Thermosensitive Chitosan Hydrogel for Implantable Drug Delivery: Blending PVA to Mitigate Body Response and Promote Bioavailability

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ABSTRACT: Thermosensitive hydrogels promise to be the injectable implants for long-term controlled drug release; however, body response to the implanted hydrogels and its unpredictable impacts on drug release complicates their applications. In the present study, hydrophilic polymer poly(vinyl alcohol) (PVA) was blended into the thermosensitive hydrogel composed of chitosan and glycerophosphate to mitigate the body responses and promote the drug bioavailability. The effects of PVA on the surface properties of the hydrogel were evaluated by zeta-potential, water contact angle, and cell attachment. Body responses were explored by histological examination via subcutaneously implanting the hydrogels into Sprague-Dawley rats. Drug release *in vivo* and bioavailability were determined with cyclosporine A (CsA) employed as the model drug. The results showed that, on one hand, the presence of PVA improved the surface hydrophilicity of the hydrogel and inhibited the cell attachment on the hydrogel, which alleviated the further cell infiltration and tissue integration in body; and on the other hand, blending of PVA led to the more rapid gel formation and more compact network, which resisted the dehydration and survived the hydrogel from cell division. These advantages benefited the controlled release and absorption of CsA, and contributed to the higher drug bioavailability. © 2012 Wiley Periodicals, Inc. J Appl Polym Sci 125: 2092–2101, 2012

Key words: drug delivery systems; hydrogel; hydrophilic polymer; biocompatibility; implant

INTRODUCTION

Hydrogels have been extensively researched as implantable drug delivery matrix over the last few decades due to their biocompatible properties and the great potentials in controlled drug release.[1,2] The *in situ* forming hydrogel, which can be introduced into the body as injectable fluid prior to solidifying or gelling within the local tissue, enables the injectable implant with a minimally invasive manner and provides a more simple and safe method for implantation.[3,4] Thermosensitive hydrogel was widely reported as *in situ* implant for the fact that body temperature is always higher than room temperature. Several thermosensitive formulations have been developed based on various polymers.[5–7] These formulations formed semi-solid gels as expected *in vivo*, and maintained their integrity for more than one month and allowed sustained drug release.

In spite of the rapid development and significant progress in implantable drug delivery based on thermosensitive hydrogel, there still lie several key issues that handicapped the applications, including the body responses and their unpredictable impacts on drug release.[8–10] Our previous studies on thermosensitive hydrogel composed of chitosan and glycerophosphate (CS/GP) manifested an acute inflammatory response which transformed the network structure and composition of the hydrogel.[11] CS, a natural polymer which proved good biocompatibility and biodegradability, is considered to be a potential candidate for various biomedical and pharmaceutical applications.[12,13] Incorporated with GP, the pH-sensitive solution of CS exhibited the thermosensitivity at physiological pH.[14–16] After injection into Sprague-Dawley (SD) rats, it showed that the CS/GP hydrogel caused immediate body responses in terms of inflammatory cell infiltrating, tissue encapsulating, and the vascularization in tissue.[11] The body response caused the dehydration of the hydrogel and the compaction of the network initially. The cell infiltrating and tissue encapsulating divided the hydrogel into small pieces afterward and made the mass transport paths more tortuous. Moreover, the tissue encapsulation acted as the drug release barrier delaying the subsequent absorption. Therefore, mitigating the undesirable body responses toward the implants, as well as improving the controlled release, became one of the

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most important issues for the applications of the hydrogel implants.

The unfavorable interaction between an implant and the surrounding tissue firstly occurs as the cell attaching on the interface,[17] which is considered to be mainly dependent on the adsorption of adhesive proteins on the cell surface. Hydrophilic polymers can handicap the protein adsorption at their surface[18] so as to modulate the cell attachment behavior. Therefore, we came to the idea of blending hydrophilic polymers into the thermosensitive hydrogel to mitigate the body response and to promote the drug bioavailability. Poly(vinyl alcohol) (PVA), a kind of widely used hydrophilic polymer with excellent weight-bearing properties and biocompatibility,[19,20] was incorporated into the CS/GP thermosensitive solution to modify the hydrophilicity and strength of the hydrogel network in this study. PVA has been reported as a coating on the surface of some implant devices to enhance the biocompatibility and inhibit protein adsorption and cell adhesion,[21] and also was used for CS/PVA blended membranes to achieve better mechanical properties.[22–24] In this work, we proposed that PVA would improve the surface/internal properties of the CS/GP hydrogel and further impact on the body response as well as the drug release behavior. The effects of PVA on the surface properties of the hydrogel were investigated through the zeta-potential analysis, water contact angles measurement, and attachment of fibroblast cells L929 on the CS/GP/PVA hydrogel. The body responses were studied by histological examination via subcutaneously implanting CS/GP/PVA hydrogels into the SD rats. The variations of the CS/GP/PVA implants in the body were traced and compared with the CS/GP implants, and the effects on the *in vivo* drug release were further evaluated and discussed.

MATERIALS AND METHODS

Materials

CS ($M_w \sim 7.5 \times 10^5$, degree of deacetylation was 92.8%) was acquired from Lizhong Chitin Co. Ltd. (Qingdao, China). PVA powder (degree of polymerization was about 1100), pentobarbital sodium salt and β-Glycerol phosphate disodium salt pentahydrat were obtained from Sigma-Aldrich Co. LLC. (St. Louis, MO, USA). Cyclosporine A (CsA, 99%, pharmaceutical grade) was offered by Xianghe Fine Chemical Co. Ltd. (Wuhan, China). All the other chemicals used in this work were pharmaceutical or reagent grade.

Preparation and gel forming of CS/GP/PVA solution

The CS/GP/PVA solution was prepared according to the work of Zan et al.[25,26] In brief, 1.5% (w/w) CS solution was prepared by dissolving CS into a 0.1 mol L^{-1} acetic solution at room temperature. PVA powder was dissolved into the CS solution at 80°C with stirring until completely dissolved. The solution was cooled to 25°C and GP was slowly added until its concentration reached 4% (w/w). Finally, the pH of the solution was adjusted to 7.3 with Na_2HPO_4. The CS/GP solution was similarly prepared with the absent step of adding PVA. CsA, a potent immunosuppressive agent preventing allograft rejection in organ transplantation, was employed as a model drug in the present study. The CsA powder was evenly dispersed into the CS/GP/PVA or CS/GP solution at the concentration of 5 mg CsA per milliliter solution.

The gel forming process was studied by viscosity tracing in a 37°C bath with a viscometer (DV-E Brookfield Inc, Middleboro, MA, USA), in order to insure that the solution would form gel quickly in the body. Gel forming caused the sharp rise of the viscosity of the solution and the viscosity approaching infinity was considered to indicate the completion of gel-forming.[25] Before gel forming, zeta-potential, the electric charge on the polymer colloid surface of the CS/GP and CS/GP/PVA solution was measured by zeta potential analyzer (ZetaPALS, Brookhaven Inc, Holtsville, NY, USA). The measurements were carried out at 20°C and pH of 7.3. After the gel was completely formed, the water contact angles of the hydrogel surface were measured by optical contact angle measurement system (OCAH200, Dataphysics Instruments GmbH, Germany). The hydrogel sample was slice of 3 × 3 cm and a 15 μL deionized water droplet was made by placing the syringe tip close to the hydrogel surface.

Cell attachment

To investigate the cell attachment on the hydrogel surface, the L929 murine fibrosarcoma cells, which could live either when they were adherent or suspended, were cultured in the culture medium (RPMI 1640) supplemented with 10% (v/v) fetal calf serum (FCS) at 37°C in an atmosphere of 5% CO_2 and 95% humidity. The cells were seeded and cultured for days at an initial density of 5×10^5 cells on a 60 Φ dish which was coated by the hydrogel on the bottom. The cells attached on the hydrogel were fixed in 2.5% glutaraldehyde solution, dehydrated using a graded series of ethanol solutions, and then dried with 1,1,1,3,3,3-hexamethyldisilane.[21] The morphology of the cells was observed by scanning electron microscope (SEM, KYKY 2800, KYKY Technology Development Ltd., China) with the acceleration voltage of 20 kV.

LIVE/DEAD staining assay (2 m*M* calcein-AM and 4 m*M* ethidium homodimer in 1 mL PBS) was

conducted to evaluate the viability of L929 cells attached. The living cells stained with calcein fluorescently showed green color and the dead cells stained with ethidium homodimer became red.

In vitro incubation and drug release test

The CS/GP/PVA or CS/GP solution was filled in dialyzer bag (molecular weight cut-off 14,000, Sigma) and the dialyzer bag was placed into the tube containing 40 mL phosphate buffered saline (PBS, 0.1 mol L^{-1}, pH 7.4), which was incubated in a 37°C shaking bath (100 rpm min^{-1}). The hydrogel samples were taken out at certain intervals for water ratio measurement and morphological observation. The PBS was replaced everyday. For *in vitro* drug release test, sodium dodecyl sulfate (SDS) was added into the PBS at the content of 5% (w/v), in order to ensure the sink condition. The receive solution was taken out and fresh solution was refilled at certain time intervals. The CsA concentration of the receive solution was determined by high performance liquid chromatography (HPLC) with an internal standard of tamoxifen.[27] All the experimental data represent the mean ± SD from three paralleled groups ($n = 3$).

In vivo injection and histological examinations

The CS/GP and CS/GP/PVA solutions were subcutaneously injected into the back of the SD rats (male, 150–200 g). Each rat received 4–6 injections with a solution volume of 0.5–1 mL. At regular intervals, the implanted hydrogels were retrieved together with surrounding fibrous tissues by surgery for further studies. The rats were anesthetized by injection of pentobarbital sodium salt solution (2% w/w, dosage 40 mg/kg) before the implant injection or surgery and sacrificed by cervical dislocation after all these experimental procedures. Six SD rats were used as parallel samples in each experimental group ($n = 6$) and every acquired data point represented mean ± standard deviation of the six samples. The *in vivo* experiments were carried out under the guidelines of the Chinese National Standards for laboratory animals (GB 14922-14925, 2001).

Immediately after retrieved, the specimen hydrogels which were enveloped by fibrous tissues were fixed in 5% formaldehyde solution for more than a week. The fixed hydrogels were prepared into paraffin blocks and cut into thin (5 μm) sections. The sections were stained with hematoxylin and eosin (HE), and then examined through optical microscopy.

Water ratio measurement and network morphology

The hydrogel retrieved from SD rats was carefully isolated from the surrounding tissues so that unadulterated hydrogel (cells infiltration could be negligible) was recovered for network morphology and water ratio measurement, respectively. The water ratio was also determined during the *in vitro* incubation as the contrast.

The water ratio (W_t) was defined as the weight ratio of total water in the network to the dried hydrogel skeleton,[28] i.e.:

$$W_t = (M_m - M_d)/M_d \quad (1)$$

in which the weight of the moistened sample (M_m) was measured after carefully removing the adhered water on the hydrogel surface and the weight of dried hydrogel skeleton (M_d) was measured after the hydrogel was completely lyophilized.

For morphological observation, the isolated hydrogels were fast frozen in liquid nitrogen and sectioned by cold knife to maintain the porous section without any collapse.[16,29] Then, the specimen was lyophilized, coated with gold, and then observed by SEM.

In vivo drug release

The drug loaded solution was subcutaneously injected into SD rats (50 mg CsA per kg) and formed the hydrogel *in situ*. The blood samples were drawn from the rats' eyeballs (0.5 mL each time) and CsA concentration in whole blood was analyzed (HPLC method was the same as that of *in vitro*). The pharmacokinetic parameters of CsA, i.e., the maximum blood concentration (C_{max}), the time to reach C_{max} (T_{max}), and the areas under the whole blood concentration–time curve in 42 days ($AUC_{0-42\ days}$) were estimated($n = 6$). The relative bioavailability (BA_r) between CS/GP/PVA and CS/GP was calculated. A one-way analysis of variance (ANOVA) followed by Tukey's test was used for analyzing the individual differences.

RESULTS AND DISCUSSION

Gel-forming

Gel-forming results in the dramatic increase of the solution viscosity, so that the gel-forming process could be characterized by tracing the viscosity. The viscosity (η) variations of CS/PVA and CS/GP/PVA solutions at 37°C *in vitro* with different mass ratio of PVA to CS ($R_{PVA/CS}$) were shown in Figure 1. It could be seen that the viscosities were raised sharply after a lag phase for all the solutions. The lag phase was ascribed to the heating process of the solution and the sharp raise of viscosity to infinity denoted the gel formation. The solution with higher $R_{PVA/CS}$ exhibited shorter lag time resulting in the faster gel-forming process. This result might be

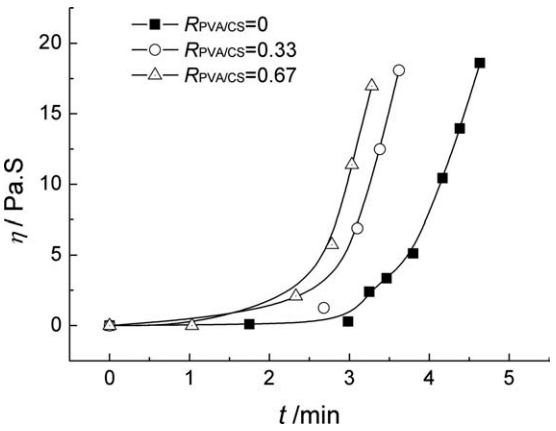

Figure 1 The viscosity (η) variations of CS/GP/PVA solutions with different ratios of PVA to CS ($R_{PVA/CS}$) at 37°C.

attributed to the impacts of PVA on the interactions between the GP molecules and the amido groups of CS. It has been reported that the polyol parts of GP enhanced the protective hydration of CS; therefore the electric interactions between GP molecules and the amido groups of CS can handicap the gelation of CS at neutral pH and convert the pH-sensitive hydrogel into thermosensitive hydrogel.[14,16] In the CS/GP/PVA solution, the additional PVA prevented the interactions between GP and CS molecules and weakened the protective hydration. When the solution was heated-up, the GP and water molecules around CS became easier to be removed and the gel formed faster through the hydrophobic attractions and hydrogen bonds between CS chains. However, $R_{PVA/CS}$ should not be higher than 0.67 because flocculation would occur below neutral pH with the higher $R_{PVA/CS}$.

The *in vivo* evaluation of gel-forming was also conducted by subcutaneous injection and surgical examination. As expected, the CS/GP/PVA solution was found to form the hydrogel *in situ* within a short time.

Surface property and cell attachment

The surface properties of the implant play the important roles because the unfavorable interaction between an implant and the surrounding tissue firstly occurs at the interface.[17] For tissue engineering, the implant surface was designed to enhance beneficial cell responses as the attachment and proliferation of osteoblasts, but to inhibit the pathogenic microbial adhesion.[30,31] However, for long-term drug delivery implants, the cell attachment and infiltration were proposed to be inhibited for maintaining the integrity and the initial network structure of the implant, because the host cell responses would induce the variation of the hydrogel network and influence the drug release.

It is considered that the cell attachment, which is largely dependent on the adsorption of adhesive proteins, is related to the properties as surface charge and hydrophilicity: neutral charged or/and hydrophobic surfaces are preferred for cell attachment.[32] Some hydrophilic polymers could handicap the protein adsorption at their surface so as to modulate the cell attachment behavior.

The surface charge of the polymer colloid (denoted by zeta-potential) and the hydrophilicity (denoted by water contact angle) of the hydrogel were investigated. Data were shown in Table I. It has been reported that the CS colloid generated positive charges due to the weakly acidic NH_3^+ groups at acidic and neutral pH.[22,25] Although the PVA was negatively charged, the presence of PVA only resulted in a quite slight reduction of the zeta-potential. This slight reduction of zeta-potential could not cause a significant impact on the cell attachment. However, as the hydrophobic CS skeleton was replaced by some hydrophilic PVA chains on the surface, the blending of PVA brought the significant reduction of the water contact angles with the increased $R_{PVA/CS}$, which indicated the surface hydrophilicity of the hydrogel was significantly improved. Therefore, the blending of PVA was supposed to impact on the cell attachment mostly due to the improvement of hydrogel hydrophilicity.

The L929 cells were cultured with the hydrogels for investigating cell attachment. The SEM images (Fig. 2) showed that the cells preferred to be attached on the surfaces of the culture dish and the CS/GP hydrogel but to be suspended in the culture medium when they were exposed to CS/GP/PVA hydrogel. Furthermore, those well attached cells were plump, but the cells on the CS/GP/PVA hydrogel surface showed a little crimpy. LIVE/DEAD staining assay results (Fig. 3) indicated that all the cells on the hydrogels and in the culture medium were viable, which illustrated the low cytotoxicity of all the hydrogels. The above experiments demonstrated that cell attachment on the hydrogel was significantly restrained in the presence of PVA. It is reasonably presumed that, by adding PVA, the undesirable blood-contact adhesion and the integration between

TABLE I
The Zeta-Potentials and Water Contact Angles on the Surfaces of the CS/GP/PVA Hydrogels with Different $R_{PVA/CS}$ ($n = 3$)

$R_{PVA/CS}$ of the hydrogel	Zeta-potential (mV)	Water contact angle (°)
0	5.06 ± 0.53	92 ± 5
0.17	4.60 ± 0.69	61 ± 5
0.33	4.58 ± 0.33	<5
0.67	4.49 ± 0.44	<5

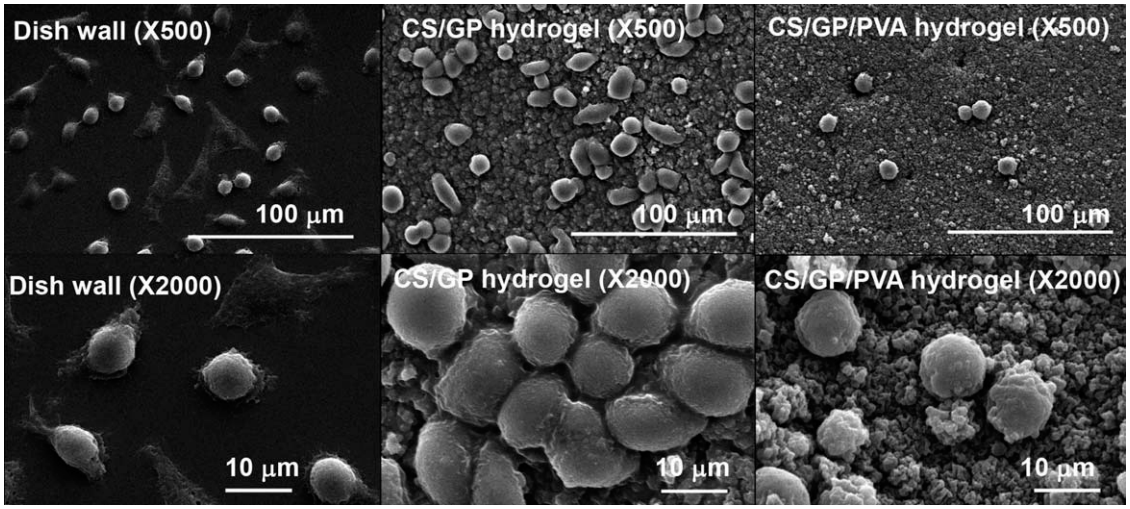

Figure 2 The SEM photos of the cells attached on the culture dish and the hydrogels.

hydrogel and inflammatory cells would be well reduced,[21] which would be discussed in the following section.

Histological examination

Our previous studies showed a relative strong body response to the implanted CS/GP hydrogel including the inflammatory cell infiltrating, the tissue encapsulating, and the vascularization.[11] All these responses were initiated by the cells' contact with the surface of the hydrogel. And the surface of CS/GP/PVA hydrogel proved to be more hydrophilic which handicapped the cell attachment *in vitro* previously. In this section, body responses to CS/GP/PVA hydrogels with different $R_{PVA/CS}$ were investigated in comparison with CS/GP hydrogel by histological examination.

The HE stained images of the interfaces between the hydrogels and tissues (Fig. 4) showed a typical process of body response to the CS/GP/PVA implants: the acute inflammatory reactions in the first week and the self-reparation by tissues encapsulating and then growing into the foreign implant thereafter. The responses to the CS/GP/PVA hydrogel in the first two weeks were similar to those to the CS/GP hydrogel as reported in our previous work. In the later 3 weeks, an integration layer with half material and half tissue was shown [Fig. 4(g–l)] due to the infiltration of the host cell and the integration of the connective tissues with biomaterials, and then significant differentials emerged between

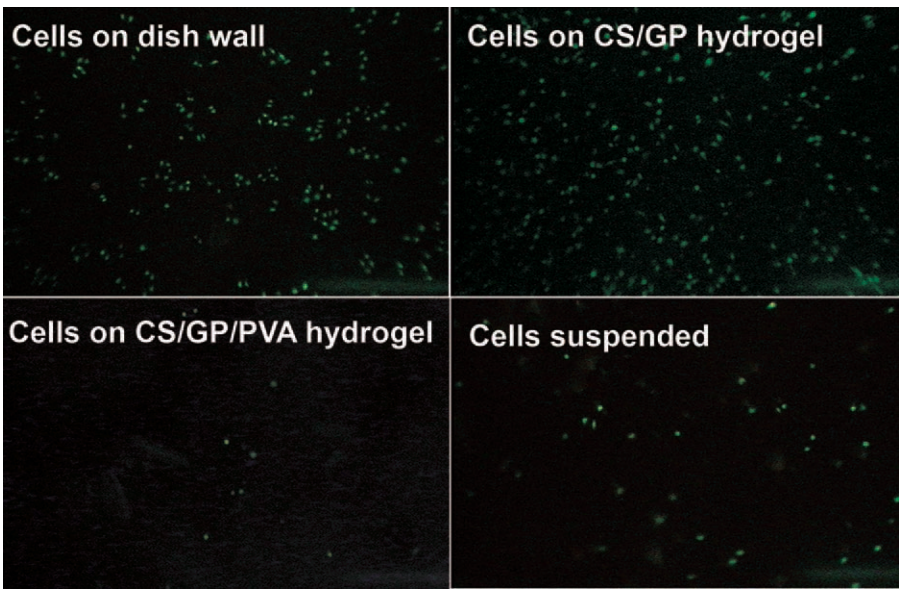

Figure 3 The viability of the cells attached or suspended. The living cells fluorescently showed green color and the dead cells showed red. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

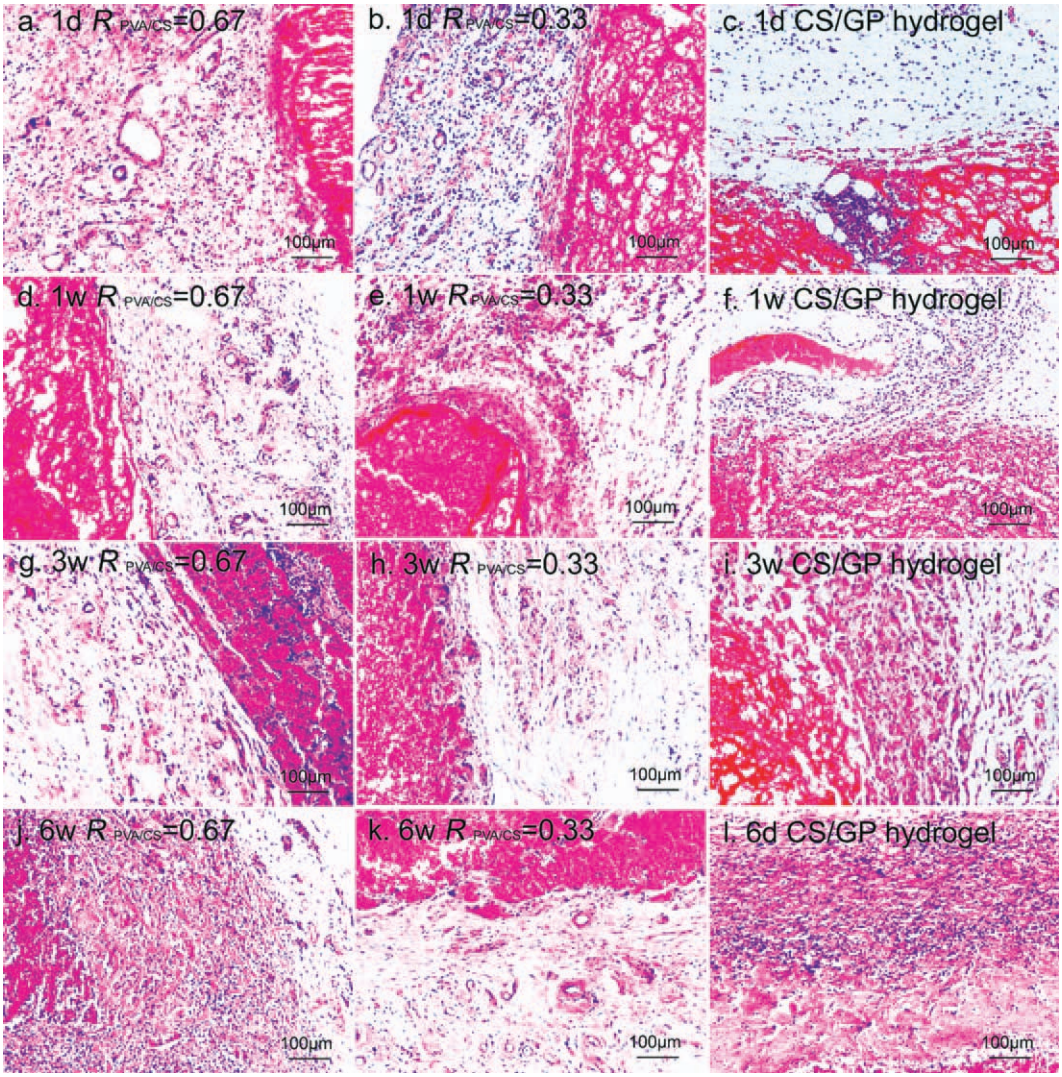

Figure 4 Histological slides of hydrogel implants with different $R_{PVA/CS}$ at the interfaces between the hydrogels and the tissues (200×). The hydrogels exhibited were implanted into the body and retrieved after gel forming in 1 day (a–c), 1 week (d–f), 3 weeks (g–i), and 6 weeks (j–l). [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

the CS/GP and CS/GP/PVA hydrogel. For the CS/GP hydrogel, the boundary of the implant was found undistinguished in the third week. The loose CS skeleton close to the hydrogel surface was divided and enwrapped by the tissues. However, the boundaries of the CS/GP/PVA hydrogels were still clear although some host cells were detected in the hydrogel, which demonstrated that the CS/GP/PVA hydrogel was more difficult to be divided and digested by the cells. Moreover, the hydrogel with the higher ratio of PVA to CS seemed to be more advantageous in protecting the integrity of the implants [Fig. 4(g–i)]. As time goes on, the CS/GP/PVA hydrogel on the surface would also be gradually wrapped and digested (Fig. 4(j–l)], resulting in the same integration layer as the CS/GP hydrogel in the sixth week.

It was assumed that the high hydrophilicity of the CS/GP/PVA hydrogel mitigated the cells' attachment and infiltration, and prevented the compact and firm hydrogel network from being segmented by tissues to some extent (discussed in section "Water ratio and network structure"), so that the CS/GP/PVA hydrogel could be held in the integrity in the initial inflammatory and self-repair stages (first four weeks) during which most of the drug was proposed to be released. Consequently, the impacts of the body response on the drug release, as well as the individual differences might be attenuated by blending PVA into the hydrogel. In addition, as the integration of the tissues and materials shown in the sixth week, it could be concluded that these implants would be finally digested after the drug was released.

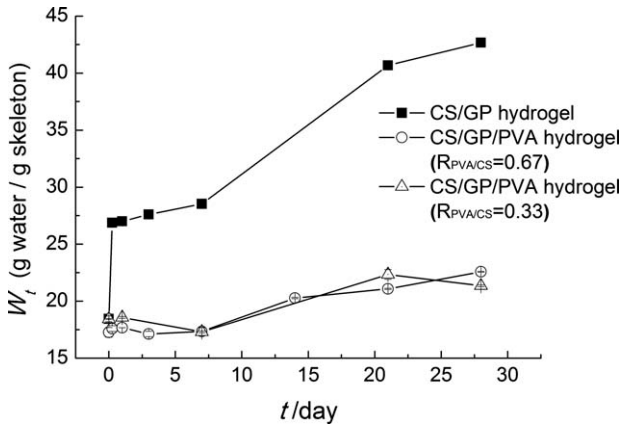

Figure 5 The total water ratio (W_t) of the hydrogels with/without PVA versus the incubating time *in vitro*. The hydrogels were incubated in the PBS. Each value represents the mean ± SD (n =3).

Water ratio and network structure

Body response to the hydrogel always results in the change of water ratio and the transformation of the hydrogel network which produce significant impacts on degradation and drug release of the implant.[1,33] In our previous work, we have found that the CS/GP hydrogel was dehydrated after gel-forming in body because of the water content differentia between the tissue and the fresh hydrogel. The change of water ratio, as well as the previously mentioned cell infiltration, caused the network transformation.

The variations of W_t of the CS/GP and CS/GP/PVA hydrogels were shown in Figures 5 (*in vitro*) and 6 (*in vivo*). The hydrogel is semi-solid composed of polymer skeleton and water. The newly formed hydrogel contained approximately 5% of skeleton material and most of the hydrogel was water, so the water ratio, as the water in the hydrogel to the dried skeleton, was very high and varied remarkably with swelling and dehydration. The water ratio of the CS/GP/PVA hydrogel varied far away from those of the CS/GP hydrogel both *in vitro* and *in vivo*. The absence of sudden increase in the first 6 h and the slower increasing thereafter made water ratio of the CS/GP/PVA hydrogel become much lower but stable *in vitro.* For the case of *in vivo* implantation, after an initial decline, the water ratio of the CS/GP/PVA hydrogel was maintained unchanged in the first week, in contrast with the continuous decline of CS/GP hydrogel. This made more water reserved in the CS/GP/PVA hydrogel although the water ratio was declined afterwards with a same rate as the CS/GP hydrogel. The results were mainly ascribed to the impacts of PVA on both the swelling behavior and the water configuration of the hydrogel.[28,34] On one hand, the hydrophilic PVA enhanced the interactions between the polymer network and the water molecules, so that more water molecules were stably bonded with the CS/PVA network. On the other hand, PVA molecules were physically entangled inside the CS network, which contributed to the more compacted structure. The more compact CS/PVA network restricted the hydrogel from both swelling and dehydration; the more bond water molecules enabled the relatively consistent water ratio of the CS/GP/PVA hydrogel. The change of PVA content in the presented range created negligible impact on the initial water ratio and the variations of water ratio both *in vivo* and *in vitro,* except that the water ratio of the hydrogel with $R_{PVA/CS}$ of 0.67 reduced more in the first 3 days *in vivo*. The reason for the phenomenon might be that the redundant PVA molecules leaked away.

The variation of water ratio caused the transformation of the hydrogel network. The SEM photos (Fig. 7) showed the similar structures of CS/GP/PVA networks ($R_{PVA/CS}$ =0.67) *in vivo* and *in vitro*, as the result of the little change of water ratio *in vivo* and *in vitro*. The initial 3D network of the CS/GP/PVA hydrogel was comparatively loose, but it became more compact soon along as the PVA chains were better arranged and entangled with the CS network *in vitro*. Throughout the observation period (4 weeks), the network with abundant microstructures and smaller pores was maintained both *in vitro* and *in vivo*. These phenomena were totally different from the CS/GP hydrogel network which presented swelled structure *in vitro* and shrunk structure with fragments *in vivo*, as shown in Figure 8 (on the 7th day after gelling).

The experimental results demonstrated that the presence of PVA contributed to a more compact and firm hydrogel network, as well as the minimized differences in network structures *in vivo* and *in vitro*. It

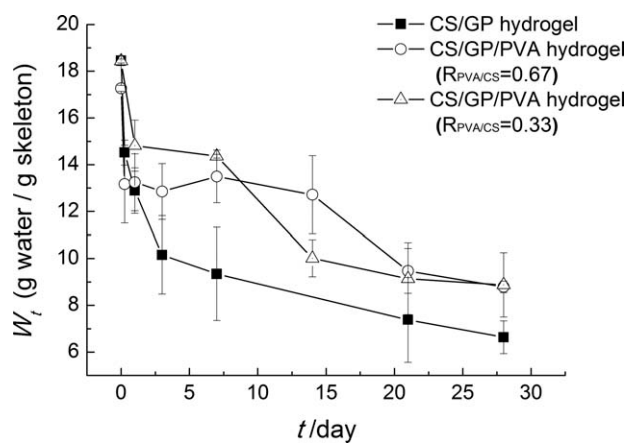

Figure 6 The total water ratio (W_t) of the hydrogels with/without PVA versus the incubating time *in vivo*. The hydrogels were formed subcutaneously and picked from the body. Each value represents the mean ± SD (n =3).

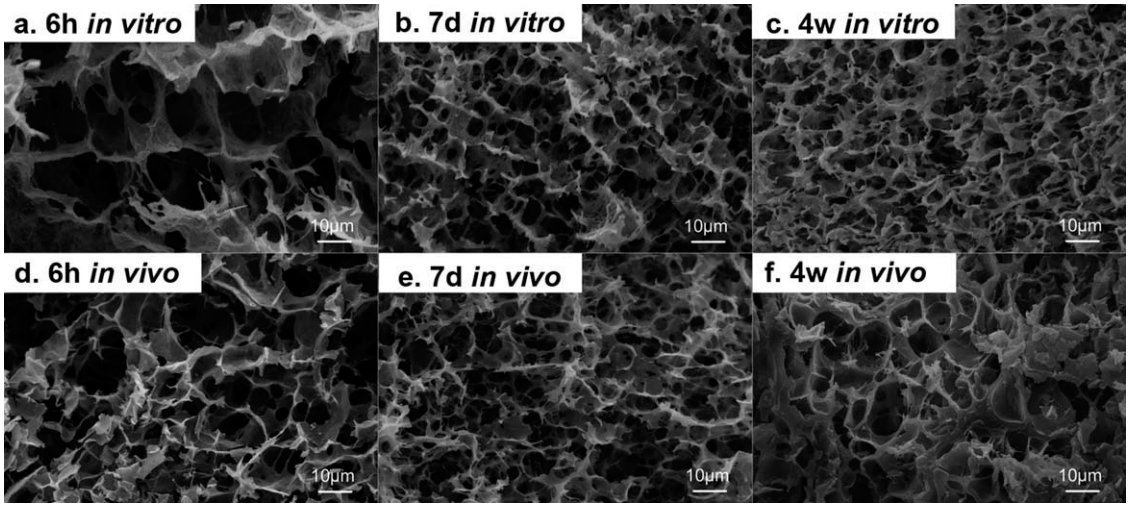

Figure 7 SEM photos of the freeze dried CS/GP/PVA hydrogels ($R_{PVA/CS} = 0.67$) *in vitro* and *in vivo*.

could also be inferred that the unpredictable variations of the hydrogel in the body would be alleviated and the *in vitro* investigations would be more advisable for analyzing the conditions *in vivo*.

Release and bioavailability of CsA

CsA should be administered for a long time to the patients after organ transplantation. As a typical fat-soluble and water-insoluble drug (Class II of BCS), it performed low oral bioavailability due to its low solubility in the gut lumen and the extensive first-pass metabolism.[35] The conventional CsA delivery system is limited in some therapies because of the systemic side effects including hypertension and notably nephrotoxicity.[36] The novel hydrogel implant is potential to improve the bioavailability of CsA and reduce the side effects owing to the long-term release manner and avoidance of first-pass metabolism.

The *in vitro* drug release profiles of the CsA from the CS/GP and CS/GP/PVA hydrogel were shown in Figure 9. Drug release rate was decreased in the presence of PVA. The hydrogel with $R_{PVA/CS}$ of 0.33 presented the zero-order release approximately. Release of CsA from the hydrogel is mainly via the dissolution of CsA in the hydrogel water and the diffusion of dissolved drug molecules through the network. For CsA is water-insoluble, it should be dissolved in the water of hydrogel before it diffuses through the network. The dissolution is dependent on the water ratio of the hydrogel and the diffusion is closely concerned with the structure of the hydrogel network. As the blending of PVA into the CS/GP hydrogel resulted in much lower water ratio and more compact network, drug release became slower. The other notable result was that the drug release of the hydrogel with higher PVA content ($R_{PVA/CS} = 0.67$) was faster in the initial 3 days. This might

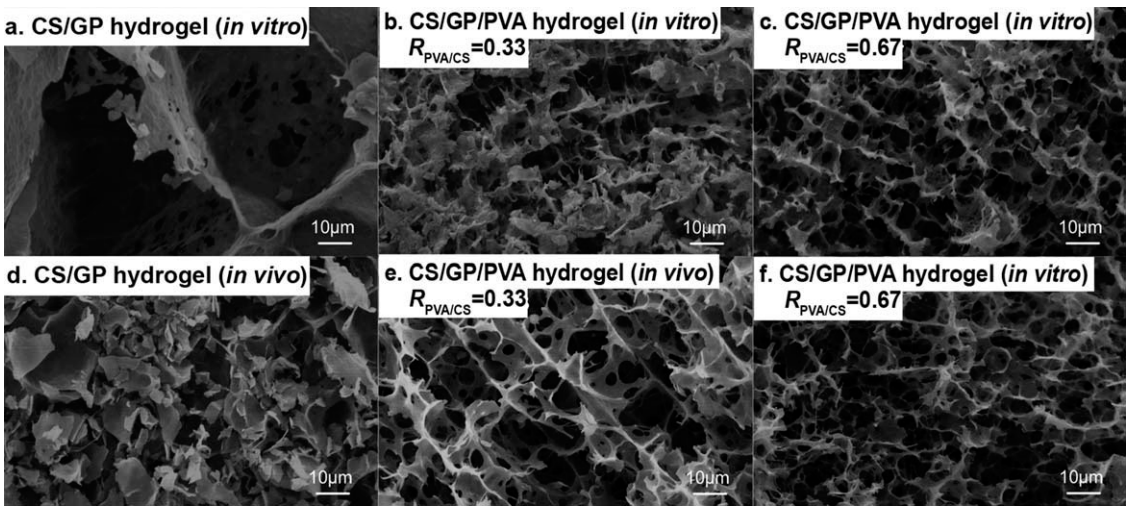

Figure 8 SEM photos of the different hydrogel networks *in vivo* and *in vitro* on the 7th day after gelling.

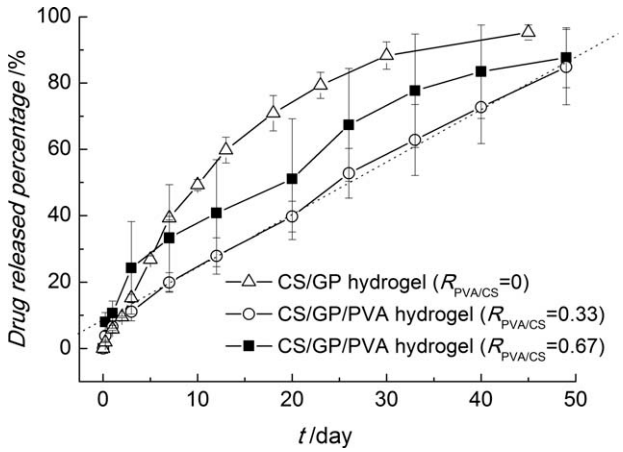

Figure 9 *In vitro* release of CsA from the CS/GP hydrogel and CS/GP/PVA hydrogel. The receive solution was PBS containing 5% (w/v) SDS. Each value represents the mean ± SD ($n = 3$).

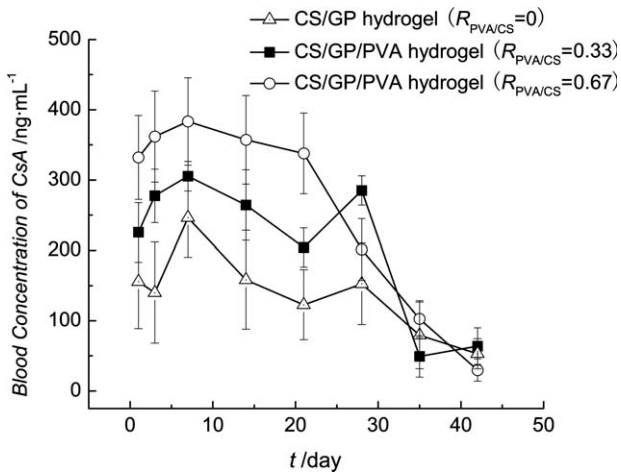

Figure 10 The blood CsA concentration–time curves in the SD rats after the subcutaneous administration of 50 mg kg^{-1} of CsA from the CS/GP hydrogel and CS/GP/PVA hydrogel. Each value represents the mean ± SD (n =6).

be the results that the water in the hydrogel with $R_{PVA/CS}$ of 0.67 was lost more in the initial days, as shown in Figure 6, and more drugs leaked out with loss of water.

The whole blood concentration–time curves after the injection of CS/GP/PVA and CS/GP hydrogels were shown in Figure 10 and the pharmacokinetic parameters were rendered in Table II. The release of CsA lasted for more than 30 days *in vivo* and the whole blood concentration within a month maintained above100 ng mL^{-1}. The T_{max} seemed to have no difference between the different types of hydrogels, but the C_{max} and $AUC_{0–42\ days}$ obtained from the group administered CS/GP/PVA hydrogels were markedly increased and higher PVA ratio resulted in much higher C_{max} and $AUC_{0–42\ days}$. The relative bioavailability (BA_r) of CS/GP/PVA hydrogels to CS/GP hydrogel was calculated to be higher than 150%, which demonstrated that the bioavailability of CsA could be significantly improved with the presence of PVA.

The *in vivo* experimental results inferred that the blending of PVA might lead to the faster drug release rate, which seemed in contrast with the *in vitro* results. The reason was that the blending of PVA resisted the water loss and maintained abundant pores in the hydrogel *in vivo*, which facilitated the dissolution and diffusion of CsA. The blending of PVA also brought the remarkable promotion of the bioavailability of CsA, which could be interpreted in terms of following aspects. First, more water was maintained within the network in which more drugs could be dissolved, and moreover, the abundant PVA, acting as the surfactant, enhanced the solubility of CsA in water; secondly, the presence of PVA attenuated the body response, especially reduced the surrounding and infiltrating of the cells to the hydrogel, which benefited the drug absorption. The cell infiltration and connective tissues integration *in vivo* transformed the hydrogel network, and built another barrier to drug absorption, and even digested some drug. The CS/GP/PVA hydrogel, with the more compact network was difficult to be compressed and unfavorable to be integrated with the tissues (section "Histological examination"). Therefore, there remained more spaces and less tortuous paths in the CS/GP/PVA hydrogel for drug diffusion, and the biologic obstruction for the drug absorption was also reduced. Summarily,

TABLE II
Pharmacokinetic Parameters of CsA Released from the CS/GP and CS/GP/PVA Hydrogels[a]

pharmacokinetic parameters	CS/GP hydrogel ($R_{PVA/CS} = 0$)	CS/GP/PVA hydrogel ($R_{PVA/CS} = 0.33$)	CS/GP/PVA hydrogel ($R_{PVA/CS} = 0.67$)
T_{max} (day)	7	7	7
C_{max} (ng mL^{-1})	246.29 ± 56.08	305.42 ± 20.91	383.20 ± 62.20
$AUC_{0–42\ days}$ (ng day mL^{-1})	5704.42 ± 943.32	8579.13 ± 1007.03	10,620.63 ± 1615.62
BA_r	–	150%	186%

[a]The rats were subcutaneously administered 50 mg kg^{-1} of CsA in the CS/GP hydrogel or CS/GP/PVA hydrogels. The values of the CS/GP/PVA hydrogels showed statistically significant differences in comparison with the CS/GP hydrogel ($P < 0.05$)

drug release from the CS/GP/PVA hydrogel and bioavailability was promoted due to the enhanced solubility of the CsA, steady diffusive paths, and decreased obstruction from the bioreactions. Consequently, higher bioavailability of CsA was achieved.

In addition, the blood concentration–time curves were fluctuated in the range of 100–400 ng mL^{-1}, within which the CsA concentrations proved to be efficacious and safe for the patients after allogeneic hematopoietic stem cell transplantation. Therefore, the hydrogel implant for CsA delivery could be considered clinically applicable.

CONCLUSION

The blending of hydrophilic polymer PVA into thermosensitive CS/GP hydrogel mitigated the body response and promoted the bioavailability of CsA in three aspects. First, the presence of PVA improved surface hydrophilicity of the hydrogel and prevented the cell attachment on the hydrogel, which alleviated the further infiltration of the host cell and the integration of the connective tissues with biomaterials; secondly, hydrogel incorporated with PVA had a more compact network, which resisted both the swelling and dehydration, and survived the hydrogel from the cell division, so that the integrity of the hydrogel was maintained for longer time; and thirdly, PVA facilitated the dissolution of CsA into the water existed in the hydrogel network. These advantages benefited the controlled drug release and the absorption of CsA, and promoted the bioavailability of CsA remarkably. Blending of hydrophilic polymer into thermosensitive hydrogel provided an advantageous way of mitigating the body response to the implanted hydrogel and promoting the bioavailability of drug.

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