International Journal of Chemical, Materials and Biomolecular Sciences
ISSN: 2415-6620
Vol:10, No:7, 2016

Controlled Release of Glucosamine from Pluronic-Based Hydrogels for the Treatment of Osteoarthritis

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***Abstract*—**Osteoarthritis affects a lot of people worldwide. Local injection of glucosamine is one of the alternative treatment methods to replenish the natural lubrication of cartilage. However, multiple injections can potentially lead to possible bacterial infection. Therefore, a drug delivery system is desired to reduce the frequencies of injections. A hydrogel is one of the delivery systems that can control the release of drugs. Thermo-reversible hydrogels can be beneficial to the drug delivery system especially in the local injection route because this formulation can change from liquid to gel after getting into human body. Once the gel is in the body, it will slowly release the drug in a controlled manner. In this study, various formulations of Pluronic-based hydrogels were synthesized for the controlled release of glucosamine. One of the challenges of the Pluronic controlled release system is its fast dissolution rate. To overcome this problem, alginate and calcium sulfate ($CaSO_4$) were added to the polymer solution. The characteristics of the hydrogels were investigated including the gelation temperature, gelation time, hydrogel dissolution and glucosamine release mechanism. Finally, a mathematical model of glucosamine release from Pluronic-alginate-hyaluronic acid hydrogel was developed. Our results have shown that crosslinking Pluronic gel with alginate did not significantly extend the dissolution rate of the gel. Moreover, the gel dissolution profiles and the glucosamine release mechanisms were best described using the zeroth-order kinetic model, indicating that the release of glucosamine was primarily governed by the gel dissolution.

***Keywords*—**Controlled release, drug delivery system, glucosamine, Pluronic® F-127, thermoreversible hydrogel.

I. Introduction

OSTEOARTHRITIS is one of joint diseases that affects more than 27 million people worldwide. The symptoms of osteoarthritis lead to difficulty in doing daily routine activities such as joint pain, loss of movement, and so on. If osteoarthritis still remains after the medication, physicians often recommend the replenishment of natural lubrication of cartilage using two popular supplements, which are chondroitin and glucosamine [1].

Recently, there are two dosage forms of glucosamine: oral tablet and solution for local injection. Although the oral dosage form is more popular, there is no proof of how much the drug gets to the target site [2]. Thus, the local injection of glucosamine can better solve the target-goal problem. However, most of the patients do not prefer to take glucosamine by this route, because of inconvenience of administration due to the requirement of doctor's visit, pain, and so on. Moreover, the patients need multiple injections which can potentially lead to the possible bacterial infection. As a result, a local delivery system that can extend the release of an active therapeutic agent is desired.

A hydrogel has been widely used in many medical applications, especially in the drug delivery. One of the important characteristics of the hydrogel is its thermo-reversibility. A drug mixed with hydrogel can be locally administered into the body in the liquid form. Once inside the body, the liquid mixture is warmed up to 37 °C and solidified into a gel, slowly releasing the encapsulated drug to the target site [3]. Pluronic® F-127, a triblock copolymer of PEO-PPO-PEO, has been widely used in many studies to control the drug release. More importantly, Pluronic® F-127 has already been proven by FDA to be used for injectable drugs [4]. To date, there is no study on the controlled release of glucosamine from a hydrogel. It would be interesting to investigate the potential use of hydrogel for the sustained release of glucosamine.

In this study, various formulations of Pluronic-based hydrogel were synthesized for the controlled release of glucosamine. Each formulation contained various concentrations of alginate, and hyaluronic acid. Alginate was used to slow down the dissolution rate of the gel [5], while hyaluronic acid could be broken down into small units of glucosamine. Hence, higher glucosamine delivery was to be expected. In addition, the physical properties of the hydrogels were characterized including gelation temperature, its morphology and hydrogel dissolution. A study on the release of glucosamine from the selected hydrogel formulations was also carried out, and these experimental data were used to develop a mathematical model to understand the mechanism of the drug release.

II. Materials and Methods

A. Preparation of Pluronic-F127 Based Hydrogel

Pluronic® F-127 stock solution at 30% w/v was prepared by dispersing 6 g of Pluronic® F-127 in 20 ml of PBS at pH 7.4. Next, a magnetic stirrer was used to mix the solution for 24 hours at 4 °C. Then, the solution was continued to be stored in

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the refrigerator to obtain the completely dissolved polymer. Afterwards, alginate, hyaluronic acid, and glucosamine were mixed with the Pluronic solution at room temperature. For the formulation containing alginate, 0.5% $CaSO_4$ solution was required to initiate the cross-link reaction. The composition in each formulation is shown in Table I (using ¼ factorial design). Each formulation was kept in at 4 °C until use.

TABLE I
COMPOSITION OF EACH FORMULATION (% W/V)

Formulation	PF-127	Alg	HA	GluN	$CaSO_4$
F0	25%	0%	0%	0%	0%
F1	25%	1%	0%	0%	0.2%
F2	25%	0%	0%	10%	0%
F3	25%	0%	0.1%	0%	0%
F4	25%	1%	0.1%	10%	0.2%

Note that PF-127 = Pluronic® F-127, Alg = alginate, HA = hyaluronic acid, GluN = glucosamine.

B. Thermoreversibility of Pluronic-Based Hydrogels

The thermoreversible properties of the pluronic-based hydrogels were assessed using their gelation temperature and their gelation time at 37 °C. Briefly, the hydrogel solution was placed in a test vial and immersed in an ice bath for 20 minutes. After that, the temperature of the solution was increased in a 1 °C increment. The gelation temperature of the hydrogel was determined by using a tube inversion method, based on flow or no flow of the solution after the vial was inverted for 2 minutes. For the determination of the gelation time at 37 °C, the liquid formulations were maintained at 4 °C for 20 minutes. Then, the solution was suddenly immersed in a 37 °C water bath. The time at which the solution solidified was recorded as the gelation time.

C. Morphology of Pluronic-Based Hydrogels

Scanning electron microscopy (SEM) was used to investigate the morphology of Pluronic-based hydrogels. Due to high water content in the gels, the hydrogels were frozen at -20°C and dried by a vacuum pump. The samples were, then, sputtered with gold before the SEM observation.

D. Dissolution of Pluronic-Based Hydrogels

Initially, the gel solution was placed in a glass vial and weighed. Phosphate buffered saline (PBS) was added into the vial to allow the hydrogel to dissolve into the PBS solution. At specific time points, the solution was discarded and the sample vial was weighed to estimate the mass loss of the hydrogels, before replenishing with the fresh PBS. This procedure was repeated until the hydrogels were completely gone.

E. Release of Glucosamine from Pluronic-Based Hydrogels

The glucosamine release experiment was similar to that of the hydrogel dissolution study. The difference was that the PBS solution would contain glucosamine released from the hydrogels. The amount of released glucosamine was determined against a calibration curve constructed using a colorimetric assay. Briefly, glucosamine was allowed to transform into a color product in the presence of ninhydrin at 100 °C. After 5 minutes, the reaction was terminated by reducing the temperature to 5 °C. The absorbance of the color product was measured at 570 nm by using a spectrophotometer [6].

F. Mathematical Modeling of Glucosamine Release

Several mathematical models were used to describe the mechanism of drug release including [7]:

- Zeroth-order model: The drug release is constant over time.

$$M_t = M_0 + Kt \quad (1)$$

where, M_t = the amount of drug in the solution at time t, M_0 = the initial amount of drug in the solution, K = the zero-order release constant, t = time.

- First-order model: The release rate depends on the drug concentration

$$\frac{dC_t}{dt} = -Kt \quad (2)$$

- Hixson-Crowell Cube-Root model describes the release system that changes according to the changes in its surface area.

$$M_0^{\frac{1}{3}} - M_t^{\frac{1}{3}} = Kt \quad (3)$$

- Higuchi model: This model is based on the hypotheses that (1) initial drug concentration in the matrix is higher that its solubility, (2) drug diffusion takes place in 1 dimension, (3) matrix swelling and dissolution are negligible, and (4) a perfect sink condition in the release environment is assumed.

$$M_t = Kt^{1/2} \quad (4)$$

- Korsmeyer-Peppas model describes a drug release from polymeric systems.

$$\frac{M_t}{M_\infty} = Kt^n \quad (5)$$

where, M_∞ is the amount of released drug at t = ∞.

TABLE II
GELATION TEMPERATURE AND GELATION TIME AT 37°C OF EACH FORMULATION

Formulation	Gelation Temperature	Gelation Time at 37°C
F0: 25% PF-127	19 – 20 °C	50 – 60 s
F1: 25% PF-127 + 1% Alg	17 – 18 °C	40 – 50 s
F2: 25% PF-127 + 10% GluN	14 – 15 °C	40 – 50 s
F3: 25% PF-127 + 0.1% HA	23 – 24 °C	80 – 90 s
F4: 25% PF-127 + 1% Alg + 0.1% HA + 10% GluN	14 – 15 °C	40 – 50 s

Note that PF-127 = Pluronic® F-127, Alg = alginate, HA = hyaluronic acid, GluN = glucosamine

G. Statistical Analysis

All the experiments in this study were performed in triplicate (n = 3) and the data were presented as mean ± standard deviation. An unpaired Student's *t*-test was used to

evaluate the mean differences of the mean values. A statistically significant difference was defined at the 95% confidence level and a $p < 0.05$ was considered significant.

III. Results and Discussion

A. Thermoreversibility of Pluronic-Based Hydrogels

The thermoreversibility of the hydrogel was assessed as the gelation temperature and the gelation time at 37°C. The gelation temperature is defined as the temperature at which the solution is solidified. Even through Pluronic has been previously shown to exhibit phase transition at a specific temperature, changing its concentration and addition other components into the mixture would shift the phase transition.

In general, hydrophilic molecules increased the gelation temperature of the hydrogel [8]. All of API (Active Pharmaceutical Ingredient) and excipients were hydrophilic molecules. According to Table II, additional substances, such as glucosamine and alginate, reduced the gelation temperature while hyaluronic acid increased the gelation temperature. Cross-linking Pluronic with alginate might have reduced the distance between each Pluronic micelle, leading to a tight structure of micellar network and easy formation of a gel matrix [9]. Although lower gelation temperature may not be desirable as these formulations would be more difficult to handle, the changes in the gelation temperature were not sufficiently significant to become problematic.

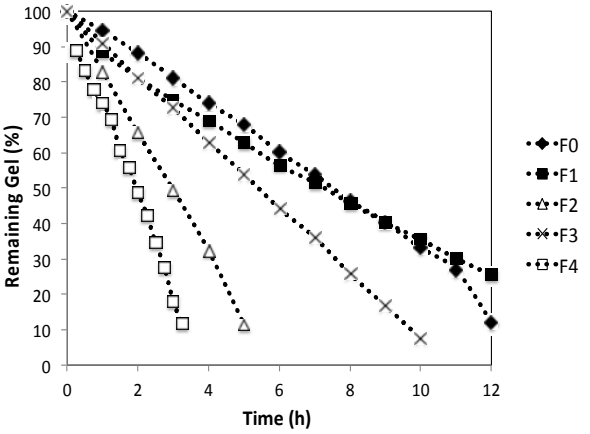

Fig. 1 Dissolution Profile of Each Formulation

All formulations displayed very fast transition to gels (Table II). The liquid hydrogel solidified into a gel in only 90 seconds. This result indicates the potential use of Pluronic®-F127 hydrogels as drug delivery vectors. The hydrogel will be administered as liquid but it will be immediately turned to a gel once it gets into the human body, allowing for the direct localization of the drug.

TABLE III
Various Kinetic Models Describing the Dissolution Rate of Each Formulation

Formulation	Zeroth-order		First-Order		Hixon-Crowell		Higuchi		Korsmeyer-Peppas	
	K (g/h)	r^2	K (h^{-1})	r^2	K ($g^{1/3}/h$)	r^2	K (h^{-1})	r^2	K (h^{-n})	r^2
F0: 25% PF-127	0.13	0.9919	0.16	0.8393	0.07	0.7717	0.58	0.8898	1.11	0.9987
F1: 25% PF-127 + 1% Alg	0.15	0.9948	0.15	0.8952	0.06	0.7991	0.54	0.9469	0.80	0.9974
F2: 25% PF-127 + 10% GluN	0.23	0.9955	0.29	0.9766	0.18	0.8764	0.57	0.9175	0.99	0.9981
F3: 25% PF-127 + 0.1% HA	0.14	0.9998	0.21	0.9064	0.08	0.7928	0.49	0.9181	1.01	0.9996
F4: 25% PF-127 + 1% Alg + 0.1%HA + 10% GluN	0.36	0.9914	0.64	0.8714	0.24	0.7521	0.69	0.8788	1.01	0.9927

Note that PF-127 = Pluronic® F-127, Alg = alginate, HA = hyaluronic acid, GluN = glucosamine, K = Rate constant

B. Dissolution of Pluronic-Based Hydrogels

The previous studies have shown that the release of drugs from a Pluronic gel is primarily governed by the gel dissolution mechanism [10]. As a result, the kinetics of the gel dissolution was thoroughly studied. The amounts of the dissolved hydrogel were collected and the plots of the dissolution curves were constructed using various well-known theoretical models described in the previous section.

Fig. 2 shows the dissolution profile of each formulation. Clearly, the gels constantly dissolved over time. It took almost 14 hours to completely dissolve Pluronic gel at 25% w/v. Adding 1% alginate could slightly extend the dissolution duration to about 15 hours, as alginate and $CaSO_4$ acted as a crosslinking agent, rendering a more tightly packed network of micelles [9]. As a result, this formulation was more difficult to be dissolved. Interestingly, the gels with glucosamine (formulations: F2 and F4) dissolved much faster than the gels without glucosamine. With a hydrophilic component, the hydrogels became more water favorable, allowing the water molecules to diffuse into the gel, untangle the micellar network, and dissolve the gel into the water phase [11].

The data of the gel dissolution were fitted with several kinetic models including the zeroth-order model, the first order model, Hixson-Crowell cube-root model, Higuchi model, and Korsmeyer-Peppas model (Table III). Only the zeroth-order model and Korsmeyer-Peppas model yielded the highest coefficient of determination (r^2) of around 0.9914 - 0.9995, indicating that the kinetics of the gel dissolution could be best described by these two models. For Korsmeyer-Peppas model, n values are more than 0.89, indicating that the model can be simplified as the zeroth-order mechanism [12]. Therefore, the hydrogels were dissolved constantly over time and the slope of the dissolution curve represented the dissolution rate of each formulation [13]. According to the zeroth-order kinetic model, F4 formulation has the highest

dissolution rate, possibly due to the presence of alginate and glucosamine which were both hydrophilic.

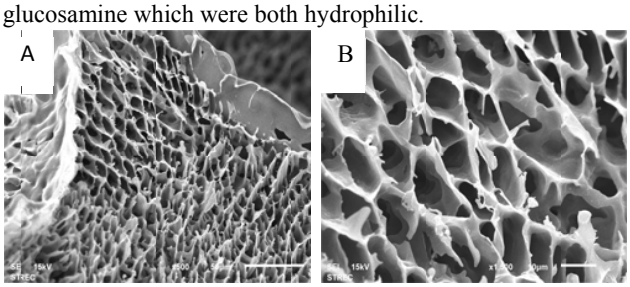

Fig. 2 SEM images of Pluronic-Based Hydrogel with magnification of (A) 500X (B) 1500X

C. Morphology of Pluronic-Based Hydrogels

Scanning electron microscopy (SEM) was performed to study the microstructure of Pluronic gels containing glucosmine. SEM images reveal a dense network of spherical nanometer-sized micelles connected together to form a honey-comb structure, and each layer was packed together (Figs. 2 (A) and 2 (B)). The pore size of honey-comb, where aqueous solution was previously located, was approximately 10 microns. In addition, no glucosamine particles were observed anywhere in the gel, indicating that glucosamine could be completely dissolved into the pore (aqueous phase), allowing for the sustained release from this network.

D. Release of Glucosamine from Pluronic-Based Hydrogels

Only formulations, F2, F3, and F4 were used in this study, since they contained 10% glucosamine. Glucosamine was allowed to continuously release from the gels at 37 °C. Their release profiles were presented in Fig. 3.

As expected, only the zeroth-order model and Korsmeyer-Peppas model yielded the highest coefficient of determination (r2) of approximately 0.9829 – 0.9997, indicating that the kinetics of the gel dissolution could be best described by these two models. The amount of hyaluronic acid release was extremely low compared to the release of glucosamine. In the other words, adding hyaluronic acid did not help much to release the intermediate in proteoglycan production pathway. For formulation F3 (with 0.1% HA) and F4 (with 1% Alg + 0.1% HA + 10% GluN), n values were greater than 0.89. Therefore, the release mechanism of glucosamine is zeroth-order model or dissolution controlled [14]. It means that glucosamine was released when the hydrogel was dissolved to the PBS phase. In addition, the slope of the graph represents the release rate of glucosamine. From Table IV, the release rate was accelerated when alginate, hyaluronic acid, and glucosamine were present because the water favorable component of the gel was increased. For formulation F2 (10% GluN), although n value was less than 0.89 but the number was very close to 0.89. Therefore, the release of glucosamine was divided into two phases. The earlier phase was governed by the burst release mechanism which was diffusion controlled. Glucosamine could diffuse into water phase by itself without relying on the hydrogel dissolution. The latter phase provided a sustained release which was dissolution controlled, similar to the zeroth-order release kinetic [15].

The amount of the glucosamine released was fitted with several kinetic models, similar to what described in the gel dissolution study (Table IV).

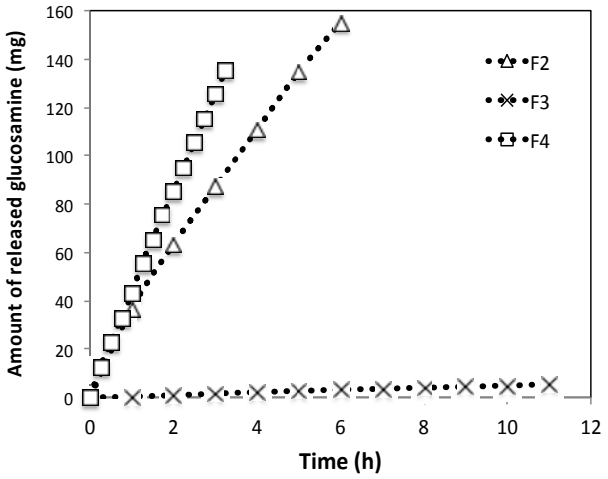

Fig. 3 Glucosamine Release Profile from Pluronic-Based Hydrogels

TABLE IV
VARIOUS KINETIC MODELS DESCRIBING THE RELEASE PROFILE OF GLUCOSAMINE FROM EACH FORMULATION

Formulation	Zeroth-order		First-Order		Hixon-Crowell		Higuchi		Korsmeyer-Peppas	
	K (mg/h)	r^2	K (h^{-1})	r^2	K ($mg^{1/3}/h$)	r^2	K (h^{-1})	r^2	K (h^{-n})	r^2
F2: 25% PF-127 + 10% GluN	27.14	0.9850	0.37	0.9852	0.87	0.9129	63.73	0.9543	0.81	0.9997
F3: 25% PF-127 + 0.1% HA	0.50	0.9987	0.22	0.9374	0.12	0.8166	1.77	0.9334	1.13	0.9829
F4: 25% PF-127 + 1% Alg + 0.1%HA + 10% GluN	42.23	0.9947	0.76	0.8963	1.19	0.8189	80.70	0.9331	0.94	0.9993

Note that PF-127 = Pluronic® F-127, Alg = alginate, HA = hyaluronic acid, GluN = glucosamine, K = Rate constant

Based on the experimental results from this study, 10 g administration of glucosamine using Pluronic-based hydrogel as a controlled release system could be extended to approximately three days. Although the sustained release of glucosamine is possible using this delivery system, longer release time is still desirable to reduce a number of glucosamine administration frequencies. Previous studies have shown that the dissolution rate of the hydrogels can be easily extended by a cross-link reaction. Therefore, a study on cross-linked hydrogels should be conducted to investigate the potential of the new drug delivery system. Appropriate cross-linking method such as photo cross-linking, temperature cross-linking, etc. should be determine to obtain an efficient delivery system for glucosamine to treat knee joint defects.

IV. Conclusion

In this study, we investigated the potential use of Pluronic-based hydrogels as a delivery vehicle to provide the sustained release of glucosamine for the treatment of osteoarthritis. Various formulations were developed containing 25% Pluronic as a base, 1% alginate and $CaSO_4$, as cross-linking agents, and 0.1% hyaluronic acid. All formulations exhibited the thermoreversible property. The gelation temperature of the hydrogels decreased when alginate and glucosamine were added. On the other hand, the gelation temperature increased with the presence of hyaluronic acid in the gel. Lower gelation temperature resulted in a faster gel formation at 37 °C. The formulation that contained alginate could extend the release period of glucosamine, while hyaluronic acid reduced the duration of glucosamine release. Although alginate displayed longer release time, the hydrogel showed undesirable appearances to be used as an injectable drug. Therefore, the formulation without alginate and hyaluronic acid was preferred. The kinetics of glucosamine release from each formulation was best fitted with the zeroth-order model and Korsmeyer-Peppas model. Because of high n values in the Korsmeyer-Peppas model, the glucosamine release from all formulations could be modeled by using the zeroth-order kinetic model for simplicity.

Acknowledgment

This work was financially supported by National Research University Project of Thailand, Office of the Higher Education Commission.

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