles (LNPs), JBNps present a breakthrough in highly efficient penetration into articular cartilage due to their nano-rod morphology. Moreover, mRNA is very vulnerable to degradation (much more than small RNAs), so we specially designed this new generation of JBNps with a pore-formation property. Thus, they can escape from early endosomes (as opposed to the late endosomes for most other delivery vehicles) and achieve maximal mRNA bioactivities. In addition, JBNps present low toxicity and do not cause immune responses due to their non-covalent structures and DNA-mimicking chemistry. As a result, we demonstrated proof-of-concept applications to deliver Anakinra mRNA to treat osteoarthritis.

METHODS: The delivery of JBNp-mRNA was first demonstrated by transfecting human chondrocytes (C28/I2) with eGFP-mRNA, and then by investigating the delivery of JBNp-mRNA-cy5 to the mouse knee joint. To treat OA, the knee joints of mice with OA as generated via the Destabilization of the Medial Meniscus (DMM) surgery were injected weekly with JBNp-Anakinra mRNAs. The extracted knee joints following this treatment regimen were imaged via Micro-CT, and sections were stained for Safranin O/Fast Green. Behavioral studies were also conducted, including thermal sensitivity (hot plate), mechanical sensitivity (Von Frey) analysis, and gait analysis.

RESULTS SECTION: JBNp can effectively deliver eGFP-mRNA into human chondrocytes (**Figure 1B**). Furthermore, JBNp-mRNA-cy5 was successfully delivered to the knee joint of DMM mice after eight weeks (**Figure 1C**). Gait analysis, including paw area, stance length, stride length, and sway length showed effective treatment of OA after injecting the JBNp-Anakinra mRNA into the DMM surgery mice (**Figure 1D**). Micro-CT images also showed less osteophyte formation in the JBNp-Anakinra mRNA treatment group (**Figure 1E**). Finally, thermal and mechanical sensitivity analysis showed that the JBNp-Anakinra mRNA treatment group significantly decreased the withdrawal latency (s) and the 50% withdrawal threshold, respectively. (**Figure 1F and Figure 1G**).

DISCUSSION: We designed and developed a nano-rod shaped delivery vehicle with a diameter of 20 - 30 nm (JBNps) capable of delivery to the intra-articular environment. This new generation of JBNps can escape the early endosome by pore-formation, showing significantly better efficacy than LNPs. Furthermore, we have engineered their self-assembly conditions to make a nano-rod shape to penetrate the thick cartilage ECM efficiently. In this proof-of-concept experiment, we selected the Anakinra mRNA to treat OA. We obtained significant results such as improved gait measures, fewer osteophytes, less cartilage degeneration, and improved thermal and mechanical sensitivity. In the future, our JBNp platform can be used for any mRNA therapeutics or as a research tool.

SIGNFICANCE/CLINICAL RELEVANCE: We have developed a new class of mRNA delivery vehicles that can achieve intra-cartilage delivery with high efficiency, enhanced endosomal escape, low toxicity, and effective therapeutic outcomes for "hard-to-penetrate" cartilage. In a preclinical model, JBNp-delivered Anakinra mRNA was shown as a promising candidate for OA treatment.

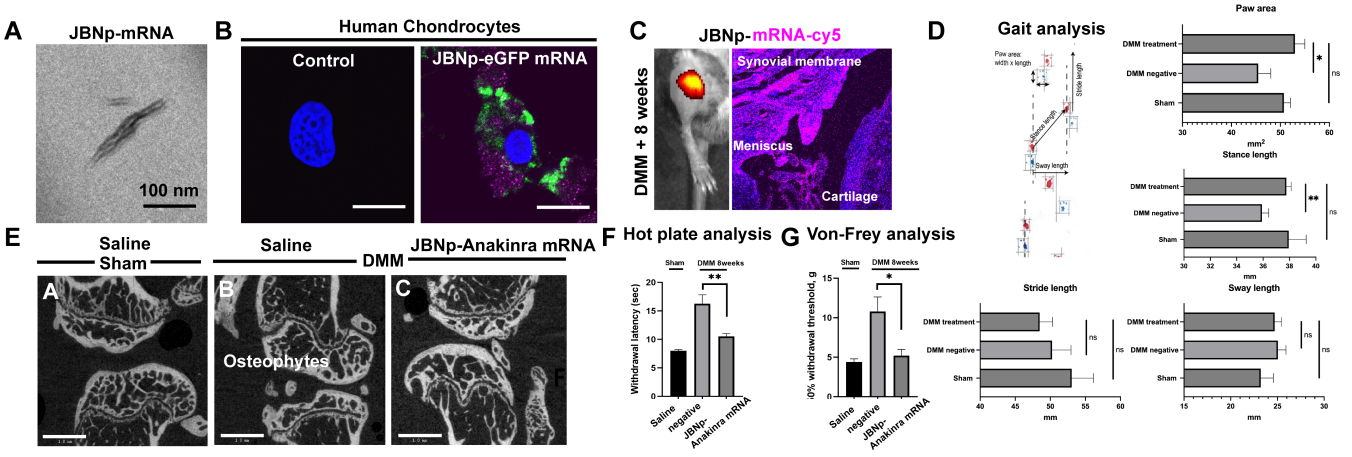


**Figure1.** (A) TEM image. (B) Cell delivery. (C) Intra-articular injection. (D) Gait analysis. (E) micro-CT. (F) Hot plate. (G) Von-Frey.