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Influence of Magnetic Nanoparticle Arrangement in the Ferrogels for Tunable Biomolecules Diffusion

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Abstract

Magnetic sensitive hydrogels (ferrogels) with tunable nanochannels were prepared by poly (vinyl alcohol) (PVA) with iron oxide magnetic nanoparticles under uniform magnetic field in the freezing-thawing process, evaluated by differential scanning calorimeter (DSC), and the influence of magnetic nanoparticle arrangement on the biomolecules diffusion behavior would been investigated. The nanochannels self-assembled by arranged magnetic nanoparticles could be tuned by manipulated the direction of magnetic field, which results in the formation of "needle-like" structures from the magnetic nanoparticles parallel or perpendicular aligned to permeation direction (anisotropic ferrogels). The effect of the biomolecules diffusion between anisotropic and isotropic (random nanoparticle dispersions without magnetic field, as control) ferrogels would be observed by the diffusion diaphragm cell. It was established that the parallel-aligned ferrogels exhibit the higher drug diffusion rate compared to the isotropic ferrogels, whereas the perpendicular aligned ferrogels display the lowest biomolecules diffusion rate. The novel ferrogels were anticipated to apply in the bio-membrane, and the nanochannels for the biomolecules (Mw: ca. 100-100k Da) diffusion could be constructed precisely by the magnetic nanoparticle arrangement.

Keywords: magnetic sensitive hydrogels, permeation, nanochannels manipulation, bio-membrane

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1. Introduction

Intelligent polymer based nanocomposites have the unique ability to change swelling behaviors, permeability and elasticity in a reversible manner by external stimulus, such as pH, thermal, electric field and magnetic field [1-19]. Magnetic-sensitive polymer is superior to that traditional stimuli response polymer because magnetic stimulation is an action-at-distance force (non-contact force) which is easier to adapting to biomedical devices [19-24]. Polymeric nanocomposites consisting of polymeric hydrogels filled with magnetic nanoparticles have been the subjects of many studies in the recent two decades, called as ferrogels. These ferrogels are successfully developed with regard to their applications to several biomedical and industrial fields such as muscle-like sift linear actuators, drug controlled release, and dialysis membrane.

Membranes are practical tools for molecular separation because they provide an energy efficient green technology. Nanoscale manipulation of the membrane nanochannels exhibits an innovative idea to control the membrane permeability. Csetneki *et al.* [4] reported thermal/magnetic sensitive nanocomposite polymer membrane (magnetic polystyrene latex-poly(N-isopropyacrylamide) with on/off switching control through thermal manipulation, which nanochannels could be opened when temperature is above the collapse transition temperature, but closed when temperature is below the collapse transition temperature. In our previous studies [19-24], we also fabricated a smart magnetic hydrogels for the development of a new magnetically induced drug delivery system. By applying direct current (DC) magnetic fields, they were able to switch the drug release profile of the hydrogels between ‘‘on’’ and ‘‘off’’ mode with the ‘‘random-dispersion’’ and ‘‘aggregation’’ of the magnetic nanoparticles, respectively

Recently, Varga et al. [25-26] reported a fascinating result to tune elastic modulus with controlled anisotropy of the ferrogel. It was established that the uniaxial field structured composites exhibit larger excess modulus compared to the random magnetic particle dispersions. The most significant effect was found that the mechanical stress of the ferrogel would be modulated under controlled anisotropy (the applied magnetic field is parallel or perpendicular to the particle alignments). On this background, we will try to design the anisotropy ferrogels, which magnetic nanoparticles are parallel or perpendicular aligned to diffusion direction, and the drug permeability of anisotropic and isotropic (random magnetic nanoparticles without magnetic field control as control) ferrogels would be studied in this work.

The main purpose of magnetic nanoparticls arrangement is to control the direction of the nanochannels (related to the diffusion direction) in the ferrogel by external magnetic field (parallel or perpendicular to the particle alignments) and thus control the permeability of the biomolecules in the ferrogels. The characterizations of PVA physical cross-linked by freezing and thawing cycles could be examined carefully by DSC analysis. The pearl-chain structures of the ferrogels developed by the magnetic field would be observed by optical microscope. The diffusion properties of the ferrogels would be evaluated by some biomolecules, such as creatinine, vitamin B12, cyotochrome c, bovine serum albumin with varied molecules weight

2. Methods

2.1 Fabrication of ferrogels with various sizes of magnetic nanoparticles

The intermolecular interactions like hydrogen bond-bridges or polymer microcrystals are responsible for the formation of the three-dimensional network structure. A so-called freezing-thawing process was used to prepare the ferrogel [27]. First, 0.05 g/mL poly(vinyl alcohol) (PVA, Fluka, M.W.: 72,000,

degree of hydrolysation: 97.5-99.5 mol%) was dissolved in 10 ml dimethylsulfoxide (DMSO) at 80°C under stirring for 6 h, and then mixed with 0.17 g/mL of magnetic particles (ca. 150-250 nm, purchased from Aldrich) at 60°C under ultrasonication for 6 h to ensure that the magnetic particles can be well dispersed. The resulting solution was then poured into plastic dish and kept frozen at -20°C for 16 h. Subsequently, the gels were thawed at 25°C for 5 h. This cyclic process including freezing and thawing was repeated for 5 times. Finally, prior to the release test, the ferrogels were washed five times and then immersed in the water for 24 hr to completely remove DMSO.

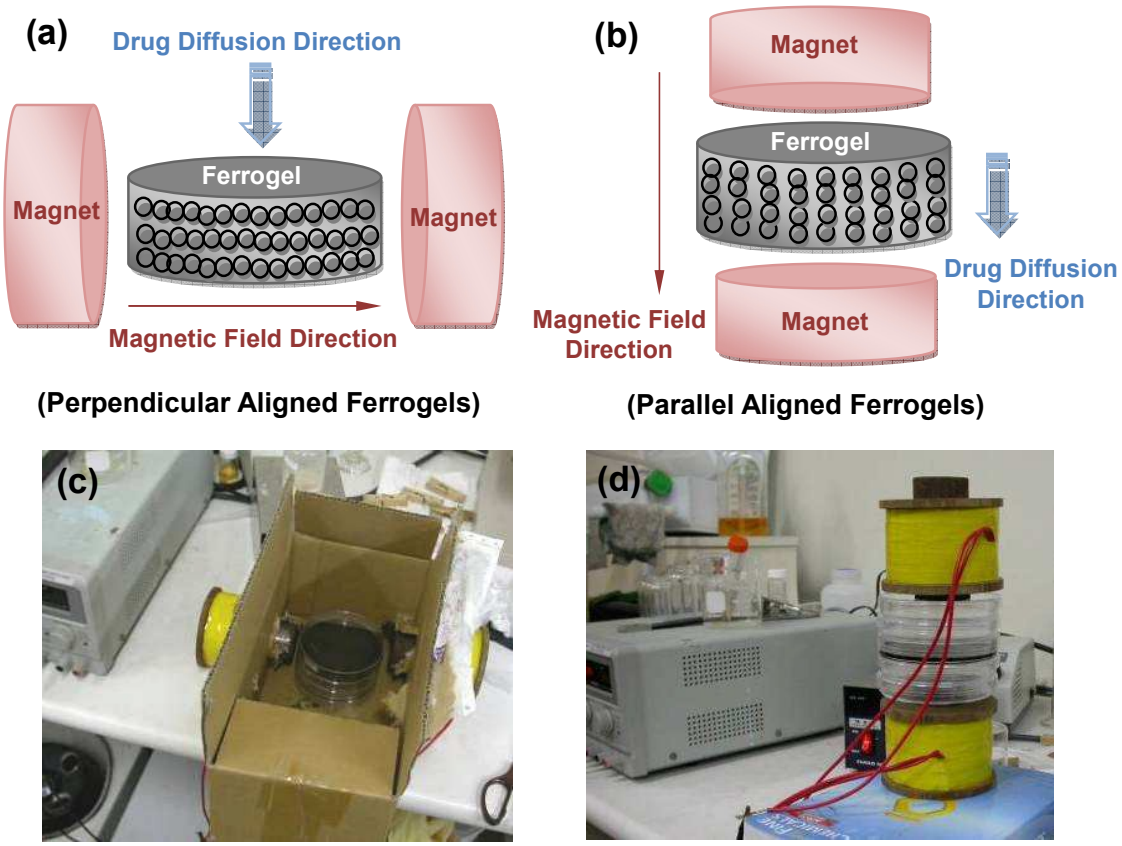

Figure 1 Schematic drawing of the experimental set-up for ferrogel preparation under uniform magnetic field: (a) and (c) the iron oxide nanopartices of the ferrogels were arranged by left-to-right electronic magnets ("perpendicular" to the drug diffusion direction); (b) and (d) the iron oxide nanopartices of ferrogel were arranged by top-to-down electronic magnets ("parallel" to the drug diffusion direction)

2.2 Fabrication of anisotropic ferrogels

The main purpose of the present work was to establish the effect of direct of magnetic fields on the drug diffusion behavior. As shown in Figure 1, PVA hydrogels loaded with randomly, perpendicularly, and parallelly distributed iron oxide nanoparticles regarding to the drug diffusion direction, were modulated by uniform magnetic field Fig. 1a and 1c represents that the iron oxide nanopartices of the ferrogels were arranged by left-to-right electronic magnets, which called as "perpendicular" to the drug diffusion direction. Fig. 1b and 1d illustrates that the iron oxide nanopartices of ferrogel were arranged by top-to-down electronic magnets, which called as "parallel" to the drug diffusion direction.

2.3 Drug diffusion test

The diffusion coefficients of the solutes were measured by the diffusion diaphragm cell (side-by-side cell) [21]. The solution in the donor side is 80 ml of isotonic phosphate buffer (PBS) (pH7.4) containing 200 ppm of the model drug (creatinine, vitamin B_{12}, cyotochrome c, and bovine serum albumin). The receptor compartment, separated by the ferrogel, was filled with 80 ml of PBS solution. The concentration of each compound in the receptor compartment was determined using a UV spectrophotometer. The permeation coefficient (P, cm^2/min) was calculated according to the following equation for the diaphragm cell:

$$\ln\left(\frac{C_{do}}{C_d - C_r}\right) = \frac{2[DH]At}{\delta V}, (P = DH) \qquad (1)$$

where C_{d0} is the initial concentration of the permeant in the donor compartment; C_d and C_r are indicative of the concentrations in the donor side and receptor side, respectively; D is the diffusion coefficient (cm^2/min) [28-231]; H is the partition coefficient,; A is the effective area of the ferrogel; δ is the thickness of the ferrogel; V is the volume of solution in the donor or receptor compartment (both are 80 ml). By plotting $\ln[C_{d0}/(C_d–C_r)]$ versus time (t), the permeability coefficient (P) can be calculated from the slope

of the line by Eq.(1). Each data point was obtained by averaging of at least three measurements.

Moreover, the dry weight (W_{dry}) of drug-free ferrogel was immersed in the release medium until equilibrium state and then the wet weight (W_{wet}) were recorded. Subsequently, the ferrogel was immersed in 10ml of vitamin B12-containing medium. Partition coefficient (H) was determined from the initial (C_0) and equilibrium (C_e) concentrations of vitamin B_{12}-containing mediums by Eq. (2) [30-31].

$$H = \frac{W_{dry}(C_0 - C_e)}{W_{wet}C_e} \quad (2)$$

2.4 Pearl-chain structure of anisotropic ferrogels test

0.01 g/mL magnetic particles were dissolved in the 0.1 g/mL PVA solution (dissolved in the DMSO at 80^{o}C beforehand). Further, the behaviors of magnetic particles would be observed directly by optical microscopic (Olympus, Japan) in the absence and present of magnetic field.

3. Results and Discussion

3.1 Physical cross-linking (freezing and thawing process) of the ferrogel

The PVA ferrogel was fabricated by physical cross-linking method (freezing and thawing process) due to the hydroxyl groups of PVA molecules participated in hydrogen bonding, displaying elastic properties. A continuous thermal analysis observed by differential scanning calorimeter (DSC) was used to evaluate the PVA crystal growing with freezing-thawing cycles. As show in Fig. 2, the number of cross-linking points and the crystal region of PVA hydrogels expanded with freezing-thawing cycles increase. DSC measurement is based on the fabrication process of the ferrogels (freezing and thawing

process). The control parameter in the DSC is (1) cooling from 25°C to -20°C (cooling rate: 1°C/min from); (2) kept frozen at -20°C for 16 hr; (3) heating -20°C to 25°C (heating rate: 1°C/min); (4) kept thawed at 25°C for 5 h. This cyclic process including freezing and thawing was repeated for 6 times.

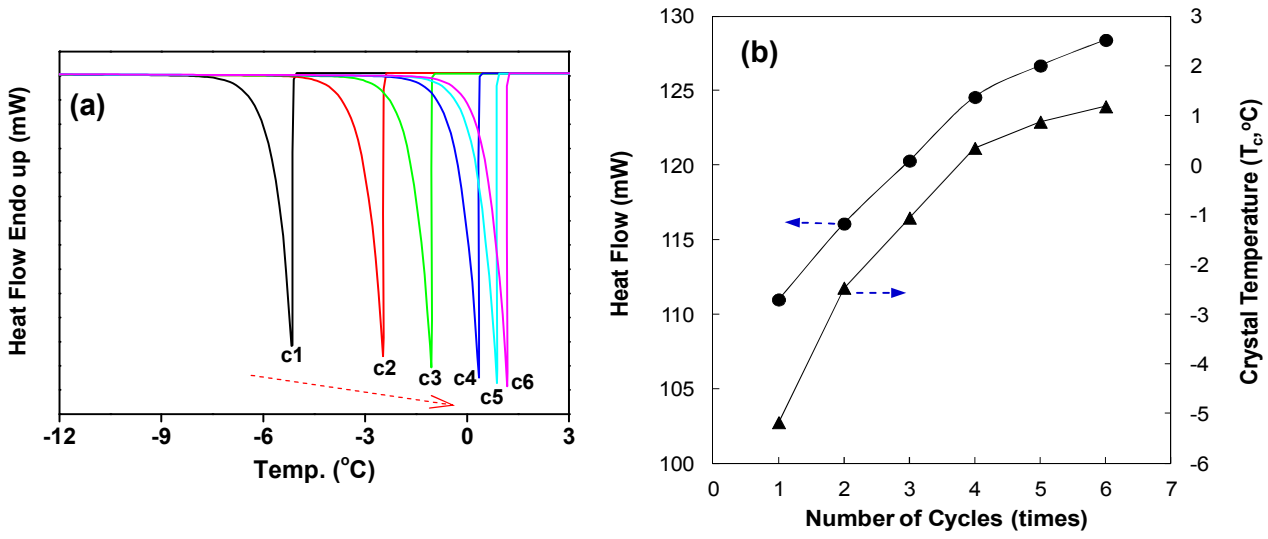

Figure 2 DSC analysis of PVA physical cross-linking by freezing and thawing cycles: (a) cooling curve; (b) integrated area of heat flow and crystal temperature (T_c) change with different cycles.

The result (Fig.2a) show that crystal temperature (T_c) of the PVA ferrogel increases with the cycles of freezing and thawing increased respectively (-5.3~1.1°C, cycle 1~6, c1~c6), indicating the plenty of cross-linked points induce the solid network of PVA ferrogel. Furthermore, the area of crystallization (integrated peak area) also enlarges with the cycles increase. It also demonstrated that the higher crystallization of the PVA ferrogel and the stronger network would be found in the higher cycles of freezing and thawing process. However, it seems to be "saturation" during the 5-6th cycles, only slightly T_c and crystallized area increase, (Fig.2b) which means the process of PVA crystallization and hydrogen bonding have been stable. Therefore, five cycles of the freezing and thawing process would be used in

this study.

3.2 Pearl-chain structure of anisotropic ferrogels

While the imposed field induces magnetic dipoles, mutual particle interactions occur if the particles are so closely packed that the local field can influence their neighbors. The particles attract with each other when aligned in an end-to-end configuration and thus a "pearl-chain structure" was developed via the attractive forces. From the optical microscopic images (Fig. 3), it was revealed that a demonstrated test that 0.01 g/mL magnetic particles were randomly distributed in the 0.1 g/mL PVA solution (dissolved in the DMSO at 80^{o}C beforehand) in the absence of MF. However, the magnetic particles attract with each other to line up in an order form in the present of MF. By controlling the magnetic nanoparticle arrangement with perpendicular and parallel to drug diffusion direction, the pearl-chain structure ferrogels were prepared for the further drug diffusion test. The isotropic ferrogel (random direction of magnetic nanoparticles) was used as reference.

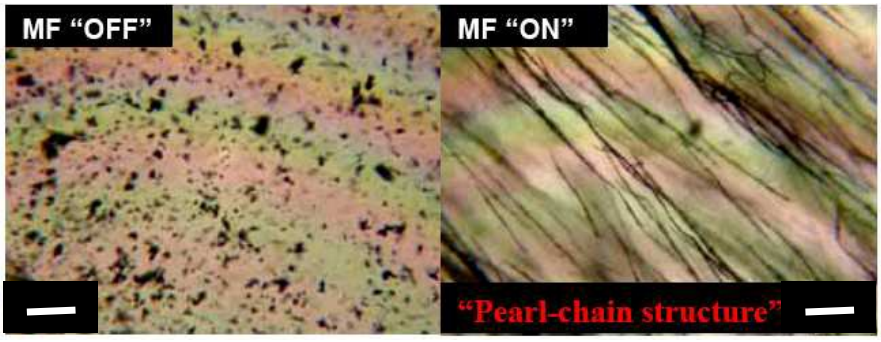

Figure 3 Pearl-chain structure was developed with the magnetic field in optical microscope (scale bar: 5μm)

3.3 Biomolecules permeated behavior in the anisotropic ferrogel

Biomolecules diffusion behaviors were described in Fig. 4. The lower initial diffusion state (the

slope in Figure 4) of the biomolecules (vitamin B12) is because the biomolecules would pass through the bumpy nanochannels in the ferrogel, which induces the biomolecules are difficult to get across. The diffusion rate of the biomolecules would increase at ca. 40 min, when the biomolecules pass though the membrane. In other hand, the result shows that the drug diffusion rate would change with the arrangement of iron oxide nanoparticles, implying the channel parallel to drug diffusion direction exhibits the highest rate of the biomolecules diffusion, whereas the perpendicular direction is the lowest. It suggests that the biomolecules action would be restricted in the perpendicular-aligned ferrogel and thus difficult to pass through, but it is freely flowing in the parallel-aligned ferrogel. Therefore, it was anticipated to control the arrangement of magnetic nanoparticles in the ferrogel could modulate the biomolecules diffusion rate. It would be useful for the application in the dialysis membrane with controlling the diffusion nanochannels by magnetic field.

The influence of the respective constituting components of the ferrogels on the permeability coefficient (P), diffusion coefficient (D), and partition coefficient (H) of the model drug (vitamin B12) will be systematically investigated in this work. When the nanochannels of the ferrogel is parallel to the drug permeation, it caused a considerable increase (2.2 times) in the P value (480×10^{-6} cm^2/min) compared to that is perpendicular to the drug permeation (223×10^{-6} cm^2/min), and 1.6 times increase compared to the random magnetic particles of the ferrogels (306×10^{-6} cm^2/min), as shown in Table 1 and Fig.4. It was believed that there exists a relationship between the P and H value of the drug inside the membrane (see Eq. (1), where P=DH). H value in the parallel ferrogel exhibits the highest drug permeation, whereas the perpendicular ferrogel is lowest, which is in the order: parallel (0.151) >random

(0.142) >perpendicular (0.135) ferrogel, as related to the formation of nanochannels morphology and the direction of drug permeation, as illustrated in Table1.

Table 1 Permeation coefficient (P), diffusion coefficient (D) and partition coefficient (H) of the vitamin B12 in the various ferrogels; the arrangement of iron oxide nanoparticles in the ferrogels are random, perpendicular and parallel to the drug diffusion direction, respectively

	Random	Perpendicular	Parallel
P (cm^2 /min) $\times 10^6$	306	223	480
H	0.142	0.135	0.151
D(cm^2 /min) $\times 10^6$	2155	1726	3179

The value of D would be used to determine the interaction between Fe_3O_4 nanochannels and drug diffusion. The diffusion coefficient (D) will play a rather important role to evaluate the behavior of drug inside the ferrogel. It could be found that the D curves show similar trend to the P. D value decreased rapidly when the ferrogel is perpendicular to the drug permeation (1726×10^{-6} cm^2/min), compared to that the parallel (3179×10^{-6} cm^2/min) and random (2155×10^{-6} cm^2/min) ferrogels. It is because the biomolecules inside the ferrogel was obstructed more strongly in the perpendicular ferrogels rather than the parallel and random ones.

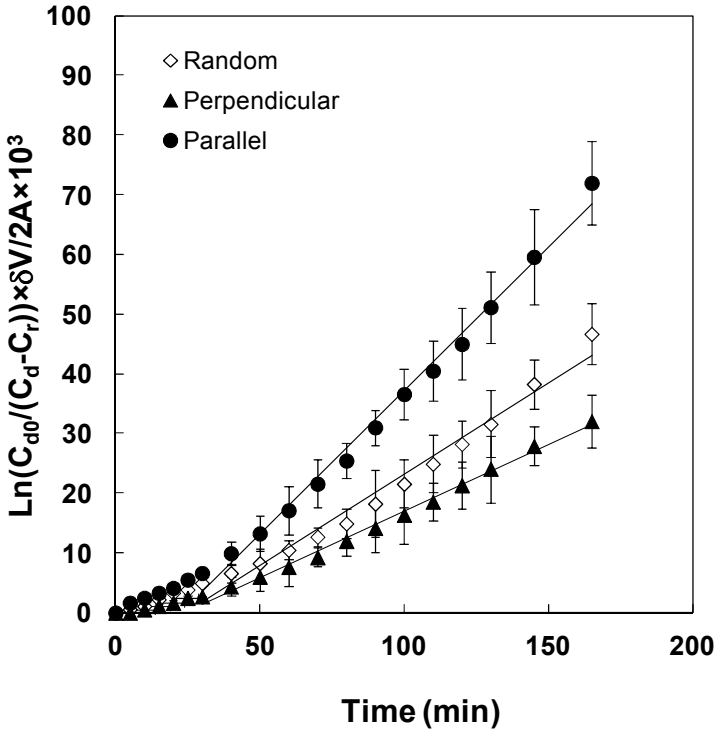

Figure 4 Biomolecules (vitamin B12) permeated behaviors in the various ferrogels; the arrangement of iron oxide nanoparticles in the ferrogels are random, perpendicular and parallel to the drug diffusion direction, respectively. There are two slopes (permeation coefficient, P) in the permeation experiment. The first one is the slower initial state of biomolecules permeation and the second one is the higher general state of biomolecules permeation. P was determined from the second linear slope.

Creatinine (MW 113), vitamin B_{12} (MW 1355), cyotochrome c (MW 12,327), bovine serum albumin (BSA, MW 65,000) were used to verify the pore size, porosity and pore geometry of the ferrogels in Figure 5. The results show that creatinine and vitamin B_{12} could rapidly pass through the ferrogels and display 1.9-2.2 times difference between the perpendicular and parallel ferrogels. However, as for the biomolecules with the lager molecular weight such as cytochrome c and BSA, they displays lower

diffusion coefficient, which means the larger biomolecules would be blocked inside the pore of ferrogels. Especially in BSA, the permeability coefficient is lower than 10×10^{-6} cm^2/min, indicated that the huge molecular weight of the protein could not pass through the pore of the ferrogel. The molecular weight of the biomolecules is suitable for the range of 100-10000 Da in this novel nanohybride ferrogels.

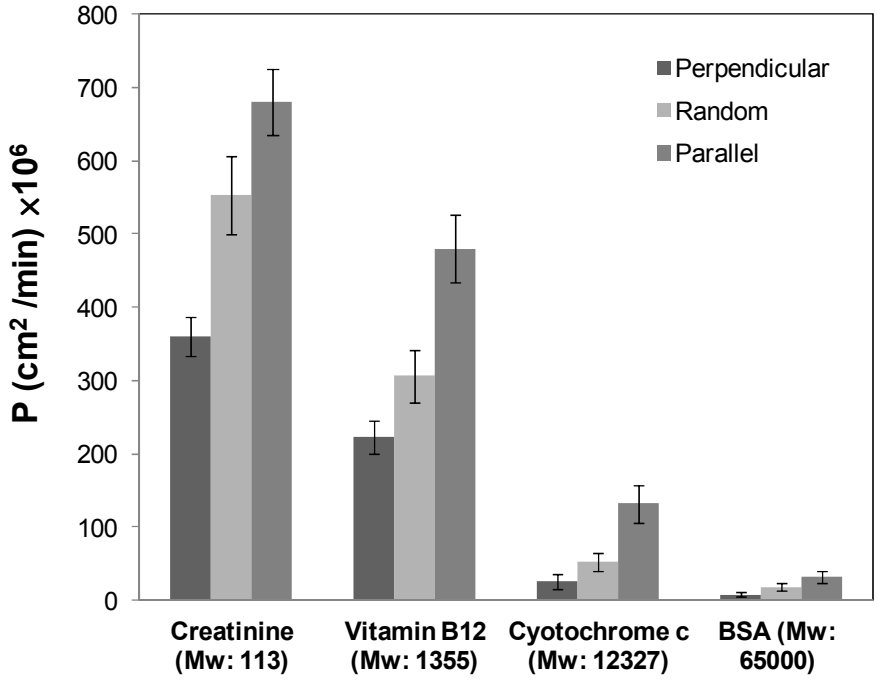

Figure 5 Permeability coefficient (P) of the varied molecular weight (Mw) of biomolecules through the PVA ferrogel

4. Conclusions

We successfully developed a novel method to control the nanochannels formation in the magnetic nanohybrid membrane by applied magnetic field. The direction of nanochannels could be manipulated by magnetic nanoparticles self-assembly (pearl-chain structure) under different direction of external

magnetic field. The result shows the drug release behavior in parallel-aligned ferrogel exhibits that the highest drug diffusion, whereas the perpendicular-aligned ferrogel is the lowest, which is in the order: parallel (anisotropic) > random (isotropic) >perpendicular (anisotropic)-aligned ferrogel. It correlates with the morphology of magnetic nanochannels and the direction of drug diffusion. It suggests lower than 100k Da of biomolecules could be used in this ferrogel platform. This effective and rapid method to arrange magnetic nanoplarticles to form the nanochannels in the membrane would be anticipated to apply in the bio-membrane and drug carriers, such as drug controlled release.

Acknowledgments

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Figure Caption

Figure 1 Figure 1 Schematic drawing of the experimental set-up for ferrogel preparation under uniform magnetic field: (a) and (c) the iron oxide nanopartices of the ferrogels were arranged by left-to-right electronic magnets ("perpendicular" to the drug diffusion direction) ; (b) and (d) the iron oxide nanopartices of ferrogel were arranged by top-to-down electronic magnets ("parallel" to the drug diffusion direction)

Figure 2 DSC analysis of PVA physical cross-linking by freezing and thawing cycles: (a) cooling curve; (b) integrated area of heat flow and crystal temperature (T_c) change with different cycles.

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Figure 5 Permeability coefficient (P) of the varied molecular weight (Mw) of biomolecules through the PVA ferrogel

Figure 1

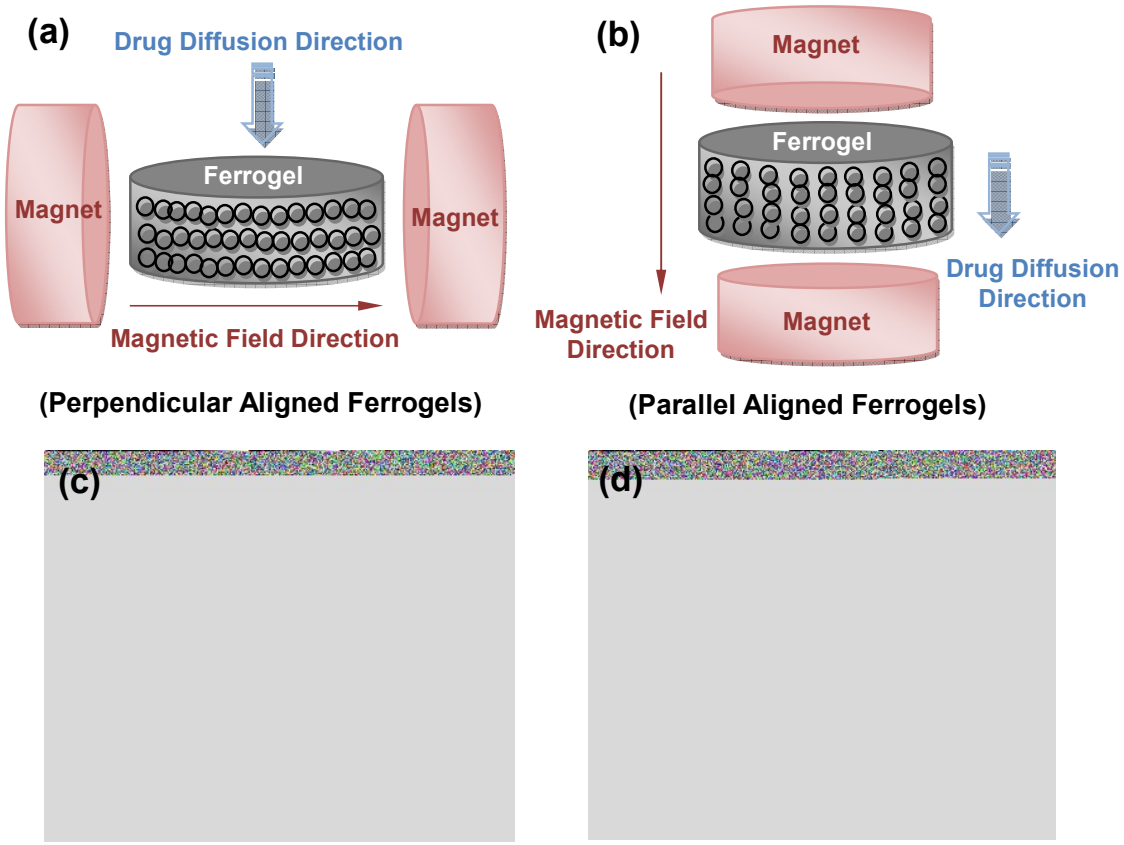

Figure 2

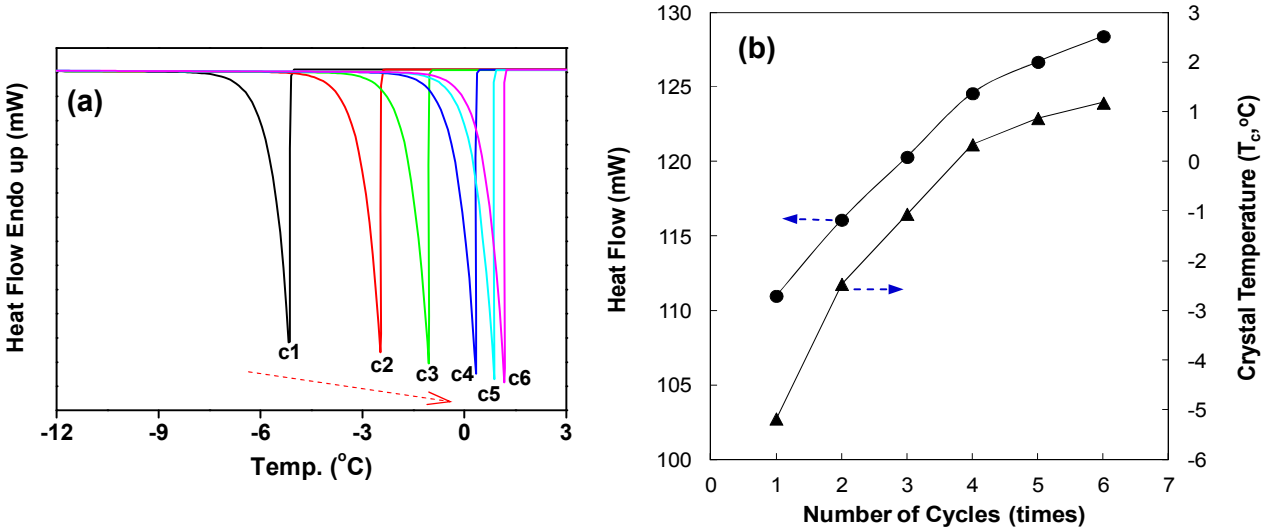

Figure 3

Figure 4

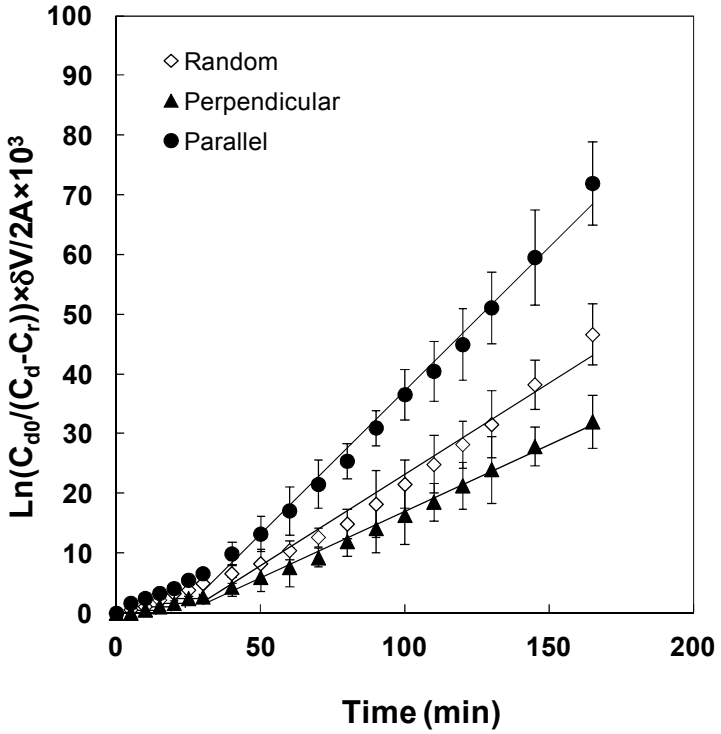

Figure 5