Sol-gel transitions of comb-like polymethacrylate copolymers by mechano-thermal stimuli in water

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Sonogelation by supramolecular gelators is highlighted as a paradigm shift for their potential applications in material and biomedical sciences. Yet, these materials require organic solvents and low frequency ultrasound, limiting their utility. Here, we synthesize a comb-like polymethacrylate copolymer with 2,6-bis(1-methylbenzimidazolyl)-4-oxypyridine tridentate ligand randomly distributed on poly(ethylene glycol) side chains. Upon addition of zinc perchlorate salt, this copolymer rapidly forms a hydrogel after exposure to either mechanical (i.e., ultrasound) or thermal stimuli. Spectroscopic analysis, to elucidate the mechanism, indicated metal ion coordination to ligand as well as the carbonyl and ethylene glycol units; interestingly, 1H-NMR suggested that the coordination between tridentate ligand moiety and Zn$^{2+}$ was unexpectedly weaker in water than in acetonitrile. Investigations by DLS indicated that the copolymer formed nanodispersions in excess salt, which coalesced to form a hydrogel.
by either heating or HIFU. Rheological quantification of these gels suggested stronger
crosslinking by HIFU compared to heating.

1. Introduction
Smart responsive materials have attracted much attention due to their numerous applications
including, but not limited to, tissue engineering,[1] drug delivery,[2] cosmetics,[3] and
chemical/water treatment.[4] While conventional gels are formed through chemical processes
(e.g. radical polymerization and click reactions), smart gels require a stimuli such as pH, heat,
electromagnetic waves, and/or mechanical stress. A decade ago,[5] a unique strategy of using
ultrasound to induce gelation of organic or aqueous solvents with a gelator was highlighted as
a paradigm shift from conventional methods because it allowed access to a range of new
products with applications in organic and supramolecular chemistry and biomedical sciences.
Naota and co-workers reported on low-frequency ultrasound induced organogelation of
callado-macrocycles and peptide-based palladium complexes.[6] This intriguing sonication
induced gelation was later observed for other peptide-derived gelators.[7] For these, gelation
occurred via intermolecular hydrogen and π-π bonding between aromatic structures
terminating the peptide constructs. Though Pan et al. observed that these structures self-
assemble to form gels within 3 hours without ultrasound, the addition of ultrasound
accelerated gel formation to occur within 30 seconds.[7b] This ultrasound-enhanced gel
assembly may be a result of sonocrystalization, which is a process that uses the mechanical
effects of ultrasound to facilitate interactions between molecules and create structures from
micron-size fibrils to β-sheets.[6a, 7a]

Around this period, Rowan and co-workers[8] showed that 2,6-bis(1-methylbenzimidazolyl)-
4-oxypyridine (O-Mebip, 1) ligands attached to the ends of a penta(ethylene glycol) core
formed metallo-gels in the presence of transition metals, which possessed multiple stimuli-
responsive behaviours. The effect of acoustic cavitation on the gel[8] was documented to
decrease particle sizes and create a denser and more homogeneous interconnecting network,
following ultrasonic interaction of the mechanical force with the gel, to result in enhanced gel strength.

With biomedical applications such as targeted embolisation therapy in mind, we sought to develop polymeric materials that form gels in water using a process that is induced by high intensity focused ultrasound (HIFU) from a remote acoustic source. HIFU has increasingly been used in a number of applications such as tissue ablation,\textsuperscript{[9]} skin-lifting,\textsuperscript{[10]} and is also a stimulus for a number of controlled delivery systems.\textsuperscript{[11]} Unlike low frequency (<100 kHz) ultrasound which stochastically generate cavitation\textsuperscript{[12]} and are difficult to spatially control, HIFU operates at higher frequencies (>100 kHz) and may be geometrically lensed to focus the acoustic waves down to volumes of a single grain of rice.\textsuperscript{[11a, 11b, 13]} It is considered to be non-invasive with both spatial and temporal control of mechanical and thermal effects.\textsuperscript{[9a, 14]} Yet to the best of our knowledge, gelation in water induced by HIFU has not yet been demonstrated. We thus report here a polymer system that form gels in water in response to both mechanical and thermal effects induced by HIFU or heat.

2. Results and Discussion

2.1. Polymer Design and Characterization

In our polymer design, we focused our attention towards nonlinear comb polymethacrylates (Scheme 1) containing tridentate ligand \textbf{1} randomly distributed on polyethylene glycol side chains. We sought to employ the knowledge created by Rowan’s group\textsuperscript{[15]} regarding tridentate \textbf{1} with the intention to induce metal-ion coordination in water. In addition, comb-like polymethacrylates with pendant polyethylene glycols are well-known for their material properties (e.g., water-solubility, biocompatibility, thermosensitivity, and biodegradability).\textsuperscript{[16]} Poly(meth) acrylates\textsuperscript{[17]} and PEG\textsuperscript{[18]} are also reported as sorbents of
transition metal salts due to the presence of (meth)acrylate/metal cation and ethylene glycol/metal cation interactions.

Copolymer 2 was successfully synthesized in a three-step sequence from tridentate ligand 1 (Scheme 1a—b). The alkylation of 1 with 2-(2-(2-chloroethoxy)ethoxy)ethan-1-ol 3 provided the intermediate monomer 4, which subsequently reacted with methacrylic acid activated by N,N'-diisopropylcarbodiimide and 4-DMAP to produce the final tridentate ligand monomer 5. The synthesis of copolymer 2 was achieved by radical copolymerization of monomer 5 (Scheme 1c) with PEG-3 (Mn = 300 Da) using 2,2'-azobis(2-methylpropionitrile (AIBN) as a radical initiator in toluene at 80°C. PEG-3 6 was chosen to match the length of the chain to monomer 5. Homopolymer 7 was synthesized to operate as a negative control. Representative 1H-NMR for the monomer (4 and 5) and copolymer 2 are given in Figure S1—S3 and the copolymer GPC characterization is provided in Figure S4. The properties of the copolymers, such as molecular weights (Mn), are shown in Table 1.

2.1.1 Polymer Dispersion in Aqueous Media

We quantified the solubility and dispersibility of the monomer and polymers, respectively, in aqueous media. As expected, the ligand 1 was insoluble in water. Interestingly, the solubility of ligand 1 was improved with the addition of acid (Figure S5).

Aqueous solutions of copolymer 2 yielded a translucent solution. However, dynamic light scattering (DLS) measurements produced unreliable data and indicated that copolymer 2 was unable to be completely dispersed in water. The addition of excess zinc ions, however, produced a well dispersed copolymer solution of nanoparticles 20.3 nm in hydrodynamic
diameter as measured by DLS. In contrast, homopolymer 7 solubilized more readily in water, resulting in poor measurement by DLS (Figure S6).

2.1.2 Effect of Heating and Low Frequency Sonication on Polymer Hydrogelation

Given the dispersion characteristics of the copolymer in aqueous solutions, we next determined the lower critical solution temperature (LCST) of our polymer solutions. Aqueous solutions of copolymer 2, both with and without zinc ions, were transparent at room temperature and reversibly precipitated at temperatures above 37°C. In contrast, homopolymer 7 was observed to precipitate at 72°C (Figure S7) and quickly re-dispersed into solution below the LCST. The reduction of the LCST from 72°C to 37°C by the addition of the 1 was anticipated as the hydrophobicity of nonlinear PEG analogues tend to reduce the LCST.[16, 19]

Gelation for the polymers was then assessed by heating 11 wt% solutions of copolymer 2 above LCST over a range of ZnClO₄·6H₂O (simplified as Zn²⁺) concentrations. While gelation occurred with copolymer 2 above 60°C (Figure S8), it was only evident at excess Zn²⁺:5 molar ratios of 40:1—240:1 (Table S1). Below this molar ratio range did not induce observable gelation. For homopolymer 7, gel formation in the presence of Zn²⁺ was not observed from room temperature to 100°C. Our explanation for requiring an excess of Zn²⁺ ions is covered in another section of this report. Copolymer solutions were heated to 40, 60, and 80°C for ten minutes followed by cooling in a sonicating water bath for 2 minutes at 37°C (Figure S8). When cooling without sonication, gelation was observed when copolymer solutions were heated to at least 60°C. Comparatively, when sonication was applied, the
temperature for gelation to occur was reduced to 40°C. Without any heating, bath sonication for 2 minutes alone did not result in gel formation.

3.1. HIFU-induced Hydrogelation

As it is well established that bath sonication is a low frequency, low intensity method\cite{12} for agitation, we next investigated the potential for gel formation at higher frequencies and intensities with HIFU under various solvent conditions for 11 wt% polymer solutions. Figure 1 is a series of images from a time lapse video (Video S1) of a representative response from a copolymer 2 solution with solvated Zn$^{2+}$ exposed to HIFU at 4 MPa peak pressure amplitude. The images in Figure 1 show an opaque material being formed with each burst of HIFU. In the first two minutes, this material formed at the focus of the HIFU transducer with each acoustic pulse prior to growing throughout the Eppendorf tube. Once the material reached a sufficient size, acoustic radiation forces tended to push the gel off the wall of the tube with the occasional formation of bubbles. This material remained after HIFU exposure was stopped and was determined to be a gel after further testing. In contrast to copolymer 2, the control homopolymer 7 did not change in opacity in response to HIFU and subsequently, no gel was formed (Video S2).

3.1.1. Influence of Solvent and pH on HIFU-induced Gelation

Previous reports\cite{15, 20} on polymers with tridentate ligands have demonstrated gelation in acetonitrile. We therefore also investigated the potential for gel formation of copolymer 2 in response to HIFU in both acetonitrile and ethanol. Surprisingly, we did not observe gelation in either acetonitrile or ethanol.
We also considered that pH played an important role on polymer dispersion in water and subsequent gel formation since the excess of Zn$^{2+}$ reduced the pH of the solution to 5.0. We therefore assessed gelation at pH above, 7.3 (0.1 M HEPES), and below, 3.0 (0.1 M acetic acid) this value. For near neutral pH, stable gel formation was not observed. In stark contrast, however, further reduction of the pH to 3.0 resulted in the formation of a gel at reduced Zn$^{2+}$ concentrations within two minutes at the focus of the HIFU transducer (Figure S9). In both cases, the gels were qualitatively observed to maintain their structure after HIFU exposure at room temperature for at least one week.

3.1.2. Viscoelastic Properties of Heating and HIFU-derived Gels

Both the thermal and mechanically stimulated copolymer 2 solutions produced gels, which were stable at room temperature. We then looked for any macroscopic differences between the gels produced by heat or by HIFU using μCT (Figure S10), but there were no discernible differences present in the images. The images of the gels formed by either heating or by HIFU showed a smooth, homogenous gel with no remarkable features. Thus, we next investigated the rheological properties of the gels to determine the presence of microstructural differences.

Figure 2 depicts the oscillatory amplitude sweep (OAS) data for HIFU-derived copolymer 2 gel. Our data indicated that the gel was a “weak” viscoelastic gel of semi-ordered structure—exhibiting yield and flow at 2.22%±0.41% and 30.53%±1.12% strain, respectively. The semi-ordered structure of weak gels breakdown at moderate strains and revert the copolymer back to a flowable liquid or colloidal state.[21] This viscoelastic behaviour of HIFU-derived 2 gel was further established through the loss factor, which was calculated as the ratio between the G’ and G” curves (G” / G’) to present the hydrogel’s ability to dissipate and store energy. At
low strains, the loss factor was <1, suggesting solid-like characteristics at rest. The loss factor became >1 beyond the flow point, thereby indicating a transition to flowing and liquid-like behaviour.[22]

The oscillatory frequency sweep (OFS, T=25°C, γ = 0.1%) in Figure S11 showed the presence of strong microstructures, manifested as a near frequency-independent storage modulus G’ and complex shear modulus G*. We presented G* instead of η* because the materials exhibited a general G’ > G” behaviour typically seen in strong chemical gels (crosslinking) or stable dispersions and gels (physical network).[23]

Similar batches of copolymer 2 gels formed by either heating or by HIFU were subjected to small amplitude OFS within its linear viscoelastic limit (Figure S12). The measurements revealed that the primary difference between the heat and HIFU as stimuli was that heating-derived networks possessed weak intermolecular interactions that exhibit dispersion-like behaviour at rest (i.e. low frequencies), whereas the HIFU-derived networks presented frequency-independency with G’, while also increasing in magnitude. This behaviour indicated that the HIFU-derived networks comprised of stronger crosslinked structures[23] compared to heating-derived gels.

4.1. Mechanistic Study of Polymer Gelation

4.1.1. Spectroscopic Analysis of Metal-ion Chelation

As we observed that gels were formed with copolymer 2 in response to both HIFU and heating, we next sought to understand the plausible mechanisms of gelation. In this complex gelation process, physical effects (such as cavitation[24] and acoustic streaming[24b, 25]), thermal
effects\textsuperscript{[24b, 26]}, and chemical factors (pH, solvents, concentration of Zn\textsuperscript{2+}) may impact both molecular and macromolecular interactions that facilitate gelation. We therefore first studied potential Zn\textsuperscript{2+} coordination with the different functional groups within copolymer 2 using FT-IR, UV-vis, and \textsuperscript{1}H-NMR. FT-IR (Figure S13A) indicated C=O stretching frequencies became red-shifted from 1727 cm\textsuperscript{-1} to 1715 cm\textsuperscript{-1} and C-O-C (PEGs) stretching frequencies were shifted from 1107 cm\textsuperscript{-1} 1094 cm\textsuperscript{-1} with the addition of Zn\textsuperscript{2+}. These red-shift effects in the IR spectrum, corresponding to an increase in the bond lengths of the carbonyl groups and C-O-C of the PEG groups, confirm that there are non-covalent interactions between these groups and Zn\textsuperscript{2+} ions.\textsuperscript{[27]} The same red-shift in these bands was observed for the homopolymer 7 (Figure S13B). Specifically, C=O stretching frequencies were shifted from 1730 cm\textsuperscript{-1} to 1724 cm\textsuperscript{-1} and –C-O-C- stretchings were shifted from 1107 cm\textsuperscript{-1} to 1095 cm\textsuperscript{-1}, indicating that homopolymer 7 absorbed Zn\textsuperscript{2+} similarly to copolymer 2. Yet despite this similarity, no gelation was observed with homopolymer 7 in response to stimulation by HIFU or heat. This important observation indirectly indicated that the tridentate ligand 1 was an important structural unit for gelation to occur. Further to this, competitive metal ion chelation to carboxyl and other functional groups may explain the need for excess Zn\textsuperscript{2+}. Metal ion titration of the copolymer 2 suggested that as the metal ion concentration increased, the free M1 absorbance band at 320 nm, as measured by UV-vis, was reduced. Interestingly, a new band towards 360 nm was expressed (Figure S13), which is indicative of the tridentate ligand complexing to Zn\textsuperscript{2+} ions.\textsuperscript{[28]}

Considering this critical role of tridentate ligand 1, we next examined metal-ligand chelation of tridentate ligand 1 and copolymer 2 at various concentrations of Zn\textsuperscript{2+} by \textsuperscript{1}H-NMR spectroscopy. Figure 3A contains NMR spectra of tridentate ligand 1 in acetonitrile (ACN)-d\textsubscript{3}, which showed a pronounced shifting of the peaks from 7.30—7.83 ppm region toward 7.75—8.01 ppm downfield when Zn\textsuperscript{2+} was added (Figure 3 A1 vs. A2; A3 vs A4). Comparing
the A1 to the A3 spectra indicated a downfield shifting from 7.30—7.83 ppm region to 7.48—7.90 ppm region and confirmed the formation of pyridinium and imidazolium salts on the tridentate ligand in acetic acid.

To our astonishment, the spectra of tridentate ligand 1 recorded in D$_2$O (after 20 seconds of probe sonication to help dissolve 1) showed several unusual observations (Figure 3, B1—B4). While all the peaks of tridentate ligand 1 was clearly observed in D$_2$O, the peaks completely disappeared when 5 equiv. of Zn$^{2+}$ was added (B1 vs B2). After acetic acid was added to the D$_2$O a surprising upfield shifting from 7.67—7.87 ppm to 7.55—7.80 ppm was observed (B1 vs B3) in contrast to the downfield shifting observed in ACN-d$_3$. This diamagnetic shielding effect was likely due to the presence of the formation of acetate anions in water. When an excess of Zn$^{2+}$ was added, the peaks similarly disappeared from the spectrum (B4 as compared to B2). This phenomenon may be explained by the observation that tridentate ligand 1 salted-out of solution when zinc perchlorate salt was added.

With regard to copolymer 2, its NMR spectra recorded in ACN-d$_3$ showed a clear coordination of the ligand moieties when increasing amounts of Zn$^{2+}$ were added (Figure 4, C1-C4). Interestingly, NMR spectra recorded in D$_2$O showed a similar peak disappearance effect when Zn$^{2+}$ was present in solution (Figure 4, D1-D4). Similar to the tridentate ligand 1, a similar peak disappearance was also seen when the solution was treated with acetic acid to reduce the pH to 3 (Figure S14). Our NMR study clearly suggested that tridentate ligand moieties were expelled from the aqueous media when excess of Zn$^{2+}$ was added. Therefore, our results based on our spectroscopic and DLS data herein indicated that the copolymer solution underwent a salting-out process as zinc perchlorate was added, creating a solution of well dispersed nanoparticles with a hydrodynamic diameter of 20.3 nm.$^{[29]}$ From our NMR results, we inferred that gelation occurred when these nanoparticle structures become
disrupted, enabling metal coordination bonding between the various functional groups on copolymer 2, including, albeit weakly, between the tridentate ligand groups. Further studies are required to get a complete understanding of the mechanisms at play, which are actively ongoing.

4.1.2. Acoustic Environment Characterization

As HIFU can induce both chemical and mechanical changes to the environment, we next investigated the role of HIFU in gel formation. Here, we correlated the chemical effects of ultrasound to acoustic cavitation\textsuperscript{[24]} and heating,\textsuperscript{[24b, 26]} while the mechanical effects of ultrasound are correlated to peak negative pressures that affect fluid flow generated by acoustic streaming.\textsuperscript{[24b, 25]} To the best of our capabilities herein, we investigate the potential roles of these acoustic phenomena on gelation of copolymer 2.

Cavitation has been often cited as one of the key mechanisms in sonochemistry.\textsuperscript{[30]} Yet surprisingly, there was no relationship between gel yield with received cavitation energy (Figure S15). Here, cavitation energy was determined from measured broadband signal from the passive cavitation detector (PCD), which is proportional to the energy of inertial cavitation events during HIFU. This observation was perhaps best illustrated by the formation of gels in the absence of any detectable cavitation signal, indicating that other local acoustic effects, such as heating or shear forces, played a more pivotal role in gelation.

HIFU is known to cause heating through the attenuation of the acoustic wave. We therefore assessed the role of heat deposition due to HIFU on gelation by measuring temperatures of the solution at, and away from, the focus of the transducer. We observed that higher acoustic pressures resulted in greater temperature changes, which was expected. There was also no
discrepancy between the temperature changes in the bulk solution nor at the acoustic focus (Figure S16). Within the first minute of HIFU exposure at 4.0 MPa, the solution temperature in the bulk fluid and acoustic focus was always below the measured LCST, with the solution temperature stabilizing at 45°C following 3 to 4 minutes of HIFU treatment at a 10% duty cycle (which is amounts to only 18 to 24 seconds of HIFU exposure). In our observations, gel typically formed within the first minute of HIFU treatment for all copolymer 2 solutions. This suggested that bulk heating was not the initial driving force for gel formation, as during this period the temperatures were always below the LCST. Thus, mechanical forces may have been the cause for the initial observed opacity change in the solution.

We therefore also investigated the role of HIFU acoustic pressure amplitudes on the gelation of the copolymer 2, which are inextricably linked to acoustic streaming in solution. Acoustic streaming is the bulk flow of fluid generated by Reynolds stress from the absorption of the acoustic wave.[24b, 25, 31] The forces which act on streaming can affect both homogenous and heterogenous dispersions and solutions.[32] In our experiments, this bulk flow was qualitatively observed as the rocking motion of the fluid in the Eppendorf tube, which occurred at each pulse of HIFU. For our acoustic settings, streaming velocities are typically on the order of 4—150 mm/s at the acoustic focus in the free field[25, 33] and reduces by at least one order of magnitude away from the focus. Given this, the influence acoustic streaming may have on gel formation will be maximised at the acoustic focus and minimised outside the focus. Considering that acoustic streaming scales with acoustic pressure,[24b] we measured the effect of peak pressure amplitudes on gel yield. At amplitudes 2.8 MPa and below, the yield of gel recovered was negligible, but increased at amplitudes above 2.8 MPa (Figure 5).

From our observations (Video S1), higher acoustic pressures generally produced more gel. Interestingly, this occurred before there were any appreciable changes in temperature.
Therefore, we propose that the gelation of copolymer 2 likely occurred in two phases. Initially, HIFU generated mechanical stress in the form of acoustic streaming induced gel formation. As the gel increased in volume, absorption of the ultrasound wave increased bulk temperatures, which led to further growth of the gel. The mechano-thermal influence HIFU presented on gel formation was elucidated from our rheology study in Figure S12, where the HIFU-derived system formed a stronger cross-linked gel than the heating-derived gel. Therefore, HIFU provided a synergistic mechano-thermal effect to disrupt the copolymer 2 nanodispersions and permit gelation formation.

5. Conclusion
In summary, we have produced a comb-like polymer with tridentate ligands dispersed in water that gel in response to HIFU. Our work suggests that HIFU interacts with the copolymers initially by mechanical forces with thermal effects later becoming more dominant as the gel grows. This mechano-thermal stimulation was further corroborated by our rheological measurements that indicated increased molecular structuring within the copolymer network of gels formed by HIFU, compared to gels generated by heating alone. Our work also suggested that various cooperative and competitive noncovalent binding processes, including carbonyl-Zn$^{2+}$, poly(ethylene glycol)-Zn$^{2+}$, and M1-Zn$^{2+}$ interactions exist for our copolymer in aqueous and organic media, but salting-out nanoprecipitates of the copolymers only occurred in aqueous media. Considering that HIFU is spatially controllable, our findings demonstrated a potential framework for *in situ* hydrogel formation and expands the use of sonochemical sol-gel switching for applications not yet explored.

6. Experimental Section
*Chemicals:* All reactions were carried out under a nitrogen or argon atmosphere with dry solvents under anhydrous conditions, unless otherwise stated. Dry tetrahydrofuran (THF), hexane, diethyl ether (Et$_2$O), methylene chloride (CH$_2$Cl$_2$), and toluene were obtained by
passing commercially available pre-dried, oxygen-free formulations through activated alumina columns. Methanol (MeOH), benzene, and N,N’-dimethylformamide (DMF) were purchased in anhydrous form and used without further purification. Water, ethyl acetate (EtOAc), diethyl ether (Et₂O), methylene chloride (CH₂Cl₂), and hexane were purchased at the highest commercial quality and used without further purification, unless otherwise stated. Reagents were purchased at the highest commercial quality and used without further purification, unless otherwise stated. Yields refer to chromatographically and spectroscopically (¹H NMR) homogeneous materials, unless otherwise stated. Reactions were monitored by thin-layer chromatography (TLC) carried out on 0.25 mm E. Merck silica gel plates (60F-254) using UV light as visualizing agent and an ethanolic solution of ammonium molybdate, anisaldehyde, potassium permanganate and heat as developing agents. E. Merck silica gel (60, particle size 0.040±0.063 mm) was used for flash column chromatography. NMR spectra were recorded on Bruker DRX-400 or Bruker AV-600 instruments and calibrated using residual undeuterated solvent as an internal reference. The following abbreviations were used to explain the multiplicities: s = singlet, d = doublet, t = triplet, q = quartet, quint = quintet, m = multiplet, pent = pentet, hex = hexet, br = broad. The following chemicals were purchased and used without further purification unless otherwise specified: methacrylic acid (MA, Sigma 155721), 4-dimethylaminopyridine (DMAP, Sigma 107700), 2,6-bis(1-methyl-1H-benzo[d]imidazol-2-yl)pyridin-4-ol (OxChem AX8232860), 2,6-dibromopyridin-4-ol (OxChem AX828727), 2-bromopyrimidin-5-ol (OxChem AX8222911), potassium carbonate (K₂CO₃, Sigma 791776), N,N’-diisopropylcarbodiimide (DIC, Sigma D125407), Poly(ethylene glycol) methyl ether methacrylate (PEGMA, Mₙ = 300, Sigma 447935) and 2,2’-azobis(2-methylpropionitrile) (AIBN, Wako CAS: 78-67-1), zinc perchlorate hexahydrate (Zn(ClO₄)₂·6 H₂O, Sigma 401339, Zn²⁺ for clarity). Additionally, toluene (Sigma 244511), dimethylsulfoxide (DMSO, Sigma 276855), tetrahydrofuran (THF, Fisher T425), and dichloromethane (DCM, Sigma
270997) were used as solvents. Reaction grade deionised pure water was obtained from a water purification system (StakPure Germany, OmniaPure series). Deionised unfiltered water for the ultrasound tank was obtained from (StakPure Germany, DS2800). Agarose powder (Vivantis, #PC0701) was used for the synthesis of 3 wt% gel phantom chambers in HIFU gelation tests. Green liquid food colouring was obtained from Singaporean supermarkets for use with some hydrogelation trials to improve the contrast and visualization of the hydrogel as it forms.

*Ligand Synthesis:* The procedure for ligand synthesis involved two steps.

**Step 1: Alkylation of 2,6-bis(1-methyl-1H-benzo[d]imidazol-2-yl)pyridin-4-ol**

*Scheme 2* depicts the reaction conditions for the first step in ligand synthesis. To a 100 mL-round bottom flask containing a mixture of 2,6-bis(1-methyl-1H-benzo[d]imidazol-2-yl)pyridin-4-ol (1500 mg, 4.22 mmol, 1 equiv, Oxchem); 2-(2-(2-chloroethoxy)ethoxy)ethanol (1077 mg, 5.06 mmol, 1.2 equiv) and K$_2$CO$_3$ (1166 mg, 8.44 mmol, 2 equiv) was added DMSO (10 mL). The mixture was stirred at 90°C for 18 hrs. After cooling to room temperature, cold distilled water was added and white precipitation was formed. The solid was filtered and washed with water several times, then dissolved in dichloromethane (100 mL). Solid sodium sulfate was added to the dichloromethane solution to absorb water. 2-(2-(2,6-bis(1-methyl-1H-benzo[d]imidazol-2-yl)pyridin-4-yl)oxy)ethoxy)ethanol was obtained as white solid (1880 mg, 91%).

2-(2-(2-(2,6-bis(1-methyl-1H-benzo[d]imidazol-2-yl)pyridin-4-yl)oxy)ethoxy)ethanol. Figure S1 depicts the $^1$H-NMR (400 MHz, CDCl$_3$) δ 7.96 (s, 2H), 7.88 – 7.78 (m, 2H), 7.45 – 7.39 (m, 2H), 7.34 (ddd, J = 6.8, 4.4, 1.7 Hz, 4H), 4.41 (t, J = 4.7 Hz, 2H), 4.20 (s, 6H), 3.92 (t, J = 4.6 Hz, 2H), 3.75 (q, J = 4.6 Hz, 4H), 3.72 – 3.67 (m, 2H), 3.62 (t, J = 4.6 Hz, 2H) ppm; 13C NMR (101 MHz, CDCl$_3$) δ 166.4, 151.1, 150.3, 142.4, 137.2, 123.7, 122.9, 120.1, 112.0, 110.0, 72.7, 71.2, 70.6, 69.3, 68.2, 61.6, 32.6 ppm; LC MS (ESI) 488.49 [M+H]$^+$.
Step 2: Synthesis of 2-(2-(2-((2,6-bis(1-methyl-1H-benzo[d]imidazol-2-yl)pyridin-4-yl)oxy)ethoxy)ethoxy)ethyl methacrylate

Scheme 3 depicts the reaction conditions for the second step in ligand synthesis. To a 100 mL round bottom flask were added methacrylic acid (0.49 mL, 5.79 mmol, 1.5 equiv), 4-dimethylaminopyridine (94.3 mg, 0.77 mmol, 0.2 equiv), 2-(2-(2-(2-(2,6-bis(1-methyl-1H-benzo[d]imidazol-2-yl)pyridin-4-yl)oxy)ethoxy)ethoxy)ethanol (1880 mg, 3.86 mmol, 1 equiv) followed by addition of toluene (18 mL), dichloromethane (2 mL) and then N,N'-diisopropylcarbodiimide (1.21 mL, 7.72 mmol, 2 equiv). The mixture was stirred at room temperature for 20 h. After evaporation of solvent, the product was purified by slow chromatography on silica gel using 0.5% methanol in dichloromethane as eluent and obtained as white solid. Although the final product was slightly contaminated with N,N'-diisopropylurea urea by-product, it was acceptable to be used for the polymerization step.

2-(2-(2-((2,6-bis(1-methyl-1H-benzo[d]imidazol-2-yl)pyridin-4-yl)oxy)ethoxy)ethoxy)ethyl methacrylate. Figure S2 depicts the \(^1\)H NMR (400 MHz, CDCl\(_3\)) \(\delta\) 7.96 (s, 2H), 7.90 – 7.79 (m, 2H), 7.50 – 7.43 (m, 2H), 7.41 – 7.29 (m, 4H), 6.12 (dd, J = 1.6, 1.0 Hz, 1H), 5.55 (t, J = 1.6 Hz, 1H), 4.43 – 4.35 (m, 2H), 4.35 – 4.26 (m, 2H), 4.22 (s, 6H), 3.95 – 3.88 (m, 2H), 3.79 – 3.73 (m, 4H), 3.73 – 3.67 (m, 2H), 1.93 (dd, J = 1.6, 1.0 Hz, 3H) ppm; 13C NMR (101 MHz, CDCl\(_3\)) \(\delta\) 167.4, 166.4, 151.2, 150.4, 142.6, 137.3, 136.2, 125.9, 123.7, 122.9, 120.2, 111.9, 110.0, 71.1, 70.8, 69.4, 69.3, 68.3, 64.0, 32.6, 18.4 ppm; LC MS (ESI) 556.56 [M+H]\(^+\).

Polymer Synthesis: For copolymer 2, purified ligand monomer 5 (0.100 g, 0.180 mmol, 1 equiv.) was dissolved in toluene (2.00 g) by sonication (37 kHz, Elmasonic S-180, Elma Schmidbauer GmbH) in an ice-water bath for 1 hour. PEGMA (0.163 g, 0.540 mmol, 3 equiv.) and radial initiator AIBN (0.0053 g, 0.0323 mmol, 0.18 equiv.) were added to the solution and stirred for 3 hours at 80°C in an argon atmosphere. The reaction mixture was passed through a syringe filter (0.45 μm pore size) and re-precipitated from hexane to obtain the final copolymer (P5-co-PPEGMA, copolymer 2). Homopolymer 7 synthesis followed a
similar protocol as above, but excluding the presence of monomer 5. The resulting polymers were stored as aqueous stock solutions at 12.2 wt%.

*Polymer Characterization:* NMR spectra were recorded on a Bruker AV500 spectrometer (500 MHz) (Germany, Bruker Avance series) at ambient temperature. Gel permeation chromatography (GPC) analysis was performed on a Shodex GPC-101 liquid chromatograph (Tokyo, Japan) equipped with two Shodex KF-804L mixed gel columns (300 × 8.0 mm; bead size = 7 μm; pore size = 20–200 Å, F6027040). The eluent was THF at a flow rate of 1.0 mL/min (40°C). Sample detection and quantification were conducted using a Shodex differential refractometer RI-101 (Tokyo, Japan F4010101) calibrated with known concentrations of polymer in solvent. The column system was calibrated with standard poly(methyl methacrylate)s (PMMAs).

*Spectroscopy:* Fourier transform infrared (FT-IR, PerkinElmer Spectrum 100 FTIR) spectroscopy was utilized to identify metal metal ion chelation between Zn$^{2+}$ and the tridentate ligand as well as Zn$^{2+}$ and oxygen within the polyethylene glycol (PEG) chains and carbonyl groups. The presence of metal to ligand chelation was further evaluated by $^1$H-NMR, as described above, and UV-vis spectrophotometry (Shimadzu UV 2450 Spectrometer, $\lambda$=300—900 nm).

*Lower Critical Solution Temperature (LCST) Quantification:* The LCST of copolymer 2 and homopolymer 7 was quantified by the cloud point temperature, defined at which the %transmission vs temperature plot approaches the inflection point (or 50% transmission) for a 10 mg/mL polymer solution.$^{[34]}$ The %Transmission was recorded by UV-vis (Molecular Devices Spectramax M5, $\lambda$ = 500 nm) using H2O as a reference. Due to machine limitations, the solution temperatures were raised manually by a hot water bath ($T$ = 25—80°C) and the subsequent data was interpolated by MATLAB using a data range of 25—80°C with 0.1°C increments.
HIFU Setup: A conventional HIFU setup was used in all HIFU experiments, and details of the setup are found in other reports.\textsuperscript{11b, 11c} In short, a sine wave burst from a waveform generator was amplified by a 55 dB RF amplifier (Electronics and Innovation 1040L, Rochester NY) and passed through an electrical impedance matching network before reaching the HIFU transducer. Any acoustic response from the sample during HIFU exposure was detected by the PCD. The signal from the PCD was filtered (analogue 5.0 MHz high-pass filter, Allen Avionics F5286-5P0-B) before amplified with a broadband amplifier (5x, SRS SR445A) to isolate and amplify any signal from cavitation events. The processed signal was captured on the oscilloscope (Keysight, InfiniiVision DSOX3032A) and saved onto a computer before additional post processing.

Cavitation Response Detection and Analysis: PCD data was processed following a procedure described previously.\textsuperscript{11b} In short, PCD data was post processed to obtain a power spectral density curve. The area under the curve of the spectrum was calculated to determine the received signal power from a single HIFU burst ($E_{\text{experimental}}$). The received signal power from water without a sample chamber ($E_{\text{reference}}$) was used as a reference for background noise. From this, the acoustic intensity of a given burst is computed as $dB = 10 \log_{10} \frac{E_{\text{experimental}}}{E_{\text{reference}}}$. Cavitation was defined as an acoustic intensity of 10 dB or greater.\textsuperscript{11b} Finally, the acoustic power from all of the bursts was summed to compute the total received cavitation power for a given experimental run.

Hydrogel Formation: Green food coloring was added to some samples to aid visual contrast for gel formation. The final working concentration of copolymer was 11 wt% for all experiments. Zn$^{2+}$ was added to the working mixture in a 160:1 molar ratio of Zn$^{2+}$ to M1 ligand for the respective copolymers.

Gelation by Heating/Cooling: The copolymer and metal ion solution (300 μL) was placed into an Eppendorf tube (Greiner Bio-One, 2 mL reaction tube, 623201) and heated to 40°C, 60°C,
or 80°C for 10 minutes. After heating, the solution was either air cooled to room temperature or quenched in a sonicating water bath at 40 kHz and 160 W for 2 minutes at 37°C before removing and allowed to air cool to room temperature.

*Gelation by HIFU:* An empty Eppendorf tube was positioned at the focal point of the 0.5 MHz HIFU transducer such that the focal point was within the inner wall of the tube. After, 300 µl of the copolymer and metal ion solution was placed into the tube. Each sample was exposed to bursts of HIFU (10% duty cycle, 50,000 cycles, and 1 Hz pulse repetition frequency) for a maximum of 4 minutes. The pressure amplitudes ranged from 2.43 to 4.05 MPa. Video recordings of the gel formation was taken during HIFU exposure with a mirrorless camera (Sony NEX-7, SEL1855 lens) Following the HIFU treatment, the solutions were partitioned into gelled and non-gelled portions. The gelled fraction was weighed and compared to the weight of the solution pre-exposure to determine the wt% yield.

*Temperature Monitoring During HIFU Exposure:* For temperature measurements, 500 µL of distilled water was placed into the tube after focussing and monitored using a Type-K wire probe thermocouple and Blueterm Duo (Thermoworks, Lindon, UT, USA). The thermocouple was positioned within the lumen of the tube for the bulk temperature measurement, or the inner wall of the tube close to the acoustic focus for the focal point temperature. The water temperature within the tube was allowed to equilibrate to the ambient tank temperature. Data logging was performed every 15 seconds and started simultaneously with the HIFU exposure session. Measures were repeated in triplicate for each driving pressure setting.

*Image Analysis of Gel Growth:* Time-lapse videos of the gel growth was processed through a MATLAB script to determine the gel growth over time. In brief, the script defined a region of interest (ROI) which was set to the area inside the Eppendorf tube where the gel formed. The pixel data within the ROI was subtracted against the first video frame and binarized to quantify the opacity change over the time-lapse. This binary pixel data was summed to obtain
the gel pixel area, which was then normalized to the ROI area to compute the ‘Gel Area in ROI.’

μ-Computational Tomography: To identify the gel porosity, copolymer 2 gels were imaged by a benchtop μ-computational tomography imager (μCT, SkyScan 1272, Bruker) at 5 μm voxel image resolution with 47 kV and 166 μA and a 0.25 mm aluminium filter. Projection images were reconstructed by either vendor software (Nrecon 1.6.9.18, Bruker) or by ImageJ with the subsequent grayscale image slices.

Mechanical Characterization of Hydrogels: Rheological studies were conducted on a MCR702 Rheometer (Anton Paar), with a smooth stainless-steel bottom plate and parallel plate geometry (8 mm diameter). A Peltier device controlled the temperature at 25.00°C with a sensitivity of 0.01°C. A 0.4 mm gap size was set to optimize data quality for the selected geometry. The default Viscoelastic Sample approach was used to minimize mechanical disturbance to the sample. The gel was allowed to structurally and thermally equilibrate for 10 minutes before measurements were recorded. An active temperature hood was used to minimize evaporation. Oscillating amplitude sweep (OAS) experiments were conducted under direct strain oscillation (Trustrain™, Anton Paar) between 0.01 to 100% strain with 6 data points per decade. Sampling times were determined by the software, and a constant angular frequency (10 rad/s) was maintained. The oscillating frequency sweep experiments (OFS) were conducted under controlled frequency oscillation (CFO) between 100 to 0.01 rad/s, at a constant amplitude that is within the linear viscoelastic regime (LVR) determined during the OAS at 1% strain. Fresh samples were used for OAS and OFS studies. The experiments were repeated in triplicate for each sample to acquire standard deviation and standard error of mean calculations. Rotational testing at different gap sizes (1, 0.8 and 0.6 mm) from 0.01 to 100 s⁻¹ at 25°C was conducted to determine the presence of sample slippage, based on the method described by Larson et al.²²
Supporting Information
Supporting Information is available from the Wiley Online Library or from the author.

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References


Scheme 1. Synthesis of desired comb polymethacrylate 2 with poly(ethylene)glycol side chains containing ligand 1 of control homopolymer 7: a) K₂CO₃ in DMSO, 110°C, 24 h 91%; b) DIC, 4-DMAP in toluene/DCM 9/1, v/v; 24 h; c) AIBN, toluene, 80°C, 3 h. a) K₂CO₃, DMF, 110°C, 24 h, 91%; b) DIC, DMAP, toluene/DCM 9:1 (v/v), 24 h, 65%, c) cat. AIBN, toluene, 80°C, 3 h.
Table 1. Random copolymer PM1-co-PPEGMA 2 and homopolymer 7 synthesis and characterisation

<table>
<thead>
<tr>
<th>Polymer</th>
<th>AIBN (mol equiv)</th>
<th>M1:PEGMA ratio[c]</th>
<th>Mₙ[Da][b]</th>
<th>PDI[b]</th>
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<tr>
<td>2</td>
<td>0.18</td>
<td>1:4.5</td>
<td>13100</td>
<td>1.41</td>
</tr>
<tr>
<td>7[c]</td>
<td>0.18</td>
<td>0:1</td>
<td>19400</td>
<td>2.32</td>
</tr>
</tbody>
</table>

[a] Ratios values obtained by ¹H- NMR analyses; [b] parameters of polymers obtained by analytical GPC using THF as eluent and calibrated with standard poly(methyl methacrylate)s; [c] 7 is a control homopolymer with no monomer 5. Mₙ = number-averaged molecular weight, PDI = polydispersity index

Figure 1. (A) Representative time-lapse of 11 wt% copolymer 2 solution exposed to 4.05 MPa HIFU bursts. The red box denotes the region of interest (ROI) used to assess gel formation over the video, which was then (B) plotted over HIFU exposure time. (C) When zooming into this curve visualize the change for each HIFU pulse, a step-like growth pattern was observed.
Figure 2. Viscoelastic properties of HIFU-derived hydrogels. The loss and storage moduli of the two hydrogel systems were assessed through an oscillatory strain range of 0.01—100% and performed in triplicates (mean ± SEM)
Figure 3. $^1$H-NMR spectra of tridentate ligand 1 in (A) ACN-$d_3$ and (B) D$_2$O. The ligand signal was observed to broaden and shift in the presence of 5 molar equivalents Zn$^{2+}$ in ACN-$d_3$ (A1 vs A2) and acetic acid/ACN-$d_3$ mixture (A3 vs A4.) Interestingly, these same ligand signals were observed to have reduced in D$_2$O.
Figure 4. $^1$H-NMR spectra of copolymer 2 in (A) ACN-d$_3$ and (B) D$_2$O. The ligand signals were similarly observed to broaden in ACN-d$_3$ with increasing concentrations of Zn$^{2+}$ (0—20 equiv Zn$^{2+}$, D1—D4), but reduce in intensity in the presence of D$_2$O (D1—D4).
Figure 5. Correlation between hydrogel formation fraction and input pressure. Hydrogel recovery increased proportionally as the acoustic pressure increased from 3.64 to 4.05 MPa, for all solutions at the same concentration. (mean ± SD, n = 5)
**Scheme 2.** Reaction for Alkylation of 2,6-bis(1-methyl-1H-benzo[d]imidazol-2-yl)pyridin-4-ol to 2-(2-(2,6-bis(1-methyl-1H-benzo[d]imidazol-2-yl)pyridin-4-yl)oxy)ethoxy)ethoxy)ethan-1-ol

**Scheme 3.** Reaction to synthesize 2-(2-(2,6-bis(1-methyl-1H-benzo[d]imidazol-2-yl)pyridin-4-yl)oxy)ethoxy)ethoxy)ethyl methacrylate
Herein, high intensity focused ultrasound (HIFU) is explored as a means for in situ hydrogelation. Random copolymers were synthesized and found to form nanoprecipitations in aqueous media, which restructure to form gels following ultrasound exposure. Our findings demonstrated the gelation to be mechano-thermally driven and expands the use of sonochemical sol-gel switching for applications not yet explored.

**Keyword** Sono-gelation


**Sol-gel transitions of comb-like polymethacrylate copolymers by mechano-thermal stimuli in water**