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DEVELOPMENT OF A LIPOSOME-BASED IN SITU GEL FOR SUSTAINED RELEASE OF METRONIDAZOLE IN PERIODONTAL THERAPY

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ABSTRACT

Periodontitis is a chronic inflammatory disease characterized by bacterial infection and progressive destruction of the periodontal tissues. Conventional systemic therapy with metronidazole is often associated with limited bioavailability at the infection site and potential systemic side effects. The present study aimed to develop and characterize an innovative liposome-based in situ gel formulation for localized and sustained delivery of metronidazole in periodontal therapy. Metronidazole-loaded liposomes were prepared using thin-film hydration and optimized for particle size, encapsulation efficiency, and stability. The optimized liposomal formulation was then incorporated into a thermosensitive in situ gel matrix designed to undergo sol–gel transition at physiological temperature. The prepared in situ gel was evaluated for pH, viscosity, gelation temperature, drug release kinetics, and antimicrobial activity against Porphyromonas gingivalis and Prevotella intermedia. Results demonstrated high encapsulation efficiency, uniform nanosized liposomes, and sustained drug release over an extended period compared to conventional formulations. The liposomal in situ gel exhibited excellent mucoadhesive strength and significant antimicrobial activity, confirming its potential for site-specific drug delivery in periodontal pockets. This novel formulation provides a promising strategy to improve therapeutic outcomes, minimize systemic exposure, and enhance patient compliance in periodontitis management.

I. 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

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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°C9 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 longterm 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.

2. 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 (KH2 PO4) 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 CaCl2 /H2 O/Ca(NO3) 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 nonflowing 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.

3. 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 lowviscosity 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,b]	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

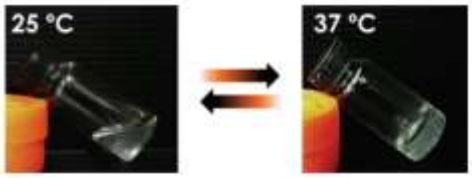

Figure 1. Sol-gel transition images of metronidazole-thermosensitive hydrogel composed of silk fibroin, methylcellulose, and Pluronic F127 by vial inversion method at 25°C and 37°C

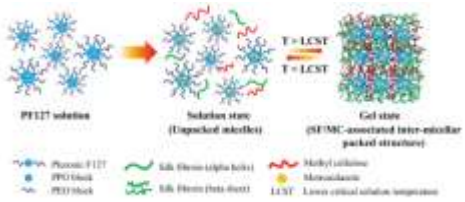

Figure 2. Mechanism of the formation of a packed micellar structure of MTZ-thermosensitive hydrogel MTZ: Metronidazole, PPP: Poly propylene oxide, PEO: Poly ethylene oxide, PF127: PF: Pluronic F127, MC: Methylcellulose, SF: Silk fibroin, LCST: Lower critical solution temperature

4. CONCLUSION

The developed liposome-based in situ gel formulation of metronidazole successfully demonstrated its potential as a localized drug delivery system for periodontitis. Incorporation of liposomes into the in situ gel matrix enabled controlled and sustained drug release, superior antimicrobial efficacy, and improved retention in the periodontal pocket. The formulation also exhibited favorable physicochemical properties, including appropriate gelation temperature, viscosity, and mucoadhesive strength, making it suitable for clinical application. By enhancing drug concentration at the infection site and reducing systemic side effects, this approach offers a significant advantage over conventional periodontal therapies. Future in vivo and clinical studies are warranted to establish its therapeutic efficacy and long-term safety, ultimately contributing to the advancement of targeted periodontal drug delivery systems.

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