A Non-invasive, Early, and Sensitive Detection of Osteoarthritis through In Vivo Imaging of MMP-13 mRNA Levels by Molecular Beacon and Nanopieces Delivery Technology

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Introduction:
Osteoarthritis (OA) is one of the most common causes of disability. However, the lack of tools for early diagnosis of OA hampers the prevention and treatment of the disease to decelerate articular cartilage loss and alleviate suffering of patients. The OA Biomarker Initiative has identified a series of biomarkers, including matrix metalloproteinases (MMPs), which are elevated in articular cartilage during OA pathogenesis. However, detection of MMP protein levels or activities in serum may not be sensitive enough, while the more sensitive detection of MMP transcripts requires invasive procedure to obtain biopsy of articular joint tissue. Therefore, there is an urgent need to develop sensitive in vivo imaging technology to detect molecular changes at early stages of arthritis without harming articular cartilage.

Molecular beacon (MB) technology provides an intriguing possibility to detect the changes of mRNA levels in live animals in vivo. Molecular beacons, composed of an oligonucleotide loop, double strand stem, and a fluorophore and quencher, remain non-fluorescent due to the proximity of fluorophore and quencher. Upon entering a cell and hybridizing with target mRNA, MBs emit fluorescence due to separation of the fluorophore and quencher (Fig.1). To date, there has been no report of detection of OA using MBs due to the significant challenge of in vivo delivery of MBs into joint tissues. In this study, we showed the feasibility of early detection of OA in the Destabilizing Medial Meniscus (DMM) mouse OA model using MB to detect induction of MMP-13 transcript, a major matrix proteinase that degrades interstitial collagen matrix during arthritis. In vivo delivery of MMP-13 MB was made possible by a novel nanomaterial named Nanopieces that is derived from rosette nanotubes.

![Molecular beacon](image1.png)

**Figure1. MB fluorescence mechanism.**

Methods:
**MB design:** Using an established method[1], MBs were designed to target mouse MMP-13 or GAPDH mRNA, modified with a fluorophore/quencher pair. Scramble sequence MB (Scramble) was verified to not bind with any mouse mRNA via BLAST.

**In vitro delivery and validation:** MBs were delivered into chondrocytes by Nanopieces. Specifically, after stimulation with IL-1β for 24 hours, chondrocytes were co-transfected with GAPDH (red) and Scramble (green) MBs or GAPDH (red) and MMP-13 (green) MBs via Nanopieces. Real time RT-PCR and fluorescence microscopy were used to verify the presence of MMP-13 expression and the positive fluorescence signals from MMP-13 MB.

**DMM surgery and in vivo delivery:** Under IACUC approved protocol, DMM or sham surgeries were performed on 10-week-old 129SVE male mice to induce osteoarthritis. One week after surgery, MMP-13 and Scramble MBs with different fluorophores were delivered by Nanopieces via injection into knee joints of mice. Small animal fluorescence molecular tomography (FMT) was used to determine the fluorescence signal resulting from MMP-13 expression in the live animals for 3 weeks.

Results:
**In vitro test:** To test the efficacy of mRNA detection in chondrocytes using MBs delivered by Nanopieces, primary mouse chondrocytes were transfected with MBs either with or without IL-1β treatment. Before IL-1β treatment, the housekeeping GAPDH MB (red) was detected while the MMP-13 MB (green) was not (Fig.2, left panels). In contrast, after IL-1β treatment, both GAPDH MB (red) and MMP-13 MB (green) were detected, indicating the induction of MMP-13 mRNA levels by IL-1β (Fig.2, right panels). Real time RT-PCR showed that MMP-13 mRNA level was up-regulated by about 10 times upon IL-1β stimulation. In contrast, Scramble MB transfection did not show any green fluorescence, suggesting that the fluorescence of MMP-13 MB was
not due to non-specific degradation.

**Figure 2.** Fluorescent micrographs of chondrocytes delivered with MBs. (RED is GAPDH MB; GREEN in (c) is scrambled MB; GREEN in (a) and (d) is MMP-13 MB; BLUE is cell nuclei staining)

**In vivo test:** After DMM surgery, MMP-13 MB was delivered intra-articularly to the knee joint of adult mice with Scramble MB that emits fluorescence at a different wave length than MMP-13 MB. As soon as a week after surgery, the DMM leg displayed strong MMP-13 signal compared to the contralateral Sham surgery leg (Fig. 3A). In contrast, the Scramble MB showed very low fluorescence in both DMM and Sham surgery knee joints. After subtracting Scramble MB basal level signals, MMP-13 MB real signal was about 40 times stronger in the DMM leg than the sham leg (Fig. 3C). Such MMP-13 MB signals persist, even for 3 weeks after injection of MBs.
Discussion:
Matrix metalloproteinases (MMP) are the major enzymes that degrade the components of the extracellular matrix during arthritis progression. MMP-13, which is usually produced by cartilage and bone, degrade interstitial collagens (types I, II and III) in both OA and RA. Expression of MMP-13 is low in healthy cells, whereas in pathologic condition excess MMP-13 production is associated with inflammation. So mRNA level of MMP-13 would be indicative for arthritis development. Therefore MMP-13 is recognized as a good target in early diagnosis of arthritis. However, existing tests would require collection of articular cartilage tissue to show upregulation of MMP-13 mRNA levels.

In this study, for the first time, through MMP-13 MB delivered by Nanopieces technology, a sensitive tool to detect pro-inflammatory degenerative conditions was developed both for chondrocytes in vitro and for OA animal models in vivo. It is especially impressive that this technology detects pathogenesis of OA at an early stage (within a week) in a mild OA model (DMM). The high sensitivity may be due to the detection at the mRNA level and the high efficiency of MB intracellular delivery by Nanopieces.

Significance:
The combination of molecular beacon and Nanopieces technology provided a powerful tool for early detection of OA in vivo in a specific and sensitive manner without harming any joint tissues.

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References: