AAPS PharmSciTech, Vol. 12, No. 1, March 2011 (© 2010)
DOI: 10.1208/s12249-010-9565-y

Research Article

Theme: Advances in Pharmaceutical Excipients Research and Use: Novel Materials, Functionalities and Testing
Guest Editors: Otilia Koo, Thomas Farrell, Allison Radwick, and Sameer Late

The Biodegradation of Zein *In Vitro* and *In Vivo* and its Application in Implants

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Received 23 August 2010; accepted 30 November 2010; published online 24 December 2010

Abstract. A unique polymer-based sustained-release implant drug delivery system was prepared by using biocompatible and biodegradable Zein as the skeleton meterial. After preparing Zein colloids, the Zein-loaded implant rods were formulated by injection molding followed by evaporating the solvent, and being coated with poly(lactic-*co*-glycolic) acid (PLGA) solution. Drug release kinetics was examined by using Fluorouracil (5-FU) as model drug. Nearly zero-order release was achieved for the model drugs for a period of 0–25 days when the implants were incubated in distilled water at 37°C. And then the degradation kinetics of the rods *in vivo* and *in vitro* were evaluated, which indicated that Zein could be absorbed by body and has good degradation property. The effects of different ratios of Zein/5-FU and the rods' diameter on drug release were studied, respectively. The plasma concentration of 5-FU in the implants were determined by HPLC after implanting a single dose of the implants in rats. All data were subsequently processed by using the computer program 3P97, and the values were showed as follows: the area under the plasma concentration–time curve (AUC) value was 321.88 (μg/ml)×day, and the mean residence time (MRT) value was 23.05 days. The sustained-release implants of Zein/5-FU were successfully formulated. The uniqueness of the article is that Zein has been used as a skeleton material in implant delivery system for the first time and zero-order release kinetics has been obtained successfully.

KEY WORDS: degradation kinetics; implants; *in vivo* characteristics; zein.

INTRODUCTION

Biodegradable and biocompatible polymeric systems have been well studied as sustained-release drug implants in recent years (1,2). These types of sustained-release implants are especially attractive for post-surgical treatment of cancer (skin cancer, gastric cancer, and so on).

By taking advantage of surgical operation, drug-loaded sustained-release polymer implants may be administrated directly at the cancer site so that the systemic toxicity and side effects can be minimized. Currently, the most commonly used biodegradable materials include poly-L-lactide acid (PLA), PLGA, and poly-caprolactone (PCL) (3), etc., which are not only expensive but also have their own shortcomings. In the degradation process, lactic acid produced by PLA in the body will make the organs suffer from aseptic inflammation, the PLGA skeleton is prone to be corroded and the pH value of the environment will reduce immediately, which has side effects on sensitive protein. PCL has a long period of degradation *in vivo*, and the accumulation of the degraded products may produce toxic effects (4,5). In addition, the natural polymeric substances such as gelatin, albumin, and casein can be dissolved in the aqueous environment rapidly which would cause drug burst (6,7). Therefore, pharmaceutical excipients with the properties of biocompatibility, biodegradability, and nontoxic adverse reactions should be actively developed and found to enable the implanted drug delivery systems to be better applied.

Zein is a natural protein Gorhamin isolated from the corn in 1821, which is rich in glutamic acid (21–26%), leucine (20%), proline (10%), and alanine (10%) but lacks the basic and acidic amino acids, especially, the tryptophan and lysine. Its molecular weight is about 22 kDa (8). It is water insoluble and ethanol soluble, which depends on the characteristics of its amino acid composition (9,10). Zein has once served as tablet coating material in the pharmaceutical field (11–13). In recent years, as drug delivery materials, Zein has attracted the attention of pharmaceuticals workers (14–16). In our recent work, it can be found that Zein has good biocompatibility and biodegradability (17), apart from the reported good film properties. We found that Zein was an excellent skeleton material to support sustained-release implants. First, its biodegradability makes it possible for implant applications.

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And second, its spiral structure enables sufficient amount of drug to be loaded.

Fluorouracil (5-FU) was used as model drugs to examine the Zein-involved formulation approach in the present study. 5-FU is a poor water soluble compound and one of the most frequently used chemotherapeutic drug for cancer therapy whose efficacy in treatment of cancer can be greatly improved by the commercially available first-order release kinetics sustained-release implant (Sinofuan®). Its hydrophobic nature may serve as a type of model drug to examine the applicability of the Zein implantation in sustained-release system. The uniqueness of the article is that Zein has been used as a skeleton material in implant delivery system and zero-order release kinetics has been obtained successfully. Compared with the commercial products, the skeleton material used in this study is cheap and biodegradable.

In this work, the Zein-loaded sustained-release implants will be prepared and then characterized by the degradation kinetics, scanning electron microscopy(SEM), *in vitro* release profile and *in vivo* release kinetics.

METHODS

Samples

Zein was purchased from Bache Pharmaceutical Adjuvant Factory (Wujiang, China). PLGA was purchased from Ji-Rui Biomaterials Co., Ltd (Hefei, China). Sprague–Dawley (SD) rats were provided by Anhui Medical University (Hefei, China). Fluorouracil with the purity of 99.1% was purchased from Auto Chemical Engineering Tech. Co., Ltd. (Nanjing, China). High-performance liquid chromatography (HPLC) grade methanol was supplied by Merck (Darmstadt, Germany). All other chemicals and solvents were of analytical reagent grade.

Implant Preparation

Zein and 5-FU powder were mixed with 95% ethanol, and then the mixture was heated in thermo-tank (Sangli Electronic Equipment Factory, Nanjing, China) at 50±0.5°C for 30 min. Finally, the different ratios of Zein/5-FU colloids can be obtained. Then the colloid was injected into a tailor-made mold (length 1.00±0.001 cm, diameters 0.10, 0.15, and 0.20± 0.001 cm) of polytetrafluoroethylene followed by evaporating the solvent and dried with vacuum oven at 50°C for 8 h. Zein colloids were molded into rod-shaped and were pushed out from the molds and stored in a desiccator. And then coated them with 20% PLGA solution (*w*/*v*, chloroform as the solvent). The drug-free implants were prepared as above.

All the formulations examined in this study were summarized in Table I.

Table I. Formulations of Drug-Loaded Zein-Based Implants

Sample	Zein/5-FU	Coating weight (%)
1	40/60	0
2	50/50	0
3	60/40	0
4	50/50	10
5	50/50	15
6	50/50	20

Degradation Kinetics

First, the *in vitro* degradation kinetics of drug-free implants was investigated by incubating the samples in a PBS buffer (pH 7.4). Briefly, several accurately weighed samples were placed in test tubes containing 2 ml of the PBS and shaked at 37°C for 2, 4, 6, 8, 12 weeks, respectively. The incubation was terminated at scheduled date, and the remaining solid samples were vacuum-dried and weighted. The sample degradation rates were calculated according to the following equation:

$$\text{System degradation rate} = (W_0 - W_r)/W_0 \times 100\% \quad (1)$$

Where W_0 is the initial sample weight and W_r is the remaining sample weight after dried with vacuum oven.

And then, the *in vivo* degradation kinetics of drug-free implants was investigated by implantation and retrieval of the system at 2, 4, 8, 12, and 25 weeks. For implantation of the drug-free rods in the SD rats, surgical procedures were performed on mature adult SD rats (weighed 250∼300 g). Under general anesthesia, subcutaneous pockets were made by one midline incisions of 5 mm on back and parallel to the spine, and the cylindrical rod was implanted under sterile conditions. A total of 12 samples were implanted in 12 rats. At 2, 4, 8, 12, and 25 weeks after implantation, three animals were euthanized, and the samples was retrieved. After retrieval, all the samples were rinsed with distilled water followed by ethanol and dried with vacuum oven and weighted. The calculation of the system degradation rate was the same as the above Eq. 1.

Morphologies of Zein Rods

SEM was used to study the surface of the rods. The samples were coated with a thin layer of gold before examination, using a HITACHI S-4800 scanning electron microscope (HITACHI Company, Japan) at an acceleration voltage of 5 kV.

Determination of Drug Loadings

One implant was crushed and dissolved in distilled water. After filtration of the solution, the drug content of 5-FU was

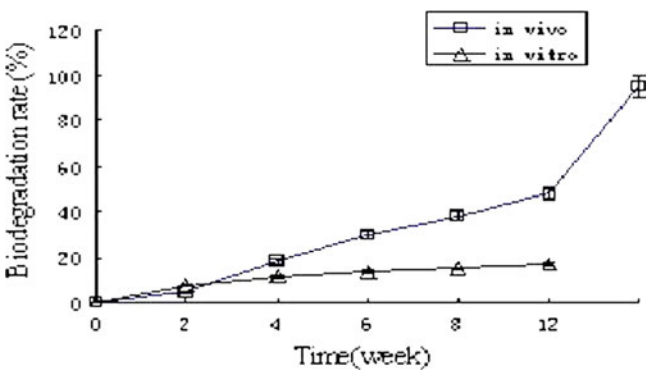

Fig. 1. Biodegradation rate of zein rods *in vivo* and *in vitro*

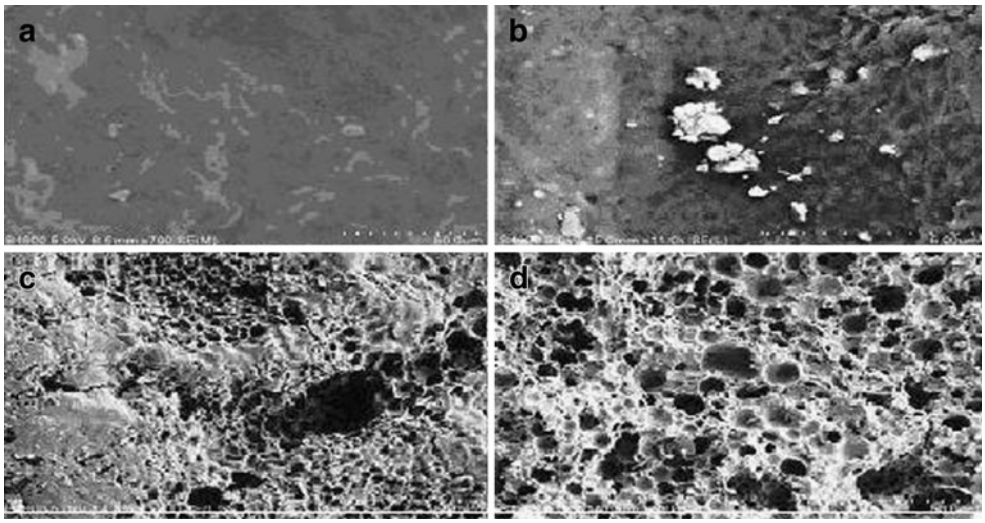

Fig. 2. Surface changes of zein rods during *in vivo* degradation for **a** 2, **b** 4, **c** 8, and **d** 12 weeks

determined spectrophotometrically at 263 nm (UV-visible spectrophotometer, Puxitongyong, Beijing, China). The assay was carried out in triplicate for each batch.

In Vitro Studies of Zein-Based Implants

Release of 5-FU from the implants were observed in triplicate in Dissolution Tester (Tianjin, China) under sink conditions. The implants were placed in flasks with distilled water (37±0.5°C). At specified time intervals, 2 ml of the aqueous solution was removed (added into the flasks with 2 ml fresh water immediately) and diluted to 10 ml. After filtration of the solution, the amount of 5-FU released was determined spectrophotometrically as explained above.

In Vivo Pharmacokinetic

Healthy SD rats weighed 250±10 g (male and female) were randomly divided into experimental group (six rats in each group).

Rods were administered in each rat at a single dose of 300 mg/kg. The drug-loaded implants were implanted subcutaneously in rats on back and parallel to the spine. Then, blood plasma (0.5 ml) was sampled from the rat retro orbital sinus into Heparin EP tubes, after 1, 3, 5, 7, 9, 11, 13, 16, 19, 20, 25 days of implantation. Blood samples were centrifuged at 4,000 rpm for 10 min. The plasma was harvested and frozen at −20°C until assay was performed using $(NH_4)_2SO_4$ precipitation and reverse phase-high performance liquid chromatography method attached with spectrophotometric detection at 263 nm and Aglent C_{18}(4.6×250 mm, 5 μm). The mobile phase composition was 2:98, *v*/*v* for methanol:0.1% acetic acid solution. All data were subsequently processed by the 3P97 program (Designed by Chinese Pharmaceutical Association).

RESULTS AND DISCUSSION

Degradation Kinetics

Figure 1 shows that biological erosion of drug-free zein-based rods is 4.60% *in vivo* and 7.70% *in vitro*, respectively, which were calculated by the Eq. 1. This may be mainly due to the hydration *in vivo* at the beginning of degradation, which caused high concentration of some small degraded amino acid residues *in vivo* and made a lower biodegradation rate. While the biodegradation rate for 12 weeks of *in vivo* and *in vitro* were 48.10% and 17.00%, respectively. This may be the enzyme, microorganisms as well as giant cell phagocytosis in the body, which caused the break in Zein protein structure and produced small molecule amino acid residues and then absorbed by the body. Additionally, there was no inflammation found in the implantation site.

Furthermore, the rods' surface modification after degradation *in vivo* was investigated by SEM (Fig. 2). No apparent changes in surface morphology of the rods were showed until 2 weeks had elapsed. However, a few concave-shaped absorptions were observed after 4 weeks. After 8 weeks, more concave-shaped absorptions were observed on the zein rods surface. Twelve weeks later, the number of the capillary vessels were increased on the membrane, and the concave-shaped absorption surface were more evident. The above-

Table II. Drug-Loading Capacity and Yield of Drug Loading of the Zein-Based Implants

Sample	Zein/5-FU	Appearance	Targeted drug-loaded rate (%)	Actual drug-loaded rate (%)	Yield (%)
1	40/60	Rough	60	58±0.31	96.7±0.52
2	50/50	Smooth	50	48±0.28	96.0±0.47
3	60/40	Smooth	40	36±0.21	90.0±0.35

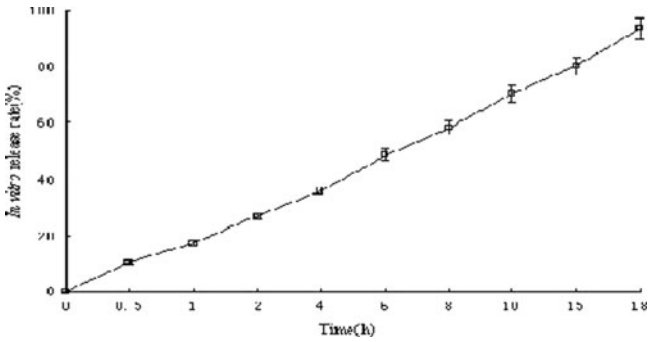

Fig. 3. *In vitro* release of rods (Zein/5-FU=50:50) without coated weight

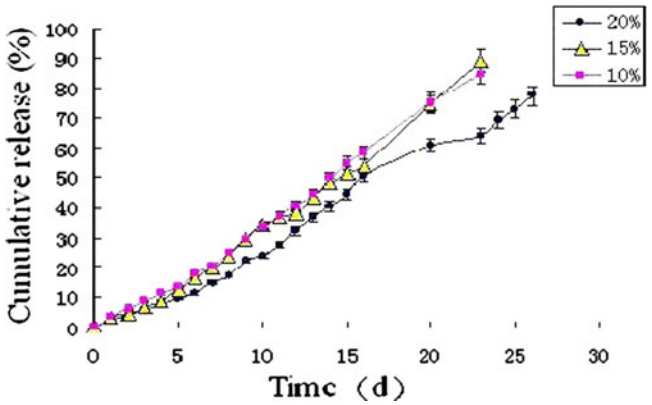

Fig. 4. *In vitro* release rate of 5-FU in implants with different coated weight (10%, 15%, and 20%)

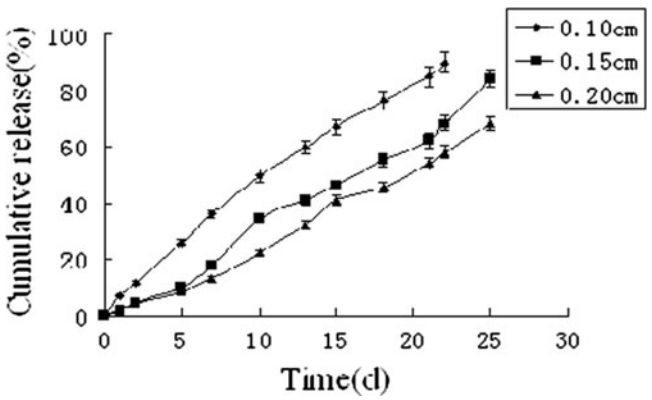

Fig. 5. *In vitro* release of implants with different diameters

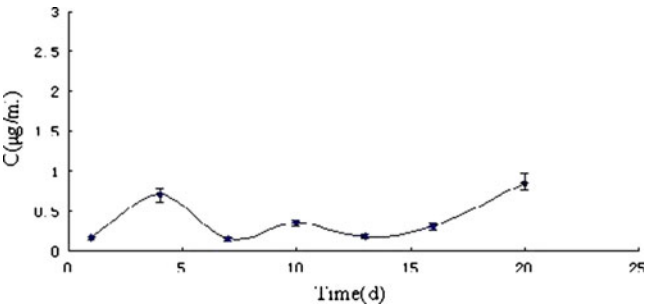

Fig. 6. Drug concentration–time curve in rats *in vivo*

mentioned phenomenon agreed with the reported literature that Zein was absorbed by body which in turn indicated that Zein has good degradation property and can be deeply studied for implants (18). The authors decided to further investigate Zein-based sustained-release implant systems.

Drug Loading Capacity and Coating Weight

The projected and actual drug loads of some Zein-based implants and the yield of the drug are summarized in Table II. For 5-FU, the drug loading yield was 96.0% for the samples projecting 50% drug loads (the drug reached 50% of the total mass of the rods). For the samples with higher projected 5-FU loads, 60%, the drug-loading yield raised to 96.7%. However, the appearance of the prepared rods became rough when the ratio of Zein/5-FU was 40/60. Hence, according to Table II, the optimal Zein/5-FU ratio of the rods should be about 50/50. The *in vitro* release of rods (Zein/5-FU=50:50) was summarized in Fig. 3, of which the rods did not meet the demand of long-term treatment of implants. So, to achieve the design goals, a 20% PLGA solution was chosen to coat the formulated rods. The *in vitro* release rate of 5-FU in implants with different coated weight were determined in Fig. 4. The experimental data showed that *in vitro* release became slower with the increase of coating weight. Hence, the maximum coating weight of the rods was determined to be approximately 10%.

Effect of Different Diameter of Drug

In vitro release of implants (length 1.00±0.001 cm, diameters 0.10, 0.15, and 0.20±0.001 cm) were determined (Fig. 5).

The results showed that larger diameter caused a slower and fluctuating *in vitro* release. Thus, the rods of model diameter 0.10 cm were selected for further studies.

Table III. *In Vitro* Release Kinetic Equations of Coated Implants with Different Diameters

		Result	
Diameter (cm)	Methods	Equation	*R*
0.10	Zero-order model	$M_t/100 = 0.0414 \times t + 0.558$	0.9959
0.15	Zero-order model	$M_t/100 = 0.0307 \times t - 0.0585$	0.9838
0.20	Zero-order model	$M_t/100 = 0.0227 \times t - 0.376$	0.9819

Table IV. Pharmacokinetic Parameters of Coated Implants in Rats

AUC (SO) (μg/ml)*day	C (max) (μg/ml)	S2	MRT (d)	VRT (d*d)
321.87	0.26	239,735.84	23.05	12.87

In Vitro Release Mechanism

For sustained release formulations, the following formula is often used to express the release behavior *in vitro*.

$$\text{Zero} - \text{order model: } M_t/M\infty = k \times t + k_0 \qquad (2)$$

Where $M_t/M\infty$ is the fractional drug release at time t and k_0 is the kinetic constant. The above zero-order model is used in this article to express the release behavior of 5-Fu from zein rods made of different diameter and the results were showed in Table III, from which we concluded that the rods of smaller diameter showed better linear relationship and rods having diameter of 0.10 cm had the best correlation coefficient.

These results suggested that the drug release *in vitro* was by the diffusion mechanism, which is governed by Fick's diffusion law:

$$dM/dt = (A/h) \times D \times (C_S - k \times C_e) \qquad (3)$$

Where dM/dt is the fractional drug release at certain time t, A is the surface area of membrane, D is the kinetic constant, C_s is saturated solubility of drugs, K is the diffusional exponent for drug release and C_e is the drug content released. According to the Fick's law, the *in vitro* release is inversely proportion to the diameter. That is because the drug diffusion coefficient (D) becomes smaller which in turn led to the reduction of dM/dt.

In Vivo Studies

Data of plasma concentration in rats after subcutaneous implantation are shown in Fig. 6. A stable plasma concentration was maintained for 20 days. All data was evaluated using the 3P97 progam and the Statistical Moments was also used to calculate the results in this article. The results are shown in Table IV, respectively, from which we found out the value of AUC in rats was 321.87 (μg/ml)×day and the $C_{\max}$ was 0.26 μg/ml and the MRT was 23.05 days, respectively, which indicate that the Zein/5-FU implants have apparent effect of sustained-release in the body.

CONCLUSION

In the present paper, the biodegradable property of Zein was investigated, and the degradation *in vivo* was observed obviously after implanting rod-shaped implants in rats for 2 weeks.

A unique Zein-based sustained-release delivery system for implants loaded with 5-FU was successfully formulated. By changing the diameter and the drug content of the implants, different drug release kinetics could be obtained. A zero-order release profile of the rod-shaped implants coated with PLGA was obtained when 1:1 of Zein to 5-Fu, 10% of PLGA solution were used.

The data of the experiments of subcutaneous implantation in rats showed a stable plasma concentration was maintained for 20 days.

In addition to nearly zero-order and burst-free release kinetics and a stable drug concentration in plasma, this formulation approach is simple and does not require complicated manufacture processing, thus can easily be scaled up. However, further research is still needed to confirm the mechanism by which 5-FU kept in the Zein rod.

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