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ORIGINAL ARTICLE

Development of Metronidazole-loaded *In situ* Thermosensitive Hydrogel for Periodontitis Treatment

Periodontit Tedavisi için Metronidazol Yüklü Yerinde Termosensitif Hidrojel Geliştirilmesi

Duy Toan PHAM[1], Premchirakorn PHEWCHAN[2], Kanchana NAVESIT[2], Athittaya CHOKAMONSIRIKUN[2], Thatawee KHEMWONG[3], Waree TIYABOONCHAI[2,4]*

[1]Can Tho University College of Natural Sciences, Department of Chemistry, Can Tho, Vietnam
[2]Naresuan University Faculty of Pharmaceutical Sciences, Department of Pharmaceutical Technology, Phitsanulok, Thailand
[3]Naresuan University Faculty of Dentistry, Department of Diagnostic Dentistry, Phitsanulok, Thailand
[4]Mahidol University Faculty of Science, Department of Chemistry and Center of Excellence for Innovation in Chemistry, Nakhon Pathom, Thailand

ABSTRACT

Objectives: Periodontal treatment focuses on the thorough removal of specific periodontal pathogens, mainly anaerobic Gram-negative bacteria, by mechanical scaling and root planning. In case the periodontal abscess is detected after treatment, a high dose of antimicrobial agents is commonly applied via oral administration. However, this approach increases the risk of antibiotic resistance and systemic side effects and decreases efficacy. To overcome the aforementioned issues, this study focused on the development of thermosensitive hydrogel to deliver the antibiotic drug metronidazole (MTZ) directly and locally to the oral infection site.

Materials and Methods: The thermosensitive hydrogels were prepared by blending 28% w/v Pluronic F127 with various concentrations of methylcellulose (MC) and silk fibroin (SF). The gel properties, such as sol-gel transition time, viscosity, and gel strength, were investigated. The drug dissolution profiles, together with their theoretical models and gel dissolution characteristics, were also determined.

Results: All hydrogel formulations exhibited sol-gel transitions at 37˚C within 1 min. An increase in MC content proportionally increased the viscosity but decreased the gel strength of the hydrogel. By contrast, the SF content did not significantly affect the viscosity but increased the gel strength of the hydrogel. The thermosensitive hydrogels also showed prolonged MTZ release characteristics for 10 days in phosphate-buffered saline (PBS) at pH 6.6, which followed the Higuchi diffusion model. Moreover, MTZ-thermosensitive hydrogel exhibited delayed dissolution in PBS at 37˚C for more than 9 days.

Conclusion: MTZ-thermosensitive hydrogels could be considered a prospective local oral drug delivery system to achieve efficient sustained release and improve the drug pharmacological properties in periodontitis treatment.

Key words: Thermosensitive hydrogel, metronidazole, silk fibroin, sol-gel transition, periodontitis

ÖZ

Amaç: Periodontal tedavi, özellikle anaerobik Gram-negatif bakteriler olmak üzere spesifik periodontal patojenlerin mekanik ölçekleme ve kök planlaması ile tamamen ortadan kaldırılmasına odaklanmaktadır. Tedaviden sonra periodontal apse saptanması durumunda, oral yoldan yüksek dozda antimikrobiyal ajanlar yaygın olarak uygulanır. Ancak, bu yaklaşım antibiyotik direnci ve sistemik yan etki riskini artırmakta ve etkinliği azaltmaktadır. Bu çalışma, bahsedilen bu sorunların üstesinden gelmek için antibiyotik ilaç metronidazolün (MTZ) doğrudan ve lokal olarak oral enfeksiyon bölgesine verilmesi için ısıya duyarlı hidrojel geliştirilmesine odaklanmıştır.

Gereç ve Yöntemler: Isıya duyarlı hidrojeller, %28 a/h Pluronic F127'nin çeşitli konsantrasyonlarda metilselüloz (MC) ve ipek fibroin (SF) ile karıştırılmasıyla hazırlanmıştır. Sol-jel geçiş süresi, viskozite ve jel kuvveti gibi jel özellikleri araştırılmıştır. Teorik modelleri ve jel çözünme özellikleri ile birlikte ilaç çözünme profilleri de belirlenmiştir.

Bulgular: Tüm hidrojel formülasyonları, 1 dakika içinde 37˚C'de sol-jel geçişleri sergilemiştir. MC içeriğindeki bir artış, orantılı olarak viskoziteyi artırmış, ancak hidrojelin jel gücünü azaltmıştır.

*Correspondence: wareet@nu.ac.th, Phone: 66814211464, ORCID-ID: orcid.org/0000-0002-8570-0465
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Sonuç: MTZ-ısıya duyarlı hidrojeller, periodontit tedavisinde verimli sürekli salım sağlamak ve ilaç farmakolojik özelliklerini geliştirmek için olası bir yerel oral ilaç dağıtım sistemi olarak düşünülebilir.

Anahtar kelimeler: Isıya duyarlı hidrojel, metronidazol, ipek fibroin, sol-jel geçişi, periodontit

INTRODUCTION

Periodontitis is a pathological inflammatory condition of periodontal tissues, including gingiva, periodontal ligament, cementum, and alveolar bone. The major cause of this condition relates to the dysbiosis condition, which is mainly associated with anaerobic Gram-negative bacterial loads.[1] Thus, periodontitis treatment mainly focuses on the reduction or eradication of periodontal pathogens.[2] The first step of periodontal treatment involves scaling and root planing through the elimination of subgingival calculus by mechanical removal.[3] However, in some cases, conventional therapy alone is insufficient because bacterial endotoxin has penetrated the root surface.[4] Therefore, combined treatment with antimicrobial agents, such as local antiseptic agents or systemic antibiotics, is essentially administered to increase treatment efficiency. Presently, metronidazole (MTZ) is one of the most widely used antibacterial compounds, which efficiently inhibits anaerobic microorganisms, in periodontal treatment.[5,6] However, oral administration of MTZ to deliver antibiotics directly to the infected site is difficult, thus leading to an insufficient concentration of the drug within the periodontal pocket. Moreover, the use of high doses of MTZ causes various side effects, such as gastrointestinal disorders, development of resistant bacterial strains, and supra-infection.[7] To this end, novel approaches for advanced periodontal treatments are necessary.

Local drug delivery administration to the oral cavity is a potential approach to overcome the aforementioned challenges. This route provides a high concentration of antimicrobial compounds directly to the infected site and minimizes their potential systemic side effects. Nevertheless, for the local route to be effective, the capability to precisely control drug release at the target site is crucial. For this issue, a drug delivery system, such as thermosensitive hydrogel that can favorably carry, protect, and release the drug, proves its benefits.

In the recent decade, thermosensitive hydrogels have been increasingly utilized as carriers for the local delivery of drugs to the sites of action. An ideal thermosensitive hydrogel should exhibit a suitable sol-gel transition behavior, in which the hydrogel remains in the solution state below body temperature and forms a gel at body temperature.[8] One of the most commonly used materials for sol-gel reversible hydrogels is Poloxamer 407 with the registered trademark of Pluronic F127 (PF127). PF127 is considered a biocompatible polymer and has been approved by the US Food and Drug Administration (FDA).[9] PF127 is a triblock copolymer of poly(ethylene oxide)-poly(propylene oxide)-poly(ethylene oxide) (PEO-PPO-PEO) that exhibits a phase transition temperature of approximately 25˚C-32˚C[9] during micellization or micelle aggregation.[10] However, because of their low mechanical strength and low molecular weight (MW), PF127 hydrogels are prone to rapid erosion, thus exhibiting low stability under physiological conditions. Consequently, burst and uncontrolled drug release occur, which further reduces system efficiency.[10,11] To overcome these, we proposed a novel approach of co-incorporating methylcellulose (MC) and silk fibroin (SF) into the PF127 thermosensitive hydrogels to improve their properties.

MC, a water-soluble cellulose derivative, has the potential characteristic to induce reversible sol-gel transitions through hydrophobic interactions in an aqueous solution with increasing temperature.[12] MC is recognized by the US FDA as a highly biocompatible material.[13] MC has been used for biomedical applications, including dermal wound repair, regenerative medicine, cell sheet engineering, and bone regeneration.[12,14] SF from *Bombyx mori* silkworms has also been widely investigated as a biomaterial because of its unique properties, including excellent mechanical properties, controllable degradability, and biocompatibility.[15-21] SF can generate sol-gel transition through β-sheet assembly under physiological conditions,[22] such as ionic surfactant, pH, concentration, and temperature.[23,24] Therefore, we hypothesized that MTZ-thermosensitive hydrogel based on PF127 with SF and MC could improve the properties of hydrogels, including increased gel strength, slow erosion, and sustained drug release, to complement periodontitis treatment.

This study aimed to develop MTZ-thermosensitive hydrogels composed of the combination of PF127, MC, and SF, at various ratios and concentrations, for intraperiodontal pocket local drug administration. The hydrogels were prepared using the physical mixing method. The hydrogel gelation time was investigated by sol-gel transition at different temperatures. The hydrogel viscosity was determined at storage temperature to ensure that the hydrogel can be administered after long-term storage. Furthermore, the gel strength was investigated at 37˚C to determine its suitability for application to the oral cavity. Finally, the drug dissolution profiles were investigated in phosphate-buffered saline (PBS) at pH 6.6 to determine the effect of biocompatible polymer content on the drug release rates from hydrogels.

MATERIALS AND METHODS

Materials

Silk yarns of *B. mori* were obtained from Bodin Thai Silk Khorat Co., Ltd. (Khorat, Thailand). PF127 (MW: 12.500 g/mol) was purchased from BASF Corporation (Bangkok, Thailand). MC (M0512; viscosity: 4.000 cP, MW: 88.000 g/mol, and DS: 1.5-1.9) was obtained from Sigma-Aldrich (St. Louis, MO, USA). MTZ injection (5 mg/mL) was purchased from Utopan Co., Ltd. (Samutprakan, Thailand). Sterile water for injection was obtained from A.N.B. Laboratories Co., Ltd. (Bangkok, Thailand). Methanol [high-performance liquid chromatography (HPLC) grade] was purchased from Sigma-Aldrich (St. Louis, MO,

USA). Triethylamine [(TEA); DNA and peptide synthesis grade] was purchased from Loba Chemie Pvt. Ltd. (Mumbai, India). Potassium phosphate monobasic (KH_2PO_4) ReagentPlus® was purchased from Elago Enterprises Pty. Ltd. (Cherrybrook, NSW, Australia).

SF extraction

SF was extracted and characterized following a previous report.[15] Briefly, degummed silk cocoon (5 g) was cut into small pieces, added to a mixed solution of $CaCl_2/H_2O/Ca(NO_3)_2$/EtOH at a weight ratio of 30:45:5:20, and heated until a clear solution was obtained. To remove the residual salts, the SF solution was dialyzed against distilled water using a SnakeSkin pleated dialysis tube (10.000 MWCO) for 3 days. Then, the SF solution was centrifuged at 10.000 rpm, 4˚C, for 30 min to eliminate the silk aggregates. To obtain SF in a dry powder form, the SF solution was subjected to a freeze dryer (Heto PowerDry LL3000, Thermo Fisher Scientific, Waltham, MA, USA) at $1x10^{-4}$ $1x10^{-4}$ Torr and -55˚C. Finally, the lyophilized SF powder was preserved in a plastic bag at -20˚C.

Preparation of MTZ-thermosensitive hydrogel

The polymer solutions were separately prepared before hydrogel preparation. The SF solution was prepared by dissolving the freeze-dried SF in sterile water. The MC and PF127 solutions were prepared by separately dispersing the powders in water with gentle mixing, followed by storing them in a refrigerator until the solutions were clear.

The MTZ-thermosensitive hydrogel was prepared using the physical mixing method. MTZ was mixed with the SF solution under gentle stirring at room temperature for 10 min. Then, the MC and PF127 solutions were added to the mixture with gentle stirring for 5 min. The final volume was adjusted with sterile water and further stirred for 30 min. Hydrogels were prepared by varying the concentrations of SF and MC. Meanwhile, the concentrations of PF127 and MTZ were kept constant at 28% and 0.05% w/v, respectively (Table 1).

Table 1. Metronidazole-loaded thermosensitive hydrogel formulations with different amounts of silk fibroin and methylcellulose with 28% Pluronic F127 and 0.05% metronidazole

Formulation		MC (% w/v)	SF (% w/v)
PF/MC	PF/MC 0.25	0.25	-
	PF/MC 0.5	0.5	-
	PF/MC 0.75	0.75	-
PF/SF	PF/SF 0.25	-	0.25
	PF/SF 0.5	-	0.5
	PF/SF 0.75	-	0.75
PF/MC/SF	PF/MC/SF 0.25	0.25	0.25
	PF/MC/SF 0.5	0.25	0.5
	PF/MC/SF 0.75	0.25	0.75

PF: Pluronic F127, MC: Methylcellulose, SF: Silk fibroin

Determination of the sol-gel transition of MTZ-thermosensitive hydrogel

The vial inversion method was employed to determine the occurrence of sol-gel transition. Sol formation was observed as flowing liquid and gel formation was observed as a non-flowing gel when the vial was inverted. Then, 1 mL of the thermosensitive hydrogel solution was transferred to a sealed test tube, which was subsequently immersed in a water bath at 4˚C±0.5˚C, 25˚C±0.5˚C, and 37˚C±0.5˚C. The gelation time of thermosensitive hydrogel was determined as the initial time point that the solution did not move when the vial was inverted.

Viscosity test of MTZ-thermosensitive hydrogel

The viscosity of MTZ-thermosensitive hydrogel solution was measured at 4˚C±0.5˚C by a Brookfield rotational rheometer model DV-III (Brookfield Engineering Labs, Middleborough, MA, USA) fitted with a parallel plate configuration (40 mm in diameter) with a rotation rate of 6 rpm. The sample (i.e., 0.5 mL of the hydrogel) was placed on the parallel plate with a temperature controller and subjected to the viscosity test.

Gel strength test of MTZ-thermosensitive hydrogel

The gel strength of the samples was analyzed by a texture analyzer (TA.XT.Plus, Charpa Techcenter Co., Ltd., Bangkok, Thailand). Then, 1 mL of the hydrogel solution was placed in a 5-mL vial to prevent air from coming in contact with the samples and ensure the generation of a smooth upper surface. Subsequently, the hydrogel solution was incubated at 37˚C±0.5˚C for 15 min to form a hydrogel. The hemispherical probe P/0.5 HS (50 mm in diameter) was compressed into the hydrogel sample to a defined depth of 4 mm. Three replicate analyses were performed at 37˚C±0.5˚C for each formulation under the same conditions.

MTZ dissolution test and hydrogel erosion study

The drug dissolution profiles were evaluated by the *in vitro* dissolution method at 37˚C±0.5˚C. Then, 1 mL of the MTZ-thermosensitive hydrogel was transferred to a 10-mL vial and kept at 37˚C for 15 min to form a hydrogel. Subsequently, 1 mL PBS pH 6.6 was added to the vial as a dissolution medium (sink condition, as MTZ aqueous solubility is 10 mg/mL at pH 2.5-8) and the temperature was kept at 37˚C in an incubator with shaking at 50 rpm. At a predetermined time point, 0.5 mL of the sample was withdrawn and replaced immediately with the same amount of free medium pre-warmed to 37˚C±0.5˚C. The amount of MTZ in the sample was analyzed by HPLC, modified from Trivedi et al.[25] Briefly, the samples were diluted with a mobile phase (20 mM KH_2PO_4/methanol at a ratio of 70:30 (v/v) with TEA 0.1% v/v) and subjected to HPLC (Shimadzu, Tokyo, Japan) equipped with an SPD-20A ultraviolet-visible detector. The separation was done with a VertiSep C18 column (5 µm, 4.6 mm×250 mm; Sigma-Aldrich, St. Louis, MO, USA) at a flow rate of 1.0 mL/min and a detection wavelength of 364 nm. The standard curve was constructed in the range of 5-100 µg/mL with a regression equation of y: 10.706x-2.950.1 and a correlation coefficient (R^2) of 0.9997. The amount of MTZ was calculated based on the calibration curve, and the results were

reported as the average cumulative drug release percentage of three determinations.

To elucidate the drug release mechanism, the correlation coefficients (R^2) for various models, such as zero-order, first-order, and Higuchi models, were tested for all samples using Microsoft Excel 2018. The release model having the highest R^2 value was considered the fitted model to assess the drug release kinetics.

In terms of hydrogel erosion study, the dissolution study was conducted with the same experimental settings. At a predetermined time point, 0.5 mL of the sample was withdrawn and replaced with a fresh sample. The remaining hydrogels after erosion were collected and photographed.

Statistical analysis

Experiments were performed in triplicate, and the data were expressed as the mean ± standard deviation. Analysis of variance followed by Tukey's post hoc test was used to determine the differences between groups, with $p<0.05$ for statistically significant comparisons.

RESULTS AND DISCUSSION

Sol-gel transition time of MTZ-thermosensitive hydrogels

To determine the suitability for in situ application, as well as the storage conditions, of the formulations, the sol-gel transition tests were conducted at three different temperatures, namely, the storage temperature of 4°C, room temperature of 25°C, and application periodontal pocket site temperature of 37°C.

In our preliminary study, by varying the PF127 concentrations from 10% to 30% w/v, we observed that the 28% PF127+0.05% MTZ solution transformed into a hydrogel within 1 min at 37°C. At a lower PF127 concentration of 18%, only the blank PF127 solution transformed into a hydrogel, whereas the PF127+0.05% MTZ solution remained in the solution form. This finding indicates that PF127 and the drug MTZ might interact via non-covalent bonds, consequently disrupting the hydrophobic interaction during the PF127 gelation process and resulting in the increased sol-gel transition temperature of PF127.[26] Therefore, 28% PF127 was selected for further studies. However, the PF127 hydrogel had low mechanical properties, which might result in hydrogel leaking from the periodontal pocket after administration, as well as rapid drug release. Therefore, to increase the mechanical properties of PF127 hydrogels, MC and SF were used as gel strength enhancers.

To this end, three groups of formulations, namely, PF/MC, PF/SF, and PF/MC/SF, at three different concentrations (i.e., 0.25%, 0.5%, and 0.75% w/v) of the enhancer were prepared (Table 1). Then, the gelation time at 4°C (storage temperature), 25°C (room temperature), and 37°C (body temperature) was determined (Table 2). At 4°C, all samples remained in low-viscosity liquid form for at least 6 months, indicating their stability for long-term storage. At 25°C, the gel was formed after at least 15 min, allowing adequate time for periodontal administration. After being administered to the site of action, at 37°C, the solutions formed a gel almost immediately within less than 1 min (Figure 1), which further stick to the dental cavity for a longer time. Thus, the drug could perform its action without being washed down to the gastrointestinal tract, consequently enhancing its efficacy and reducing the systemic side effects. Ultimately, the thermosensitive hydrogel should be stored at 4°C (solution form) and quickly applied (i.e., not more than 15 min) to the periodontal pocket site after retrieving it from the fridge.

The mechanism of sol-gel transition of thermosensitive hydrogel is shown in Figure 2. First, at a concentration higher than the PF127 critical micelle concentration [(CMC); 1-7 g/L][27], the polymer formed polymeric micelles consisting of a PPO hydrophobic core enclosed by hydrophilic PEO blocks. MTZ mostly resided in the PPO hydrophobic core. When the temperature increased to a higher value (37°C in our case) than the PF127 lower critical solution temperature, the polymer solubility decreases through partial dehydration, leading to the decrease in PF127 CMC (0.09 g/L at 37°C). Consequently, more micelles were formed, leading to the formation of a packed micellar structure because of enhanced particle contact and, ultimately, the formation of a gel. The MC and SF molecules could entangle with the hydrophilic PEO shell of the PF127 micelles

Table 2. Sol-gel transition time of thermosensitive hydrogels at 4°C, 25°C, and 37°C. The results are expressed as the mean ± standard deviation (n=3). The time unit at 25°C and 37°C is minute and second, respectively

Formulation[a,c]	Gelation time		
	4°C[b]	25°C (min)	37°C (s)
PF/MC 0.25	N/A	17±1	33±2
PF/MC 0.5	N/A	39±2	48±3
PF/MC 0.75	N/A	>120	61±3
PF/SF 0.25	N/A	26±1	35±2
PF/SF 0.5	N/A	20±2	28±2
PF/SF 0.75	N/A	16±1	25±2
PF/MC/SF 0.25	N/A	19±1	35±2
PF/MC/SF 0.5	N/A	28±2	30±2
PF/MC/SF 0.75	N/A	40±2	29±1

[a]Thermosensitive hydrogel composed of 28% PF127 and 0.05% w/v MTZ, [b]N/A: Could not form a hydrogel for at least 6 months, [c]For PF/MC/SF formulations, the MC concentration was fixed at 0.25% w/v. PF: Pluronic F127, MC: Methylcellulose, SF: Silk fibroin, MTZ: Metronidazole

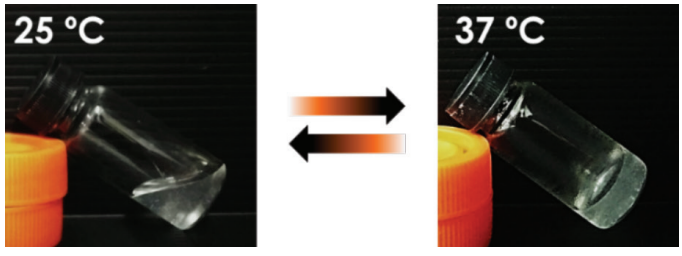

Figure 1. Sol-gel transition images of metronidazole-thermosensitive hydrogel composed of silk fibroin, methylcellulose, and Pluronic F127 by vial inversion method at 25°C and 37°C

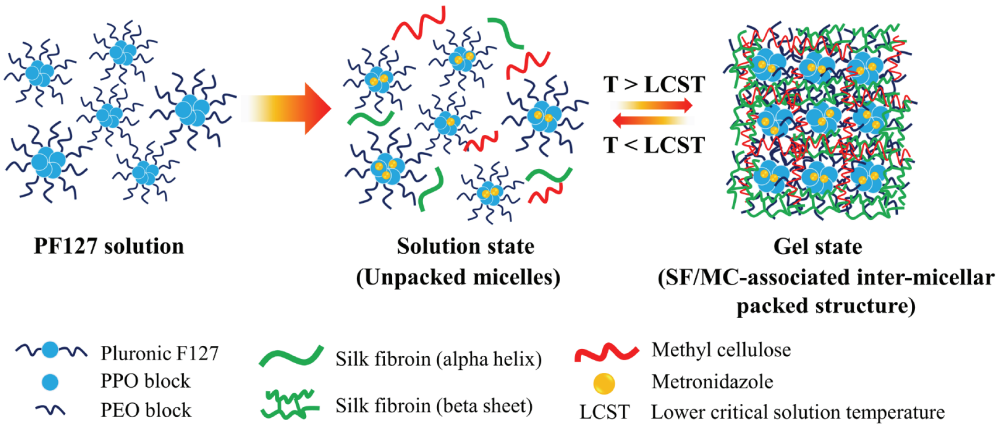

Figure 2. Mechanism of the formation of a packed micellar structure of MTZ-thermosensitive hydrogel

MTZ: Metronidazole, PPP: Poly propylene oxide, PEO: Poly ethylene oxide, PF127: PF: Pluronic F127, MC: Methylcellulose, SF: Silk fibroin, LCST: Lower critical solution temperature

by intercellular packing, thus leading to the enhancement of the gel properties.[10]

Viscosity of MTZ-thermosensitive hydrogel

The viscosity of all hydrogels in solution form was determined at 4˚C to ensure that the hydrogels can be applied to the periodontal infection site (Figure 3). All hydrogel formulations had a viscosity of less than 500 cP, indicating a low viscosity, which ensures easy administration to the lesion site. Moreover, the MC content could influence the viscosity of the hydrogel. When the MC content increased from 0.25% to 0.75% w/v, the viscosity of PF/MC dramatically increased from 158.00 cP to 476.17 cP. Meanwhile, with an increase in SF content, both PF/SF and PF/MC/SF hydrogels showed no observable changes in viscosity. This finding can be explained by the inherent hydrophilicity of MC and SF. MC can form hydrogen bondings with water and PF127, consequently increasing the gel connecting networks and resulting in enhanced viscosity. By contrast, less hydrophilic SF might have limited interactions with the polymer compared with MC, thus not affecting the gel viscosity.

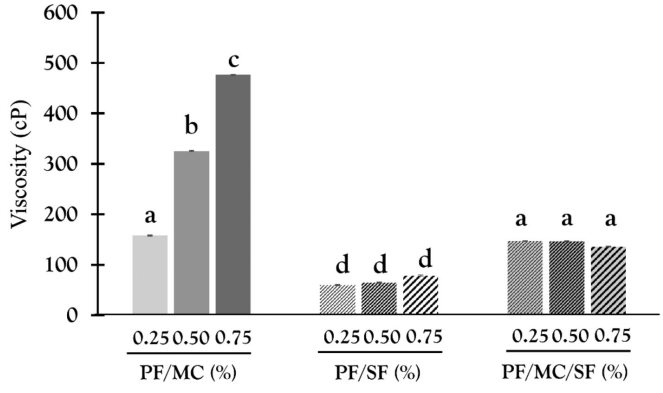

Figure 3. Viscosity of MTZ-thermosensitive hydrogel at 4˚C. The data are expressed as the mean ± standard deviation (n=3). Different letters (a, b, c, d) denote the statistical differences, $p<0.05$

MTZ: Metronidazole, PF: Pluronic F127, MC: Methylcellulose, SF: Silk fibroin

Gel strength of MTZ-thermosensitive hydrogel

The gel strength of all hydrogels was determined at 37˚C, and the results are shown in Figure 4. When increasing the MC concentration from 0.25% to 0.75%, the PF/MC hydrogel gel strength proportionally decreased from 2,234.97 g to 1,143.70 g. By contrast, the PF/SF hydrogel gel strength slightly increased from 1,809.20 g to 2,196.17 g when the SF concentration increased from 0.25% to 0.75%. Thus, the hydrophobic SF possibly enhanced the mechanical strength of the hydrogels by forming a rigid β-sheet, which is induced by heat at 37˚C. Meanwhile, the hydrophilic MC increased hydrogel flexibility by forming hydrogen bondings with water and hydrophilic PEO shell of polymeric micelles.

Notably, when combining both MC and SF in the formulations, the gel strength of PF/MC/SF decreased slightly with the increase in SF concentrations. This phenomenon can be explained by the interactions between MC and SF, as MC could swell and coat the SF surfaces, altering its properties. Nevertheless, more experiments are needed to explain this issue.

Drug dissolution study and erosion behavior of MTZ-thermosensitive hydrogels

To mimic the conditions of periodontal infection, a sink condition with PBS at pH 6.6 and 37˚C was selected to be the medium for analyzing the dissolution behavior of MTZ-thermosensitive hydrogels. Notably, all PF/SF formulations were dissolved rapidly in the dissolution medium, and all PF/MC formulations were not strong/rigid enough for the experiments. Thus, both MC and SF are necessary to improve the gel properties, as MC enhances gel viscosity and SF increases gel strength. Figure 5A shows the cumulative release percentages of MTZ over time from PF/MC/SF formulations at different SF concentrations of 0.25%, 0.5%, and 0.75% w/v. With no significant differences, all formulations showed a sustained dissolution profile of MTZ up to 80% within 10 days. Furthermore, these dissolution data were fitted nicely with the Higuchi model (Table 3), with R^2 values of >0.98, indicating that the drug was released via the diffusion-controlled mechanism.

As expected, hydrogel erosion in the dissolution medium was observed. As shown in Figure 5B, the hydrogels were dissolved sustainably, with a remaining weight of approximately 40% after 9 days of the dissolution study (data not showed). This finding can be attributed to the hydrophilicity of both PF127 and MC, which are subjected to dissolution in an aqueous medium.[28,29] Because the drug MTZ mostly resided in the PPO hydrophobic core of PF127 micelles, its diffusion-controlled release profile is governed by the dissolution of PF127 and/or MC.[28] Therefore, this fact benefits periodontitis treatment, as the systems release a high initial dose in the first day (approximately 30%) that is adequate for fast action, followed by a decreasing release rate over the next 9 days that is suitable for maintaining the drug efficacy without the need for readministration.

Table 3. Kinetic release modeling of the metronidazole-thermosensitive hydrogels

Formulation[a]	R^2 value		
	Zero-order model	First-order model	Higuchi model
PF/MC/SF 0.25	0.7139	-0.5380	0.9872
PF/MC/SF 0.5	0.7331	-0.5090	0.9865
PF/MC/SF 0.75	0.7012	-0.5420	0.9811

[a]Thermosensitive hydrogel consisted of 28% PF127, 0.05% w/v MTZ, and 0.25% w/v MC. PF: Pluronic F127, MC: Methylcellulose, SF: Silk fibroin, MTZ: Metronidazole

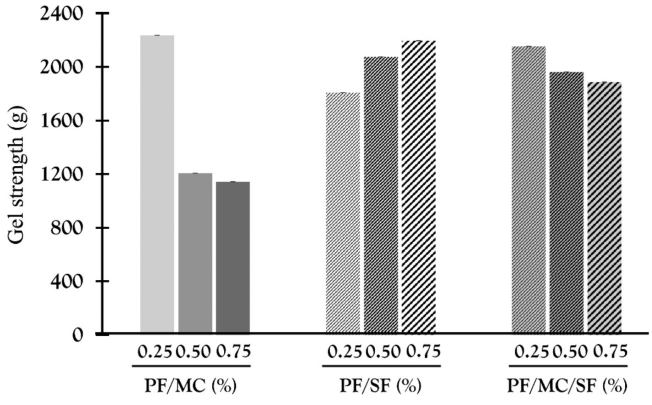

Figure 4. Gel strength of MTZ-thermosensitive hydrogel at 37°C. The data are expressed as the mean ± standard deviation (n=3). All data are significantly different from each other ($p<0.05$)

MTZ: Metronidazole, PF: Pluronic F127, MC: Methylcellulose, SF: Silk fibroin

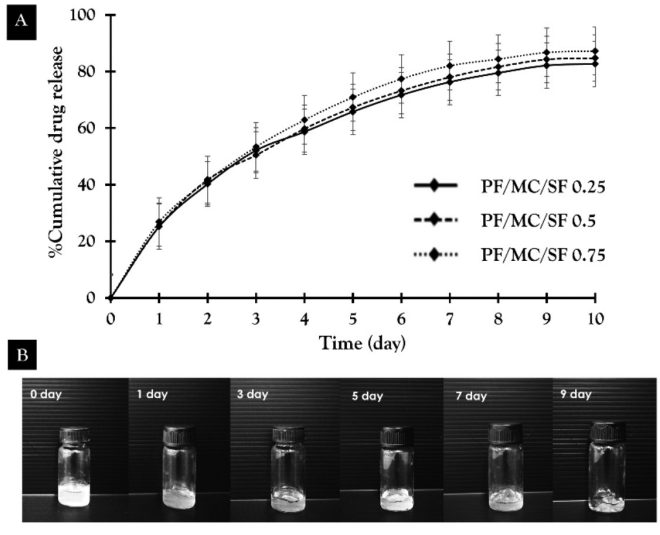

Figure 5. (A) Cumulative release of MTZ from thermosensitive hydrogel in phosphate-buffered saline (PBS) pH 6.6 within 10 days and (B) erosion of MTZ-thermosensitive hydrogel (PF/MC/SF 0.75) in PBS pH 6.6 within 9 days. The data are expressed as the mean ± standard deviation (n=3)

MTZ: Metronidazole, PF: Pluronic F127, MC: Methylcellulose, SF: Silk fibroin

MTZ is commonly used to inhibit infection from anaerobic microorganisms, such as *Porphyromonas gingivalis*. The minimal inhibitory concentration (MIC) of MTZ against this bacteria isolated is in the range of 0.063-0.514 µg/mL.[30,31] From our data, the amount of MTZ release in the first time point (the first day) was approximately 120 µg/mL for all formulations, which was more than 200 times higher than the MIC of MTZ against *P. gingivalis*. Moreover, the amount of MTZ release on the ninth day was approximately 13 µg/mL, which was still more than 20 times higher than the required MIC. Thus, the release amount of the drug reached the therapeutic concentration for at least 9 days

CONCLUSION

This study successfully developed and characterized the novel MTZ-loaded PF/MC/SF thermosensitive hydrogels as in situ drug delivery systems for periodontitis treatment. The hydrogels remained in low-viscosity solution form for at least 6 months at 4°C (storage temperature) and rapidly formed a hydrogel at 37°C within 1 min after injection into the dental pocket. SF significantly enhanced the gel strength, whereas MC increased the gel viscosity. Furthermore, the hydrogels exhibited sustained MTZ release for 10 days, which reduces administration frequency. The drug dissolution profile of PF/MC/SF was governed by two different mechanisms, namely, hydrogel erosion by fluids at the administration site (periodontal pocket) and diffusion of the drug through the hydrogels. In conclusion, these novel thermosensitive hydrogels have considerable potential to control drug release in the periodontal pocket, which might improve its pharmacological properties. Thus, these novel thermosensitive hydrogels could be further investigated *in vitro* and *in vivo* for application to clinical treatment in the future.

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