Injectable Superparamagnetic Ferrogels for Controlled Release of Hydrophobic Drugs

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In the past two decades, owing to the significant technological and biomedical applications, considerable attention has been attracted to the development of intelligent hydrogels that respond to external stimuli such as temperature, pH, electric field, specific analytes and enzymes.[1–7] Ferrogels consisting of magnetic nanoparticles embedded in polymer gels are an important category of stimuli-sensitive hydrogels that respond to external magnetic stimuli.[8–15] Their unique magnetoelastic property endows ferrogels with great potential for magnetic-controllable drug-delivery systems. However, compared to drug-release studies of other stimuli-sensitive hydrogels, about which many papers have been published,[16–18] this field has not been explored sufficiently to date. Recent studies by several groups on various types of ferrogels for in vitro release have implemented magnetically controlled drug release.[19–24] Nevertheless, nearly all reported studies are focusing only on water-soluble drugs, due to the overall hydrophilic nature of ferrogels. Effective incorporation of important hydrophobic drugs in ferrogels still remains a challenge. Here, we address this problem by employing Pluronic copolymers as a gelling material that is biocompatible and has been extensively used in the pharmaceutical research for wound coating, burn treatment, and controlled drug release, due to its stability and low toxicity.[25–28] Pluronic is a group of ABA-type triblock copolymers consisting of one poly(propylene oxide) (PPO) block and two poly(ethylene oxide) (PEO) blocks. Above a certain critical concentration and temperature, Pluronic copolymers associate into micelles via hydrophobic interaction, as the PPO segments form a nearly water-free central core surrounded by a hydrated PEO corona.[29–31] Many research groups have studied this micellar structure and shown that low-molecular-weight hydrophobic compounds can be solubilized in the PPO core of Pluronic micelles, resulting in enhancement of the solubility of hydrophobic molecules in aqueous solutions.[32–35]

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We have developed, for the first time, a new type of ferrogel consisting of superparamagnetic iron oxide nanoparticles (SPIONs) and Pluronic F127 (PF127) copolymer (abbreviated as SPEL). Pluronic copolymers possess a unique viscosity-adjustable property, which renders PF127 gels easy to handle compared to conventional crosslinked hydrogels based on, amongst others, poly(vinyl alcohol), gelatin, and poly (*N*-isopropylacrylamide).[36,37] As an example, PF127 aqueous solutions (18–30% (w/v)) appear to be fluid at low temperatures (0–5 °C), and can transform into a gel-like cubic liquid crystal within a few seconds when heated.[38–40] Such a fast sol–gel transition renders PF127 gels particularly useful as an injectable vehicle for local delivery of various therapeutic agents.[41–45] Ordinary ferrogels based on crosslinked polymeric structures are always in solid form, often requiring surgical insertion for in vivo applications, giving rise to tissue irritation and trauma. In comparison, SPEL features a temperature-dependant reversible sol–gel transition. Since this transition temperature can be adjusted by varying the concentration of PF127,[36,37] it is possible to apply SPEL as an injectable drug-delivery system for magnetically modulated release of hydrophobic bioactive agents on pathological sites.

SPIONs were prepared through the high-temperature decomposition method, and were obtained as colloidal particles dispersed in common nonpolar or weakly polar solvents, such as hexane, tetrahydrofuran, chloroform, etc.[46] Subsequently, these SPIONs were transferred to an aqueous solution with the assistance of PF127, as described elsewhere.[47] A representative transmission electron microscopy (TEM) image shows that SPIONs in tetrahydrofuran are highly monodisperse, with an average particle diameter of 10.6 nm ($\sigma = 4{,}7\%$, Fig. 1a). In our previous work, we demonstrated that SPIONs are coated by a hierarchical layer composed of PF127 and oleic acid.[47] The PEO segments of PF127 endow SPION with hydrophilicity. The resulting water-soluble SPIONs were introduced to the concentrated Pluronic aqueous solution at low temperature (4 °C).[36] Upon gelation at elevated temperatures, the PF127 micelles organized into an ordered structure, shown by TEM (Fig. 1b). Because the specimen was not stained, only the micelles with encapsulated SPIONs are visible. The size of an individual SPION-containing micelle is around 13 nm,which is very similar to the reported diameter of an empty micelle formed in a concentrated PF127 solution (>20%) with similar concentration used in this work.[48] The encapsulation of SPIONs inside the micelle did not significantly perturb micellar morphology or size. The magnetic properties of both SPION and SPEL were examined at room temperature using a vibrating sample magnetometer.

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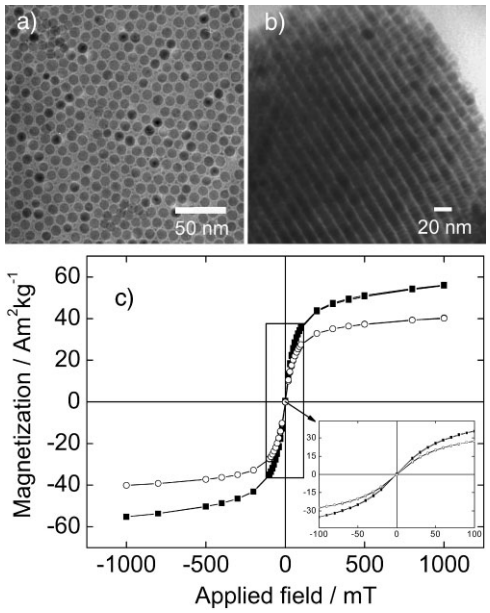

Figure 1. a) TEM image of SPIONs dried on a carbon-Formvar-coated 200 mesh copper grid. b) TEM image of SPEL dropped on the same type of grid at 4 °C and allowed to dry at 37 °C; c) Room-temperature magnetization curves of SPIONs (-■-) and SPEL (-○-).

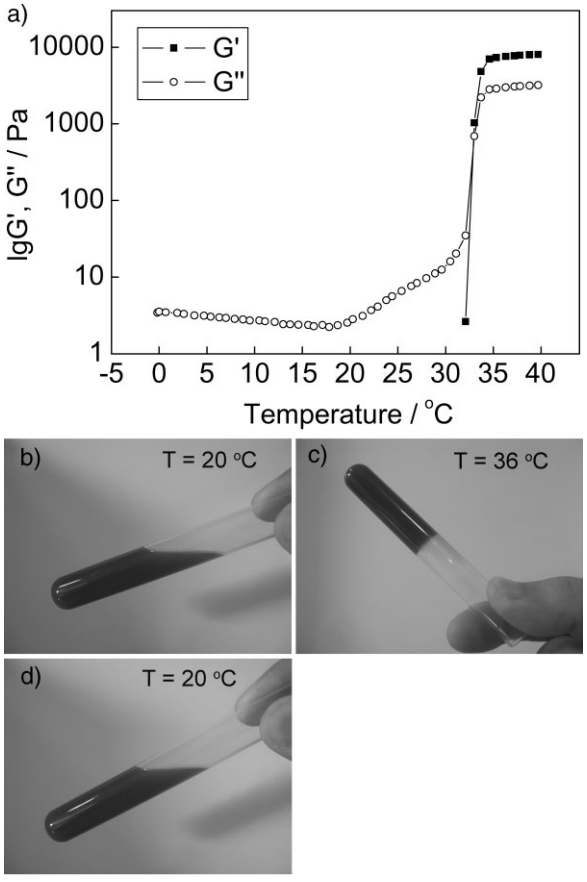

Figure 2. a) Rheological data obtained from SPEL containing 17.5% (w/v) PF127. Photographs of SPEL b) below the transition temperature, c) above the transition temperature, and d) taken for the same sample when chilled again.

Magnetization measurements have shown that iron oxide nanoparticles retain superparamagnetism after incorporation into the ferrogel, indicating that the magnetic moments of particles can easily reorient upon removal of the magnetic field (Fig. 1c). Saturated magnetizations (*Ms*) of SPIONs and SPEL are 54.1 and 39.4 A m^2 kg^{-1}, respectively. The lower *Ms* value of the SPEL was expected, due to the formation of the magnetic-dead layer on the surface of SPIONs after transfer to aqueous phase.[49] Initial magnetic susceptibility χ_0 (obtained by measuring the slope at the *M*–*H* curve origin) for SPEL is lower than that of SPIONs. This indicates a less effective coupling between particles incorporated in SPEL, caused by increased first-neighbor distances.[50]

The rheological response as a function of temperature was examined on SPEL with typical PF127 concentration at 17.5% (w/v). Figure 2a shows the temperature-dependant rheological data of the SPEL, where elastic modulus G' and viscous modulus G'' are plotted as functions of temperature. It was found that at low temperatures the ''sol'' behaves as a viscous liquid ($G'' > G'$), while at high temperatures the behavior becomes elastic ($G' > G''$). The critical temperature at which the PF127 solution forms a gel depends on the concentration of the polymer solution.[39] The transition temperature was determined at 32.5 °C for the SPEL containing 17.5% (w/v) PF127, as indicated by the crossing of G' and G'' curves in Figure 2a. The photographs in Figure 2 show the reversible sol–gel transformation of an aqueous solution containing 17.5% (w/v) PF127 and SPIONs (4 mg Fe mL^{-1}) before and after heating above the critical transition temperature. The solution remains as a ''sol'' at room temperature (Fig. 2b), and gelation occurs when the solution is warmed up to human-body temperature, resulting in a solid gel, as seen in Figure 2c. The fluidity is thereafter restored when the gel is cooled down (Fig. 2d). In addition, the SPEL remains stable for at least five months when stored above the gelation temperature (data obtained so far).

For in vitro release tests, indomethacin (IMC) was selected as a model drug. IMC has a very low solubility in water,[51] whereas in the SPEL containing 17.5% (w/v) PF127 the concentration of IMC can be significantly increased, due to incorporation into hydrophobic cores of micelles. TEM imaging (Fig. 1b) shows that SPEL constitutes of micelles arranged in a cubic liquid-crystalline structure. As depicted in Figure 3a, each micelle comprises a hydrophobic core (PPO), in which the drug molecules are solubilized, and a hydrophilic corona (PEO), acting as the scaffold of the ferrogel. SPIONs are encapsulated inside of some micelles during the phase transfer of nanoparticles. The release profiles of IMC from ferrogel are shown in Figure 4, both in the absence and presence of a magnetic field (MF). For the release test without a MF (Fig. 4a), the drug is continuously released to up to 95% in 7 000 minutes (ca. 5 days). The drug release depends mainly on the diffusion of IMC molecules from the hydrophobic PPO cores through water channels in the SPEL, and also, to some extent, on dissolution of PF127 gel.[43] The release profile was a quasi-linear curve, and no significant initial burst could be observed. The release test was

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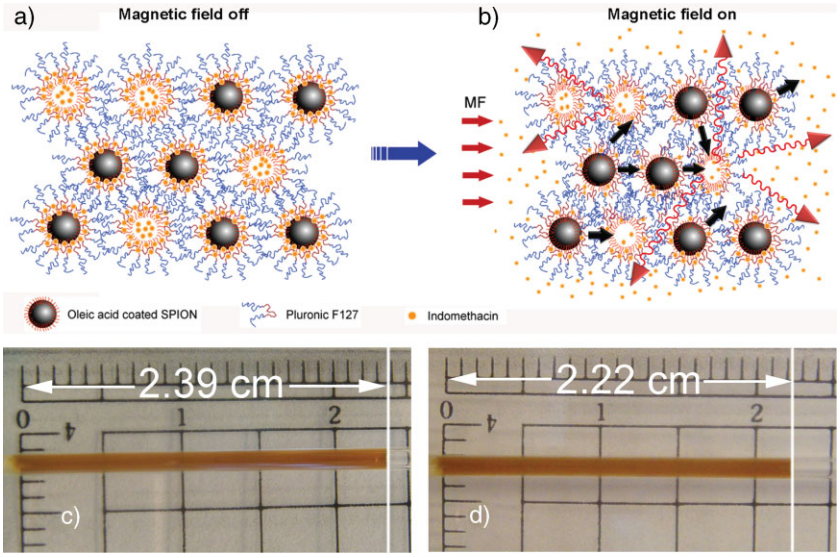

Figure 3. Schematic representation of ordered microstructure of SPEL: a) before applying the MF, IMC drug molecules are encapsulated in the hydrophobic moiety of micelles; b) when the MF is on, SPIONs orient and approach each other, squeezing the micelles and leading to enhancement of IMC release. Corresponding photographs of SPEL in a capillary c) before and d) after applying a MF.

also conducted in the presence of a MF (300 mT, see Supporting Information). We found that in that case, the IMC was released much faster. The half-time ($t_{1/2}$) of drug release is reduced to 1500 minutes, compared to the 3195 minutes when recorded without the MF (Fig. 4).

Under the influence of a MF, the release rate of conventional crosslinked ferrogels is decreased, as reported elsewhere.[20–22] Such a contradictory performance results from the essential difference of the microstructure between the crosslinked ferrogel and the SPEL.[24] The crosslinked ferrogels contain interlaced polymer chains with magnetic particles embedded. Numerous channels and pores in the gel serve as a reservoir and an escaping pathway for the drug. When subjected to a MF, magnetic particles are attracted, pulling the polymeric scaffold. As a consequence, the pore size is reduced and the diffusion of the drug through the pores is hindered. On the other hand, as mentioned before, the SPEL contains large amounts of close-packed micelles, and therefore no pore opening or closure are expected, as what crosslinked ferrogels behave. The hydrophobic drugs are encapsulated in PPO cores, and the interconnecting aqueous moiety exists in between the micelles, functioning as a pathway for diffusion of drug molecules. As depicted in Figure 3b, while applying a MF, hydrophobic cores in the ferrogel are "squeezed" when SPIONs tend to orient and approach each other, so that the local concentration of IMC is increased, producing a large concentration gradient between PPO cores and water channels in the SPEL, pumping out many more drug molecules. Therefore, under the applied field, the release rate is enhanced due to the constriction of the SPEL. To further clarify the release mechanism, we introduced a small amount of SPEL into a capillary with the inner diameter of 1 mm, and measured the length of the gel before and after applying a MF (Fig. 3c and d). Being exposed to the MF (300 mT) for 2.5 h, the length of the SPEL decreases by 7%, from 2.39 to 2.22 cm. Taking into account that about 80% of the SPEL is incompressible water, the volume of the polymeric matrix is decreased by 35% when subjected to a MF. It is therefore expected that such a dramatic field-dependent volume change within a few hours gives rise to the enhancement of drug-release rate at the initial period of the release test.

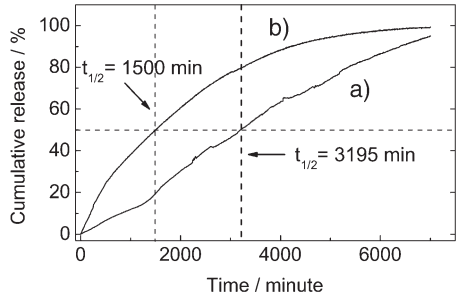

Figure 4. Release profiles of IMC from SPEL in PBS (pH 7.4) at 37 °C with a) MF off; b) MF on.

In summary, a novel type of SPEL has been prepared from SPIONs and PF127 gel. Hydrophobic drugs (e.g., IMC) can be loaded in the SPEL in a "sol" state at low temperatures, and the drug-loaded "sol" can be injected into tissues. After injection, at the human-body temperature, the "sol" rapidly becomes a gel, which acts as a matrix for delivering hydrophobic drugs. The release rate of the drug from SPEL can be tuned with an external magnetic field. Therefore, this novel ferrogel can be used as an injectable matrix for magnetically controlled release of hydrophobic bioactive agents.

Experimental

Preparation of Water-Soluble SPIONs: The Pluronic-coated SPION were prepared according to our previous study [47]. Briefly, SPIONs were synthesized through decomposition of iron oleate complex ($Fe(Oleate)_3$) in a high-boiling-point solvent (dioctyl ether, $T_B = 287$ °C). Subsequently, a tetrahydrofuran suspension of SPIONs was mixed with an equal volume of aqueous solution of PF127 (10%, w/v) followed by vigorous agitation for 30 min. The solution obtained was subjected to evaporation to remove the organic solvent, resulting in a stable aqueous suspension of SPIONs.

Preparation of IMC-Loaded SPEL: An aqueous suspension of SPIONs was mixed with a PF127 solution containing a desired amount of IMC at 4 °C, to obtain a solution containing PF127 (17.5%, w/v), SPIONs (4 mg Fe mL^{-1}), and IMC (1 mg mL^{-1}). The mixture was votexed to form a homogeneous suspension and the temperature was increased to 35 °C. Gelation occurred within a few seconds of warming. The SPEL was cooled down again and the previous two steps were cycled for three times.

Characterization: Transmission electron microscopy (TEM) images were taken using a JEOL JEM-2000EX. The magnetization measurements were carried out at room temperature using a vibrating sample magnetometer (VSM-NUVO, MOLSPIN, Newcastle Upon Tyne, UK) with

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a maximum applied field of 1 T. The change in viscosity of the ferrogel at increasing temperature was observed using the AR2000 rheometer (TA Instrument). Briefly, a small amount of SPEL (1.30 mL) was pipetted on the sample holder and a conical titanium plate ($\phi = 40$ mm) was loaded over the sample. The gap between the sample holder and the titanium plate was maintained at 800 μm for all the measurement performed. Maintaining a constant shear force, the sample was heated at a rate of 1 °C min^{-1}.

In vitro Release of IMC: A Franz diffusion cell was used for studying the drug release. The donor chamber was separated from the receiving chamber by a cellulose membrane (MWCO: 1.2–1.4 kDa, Spectrum Inc., Breda, the Netherlands). The IMC-loaded SPEL was injected into the donor chamber at 4 °C, and the temperature of the solution was slowly increased to 37 °C. The receptor chamber was filled with 10 mL of phosphate buffer saline (PBS, pH 7.4, Medicago AB, Uppsala, Sweden). The release experiments were carried out thermostatically at 37 °C. A permanent magnet with field of 300 mT was used in order to examine the effect of the MF on drug-release rate. Two comparative sets of experiments were undertaken; one without the MF, as a control, and one with a MF. The IMC concentration was recorded using an UV-visible spectrophotometer (Varian Cary 100 Bio, $\lambda = 319$ nm) equipped with an autosampler. The data points were collected automatically every 5 min.

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