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Improved magnetic regulation of delivery profiles from ferrogels

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Abstract

While providing the ability to magnetically enhance delivery rates, ferrogels have not been able to produce the various types of regulated delivery profiles likely needed to direct complex biological processes. For example, magnetically triggered release after prolonged periods of payload retention have not been demonstrated and little has been accomplished towards remotely controlling release rate through alterations in the magnetic signal. Also, strategies do not exist for magnetically coordinating multi-drug sequences. The purpose of this study was to develop these capabilities through improved ferrogel design and investigating how alterations in the magnetic signal impact release characteristics. Results show that delivery rate can be remotely regulated using the frequency of magnetic stimulation. When using an optimized biphasic ferrogel design, stimulation at optimized frequencies enabled magnetically triggered deliveries after a delay of 5 days that were 690- to 1950-fold higher than unstimulated baseline values. Also, a sequence of two payloads was produced by allowing one payload to initially diffuse out of the ferrogel, followed by magnetically triggered release of a different payload on day 5. Finally, it was demonstrated that two payloads could be sequentially triggered for release by first stimulating at a frequency tuned to preferentially release one payload (after 24 hours), followed by stimulation at a different frequency tuned to preferentially release the other payload (After 4 days). The strategies developed here may expand the utility of ferrogels in clinical scenarios where the timing and sequence of biological events can be tuned to optimize therapeutic outcome.

Graphical Abstract

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Keywords

stimuli responsive; ferrogel; drug delivery; controlled release

1. Introduction

The biological processes underlying many injuries and diseases can often be characterized as sequences of biological events. The delivery of therapeutics can be used to medically regulate these biological processes. However, control over sequential biological processes requires drug deliveries where the timing, sequence, dose, and localization of therapeutic payloads are tightly controlled [1–5]. Polymeric depots such as hydrogels can provide localized deliveries at the implantation site [6] and have been used in numerous applications (e.g., chemotherapies [2], immunotherapies [7], wound healing [8, 9], and tissue engineering [10–12]). Polymeric depots can also be formulated to provide various temporal delivery profiles [13] and multi-drug deliveries where different therapeutics release at different rates [3, 9, 14]. However, these deliveries are typically predetermined and do not provide the adaptability required to optimize temporally complex, multi-drug therapies. Additionally, in clinical settings, on-demand flexibility is desired to provide patient-specific drug delivery regimens and to alter the course of therapies according to updates in prognoses [15, 16].

Stimuli-responsive materials can deliver therapeutic payloads in response to external signals—for example, pH [17], temperature [18], electric fields [19], optical signals [20], and magnetic fields [21, 22]—so they offer potential for on-demand control over the timing of deliveries (i.e., triggered release). Additionally, it may be possible to tune these stimuli-responsive materials so that stimuli signal parameters (e.g., amplitude, duration, frequency, polarity, etc.) can control delivery rates. Magnetically responsive hydrogels (i.e., ferrogels) provide potential platforms for flexibly coordinating the timing and rate of these deliveries. Ferrogels can be made by integrating magnetic particles, such as magnetic oxides, within the hydrogel's polymer matrix [15]. Owing to their biocompatibility [23], these magnetic oxide particles have been extensively used in medical applications (e.g., cell tracking [24], hyperthermal treatments [25], magnetic resonance imaging (MRI) [26], and magnetic biosensing [27–29]). Additionally, incorporation of iron oxide particles into hydrogels can enhance adhesion of cells [30], which is critical in a wide variety of biomedical applications [31]. Ferrogels can be triggered to release payloads by stimulating at high-frequencies (100s of kHz) as a result of heating magnetic oxide particles [32], though this can damage sensitive payloads if temperatures rise by 3° to 5°C. This limits the amount of stimulation that can be used and thus limits the dosing range [15].

Ferrogels can also provide on-demand release through magnetically triggered gel deformations [23, 33]. Critically, these gels respond to graded magnetic fields of lower frequency and do not generate heat when stimulated. The responsivity and efficiency of magnetically triggered deliveries can be enhanced by designing the gel to have a more deformable macroporous structure [22]. These macroporous ferrogels can enable efficient, responsive, non-thermal, localized, remote-controlled (on demand) drug deliveries. However, explicit control over the rate and timing of release from macroporous ferrogels is not currently well controlled. Specifically, while they can be magnetically deformed which enhances convective release, it has yet to be demonstrated that alterations in the magnetic signal can be used to explicitly regulate release rates. This is critical for on-demand control over dosing. Additionally, regulation of the timing of release has been limited to experiments conducted over hours and has not been demonstrated over the course of days or weeks. This is critical in instances where extended delays in delivery are required. Finally, many therapies utilize multiple therapeutics. However, it has yet to be demonstrated that macroporous ferrogels can be used to magnetically coordinate multi-drug deliveries (e.g., multi-drug sequences). The objectives of this work were to achieve better regulation over release profiles from ferrogels by devising strategies for (i) magnetically controlling release rate, (ii) delaying triggered release for extended periods of time, and (iii) coordinating sequential, multi-drug deliveries.

2. Materials and methods

2.1 Materials

Sodium alginate was purchased from Pronova Biopolymers (Oslo, Norway) with an average molecular weight of ~250 kDa and with high guluronate content (Protoanal LF 20/40). Adipic acid dihydrazide (AAD), 1-ethyl-3-(dimethylaminopropyl) carbodiimide (EDC), MES, 1-hydroxybenzotriazole (HOBt), iron (II,III) oxide powder (< 5 micron), mitoxantrone, phosphate buffered saline (PBS), bovine serum albumin (BSA), Sigmacote, Fluorescein isothiocyanate (FITC)-dextran, Rhodamine-dextran, and FITC-Diethylaminoethyl (DEAE)-dextran were all purchased from Sigma-Aldrich (St. Louis, MO). Mouse Bone Morphogenetic Protein-2 (BMP-2), Thymus Chemokine-1 (TCK-1), and Enzyme-linked Immunosorbent Assay (ELISA) kits and kit reagents were purchased from R&D Systems (Minneapolis, MN).

2.2. Ferrogel fabrication and imaging

The monophasic and biphasic ferrogels used in these studies were fabricated and imaged in a manner described in Cezar et al. [34], where they were optimized to exhibit a balance of magnetic force vs. gel stiffness for efficient deformation. Briefly, monophasic ferrogels were made by adding a solution of MES buffer, alginate, HOBt, AAD, iron oxide, and EDC to frozen (−20°C) 10 × 5 mm cylindrical Teflon molds (Fig. 1A). Biphasic ferrogels were made using the same pre-gel solution, but were cast between two glass plates separated by 2-mm spacers with a magnet placed against one glass plate as to pull the iron oxide particles towards one side of the gel (Fig. 1B). Both monophasic and biphasic ferrogels were formulated to yield gels with 1 wt% alginate with 2.5 mM AAD crosslinker and 7 wt% iron oxide. Ferrogels were thoroughly rinsed to remove residual agents and lyophilized overnight

for Scanning Electron Microscopy (SEM, Tescan Vega Environmental SEM equipped with a Bruker XFlash 5030 Energy Dispersive Spectrometer for elemental mapping) imaging and drug loading (by adding concentrated drug solutions directly to the ferrogel).

2.3. Custom magnetic stimulation setups

Two different magnetic stimulation setups were employed. The first setup was designed for prolonged magnetic stimulations at a set frequency. This setup consisted of a platform upon which ferrogel samples could be placed and under which a platform with 1.27×1.27 -cm cylindrical neodymium magnets (K&J Magnetics, Inc., Pipersville, PA) could be cyclically raised and lowered (Fig. 2A). When the magnet-containing platform was lowered (Fig. 2A, i) the ferrogel samples (contained in scintillation vials) were subjected to near-zero magnetic gradients and were therefore not compressed (Fig. 2A, ii). When raised (Fig. 2A, iii), ferrogels were exposed to stronger magnetic gradients (with maximum fields around 5.6 kGauss) by virtue of being proximal to neodymium magnets (with 6.6 kGauss surface fields), thus deforming the ferrogels (Fig. 2A, iv). The magnet-containing platform was connected to a pulley system and cyclically pulled up and down by being tethered to a laboratory rocker. The rocker was set to a speed at which the neodymium magnets were brought to the ferrogels once per second. Note that the produced magnetic signal in this setup was not sinusoidal *per se*. Rather, ferrogels were exposed to positive (north) magnetic gradient once per second. Thus, ferrogels were technically exposed to a rectified sinusoidal magnetic gradient whose period was 2 s (i.e., 0.5 Hz). For the purposes of nomenclature, this signal will be referred to as a *0.5 Hz rectified sinusoid*.

The second setup was designed to provide briefer magnetic stimulations but at a wide range of stimulation frequencies (1 Hz to 550 Hz non-rectified sinusoidal magnetic gradients, see Figure S1 in Supplementary Material for a few examples of field vs. time). In this setup (Fig. 2B), a custom-built 286-turn electromagnet was built (Dura Magnetics, Inc., Sylvania, OH) with 0.5-inch Hyperco 50 core which drew current from a 750 VA programmable AC/DC power source (APS-1102, GW Instek, New Taipei City, Taiwan). Maximum AC current from this source was 10 A which resulted in surface fields of approximately 5.6 kGauss. The high sinusoidal currents running through the electromagnet generated heat that could harm the electromagnet and have an impact on drug release from ferrogels (i.e., increased diffusion at increased temperatures). Therefore, the electromagnet was cooled by running 3°C water across its electrically insulated coil. Warmed water exiting the electromagnet was passed through a heat exchange and back through a circulating water bath's cooling unit before being run across the electromagnet's coil again. This cooling system maintained temperatures on top of the electromagnet (where the ferrogel samples were placed) between 10 °C and 13°C during 10-minute magnetic exposures. Note that this electromagnet was never used for more than 10 minutes at a time and was left to fully cool (~20 minutes) between subsequent exposures. The signals produced by this electromagnet will be referred to simply by the frequency of the signal used (e.g., a 20 Hz signal).

2.4. Dextran release experiments

Ferrogels were produced and lyophilized as described above and were loaded with concentrated solutions of dextran. These dextrans included "low affinity" dextrans that were

relatively small and almost neutrally charged. It was estimated that these 4 kDa FITC-dextran would have average net charges of -2 due to FITC labeling (Fig. 3A). They would therefore not be electrostatically attracted to the negatively charged alginate polymer constituting ferrogel matrices and would exhibit relatively small degrees of matrix entanglement due to their relative small size. Also, “high affinity” dextrans were used that were relatively large and positively charged (due to DEAE side group substitutions). It was estimated that these 150 kDa FITC-DEAE dextrans would have average net charges of +30 due to FITC labeling and DEAE-substituted side groups (Fig. 3A). They would therefore be electrostatically attracted to the negatively charged alginate matrix and would exhibit more matrix entanglement than the 4 kDa “low affinity” dextrans.

For loading dextran in ferrogels, it was determined that lyophilized monophasic ferrogels would absorb up to 200 μL of liquid. Biphasic ferrogels could absorb less volume (75 μL) due to their smaller size. To facilitate direct comparisons between monophasic and biphasic ferrogels, they were loaded with the same amount of dextran per mass of gel (8:3 monophasic:biphasic mass ratio): thus, 4 mg of dextran in monophasic ferrogels and 1.5 mg in biphasic ferrogels. Thus, monophasic ferrogels were loaded by adding a solution containing 4 mg dextran in 200 μL of PBS (0.02 mg μL^{-1}) dropwise to the top and bottom of a lyophilized gel. Biphasic ferrogels were loaded by adding a solution containing 1.5 mg dextran in 75 μL PBS (0.02 mg μL^{-1}) dropwise to the iron-oxide-free region of the gel.

This loading was performed with ferrogels placed individually in silicone-treated scintillation vials. This silicone-treatment was performed to limit dextran adsorption to the walls of the vial. After loading, scintillation vials were capped and left overnight at room temperature to allow the dextran to fully integrate into the gels. Then, to remove any dextran that was not well integrated, ferrogels were rinsed in 5 mL of PBS with 1% BSA for 1 hour prior to experimentation. Experiments began by adding release media (2 mL of PBS with 1% BSA) to vials containing ferrogels. At various time points, all 2 mL of release media were removed, reserved, and replaced with fresh release media. Reserved media were assessed for dextran content by quantifying FITC fluorescence (488 nm excitation and 525 emission) against a standard curve using a BioTek Cytation 3 multi-mode plate reader.

One experiment involved loading biphasic ferrogels with both 150 kDa FITC-DEAE-dextran (high affinity) and 10 kDa Rhodamine-dextran (low affinity) for dual dextran release. First, 1.5 mg of FITC-DEAE-dextran in 37.5 μL PBS was added dropwise to ferrogels and allowed to absorb overnight. Ferrogels were then rinsed for 2 days in 5 mL of PBS with 1% BSA to remove unincorporated dextran. Ferrogels were then allowed to slightly dry in the refrigerator for 1 hour. Then 1.5 mg of Rhodamine-dextran in 37.5 μL PBS was added dropwise to ferrogels. This particular loading was designed to enhance initial release of rhodamine-dextran followed FITC-DEAE-dextran. Time-course experiments began as described above. FITC-DEAE-dextran release was quantified by reading FITC fluorescence (488 nm excitation and 519 emission) and rhodamine-dextran by reading rhodamine fluorescence (555 nm excitation and 590 nm emission) against standard curves.

2.5. Protein release experiments

In experiments involving the release of only BMP-2, ferrogels were loaded with 1 μg of protein. Thus, biphasic ferrogels were loaded dropwise with 1 μg BMP-2 in 75 μL PBS and monophasic ferrogels with 1 μg BMP-2 in 200 μL PBS. Ferrogels were allowed to absorb BMP-2 overnight in sealed and silicone-treated scintillation vials. Ferrogels were rinsed 3 times (24 hours each) in 2 mL of PBS with 1% BSA prior to running time course release experiments to remove unincorporated protein. At time zero, ferrogels were submerged in 2 mL of PBS with 1% BSA. At each time point, all 2 mL of media were removed, stored at -20°C , and replaced with fresh media. The BMP-2 content of these samples was assessed by thawing collected samples and then performing ELISA. In the experiment involving the release of both TCK-1 and BMP-2, biphasic ferrogels were loaded dropwise with a solution containing 1 μg of TCK-1 and 1 μg of BMP-2 in 75 μL PBS and rinsed for 3 days in PBS with 1% BSA. Time-course samples were collected as described above and TCK-1 and BMP-2 content was assessed using ELISA.

2.6 Frequency vs. release rate characterizations

Monophasic and biphasic ferrogels were fabricated, placed in silicone-treated scintillation vials, loaded with dextran, and rinsed as described above (sections 2.2 and 2.4). In one series of experiments, mitoxantrone—a common chemotherapeutic—was added to monophasic ferrogels using a solution containing 250 μg in 200 μL PBS. In all experiments involving use of the electromagnet, the electromagnet's cooling system was turned on and allowed to cool to 10°C prior to experimentation, requiring about 20 minutes. A scintillation vial containing a rinsed ferrogel was placed on the cooled electromagnet (Fig. 2B) such that the cylindrical ferrogel was directly aligned with the cylindrical electromagnet's core. 2 mL of PBS with 1% BSA was gently added to the vial and magnetic stimulation immediately began, lasting for 10 minutes. Samples of the experimental media were taken immediately after 10 minutes and sample contents were quantified for release. FITC-dextran release was quantified on a plate reader by measuring fluorescence (488 nm excitation and 519 emission) against a standard curve. Mitoxantrone release was quantified by measuring optical absorbance at 610 nm against a standard curve. Rates of release were computed by taking the amount of release and dividing by the 10-minute experimental duration. The electromagnet was allowed to cool back down to 10°C (typically from $\sim 13^{\circ}\text{C}$, requiring 20 minutes) before being used in a subsequent experiment.

2.7. Data representation and statistical analyses

All quantitative data presented in this work are represented as means $\pm$ standard deviations. The majority of statistical comparisons in this work were limited to a single comparison. For these single comparisons, a student t-test was applied to calculate significance with p -values of less than 0.05 being our benchmark for significance. For instances where multiple comparisons were made, one-way Analysis of Variance (ANOVA) with a Tukey's post hoc test was utilized. The following convention was used for indicating the level of significance: *, **, and *** indicate $p < 0.05$, 0.01, and 0.001, respectively. "n.s." indicates that this benchmark for statistical significance was not met ($p > 0.05$).

3. Results and discussion

3.1. Release from and limitations associated with monophasic ferrogels

Experiments were conducted to assess how the relative affinity of a model drug to the ferrogel's matrix and the timing of magnetic stimulation influenced release characteristics. Monophasic ferrogels were loaded with either dextran expected to interact with the negatively charged alginate constituting the ferrogel matrix to a lesser degree (Fig. 3A, "low affinity" dextran) or to a higher degree (Fig. 3A, "high affinity" dextran) (see Section 2.4 in Materials and methods). The release of dextran from these monophasic ferrogels (Fig. 3B, i) was modulated through magnetic compression (Fig. 3B, ii). When applied at early time points, continuous magnetic stimulation provided enhanced release compared to unstimulated ferrogels for low affinity dextrans (Fig. 3C, comparing solid and dashed blue curves). However, high affinity dextran release was statistically similar for magnetically stimulated and unstimulated control gels (Fig. 3C, red curves). Additionally, magnetically enhanced release could only be achieved when stimulation was applied at early time points. For example, when attempting to magnetically trigger release both low and high affinity dextran after a 120-hour delay, no statistical difference was observed between magnetically stimulated and unstimulated ferrogels (Fig. 3D, curves from 120 to 168 hours). For low affinity dextran, this inability to trigger release was likely due to diffusive depletion of dextran prior to magnetic stimulation (Fig. 3D, blue curves reaching ~80% release prior to 120 hours). For high affinity dextran, while there was ample dextran left to magnetically release at 120 hours (Fig. 3D, red curves reaching ~10% prior to 120 hours), it appears that magnetic compressions were insufficient to overcome the interactions between the high affinity dextran and the alginate matrix.

The inability to delay triggered release events presents a practical limitation that is antithetical to the advantages ferrogels could bring to therapies [22, 34–36]. For example, biomaterials scaffolds can be used to direct bone regeneration [5] and bone growth can be enhanced when these scaffolds are loaded with osteo-differentiation factors (e.g., bone morphogenetic protein 2 (BMP-2)) [10, 12, 37]. However, it has been shown that delaying the presentation of BMP-2 by 5 to 10 days further enhances healing outcome [38]. This may be the result of delaying osteo-differentiation until after the inflammatory response subsides and/or allowing time for a strong population of bone progenitor cells to establish themselves at the injury site [5, 38]. In either case, the optimal time for BMP-2 delivery is likely differs by patient and injury/disease. Thus, using ferrogels to magnetically control the time at which BMP-2 is delivered at the bone injury site could improve bone injury treatments.

Unfortunately, BMP-2 could not be triggered to release at higher rates than baseline when magnetic stimulation was applied in a delayed manner (Fig. 3E, comparing solid and dashed curves from 194 to 240 hours) even though there was ample BMP-2 left in the gel prior to magnetic stimulation (99% at 194 hours). Because heparin-binding BMP-2 has a high affinity to heparin-mimicking alginate, it was expected that BMP-2 would remain in the gel for prolonged periods of time. But again, just as with the high affinity dextran, magnetically induced deformations were insufficient to overcome interactions between BMP-2 and the alginate matrix.

To the best of our knowledge, ferrogels have not previously been shown to provide triggered release after multi-day waiting periods, which is consistent with our findings. Previous reports of triggered delivery from ferrogels have demonstrated magnetically enhanced release only at relatively early time points [22, 23, 34, 39–42]. While magnetically enhancing release rates directly after implantation has treatment applications, in many situations delayed and pulsatile delivery profiles may be desirable. A few studies have demonstrated pulsatile release profiles (i.e., repeated switching from high and low rates of release), but these demonstrations were only observed when magnetically stimulating within the first hundreds of minutes after the start of the experiments [21, 32, 43]. Strategies to improve triggered release after prolonged delay would have broad clinical utility. For example, beyond bone injury treatments, delayed deliveries may enhance outcome in chemotherapies [1, 2, 44–46], wound healing [9, 14], and tissue vascularization [3, 4]. Because of this broad therapeutic potential, the remainder of this study focused on developing strategies for achieving magnetically triggered enhancements in release rate after prolonged delay times.

3.2. Enhancing release characteristics through stimulation frequency

One strategy for achieving delayed enhancements in release, when there still remained ample agent available for release (e.g., high affinity factors), was to optimize the manner in which ferrogels were stimulated. It was hypothesized that the rate at which a ferrogel was magnetically compressed could influence the release rate. For example, higher frequencies of stimulation would result in higher rates of deformation and thus higher rates of drug being purged from the ferrogel. Though, at some stimulation frequency, the ferrogel might not be able to rebound between subsequent stimulations and deformations would become less pronounced as frequency increased. Thus, we hypothesized that the rate of release would increase vs. frequency but then reduce beyond an optimal frequency. To investigate this hypothesis, experiments were conducted to investigate how the frequency of magnetic stimulation influenced the release of various molecules from monophasic ferrogels. First, the electromagnet used in these studies (Fig. 2B) was tuned so that it would provide similar surface fields across a wide range of stimulation frequencies (Fig. 4A, i). Additionally, the electromagnet's cooling system was tested to ensure that the temperature at the surface of the magnet (where the ferrogel samples were placed) was maintained within a consistent window over a wide range of frequencies. As designed, these 10-minute release experiments started at around 10°C and heated to no more than 13°C at each magnetic frequency used (Fig. 4A, ii).

Release rates from ferrogels varied as a function of magnetic stimulation frequency. Both low and high affinity dextrans (1) released at relatively high rates at 1 Hz, (2) released at rates similar to diffusion from 2 to 5 Hz, and (3) released at optimally high rates at around 50 Hz (Fig. 4B). Note that diffusion based release (i.e., release under no magnetic stimulation) was measured at 9°C and room temperature since it was determined that ferrogels would experience temperatures within this range since during stimulation. That is, the surface of the electromagnet was maintained at around 10°C to 13°C during experimentation, but ferrogels were otherwise exposed to ambient room temperature in all other directions.

Interestingly, release rates exhibited local maxima at both 1 Hz and 50 Hz (Fig. 4B). However, ferrogels only appreciably deformed in response to each magnetic cycle at lower frequencies and did not appreciably deform/rebound at or above 50 Hz (Movie S1, Supplementary Material). It appears that this appreciable compression/rebounding at 1 Hz resulted in efficient convective purging of dextran from ferrogels. The mechanism(s) resulting in efficient release rates at higher frequencies (~50 Hz) are not clear. It is possible that 50-Hz agitation of ferrogel matrices results in lower dextran on-rates (k_{on}), higher off-rates (k_{off}), and thus higher overall disassociation constants ($K_d = k_{\text{off}}/k_{\text{on}}$). That is, electrostatic binding sites about the ferrogel matrix may become moving targets when the gel is magnetically vibrated, resulting in poorer binding and enhanced release rates. While this is an area of ongoing investigation, the ability to use magnetic frequency to remotely regulate the rate of drug release could have clinical utility. Because of this potential, a clinically relevant therapeutic was used to see if its release rate could be regulated via magnetic frequency. Mitoxantrone, a common chemotherapeutic, was released from ferrogels at rates dependent on the frequency of magnetic stimulation (Fig. 4C). Maximal release rates were observed for mitoxantrone at 20 Hz (Movie S2, Supplementary Material).

3.3. Enhancing release characteristics through ferrogel design

Another strategy for achieving delayed enhancements in release was to employ a ferrogel design that could more efficiently deform when magnetically stimulated. In monophasic ferrogels, increased iron-oxide concentrations can result in stronger magnetic body forces exerted on the gel but this also increases gel stiffness, rendering the ferrogel less compressible. In previous work, this issue was resolved by designing a biphasic ferrogel [34] containing an iron-oxide-laden region and an iron-oxide-free region (Fig. 5A, i: yellow (Fe) and aqua (C) regions, respectively). This design allowed for high concentrations of iron-oxide (and thus strong magnetic force generation) from the iron-oxide-laden region of the gel combined with efficient compression of the soft, drug-loaded, iron-oxide-free region (Fig. 5A, ii). In the current study, in a head-to-head comparison, when stimulated under identical magnetic conditions (1 Hz magnetic signals), biphasic ferrogels delivered both high and low affinity dextrans at higher rates compared to monophasic ferrogels (Fig. 5B). These results verify the improved efficiency that the biphasic design provides. Additionally, biphasic ferrogels were still capable of delivering both low and high affinity dextrans at frequency-dependent rates (Fig. 5C).

It is notable that ferrogel performance could be further enhanced by incorporating particles with higher magnetic moments [47] and by optimizing ferrogel formulation (e.g., fine-tuning gel mechanics by optimizing gel polymer and crosslinker amounts [48] and magnetic particle concentrations [34]). The iron oxide particles used here were previously demonstrated to form ~ 5 μm aggregates which outperformed smaller, chemically synthesized, well-dispersed 50 nm iron oxide nanoparticles [34] since larger particles have higher saturation magnetization values [49]. However, future experiments must be conducted in order to characterize how particle fillers with various magnetic properties impact ferrogel drug delivery performance vs. field frequency.

While the approach adopted here is based on optimizing soft, highly deformable ferrogel structures, it remains to be seen how the use of biphasic gel ferrogels and magnetic fields of various frequencies impacts performance when using ferrogels that exhibit different mechanical properties. That is, these studies have been limited to a one formulation of a macroporous monophasic ferrogel and one formulation of a macroporous biphasic ferrogel. These specific gel formulations produced very deformable ferrogels with Young's moduli of 1.25 kPa and 0.5 kPa, respectively [34]. Moving forward, it will be important to more generally characterize how gel mechanical properties (based on gel formulation) impact release vs. magnetic stimulation frequency. There is both a theoretical basis for [50] and experimental evidence of [30, 34] increased gel stiffness resulting from increases in magnetic particle concentrations. However, (i) softer ferrogels do not necessarily provide enhanced magnetically triggered drug release [34] and (ii) the relationship between drug release vs. frequency is likely dependent on other gel characteristics beyond stiffness. For example, ferrogel shape can impact how the applied magnetic field is distributed within the ferrogel's volume, impacting gel deformation [50] and thus may have an impact on drug release. Additionally, ferrogel deformation is highly dependent on magnetic particle rigidity, shape, and their magnetic interactions with other particles and polymers within the gel structure [51]. Taken altogether, this motivates the need for future experiments investigating the interplay between ferrogel composition and drug release.

3.4. Delayed release profiles from biphasic ferrogels using more efficient stimulation profiles

Experiments were conducted to determine if more efficient magnetic signals (i.e., at specified frequencies) and a more efficient ferrogel design (i.e., biphasic) could be used to achieve magnetically triggered enhancements in release after several days of unstimulated baseline release. High affinity dextran was used as the payload because it remained in gels for prolonged periods of time (Fig. 3D, red curves). To minimize baseline release prior to magnetic stimulation, biphasic ferrogels were rinsed for 2 days to remove any dextran that was not well-integrated into the gel (Fig. 6A, i: from -48 h to 0 h). Biphasic ferrogels were then allowed to diffusively release dextran for 120 hours (5 days) and were then magnetically stimulated from 120 to 128 hours. To avoid overheating of the electromagnet, a piecemeal stimulation profile was adopted: ferrogels were exposed to a 200 Hz signal for 10 minutes every hour on the hour using the electromagnet and were otherwise exposed to a 0.5 Hz rectified sinusoid using the platform stimulator (Fig. 6A, ii). The 200 Hz signal was used because it resulted in relatively high release rates for high affinity dextran (Fig. 5C, rightmost data point on red curve). When applied after a 5-day delay, this magnetic signal greatly enhanced release above baseline levels (Fig. 6B, comparing red to black curves after 120 hours). The highest rate of release recorded prior to magnetic stimulation was 0.02 μg per hour, but after 120 hours magnetically stimulated release rates were recorded between 13.8 and 39.0 μg per hour. This represents a 690- to 1950-fold increase in release rate when magnetically stimulated. This level of enhanced release above baseline and the length of time prior to triggered release have not been previously demonstrated and (as discussed earlier) has potentially broad clinical utility.

3.5. Sequential release profiles from biphasic ferrogels

Many therapies require directing sequences of biological events. For example, a strategy for regenerating injured or defective tissues is first to recruit cells to an implanted 3D scaffold and then differentiate those recruited cells into tissue-specific cell types. For regenerating bone tissues, this sequence could be directed by first releasing a signaling factor capable of recruiting bone progenitors (such as TCK-1/CXCL7 [52]) and delaying the release of osteo-differentiation factor (such as BMP-2 [37, 53, 54]). To produce this delivery sequence, both proteins were loaded into biphasic ferrogels. During the 5 days prior to magnetic stimulation, TCK-1 released at much higher rates (Fig. 7, blue curve before 120 h). The strong BMP-2 retention was consistent with past studies [17]. When applied, magnetic stimulation resulted in enhanced BMP-2 release rates (Fig. 7, red curves after 120 h). Note that the magnetic signals used here were the same as those which failed to enhance release above baseline levels from monophasic ferrogels (Fig. 3E). Therefore, the biphasic ferrogel design was sufficient to enable triggered BMP-2 release without optimizing the magnetic signal.

Finally, experiments were conducted to determine if a sequence of two different payloads could be triggered for release through the application of two different magnetic signals. It was first observed that 1 Hz stimulation resulted in low affinity dextran released at higher rates than high affinity dextran (Fig. 8A, comparing blue and red bars at 1 Hz). It was also observed that 20 Hz stimulation resulted in high affinity dextran released at higher rates than low affinity dextran (Fig. 8A, comparing red and blue bars at 20 Hz). Therefore, biphasic ferrogels were loaded with both a rhodamine-labeled low affinity dextran and a FITC-labeled high affinity dextran. For the first 24 hours, no magnetic stimulation was applied and both low and high affinity dextrans did not release at appreciable rates (Fig. 8B, i: blue and red curves at low values before day 1). On day 1, a magnetic signal was applied that alternated between a 1 Hz signal on the electromagnet and 0.5 rectified sinusoid on the platform system (Fig. 8B, ii). This magnetic signal resulted in dramatic enhancement in low affinity dextran release (Fig. 8B, i: blue curve transitioning from roughly 0 to 100% release from 24 to 26 hours). As expected, this magnetic signal also slightly enhanced release of the high affinity dextran, though it left nearly 93% of the high affinity dextran in the gel. From days 1 to 4, no magnetic stimulation was applied, resulting in flatline rates of release. On day 4, a magnetic signal was applied that alternated between a 20 Hz signal on the electromagnet and a 0.5 rectified sinusoid on the platform system (Fig. 8B, iii). This resulted in enhanced release of the high affinity dextran on day 4 (Fig. 8B, i: increase of red curve from 7% to 23% release on day 4). These studies demonstrate that magnetic stimulation frequency can not only be used to regulate the release rate of individual payloads (Figs. 4B, 4C, and 5C), but can also be used to select the release of a specified payload over another payload.

4. Conclusion

These studies confirmed that existing magnetic stimulation strategies are incapable of triggering payload release after prolonged periods of retention from ferrogel systems that deform in response to magnetic fields. However, stimulation of biphasic ferrogels with more

optimized magnetic signals enabled triggered deliveries after prolonged delay times. Triggered release rates were 690 to 1950 times greater than baseline release levels when magnetically stimulated on day 5. The frequency of magnetic stimulation could be used to remotely regulate release rate. A sequence of two potential therapeutics (TCK-1 followed by BMP-2) could be delivered by allowing TCK-1 to initially diffuse out of the ferrogel, followed by magnetically triggered delivery of BMP-2. Finally, two model drugs (low and high affinity dextrans) were sequentially triggered for release by stimulating at different times with different frequencies that were tuned to preferentially release one over the other. Taken altogether, these studies expand the potential of ferrogels for providing on-demand, remotely triggered control over drug delivery profiles.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Fig. 1. Fabrication of ferrogels in monophasic and biphasic formats

Schematics of the monophasic ferrogel's cryogelation process (A) and biphasic ferrogels freeze-dry fabrication process (B).

Fig. 2. Schematics of the two types of experimental setups used in this study to magnetically stimulate ferrogels

(A) For experiments requiring prolonged cyclic magnetic stimulation, a platform was used that was mounted with a neodymium magnet. This platform was attached to a laboratory rocker via a pulley system so that the magnet would bob up and down periodically away from the sample (i) (thus not stimulating the ferrogel (ii)) and near the sample (iii) (thus magnetically compressing the ferrogel (iv)). (B) For experiments requiring magnetic stimulations at frequencies ranging from 1 to 550 Hz, an electromagnet was used. This electromagnet was excited by an AC current from a programmable power source and was cooled using a circulating water bath and heat exchange.

Fig. 3. Magnetic stimulation can be used to enhance release rates initially from monophasic ferrogels, but cannot necessarily enhance release rates when stimulation is delayed

(A) A table providing theoretical size, DEAE substitution, and estimated net charge for the FITC-labeled dextrans used in this study. (B) (i) SEM image with elemental mapping of a dehydrated monophasic ferrogel. (ii) Photographs of a monophasic ferrogel loaded with 4 kDa FITC-dextran before (top) and during (bottom) magnetic compression. (C) Cumulative release vs. time for low (blue) and high (red) affinity dextrans when monophasic ferrogels were either not magnetically stimulated (solid) or continuously stimulated with a 0.5 Hz rectified magnetic gradient (dashed). (D) Cumulative release vs. time for low (blue) and high (red) affinity dextrans when monophasic ferrogels were either not magnetically stimulated (solid) or stimulated with a 0.5 Hz rectified magnetic gradient from 120 to 168 hours (dashed). (E) The cumulative and percent BMP-2 release (left and right vertical axes, respectively) versus time when monophasic ferrogels were either not stimulated (solid) or stimulated with a 0.5 Hz rectified magnetic gradient from 192 to 240 hours (dashed). In parts C through E, statistics were applied comparing stimulated release to unstimulated release ($N = 4$).

Fig. 4. The frequency of magnetic stimulation can be used to regulate and optimize the rate of drug delivery

(A) Measured electromagnet surface field (i) and temperature (ii) vs. magnetic stimulation frequency. (B) Release rate from monophasic ferrogels after 10-minutes of magnetic stimulation for low (blue) and high (red) affinity dextran vs. frequency. At left, bar graphs of release rate from unstimulated ferrogels at 9°C and room temperature for low (blue) and high (red) affinity dextran. (C) Release rate vs. frequency (left) and diffusive release (right) for mitoxantrone. For parts B and C, asterisks code for *p*-values associated with comparing release rate at a given frequency to unstimulated diffusive release ($N = 6$).

Fig. 5. Biphase ferrogels deliver drugs at more efficient rates compared to monophasic ferrogels and exhibited frequency-dependent release rates

(A) (i) SEM image with elemental mapping data of a biphase ferrogel. (ii) Photographs of a biphase ferrogel loaded with 4 kDa FITC-dextran before (top) and during (bottom) magnetic compression. (B) A comparison of the release rates of low and high affinity dextrans after 10 minutes of magnetic stimulation at 1 Hz from monophasic (light grey) and biphase (dark grey) ferrogels. (C) Release rate from monophasic ferrogels after 10-minutes of magnetic stimulation for low (blue) and high (red) affinity dextrans vs. frequency. In parts B and C, $N = 4$.

Fig. 6. Magnetically triggering of release after a prolonged retention can be achieved by stimulating biphasic ferrogels with more efficient magnetic stimulation schemes

(A) (i) A timeline outlining how experiments were conducted to explore delayed dextran release. (ii) A graph detailing the magnetic stimulation profile used in these experiments alternated between 200 Hz and a 0.5 Hz rectified sinusoid. (B) Cumulative (i) and rate of (ii) high affinity dextran release vs. time from biphasic ferrogels that were either not stimulated (solid black curves) or magnetically stimulated (dashed red curves) from 120 to 128 hours. $N = 4$.

Fig. 7. A sequence of deliveries can be achieved by first allowing one payload to diffuse out of the gel followed by magnetically triggered release of a higher affinity payload

(A) Cumulative release of TCK-1 (blue) and BMP-2 (red) over time when biphasic ferrogels were loaded with both TCK-1 and BMP-2 and magnetically stimulated at 8 hours starting at 120 hours. Starting at 120 hours, ferrogels were exposed to a 0.5 Hz rectified sinusoid. (B) Rate of release for TCK-1 (blue) and BMP-2 (red) over time corresponding to the experiment detailed in part A. N = 4.

Fig. 8. Different payloads can be preferentially released at different times by triggering with magnetic signals composed of different frequencies

(A) Release rate of low (blue) and high (red) affinity dextrans from biphasic ferrogels when stimulated at either 1 Hz or 20 Hz. (B) (i) The cumulative release of low (blue) and high (red) affinity dextrans over time from biphasic ferrogels when magnetically stimulated on day 1 (blue window) and on day 4 (red window). Ferrogels were stimulated on day 1 with a magnetic signal that alternated between 1 Hz and a 0.5 Hz rectified sinusoid (ii) and on day 4 with a signal that alternated between 20 Hz and a 0.5 Hz rectified sinusoid. N = 4.