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Title: Injectable and Thermally Contractible Hydroxypropyl Methyl Cellulose/Fe₃O₄ for Magnetic Hyperthermia Ablation of Tumor

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Keywords: Thermally contractible, Hydroxypropyl methyl cellulose, Magnetic hyperthermia, Tumor therapy

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Abstract: The development of efficient strategies for the magnetic hyperthermia ablation of tumors remains challenging. To overcome the significant safety limitations, we developed a thermally contractible, injectable and biodegradable material for the minimally invasive and highly efficient magnetic hyperthermia ablation of tumors. This material is composed of hydroxypropyl methyl cellulose (HPMC), polyvinyl alcohol (PVA) and Fe₃O₄. The thermal contractibility of HPMC/Fe₃O₄ was designed to avoid damaging the surrounding normal tissue upon heating, which was confirmed by visual inspection, ultrasound images and computed tomography (CT) images. The efficient injectability of HPMC/Fe₃O₄ was proven using a very small needle. The biosafety of HPMC/Fe₃O₄ was evaluated by MTT and biochemical assays as well as flow cytometry (FCM). All the aforementioned data demonstrated the safety of HPMC/Fe₃O₄. The results of in vitro and ex vivo experiments showed that the temperature and necrotic volume of excised bovine liver were positively correlated with the HPMC/Fe₃O₄ weight, iron content and heating duration. The in vivo experimental results showed that the tumors could be completely ablated using 0.06 ml of HPMC/60%Fe₃O₄ after 180 s of induction heating. We believe that this novel, safe and biodegradable material will promote the rapid bench-to-bed translation of magnetic hyperthermia technology, and it is also expected to bring a new concept for the biomaterial research field.

Dear Prof. Zhuang Liu,

Thank you very much for your email informing us of the “Revision” decision for our manuscript entitled “Injectable and Thermally Contractible Hydroxypropyl Methyl Cellulose/Fe₃O₄ for Magnetic Hyperthermia Ablation of Tumors”. We greatly appreciated the reviewers’ comments and suggestions for further improving the quality of this work.

We have carefully considered the kind comments and suggestions of the editor and reviewers, performed additional experiments to answer the reviewer’s comments and carefully revised the manuscript accordingly.

Your kind consideration will be greatly appreciated.

Sincerely yours,

Prof. Yuanyi Zheng

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Response to Reviewers

Reviewer #1:

Comment: *The authors have made a good revision to their manuscript. They have made clear explanation and also added some supplementary data to support their conclusion. I think that the present form can be accepted for publication on Biomaterials.*

Response: We thank the reviewer for the kind and encouraging comments.

Reviewer #3:

Comment 1: *The advantage and novelty of HPMC/Fe₃O₄ are still not presented clearly. The author presented that the phenomenon of "tail" was different with general leakage of injected magnetic hydrogel from the tumor tissues. The main novelty of this work is to overcome the tail formation. However, the potential risk induced by tail-like shape after injection of PLGA/Fe₃O₄ or CPC/Fe₃O₄ was not occurred either in the previous publications or in this manuscript. To testify the advantage of HPMC/Fe₃O₄, the hydrogels to form the tail-like formation without shrink after ablation should be used as a control for a purpose of comparison.*

Response: Thank you for the comments. Based on the consideration that the formation of the "tail" will cause some potential safety problems especially when it is close to some important organs, we came out this idea, designed and successfully prepared this contractible biomaterial. The novelties are not only limited to overcome the "tail" problem, but also to contribute to the biomaterial researches field a concept of "thermally contractible biomaterial". As we know, the "heat shrink tubing" was developed and has been widely used in many different industries and environments such as: electrical insulation, strain relief, environmental protection, splice sealing, mechanical protection, wire bundling, identification, etc. We think the new comcept of thermally contractible biomaterial might be as useful as the heat shrink tubing in future, although we haven't figured out more useful applications except in overcoming the "tail" problem.

In our previous studies, we have noticed this formation of tail, such as: the Figure 2(i) in the reference (Y Chen et al. *Advanced Materials*. 2014; 26(44):7468-7473) has already showed the formation of tail (right picture). And Luis Solorio et al. have also showed a picture (Figure 4, *Journal of*

Controlled Release. 2010;143:183–190), in which we could see the significant tail-like formation (left picture).

Furthermore, the other potential applications of the contractible biomaterial might include: Its good repeatable heating capability could permit the repeatable ablation therapy of tumor recurrence or residue tumor.

We thank the reviewer for the suggestion that “the hydrogels to form the tail-like formation without shrink after ablation should be used as a control”, while it is difficult to prepare such a control since the key point is to prepare a tail-like formation which is close to some important organs, such as the bile duct which is very sensitive to heat and very easy to be damaged. While the bile duct in mouse is much smaller and it is almost impossible to be observed by ultrasound imaging, which means we can’t precisely guide the injection of the hydrogel into the place where we expected. Since the main idea of this study is to present a new concept of “thermally contractible biomaterials”, we think we have demonstrated this concept. For the concerns about “how efficiently the concept is”, we will go on this study in our future work. Thanks.

Comment 2: *Does the formation of tail make the injected hydrogel out of tumor tissue after a direct injection compared with the leakage phenomenon? The potential safety problem is negligible if the tail was not leaked out of tumor. In contrast, the contractibility of HPMC/Fe₃O₄ induced by the ablation-generated heat possibly cause the inhomogeneous magnetic ablation within the tumor. The edge of tumor tissues has a potential for recurrence if the temperature in the center and edge of tumor are different due to the shrinkage of HPMC/ Fe₃O₄.*

Response: Thank you for the comments. Yes, sometimes, the formation of tail which was stuck to the needle could make the injected hydrogel out of tumor tissue when we took out the needle after injection. While in our previous studies and this study, we employed the subcutaneous tumor models which were easily to be handled and the formation of "tail" could be further decreased by hand pressure on the skin. And the more or less formation of tail seems to be related to the injection skill of the operators too. Some students did a better job than the others did. And even for one same operator, there were differences. As we can see from the Figure 4 in reference (*Luis Solorio, Agata A. Exner, et al. Journal of Controlled Release. 2010;143:183–190*), some implants had tails, some didn't.

We can definitely understand the reviewer's consideration about the possible tumor residue. We have performed in vitro studies using the excised bovine liver to figure out the ratios between the volume of the HPMC/Fe₃O₄ implant and the ablation volume. Based on the ratio, we selected the best volume size, which could cover the whole tumor for the in vivo tumor ablation experiment, to avoid the possible tumor residue. Although we didn't find significant increase in tumor recurrence in this study, we agree with the reviewer that the safety risk should be further conducted in our future studies. Furthermore, as any other hyperthermia methods, there are always potentials for recurrence. The advantage of HPMC in combined hyperthermia and chemotherapy might add value to this contractible magnetic ablation. Furthermore, HPMC/Fe₃O₄ can be used repeatedly (Figure S1) for ablation which might add value to the further ablation of residual tumor or tumor recurrence. Moreover, the intratumoral injection of HPMC/Fe₃O₄ could be guided by real-time ultrasound imaging, which permits us to select the injection site and the injection dosage according to the tumor shape under ultrasound imaging to further avoid the potential tumor residue.

Comment 3: *The syringe can be remained in the injection site for a while until a complete phase transition of Fe₃O₄ hydrogel if the trail phenomenon was induced by*

pulling out the syringe.

Response: Thank you for the suggestion. Yes, your suggestion might decrease the formation of tail if the syringe can be remained in the injection site for a while until a complete phase transition. While on the other hand, if we take out the needle after the complete phase transition, the solidified hydrogel will stick to the needle which might make the hydrogel out of the tumor when taking out the needle. As we mentioned above, the formation of "tail" might be related to the skill of the operators too. The reason will be studied, and we wish a good finding in future to reduce the phenomenon of tail formation. Thanks.

Comment 4: *It's better to convert 60% Fe_3O_4 of HPMC hydrogel to the unit of concentration, such as mg/mL, rather than the percentage. It is not clear for the exact amount of Fe_3O_4 injected to tumor because the author did not present the injected volume of HPMC hydrogel in the manuscript. In addition, how much percent of HPMC, PVA and water in the hydrogel if Fe_3O_4 is 60 wt%. Is the polymer amount enough to hold so much Fe_3O_4 stable by hydrophobic interaction?*

Response: Thank you for the suggestion. The reasons for our using percentage instead of mg/mL are as follows: First, as the reports in literature, the percentage was used for the description of HPMC (Hui-Huang Chen. *Food Hydrocolloids*. 2007; 21; 1201–1208; Cristhian J et al. *Pharmaceuticals*. 2016; 10; 5) and for the description of PLGA or CPC implant (Yu Chen , Ling Jiang et al. *Advanced Materials*; 2014; 267468-7473. Gao W, Zheng Y et al. *Acta biomaterialia*, 2016; 29; 298-306. Xu C, Zheng Y, et al. *ACS applied materials & interfaces*, 2015; 25; 13866-13875.) Second and most important, when HPMC was dissolved into water, it significantly increased the volume of the water to form the hydrogel, which makes it be difficult to calculate the accurate mg/mL for the final product which was used for injection. For example, we added 0.20g HPMC, 0.06g PVA and 0.38g Fe_3O_4 to 1 mL of water to prepare the 60% Fe_3O_4 of HPMC hydrogel. The final volume for the hydrogel is about 1.8 mL instead of 1mL because of the reswelling ability of the HPMC. Since the

ratios of the HPMC, PVA, Fe_3O_4 and water for any volume of the 60% Fe_3O_4 is the same and based on the consideration that the volume changed significantly and the small amount of water about 0.033 mL in total for the 0.06 mL 60% Fe_3O_4 of HPMC hydrogel will come out of the hydrogel after magnetic heating, we ignored the water volume in calculation. We appreciate the reviewer for the reminding of the accurate percentage for all the components in the hydrogel, more descriptions about the percentage have been added to the method paragraph in the revised manuscript.

For the in vivo experiment, we injected 0.06 ml of HPMC/60% Fe_3O_4 into the tumor. In this study, we found that the HPMC could hold the Fe_3O_4 very stable. The possible mechanism might owe to the special adhesion performance of HPMC (*Chen CP et al. International journal of molecular sciences. 2015;16:20859-72.*). Thanks.

Comment 5: *I think the author did not fully understand my previous comment 4. The heating efficiency of iron-based nanoparticles as a thermal seed can be improved by adjustment of various parameters of nanoparticles, such as particle size, size distribution, particle shape, composition, doping level of ally. The development of the magnetic nanoparticles with high heating efficiency has been reported to reduce dosage and potential toxicity compared with traditional Fe_3O_4 . The toxicity of Fe_3O_4 has been testified long time ago which is dependent on the coating and concentration.*

Response: Thank you for the comments. We agree with the reviewer that magnetic nanoparticles with high heating efficiency have been reported to reduce the dosage and potential toxicity compared with traditional Fe_3O_4 . The reason for our choosing Fe_3O_4 is based on the consideration of the low cost, easy obtainability as well as its acceptable magnetic and inductive heat properties, which is still the first choice for many studies. And since Fe_3O_4 has already been approved by FDA for clinical application, it should be much easier for the bench to bed translation than the other new magnetic nanoparticles. For the clinically used Fe_3O_4 MRI contrast agent, such as FERAHEME® (ferumoxytol), the recommended dose for i.v. injection is an initial 510 mg dose followed by a second 510 mg dose 3 to 8 days later. In this study, only

about 13 mg Fe_3O_4 was used for the 0.06ml HPMC/ Fe_3O_4 , thus we think the toxicity of Fe_3O_4 could be neglected especially for the local tumor therapy. Thanks.

Comment 6: *The author mentioned 0.06 mL for about 2 cm³ tumor. The injection volume is only 3 μL if in-vivo anti-cancer test started when the tumor volume reached 100 mm³. Such a small volume of hydrogel will cause serious experimental error for intratumor injection. Meanwhile, the volume of injected hydrogel is too small to cover the tumor tissues for homogenous ablation.*

Response: Thank you for the comments. In this study, we employed subcutaneous tumor models, which are easy to be handled, for the in vivo experiment. And the injection is monitored by real time ultrasound imaging since the developed hydrogel is visible in ultrasound imaging. Thus, we think the potential experimental error is small and the data showed good repeatability. Furthermore, we found the temperature for hydrogel is controllable which might further improve its safety. The following figure showed the temperature could be controlled at an expected degree.

Based on the consideration that the lower dose the better safety, which is the most important factor for a successful clinical translation, we think the very small dose might add value to the possibility for the clinical translation. We do understand your consideration about the inhomogeneous ablation and we do believe this technique should have the same limitations like all the hyperthermia techniques, such as inhomogeneous ablation. We plan to combine the magnetic hyperthermia with chemotherapy in our future studies to further improve its efficiency. Thanks.

Comment 7: *The basic property of Fe_3O_4 should be presented, such as nanoparticle surface coating.*

Response: Thank you for the comments. We have called the company from which we bought the Fe_3O_4 nanoparticles and more descriptions about the basic properties of the Fe_3O_4 (1317-61-9, Chengdu AikeDa Chemical Reagent Co., Ltd, Sichuan, China) have been added to the revised manuscript, such as: The Fe_3O_4 nanoparticles were made by chemical synthesis without surface coating. The relative molecular mass is 231.53, the particle size was 20-50nm, the specific saturation magnetic intensity was 90-115emu/g and the PH value was 5-7. Thanks.

Comment 8: *The significance of rheological property of HPMC/ Fe_3O_4 was to demonstrate the flowability at room temperature, phase transition temperature and the gel strength of hydrogel at body temperature. These parameters are crucial for an injectable and temperature-responsive hydrogel system.*

Response: Thank you for the very good suggestions. We have performed additional experiments to answer these questions. The rheological measurements were conducted on an RS150 rheometer (Thermo-Haake, Germany) with a 20 mm parallel plate (Ti, gap 0.3 mm) at room temperature. The method is similar to the method in literature (Zhang C, et al. ACS applied materials & interfaces, 2016, 8(5): 3349-3358.). The shear stress was 160 ± 4 Pa at 1 rpm and the viscosity was 1063 ± 40 (Pa·s) at 0 rpm, respectively. The rheological curve of shear stress to shear rate in figure 1 showed that the shear stress increased with the shear rate. In figure 2, we can see that the viscosity of the HPMC/ Fe_3O_4 decreased with the shear rate, and the decrease was obvious when the shear rate was less than 200 s^{-1} .

Figure 1

Figure 2

Based on these data, we think that the viscosity of the HPMC/Fe₃O₄ was suitable for the injection. And it was reported that the phase transition temperature of HPMC is its the gelation temperature, which is between 50 and 90 °C (*Lopes da Silva, et al. Rheology of fluid and semisolid foods. Gaithersburg, Maryland: Aspen Publication, Inc, 1999: 219-318; Chen H H. Food hydrocolloids, 2007, 21(7): 1201-1208*). Thank you.

Comment 9: *What's the significance to measure the porous structure of HPMC/Fe₃O₄ using SEM? Is it possible to drop the hydrogel on the TEM grid after appropriate dilution? Based on the author's explanation, it seems that HPMC/Fe₃O₄ is in a semi-solid state rather than a fluid state at room temperature.*

Response: Thank you for the comments. At the beginning, the SEM was designed to characterize the morphological features of the HPMC/Fe₃O₄, while when we found the porous structure by SEM, we think it might add value to the HPMC/Fe₃O₄ in drug delivery in future chemotherapy combined hyperthermia therapy since the porous structure is very helpful for drug loading and controlled drug release.

Theoretically, it might be possible to drop the hydrogel on the TEM grid after appropriate dilution. While in fact, we tried and failed in the preparation of very thin slice and good quality slice for the TEM observation. HPMC/Fe₃O₄ is not in an fluid state, it is semifluid at room temperature and will turn into solid at room temperature in about 24 hours. Thank you.

Comment 10: *There is no obvious ultrasound contrast to localize the position of*

1 *injected HPMC/Fe₃O₄ in Figure 3b. It's seemed that the tail of injected HPMC/Fe₃O₄*
2 *is out of tumor in Fig. 3c. No matter how contractible HPMC/Fe₃O₄ is, the ablation*
3 *followed by injection will cause the potential extra damage to the tail covered other*
4 *healthy tissues.*

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10 **Response:** Thank you very much for the reminding. The obscure ultrasound contrast
11 might owe to the over compress of the image. We have replaced it with a better
12 quality image, in which the position of the injected HPMC/Fe₃O₄ in Figure 3b should
13 looks more clear. The corresponding CT images in Fig. 3c also showed the “tail” of
14 the injected HPMC/Fe₃O₄ was not outside of the tumor (Please see the following
15 picture) (Yellow continuous line: tumor; Red continuous line: HPMC/Fe₃O₄ implant;
16 Red arrow: injection site). We think the contractability could minimize the potential
17 extra damage to the healthy tissue at some extent. Thanks.

40 **Comment 11:** *Please Google it if you don't understand specific loss power or specific*
41 *absorption rate. This parameter was frequently used in many literatures about*
42 *magnetic hyperthermia to describe the power achievable per gram of iron in the*
43 *ferrofluid. In my option, the measurement of SLP value for the iron oxide*
44 *nanoparticles-based thermal therapy is necessary.*

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51 **Response:** Thank you for the comments. Finally, we got access to Google by VPN.
52 We have looked up the references and have requested any possible labs which might
53 have the equipments for help, such as College of Materials Science and Engineering,
54 Chongqing University; the Center of Material Analysis and Testing of Chongqing
55 University of Science & Technology; Shanghai Institute of Ceramics; Shanghai
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Jiaotong University Sixth Affiliated Hospital, etc., while none of them have the equipment to measure the SLP value. We are sorry for not being able to provide a more positive response. We hope that the reviewer understands. Alternatively, we employed the thermo gravimetric analysis to evaluate the mass change and thermal change of the iron oxide (Fe_3O_4) in the process of heating. From the thermogravimetric curve of Fe_3O_4 in figure 1, we can see that the mass loss of Fe_3O_4 started at 34°C . The thermogravimetric curve tended to be stable at 44°C - 100°C which means that the mass loss of the Fe_3O_4 at steady rates. The Fe_3O_4 mass loss just decreased by 0.06% in the process of heating to 100°C . The differential scanning calorimetry (DSC) curve of heat flow in figure 2 showed that the endothermic signal decreased, which means that the Fe_3O_4 was endothermic in the process. The above data showed the mass loss of the iron oxide was very little and there was no significant thermal loss during heating.

Thank you.

(figure 1)

(figure 2)

Comment 12: *It seems that f and H in the AMF occurred in this manuscript are 626 kHz and 7.2 kA/m. Please confirm your calculation whether $H \times f = 6.3 \times 10^8$ or not. Meanwhile, for a coil with 3 cm diameter, the peak to peak voltage would be extreme high at a current of 28.6 A which is likely to generate extra heat due to eddy current, and lead the animal in danger. Please confirm the f and H values.*

Response: Thank you for the reminding. We are sorry for the unclear description and not very accurate method for the calculation of the $H \times f$, which made the reviewer's misunderstanding. The maximum f and H and current values for the instrument we used are 6.26×10^5 Hz, 259 A/m and 28.6 A respectively, the maximum $H \times f$ (A/m/s) $= 259 \text{ A/m} \times 6.26 \times 10^5 \text{ Hz} = 1.62 \times 10^8 \text{ A/m/s}$. While, in this study, the values of the f and H and current used for the magnetic hyperthermia were 5×10^5 Hz, 205 A/m and 22.5A, respectively, the value of $H \times f$ (A/m/s) $= 205 \text{ A/m} \times 5 \times 10^5 \text{ Hz} = 1.02 \times 10^8 \text{ A/m/s}$. All of which less than the limitation of the safe values of Hf dose. (*Rudolf Hergt et al, Journal of physics condensed matter, 2006; 18; 2919–2934 R. Hergt et al, Journal of Magnetism and Magnetic Materials 2004; 270; 345–357.*) The Field amplitude H was calculated by the formula of $H = N \times I / L_e$, N is the number of turns of excitation coils; I is the excitation current; L_e is the length of magnetic circuit. (*James K R, Dowling D R. The Journal of the Acoustical Society of America, 2008, 124(3): 1465-1476.*) Although we didn't find any death during the magnetic heating process, we do understand the importance of safety in the clinical translation of this technology. We will optimize our apparatus and perform additional experiments to test the safety in future studies. Thank you.

Comment 13: *It is difficult to summarize the key points of this paper from such a simple conclusion. Please revise the conclusion again.*

Response: Thank you very much for the comments. We have revised the conclusion as follows: In conclusion, in this study, we developed a thermally responsive contractile material using HPMC and Fe_3O_4 with good injectability, and have confirmed its safety as well as the high ablation efficiency for the minimally invasive magnetic ablation of tumors.

1 We believe that this novel, safe and biodegradable material will promote the rapid
2 bench-to-bed translation of magnetic hyperthermia technology, which is expected to
3 substantially broaden this biomaterial's applications.
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7 **Comment 14:** *There are still English grammar and language expression mistakes in*
8 *the manuscript that will confuse the reader.*
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10 **Response:** Thank you for the comments. We have forwarded your comment to the
11 English service company and requested them to further correct the English grammar
12 and language expression mistakes in the revised manuscript.
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20 **Comment 15:** *Some of references are still not well-matched to the contents in the*
21 *manuscript. Please confirm them again.*
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25 **Response:** Thank you for the comments. We have carefully reviewed the references
26 and deleted the not well-matched references in the revised manuscript.
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DOI: 10.1016

Article type: Communication

**Injectable and Thermally Contractible Hydroxypropyl Methyl Cellulose/Fe₃O₄
for Magnetic Hyperthermia Ablation of Tumors**

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Injectable and Thermally Contractible Hydroxypropyl Methyl Cellulose/Fe₃O₄ for Magnetic Hyperthermia Ablation of Tumors

Abstract: The development of efficient strategies for the magnetic hyperthermia ablation of tumors remains challenging. To overcome the significant safety limitations, we developed a thermally contractible, injectable and biodegradable material for the minimally invasive and highly efficient magnetic hyperthermia ablation of tumors. This material is composed of hydroxypropyl methyl cellulose (HPMC), polyvinyl alcohol (PVA) and Fe₃O₄. The thermal contractibility of HPMC/Fe₃O₄ was designed to avoid damaging the surrounding normal tissue upon heating, which was confirmed by visual inspection, ultrasound images and computed tomography (CT) images. The efficient injectability of HPMC/Fe₃O₄ was proven using a very small needle. The biosafety of HPMC/Fe₃O₄ was evaluated by MTT and biochemical assays as well as flow cytometry (FCM). All the aforementioned data demonstrated the safety of HPMC/Fe₃O₄. The results of in vitro and ex vivo experiments showed that the temperature and necrotic volume of excised bovine liver were positively correlated with the HPMC/Fe₃O₄ weight, iron content and heating duration. The in vivo experimental results showed that the tumors could be completely ablated using 0.06 ml of HPMC/60%Fe₃O₄ after 180 s of induction heating. We believe that this novel, safe and biodegradable material will promote the rapid bench-to-bed translation of magnetic hyperthermia technology, and it is also expected to bring a new concept for the biomaterial research field.

Key words: Thermally contractible, Hydroxypropyl methyl cellulose, Magnetic hyperthermia, Tumor therapy

1. Introduction

Hyperthermia ablation is a minimally invasive therapeutic technique that promotes tumor regression and has been widely used in clinical applications [1-3]. Methods of hyperthermia ablation include the use of microwaves (MW), radiofrequency (RF), and high-intensity focused ultrasound (HIFU) [4, 5], but these strategies have limitations, as they can cause skin or muscle burns, ecchymosis or mass formation, which restrict the extent of their clinical application [6]. The magnetic hyperthermia technique, based on converting electromagnetic energy into heat [7], is a promising tumor therapy technique [8] that does not readily damage the skin or muscles and can prevent unnecessary heating in healthy tissues because only the magnetic nanoparticles absorb the magnetic field [9]. Additionally, magnetic hyperthermia is not influenced by unavoidable factors such as the presence of bone and gas, which are limitations of HIFU, or by the depth limitation of lasers due to the properties of the electromagnetic energy of the magnetic materials.

To overcome the limitations of the currently used magnetic hyperthermia technique, especially the diffuse leakage of materials into the surrounding tissue when they are injected inside the tumor, our team developed an injectable phase-transitional magnetic media that is S-Fe/PLGA (poly lactic-co-glycolic acid) [10] and Fe_3O_4 /MCPC (calcium phosphate cement) [11]. These materials can be locally injected and can confine the iron particles inside the target tissue to achieve targeted magnetic ablation. However, after further investigation, several limitations of these newly developed materials were uncovered.

First, although the developed phase-transitional PLGA/ Fe_3O_4 or MCPC/ Fe_3O_4 implant material could confine the iron particles inside the implant, thus preventing diffusion into the surrounding tissue and even to distant organs, we found in the following experiments that the implant formed a “tail”-like shape after injection. The heat generated by the “tail”, which protruded from the tumor, could still cause some potential safety problems when the “tail” is close to important organs or tissues. Second, MCPC/ Fe_3O_4 requires a large needle (14G needle with an inner diameter of 1.6 mm [11]) for injection, which might increase the safety risk during deep tissue injection. Third, although the N-methylpyrrolidone (NMP) used in the preparation of S-Fe/PLGA has low toxicity and its use is permitted in the application of tumor

1 therapy. However, NMP, as an organic solvent, might be teratogenic and decrease
2 the physical development and viability of a fetus in the womb [12]. Finally, when the
3 amount of Fe_3O_4 in the S-Fe/PLGA or Fe_3O_4 /MCPC implant raised to 60% to
4 increase the heating efficiency, the implants were easily broken up into powder after
5 heating for 3 min (**Fig. S1**), which may decrease the repeatability or the safety of the
6 ablation therapy. Thus, to avoid the “tail” formation and to improve the injectability
7 without using toxic materials are important for the bench-to-bed translation of these
8 newly developed magnetic materials. Based on these limitations, we hypothesized that
9 a thermally contractible magnetic material might eliminate the “tail” problem
10 (**Scheme 1**), and the safety should be further improved if the injectability is improved
11 and non-toxic materials are used.
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21 In this study, we employed hydroxypropyl methyl cellulose (HPMC), Fe_3O_4 and
22 polyvinyl alcohol (PVA) to develop a new method for the magnetic ablation of
23 tumors in vivo. By applying an alternating magnetic field (AMF), this material was
24 expected to in situ heat generation of tumor ablation therapy, minimize the
25 invasiveness, and avoid potential damage to nearby normal tissue for its thermal
26 contractibility. In addition to its efficient therapy, injectability and contractibility, the
27 main components (HPMC, Fe_3O_4 and PVA) used in the preparation of this material
28 are known to have good safety profiles and should further improve the safety of this
29 therapy. We learnt that Fe_3O_4 is a widely used biocompatible magnetic material [13,
30 14] and can generate heat under an AMF [15]; HPMC is the most widely used
31 biodegradable polymer with pharmaceutical applications in oral and transdermal drug
32 delivery systems [16]; and PVA is a water-soluble synthetic polymer that possesses
33 good biocompatibility and biodegradability [17]. The good properties of these
34 materials could minimize the potential adverse effects and enhance the safety
35 associated with the long-term retention of foreign materials in tissue, as well as
36 potentially helping to achieve an optimal treatment technology using magnetic
37 hyperthermia. Moreover, the newly developed material also showed the ability to be
38 repeatedly heated, which might be useful for the ablation of tumor residues or local
39 tumor recurrence without having to perform another interventional procedure.
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2. Experimental Section

2.1 Preparation and Synthesis of HPMC/Fe₃O₄ The Fe₃O₄ nanoparticles (CAS:1317-61-9, 10-50 nm, Chengdu AlikeDa Chemical Reagent Co., Ltd., China) used in this study was made by chemical synthesis without surface coating, it was insoluble in water. The relative molecular mass of Fe₃O₄ is 231.53 and the specific saturation magnetic intensity was 90-115emu/g. Fe₃O₄ and HPMC (CAS: 9004-65-3, molecular mass : 1261.4387, Shijiazhuang DingShengFumei Technology Development Co., Ltd., China) were adequately mixed in a beaker, which was then placed in an orbital shaker incubator (37.0°C, 150 rpm, 48 h) for full blending. To prepare the solution, PVA (fully hydrolyzed; Sigma-Aldrich, USA) was dissolved in axenic PBS at a solid-liquid (S/L) ratio of 0.06 and stored at 4°C in a refrigerator. The prepared HPMC/Fe₃O₄ was then mixed thoroughly with the PVA solution and was ready for use.

2.2 Morphological and Structural Analysis The morphological features of HPMC/60%Fe₃O₄ were observed by scanning electron microscopy (SEM; Ametek EDAX, S-3700, 371027-02, USA; accelerating voltage: 15-20 kV) at 1000×, 5000× and 15000× magnification and energy-dispersive spectroscopy (EDS; Ametek EDAX, S-3700, 371027-02, NJ, USA; accelerating voltage: 15 kV) at 500× magnification. The samples were dried to improve their surface conductivity.

2.3 Heating Device and Temperature Measurements The HPMC/Fe₃O₄ prepared for in vitro and in vivo tests was heated in the center of a custom-built magnetic hyperthermia analyzer (frequency: 626 kHz; output current: 28.6 A; coil diameter: 3 cm) [10], and the peak surface temperature was continuously recorded by a far-infrared thermometer (Fluke Ti32, Fluke International Corporation, Washington, USA). The thermal images were analyzed by Smart View 3.11 software (Fluke International Corporation, USA).

2.4 Bi-modal Observations of the Contractibility of HPMC/Fe₃O₄ The contractibility of the HPMC/Fe₃O₄ material was monitored through macroscopic measurement of the material length and volume. The HPMC/Fe₃O₄ volume changes

before and after heating in excised bovine liver were observed via ultrasound imaging (Esaote MyLab 90, L5-15 MHz, Italy). Volume changes in tumor-bearing mice were observed via ultrasound and micro-CT imaging (Quantum FX CT, Perkin Elmer, USA). The HPMC/Fe₃O₄ volume was estimated using the formula $V = D_L D_S^2 \pi / 6$, where D_L is the long diameter and D_S is the short diameter of the tumor measured using a Vernier caliper [18].

2.5 In vitro and vivo biosafety

2.5.1 In vitro biosafety: Normal human smooth muscle cells (SMCs) were cultured in a culture bottle containing medium. After culturing for 24 h, 1 ml of the prepared HPMC/Fe₃O₄ suspension at a concentration of 200 µg/ml was added to the culture bottle to incubate with the SMCs for 24 h. After incubation for 24 h, the bottom of the bottle was repeatedly rinsed with sterile PBS, and the cells were then observed under a microscope.

2.5.2 Cytotoxicity based on the MTT assay: The SMCs were cultured in 96-well plates (number of cells per orifice: approximately 6000) and incubated in an incubator (37°C, 5% CO₂). After incubation for 24 h, different concentrations of HPMC/Fe₃O₄ were prepared in a solution medium (150, 200, 250, 300, and 350 µg/ml) and added into the wells of the experimental group to replace the previous medium. After incubation for an additional 24 h, 20 µl of MTT solution at a concentration of 5 mg/mL was added into every well. After incubation for 4 h, the medium in the plate was carefully removed, and 100 µl of dimethyl sulfoxide (DMSO) was added into each well. The colorimetry of the samples was determined on an enzymatic reader, and the absorbance was measured by an enzyme-linked immunosorbent assay at an optical density (OD) of 490 nm. The cell survival rate was calculated using the formula $(R) = (OD_e - OD_b) / (OD_c - OD_b)$, where OD_e, OD_c and OD_b represent the ODs of the experimental, control and blank groups, respectively.

2.5.3 Flow cytometry evaluation of cell apoptosis: The SMCs were inoculated in a 6-well plate with PBS for 24 h. After incubation for 24 h, the prepared HPMC/Fe₃O₄ suspension in a solution medium (200 µg/ml) was added into the experimental well. After incubation for an additional 24 h, the cells were collected and preserved by the addition of 1 ml of PBS into the tubes. The samples were then prepared for the cell apoptosis test.

2.5.4 In vivo biosafety: The safety of the HPMC/Fe₃O₄ material was evaluated in biochemical assays using mice serum at different time points (1 h, 1 day, 7 days and 14 days) after the injection of HPMC/Fe₃O₄. The serum of the mice was analyzed on an automated biochemical analyzer (Hitachi 7600-110, Japan). The serum biochemical indexes included aspartate aminotransferase (AST), alanine aminotransferase (ALT), lactate dehydrogenase (LDH), alkaline phosphatase (ALP), blood urea nitrogen (BUN), and serum creatinine (sCr).

2.6 Creation of the Nude Mouse MB-231 Human Breast Cancer Xenograft Model MB-231 cancer cells were incubated and obtained by serial subcultivation in vitro, collected by digestion and centrifugation, and dispersed with axenic PBS. The cells were subcutaneously injected into the backs of female SPF nude mice (age: 4-5 weeks) with a mean body weight of 19 g (1×10^7 cells/100 ml/mouse). Injection and ablation occurred four weeks later, and the prepared mice were then used for the experiments. All the animal were purchased and bred in the Experimental Animal Center of Chongqing Medical University, and all the experimental operation followed the guidelines of Chongqing Medical University.

2.7 In Vivo Therapeutic Efficiency The intratumoral injection of HPMC/Fe₃O₄ was performed along the long axis of the tumor under the guidance of real-time ultrasound after the mice had been anesthetized. The peak surface temperature of the tumors was continuously recorded using a far-infrared thermometer (Fluke Ti32, Fluke Corporation, USA). After magnetic hyperthermia ablation treatment, the tumors and the body weight changes of the mice were observed by ultrasound, and the tumor

volumes were macroscopically measured each day. The tumor volumes were estimated using the formula $V = D_L D_S^2 \pi / 6$, where D_L is the long diameter and D_S is the short diameter of the tumor measured using a Vernier caliper [19]. After ablation treatment, two mice in every group were randomly selected and euthanized for pathological analysis of the tumors by hematoxylin and eosin (H&E) staining. After 15 days, the other mice in the experimental group were fed to observe the health status and recurrence of the tumors. The mice in the two control groups were euthanized.

3. Results and Discussion

3.1. Synthesis

To prepare the thermally responsive, contractible and injectable magnetic material, five percentages of Fe_3O_4 , HPMC and PVA corresponding to five different mass ratios were physically mixed for preparation of HPMC/ Fe_3O_4 . First, the Fe_3O_4 nanoparticles were sufficiently mixed with HPMC, and the mixture was then placed in an orbital shaker incubator for full blending. Second, PVA was sufficiently dissolved in axenic PBS at an S/L ratio of 0.06 using a magnetic stirring apparatus. Third, the mixture of HPMC and Fe_3O_4 was mixed thoroughly with the PVA solution, and the resulting HPMC/ Fe_3O_4 material was ready for use. It has been well documented in the literature that HPMC can be blended with PVA [20, 21]. The miscibility of the blend might be attributed to a specific interaction, such as hydrogen bonding between HPMC/PVA [22]. Moreover, the PVA solution is an essential ingredient for preparation of HPMC/ Fe_3O_4 material; and PVA is a semicrystalline copolymer of vinyl acetate and vinyl alcohol [23] that has a two-dimensional, hydrogen-bonded network, sheet structure and good thermal stability and chemical resistance [17, 24].

3.2. In Vitro Heating Efficiency and Selection of the Appropriate Proportion of HPMC/ Fe_3O_4

The sample tubes containing PBS and HPMC/Fe₃O₄ were placed in the center of the heating coil, and the obtained thermal images (**Fig. 1a**) showed that HPMC/Fe₃O₄ could heat the PBS with the application of AMF. The surface temperature of 0.05 ml of HPMC/Fe₃O₄ increased with the Fe₃O₄ content and heating time (**Fig. 1b**). From above, we drawn the conclusion that the HPMC/Fe₃O₄ material has a high in vitro efficiency.

In our pre-experiment, we found that the temperature of HPMC/Fe₃O₄ quickly increased when the percentage of Fe₃O₄ exceeded 70% in the HPMC/Fe₃O₄ material, and a larger needle was required for injection when the concentration of Fe₃O₄ was below 50%, which may have been due to the HPMC's high reswelling and thickening characteristics [25]. As shown in **Fig. S2**, 0.1 ml of HPMC-50% Fe₃O₄ required 78±2.10 s to reach 70°C, whereas 0.1 ml of HPMC-90% Fe₃O₄ required only 13.0±2.97 s. The temperature increased so quickly that was difficult for us to control the temperature, which may cause damage to surrounding normal tissue. Considering the above findings, we believed that an Fe₃O₄ percentage in HPMC/Fe₃O₄ of 60% was more suitable for subsequent experiments in this study (the mass ratio of Fe₃O₄, HPMC and PVA was about 6:3:1 for the preparation of the HPMC/Fe₃O₄).

Smaller needles more easily to achieve microinvasive or noninvasive treatments, thereby reducing injury. As shown in **Fig. 1c and Movie S1**, the HPMC-60%Fe₃O₄ material could be smoothly injected using a small needle. The injectability of this material is attributed to the miscibility of HPMC and PVA, and their interaction could reduce the injection resistance of the magnetic particles [26, 27]. The possible mechanisms for this phenomenon might involve secondary forces without covalent bonding [28]. After a comprehensive consideration of the results, it was concluded that 60% Fe₃O₄ was chosen for the preparation of HPMC/Fe₃O₄.

3.3. Morphological Characteristics and In Vitro Stability

The prepared HPMC/Fe₃O₄ material was dried to observe its morphological characteristics using SEM and EDS. The SEM (**Fig. 2a**) images revealed that the HPMC/Fe₃O₄ matrix was porous, the porous structure might add value to the HPMC/Fe₃O₄ in drug delivery in future chemotherapy combined hyperthermia therapy. The EDS images (**Fig. 2b**) demonstrated that the Fe₃O₄ nanoparticles were well mixed within the HPMC. As observed in **Fig. 2b**, the constituent elements of HPMC/Fe₃O₄ are carbon, oxygen and iron, which are all safe for use in the body. The corresponding percentages in **Fig. 2c** obtained from the elemental energy spectrum. The HPMC/Fe₃O₄ material co-incubated with serum containing PBS in a 37°C thermostatic bath to test the vitro stability or Fe₃O₄ release of HPMC/Fe₃O₄. As shown in **Fig. S3**, the sample was macroscopically observed every 5 h. Within 25 h, the HPMC/Fe₃O₄ matrix was relatively stable, and no Fe₃O₄ was released from the surface of the HPMC/Fe₃O₄ matrix.

3.4. Thermally Responsive Contraction

The thermally responsive contraction ability was investigated in excised bovine liver, tumor-bearing mice and an agar phantom. The HPMC/Fe₃O₄ was injected into the excised bovine liver and tumors, which were then heated in an AMF. Ultrasound images of the HPMC/Fe₃O₄ in the excised bovine liver (**Fig. 3a**) and tumors (**Fig. 3b**), and the CT images of the tumors (**Fig. 3c**) revealed that the implant had contracted and that its volume had obviously decreased after heating in an AMF. The CT images (**Fig. 3c**) also showed that the irregularly shaped HPMC/Fe₃O₄ material became relatively regular and that its “tail” disappeared after heating. The HPMC/Fe₃O₄ matrix was heated in an agarose gel module for 2 min with an AFM to calculate its contractible properties. The two “tails” of the HPMC/Fe₃O₄ material obviously contracted after heating (**Fig. 3d**). The length of one “tail” was 1.0±0.15 cm before AMF application and decreased to 0.5±0.03 cm after heating for 30 s. As shown in **Fig. 3e**, the volume of the HPMC/Fe₃O₄ matrix decreased from 3.02±0.45 cm³ to 0.27±0.12 cm³ over 2 min, decreasing with the heating duration. The

contraction-recovery ability of HPMC/Fe₃O₄ was also evaluated in an agarose gel module with an AMF. In **Fig. S4**, the volumes of the HPMC/Fe₃O₄ material were 1.20±0.12 cm³ and 0.07±0.02 cm³ at 0 s and 60 s, respectively. However, the volumes increased to 0.61±0.75 cm³ and 0.97±0.11 cm³ after the HPMC/Fe₃O₄ material was incubated with PBS at 37°C for 24 h and 48 h, respectively. The volume of the HPMC/Fe₃O₄ material was reduced (0.06±0.01 cm³) again after heating and then increased (0.77±0.09 cm³) after incubating for 48 h. The reason for this amazing phenomenon is that HPMC, as a cellulose material, possesses the swelling ability[29] and thermal contraction ability.

The interactions between the hydrophobic polymer chains and water proved to be involved in the gelation process [30]. The mechanism for the thermally contractible behavior of HPMC should be related to the properties of HPMC gelation. Therefore, the potential contraction mechanism can be explained via the hydrophobic interactions of the hydrophobic groups (such as methoxy groups) on the polymer chain [31, 32], which is generally believed to proceed as follows [33, 34]. 1) The HPMC macromolecules combine with water molecules to form a network structure at low temperature. 2) As the temperature increases, the break of the hydrogen bonds between the water and HPMC molecules resulting in the destruction of the network structure and the exposure of hydrophobic methyl groups. 3) Increasing numbers of hydrophobic methyl groups gathered to forming a hydrophobic aggregate. When the temperature reached the gelation temperature, the strong hydrophobic effect by the many hydrophobic aggregates caused the polymer chains of HPMC to gather together, resulting in the structure of the HPMC more and more tightly, which macroscopically performed the contractibility of the HPMC/Fe₃O₄.

3.5. Biosafety

Biosafety has always been the greatest factor restricting many drugs and materials that are widely used in clinical applications. To evaluate the cytotoxicity of HPMC/Fe₃O₄, we incubated the materials with normal human SMCs. As shown in **Fig. 4a**, some of

the HPMC/Fe₃O₄ particles (most may be the Fe₃O₄ particles) were engulfed by the cells as a result of the cellular endocytosis [35, 36] and some gathered around the cell membranes, but no cell death was observed. Moreover, the MTT colorimetric assay is a common cell detection method often be used to assess cytotoxicity in drug testing [37]. As shown in **Fig. 4b**, the cell viability was 100% in the control group, and the values were 92.6±2.3%, 91.6±2.7%, 88.9±3.9%, 88.5±5.1% and 86.6±3.5% in the five experimental groups (50-90% Fe₃O₄), respectively. Based on the cell apoptosis results measured by flow cytometry (FCM) (**Fig. 4c**), the proportion of living cells was 91.72±1.59% in the control group and 90.85±1.48% in the experimental group. There was no statistically significant difference between the two groups at each developmental stage of the cell cycle (P>0.05). The cell experiment indicated that HPMC/Fe₃O₄ had no significant toxic effects on the SMCs. Then the mice subjected to intratumoral administration of HPMC/Fe₃O₄ and the serum biochemical indexes were detected at different time points to evaluate the biosafety in vivo. The levels of ALT, AST, LDH, ALP, BUN and sCr in the treated mice exhibited no statistically significant differences (P>0.05) compared with the control group at the time points of 1 h, 1 days, 7 days and 14 days after injection of the HPMC/Fe₃O₄ (**Fig. S5**). Furthermore, the heart, liver, spleen, lung and kidney of these mice were then removed at time points of 1 h, 1 day and 7 days for H&E staining. No materials were detected in these tissues, and no cell damage was observed (**Fig. S6**). From which we concluded that HPMC/Fe₃O₄ implant has no significant toxicity to tumor-bearing mice, and it was relatively safe for in situ injection.

The in vivo and in vitro experiments demonstrated the high biological safety of the HPMC/Fe₃O₄ implant, the reason may be that the components of HPMC/Fe₃O₄ are all safe for use in the body. The Fe₃O₄ particles have been authorized by the FDA for use in clinical applications [38]. HPMC, a non-toxic and inexpensive compound also known as hypromellose [39], is a semi-synthetic hydrophilic polymer that is widely used as an ophthalmic lubricant [39] and a controlled-delivery component for transdermal and oral medicines [40, 41] and in dermatology [42]. The PVA is a

1 non-toxic, biodegradable and biocompatible polymer [23, 43] which has been widely
2 used in different areas of the biomedical field [44].
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5 The levels of AST, ALT and LDH at 1 day post-injection were higher in the treated
6 mice compared with the control, but these indicators all decreased at 7 days and 14
7 days. Although the serum biochemical indexes in the treated mice showed no
8 statistically significant differences compared with the control group, the increased
9 levels of ALT, AST and LDH at 1 day post-injection require further investigation.
10 The liver indexes require long-term observations of large sample sets and further
11 analysis to exclude potential damage caused by the injection and ablation. Although
12 our material proved to be safe and non-toxic in vitro cell experiments and in vivo
13 animal experiments, its safety in the human body remains unknown, and future
14 monitoring and verification are needed.
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27 **3.6. Heating and Ablation Efficiency of HPMC/Fe₃O₄ in Excised Bovine Liver**

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31 The exposure of magnetic HPMC/Fe₃O₄ to an AMF quickly heated the PBS in our
32 previous experiments. To study the heating efficiency in excised tissue, different
33 doses of HPMC/Fe₃O₄ (0.02 ml - 0.1 ml) were injected along the long axis of the
34 prepared excised bovine liver and then the bovine liver was exposed to an AMF for
35 different time periods (1min - 5min). The ablation range (the distance from the outer
36 edge of the black implant to the outer edge of the necrotic area) of the bovine liver
37 was macroscopically measured using a ruler (**Table S1**). The peak surface
38 temperature of the liver was continuously recorded using a far-infrared thermometer
39 (Fluke Ti32, Fluke Corporation, USA). As shown in **Fig. 5a and b**, the thermal
40 images showed that the surface temperature increased with heating time and injection
41 dosage. The temperature of the bovine liver with 0.02 ml, 0.04 ml, 0.06 ml, 0.08 ml
42 and 0.1 ml of HPMC/Fe₃O₄ gradually increased from 24.9±1.5°C to 98.3±8.6°C
43 within 5 min. In **Fig. 5c and d**, the macroscopic images showed the ablation range
44 extended with the prolongation heating time the addition of the injection dosage. The
45 time-ablation distance curves exhibited the increasing trends of the ablation distance.
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On the one hand, the results demonstrated that magnetic HPMC/Fe₃O₄ with an applied AMF could quickly heat the bovine liver tissue and generate coagulation necrosis, the heating and ablation efficiency of HPMC/Fe₃O₄ in bovine liver increased with the heating time and the injection dosage. On the other hand, this experiment could provide the reference data for us to select the appropriate injection dosage and heating time according to the tumor size, and then achieve the purpose of best ablation effect without damaging normal tissue. We recognized that these experimental data were not very perfect, lots of living animal experiments will be carried out in the future to optimize it.

3.7. Therapeutic Efficacy in Tumor Model

To investigate the therapeutic efficacy, the tumor-bearing mice were randomly divided into three groups. Mice in the treated group were subjected to intratumoral injection of HPMC/Fe₃O₄ and exposure to an AMF with magnetic heating. For comparison, we prepared the following control groups: mice without any treatment, and mice subjected to intratumoral administration of HPMC/Fe₃O₄. A far-infrared thermometer was applied in situ to monitor the temperature changes and acquire thermal images every 10 s (**Fig. 6a**). Tumor coagulation necrosis reportedly occurs at temperatures above 47°C lasting for a few minutes of exposure [45, 46]. In our experiment, the temperature of the tumor tissues increased rapidly, and the high-temperature area quickly extended to more than 60°C at the location of the injection site (**Fig. 6a and b**). Surprisingly, the extended high-temperature area covered almost the whole area of the tumor location in 3 min. Furthermore, the quantitative time-temperature curve from the analysis of the thermal images revealed the increasing tendency of the temperature in tumor tissues (**Fig. 6b**). The in vivo therapeutic efficiency of the HPMC/Fe₃O₄ implant after magnetic field irradiation was evaluated by measuring the tumor volumes. Remarkably, the tumor shrank significantly on day 5 after ablation and then disappeared on the 15th day with further observation (**Fig. 6c**). During the experiment, the skin color of the tumor tissue in the

experimental group immediately turned pale after local application of an AFM for 3 min. As shown in **Fig. S7**, the tumor turned black after 1 day post-ablation, and a clear boundary between the non-ablated and ablated tissue was present at 5 days after magnetic hyperthermia ablation. At 10 days, most of the black necrotic tissue had desquamated from the surrounding skin, and the tumors were obviously diminishing. Surprisingly, the black necrotic tumor tissue detached from its original position at 15 days. However, the tumor volumes in the control groups significantly increased with time. The treatment process of the tumors was also monitored by ultrasound. The ultrasound images (**Fig. 7a**) showed that the tumor appeared hypoechoic, the material was hyperechoic, the shape of the tumor was elliptical, and the shape of the HPMC/Fe₃O₄ material was irregular in the longitudinal and transverse sections. After heating for 3 min, the ultrasound images revealed that the HPMC/Fe₃O₄ implant had obviously contracted. The echo of the tumor tissue around the material was higher than that of before heating, which exhibited the ablation process of the tumor. The time-volume curves showed a decreasing trend of the tumor volume in the treatment group and an increasing tendency in the two control groups (**Fig. 7b**). The microscopic therapeutic outcome for the tumors was evaluated using typical H&E staining. The tumor tissues in the treated group that had received HPMC/Fe₃O₄ followed by magnetic ablation suffered from apoptosis and coagulation necrosis, and their cytoplasm was uniformly stained red. Comparatively, the control groups showed no signs of cell apoptosis, and their cells had integrated nuclei (**Fig. 7c**). Some tumor cells did not suffer absolute coagulation necrosis (cells stained dark blue, below the red dotted line in Fig. 7c) in the experimental group, but all contained non-intact nuclei and altered cell shapes, indicating that they were suffering from apoptosis and death. The body weights of the ablated mice were recorded every day. As shown in **Fig. S8**, the body weights of the treated mice decreased at 5 days, 10 days and 15 days after ablation, which was consistent with the time points of the significant reductions in tumor volume in Fig. S7. The weight of the mice after ablation in an AMF had no significantly statistical difference compared with that of the blank control group.

1 In this study, HPMC/Fe₃O₄ exhibited high anticancer efficiency in the model tumor
2 exposed to an AMF, and Fe₃O₄ played an essential role in this effect. The Fe₃O₄
3 particles, which acted as perfect thermoseeds for local magnetic hyperthermia
4 treatment of tumor were used in our study, have superior magnetic and inductive heat
5 properties [47] and can cause an obvious thermal effect under the action of a magnetic
6 field [7, 48]. It was reported that cell death or coagulation necrosis were induced
7 through a necrotic pathway at temperatures above 47°C [49]. In our experiments, the
8 heating temperature could exceed 60°C, the tumor cells appeared apoptotic and
9 coagulative necrosis occurred in the tumor tissues. Moreover, cancerous tissues have
10 been reported to exhibit higher sensitivity to hyperthermia than normal tissues [50].
11 Therefore, the magnetic HPMC/Fe₃O₄ implant responded to an external AMF and
12 exhibited high efficiency for tumor therapy.
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26 **4. Conclusion**

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29 In this study, we developed a thermally responsive contractible material using HPMC
30 and Fe₃O₄ with good injectability, and have confirmed its safety as well as the high
31 ablation efficiency for the minimally invasive magnetic ablation of tumors. The
32 undiscovered mechanism for the contractibility of the HPMC/Fe₃O₄ might owe to the
33 hydrophobic interactions between the cage-like hydrogen bonds of water and the
34 methoxy groups on the polymer chains of HPMC. We believe that this novel material
35 will further improve the safety to promote the rapid bench-to-bed translation of
36 magnetic-hyperthermia technology, and the developed “thermally contractible
37 biomaterial” is also expected to bring a new concept for the biomaterial research field.
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49 **Author contributions**

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52 All authors contributed to the written manuscript and have all approved the final
53 version of the manuscript.
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58 **Acknowledgments**

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Figures

Fig. 1. Heating and injection efficiency of HPMC/ Fe_3O_4 with different ferrous ferric oxide contents. (a) Thermal images of HPMC/ Fe_3O_4 with different ferrous ferric oxide contents at different times. (b) Corresponding temperature-time curves of HPMC/ Fe_3O_4 with different ferrous ferric oxide contents. (c) Photographs of the injection of HPMC/ Fe_3O_4 .

Fig. 2. Morphological characterization of the HPMC/Fe₃O₄ matrix. (a) SEM images of the HPMC/Fe₃O₄ matrix. (b) Elemental energy spectrum from EDS. The darker areas (*) reflect the rough surface of the matrix. (c) Corresponding elemental energy spectrum from the EDS map scan.

Fig. 3. Thermally responsive contraction ability. (a) Ultrasound images of the volume changes of the HPMC/Fe₃O₄ material in the excised bovine liver before and after ablation (L-axis: long axis; S-axis: short axis). (b) Ultrasound images of the volume changes of the HPMC/Fe₃O₄ material in tumors before and after ablation (L-axis: long axis; S-axis: short axis). (c) CT images of the volume changes of the HPMC/Fe₃O₄ material in the tumors before and after ablation (red dotted line: HPMC/Fe₃O₄ implant; yellow arrows: "tail" of the HPMC/Fe₃O₄ implant). (d) Visual observation of the length changes of the "tail" before and after AMF (red dotted line: "tail" of the HPMC/Fe₃O₄ implant; m: length of the "tail" before heating (10±1.5 mm); n: length of the "tail" after heating for 30 s (5±0.3 mm)). (e) Volume-time curve for the HPMC/Fe₃O₄ matrix.

Fig. 4. Cytotoxicity of HPMC/Fe₃O₄. (a) Microscopic structure of SMCs incubated with HPMC/Fe₃O₄ for 24 h. (b) Cell viability-concentration curve from the MTT assay for HPMC/Fe₃O₄ with different ferrous ferric oxide contents. (c) Cell apoptosis results measured by FCM.

Fig. 5. Heating and ablation efficiency of HPMC/Fe₃O₄ in excised bovine liver. (a) Thermal images of different weights of HPMC/Fe₃O₄ at different magnetic heating time points. (b) Corresponding temperature-time curves for Fig. 5a. (c) Macroscopic changes in the ablated tissue area for different weights of HPMC/Fe₃O₄ at different magnetic heating time points. (d) Corresponding ablation distance-time curves for Fig. 5c.

Fig. 6. Magnetic hyperthermia ablation efficiency in vivo. (a) Thermal images of tumors containing 0.06 ml of HPMC/ Fe_3O_4 after magnetic heating for 180 s. (b) Corresponding temperature-time curve. (c) Macroscopic changes in tumors at the time points of 5 days and 15 days after magnetic hyperthermia ablation treatment.

Fig. 7. Magnetic hyperthermia ablation efficiency in vivo. (a) Ultrasound images of 0.06 ml of HPMC/Fe₃O₄ injected inside a tumor before and after 180 s of magnetic induction heating, and the area changes of the HPMC/Fe₃O₄ material in the tumor at the time points of 7 days and 14 days after magnetic heating (L-axis: long axis; S-axis: short axis; T: tumor; M and red continuous line: HPMC/Fe₃O₄ implant; Mu and pink dotted line: leg muscle of the mice; blue dotted line: tumor tissue after ablation; red arrow: the pit was caused by the disappearance of tumor). (b) Tumor volume-time curves showing changes in the treated and untreated groups. (c) Microscopic images of the tumor tissue (H&E staining) showing coagulation necrosis of the ablated tumor and the clear boundary between the coagulation necrosis and the liver tumor tissue (red dotted line). The scale bar is 100 μ m.

Scheme 1. Injectable and thermally contractible HPMC/Fe₃O₄ for the magnetic hyperthermia ablation of tumors. (a) Injection of the HPMC/Fe₃O₄ material into the tumor with the formation of “tail”-like leakage. A₁) Corresponding diagram for Scheme 1a of the HPMC/Fe₃O₄ material inside the tumor and the adjacent normal structures: the “tail”-like leakage is close to the important normal structures (green, red and blue vessels). (b) HPMC/Fe₃O₄ inside the tumor under an AMF: HPMC/Fe₃O₄ contracts after heating (red color represents the increased temperature of HPMC/Fe₃O₄). B₁) Corresponding diagram for Scheme 1b of the HPMC/Fe₃O₄ material inside the tumor and the adjacent normal structures: HPMC/Fe₃O₄ contracts, and its “tail” disappear after heating. The HPMC/Fe₃O₄ implant is placed away from the important structures, which avoids damage to the important structures. (c) The tumor disappears with the degradation of HPMC/Fe₃O₄. c₁) Corresponding diagram of HPMC/Fe₃O₄ degradation. (d) Molecular formula of HPMC.