Evaluations of therapeutic efficacy of intravitreal injected polylactic-glycolic acid microspheres loaded with triamcinolone acetonide on a rabbit model of uveitis

Wenchang Li · Bing He · Wenbing Dai · Qiang Zhang · Yuling Liu

Received: 1 July 2012 / Accepted: 3 July 2013
© Springer Science+Business Media Dordrecht 2013

Abstract Conventional treatments of uveitis are not ideal because of the short period of therapeutic efficacy. In this study, biodegradable polylactic-glycolic acid microspheres loaded with triamcinolone acetonide (TA) were prepared to achieve sustained drug release and their therapeutic efficacy was investigated on a rabbit model of uveitis. TA-loaded microspheres (TA-MS) were prepared by the solvent evaporation method and characterized for encapsulation efficiency, particle size, morphology and in vitro release. The therapeutic efficacy was studied on the rabbit experimental uveitis model based on scoring of the inflammation, aqueous leukocyte counting, aqueous protein determination and histological examination. The TA-MS exhibited smooth and intact surfaces with an average diameter of 50.87 μm. The drug-loading coefficient and encapsulation efficiency were 15.2 ± 0.6 % and 91.24 ± 3.77 %, respectively. The drug release from TA-MS lasted up to 87 days, but only 46 days for TA suspension. The change in surface morphology also showed sustained drug release from TA-MS. TA-MS exhibited improved therapeutic efficacy in lipopolysaccharide-induced uveitis compared to TA suspension, especially in regard to the inhibition of inflammation. The TA-MS had a longer-term therapeutic effect on intraocular inflammation in LPS-induced uveitis in rabbits compared to TA suspension. The results suggested that TA-MS can be developed as a potential sustained-release system for the treatment of uveitis.

Keywords Uveitis · Microspheres · Triamcinolone acetonide · Lipopolysaccharide · Intraocular

Abbreviations
TA Triamcinolone acetonide
MS Microspheres
TA-MS TA-loaded microspheres
PLGA Polylactic-glycolic acid
LPS Lipopolysaccharide
AS Aseptic saline
EIU Endotoxin-induced uveitis
SEM Scanning electron microscopy
PVA Polyvinyl alcohol
MS-blank TA-unloaded microspheres
HPLC High-performance liquid chromatography
EE Encapsulation efficiency
PBS Phosphate-buffered saline
Introduction

Uveitis is a common disease which comprises a large group of ocular inflammatory disorders affecting not only the uvea but also the adjacent tissues such as retina, optic nerve, and vitreous [1]. Control of inflammation is imperative to ameliorate the individual and socioeconomic impacts of vision loss from this disorder [2, 3]. However, an effective treatment with minimum side effects for this disease is still challenging.

Triamcinolone acetonide (TA) is a water-insoluble steroid that can remain for a longer duration in the vitreous cavity and may have longer action following intravitreal administration compared to other steroids [4]. In recent years, the use of TA has increased dramatically in the treatment of several ocular diseases, including cystoid macular edema [5, 6], age-related macular degeneration [7], and posterior uveitis [8]. The intravitreally injected TA (IVTA) is rapidly eliminated by the eye’s natural circulatory process, so frequent injections are needed, which is unpopular with many patients. Large dose injections are therefore often required to guarantee the effect (usually 25 mg/eye) [9]. However, there is concern that increased doses confer increased ocular complications or side effects including raised intraocular pressure (IOP), cataract formation, endophthalmitis, pseudoendophthalmitis and retinal detachment [10]. Salvolini et al. [11] have shown that a dose as low as 4 mg of TA injected into rabbit vitreous had a local toxic effect in terms of IOP elevation, occurrence of endophthalmitis and changes in the retinal morphology. They emphasized that a micronized triamcinolone injection showed a less toxic effect in situ, suggesting that an alternative preparation of TA seemed to be safer for clinical use [11]. Overall, a more rational and optimized intravitreal drug delivery system loaded with TA to prolong the therapeutic effects and reduce the side effects and frequency of intravitreal injection is mandatory.

Drugs for uveitis treatment can be delivered systemically, topically, periocularly or intraocularly [12]. All of the abovementioned delivery pathways can entail significant systemic side effects, particularly if administered for prolonged durations. Topical medications, which have the least side effects, do not penetrate well into the posterior segment and are

unsuitable for posterior uveitis, which is often sight-threatening. Intraocular or periocular injections can deliver relatively high doses of drug to the eye with few or no systemic side effects. However, such injections are associated with significant complications and must often be repeated at regular intervals [12]. Thus, an increasing number of sustained-release drug delivery devices using different mechanisms and containing a variety of agents have been recently developed to treat uveitis, including retisert, iluvien, surodex, ozurdex, vitrasert, vesiremia delivery system, cortixject implant, particulate drug delivery systems, liposomes and trans-scleral iontophoresis [12].

Microspheres (MS) are defined as biodegradable colloidal systems made of solid polymers falling in the size range 1–1,000 μm [13]. They comprise a polymeric matrix with drug molecules distributed within [14]. MS have been widely utilized as sustained-release drug delivery systems for a variety of drugs. They are suitable for different routes of administration [15–19], and yet related research on their application in intravitreal injection is sparse.

In the present study, microspheres loaded with TA (TA-MS) were prepared, characterized and investigated in comparison with TA suspension, with the hypothesis that the sustained release of TA from MS may lead to excellent and persistent therapeutic efficacy.

Methods

Materials

TA was obtained from JIDA Pharmaceutical Group (Kunming, China), and polylactic-glycolic acid (PLGA) (75/25, MW = 15,000) was supplied by Daigang Technology Co., Ltd. (Jinan, China). Polyvinyl alcohol (PVA) was the product of Beijing Organic Chemical Plant (Beijing, China), and lipopolysaccharide (LPS) was purchased from Sigma (St. Louis, MO, USA). Aseptic saline (AS) was from Shuanghe Pharmaceutical Group (Beijing, China).

Animals

New Zealand white rabbits weighing 2–3 kg were supplied by the Laboratory Animal Center in Peking University (Beijing, China) and managed according to
the guidelines of the International Guiding Principles for Animal Research (WHO Chronicle 39:51–56, 1985; A CIOMS Ethical Code for Animal Experimentation). The protocols were approved by the Peking University Animal Ethics Committee. The rabbits were fed a regular diet, with no restrictions on the amount of food or water consumed.

Preparation of TA loaded/unloaded microspheres and TA suspension

TA-MS were prepared by the solvent evaporation method [20]. Briefly, 48 mg TA and 240 mg PLGA were dissolved in 2 ml methylene chloride. The solution was emulsified in 10 ml distillated water containing 0.5 % PVA and stirred at 2,000 rpm for 15 min. The resulting emulsion was then mixed with 50 ml distilled water containing 0.1 % PVA and stirred at 500 rpm at room temperature for another 3 h to allow evaporation of the organic solvent. After being centrifuged at 4,000 rpm for 10 min, the resulting MS containing TA were harvested and washed three times with distilled water to remove residual organic solvent. TA-unloaded microspheres (MS-blank) were also prepared as negative control. TA suspension was prepared by diluting the TA crystalloid with AS to a final concentration of 15 mg/ml. Microspheres were then freeze-dried overnight and stored at **–20 °C** for further experiments.

Characterization of TA-MS and MS-blank

To determine the TA content of the prepared TA-MS, 10 mg TA-MS were dissolved in 400 μl methylene chloride before dilution in methanol to a final volume of 25 ml. 20 μl of the solution was injected into a high-performance liquid chromatography (HPLC) system. A Phenomenex C18 column (5 μm, 4.6 × 250 mm) (Phenomenex, Torrance, CA, USA) was maintained at 30 °C, and the mobile phase was a mixture of methanol and water (70:30, v/v), with a flow rate of 1.0 ml/min. The UV detector was set at 240 nm. The encapsulation efficiency (EE) was calculated by the following equation [21]:

\[
EE\% = \frac{\text{actual amount of drug in microspheres}}{\text{theoretical amount of drug in microspheres}} \times 100\%
\]

Optical (Olympus BX51; Olympus, Tokyo, Japan) and scanning electron microscopy (SEM) (JEOL JSM-5600LV; JEOL, Tokyo, Japan) were used to study the morphology and surface characteristics of the microspheres. Mean diameters and particle size distribution of microspheres were measured by a laser particle size analyzer (BT-9300H, Bettersize, Dandong China).

In-vitro release studies

10 mg of TA-MS were suspended in 6 ml phosphate-buffered saline (PBS, pH 7.2–7.4) in capped test tubes. The tubes were kept under constant shaking (60 rpm) at 37 °C. At each time interval, 4 ml supernatant solution containing released TA was aspirated from each tube after centrifugation and the TA concentration was examined. Based on the concentration of released TA in the aspirated 4 ml supernatant solution, the total TA released from 6 ml TA-MS suspension within that period was calculated as percentage of total TA amount in 10 mg TA-MS. 4 ml fresh PBS buffer was then added to each tube to make up 6 ml and incubated at 37 °C for another time interval. The drug concentration was determined by HPLC as described above. TA suspensions were subjected to the same procedure as the control group at the same time intervals.

Evaluation of therapeutic efficacy

**Intravitreal injection of TA-MS, MS-blank, TA suspension and AS in rabbit eyes**

Twenty-four rabbits were anesthetized by intravenous injection of 20 % urethane (1 g/kg body weight), followed by an eye drop of 0.5 % procaine hydrochloride, and randomly divided into four groups (six animals in each group) named AS control group, MS-blank group, TA suspension group, and TA-MS group, respectively. 0.1 ml of AS, MS-blank (100 mg/ml in AS), TA suspension (15 mg/ml in AS) and TA-MS (100 mg/ml in AS) were intravitreally injected into the eyes of the corresponding groups at the beginning of the study.

**Establishment of uveitis rabbit model**

Endotoxin-induced uveitis (EIU) has been widely recognized as an experimental uveitis model that can
be created by either intravitreal, intracorneal, intravenous, intraperitoneal or foot-pad injection of LPS [22–
24]. The rabbit EIU model was established by intravitreal injection of LPS as previously described [25]. In brief, LPS was dissolved in AS to a final concentration of 0.01 mg/ml. 0.1 ml of LPS solution was intravitreally injected into both eyes of each rabbit at the time points of 2 and 8 weeks (three animals per group at each time point).

**Scoring of the inflammation, aqueous leukocyte counting, aqueous protein determination and histological examinations**

Slit-lamp examinations and indirect ophthalmoscopy were used for observing the physiological changes of uveitis at 3, 6, 12, and 24 h after LPS injections. The scoring of uveitis was performed for all of the rabbits 24 h after intravitreal administration of LPS at the time points of 2 and 8 weeks (three animals per group at each time point), according to the scoring system of clinical evaluation of uveitis as reported previously [25]. The grade of uveitis was in accordance with the severity of inflammation; the grade was given from 0 to 3 when iris hyperemia was absent, mild, moderate or severe. Scores above 3 represent severe inflammation.

The aqueous protein level, leukocyte counts, and histopathology were examined in each group at 24 h after the LPS injections at the time points of 2 and 8 weeks (three animals per group at each time point). After anesthetization, aqueous humor was drawn from each eye of the experimental rabbits, using a heparin-rinsed glass syringe connected to a 27-gauge needle. Aqueous leukocyte counts and protein concentrations were measured as described previously [26], and the inhibition rate was calculated by subtracting the data of the test group from that of the control, then dividing by that of the control. The ratio of aqueous leukocyte counts or protein concentrations in the experimental eyes to that in the control eyes was also determined.

After the aqueous humor was taken, the animals were immediately killed. The eyeballs were enucleated, fixed in 10 % formalin solution, dehydrated with ethanol, cleared, and embedded with paraffin. The retinas and ciliary bodies were cut into 5 μm-thick sections and stained with hematoxylin–eosin (H&E) for histological examination under the light microscope.

**Statistical analysis**

The Mann–Whitney U test or analysis of variance (ANOVA) was used to determine whether differences existed between experimental mean values. $P < 0.05$ was considered significant. All statistical analysis was done with StatView software (SAS Institute, Cary, NC, USA).

**Results**

Characterization of TA-MS, MS-blank and TA suspension

The prepared microspheres were spherical in shape with smooth and intact surfaces (Fig. 1). The mean size was 50.87 μm in diameter with mean span value of 0.70, and the mass of TA in each 10 mg of TA-MS was 1.52 ± 0.06 mg, with an encapsulation efficiency of 91.24 ± 3.77 %.

In-vitro release profiles of TA from 10 mg TA-MS (approximately containing 1.5 mg TA based on the encapsulation efficiency) and 1.5 mg TA suspension were compared. 91.65 ± 3.70 % TA was slowly dissolved from the TA suspension on the 46th day of incubation, compared with 64.25 ± 4.27 % released from TA-MS. On the 87th day of incubation, 94.79 ± 7.48 % TA was released from TA-MS (Fig. 2).

SEM examinations revealed that the morphology of TA-MS underwent great changes during the release process. Figure 1a, b show the TA before being loaded into microspheres. Figure 1c–j show morphological changes of TA-MS after incubation in PBS for various periods at 37 °C. Figure 1c, d show the spherical shape of TA-MS on day 0 of incubation in PBS at 37 °C. Obviously increased porosity and roughness of the TA-MS were observed on the 30th day of incubation, although the spherical shape of the TA-MS were still maintained on the 30th day of incubation (Fig. 1e, f). Microsphere morphology became significantly different on the 60th day of incubation, with marked erosion and pitting (Fig. 1g, h). However, some of the TA-MS became degraded to a large extent (marked mass loss), with their internal structures revealed. After 87 days of incubation, the TA-MS lost their spherical shapes. Their surfaces became largely crumpled, wrinkled, and collapsed (severe mass loss), leaving only eroded polymeric structures (Figs. 1i, j).
Fig. 1  Typical SEM graphs of TA-MS.  

**a** TA before loading into microspheres.  
**b** Higher magnification of (a).  
**c** TA-MS in PBS.  
**d** Higher magnification of (c).  
**e** TA-MS after incubation in PBS at 37 °C for 30 days.  
**f** Higher magnification of (e).  
**g** TA-MS after incubation in PBS at 37 °C for 60 days.  
**h** Higher magnification (g).  
**i** TA-MS after incubation in PBS at 37 °C for 87 days.  
**j** Higher magnification of (i).
LPS injection induced EIU in rabbit

After intravitreal injection with LPS, all eyes developed epiphora, blepharospasm, ciliary congestion, and iris hemangiectasia within 3 h (data not shown). 6 h after the injection, aqueous flare and leukocytes could be observed, while at 12 h post-injection the inflammation appeared more severe, characterized by miosis, iris opisthosynechia, occlusion pupillae, flocculation exudation, and vitreous opacity (data not shown). The uveitic scores were evaluated at 24 h after LPS injection. Because of the severe inflammatory reaction, the fundus of the eye could not be seen clearly, but none of the 48 eyes displayed hypopyon.

Therapeutic evaluation of TA-MS and TA suspension in EIU eye

Uveitic scores of the TA-MS group and TA suspension group were significant lower than the AS control group when LPS was injected 2 weeks later than intravitreal injections of AS, TA suspension, TA-MS and MS-blank (Table 1). However, when LPS was injected 8 weeks after the injections of different administrations, no significantly different uveitic scores were observed between the AS control group and the TA suspension group, but the TA-MS group still had significantly decreased uveitic scores (Table 1). Aqueous leukocyte cell counts were significantly lower in the TA-MS group and TA suspension group than the AS control group when LPS was injected 2 weeks after AS, TA suspension, TA-MS and MS-blank injections (Table 3). Only the TA-MS group had a decreased aqueous protein concentration when LPS was injected 8 weeks after the injections of different administrations (Table 3). There were no significant differences between the MS-blank group and AS control group during the studies.

Aqueous protein concentrations were significantly lower in the TA-MS group and TA suspension group than the AS control group when LPS was injected 2 weeks after AS, TA suspension, TA-MS and MS-blank injections (Table 3). Only the TA-MS group had a decreased aqueous protein concentration when LPS was injected 8 weeks after the injections of different administrations (Table 3). There were no significant differences between the MS-blank group and AS control group during the studies.

Histological observations

When LPS was injected 2 weeks after intravitreal injections of different administrations, marked polymorphonuclear cell infiltrations were observed in ciliary processes and retina in the AS control group (Fig. 3a, b, arrows) as well as the MS-blank group (Fig. 3c, d, arrows) after 24 h of LPS injection. The ciliary processes were markedly swollen and surrounded with inflammatory cell infiltration and pink-staining unorganized material (fabric exudation). In some parts of the retina, there were numerous polymorphonuclear cells, which are typical of acute lymphocytes, lymphocytes, plasma cells and monocytes. However in the TA suspension group (Fig. 3e, f) and TA-MS group (Fig. 3g, h), a small quantity of inflammatory cells surrounded the relatively normal intraocular architecture, and the inflammation was obviously inhibited compared to the AS control group. When LPS was injected 8 weeks after intravitreal injections of different administrations, marked polymorphonuclear cell infiltrations were observed in ciliary processes and retina in the AS control group (Fig. 3a, b, arrows), MS-blank group (Fig. 3c, d, arrows) and TA suspension group (Fig. 3e, f, arrows) after 24 h of LPS injection, but the inflammation was
obviously inhibited in the TA-MS group (Figs. 4g, h) compared to other groups.

**Discussion**

TA was chosen in our experiment because TA is one of the most extensively used drugs in the treatment of several ocular diseases which are characterized by inflammation, edema and neovascularization [18]. However, while the drug is effective, the drug delivery system is not ideal and many side effects and complications can occur. Novel and more efficient delivery systems are desirable [21, 25].

MS were chosen as the drug carriers and were well prepared in the present study. The size determined by a laser particle size analyzer was similar to that shown in the SEM graph. MS have been used as carriers for different drugs, including antimicrobial, chemotherapeutic, and anti-inflammatory agents such as corticosteroids and dexamethasone [18, 27]. Growing evidence has also indicated the usefulness of biodegradable MS for vitreoretinal drug delivery, offering an excellent alternative to lessen the risk associated with multiple intravitreous injections [19]. These erodible devices have the inherent advantage over non-erodible systems in that they gradually disappear from the site of implantation. In addition, MS have also the advantage over larger devices in that they can be delivered by a simple injection, and, as a result, fulfil most of the requirements for an ideal intravitreous-delivery carrier system. On the other hand, the conventional pellet-shape implants require an expensive engineered delivery applicator of invasive nature due to its usually large-gauge needles and unfriendly scleral placing requirement [28].

### Table 1 Uveitic scores for the four groups of rabbits following clinical uveitis evaluation

<table>
<thead>
<tr>
<th>Time (weeks)</th>
<th>AS control</th>
<th>MS-blank</th>
<th>TA</th>
<th>TA-MS</th>
</tr>
</thead>
<tbody>
<tr>
<td>2 weeks</td>
<td>5.00 ± 1.26</td>
<td>5.00 ± 0.63</td>
<td>2.50 ± 1.38*</td>
<td>2.17 ± 1.16*</td>
</tr>
<tr>
<td>8 weeks</td>
<td>5.50 ± 0.54</td>
<td>5.83 ± 0.41</td>
<td>4.67 ± 0.82</td>
<td>2.33 ± 0.82*</td>
</tr>
</tbody>
</table>

0 = normal to 6 = most severe; n = 6 eyes

IR inhibition rate

* P < 0.05 versus AS control

### Table 2 Effect of intravitreal injection of TA-MS on leukocyte counts in the aqueous humor of rabbit

<table>
<thead>
<tr>
<th>Time (weeks)</th>
<th>AS control</th>
<th>MS-blank</th>
<th>TA</th>
<th>TA-MS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cells (μl)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2 weeks</td>
<td>2271 ± 1133</td>
<td>1979 ± 834</td>
<td>708 ± 385</td>
<td>646 ± 366</td>
</tr>
<tr>
<td>8 weeks</td>
<td>1542 ± 883</td>
<td>1771 ± 1082</td>
<td>1396 ± 503</td>
<td>667 ± 638</td>
</tr>
</tbody>
</table>

IR inhibition rate

* P < 0.05 versus AS control

### Table 3 Effect of intravitreal injection of TA-MS on protein content in the aqueous humor of rabbit

<table>
<thead>
<tr>
<th>Time (weeks)</th>
<th>AS control</th>
<th>MS-blank</th>
<th>TA</th>
<th>TA-MS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pro (mg/ml)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2 weeks</td>
<td>25.13 ± 4.13</td>
<td>22.17 ± 6.08</td>
<td>8.05 ± 2.48</td>
<td>7.53 ± 5.62</td>
</tr>
<tr>
<td>8 weeks</td>
<td>16.48 ± 3.08</td>
<td>13.34 ± 4.60</td>
<td>14.98 ± 1.53</td>
<td>5.29 ± 2.82</td>
</tr>
</tbody>
</table>

IR inhibition rate

* P < 0.05 versus AS control
biodegradable MS as drug delivery systems offers several important advantages, including controlled drug release and improved biodegradability and biocompatibility [18, 27, 29, 30]. Drug release from microspheres depends on several factors such as polymer composition and molecular weight, drug hydrophilicity, drug loading, as well as microsphere size [31]. Furthermore, the drug release rate from small microspheres is faster than from larger ones [32].

The formulation of MS in our study was monitored to make sure the mean size of the microspheres was approximately 50 μm in diameter, which was adequate for intravitreal administration through a 25G syringe needle [19]. Due to the limitation of the volume of vitreous cavity (1.5 ml in rabbits), an intravitreal injection volume of 0.1 ml solution was considered suitable and was adopted in the present study [33].

An in vitro release test demonstrated the long-term release behavior of TA-MS compared to TA suspension, which showed that TA was released from TA-MS for a period of 87 days, significantly longer than the 46-day duration from TA suspension under the same conditions (Fig. 2). Generally, a release test for controlled drug delivery systems should continue until at least 80 % of the loaded drug is released; more than 90 % of TA was released from TA-MS and TA suspension in our study. The release experiments were carried out where the drug concentration in the release medium did not exceed 10 % of the saturation concentration (released TA concentration <15 μg/ml, data not shown) [21]. TA-MS degradation was investigated using SEM; Fig. 1 shows TA-MS after incubation in PBS for various periods at 37 ºC. TA was released with TA-MS degradation, which is responsible for the sustained release, illustrated by TA-MS shrinkage in the in vitro release test, which is responsible for the sustained-release.

Rabbit eyes are particularly sensitive to LPS from Escherichia coli, Salmonella typhi, and Klebsiella pneumoniae, and the inflammatory response is similar among them [34]. We used a dose of 0.1 ml 0.01 mg/ml LPS as previously described [25]. Based on the results of the in vitro release test of TA-MS and TA suspension (Fig. 2), time intervals of 2 and 8 weeks between TA-MS injection and LPS injection were investigated using SEM; Fig. 1 shows TA-MS after incubation in PBS for various periods at 37 ºC. TA was released with TA-MS degradation, which is responsible for the sustained-release. Generally, a release test for controlled drug delivery systems should continue until at least 80 % of the loaded drug is released; more than 90 % of TA was released from TA-MS and TA suspension in our study. The release experiments were carried out where the drug concentration in the release medium did not exceed 10 % of the saturation concentration (released TA concentration <15 μg/ml, data not shown) [21]. TA-MS degradation was investigated using SEM; Fig. 1 shows TA-MS after incubation in PBS for various periods at 37 ºC. TA was released with TA-MS degradation, which is responsible for the sustained release, illustrated by TA-MS shrinkage in the in vitro release test, which is responsible for the sustained-release. Rabbit eyes are particularly sensitive to LPS from Escherichia coli, Salmonella typhi, and Klebsiella pneumoniae, and the inflammatory response is similar among them [34]. We used a dose of 0.1 ml 0.01 mg/ml LPS as previously described [25]. Based on the results of the in vitro release test of TA-MS and TA suspension (Fig. 2), time intervals of 2 and 8 weeks between TA-MS injection and LPS injection were investigated using SEM; Fig. 1 shows TA-MS after incubation in PBS for various periods at 37 ºC. TA was released with TA-MS degradation, which is responsible for the sustained-release. Generally, a release test for controlled drug delivery systems should continue until at least 80 % of the loaded drug is released; more than 90 % of TA was released from TA-MS and TA suspension in our study. The release experiments were carried out where the drug concentration in the release medium did not exceed 10 % of the saturation concentration (released TA concentration <15 μg/ml, data not shown) [21]. TA-MS degradation was investigated using SEM; Fig. 1 shows TA-MS after incubation in PBS for various periods at 37 ºC. TA was released with TA-MS degradation, which is responsible for the sustained release, illustrated by TA-MS shrinkage in the in vitro release test, which is responsible for the sustained-release. Rabbit eyes are particularly sensitive to LPS from Escherichia coli, Salmonella typhi, and Klebsiella pneumoniae, and the inflammatory response is similar among them [34]. We used a dose of 0.1 ml 0.01 mg/ml LPS as previously described [25]. Based on the results of the in vitro release test of TA-MS and TA suspension (Fig. 2), time intervals of 2 and 8 weeks between TA-MS injection and LPS injection were investigated using SEM; Fig. 1 shows TA-MS after incubation in PBS for various periods at 37 ºC. TA was released with TA-MS degradation, which is responsible for the sustained-release.
sustained release of TA from MS would lead to persistent therapeutic efficacy. The sustained TA-MS effectively reduced intraocular inflammation in rabbit eyes with LPS-induced EIU. The anti-inflammatory duration of TA-MS was longer than that of TA suspension. The results suggest that MS might be used as a potential sustained-release drug delivery system for improved uveitis therapy.

**Acknowledgments**  This work was supported by the Program for New Drug R&D (No. 2009ZX09310-001) and Innovation Team of Ministry of Education (No. BMU20110263). The authors thank Dr. Jiying Wang for his critical final manuscript revision.

**Conflict of interest**  The authors declare no conflict of interest.

**References**