Novel Drug-Loaded Gelatin Films and Their Sustained-Release Performance

Mei Lin,1,2 Sheng Meng,1 Wei Zhong,1 Rui Cai,1 Qiangguo Du,1 Piotr Tomasik3

1 Key Laboratory of Molecular Engineering of Polymers of Ministry of Education, Department of Macromolecular Science, Fudan University, Shanghai 200433, China
2 School of Pharmacy, Fujian Medical University, Fuzhou 350004, China
3 Department of Chemistry, Agricultural University, 31-120 Cracow, Poland

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Abstract: A new particulate drug delivery system with gelatin matrix containing Ibuprofen as a model drug molecule was developed for an epidermis drug prolonged release. Gelatin films containing Ibuprofen-loaded poly-ε-caprolactone (PCL) microspheres have been developed on evaporation of organic solvent from an oil-in-water emulsion followed by cross-linking. The microspheres were characterized for particle size, encapsulation efficiency, and surface morphology. Water uptake, matrix erosion, and drug release profile of the microsphere-film system were investigated. The results indicated that drug-loaded microspheres introduced in this system successfully prolonged drug release time. This kind of microsphere-film system combined good adhesion, typical for gelatin films, with the sustained release performance of PCL microspheres. © 2009 Wiley Periodicals, Inc. J Biomed Mater Res Part B: Appl Biomater 90B: 939–944, 2009

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INTRODUCTION

In recent years, biodegradable polymers evoked considerable interests in drug delivery systems and tissue engineering. Gelatin (GT) is a heterogeneous mixture of high-molecular mass polypeptides derived from partial hydrolysis of native collagens, which are the most abundant structural proteins found in animal skin, tendon, cartilage, and bone.1 It is characterized by a sharp sol–gel transition of its water solution. It readily forms gels below 35°C.2 Because of its biodegradability, biocompatibility, and nonimmunogenicity, GT is widely used in pharmacy and medicine as a carrier in controlled release drug delivery systems, dressings for wound healing, and so on.3–6 GT readily forms films and it is frequently utilized as films7,8 or microspheres. For its solubility in water and poor mechanical performance often limiting its applications, cross-linking of GT was used to make it water insoluble and to improve its mechanical property as well as sustained release performance under physiological conditions.1,2,9,10

A diversity of drug carrying devices, such as microspheres, nanospheres, films, and so on, has been developed for controlling drug delivery following particular requests and conditions of their application.11–15 For instance, drug-loaded nanospheres permeate cells for cellular internalization and permeate connective tissue, thus they deliver a drug efficiently to the targeted tissue without clogging capillaries by injection or ingestion,16 whereas films containing dispersed medication can be implanted to release slowly the drug at specific site. Biodegradable polymers, such as poly-ε-caprolactone (PCL), are often used to encapsulate drugs in forms of microspheres and nanospheres.17–19

This article presents the study and applications of an adhesive and biodegradable drug sustained delivery material made of PCL microparticles dispersed in gelatin films. This kind of material is designed for treating cankers and as a component of wound healing dressings. In contrast to conventional drug-loaded gelatin films, the new system has two levels of structural hierarchy: the primary microspheres consist of PCL chains and the secondary network consists of cross-linked GT chains. The majority of the encapsulated drug molecules should diffuse through first the PCL matrix and then the cross-linked gelatin gel matrix, to pass into the phosphate buffer solution (PBS). Thus, this new system demonstrates better sustained-release property than conventional cross-linked GT films.
MATERIALS AND METHODS

Materials
Poly-ε-caprolactone (PCL, $M_w = 165,000$ and $M_w = 11,000$, respectively) was purchased from Solvay (England). Gelatin (GT, type B) was purchased from Sigma (St. Louis, MO). Ibuprofen (IB, 99.9 wt %) was a gift from Fujian Medical University, Fuzhou, China. Polyvinyl alcohol (PVA), purchased from Kuraray (Osaka, Japan), had a $M_w = 79,200$ and the viscosity of its 4% solution was in the range of 29.5 $\times 10^{-3}$ Pa s at 20°C. The degree of its hydrolysis was 78.5 $\sim$ 81.5%. Methylene chloride (dichloromethane, DCM, analytical grade) was purchased from Shanghai Chemical Reagents Company (China). Glutaraldehyde (GA, biological reagent) was purchased from Shanghai Wulian Chemical Company (China).

Microsphere Suspension Preparation
The IB-loaded microspheres were fabricated by an oil-in-water (O/W) single emulsion-solvent evaporation technique. PCL (0.2 g) and IB (0.05 g) were added into DCM (3.5 mL) and agitated until they dissolved. This solution was slowly dropped into the stirred aqueous solution (2500 rpm) with PVA or GT as emulsifier (1 wt %). The resulting O/W emulsion was gently stirred for 4 h at room temperature to evaporate the organic solvent. The produced suspension was used immediately.

Encapsulation Efficiency
The drug entrapped in the microspheres was determined in triplicate by a UV-VIS spectrophotometer (UV-2000, Unico Instruments Co., Shanghai, China). The microspheres were collected by centrifugation at 3000 rpm for 10 min, followed by freeze drying. A 5-mg sample of dried microsphere powder was dissolved in DCM. Then, 10 mL of PBS (pH 7.4) was added and the content was agitated for 5 min. A nitrogen stream was introduced to evaporate DCM. It was then centrifuged for 10 min at 3000 rpm and the drug in PBS solution was determined by the absorbance at 266 nm. The encapsulation efficiency of Ibuprofen was obtained as the mass ratio of the amount of drug incorporated in microspheres and that originally introduced.

Characterization
The particle size and the size distribution of prepared microspheres were measured with a laser diffraction particle size analyzer (LS230, Beckman Coulter). The shape and surface morphology of the produced microspheres were observed with a scanning electron microscope (SEM, Tescan-5136MM, Czech Republic).

Preparation of Drug-Loaded Gelatin Film
GT (1 g) was dissolved in water at (37 ± 1)°C, then the drug-loaded microsphere suspension was added and stirred magnetically until the solution homogenized. Next, 5 wt % of the GA solution was added to the solution to crosslink GT chains. The resulting solution was poured into a Petri dish and cooled to form gels. On drying at (30 ± 1)°C, thin films were formed. Samples weighing about 100 mg were cut from the dry film for the in vitro degradation and release study. The film with IB but without microspheres (noted as nonmicrosphere) and the film without IB (noted as blank) were also made for comparison. The estimations were run in triplicates.

Water Uptake
The films after soaking in the PBS solution for a specified time interval were weighted after excess water was wiped off from the film surface with filter paper. The swelling ratio (SR) of matrices was calculated from Eq. (1):

$$SR\% = \frac{W_{\text{wet}} - W_{\text{dry}}}{W_{\text{dry}}} \times 100$$  \hspace{1cm} (1)

where $W_{\text{wet}}$ and $W_{\text{dry}}$ are weights of the wet films and dry films measured at time $t$, respectively. They were measured by short intervals for the first several hours, and then measured by longer intervals in the following hours. The estimations were run in triplicate.

Weight Loss
The weight loss of GT film in PBS was measured as a change in dry weight after soaking for specified time periods. Percent weight loss was computed from Eq. (2):

$$\text{Weightloss}\% = \frac{W_0 - W_t}{W_0} \times 100$$  \hspace{1cm} (2)

where $W_0$ and $W_t$ are initial and dry weight at time $t$, respectively.

In Vitro Release Study
Each drug-loaded composite sample was soaked in 10 mL of PBS and incubated at (37 ± 0.1)°C with continuous shaking. After specific intervals, the soaking liquid was replaced with fresh PBS. In the beginning, the samples were measured every half hour, and then the intervals were extended from 1 to 10 h at the final stage. The release process was run for 48 h. The IB content in PBS was measured in triplicate at 266 nm.

RESULTS

Characterization of Microspheres
The composition of emulsifier, molecular weight, sizes, and encapsulation efficiencies of the microspheres prepared are summarized in Table I. The encapsulation efficiency of IB
was 60–77% and the average diameter of the microspheres was in the range of 6–47 μm. The nonencapsulated IB was dissolved in aqueous solution during the fabrication process of the microspheres and would be dispersed in the GT film matrix during the subsequent mixing and film-forming process. The size of microspheres prepared with the higher molecular weight PCL was much smaller than that prepared with the lower molecular weight PCL in 1% PVA. The composition of emulsifier also influenced the size of microspheres. Comparing PVA-PCL165 and PVA/GT-PCL165 samples, one could see that when the 1% PVA emulsifier was replaced with 0.5% PVA + 0.5% GT, the size of microspheres became larger. Thus, the size of microspheres could be controlled with emulsifier and molecular weight of polymers.

Figure 1(a–c) shows scanning electron micrographs of the surface morphology of microspheres prepared under different conditions. The size observed by SEM was consistent with that measured by light scattering method. The surface morphology of microspheres made of low-molecular weight PCL [Figure 1(c)] was coarse with a lot of pores, whereas the surface of microspheres made of high-molecular weight PCL was smooth regardless emulsifier used [Figure 1(d,e)].

**Weight Loss of GT Films With and Without Drug**

In all the cases, the amount of cross-linking agent GA added was 5% w/w GT. Figure 2 shows the weight loss of different types of GT films after 12 h storage in PBS. All

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**TABLE I. The Size and IB Loading Efficiency of Microspheres (Mean ± S.D., n = 3)**

<table>
<thead>
<tr>
<th>Samplesa</th>
<th>Emulsifier</th>
<th>$M_n$ of PCL</th>
<th>Size (μm)</th>
<th>IB Encapsulation Efficiency (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PVA-PCL165</td>
<td>1%PVA</td>
<td>165,000</td>
<td>6.1 ± 2.7</td>
<td>60.0 ± 0.5</td>
</tr>
<tr>
<td>PVA-PCL11</td>
<td>1%PVA</td>
<td>11,000</td>
<td>47.0 ± 3.0</td>
<td>75.7 ± 2.5</td>
</tr>
<tr>
<td>PVA/GT-PCL165</td>
<td>0.5%PVA + 0.5%GT</td>
<td>165,000</td>
<td>24.3 ± 3.5</td>
<td>76.5 ± 3.8</td>
</tr>
</tbody>
</table>

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* Sample name is constituted of emulsifier and PCL, and the number represents the molecular weight of PCL.
of them lost ~9% of their weights regardless of the various kinds of microspheres they contained.

**Water Uptake of GT Films**

In PBS, GT films readily took water and turned into hydrogels. Figure 3 presents the time-dependent water uptake (swelling) for the GT films with and without drug loading microspheres. The swelling ratio for the drug-free GT film was lower than that for those films containing IB. The swelling ratio for the film with the same amount of IB but without microspheres (nonmicrosphere) was the highest, whereas the percentage of water uptake of those films containing IB-impregnated microspheres was median. It also can be seen that the swelling ratio of the film with PVA-PCL11 particles was about 25% higher than that with PVA/GT-PCL165 particles.

**In Vitro Drug Release**

Figure 4 shows the IB release from various GT films. The IB release profiles resembled the water uptake profiles. The release data were statistically analyzed by paired t-test. With the level of significance set at 0.05, we found that the "nonmicrosphere" film had a significantly higher IB release rate than the sample films with IB-loaded microspheres ($p < 0.05$). IB in "nonmicrosphere" pure gelatin films readily diffused to the solution and released completely in 2.5 h, whereas the IB release of microsphere-film system could last more than 35 h.

**DISCUSSION**

GA is one of the commonly used crosslink agents for GT, making it water insoluble, more stable, and resistant to biodegradation. The weight loss of various kinds of films were similar. It suggested that the cross-linking density of those films was comparable, so the weight loss of the matrix might not influence the drug release behavior in our study.

Water uptake (swelling ratio) of hydrophilic films is one of the influencing factors for drug release behavior. The difference of water uptake of these films can be attributed to the osmotic driving force of IB. It could be anticipated that the film with more IB would dispose with a higher osmotic driving force. The total amount of IB in all the drug-loaded systems was the same; but when a part of IB was encapsulated in PCL microparticles, the percentage of the water uptake in such a system was lower than that in the "nonmicrosphere" film. The system with IB fully dispersed in the GT network would provide the highest water uptake.

It should be noted that the encapsulation efficiencies of PVA-PCL11 and PVA/GT-PCL165 were close to each other. Therefore, one could assume that the level of residual IB in GT film of these two samples should also be similar. However, the swelling ratio of the film with PVA-PCL11 was higher than that with PVA/GT-PCL165. The results could be rationalized in terms of the quality of the microsphere surface. There were numerous pores in PVA-PCL11.
PCL11 microspheres, whereas the surface of PVA/GT-PCL165 microspheres was smooth. Thus, water could easily permeate into microspheres of PVA-PCL11 facilitating diffusion of IB to GT hydrogel matrix, and the osmotic driving force for water uptake. PVA/GT-PCL165 and PVA-PCL11 microspheres are different from their fabrication conditions. For the PVA/GT-PCL165 sample, the molecular weight of PCL is 165,000 and the emulsifier is 0.5%PVA and 0.5%GT, whereas the molecular weight of PCL is 11,000 and the emulsifier is 1%PVA for the PVA-PCL11 sample. Thus, combining the results of characterization of microspheres and the release profiles, one could conclude that the parameters of microsphere preparation could influence the drug encapsulation efficiency as well as the microsphere surface morphology, and consequently influence the water uptake of the microsphere-film system. We could also predict that the higher water content would facilitate the drug release from the drug-loaded system.

The IB release profiles (Figure 4) resembled the water uptake profiles. Osmotic driving force of IB contributed to water uptake, in turn, water uptake of matrices contributes to the drug release rate.\(^{22,23}\) For instance, the sample with IB solely dispersed in GT network (nonmicrosphere), which had the highest water uptake, showed the fastest release rate.

In a microsphere-film system, drug diffusion could proceed stepwise. In the first step, diffusion from the microspheres occurred, and in the second step, the diffusion through GT networks to solution took place. The burst release of the microsphere-film system was dependent mainly on nonencapsulated drug diffusion. After the burst release in the first phase, the drug released mainly from the microspheres. The diffusion path of the IB molecules from microspheres to solution involved both PCL chains and GT network. Thus, the IB release from the microspheres would be slower than release of nonencapsulated IB. For that reason, microsphere-film systems have better sustained release performance than the "nonmicrosphere" GT film system. Because of the easy swelling characterization of the GT matrix, the IB release rate from microsphere-film systems depended chiefly on the diffusion of drug molecules across the PCL chains.

The handles of the drug-loaded microsphere-film samples in both dry and wet states are similar to those of pure GT films. Therefore, this new kind microsphere-film drug release system combined the advantages of the sustained release for small drug molecules, good adhesion, and biodegradability in one approach. It may provide a good solution to prolong drug release for those hydrophilic matrices.

CONCLUSIONS

1. Incorporation of drug-containing PCL microspheres to GT film provides the sustained release property of IB.
2. Both the molecular weight of PCL and the kind of emulsifier influence the drug encapsulation efficiency and microsphere surface morphology, and consequently influence the release behavior of the microsphere-film system.
3. The new microsphere-film drug release system has combined the advantages of gelatin films and PCL microspheres, that is, they offer both better adhesion and sustained release performance.

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REFERENCES


