Design of injectable and renal-clearable sensor of joint health

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Introduction: Joint infections are increasing over time,[1] due to an increase in joint arthroplasties. The primary surgical management strategy for joint infection is arthroscopic lavage (washout), during which time the infected joint is rinsed with antibiotics. Even after washout, ~30% of patients struggle with persistent, lingering infections and must undergo repeated washouts with significant wait times in-between to determine the success, or failure, of each procedure, increasing the risk of mortality.[2] Unfortunately, there are no diagnostic strategies that can be performed in conjunction with arthroscopic lavage to determine the success of this procedure, and reduce repeated office visits and arthrocenteses for the patient. In this study, we aimed to design an injectable and renal-clearable sensor of joint health that could be detected in a patient's own urine. To accomplish this goal, we considered the renal filtration cutoff (<5.5nm) and developed a material system whereby injectable microgels (large, ~30 µm) are linked to nanoparticles (small, ~2 nm) via an enzyme-cleavable linker (**Fig. 1A**). As a proof-of-concept, we designed this linker to be responsive to proteinase 3 (PR3), an enzyme upregulated in the knee joints of humans with periprosthetic joint infections (**Fig. 1B**).[3] Overall, we hypothesized that small volumes (<10 µL) of the peptide-NP conjugates could be colorimetrically detected in synthetic urine, mimicking the at-home detection of excreted particles (**Fig. 1C-E**).

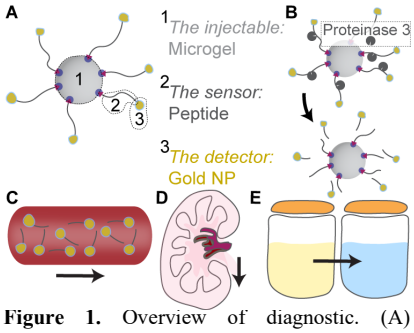

Figure 1. Overview of diagnostic. (A) Components of the diagnostic. (B) Proteinase 3-cleavable peptides release nanoparticles into synovial capillaries (C), which filter through the kidneys (D). (E) Colorimetric detection in urine.

Methods: Microgel synthesis and quantification. Methacrylated hyaluronic acid (MeHA) was synthesized as previously.[4] MeHA (3 wt%; **Fig. 2A**) was mixed with crosslinker (dithiothreitol; DTT) and FITC-SH (fluorescent proxy for DBCO-SH) in a basic buffer (pH, 9.5) and injected dropwise into with mineral oil and span80 (2 wt%). The solution was stirred at 37°C to facilitate droplet formation and crosslinking. Microgels were pooled and washed, followed by imaging on a confocal microscope (Nikon AXR). Images were binarized to quantify microgel diameter in FIJI. Design and validation of proteinase 3-responsive peptide. A custom proteinase-3 responsive peptide was synthesized as in [5], with a N-terminal azide group and C-terminal cysteine (thiol) (CPC Scientific). HPLC confirmed sample purity. Synthesis of peptide-NP conjugates. An aqueous solution of gold(III) chloride trihydrate ($HAuCl_4$, 20 mM) was mixed with DI water, L-Glutathione reduced (GSH, 20 mM), and the custom proteinase 3-responsive peptide (20 mM). The volume ratio of the peptide to GSH was 1:10 peptide: GSH. Quantification of peptides-nanoparticle (NP) conjugates. The peptide-NP conjugates were characterized with transmission electron microscopy (TEM) by drop casting a sample onto a copper grid, and imaging with the FEI Tecnai F30 (300 kV). FIJI software was used to quantify NP size. Colorimetric readout of peptide-NP concentration. To colorimetrically detect the gold NPs, 5 different solutions were made including: 0, 2.5, 5, 7.5, or 10 µL NP with hydrogen peroxide, and tetramethylbenzidine (TMB). Statistics. All data reported as mean +/ std dev. n values in figure caption.

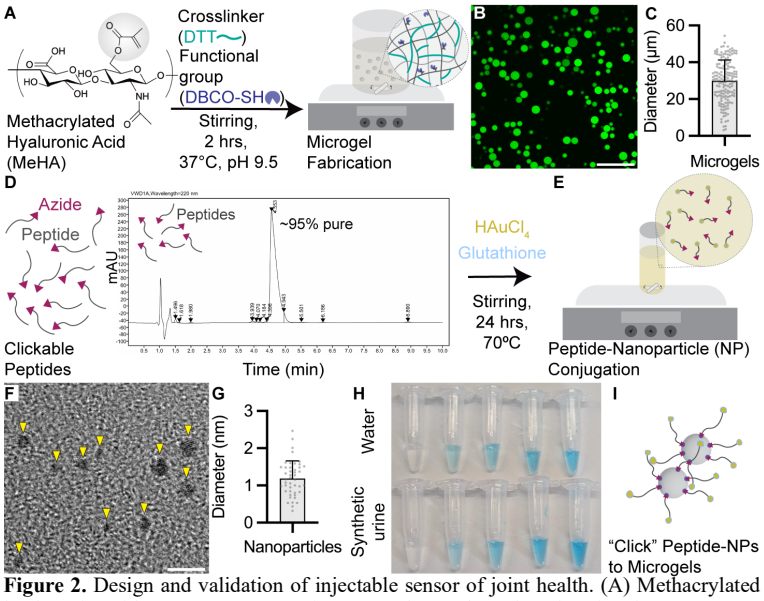

Figure 2. Design and validation of injectable sensor of joint health. (A) Methacrylated hyaluronic acid (MeHA). Grey region notes the methacrylate functional group. DTT (dithiothreitol) crosslinker and thiolated DBCO (DBCO-SH) are mixed with MeHA solution and added dropwise to oil bath. Microgels are formed via Michael addition reaction. (B) FITC-SH was incorporated into the MeHA network, in lieu of DBCO-SH to fluorescently label the microgels. Scale bar = 200 µm. (C) Quantification of microgel diameter. Data = mean +/- std dev. n = 166 microgels. (D) HPLC quantification of custom peptide purity with terminal azide group. (E) The peptides were stirred at 70°C with gold chloride to create the peptide-nanoparticle (NP) conjugates. (F) Transmission electron microscopy (TEM) analysis of the peptide-NP conjugates. Scale bar = 5 nm. (G) Quantification of peptide-NP conjugates. Data = mean +/- std dev. n = 45. (H) Colorimetric assay with peptide-NP conjugates performed in synthetic urine or water. Left to right: increasing concentration of NPs. (I) Future work will click peptide-NP conjugates to the DBCO-microgels for intraarticular delivery and sensing.

Results: The microgels appeared as distinct spherical units (**Fig. 2B**), suggesting completion of the reaction after collecting and washing the gels in a pH 7 solution, and averaged around 30 µm in diameter (**Fig. 2C**). The custom peptide synthesis led to the effective generation of a pure (~95%) PR3-responsive sequence with terminal azide, allowing addition to DBCO-moieties (**Fig. 2D**). Since the peptides also included a terminal -cys group, we could selectively add gold nanoparticles to one end (**Fig. 2E**).[6] The lattice structure of the gold nanoparticles was clearly visible under TEM (**Fig. 2F**). The nanoparticles had an average diameter of 1.2 nm (**Fig. 2G**), underneath the renal filtration cutoff. We then confirmed that the synthesized gold NPs could indeed lead to colorimetric detection within a (synthetic) urine sample. Even 2.5 µL of peptide-NP conjugates (second column) led to a color chance with this simple assay (**Fig. 2H**).

Discussion: We developed a modular, injectable biosensor of joint health that includes a microgel, peptide sequence, and nanoparticle. Now that both the DBCO-microgels and Azide-peptide-NPs have been validated, we can rapidly click these together to form our injectable sensors (**Fig. 2I**). Once injected into the joint, we expect PR3 to cleave the peptides, releasing the nanoparticles. Free nanoparticles will then enter the synovial vasculature, filter through the renal system and out of the body via excreted urine. Future work will assess clearance from murine knees, and the ability to sense these nanoparticles from collected urine samples. We will also further tune the peptide sequence to enhance the sensitivity and specificity of this probe.

Significance: This injectable diagnostic explores a new delivery and sensing strategy, reliant on size-based filtration of small (<3nm) particles from the joint. Currently, there is no known at-home urine-based diagnostic of joint health, and this technology fills a critical gap in the field of joint infection.

References: [1] Shichman, *et al.,* JBJS, 2023. [2] Kim, *et al*., J of Arthroplasty, 2019. [3] Li, *et al*., JBJS, 2021. [4] Zlotnick, *et al.*, Adv Mat, 2020. [5] Hajjar, *et al.*, J Med Chem, 2006. [6] Loynachan, *et al.*, Nat Nanotech, 2019.

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