Effect of an Oscillating Magnetic Field on the Release Properties of Magnetic Collagen Gels

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The paper describes the effect of an oscillating magnetic field (OMF) on the morphology and release properties of collagen gels containing magnetic nanoparticles and microparticles and fluorescent drug analogues. Collagen gels were prepared through fibrillogenesis of collagen in the presence of iron oxide magnetic particles averaging 10 nm or 3 μm in diameter and rhodamine-labeled dextran (Dex-R) of molecular weights between 3000−70 000 g/mol. Dextran molecules effectively simulate protein-based drugs, since they have similar molecular weights and dimensions. The paper discusses the effect of an OMF on the release properties of the gels and proposes an empirical model to predict the release rate. It also demonstrates the self-repair capability of collagen gels following the structural damage caused by an OMF.

Introduction

The ability to control the release of drug from drug carriers has been a major goal in drug delivery research over the last two decades.[1] Drug delivery devices which are capable of adjusting drug output to meet a physiological need in response to an externally or internally generated signal were developed. Recently introduced methods to trigger drug release from drug carriers are based on the response of the devices to changes in pH[5−8] and temperature.[5,6,9] Collagen-based materials have been widely used as drug delivery carriers due to their biocompatibility, biodegradability, low antigenicity, and low inflammatory effects.[1−4] Additionally, collagen can be shaped in a variety of forms including sheets, scaffolds, dressings, gels, and pellets.[2] Previously, we showed that it is possible to attenuate the release properties of collagen gels by controlling their cross-linking level and by adding molecules to the gel formulation that increase the stability of collagen gels and their resistance to enzymatic degradation.[10] In this paper, we describe the enhancement of the drug release rate from collagen gels by using an oscillating magnetic field (OMF). The oscillating magnetic field provides an external means to control the release of drugs from collagen gels.[5,6]

The use of an OMF to modulate the rate of drug delivery from polymer matrices was previously shown by Langer et al.[11−15] Their work involved the use of ethylene−vinyl acetate copolymer matrices containing millimetric magnetic steel beads[16] or nickel coated samarium cobalt (SmCo5) magnetic particles.[11−15] The oscillating magnetic field was generated by a plate demagnetizer or by moving a permanent magnet back and forth. A similar system was used to deliver insulin from alginate microspheres containing micrometric strontium ferrite microparticles.[17] In this paper, we describe the use of an OMF which is generated by an electromagnet. The experimental system is much simpler than the one used by Langer and co-workers, since it does not involve the use of moving parts. This eliminates possible effects of mechanical movement of the gels on their release properties. The current study makes use of super-paramagnetic nanoparticles or microparticles to enhance the rate of release of dextran rhodamine (Dex-R), which serves as a drug analogue, from collagen gels. The use of nanoparticles is advantageous, since these particles show very low coercivity.[18] As a result, they vibrate freely in collagen gels when the external magnetic field is applied and lose their magnetic moment as soon as the magnetic field is turned off. This provides a shut off mechanism and improves the controlled release properties of the gels. In this manuscript,

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we describe the effects of magnetic particle size and concentration and the drug analogue molecular weight on its release rate from magnetic collagen gels. In addition, we propose an empirical kinetic model to describe the release rate of Dex-R from collagen gels containing magnetic nanoparticles or microparticles when subjected to an oscillating magnetic field.

Numerous theoretical models were previously developed to describe the release of encapsulated substances from carriers.[20−25] The release rate of encapsulated substances from collagen matrices depends on multiple parameters including the diffusion rate of an external aqueous medium into the matrix, the relaxation of the matrix, liberation of the encapsulated substance due to hydrolytic or enzymatic degradation of the matrix, the diffusion rate of the encapsulated substance from the bulk of the matrix to its surface, possible phase transfer processes in the matrix, and the diffusion rate of encapsulated substances across the boundary layer.[26−31] In this paper, we utilize a simple model to describe the release rate of Dex-R from magnetic collagen gels, which is largely based on a detailed study recently reported by Tsafriri and co-workers.[31] While the release properties are affected by multiple parameters that are difficult to measure and quantify, it is greatly simplified when entrapped molecules do not interact with the carrier once released. Experimentally, this situation is possible when the molecules are released from a carrier to a diluted solution. Under these conditions, the release rate of entrapped molecules is limited by diffusion. It is therefore anticipated that molecules weakly associated with the carrier will be quickly released, creating an initial release burst. The initial release burst will be followed by a slower release component which could be affected by an external force like an oscillating magnetic field, as used in our study.

Materials and Methods

Collagen type I from rat tail tendon was purchased from Upstate. *N*-(3-Dimethylaminopropyl)-*N*′-ethyl-carbodiimide, *N*-hydroxysulfo-succunimide sodium salt (EDAC/NHS) was purchased from Fluka. 2-Morpholinoethanesulfonic acid (MES) buffer was purchased from Sigma-Aldrich. Dextran-tetramethylrhodamine (Dex-R) was purchased from Molecular Probes−Invitrogen. Phosphate-buffered solutions were purchased from Gibco−Invitrogen. Magnetic microparticles (2.8 μm average diameter) were acquired from Bangs Laboratories, Inc. Magnetic nanoparticles were provided by Professor O'Connor's laboratory. The synthesis and the chemical and physical properties of the magnetic nanoparticles are described by Prof. O'Connor.[18,19] The particles are single crystalline and are nonaggregated.[19] They are super-paramagnetic and water soluble. The particle size is 10 nm, and the size distribution is 8−10 nm. The magnetic data show that the saturation magnetization (M_s) at room temperature is 83.5 emu/g, close to the value of 92 emu/g reported for bulk material. The ZFC/FC curves of the iron oxide nanoparticles were measured in a field of 100 Oe on a superconducting quantum interference device (SQUID) magnetometer. These curves diverge at 228 K. The absence of a well-defined maximum in the ZFC curve indicates that the nanocomposite exhibits a blocking temperature above room temperature.

Preparation of Magnetic Collagen Gels. The collagen gels were prepared in 1 mL Eppendorf vials by the fibrilogenisis method in which collagen molecules spontaneously self-assemble into higher-order structures when the pH of collagen solutions is raised to 7.4 at 37 °C.[32] Entrapping magnetic particles in the collagen gels was realized by heating to 37 °C a mixture containing 125 μL of 4 mg/mL collagen solution and 12.5 μL of 4×10^{14} particles/mL nanoparticle suspension or 30 μL of 3.7×10^6 particles/mL microparticle suspension at pH 4.0. A 45 μL portion of 1 mg/mL Dex-R in phosphate buffer solution at pH 7.4 was added to the samples to form Dex-R- and magnetic-particle-containing collagen gels. The conical shaped collagen gels had a thickness of 1.0 cm. The gels were incubated with a phosphate buffer solution for 2 h and washed to remove loosely bound particles and Dex-R molecules. The amount of iron oxide in the gels was kept constant at 0.6 mg/mL when the effect of magnetic particle size on the release properties of the gels was determined. In experiments that tested the effect of nanoparticle concentration on the release rate, the concentration of the nanoparticles in the gels was varied between 0.1 and 6 mg/mL.

Stabilization of Gels by Cross-Linking. To further stabilize the Dex-R- and magnetic-particle-containing collagen gels, the gels were cross-linked by adding 0.5 mL of *N*-(3-dimethylaminopropyl)-*N*′-ethyl-carbodiimide, *N*-hydroxysulfo-succunimide sodium salt (EDAC/NHS; 40 mM/5 mM) solution freshly prepared in 2-morpholino-ethanesulfonic acid (MES) buffer (50 mM, pH 5.0) at room temperature. The EDAC/NHS induces the formation of amide linkages between free amino and carboxyl groups of collagen molecules.[32,33] The advantage of using this chemical is that EDAC/NHS is a zero-length cross-linker; that is, it is not linked between the protein fibers and all of the reaction products can be removed by washing the gels, resulting in a biocompatible product.[34] The gels were incubated with EDAC/NHS overnight. The reaction was stopped by washing the gels thoroughly with a phosphate buffer solution (PBS).

Scanning Electron Microscopy (SEM). The morphology of collagen matrices was visualized by an environmental scanning electron microscope (Phillips XL-30). The specimens were prepared by dehydrating the collagen gels using water/ethanol mixtures of increasing ethanol percentage. This was followed by critical point drying (Ladd 28,000). In the process, water is removed from the gels while preserving the gel microstructure. The dried gels were mounted on stubs and coated with Pd/Au using an ion sputter (Hummer II Sputter Coater) to facilitate contrast imaging.

Oscillating Magnetic Field (OMF) Studies. Collagen gels were subjected to an oscillating magnetic field (magnetic field variation, sinusoidal; magnetic field peak-to-peak value, 0.14 T; magnetic field variation frequency, 0.3 Hz). The sinusoidal magnetic field was produced by an electromagnet using a bipolar power supply/amplifier (Kepco, model BOP 50M4) driven by a wave generator (Agilent 33250A). The samples were placed at the edge of the electromagnet poles where the magnetic field gradient is maximum. A gauss meter (Lakeshore 450) was used to check the amplitude of the alternative magnetic field.

Release Measurements. Cross-linked magnetic collagen gels containing Dex-R were first incubated with 1 mL of phosphate buffer solution at pH 7.4 for 2 h at room temperature to remove loosely bound Dex-R and magnetic particles. The gels were washed, incubated with a fresh buffer solution, and placed at the edge of the electromagnet for the release experiments. Up to eight gels could be placed in the magnetic field simultaneously while keeping identical

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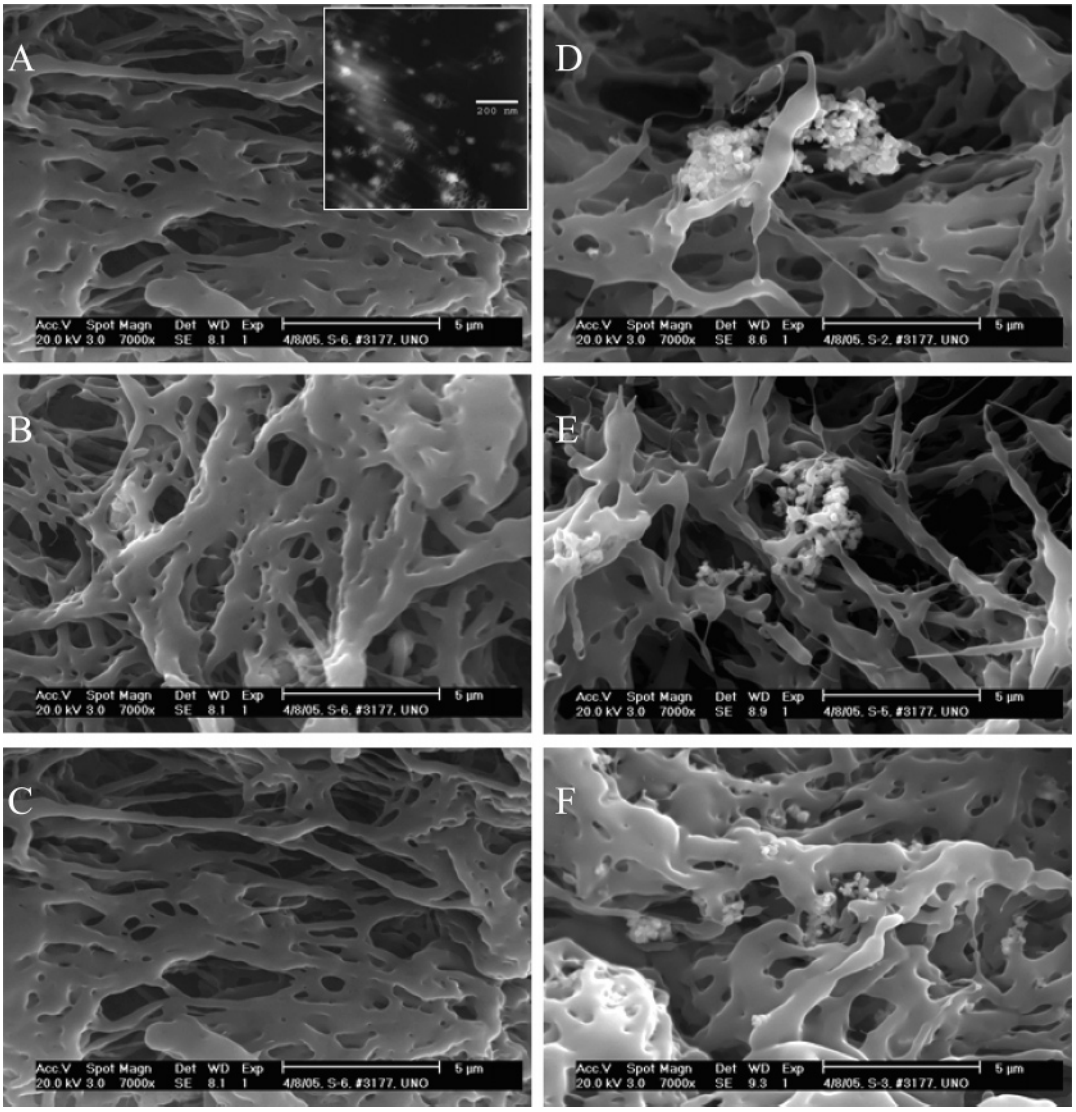

Figure 1. SEM images of collagen gels loaded with magnetic nanoparticles (A−C) and magnetic microparticles (D−F). Inset: A high resolution TEM image of magnetic nanoparticles entrapped in a collagen gel. Images A and D were taken prior to exposure of collagen gels to an OMF. Images B and E were taken immediately following the application of the OMF on the gels. Images C and F were taken after 12 h of rest.

magnetic field conditions. The release rate of Dex-R from the gels was followed by removing 100 μL aliquots from the samples at different time intervals and measuring their fluorescence intensity using a microplate reader (Molecular Devices, SpectraMax M2). The fluorescence measurements were taken at 580 nm using an excitation wavelength of 535 nm. Comparative release measurements from gels in the absence and presence of an OMF were carried out at room temperature to determine the effect of an OMF on the release rate of Dex-R from the collagen gels. To investigate the effect of the temperature on the release properties of collagen gels, the fluorescence intensities of Dex-R for samples at room temperature were measured in the absence of an OMF and compared with measurements for samples kept at 37 °C.

Results and Discussion

Morphology of Collagen Gels Containing Magnetic Nanoparticles and Microparticles. Figure 1 shows the morphology of collagen gels that contain magnetic nanoparticles (A−C) and microparticles (D−F). Images A−C show collagen gels that contain magnetic nanoparticles prior to the application of an oscillating magnetic field (OMF) (A), immediately following the application of an OMF on the gel for 12 h (B), and after 12 h of rest (C). A transmission electron microscopy (TEM) image of the magnetic nanoparticles entrapped in the gel is shown as an inset in Figure 1A. The TEM image shows well dispersed nanoparticles in the gel. The 7000× scanning electron microscopy (SEM) images confirm that the entrapment of nanoparticles does not alter the fiber structure of collagen gels and that the application of an OMF on nanoparticle-containing collagen gels does not affect the gel morphology. Images D−F show collagen gels that contain magnetic microparticles prior to the application of an OMF (D), immediately following the application of an OMF on the gel for 12 h (E), and after 12 h of rest (F). Image D shows that the magnetic microparticles aggregate between the collagen fibers but the collagen gel appears to maintain high structural integrity with no apparent holes or gaps. The aggregation of magnetic microparticles in the gel is attributed to magnetic interactions. These interactions are greatly reduced when nanoparticles are encapsulated in the gels, which may be the result of their enhanced super-paramagnetic properties.[18] Image E shows a significant physical deformation of the gel in localized areas where aggregates of microparticles are found following the application of an OMF for 12 h. The gel has bigger holes and the fibers seem to be thinner than before the application of an OMF. This indicates mechanical damage to the gel. The OMF

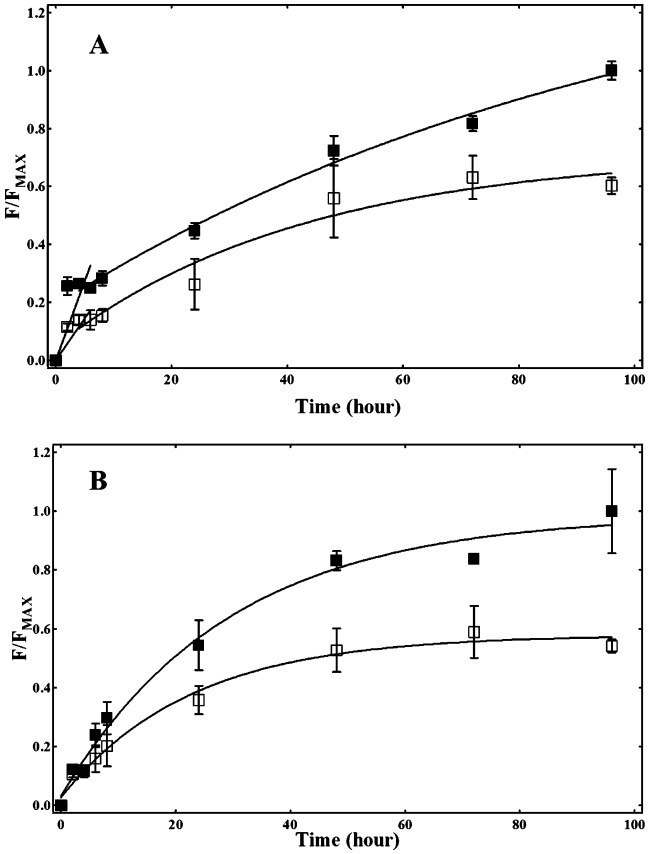

Figure 2. Release of the rhodamine-labeled dextran (Dex-R) from collagen gels containing (A) magnetic nanoparticles and (B) magnetic microparticles in the absence (□) and presence (■) of an OMF. The Dex-R molecular weight was 70 000 g/mol. The experiments were carried out at room temperature.

causes the particles to vibrate in a horizontal movement that pushes the fibers in the microenvironment near the particles. This creates a bigger area for diffusion of molecules which explains the effect on the release, as seen in Figure 2. Our conclusion therefore is that the particles' vibration pushes the fibers. We have tested the effect of the OMF on nanoparticles and microparticles not entrapped in gels and recorded their vibration as a function of the OMF intensity (the movie can be seen in the Supporting Information). This movement, even though limited by the collagen fibers, can cause condensation of the fibers away from the particles and therefore bigger holes and more area for drug diffusion out of the gels. Image F indicates that the damage is reversible and the collagen fiberlike structure is restored over time once the OMF is turned off. This demonstrates the utility of collagen gels as drug carriers as they show self-repair capabilities. It appears however that using magnetic nanoparticles to induce drug release by an OMF is advantageous, since physical deformation of collagen gels is negligible at all times.

Release Properties of Magnetic Collagen Gels in the Presence of an OMF. As previously mentioned, collagen gels were loaded with rhodamine-labeled dextran (Dex-R) in order to analyze the effect of the OMF on the release properties of collagen gels. The release rate of Dex-R was measured in the absence and presence of an OMF as described in the Experimental Section. Parts A and B of Figure 2 show Dex-R release curves of gels containing magnetic nanoparticles and microparticles, respectively. The fluorescence readings were normalized to the maximum fluorescence intensity of the solution in which the released Dex-R accumulated in the presence of OMF. The temporal dependence of the fluorescence intensity of released Dex-R is described as follows:

$$F(t) = k_1 t + B \exp(-k_2 t) \quad (1)$$

The equation consists of two kinetic terms. The first term accounts for the diffusion of weakly associated Dex-R molecules from collagen gels. These molecules are entrapped close to the surface and readily diffuse to the solution once the gels are subjected to an OMF. The second term accounts for strongly bound Dex-R molecules that could only be released from the gels due to large morphological changes induced by prolonged exposure to OMF. B is the effective concentration of strongly bound Dex-R molecules, while k_1 and k_2 are the corresponding release rate constants. The theoretical curve fits are shown in Figure 2. Figure 2A describes the effect of an OMF on collagen gels containing nanoparticles. An initial release burst is seen in this system in the absence and presence of an OMF. The application of an OMF in this system affects mainly the magnitude of the initial release burst, which is almost doubled in the presence of an OMF. It appears that a larger number of molecules diffuse freely from the gels when subjected to an OMF. The release of Dex-R following the initial burst is slower than that observed when microparticles are entrapped in collagen gels. This is explained by the minor morphological changes induced by an OMF in collagen gels containing nanoparticles. Figure 2B describes the effect of an OMF on the release rate of Dex-R from collagen gels containing magnetic microparticles. It is interesting to note that the initial release burst is smaller in this system compared to the amount of Dex-R released over time particularly when the gels are exposed to an OMF. It seems that the morphological changes induced by an OMF result in continuous Dex-R release which overwhelms the initial burst. As a result, the release rate of Dex-R from gels containing microparticles is well described by a single-exponential kinetic term.

Effect of Temperature on the Release Properties of Magnetic Collagen Gels. It is well-known that heat is generated when magnetic particles are subjected to a variable magnetic field. Magnetic induced hyperthermia has been used as therapy for cancer treatment.[35−38] This raised the concern that the Dex-R release profiles observed might be due to changes in the temperature of the gels induced by the movement of magnetic particles when submitted to an OMF.

The effect of the temperature on the Dex-R release from the magnetic gels in the absence of an OMF is presented in Figure 3. The results show an increase in the release of drug analogue for gels incubated at 37 °C compared to gels kept at room temperature. However, when measuring the temperature of the gels directly, during a period of 3 days, the temperature of the magnetic gels submitted to an OMF at room temperature was constant and did not increase ($\Delta T \leq 3$ °C). These results indicate that although changes in temperature have an effect on the release, the OMF as applied in our experimental setting does not change the temperature of the gels and therefore the effect of the OMF on the release is not due to damage caused by the increase in temperature. There might be a local increase in the temperature in the microenvironment surrounding the magnetic nanoparticles, but it is not enough to cause change in the gel temperature or damage to the gel. The fact that release was observed only when the sample was positioned at the edge of the magnet but not

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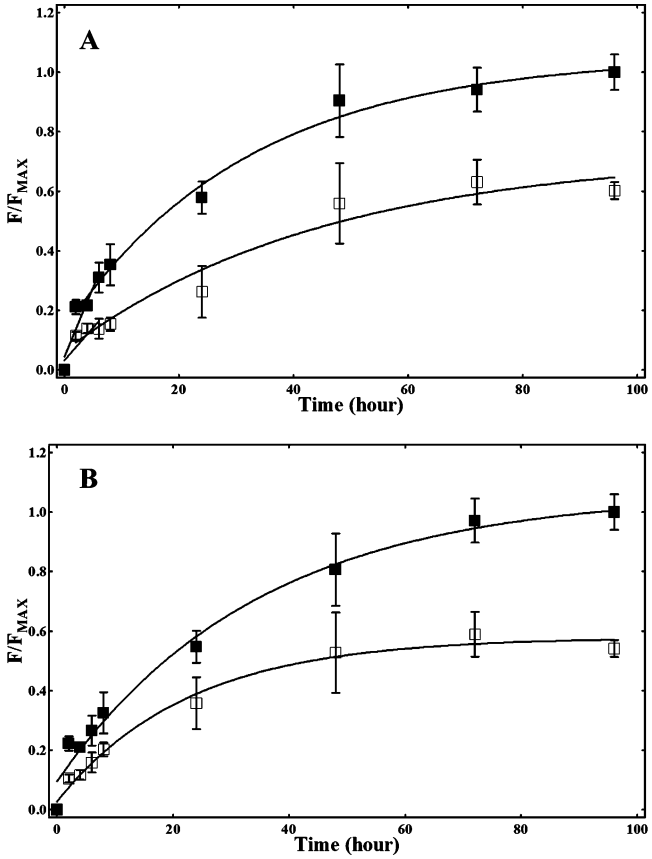

Figure 3. Release of the rhodamine-labeled dextran (Dex-R) from collagen gels containing (A) magnetic nanoparticles and (B) magnetic microparticles in the absence of an OMF and at (□) room temperature and (■) 37 °C. The Dex-R molecular weight was 70 000 g/mol.

when it was positioned at the center also indicate that the effect on the release is due to particle movement and not change in temperature.

Effect of Particle Concentration. Since the release rate of Dex-R from magnetic collagen gels is induced by morphological changes in the gels when subjected to an OMF, it is reasonable to expect that changes in the particle concentration would also affect the release rate of Dex-R from magnetic collagen gels. Collagen gels prepared with increasing concentration of magnetic nanoparticles were exposed to an OMF for 12 h at room temperature. Control experiments were carried out for gels containing the same levels of Dex-R and magnetic particles under the same conditions but in the absence of an OMF. Figure 4 describes the percent enhancement (% E) of the release rate of Dex-R from magnetic collagen gels when subjected to an OMF as a function of magnetic nanoparticle concentration in the gels. The percent enhancement was calculated by the following equation:

$$\% \mathrm{E} = \left(\frac{[F_{\max}(\mathrm{OMF})] - [F_{\max}(\text{no-OMF})]}{F_{\max}(\text{no-OMF})} \right) \times 100 \quad (2)$$

where $F_{\max}$(OMF) and $F_{\max}$(no-OMF) are the maximum fluorescence intensities of solutions containing released Dex-R in the presence and absence of an OMF. The release rate of Dex-R from the gels increased with increasing magnetic particle concentration up to a concentration of 1 mg/mL. A higher concentration of nanoparticles did not result in an increased release rate probably due to steric hindrances. The nanoparticles may have blocked the pores in the collagen gels and thus slowed the release of Dex-R from the gels.

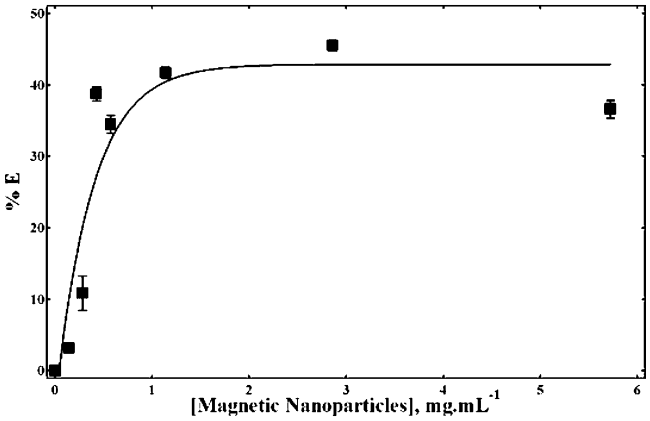

Figure 4. Effect of the magnetic nanoparticle concentration on the release rate of Dex-R from magnetic-nanoparticle-containing collagen gels. The Dex-R molecular weight was 70 000 g/mol. The experiments were carried out at room temperature.

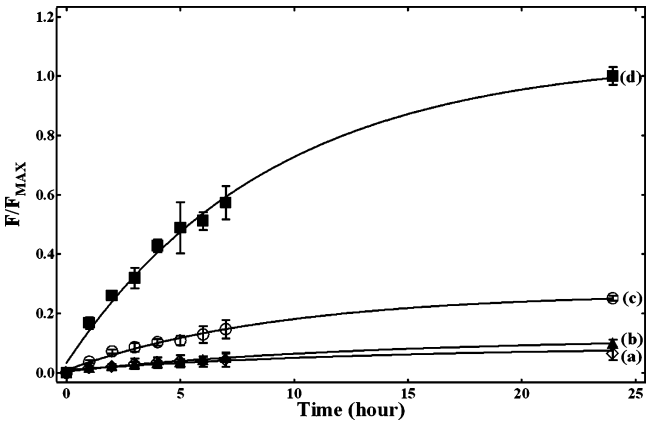

Figure 5. Effect of the molecular weight of Dex-R on its release from collagen gels containing magnetic nanoparticles when exposed to an OMF. The Dex-R molecular weights were (■) 3000 g/mol, (○) 10 000 g/mol, (▲) 40 000 g/mol, and (◇) 70 000 g/mol. The experiments were carried out at room temperature.

Effect of the Molecular Dimensions of the Encapsulated Substance on the Release Properties of Magnetic Collagen Gels. As previously mentioned, Dex-R was chosen as a model drug because it effectively simulates protein-based drugs.[39] It is reasonable to expect that the molecular dimensions of encapsulated drugs as well as the nature of their interactions with collagen would have a profound effect on the release properties of collagen gels. To demonstrate this effect, we prepared magnetic collagen gels that contained Dex-R of molecular weight ranging from 3000 to 70 000 Da. Figure 5 describes the release rate of Dex-R of increasing molecular weights from magnetic collagen gels when subjected to an OMF. The fluorescence intensities of the solutions containing released Dex-R were normalized to the maximum fluorescence intensity of released Dex-R of 3000 Da (curve d). Curves a, b, and c describe the release rate of Dex-R of 70 000, 40 000, and 10 000 Da, respectively, from magnetic collagen gels. As expected, the release rate of Dex-R from magnetic collagen gels increased with decreasing molecular weight of Dex-R. This could be attributed to the increased diffusion rate due to the decrease in size and molecular weight as well as the decreased stability of collagen gels that are known to be stabilized by carbohydrate−protein interactions.[10] The theoretical curve fits in Figure 5 were calculated on the basis of eq 1. The release rate increase could be accounted for by increasing

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the rate constant for diffusion of Dex-R molecules from the gels. It must be noted however that kinetic experiments with Dex-R of 3000 Da were difficult to perform, since the 3000 Da Dex-R diffused freely from the collagen gels even in the absence of an OMF.

Summary and Conclusions

This study describes the effect of an oscillating magnetic field (OMF) on the release rate of Dex-R from collagen gels that contain magnetic particles. Electron microscopy analysis of collagen gels containing iron oxide magnetic nanoparticles and Dex-R reveals that the presence of magnetic nanoparticles or Dex-R did not negatively affect the morphology of collagen gels when exposed to an oscillating magnetic field (OMF). In contrast, collagen gels that contained magnetic microparticles were physically deformed when the gels were exposed to an OMF. However, the fiberlike structure of the affected collagen gels was restored following 12 h of rest. Since the average temperature on the gel did not change over 72 h of exposure to the OMF, we conclude that, under our experimental conditions, the effects of the OMF on the release as well as the structural changes in the gel as a result of exposure to the OMF are not due to the increase in the temperature caused by the oscillation of the magnetic particles. A simple kinetic model was introduced to predict the release rate of Dex-R molecules from collagen gels that contain magnetic particles. The release rate is effectively described by an equation that contains two rate components. One accounts for an initial release burst of loosely associated molecules from the gels, while the second term accounts for the slow diffusion of entrapped Dex-R molecules following the initial release burst. Our results suggest that application of an OMF affects gels that contain magnetic nanoparticles and microparticles differently. The OMF affects mainly the magnitude of the initial release burst in gels containing nanoparticles. On the other hand, the OMF increases the amount of released Dex-R from gels containing magnetic microparticles. Magnetic collagen gels that contain nanoparticles would have a distinct advantage in drug delivery applications requiring a strong initial release burst which is followed by a very slow release component. On the other hand, magnetic collagen gels containing magnetic microparticles should be used when a continuously increasing drug level is sought. It must be noted however that the use of magnetic nanoparticles in magnetic collagen gels leads to a higher gel stability, since no morphological deformation is seen during the exposure of the gels to the OMF at all times. Experiments were carried out to determine the effect of magnetic nanoparticle concentration on the release rate of Dex-R from collagen gels when subjected to an OMF. The release rate of Dex-R increased with increasing magnetic nanoparticle concentration up to a concentration of 1 mg/mL. Higher concentrations of magnetic nanoparticles resulted in gel blockage and decreased release rate. The molecular weight of dextran also affected the release properties of collagen gels. The release rate increases with decreasing molecular weight. This was attributed to the increased diffusion rates of smaller Dex-R molecules and to the decreased stability of collagen gels that are known to be stabilized by carbohydrate−collagen interactions. While theoretically the release rate of collagen gels depends on multiple parameters, our study shows that under our experimental conditions the release rate of Dex-R from collagen gels could be effectively described using a simple kinetic model. Future studies will focus on the development of an instrumental capability to attenuate the frequency and amplitude of the oscillating magnetic field in order to better control the release rate. In addition, a new experimental system will be developed to enable the use of an oscillating magnetic field to release drugs from implanted collagen gels in vivo.

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Supporting Information Available: A movie (AVI) showing the movement of the magnetic particles under the influence of the oscillating magnetic field. This material is available free of charge via the Internet at http://pubs.acs.org.

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