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ORIGINAL ARTICLE

Self-assembled L-alanine derivative organogel as in situ drug delivery implant: characterization, biodegradability, and biocompatibility

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Abstract

Objective: The purpose of this work is to prepare and characterize the novel in situ forming implants, obtained through self-assembling of N-stearoyl-L-alanine methyl ester (SAM) in pharmaceutical oils, and to evaluate the biodegradability and biocompatibility of this organogel system. *Methods*: Minimum gelation concentration was used to measure the gelling ability of gelator SAM in different oils to select the optimal oil for further research. Phase transition temperatures of SAM/soybean oil organogels were determined by differential scanning calorimetry. Comparative studies on the in vitro degradation and in vivo degradation of SAM/soybean oil organogels in mice were investigated. Cytotoxicity tests and histological analysis of SAM/soybean oil organogels were studied by using mouse fibrosarcoma cells and mouse, respectively. *Results*: As an organogelator, SAM could gel a variety of oils at different minimum gelation concentration. Among them, it had the best-gelling ability in soybean oil, and the SAM/soybean oil organogel could be turned into gels abruptly at body temperature when the concentration of SAM was higher than 5% (w/v) to be used as an injectable system. The in vitro degradation rate of organogel was inversely proportional to the organogelator concentration, whereas the degradation rate in vivo was much higher than in vitro, and gels were almost disappeared after 6 weeks. The selected formulation showed excellent biocompatibility as tested by in vitro cytotoxicity and in vivo histological evaluation. *Conclusion*: SAM/soybean oil organogel has excellent biodegradability and biocompatibility, which indicates that it has a great potential for safe in situ forming drug delivery.

Key words: *Biocompatibility; cytotoxicity; degradation; histological analysis; organogel; phase transition temperature*

Introduction

Biodegradable injective in situ gelling systems were developed at the beginning of 1980s as a predominant drug depot system for parenteral sustained release drug delivery. These systems are made of biodegradable components, which can be injected via a syringe into the body as liquid and, once injected, solidify to form a semisolid depot and thus are attractive starting points for new drug delivery system material[1,2]. They possess many advantages, such as ease of application, localized delivery for a site-specific action[3–5], prolonged delivery periods, decreased body drug dosage with concurrent reduction in possible undesirable side effects common to most forms of systemic delivery, and improved patient compliance and comfort[6].

Biodegradable injective in situ gelling systems can be divided into two categories according to their different solvents: hydrogels and organogels. Recently, injectable hydrogels, assembled by many macromolecular copolymers, such as modified or unmodified chitosan, poly(lactic acid) (PLA)/poly(DL-lactide-co-glycolide) (PLGA), and triblock copolymers composed of ABA or BAB types, where A is polyethylene glycol (PEG) and B

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is PLGA, have been reported. Micromolecular and protein had been loaded in ABA and BAB triblock copolymer hydrogels for advanced researches[7–9]. The PLGA–PEG–PLGA (15/1) copolymer displayed a fast degradation rate in vitro for 15–22 days[8]. Furthermore, the in vitro and in vivo biocompatibilities of mPEG–PLGA–mPEG gel were compared, and the copolymer-based formulations showed excellent biocompatibility as tested by 3-(4,5)-dimethylthiahiazo (-z-y1)-3,5-di-phenytetrazoliumromide (MTT) assay and in vivo histological evaluation[10]. However, the biggest problem of such hydrogel system is that the acidic metabolites hydrolysis by lactide/glycolide copolymers can result in the labilization and denaturation of polypeptides and proteins, which would cause some security risks[11]. Although a number of studies have been devoted to solve this problem, the biocompatibility of this drug delivery is still a main barrier to limit its wide use in pharmaceutics.

To improve the biocompatibility of injective in situ gelling systems, many organogels have been used for attractive drug delivery in the last few decades. Organogels are semisolid systems, in which an organic liquid phase is immobilized by a three-dimensional network composed of self-assembled, intertwined gelator fibers[12]. Organogelators are mostly low-molecular weight (MW) molecules with the capacity of self-assembling in organic liquids at low concentrations. The gels are usually prepared by dissolving a gelator in an organic solvent on heating and then gelling sol on cooling. Upon cooling, noncovalent intermolecular interactions, such as H-bonds, π–π stacking, electrostatic interactions, and London dispersion forces, establish between the molecules, leading to various supramolecular entangled structures ranging from fibers to strands or tapes. The resulting three-dimensional network prevents the solvent from flowing and confers solid-like properties to the system[13]. Up to now, a wide variety of gelators were used to prepare sustained release drug delivery organogel system, such as glyceryl fatty acid esters[14], amino acid derivatives[15,16], fatty acid derivatives[17–19], lecithin[20–22], and poly(ethylene)[23].

As low-MW organogelators, amino acid derivative organogelators have been used in long-term delivery of drug as subcutaneously injected in situ forming organogels. It was reported that fluorescein isothiocyanate dextran could be released less than 6% after 20 days in vitro from *N*-lauroyl-L-alanine methyl ester/soybean oil organogel[24]. Six different amphiphilic organogelators based on L-alanine were synthesized and six organogels in safflower oil with these organogelators were prepared, respectively, by Plourde et al.[25] Among them, sustained release of leuprolide could be achieved from *N*-stearoyl-L-alanine methyl ester (SAM)/safflower oil organogel for 14–25 days following subcutaneous injection accompanied by sustained castration lasting up to 50 days[16]. And the same system was also used in Alzheimer's disease treatment, by loading rivastigmine as an in situ forming implant for long-term drug delivery, which had the lowest burst in vitro (<15% in 24 hours) and provided prolonged drug release within the therapeutic range for 11 days in vitro[12]. However, most studies were all focused on their ability to prolong the drug release behaviors. No work has evaluated the biocompatibility and biodegradability of organogels based on amino acid derivative organogelators.

So, in this work, the SAM/soybean oil organogel was prepared, its gelation properties were determined through inverse flow method, and the phase transition temperatures of organogels were studied. Their in vitro and in vivo degradation behaviors were investigated and the biocompatibility was first assessed by cytotoxicity tests of extraction solution and histological evaluation of the tissues adjacent to the implants. The intention of this research is to point out the biodegradability and biocompatibility of SAM organogel, which helps to provide much more powerful evidence to prove that the SAM organogel can be used as biodegradable sustained drug delivery implant.

Materials and methods

Materials

The organogelator SAM used in this study was synthesized as described by Hoarau et al.[26] and Motulsky[27]. Four vegetable oils and medium chain triglycerides (Mig812) were purchased from Tieling Beiya Medicinal Oil Co. Ltd., Tieling, China and Sasol German Co. Ltd., Hamburger, Germany, respectively. Isopropyl palmitate (IPP) and isopropyl myristate (IPM) were obtained from Beijing Hanlin Chemical Co. Ltd., Beijing, China. *N*-Methyl-2-pyrrolidone (NMP) was purchased from Tianjin Bodi Chemical Co. Ltd., Tianjin, China. 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) and RPMI1640 medium were purchased from Sigma Chemical, St. Louis, MO, USA. All other chemicals used were of analytical grade.

Preparation of organogel

The injectable organogels were prepared in the following steps. First, the organogelator SAM was added to the oil phase at different concentrations (w/w). Then, the mixture was heated to 70°C and swirled to form a clear oil sol. Upon cooling, the sol changed into the SAM organogel, which was a translucent or opaque gel. Unless otherwise specified, 35% (w/w) NMP (when containing 12% SAM) was added to the gel, to keep the organogel system in the sol state, which could be easily injected. Finally, the gel was heated and swirled again to form a homogenous formulation.

Gelation properties

The gelation properties of SAM organogel were denoted by minimum gelation concentration (MGC, g/L), which can evaluate the gel strength of SAM in different oils. Many oils such as silicone oil, Mig812, IPM, IPP, castor oil, sunflower oil, peanut oil, olive oil, and soybean oil were selected to study the MGC of SAM.

The MGC was recorded by the inversion flow method[27]. The test was carried out as follows: the organogelator was mixed in a closed-capped tube with an appropriate amount of oil phase to develop a concentration of 2% (about 20 mg of SAM and 1.0 mL of oil phase were used). The mixture was heated until the solid dissolved. The test tube was cooled in water bath at 25 ± 0.2°C. After 4 hours, invert the tube vertically. If the sample flew along the tube, 0.1 mL corresponding oil was added and the above steps should be repeated; if the sample did not flow, 5 mg SAM should be added and these steps were repeated. Every sample had 4 hours to completely form gel or sol. The concentration which can exactly emerge as gel was the value of MGC in relevant oil phase.

Phase transition temperatures of SAM/soybean oil organogel

Differential scanning calorimetry (DSC) was used to determine the phase transition temperatures of organogels. DSC analysis was performed using a Thermal Analysis System TA-60WS (Shimidazu Co., Kyoto, Japan). Samples composed of 3–15% SAM/soybean oil organogel were melted at 70°C and a drop of about 5 mg sol was placed in an aluminum pan. After cooling, the cup was sealed. The sample and aluminum pan were stored overnight at 4°C. The DSC chamber was preset at 20°C before loading the sample. The sample was heated to 80°C at a heating rate of 5°C/min and then cooled to 20°C at the same rate. At least, for each sample two heating and cooling scans were recorded.

In vitro degradation of SAM/soybean oil organogel

Degradation on single side

Different concentrations of SAM (5–12%) were weighted in ampules (d = 16.2 mm) and dissolved in 1 mL soybean oil at 70°C. After cooling to room temperature, the samples were stored at 4°C overnight. One milliliter hydrolytic medium, preheated to 37°C [phosphate buffer solution (PBS), pH 7.4], was added into each ampule. These ampules were incubated in a water bath at 37°C under a mild shaking motion for 50 days. Samples were taken at predetermined time intervals. After the medium was removed, the ampule and gel were washed with distilled water, dried in a desiccator which contained allochroic silica gel and phosphorus pentoxide for 4 hours, and weighted. Finally, fresh medium with the same volume was added, and the operation above was repeated[28]. The time-dependent hydrolytic degradation behavior was evaluated in terms of the weight loss of the organogel on single side (d = 16.2 mm). The weight loss (W_L) was calculated as follows[29]:

$$W_L\,(\%) = \frac{W_0 - W_d}{W_0 - W_{am}} \times 100, \tag{1}$$

where W_0 is the initial weight of the specimen (gel and ampule), W_d the weight of the specimen dried after different degradation time, and W_{am} the weight of blank ampule.

Degradation on both sides

A new method was first developed to simulate the hydrolytic degradation in vitro, by which the gel can be degraded on both sides. The pre-prepared sol sample was poured into an oblate mold (d = 14.0 mm) at high temperature. After gelling, this system was stored overnight at 4°C. The gel was transferred to 20 mL PBS which was preheated to 37°C and shaken for 30 minutes to remove the fragments on gel block. The smooth gel was taken out, washed, and dried. The weight of this gel was the initial mass of pre-degrade gel. After weighting, the sample was immersed in equivalent volume of fresh PBS and the gel was taken at predetermined time intervals, washed with distilled water, dried in a desiccator for 4 hours, and weighted. The weight loss (W_L) was calculated as follows:

$$W_L\,(\%) = \frac{W_0 - W_d}{W_0} \times 100. \tag{2}$$

W_0 is the initial weight of the gel (first 30 minutes) and W_d the weight of the gel dried after different degradation time.

Surface morphology of undegraded and degraded organogels

The surface morphology of SAM organogel was studied by scanning electron microscopy (SEM) using a JSM 5300 scanning microscope (JEOL 5300, JEOL Ltd., Kyoto, Japan). The degraded organogel for 40 days was removed from immersion solution, frozen in liquid nitrogen, and freeze-dried. Undegraded and degraded gel samples were mounted on the metal stubs and sputter coated with gold for 5 minutes prior to examination under SEM[30].

In vivo degradation of SAM/soybean oil organogel

In vivo degradation studies were performed on male Kunming mice (20–25 g) obtained from the Experimental Animal Center of Shenyang Pharmaceutical University of Traditional Chinese Medicine (Shenyang, Liaoning, China). The animal experimentation was approved by the Animal Ethics Committee of Shenyang Pharmaceutical University. The animals should be familiar with the environment for a few days before the experiment. Eighteen mice were randomly divided into six groups. After being housed for a week under controlled conditions, the mice were given a single subcutaneous injection of 12% (w/v) SAM organogel formulation (200 μL, contained 35% NMP) in the dorsal area with a 25-gauge syringe. To keep the sol estate, 35% (v/v) NMP was added to the gel. According to the reference, NMP can hinder the forming of intermolecular hydrogen bonds between organogelator molecules so as to decrease the gel's viscosity and facilitate injection through conventional needles[31]. After 2 hours, 3, 7, 14, 28, and 42 days, the mice were killed by cervical dislocation. The subcutaneous gels were taken out, washed with distilled water, dried in a desiccator for 4 hours, and weighted. The mass of 2 hours was regarded as the initial weight (W_0). The weight loss (W_L) was calculated as Equation (2).

Cytotoxicity tests

Cytotoxicity test was an in vitro method to evaluate the biocompatibility of biomaterials. The cytotoxicity of SAM/soybean oil organogel was investigated by MTT assay using mouse fibrosarcoma cells (L929). Because living cells can reduce a water-soluble yellow dye MTT to a water-insoluble purple formazan product by mitochondrial succinate dehydrogenases, the absorbance of the formazan product at 570 nm can be used to measure the cell viability[10].

Mouse fibrosarcoma cells were cultured in RPMI1640 medium [100 units/mL penicillin, 100 μg/mL streptomycin, 0.2% $NaHCO_3$, and 10% (v/v) heat-inactivated fetal bovine serum] and incubated at 37°C in a HIRASAWA CPD-170 CO_2 incubator with humidified 5% CO_2/95% air atmosphere. L929 cells were seeded in a 96-well plate at a density of 2×10^4 cells per well. After incubation for 24 hours, the culture medium was removed and replaced with the diluted extraction medium of different ratios. The sterilized organogel (12% SAM, 35% NMP) was immerged into PBS at 37°C for 10 days to obtain the extraction solution. The gel extracts were sterilized by 0.22μm membrane and diluted to 1:5, 1:10, 1:20, 1:40, and 1:80 with RPMI1640 medium, respectively. The different concentration extraction mediums were added into 96-well plates containing incubated L929 cell. Cells treated with the same amount of growth medium were used as a control group. After incubation for 48 and 72 hours, the morphology of cells was observed using an optical microscope equipped with a digital camera, and 50 μL MTT solution was added to each well. After 3 hours of incubation at 37°C, 100 μL of dimethyl sulfoxide was added to dissolve the formazan crystals. The dissolved solution was swirled homogeneously for about 10 minutes using a shaker. The optical density of the formazan solution was detected by Bio-Rad 550 ELISA reader (Bio-Rad Laboratories, Inc., Hercules, CA, USA) at 570 nm.

Histological analysis

The research on in vivo biocompatibility of the SAM/soybean oil organogel system was carried out through histological analysis of mice's (20–25 g) skin tissue obtained from the injection site. The formulations were prepared under aseptic conditions after the sterilization of each constituent. Animals were injected subcutaneously on dorsa with 200 μL of 12% SAM in soybean oil with 35% NMP. After 3, 7, 14, 28, and 42 days, the animals were killed. Skin tissues were collected and fixed in 10% neutral buffered formalin for 48 hours, processed, and embedded in paraffin. Transverse sections were prepared through microtome and mounted on glass slides followed by counter staining with hematoxylin–phloxin–safran standard procedure[32], employing hematoxylin (nucleus coloration) and phloxin (cytoplasmic coloration) for cellularity and cell number, and safran for collagen deposition and fibrous tissue formation[33]. The slides were examined under a light microscope for any signs of acute and chronic inflammations, such as tissue granulation, fibrous capsule formation, and inflammatory cell infiltrate.

Results and discussion

Gelation properties

To evaluate the gelling ability of gelator SAM and choose the optimal oil to prepare the organogel, the gelation properties of SAM in different oils were determined. According to the research, SAM is readily soluble in different oils when temperature increases. In addition, when the oils were slowly cooled to room temperature, translucent gels could be obtained within a few minutes. It can be concluded that SAM was a powerful organogelator in different organic oils and vegetable oils. Results of gelation tests are summarized in Table 1, in which the values denote the MGC (g/L) in different oils.

It can be seen from Table 1 that the amount of SAM to gel 1 L silicone oil, Mig812, sunflower oil, olive oil,

Table 1. Minimum gelation concentration (MGC) in different oils at 25°C (g/L).

Solution	MGC (g/L)
Silicone oil	9.17
Mig812	19.09
IPM	29.80
IPP	31.82
Castor oil	–
Soybean oil	20.0
Sunflower oil	20.83
Olive oil	25.42
Peanut oil	27.27

'–' means value higher than 50.

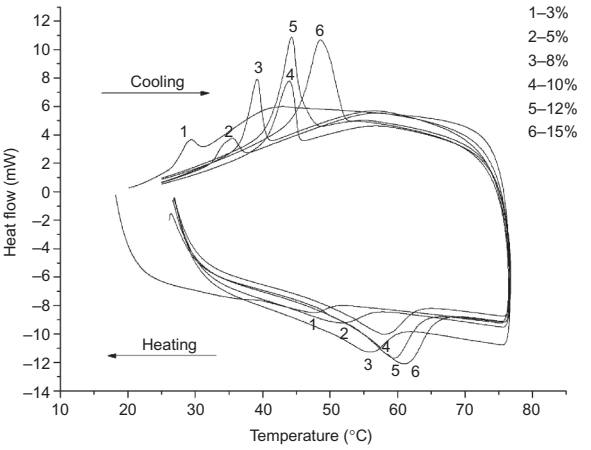

Figure 1. Typical gel–sol transitions (heating) and sol–gel transitions (cooling) for SAM/soybean oil organogel with different concentrations measured by differential scanning calorimetry (DSC) using a 5°C/min cooling and heating rate.

peanut oil, and soybean oil was 9.17, 19.09, 20.83, 25.42, 27.27, and 20.0 g, respectively. It could not gel castor oil even at an amount less than 50 g/L. Relatively, the MGC in IPM and IPP were a little higher. It was clear that organogelator SAM can form a stable physical organogel and gelatinize a number of oils even at a very low concentration.

At MGC, the appearance of organogels in oils was different. The gels were opaque in IPP, IPM, and Mig812, whereas translucent in other oils. According to the study by Luo, the size of organogel networks depended on the nature of the liquid[34]. It may be due to the different size and density of their networks that leads to the different gel configuration. Meanwhile, the nature of oils may also affect the gel strength of organogelator in corresponding oil.

Among the vegetable oils, soybean oil, sunflower oil, olive oil, and peanut oil have an ascending sequence of MGC. Different MGC may be due to different ingredients in these oils. Silicone oil is composed of polysiloxane of different chain structures. Medium- or long-chain fatty acid esters build up Mig812, IPM, and IPP. However, vegetable oils are all made up of unsaturated fatty acids (oleic acid and linoleic acid) and saturated fatty acids (stearic acid and palmitic acid). The concentrations of saturated and unsaturated fatty acids in the four oils are different, which caused the different MGC. But the most powerful gelling ability of SAM emerged in soybean oil. And taking into account the clinical use, soybean oil is the most wildly used and safest for injection. Overall, soybean oil was selected to prepare the SAM organogel for further studies.

Phase transition temperatures of SAM/soybean oil organogel

To study the effect of SAM on soybean oil and monitor the heat flow associated with the melting and gelling enthalpy as well as the process of phase transition, DSC studies were employed. Enthalpy associated with the melting and gelling of the different proportions of SAM gel was measured by the integration of the heat flow curve versus time (Figure 1).

It indicates that peaks associated with endothermic gel–sol transitions are broader than those associated with exothermic sol–gel transitions. However, in both endothermic and exothermic processes, the baseline slope prior to the onset and later to the endpoint made it difficult to determine the regions of gel–sol transition and sol–gel transition. Moreover, phase transition temperatures (T_t) measured by DSC corresponded to the temperature at which the three-dimensional network melted or shaped[35]. Therefore, to ensure an accurate determination of the enthalpy and phase transition temperature, the temperature corresponding to the peak dot was selected as the transition temperature.

Figure 2 shows the variation of sol–gel transition temperatures (T_{sg}) or gel–sol transition temperatures (T_{gs}) as a function of gelator concentration in soybean oil. The temperature at which transition occurred increased with gelator concentration and then leveled off, depending on the concentration of gelator in formulation[36]. It was clear that the curves of T_{sg} and T_{gs} were almost parallel to each other and gels containing the same concentration exhibited strong hysteretic behavior with T_{gs} exceeding T_{sg} by at least 10°C. At the temperature between T_{sg} and T_{gs}, the samples can still remain in the gel state for at least a month. Nevertheless, this characteristic made the sample more prone to transit to gel in vivo and enhanced its stability, because at body temperature the gel was easy to form and hard to revert. This made the organogel more stable at body temperature with comparatively low concentration. It

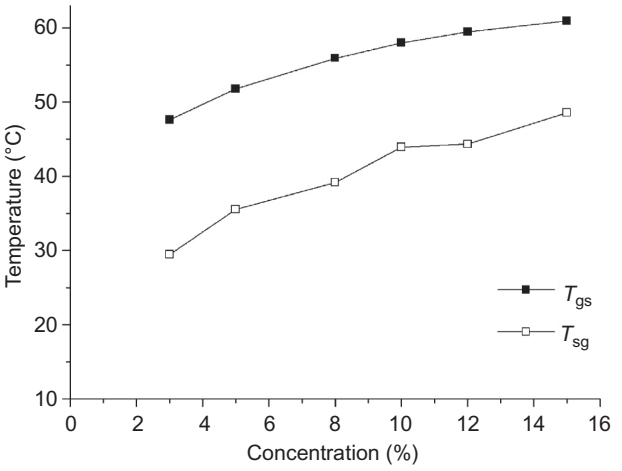

Figure 2. T_{gs} and T_{sg} as a function of SAM concentration in soybean oil obtained by DSC.

indicated that this system was more suitable to apply as injectable in situ implant system.

In vitro degradation

Two methods (one side and both sides) were developed to investigate the in vitro degradation of SAM/soybean oil organogel with different concentrations. Weight loss of organogel in pH 7.4 PBS as a function of immersion time is presented in Figure 3. The degradation rates of organogel, investigated by one-side method and both-sides method, are given in Figure 3A and B, respectively. It could be seen that with the prolongation of immersion time, the weight loss of specimens significantly increased. The gradual degradation of organogel in PBS implied that the SAM/soybean organogels were hydrolytically degradable.

By both methods, it was clear that degradation of the organogels depended on the amount of SAM used in their preparation. With the increase of SAM concentration, the degradation rates of organogel decreased in both methods. Compared to its counterparts formulated with high concentrations, the 5% SAM organogel exhibited a considerably faster degradation profile.

Figure 3B shows that the degradation of 8%, 10%, 12%, and 15% SAM organogel was 35.40%, 31.32%, 25.39%, and 17.50% in 40 days, respectively. In general, higher SAM concentration can lead to higher density of the fiber network[16]. According to the DSC analysis, the phase transition temperature of the system increased with the concentration of SAM in soybean oil, as well as the gel strength[27]. Accordingly, the degradation behavior of organogels was dependent on the density of three-dimensional network structures, and greater

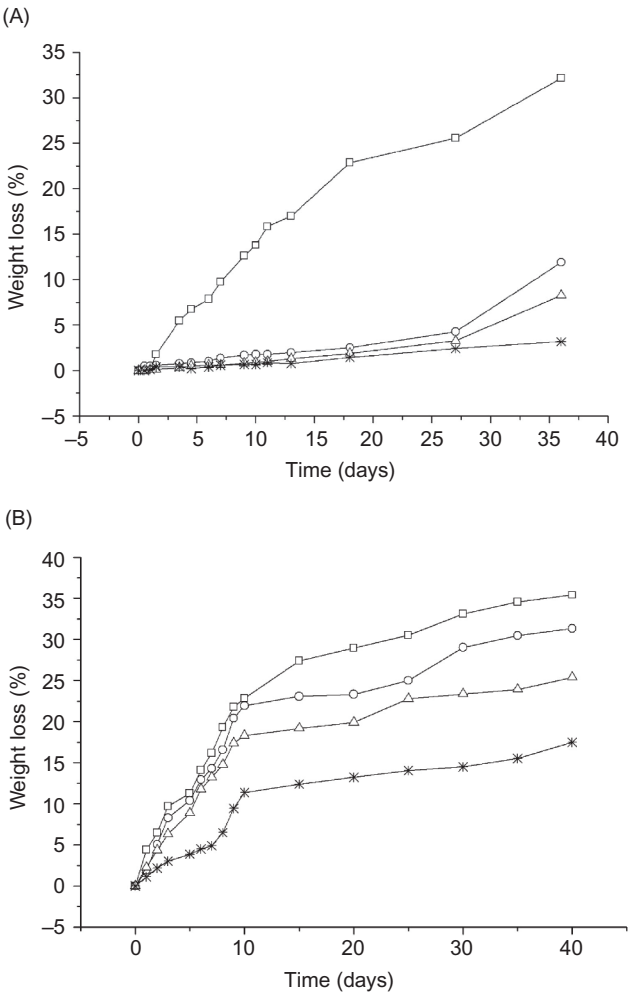

Figure 3. (A) Weight loss of SAM/soybean oil organogels as a function of immersion time by one-side method. Organogel: 5% SAM (□); 8% SAM (○); 10% SAM (Δ); 12% SAM (×). (B) Weight loss of SAM/soybean oil organogels as a function of immersion time by both-sides method. Organogel: 8% SAM (□); 10% SAM (○); 12% SAM (Δ); 15% SAM (×).

extent of the network typically produced more stable organogels so that it could block off more water molecules from entering into gel, leading to a lower degradation rate.

It can be seen from comparison of the degradation rates of the two methods that the latter was distinctly higher than the former. At the same concentration (12%), the weight loss was 3.15% and 25.39% on one side and both sides, respectively. When the concentration was 15%, there was nearly no degradation on one-side method. This difference indicated that the both-sides method was appropriate to accurately simulate the degradation of the gel. It can be explained that the full area surface of gel was taken into account in bothsides method, so it is consistent with the in vivo processes. From this, the both-sides method could be

described as 'full side method'. Moreover, the degradation behaviors tested by the two methods were also different. In the first method, the rate of degradation within 10 days was lower than that in following days. Regarding the second method, the results were exactly opposite. The high degradation rates emerged in first 10 days. It can be concluded that this was probably because of the transfer of organogel from mold to immersion solution by both sides. The transfer caused the gel to form rough surface, which can help water to enter the surface of organogel. When water entered into gel, oil embedded in the network structure was replaced by water molecules. With more and more oil effusing, there were apparent oil drops floating on the immersion solution, and white solid emerged on the gel's surface. This was likely the mechanism of organogel degradation.

To observe the morphology of the degradation of SAM organogel more clearly, the undegraded and degraded organogels were compared by SEM. Different from the smooth surface with intact network connection of undegraded organogel (Figure 4A), it obviously showed that the micrograph of gel surface was a porous network of interconnected structures, and the ruptures distinctly appeared on the surface (Figure 4B). According to Figure 4A, it is obvious that the organogel is assembled with the tridimensional network by organogelator. With the degradation in vitro, the consequent network was ruptured and disappeared on surface. According to the mechanism of in vitro degradation, with the immersion of solution, networks formed by organogelators were cracked by degrees, which caused the departure of oil and degradation of organogels.

In vivo degradation

To investigate the in vivo degradation of 12% SAM/soybean oil organogels, the sol was injected subcutaneously into mice. As a function of time, the weight loss of organogel in mice is presented in Figure 5. It is clear that the organogel underwent fast mass loss in the first 2 weeks, followed by a more moderate degradation. In the experiment, organogels gradually reduced, and almost disappeared after 6 weeks. This implied that the SAM/soybean oil organogel was biodegradable. Apparently, the degradation rates were different between in vivo and vitro. After 6 weeks, the weight loss in vivo and in vitro was 94.57% and 25.39%, respectively. Owing to the multiple enzymes in tissue, the organogels were eroded and fragmented quickly. Moreover, after the NMP released into body fluid, apertures were formed and resulted in the fast degradation.

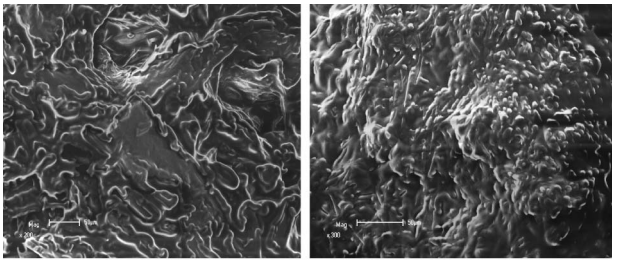

Figure 4. SEM micrographs of undegraded organogel (A) (magnification: 200×) and degraded, after 40 days, organogel (B) (magnification: 300×).

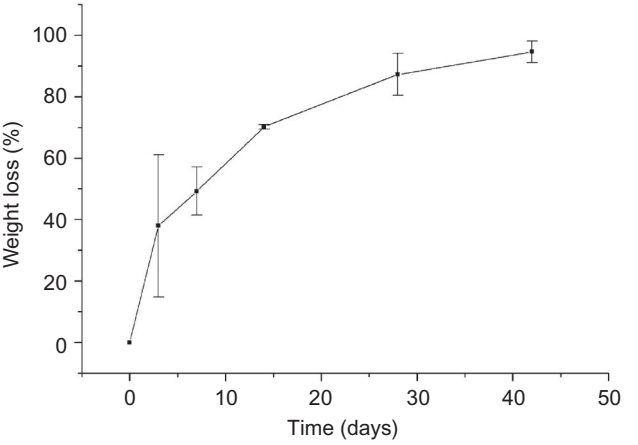

Figure 5. Weight loss of SAM/soybean oil organogels as a function of inject time by in vivo degradation. Data are presented as mean ± SD ($n = 3$).

Cytotoxicity tests

The cytotoxicity of materials was one of the most important sources of information to tell us whether those materials were suitable for pharmaceutical and biomedical uses[37]. L929 was the fibroblast of mice subcutaneous tissue, which was widely used in cytotoxicity study of biomaterials[38].

Figure 6A and B shows the photos of cell growing after giving the extraction for 2 and 3 days, respectively. It can be seen that cells were almost fusiform or irregularly triangular in the culture periods, rarely circular. Figure 6 indicates the apparent growth and intact shape of fibroblast in 2- and 3-day periods.

Figure 7 shows the cell viability obtained from an MTT assay of L929 cells which were cultured with extracts diluted with RPMI1640 medium at different dilution ratios. It can be seen that extracts had the equivalent cell viability to PBS. The viabilities increased with dilution ratio, as well as PBS. In the 5:1

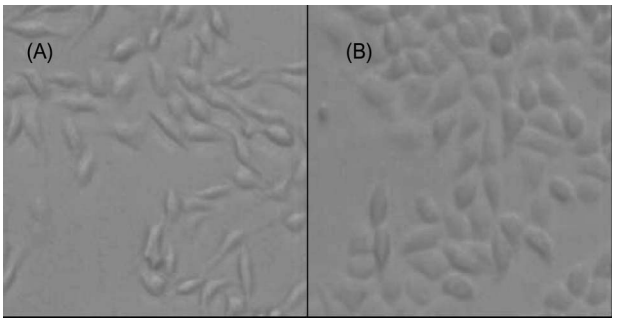

Figure 6. Microphotograph of L929 after administration of the extraction of organogel for 2 days (A) and 3 days (B).

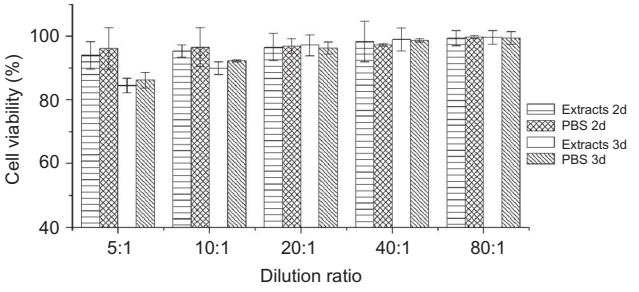

Figure 7. Cytotoxicity was expressed as cell viability in terms of dilution ratio. Comparison of cell viability between SAM/soybean oil organogel extracts and PBS for 2 and 3 days, respectively.

diluent, the cell viability ranged from 84% to 96%, indicating a very low cytotoxicity to fibroblasts. When the ratio was higher than 20:1, cell viabilities were almost close to 100%, indicating a nontoxic outcome. The cell viability decreased gradually with the increased concentration of extracts and PBS in growth medium, which was attributed to the depletion of nutrition for cell growth and proliferation[10]. In this study, no significant difference in cell viability was observed between cells treated with organogel extracts and PBS. Because PBS is noncytotoxic, it is correct to say that SAM organogel in soybean showed a good in vitro biocompatibility.

Histological analysis

In vivo biocompatibility of the SAM organogel system was evaluated by the histological responses of mice. Figure 8A is the image of normal subcutaneous histology of mice skin where no formulation was injected. The subcutaneous histology of mice skin following administration of the SAM organogel is presented in Figure 8B–J. It can be seen that 3 days after injection, an acute inflammatory response occurred at the injection site characterized by infusion of abundant neutrophils in hypoderm and dermis edema[39]. After 1 or 2 weeks, the number of neutrophils dropped and edema alleviated. After 2 weeks, the fibroblast proliferation were appeared at injection site, which indicated the acute inflammatory was began to heal[40] and meanwhile the macrophages were formed, which indicated the emergence of chronic inflammatory reaction[41]. After 4 weeks, the lymphocytes and macrophages became predominant, but the presence of these cells almost lasted for about 4–6 weeks after subcutaneous administration of the SAM organogel. After 6 weeks, the histology of the skin sample from inject site was similar to normal skin but only formed the connective tissue mass.

It can be concluded that the minimal inflammatory reaction was observed according to the histological analysis. Such a process of inflammatory response was characterized by acute inflammatory responses for 3–14 days followed by a longer duration of chronic inflammation, which depended on the existence of stimulus. With the degradation, organogel almost disappeared after 6 weeks, resulting in the termination of inflammatory responses. No signs of fibrosis, muscle damage, necrosis, and granulomatous inflammatory reaction were observed during the study, which indicated the acceptable biocompatibility of the organogel. Thus, the biocompatibility studies confirmed that the SAM/soybean oil organogel had acceptable biocompatibility for use in drug delivery system.

Conclusion

In this study, a novel, injectable in situ organogel system for parenteral drug delivery was studied. It indicated that the SAM has a powerful gelling ability in many kinds of oil, among which SAM/soybean oil organogel is degradable both in vitro and in vivo and has good biocompatibility according to cytotoxicity tests and histological analysis. Thus, the in situ organogel-forming implant based on SAM showed a great potential for a safe drug delivery, and it would be an appropriate delivery system for controlled release of therapeutic drugs. Further work will aim at the mechanism of different drug delivery made from these organogels, diffusion behavior of hydrophilic solvent between oil phase and water phase, and how relevant factors influence the release properties of the system.

Declaration of interest

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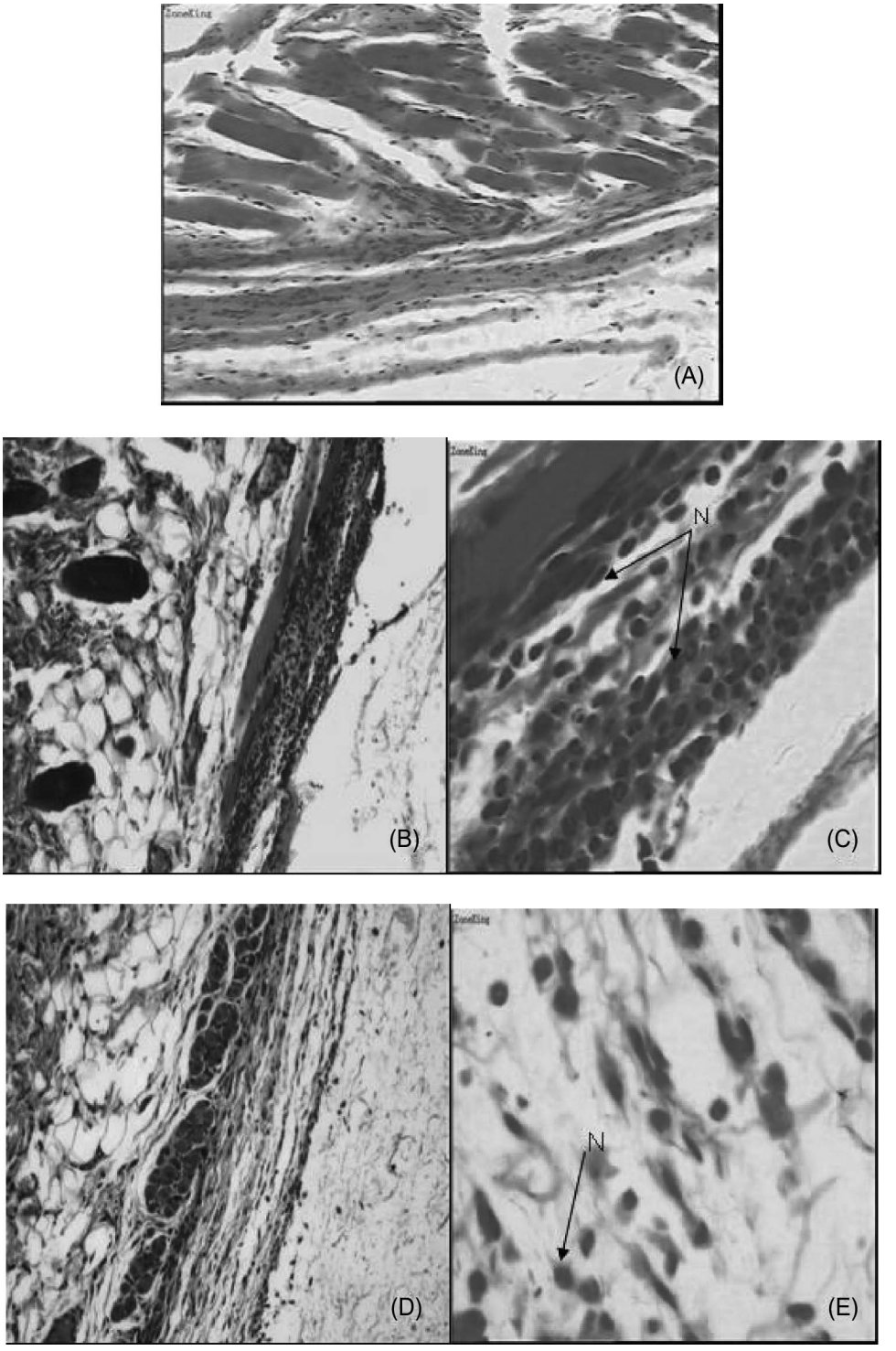

Figure 8. Light micrographs of mice skin showing subcutaneous histology. (A) Normal subcutaneous histology, (B, C) mice skin sampled from the injection site after 3 days subcutaneous administration of the SAM organogel, (D, E) mice skin sampled from the injection site after 1 week subcutaneous administration of the SAM organogel, (F, G) mice skin sampled from the injection site after 2 weeks subcutaneous administration of the SAM organogel, (H, I) mice skin sampled from the injection site after 4 weeks subcutaneous administration of the SAM organogel, (J) rat skin sampled from the injection site after 6 weeks subcutaneous administration of the SAM organogel (N: neutrophils; F: fibroblast; L: lymphocytes; M: macrophage). Original magnification (A, B, D, F, H, and J) 40× and (C, E, G, and I) 100×.

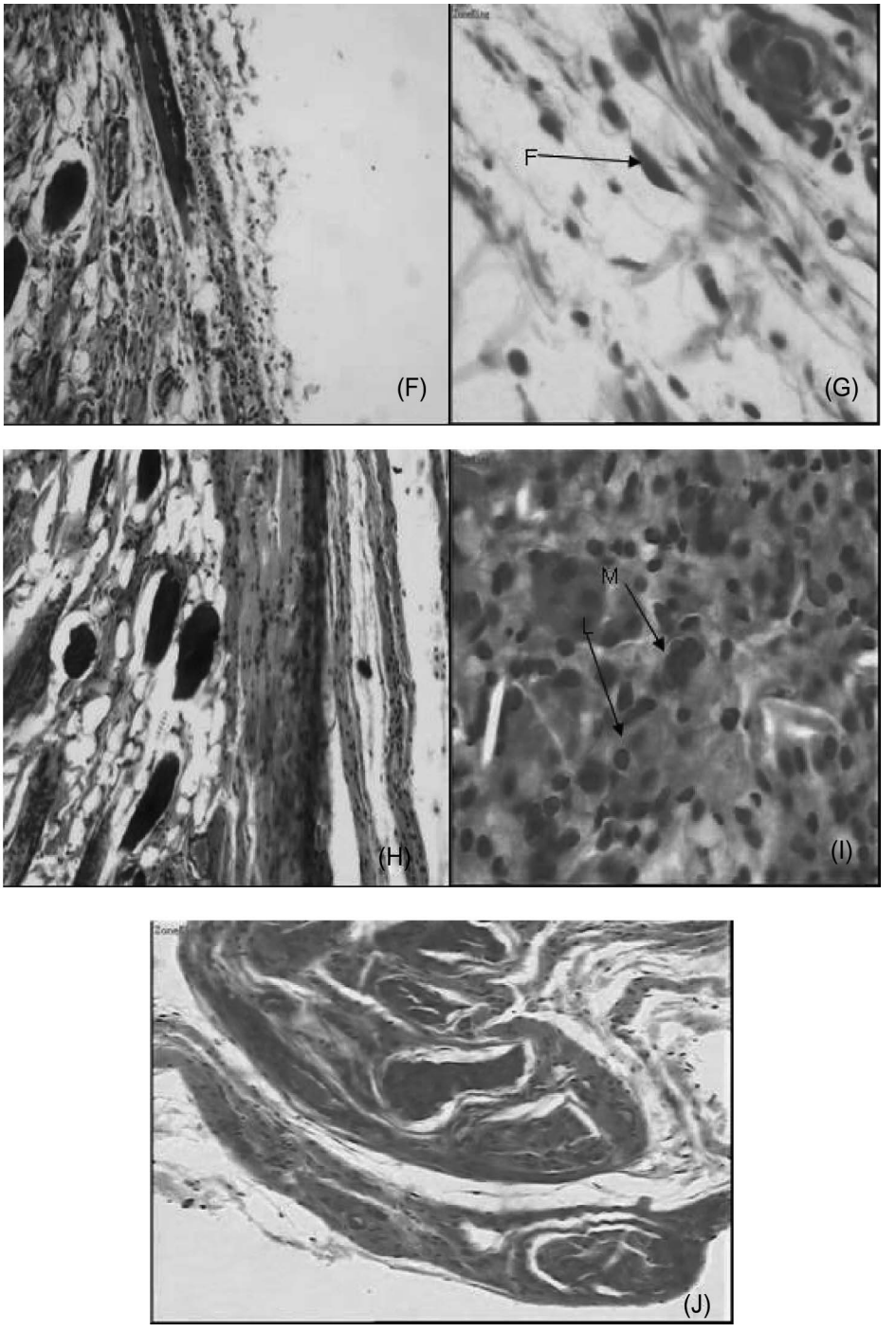

Figure 8. (*Continued*).

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