Injectable Radiopaque Hyaluronic Acid Granular Hydrogels for Intervertebral Disc Repair

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Intorduction

Injectable hydrogels have the potential to increase minimally-invasive treatment options for degenerative disc disease, a condition that affects millions of Americans each year.[1] However, many hydrogels for intervertebral disc (IVD) repair suffer from weak mechanical integrity, expulsion from the injection site, and gel migration within the disc.1 To overcome these limitations, we developed an injectable hyaluronic acid granular hydrogel with encapsulated radiopaque zirconium oxide nano-powder for IVD repair. The granular structure imparts both injectability and enhanced biomaterial integrity when compared to previously investigated polymer solutions, while the radiopacity allows for direct visualization of gel injection and retention at the injection site. The purpose of this study was to characterize the material properties of the radiopaque granular hydrogel, determine its capacity to restore disc mechanical properties ex vivo, and demonstrate feasibility of in vivo minimally invasive delivery.

Methods

Norbornene-modified hyaluronic acid (NorHA. 2 wt.%, 25% degree of modification of norbornene groups), dithiothreitol (DTT, 6mM), zirconium oxide nano-powder (ZrO_2, 30 wt.%), ammonium persulfate (APS, 10 mM), and tetramethylethylenediamine (TEMED, 10mM) were thoroughly mixed in phosphate-buffered saline (PBS) and allowed to form a bulk hydrogel over 30 min. The bulk hydrogel was then fragmented by extrusion fragmentation to obtain microgels.[2] Microgels were then jammed by vacuum-driven filtration to form an injectable radiopaque granular hydrogel (Figure 1a). Extrusion forces were measured using a custom-built setup consisting of an Arduino Uni Rev 3, round force-sensitive resistor (Interlink), and

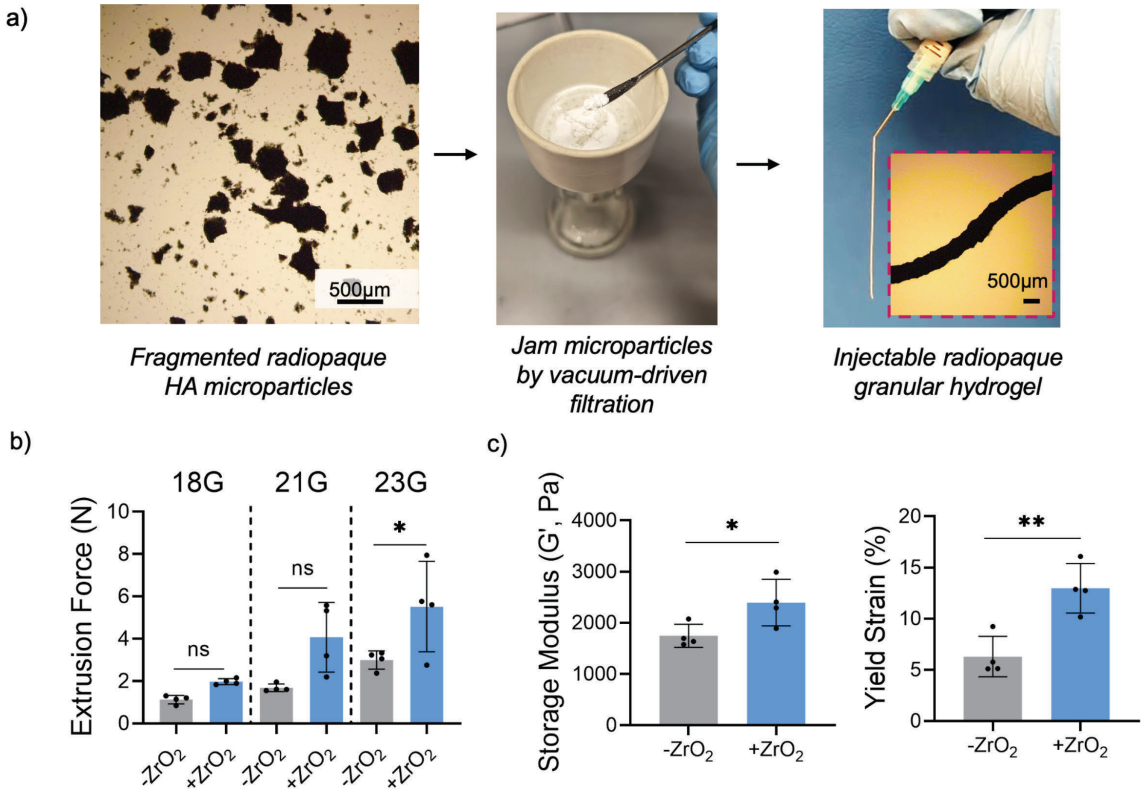

Figure 1. Radiopaque granular hydrogel **(A)** fabrication; **(B)** extrusion force; **(C)** mechanical characterization.

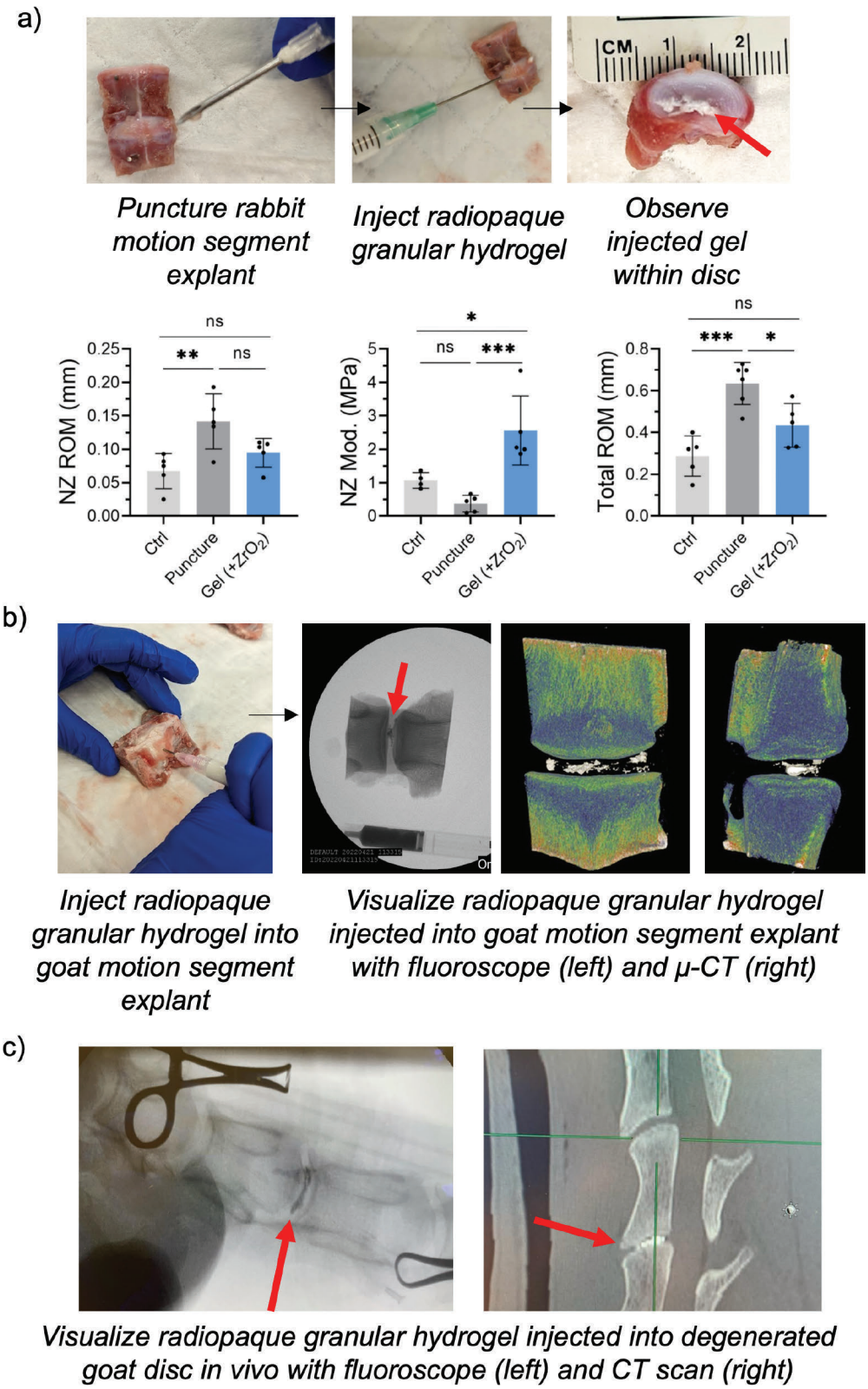

Figure 2. Radiopaque granular hydrogel evaluation for **(A)** restoration of mechanics, **(B)** imaging ex vivo and **(C)** imaging in vivo.

syringe pump.[3] Rheological properties were assessed using an oscillatory shear rheometer (AR2000, TA Instruments). Storage modulus (G') and yield strain were determined from oscillatory shear time sweeps (1% strain, 1 Hz). To mimic the loss of disc mechanical function associated with disc degeneration ex vivo, rabbit lumbar spinal motion segments were punctured with a 16G needle, and nucleus pulposus tissue was removed (Figure 2a). Radiopaque gel was injected into punctured rabbit motion segment explants using a 21G needle, and compressive mechanical properties of disc explants (i.e., neutral zone range of motion [NZ ROM], neutral zone modulus [NZ Mod.], and

total range of motion [Total ROM]) were determined using mechanical testing methods previously described.[4] To confirm adequate visualization on radiographic imaging, radiopaque gel was injected ex vivo into goat cervical motion segments using an 18G needle (Figure 2b). The goat explant was imaged using a fluoroscope as well as µCT scan to visualize the radiopaque gel within the disc. Finally, as an in vivo proof of concept, following IACUC approval disc degeneration was induced in one goat at the C4-C5 level of the cervical spine via injection of 200µL of 2U ChABC, as previously described.[5] 14 weeks following ChABC injection, a second procedure was performed to inject 500µL of the radiopaque granular hydrogel percutaneously into the C4-C5 disc space under fluoroscopic guidance using an 18G spinal needle (Figure 2c). Data is presented as mean ± standard deviation, unless otherwise indicated. Statistical analysis was conducted in GraphPad Prism 8 using ANOVA and a Tukey's post hoc comparison. For all quantified samples, $n \geq 3$, $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$, ns = not significant.

Results

ZrO_2 nano-powder was encapsulated within fragmented hyaluronic acid microgels (average ~100 µm diameter), which were then subsequently assembled into a jammed state to form a microporous injectable radiopaque granular hydrogel (Figure 1a). The inclusion of zirconia oxide nano-powder significantly increased the extrusion force of the granular gel through 21G and 23G needles, but not 18G needles (Figure 1b). The radiopaque granular gel also had a significantly higher storage modulus and yield strain compared to the granular gel without the encapsulated zirconia oxide (Figure 1c). In ex vivo mechanical studies, punctured rabbit motion segments (mimicking degenerative conditions) exhibited increased neutral zone and total range of motion as well as decreased neutral zone compressive moduli (Figure 2a). Upon ex vivo injection of the radiopaque granular hydrogel, both the neutral zone and total range of motion, as well as neutral zone modulus, were returned to healthy (control) levels (Figure 2a). Due to the high encapsulated ZrO2 content, the injected radiopaque granular hydrogel could be visualized within the goat disc ex vivo as seen by both fluoroscopy and µ-CT imaging (Figure 2b). The in vivo proof of concept study demonstrated that the radiopaque granular gel could be successfully delivered to a degenerative disc in a minimally invasive fashion under fluoroscopic control and visualized on post-operative CT imaging (Figure 2c).

Discussion

In this work we developed an injectable hyaluronic acid granular hydrogel for IVD repair. The injectable gel restored disc mechanics in punctured rabbit discs ex vivo. Though limited to an n = 1 proof-of-concept study, the injectable hydrogel was injectable percutaneously and visible through clinically-relevant imaging in a degenerated disc goat in vivo model. Future directions will include long-term in vivo outcomes in a goat model such as restoration of disc height, mechanics, and MRI signal after hydrogel injection.

Significance

The radiopaque injectable granular hydrogel explored in this study has the potential to advance injectable hydrogel biomaterials for minimally-invasive degenerative disc disease treatment.

References

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