Nanopieces nucleic acid delivery platform-based theranostics for orthopaedic imaging and therapy

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Disclosures: YC, and QC: Nanode Inc.

INTRODUCTION:

Recently, theranostic nanomedicine has emerged as a promising personalized medicine approach to combining both diagnostics and therapeutics simultaneously. It takes advantage of the high capacity of nano-delivery platform to load both imaging and therapeutic agents and deliver them to the same tissues and cells at the same time. The resulting nanoplatform-based theranostics is a powerful tool capable of performing multiple functions including diagnosis, drug delivery, therapy, and monitoring therapeutic responses. However, challenges of developing highly efficient delivery vehicles for dual cargos hinder wide use of the theranostic technology. Developing theranostic nanomedicine in orthopaedics is even more challenging because of joint tissue barrier to theranostic delivery. Orthoapedic tissues including cartilage, bone, tendon and ligament are rich in extracellular matrix that may hinder cargo delivery. Furthermore, cartilage, tendon and ligament are avascular, which are difficult for drugs to reach via vasculature transportation. We developed Nanopieces (NP), a novel nucleic acid delivery platform for diagnosis and treatment of orthopaedic diseases. Previously we demonstrated sensitive detection of joint disease gene expression in real time using NP delivered molecular beacon (MB) oligonucleotides that served as a diagnostic agent. We also demonstrated effective treatment of post-traumatic joint injury in vivo using NP delivered siRNA of a joint disease gene, which served as a therapeutic agent. Therefore, we hypothesized that it is possible to achieve diagnostic and therapeutic purposes simultaneously using co-delivery of two nucleic acids cargos, one for imaging and the other for therapeutic purpose. As the first step towards that goal, we performed dual delivery of siRNA (as therapeutic agent) and MB oligos (as diagnostic agent) into chondrogenic ATDC5 cells to achieve highly efficient gene inhibition and detection of gene expression simultaneously.

METHODS:

Molecular Beacon (MB) design: Glyceraldehyde 3-phosphate dehydrogenase (GAPDH) MB was designed to target mouse GAPDH mRNA, modified with a fluorophore/quencher pair. Scrambled sequence MB (Scramble) was verified to not bind with any mouse, rat or human mRNA via BLAST. **Nanopieces synthesis and formulation:** Nanopieces were formulated with siRNA or MB oligos using a small molecule JBaK3 (Janus Base with amine or lysine (K)), which was designed and synthesized in the lab. **In vitro NP co-delivery:** Matrilin 3 (MATN3) stably transfected chondrogenic ATDC5 cells were cultured in medium containing 1 : 1 mixture of Dulbecco's modified Eagle's medium (DMEM) and Ham's F-12 medium supplemented with 5% fetal bovine serum and ITS in culture flasks at 37°C under 5% CO_2. Transfections were performed in DMEM medium with no serum or ITS. Transfections were divided into three groups: Nanopieces co-delivery of scrambled siRNA and scrambled MB (green fluorescence), NP co-delivery of MATN3 siRNA and GAPDH MB (red fluorescence), and MATN3 siRNA and GAPDH MB only. 24 hours after delivery, fluorescence microscopy and real time RT-PCR were performed to verify the functional co-delivery of GAPDH MB and MATN3 siRNA targeting matrilin 3 mRNA separately.

RESULTS:

To determine feasibility of co-delivery of two nucleic acids (NA) using NP, we labeled RNA1 with green fluorescence and RNA2 with red fluorescence. Delivery of two RNAs separately with NP resulted in cells positive for green (RNA1), red (RNA2), and yellow (RNA1 and 2) (Fig. 1A). When we mixed two RNAs together and then incorporated with NP, only cells with yellow fluorescence were observed (Fig. 1B). Therefore, NP is capable of delivering two NAs together. Furthermore, it is critical to mix them together before NP delivery to enable co-delivery to the same cells. To determine whether we can achieve gene expression imaging and knockdown at the same time, we transfected chondrogenic ATDC5 cells that stably express a cartilage specific matrilin-3 (MATN3) gene. MATN3 siRNA was used to achieve gene specific inhibition, while a MB of the housekeeping GAPDH gene (red) was used to assess gene specific detection. Co-delivery of MATN3 siRNA and GAPDH MB by NP showed significant knocking down of MATN3 mRNA levels (Fig. 2) and GAPDH MB red fluorescence (Fig. 3B) in ATDC5 cells. In contrast, co-delivery of non-targeting siRNA and scrambled MB (green) by NP (Control) did not result in MATN3 knockdown (Fig. 2) or significant fluorescence (Fig. 3A). Thus, the MATN3 KD and GAPDH signals were specific. Furthermore, in the absence of NP, GAPDH MB and MATN3siRNA co-transfection did not result in either MATN3 KD (Fig. 2) or GAPDH fluorescence signals (Fig. 3C). Thus, NP delivery is critical to achieve dual functions of imaging and gene knockdown.

DISCUSSION:

In this proof of concept study, we demonstrated for the first time the siRNA and MB co-delivery by NP can achieve gene inhibition and detection at the same time with one theranostic. Achievement of dual functions proved that co-delivery did not interfere with the function of either siRNA or MB. Most current theranostic nanoplatforms are based on pre-existing imaging agent carriers such as iron oxide nanoparticles, quantum dots, carbon nanotubes, gold nanoparticles and silica nanoparticles. Although they may be suitable for ferrying imaging agents into the body on a transient basis, they are not biodegradable and thus may not be appropriate to deliver therapeutics that require more stringent biosafety and efficacy standards. Theranostic vehicles require infiltrating tissues and cells to deliver dual cargos in a highly efficient, long lasting, and low toxicity manner. Our data suggest that biodegradable NP-based platform may enable surgeons to perform diagnosis and treatment by co-delivering multiple NAs to cartilage cells at the same time.

SIGNIFICANCE:

The combination of siRNA and MB as theranostics opened up the possibility of gene detection during treatment. The co-delivery of theranostics by biomimetic NP into joint tissues may provide a novel solution for personalized treatment of orthopaedic diseases.

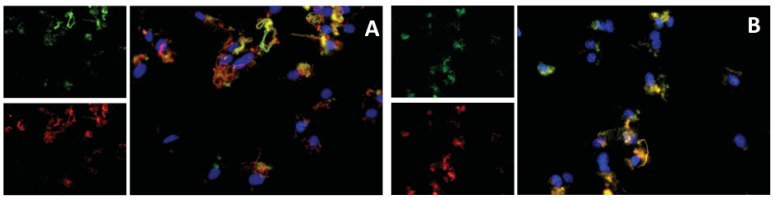

Fig.1 Separate delivery of fluorescent RNA (A) vs Co-delivery of fluorescent RNA (B)

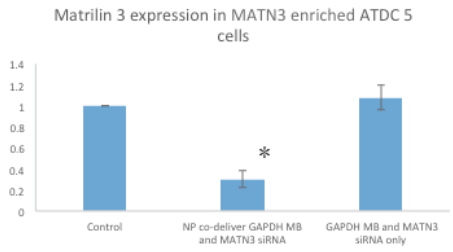

Fig. 2 Nanopieces co-delivery achieved MANT3 knockdown

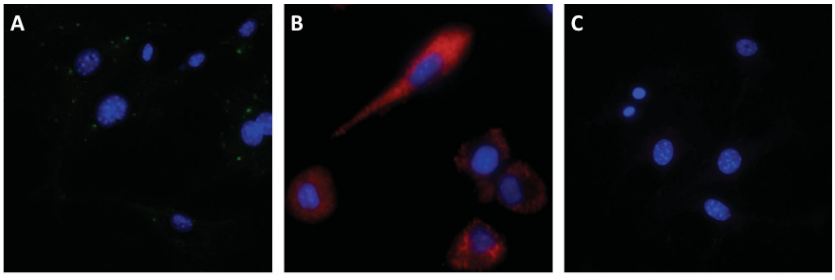

Fig.3 Fluorescence of scrambled and GAPDH MB in ATDC5 cells. A: NP co-delivery of scramble siRNA and scramble MB (green fluorescence). B: NP co-delivery of MATN3 siRNA and GAPDH MB (red fluorescence). C: MATN3 siRNA and GAPDH MB only