

Development of metronidazole-loaded thermosensitive hydrogel for local treatment of periodontitis

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ABSTRACT

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Periodontitis, a gum disease caused by bacterial infection and plaque accumulation, leads to tooth loosening and loss if untreated. In this study, thermoresponsive hydrogels for periodontal drug delivery were developed, using poloxamer 407 (PX) at 10–25% (w/w), prepared via the cold method. Polymer blends incorporating PX with methylcellulose (MC), poly(acrylic acid) (PAA), and chitosan (CS), with and without metronidazole (MT) as a model drug, were formulated and evaluated. The hydrogels exhibited gelation below 20°C and transitioned into a gel within 2 min at 37°C. Rheological analysis showed that polymer addition increased viscosity, ranked as PAA > MC > CS > PX alone, thereby influencing extrudability. The pH ranged from 6 to 8, except for the CS formulations (pH 3–4). Higher polymer concentrations and blends reduced gel dissolution in phosphate buffer (pH 7.4) and water. Drug release studies indicated that 15% PX released MT faster than 20% or 25% PX, while PAA and CS blends provided sustained release. These hydrogels remain in solution at low temperatures, are easily injectable, and transform into a gel when administered into the periodontal pocket, demonstrating potential as localized drug delivery systems for periodontitis treatment.

Keywords: periodontitis; drug delivery systems; metronidazole; thermosensitive hydrogel; poloxamer 407

1. INTRODUCTION

Periodontitis is an inflammatory gum disease caused by the accumulation of bacterial plaque, leading to the destruction of tooth-supporting structures, including the periodontal ligament and alveolar bone (Kwon et al., 2021). The progression of the disease can ultimately result in tooth loss. The primary causative bacteria are *Aggregatibacter actinomycetemcomitans* and *Porphyromonas gingivalis*, while additional risk factors include gender, smoking, alcohol consumption, diabetes, and genetic predisposition (Casarin et al., 2010; Li et al., 2024). According to the 8th National Oral Health Survey of Thailand in 2017, periodontitis is most prevalent among

individuals aged 60–74 years, with a reported prevalence of 36.3%. Among these cases, 12.2% exhibited severe periodontitis, characterized by periodontal pockets deeper than 6 mm (Piyakhunakorn & Sermsuti-anuwat, 2021).

The current treatment for periodontitis primarily involves the elimination of local contributing factors, beginning with the removal of bacterial plaque and calculus, which serve as reservoirs for pathogenic bacteria (Kwon et al., 2021). This is accompanied by patient education on proper oral hygiene practices. Once the patient achieves adequate oral hygiene control, adjunctive antibiotic therapy is introduced to further reduce the bacterial load, with amoxicillin and metronidazole being the most commonly prescribed antibiotics (Sgolastra

et al., 2012). Additionally, chlorhexidine gluconate 0.2% mouthwash is widely used as an adjunctive therapy, requiring continuous use for at least seven days (Christie et al., 1998). However, these adjunctive treatments, including antibiotic administration and mouth rinsing, require frequent application and may be limited in maintaining sustained drug activity at the target site. Therefore, the development of drug delivery systems, such as hydrogels, has been proposed to overcome these limitations and enhance treatment efficacy.

Previous research into hydrogel for oral drug delivery has shown promising results; for instance, the formulation and characterization of a thymol-chitosan hydrogel demonstrated that a loading of 0.57 ± 0.03 mg of thymol exhibited significant antioxidant activity within the oral cavity (Alvarez Echazú et al., 2017). In another study, a metronidazole-loaded gel demonstrated rapid absorption, reaching peak drug concentration within 15 min, followed by a sharp decline within 6 h. Minimal drug levels were detected at 24 and 48 h post-administration, indicating a short retention time in the gingival crevicular fluid (Sato et al., 2008). Additionally, a chitosan-based hydrogel with dental pulp stem cell-derived exosomes (DOSC-Exo/CS) showed potential for periodontitis treatment by mitigating inflammation and regulating immune responses, contributing to improved periodontal health (Shen et al., 2020). Collectively, these studies suggest that hydrogel-based drug delivery systems hold promise for further development as potential therapeutic strategies for periodontitis treatment.

A hydrogel is a polymeric network primarily composed of hydrophilic components. It consists of cross-linked macromolecules that can absorb water, hydrophilic solutions, or bodily fluids. However, instead of dissolving, the hydrogel swells to form a three-dimensional structure, stabilized by various types of cross-linking bonds (Peppas et al., 2012). Poloxamer 407 (PX) is a non-ionic triblock copolymer composed of poly(ethylene oxide)-poly(propylene oxide)-poly(ethylene oxide) (PEO-PPO-PEO) subunits. The hydrophobic poly(propylene oxide) (PPO) core is flanked by hydrophilic poly(ethylene oxide) (PEO) segments, resulting in amphiphilic characteristics. PX is among the most widely utilized copolymers due to its low toxicity, excellent compatibility with various chemical compounds, and outstanding thermoreversible gel-forming properties (Sato et al., 2008; Urbán-Morlán et al., 2008).

Methylcellulose (MC) is a methyl ether derivative of cellulose that is water-soluble and exhibits thermal gelation in aqueous solutions. It is widely used due to its chemical inertness and excellent biocompatibility. These properties make MC hydrogels particularly attractive for applications such as cell culture, as they can form robust scaffolds. Compared to other polysaccharides, MC demonstrates a thermoreversible gelation, transitioning back to a liquid state upon heating. Additionally, MC has been incorporated primarily to enhance the mechanical strength of the hydrogel, while PX remained the main gelling agent (Brady et al., 2017; Teoh et al., 2022).

Poly(acrylic acid) (PAA) is an anionic polymer whose side chains ionize at neutral pH, enabling significant water absorption, often several times its weight. This high water-binding capacity is attributed to the carboxyl groups along the polymer backbone, which strongly interact with water molecules. These functional groups readily dissociate and exhibit sensitivity to pH and ionic strength, influencing the polymer's swelling and gelation behavior (Elliott et al.,

2004; Pashirova et al., 2023). Chitosan (CS) is a naturally derived polysaccharide with notable physicochemical properties, including high biocompatibility, non-toxicity, biodegradability, and pH sensitivity. Under alkaline conditions, CS undergoes deprotonation, leading to hydrogel formation, whereas in acidic environments, it remains soluble due to the protonation of its amino groups (Lee et al., 2023).

In this study, a thermosensitive hydrogel formulation incorporating an antibiotic was developed for localized periodontal treatment. The hydrogel was formulated using PX in combination with various polymeric excipients, including MC, carbopol (a PAA derivative), and CS, to encapsulate metronidazole. The formulation was systematically evaluated for gelation behavior at different temperatures, dissolution characteristics, pH stability, injectability through an 18-gauge needle, gelation time at 37°C, and metronidazole release profiles. The objective of this approach is to enhance the efficacy of localized periodontal therapy by addressing formulation limitations and reducing the frequency of drug administration.

2. MATERIALS AND METHODS

2.1 Materials

Metronidazole (MT) and methylcellulose 4000 (MC) were donated by Pharmasant Laboratories Co. Ltd., Nonthaburi, Thailand and Colorcon Company, Mumbai, India, respectively. Poloxamer 407 (PX), carbopol 940 (PAA), chitosan (CS, degree of deacetylation $\geq 90\%$), and glacial acetic acid were purchased from Chanjao Longevity Co., Ltd., Bangkok, Thailand, Anhui Newman Fine Chemicals Co., Ltd., Anhui, China, Qingdao BNP BioScience Co., Ltd., Qingdao, China, RCI Labscan, Bangkok, Thailand, respectively. In addition, triethanolamine and disodium hydrogen orthophosphate were obtained from AnalaR®, Bang Trading Co., Ltd., Bangkok, Thailand. Potassium dihydrogen orthophosphate was purchased from UNIVAR (Ajax Finechem, Australia).

2.2 Preparation of thermosensitive hydrogel

Thermosensitive hydrogel formulations were prepared using the compositions shown in Table 1. PX solutions were prepared using the cold method, as described previously (Sherif et al., 2014). In brief, PX at concentrations of 10%, 15%, 20%, and 25% (w/w) (designated as 10PX, 15PX, 20PX, and 25PX, respectively) were formulated by dispersing 5.0, 7.5, 10.0, and 12.5 g of PX in 25 mL of cold water, ensuring complete wetting of the powder. The dispersions were then diluted with additional cold water to obtain a final sample weight of 50 g.

Mixtures of 20PX with 0.5% and 1.0% (w/w) MC, referred to as 20PX/0.50MC and 20PX/1.00MC, respectively, were prepared by dispersing 10 g of PX into 25 mL of cold water containing 0.25 or 0.50 g of dissolved MC. The resulting dispersions were subsequently diluted with cold water to achieve a final weight of 50 g. For the preparation of 20PX/0.05PAA and 20PX/0.10PAA, 10 g of PX was combined with 25 mL of cold water in which 0.025 or 0.050 g of PAA had been dissolved. The mixtures were then subsequently adjusted with cold water to reach a final weight of 50 g. In the case of 20PX/0.50CS and 20PX/1.00CS, 0.25 and 0.50 g of CS were first dissolved in 25 mL of 4% glacial acetic acid. PX (10 g) was then incorporated, followed by the addition of 4% glacial acetic acid to achieve a final weight of 50 g.

For MT-loaded formulations (MT-thermosensitive hydrogel), MT was first dissolved either in water or 4% glacial acetic acid prior to the addition of the gelling agents. The resulting formulations included 15PX/MT, 20PX/MT,

25PX/MT, 20PX/0.50MC/MT, 20PX/1.00MC/MT, 20PX/0.05PAA/MT, 20PX/0.10PAA/MT, 20PX/0.50CS/MT, and 20PX/1.00CS/MT.

Table 1. Composition and sol-gel transition of thermosensitive hydrogel

Formulations	Amount (% w/w)					Temperature (°C)	
	PX	MC	PAA	CS	MT	Solution (Sol)	Gel
10PX	10.00	-	-	-	-	N/A	N/A
15PX	15.00	-	-	-	-	30 ± 1	31 ± 1
20PX	20.00	-	-	-	-	21 ± 1	22 ± 1
25PX	25.00	-	-	-	-	16 ± 1	17 ± 1
15PX/MT	15.00	-	-	-	1.60	32 ± 1	33 ± 1
20PX/MT	20.00	-	-	-	1.60	21 ± 1	22 ± 1
25PX/MT	25.00	-	-	-	1.60	17 ± 1	18 ± 1
20PX/0.50MC	20.00	0.50	-	-	-	20 ± 1	21 ± 1
20PX/1.00MC	20.00	1.00	-	-	-	22 ± 1	23 ± 1
20PX/0.50MC/MT	20.00	0.50	-	-	1.60	20 ± 1	21 ± 1
20PX/1.00MC/MT	20.00	1.00	-	-	1.60	21 ± 1	22 ± 1
20PX/0.05PAA	20.00	-	0.05	-	-	20 ± 1	21 ± 1
20PX/0.10PAA	20.00	-	0.10	-	-	22 ± 1	23 ± 1
20PX/0.05PAA/MT	20.00	-	0.05	-	1.60	20 ± 1	21 ± 1
20PX/0.10PAA/MT	20.00	-	0.10	-	1.60	20 ± 1	21 ± 1
20PX/0.50CS	20.00	-	-	0.50	-	22 ± 1	23 ± 1
20PX/1.00CS	20.00	-	-	1.00	-	22 ± 1	23 ± 1
20PX/0.50CS/MT	20.00	-	-	0.50	1.60	22 ± 1	23 ± 1
20PX/1.00CS/MT	20.00	-	-	1.00	1.60	22 ± 1	23 ± 1

N/A: Unable to form a hydrogel within the temperature range of 15–45°C

2.3 Determination of the sol-gel transition

To evaluate the gelation temperature of thermosensitive gels, the tube inversion method was employed (Cai et al., 2012; Xie et al., 2013). Each liquid sample (2 mL) was placed in a sealed test tube and submerged in a water bath. The samples were maintained at each temperature for 5 min to reach equilibrium before assessment. The gelation temperature was identified as the temperature at which the sample remained stationary upon tube inversion. The measurements were conducted in 1°C increments. Additionally, the gelation time at 37°C, representing physiological conditions, was determined using the same approach. All experiments were performed in triplicate to ensure accuracy and reliability.

2.4 Determination of the viscosity

The rheological properties of the thermosensitive hydrogels were assessed using a rheometer (Brookfield Ametek®, Brookfield Engineering Labs Inc., Stoughton, USA) equipped with a cone-and-plate configuration. To investigate the thermal reversibility of the hydrogels, viscosity measurements were conducted through cyclic temperature sweeps ranging from 20 to 50°C for three cycles at 40 rpm. All experiments were performed in triplicate.

2.5 Determination of hydrogel erosion

The erosion of the hydrogel was assessed using the gravimetric method, as previously described (Li et al., 2014). Before use, an empty vial was weighed to record its initial weight. Then, 2 mL of the sample was added to the

vial, and the total weight, including the sample, was measured. The sample was allowed to undergo gelation at 37°C, after which 2 mL of pre-warmed phosphate buffer solution (37°C) was carefully added on top of the gel, and the vial was incubated at 37°C for 5 min. At predetermined intervals (after 5, 10, and 20 washing cycles), the buffer solution was removed, and the vial containing the remaining gel was weighed. Following each measurement, the buffer solution was replaced with fresh medium. The percentage of the remaining gel was calculated using Equation 1. All experiments were performed in triplicate.

$$\frac{(W_t - W_v) \times 100}{(W_i - W_v)} \quad (1)$$

where W_t is the weight of the vial containing the initial gel, W_i is the weight of the vial containing the remaining gel, and W_v is the weight of the empty vial.

2.6 Determination of pH

The pH of the thermosensitive hydrogels was measured using a pH meter (SevenCompact Duo, S213, P.P. Chemical and Holding Co., Ltd., Phitsanulok, Thailand).

2.7 Extrudability study

The thermosensitive hydrogels were loaded into 2 mL syringes and left at room temperature (20–25°C) for 2 h. The hydrogel was then extruded through an 18-gauge needle under applied pressure, and the flow behavior was recorded. This procedure assessed the ease of drawing and extruding the liquid through a needle.

2.8 *In vitro* release study of MT-loaded hydrogels

The release of MT from the MT-thermosensitive hydrogel was evaluated *in vitro* using the direct contact method, also known as the membrane-less diffusion approach (Boonlai et al., 2018). This technique allows the release medium to be in direct contact with the sample, making it a suitable model for simulating the *in vivo* environment of the periodontal pocket (Zhang et al., 2022). It offers the advantage of identifying formulation influences while ensuring direct interaction between the gel and the release medium. A phosphate buffer (pH 7.4) served as the release medium. All *in vitro* release studies were conducted in a water bath incubator maintained at 37°C. Each sample (1 g) was placed in a flat-bottomed vial and allowed to undergo gelation before 8 mL of pre-warmed release medium (37°C) was carefully added over its surface. At predetermined time points (15, 30, 60, 120, 240, 360, 480, and 1,440 min), 2 mL of the release medium (37°C) was withdrawn and replaced with an equal volume of fresh medium. The concentration of MT in the collected samples was determined using a UV-visible spectrophotometer (Shimadzu, UV-1800, Bara Scientific Co., Ltd., Bangkok, Thailand) at a wavelength of 320 nm. All experiments were conducted in triplicate.

2.9 Statistical analysis

Results are presented as the mean $\pm$ standard deviation (SD). Statistical analysis was performed using one-way analysis of variance (ANOVA) followed by Tukey's HSD post-hoc test ($p < 0.05$) using SPSS software version 17.0.

3. RESULTS AND DISCUSSION

3.1 Preparation of thermosensitive hydrogel

The thermosensitive hydrogel exhibited a clear physical appearance with no turbidity or phase separation in any of the samples.

3.2 Determination of the sol-gel transition of thermosensitive hydrogel

In the gelation temperature test using the tube inversion method, the gelation point is defined as the temperature at which the solution (sol) transforms into a gel. This is determined by inverting the test tube. If no flow is observed, the formulation is considered to have formed a gel at that temperature. The sol-gel transition occurs over a narrow temperature range (within approximately 1°C). This small gap between the sol and gel transition temperatures makes the formulation reliable and suitable for periodontitis treatment, as it transforms into a gel at the higher temperature within the periodontal pocket and remains there due to the cavity temperature being significantly higher than the sol temperature. For the single polymer formulations, it was found that 10PX did not form

a gel within the tested temperature range of 15–45°C (Table 1). However, in formulations containing other concentrations of PX, an increase in concentration led to a decrease in gelation temperature. At a concentration of 15PX, the solution formed a gel at 31°C, 20PX at 22°C, and 25PX at 17°C. This occurs because a higher concentration results in more polymer molecules, which facilitates the formation of complex molecular structures, thereby lowering the temperature required for gelation (Brambilla et al., 2022; Fakhari et al., 2017). When metronidazole was incorporated into the single polymer formulations (15PX/MT, 20PX/MT, and 25PX/MT), it was observed that the gelation temperature increased in the 15PX/MT formulation (from 31°C to 33°C) and in the 20PX/MT formulation (from 17°C to 18°C). However, in the 25PX/MT formulation, no change in gelation temperature was observed. In the case of gel formation in mixed polymer formulations, 20PX produced a quick gelation time while exhibiting relatively low viscosity. Therefore, 20PX was selected as the base formulation for blending with other polymers. All subsequent formulations formed a gel within the temperature range of 20–23°C. A similar gelation behavior was observed when metronidazole was incorporated into the mixed polymer formulations.

In summary, the sol-gel transition temperatures of all formulations, except for 10PX, were within the range of 17–33°C. This indicates that, when administered into the periodontal pocket, these formulations are expected to undergo a reliable sol-gel transition, as the local temperature within the oral cavity typically ranges from 33.2°C to 38.2°C, according to studies with strong or moderately strong evidence. Specifically, reported oral temperature ranges are 35.7–37.7°C in males and 33.2–38.1°C in females, thereby supporting the clinical applicability of these thermosensitive hydrogels (Sund-Levander et al., 2002). As well, the temperature within periodontal pockets has been reported to be significantly higher in the presence of major periodontal pathogens, further supporting the potential effectiveness of these formulations under clinical conditions (Rams & Slots, 2024).

Based on the gelation time test at 37°C for both single polymer and mixed polymer solutions, it was found that all formulations gelled in less than 2 min (Table 2). The gelation time ranged from 10 to 90 s. Notably, the PX10 formulation failed to form a gel even after 5 min at 37°C, indicating insufficient polymer concentration for thermogelation. The gelation time was inversely related to the concentration of PX, with higher concentrations resulting in more rapid gel formation. Additionally, the type and amount of secondary polymers influenced gelation behavior. For example, formulations containing higher concentrations of polyacrylic acid (PAA) exhibited significantly faster gelation.

Table 2. Sol-gel transition time, pH, and extrudability of thermosensitive hydrogel

Formulations	Gelation time at 37°C (s)	pH	Extrudability
10PX	> 300	6.89	Free flow
15PX	32.47	7.09	Free flow
20PX	17.72	7.15	Free flow
25PX	9.09	7.26	Free flow
15PX/MT	26.62	7.07	Free flow

Table 2. Sol-gel transition time, pH, and extrudability of thermosensitive hydrogel (continued)

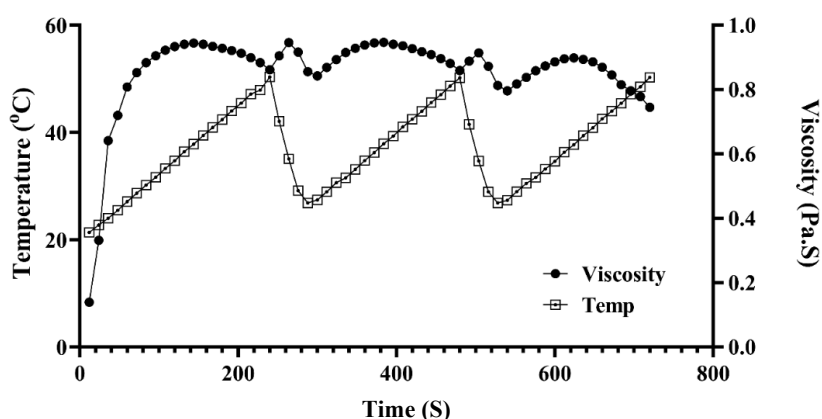
Formulations	Gelation time at 37°C (s)	pH	Extrudability
20PX/MT	27.41	7.11	Free flow
25PX/MT	12.84	7.94	Free flow
20PX/0.50MC	8.02	8.04	Free flow
20PX/1.00MC	22.94	3.54	Free flow
20PX/0.50MC/MT	18.92	3.88	Free flow
20PX/1.00MC/MT	84.5	7.18	Free flow
20PX/0.05PAA	21.88	7.25	Free flow
20PX/0.10PAA	17.71	7.33	Free flow
20PX/0.05PAA/MT	26.79	7.18	Free flow
20PX/0.10PAA/MT	23.85	7.31	Free flow
20PX/0.50CS	21.56	8.27	Free flow
20PX/1.00CS	21.57	8.61	Free flow
20PX/0.50CS/MT	23.55	3.58	Free flow
20PX/1.00CS/MT	31.19	3.96	Free flow

3.3 Determination of the viscosity of thermosensitive hydrogel

As illustrated in Figure 1, the viscosity of the hydrogel increased with rising temperature until it reached a peak, after which a gradual decline was observed. This thermoresponsive behavior is attributed to the gelation properties of PX, a synthetic triblock copolymer composed of poly(ethylene oxide) (PEO) blocks and a poly(propylene oxide) (PPO) block arranged in a PEO-PPO-PEO configuration (White et al., 2023; Zeqiri et al., 2024). Due to its amphiphilic nature, PX exhibits thermoreversible sol-gel transitions. At lower temperatures, the polymer exists in a sol (liquid) state, but as the temperature increases, micellization occurs, leading to gel formation. This transition occurs at a temperature known as the sol-gel transition temperature ($T_{sol-gel}$). Below this point, the polymer chains remain dispersed in solution (Figure 2a), while temperatures above $\sim 20^\circ\text{C}$ promote micelle formation, with PPO segments aggregating inward and PEO chains extending outward (Figure 2b). Continued temperature elevation above 30°C resulted in micelle

packing into a more complex gel network (Figure 2c). The gel achieved maximum viscosity near physiological temperature, after which a decline was observed, confirming the thermoreversible nature of PX-based hydrogels.

Figure 3 illustrates the relationship between viscosity and temperature for single-polymer formulations containing varying concentrations of PX at 10, 15, 20, and 25% w/w. An increase in PX concentration led to a proportional rise in viscosity. This effect was particularly pronounced when comparing the 15PX and 20PX formulations, where the viscosity profile of 20PX consistently exceeded that of 15PX across the entire temperature range of 20°C to 50°C . Furthermore, the viscosity of PX-based hydrogels increased with temperature up to a certain threshold, after which a gradual decline was observed. This thermoresponsive behavior aligns with the known characteristics of PX. The initial increase in viscosity is attributed to the formation of a more tightly packed gel network, facilitated by higher polymer concentrations that enhance micelle packing and gelation.

**Figure 1.** Viscosity curves of 20PX formulation through cyclic temperature sweeps ranging from 20°C to 50°C

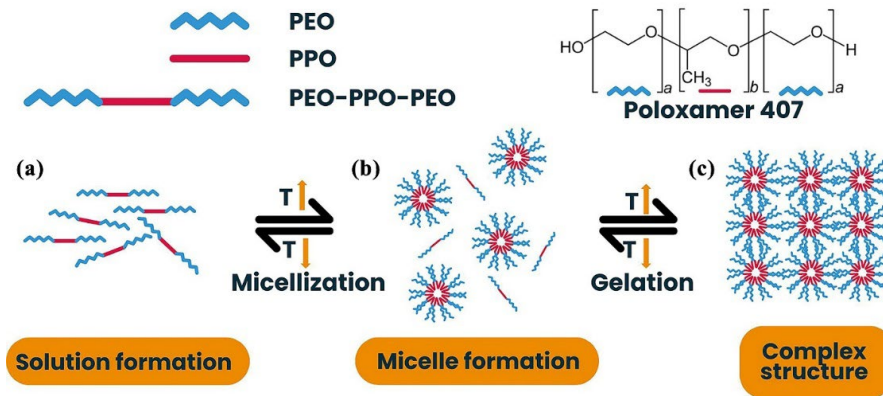


Figure 2. Mechanism of the formation of a micellar structure MT-thermosensitive hydrogel (modified from Brambilla et al., 2022)

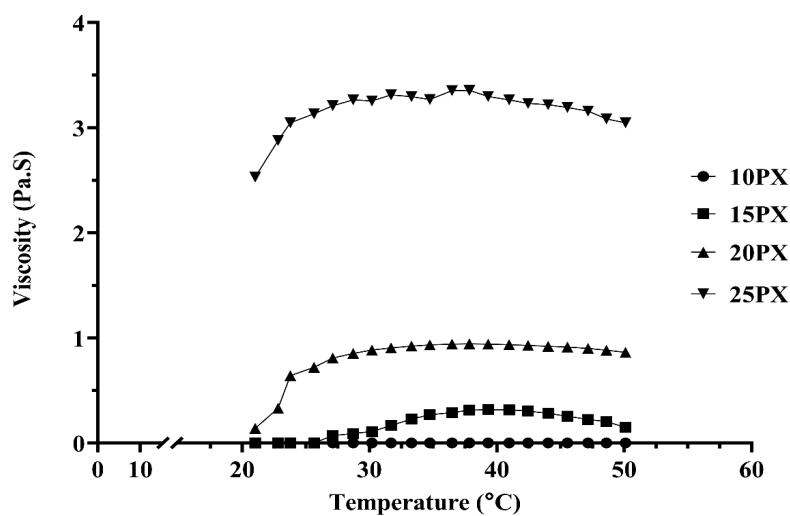


Figure 3. Viscosity profiles of PX formulations at varying concentrations over a temperature range of 20°C to 50°C

3.4 Determination of thermosensitive hydrogel erosion

The hydrogel used in periodontal pockets is eliminated through a combination of mechanisms, including enzymatic digestion, gel erosion, dissolution, and cellular uptake (Guo et al., 2024). To investigate this, we evaluated the erosion behavior of the thermosensitive hydrogels in the phosphate buffer solution (pH 7.4) for single-polymer formulations. A concentration-dependent relationship was observed, where increasing the concentration of PX led to a significant reduction in erosion. However, no statistically significant difference in erosion was found between the 20PX and 25PX formulations (Figure 4). Furthermore, the incorporation of various secondary polymers into mixed-polymer systems markedly decreased hydrogel erosion, except for the 20PX/1.00MC formulation, which did not differ significantly from the 20PX control. These findings suggest that the appropriate selection and concentration of additional polymers can effectively enhance the structural stability and reduce erosion of single-polymer hydrogel systems.

Similarly, the addition of MT significantly reduced the erosion in both single and mixed polymer formulations. The

only exception was the 20PX/0.05PAA/MT formulation, in which the presence of the drug led to a statistically significant increase in erosion. Nevertheless, more than 80% of the hydrogel remained, which is sufficient for further development as a drug delivery system in the future.

3.5 Determination of pH of thermosensitive hydrogel

The pH of the single and mixed polymer formulations was measured using a pH meter. It was found that both single polymer formulations and those mixed with MC or PAA, with or without metronidazole, had pH values ranging from 7 to 8 (Table 2). The CS-mixed formulations, with or without metronidazole, exhibited acidic pH values ranging from 3 to 4, primarily due to the incorporation of 4% glacial acetic acid as the solvent. This acidic nature contrasts with the salivary pH observed in patients with periodontitis, which averages 6.24 ± 0.11 , compared to 6.58 ± 0.58 in those with gingivitis and 7.00 ± 0.18 in individuals with healthy gingiva (Ranjith Raj & Thamaraiselvan, 2020). Although the current formulation demonstrates compatibility with the local environment of

periodontitis patients, future studies may consider adjusting the formulation's pH closer to physiological conditions. This is supported by evidence that chitosan undergoes a sol-to-gel transition around pH 6.5 (Irimia et al., 2018), which could enhance performance and patient comfort. Nonetheless, relying solely on the narrow pH discrepancy between disease states as a mechanism for controlled drug release may not be practical.

3.6 Extrudability study of thermosensitive hydrogel

The extrudability of all the single- and mixed-polymer formulations was evaluated using an 18-gauge needle, and all samples were successfully extruded (Table 2). Among the single-polymer formulations, an inverse relationship was observed between poloxamer concentration and extrudability. Specifically, formulations with lower concentrations, such as 15PX, exhibited the highest ease of

injection, followed by 20PX and 25PX. This trend correlates with the viscosity profiles of the formulations, increasing the concentration of PX leads to higher gel viscosity, thereby increasing resistance to flow and reducing extrudability.

Furthermore, when comparing 20PX to various mixed-polymer formulations containing PAA, MC, or CS, it was found that the addition of these secondary polymers reduced extrudability through the 18-gauge needle. This is due to the increased viscosity conferred by the added polymers. Higher concentrations of secondary polymers resulted in greater viscosity, making injection more difficult. Among the mixed-polymer formulations, those containing PAA were the most difficult to inject, followed by those with MC and CS. Therefore, factors affecting extrudability include the concentration of PX, the type and amount of additional polymers, and the temperature at which the test is conducted.

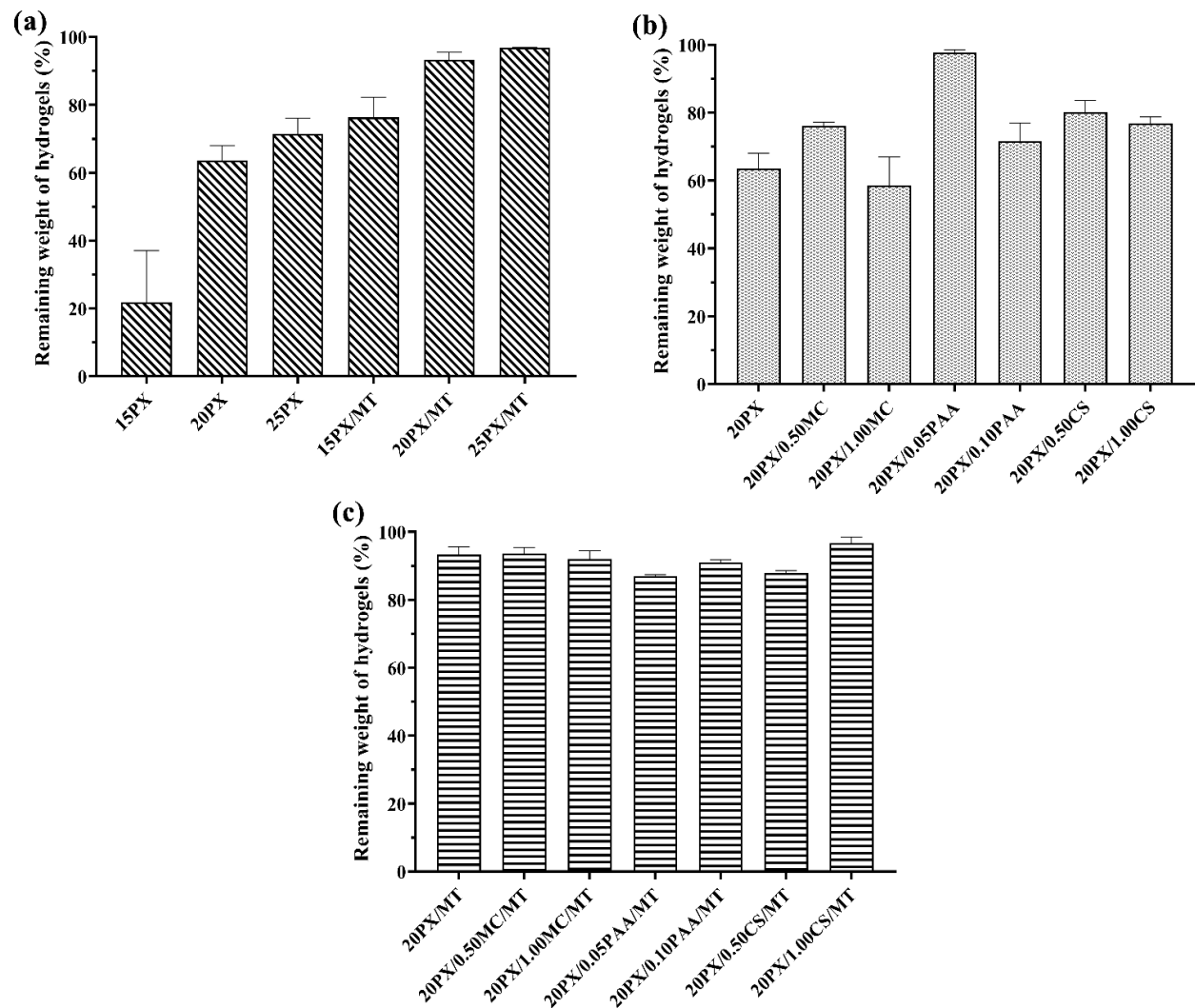


Figure 4. *In vitro* thermosensitive hydrogel erosion of (a) single polymer (PX) with/without MT, (b) mixed polymer without MT, and (c) mixed polymer with MT

3.7 *In vitro* release study of MT-thermosensitive hydrogel

It was found that for the single polymer formulations, the highest drug release occurred in the 15PX, 20PX, and 25PX formulations, in that order (Figure 5a). The formulation containing the highest concentration of poloxamer (25PX) exhibited the slowest drug release, which is likely due to the formation of a denser gel matrix that restricts drug diffusion into the phosphate buffer solution. This observation is consistent with findings from previous studies. For instance, a study on the release of ketoprofen from poloxamer gels, using a membraneless diffusion cell, reported that increasing the polymer concentration from 20% to 30% significantly reduced drug release, which was attributed to the development of a more compact gel network that limits molecular movement (Chi & Jun, 1991). Similarly, research on poloxamer-based *in situ* gelling systems highlighted that hydrophobic gels with tightly packed micellar structures result in slower drug diffusion and erosion (Abdeltawab et al., 2020). These studies emphasize that the nature, concentration, and

composition of poloxamers play a crucial role in controlling drug release behavior.

For the drug release of various mixed polymer formulations, compared with the single polymer formulation 20PX, it was found that some mixed formulations exhibited higher drug release than the 20PX formulation (Figures 5b, 5c, and 5d). This suggests that incorporating additional polymers into the single polymer formulation may enhance drug release from the MT-thermosensitive hydrogel. However, it is important to note that the mixed polymer formulations successfully enhance the hydrogel integrity in our model of *in vitro* release study of MT-thermosensitive hydrogel, where they are less likely to wash away from the dental pockets. This can be attributed to the formation of intermolecular bonds between the additive material and poloxamers, creating a tightly packed system. Notably, the enhancement of mechanical and rheological properties is associated with both the concentration and molecular weight of the additive materials (Dewan et al., 2015; Kim et al., 1998).

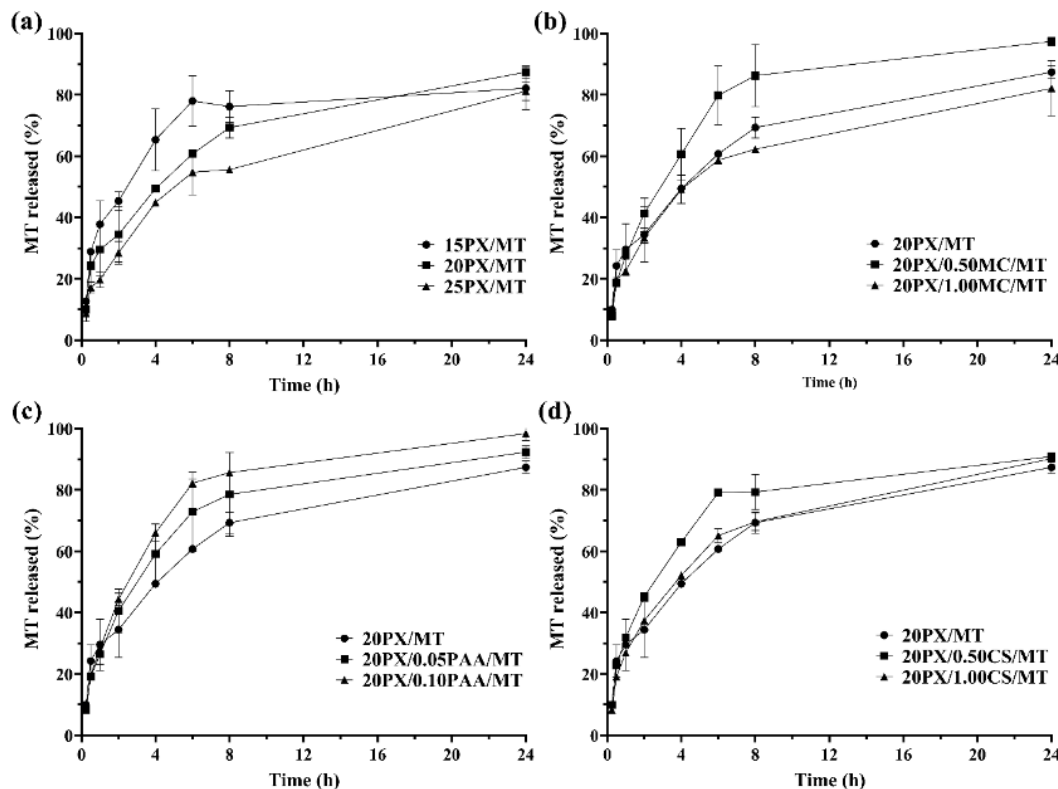


Figure 5. The release profiles of MT from 15PX/MT, 20PX/MT, and 25PX/MT (a), 20PX/0.50MC/MT and 20PX/1.00MC/MT (b), 20PX/0.05PAA/MT and 20PX/0.10PAA/MT (c), 20PX/0.50CS/MT and 20PX/1.00CS/MT (d)

4. CONCLUSION

This study successfully developed an MT-thermosensitive hydrogel for potential application in the treatment of periodontitis. The thermosensitive hydrogel formulations were prepared using PX, a synthetic triblock copolymer known for its thermoreversible sol-gel transition behavior. The thermoresponsive nature of PX was confirmed by rheological analysis, which demonstrated a reversible transition between sol and gel states. Viscosity measurements

indicated that increasing the concentration of PX led to a proportional increase in gel viscosity. Among the single-polymer formulations, 20PX exhibited significantly lower erosion than 15PX, with no statistically significant difference from 25PX. Based on these findings, the 20PX formulation was selected as the base for further modification with secondary polymers. The incorporation of other polymers improved the formulation's resistance to erosion and enhanced the drug release profile relative to the single-polymer systems. Gelation temperatures,

determined using a temperature-controlled water bath, remained within the range of 20–23°C across all formulations. Importantly, the addition of metronidazole did not alter the gelation temperature but contributed to further erosion resistance. All formulations demonstrated excellent extrudability through an 18-gauge needle and underwent rapid sol-to-gel transition at 37°C, with gelation times ranging from 10 to 90 s. The pH values of single-polymer and mixed-polymer formulations containing MC or PAA, with or without metronidazole, were in the physiologically acceptable range of pH 7–8. However, formulations containing CS, due to the use of glacial acetic acid as a solvent, exhibited acidic pH values of 3–4. Incorporating additional polymers into the single polymer formulation may enhance drug release from the MT-thermosensitive hydrogel. Overall, this study demonstrated that thermosensitive hydrogels based on PX, particularly when combined with additional polymers, are promising candidates for localized, injectable drug delivery systems in the treatment of periodontitis.

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AUTHOR CONTRIBUTIONS

Conceptualization, N.K. and S.W.; methodology, N.K. and S.W.; data curation, N.K. and S.W.; formal analysis, N.K. and S.W.; investigation, N.K., P.S. (Prapatsorn Saengtu), and P.S. (Pattamaporn Singlor); resources, S.W.; writing—original draft, N.K.; writing—review and editing: S.W. All authors have read and agreed to the published version of the manuscript.

REFERENCES

- Abdeltawab, H., Svirskis, D., & Sharma, M. (2020). Formulation strategies to modulate drug release from poloxamer based in situ gelling systems. *Expert Opinion on Drug Delivery*, 17(4), 495–509. <https://doi.org/10.1080/17425247.2020.1731469>
- Alvarez Echazú, M. I., Olivetti, C. E., Anesini, C., Perez, C. J., Alvarez, G. S., & Desimone, M. F. (2017). Development and evaluation of thymol-chitosan hydrogels with antimicrobial-antioxidant activity for oral local delivery. *Materials Science and Engineering: C*, 81, 588–596. <https://doi.org/10.1016/j.msec.2017.08.059>
- Boonlai, W., Tantishaiyakul, V., Hirun, N., Sangfai, T., & Suknuntha, K. (2018). Thermosensitive poloxamer 407/poly(acrylic acid) hydrogels with potential application as injectable drug delivery system. *AAPS PharmSciTech*, 19, 2103–2117. <https://doi.org/10.1208/s12249-018-1010-7>
- Brady, J., Dürig, T., Lee, P. I., & Li, J.-X. (2017). Chapter 7 - Polymer properties and characterization. In Y. Qiu, Y. Chen, G. G. Z. Zhang, L. Yu, & R. V. Mantri (Eds.), *Developing solid oral dosage forms* (2nd ed., pp. 181–223). Academic Press. <https://doi.org/10.1016/B978-0-12-802447-8.00007-8>
- Brambilla, E., Locarno, S., Gallo, S., Orsini, F., Pini, C., Farronato, M., Thomaz, D. V., Lenardi, C., Piazzoni, M., & Tartaglia, G. (2022). Poloxamer-based hydrogel as drug delivery system: How polymeric excipients influence the chemical-physical properties. *Polymers*, 14(17), Article 3624. <https://doi.org/10.3390/polym14173624>
- Cai, X., Luan, Y., Jiang, Y., Song, A., Shao, W., Li, Z., & Zhao, Z. (2012). Huperzine A-phospholipid complex-loaded biodegradable thermosensitive polymer gel for controlled drug release. *International Journal of Pharmaceutics*, 433(1–2), 102–111. <https://doi.org/10.1016/j.ijpharm.2012.05.009>
- Casarin, R. C. V., Del Peloso Ribeiro, É., Mariano, F. S., Nociti Jr, F. H., Casati, M. Z., & Gonçalves, R. B. (2010). Levels of *Aggregatibacter actinomycetemcomitans*, *Porphyromonas gingivalis*, inflammatory cytokines and species-specific immunoglobulin G in generalized aggressive and chronic periodontitis. *Journal of Periodontal Research*, 45(5), 635–642. <https://doi.org/10.1111/j.1600-0765.2010.01278.x>
- Chi, S. C., & Jun, H. W. (1991). Release rates of ketoprofen from poloxamer gels in a membraneless diffusion cell. *Journal of Pharmaceutical Sciences*, 80(3), 280–283. <https://doi.org/10.1002/jps.2600800318>
- Christie, P., Claffey, N., & Renvert, S. (1998). The use of 0.2% chlorhexidine in the absence of a structured mechanical regimen of oral hygiene following the non-surgical treatment of periodontitis. *Journal of Clinical Periodontology*, 25(1), 15–23. <https://doi.org/10.1111/j.1600-051x.1998.tb02358.x>
- Dewan, M., Bhowmick, B., Sarkar, G., Rana, D., Bain, M. K., Bhowmik, M., & Chattopadhyay, D. (2015). Effect of methyl cellulose on gelation behavior and drug release from poloxamer based ophthalmic formulations. *International Journal of Biological Macromolecules*, 72, 706–710. <https://doi.org/10.1016/j.ijbiomac.2014.09.021>
- Elliott, J. E., Macdonald, M., Nie, J., & Bowman, C. N. (2004). Structure and swelling of poly(acrylic acid) hydrogels: Effect of pH, ionic strength, and dilution on the crosslinked polymer structure. *Polymer*, 45(5), 1503–1510. <https://doi.org/10.1016/j.polymer.2003.12.040>
- Fakhari, A., Corcoran, M., & Schwarz, A. (2017). Thermogelling properties of purified poloxamer 407. *Heliyon*, 3(8), Article e00390. <https://doi.org/10.1016/j.heliyon.2017.e00390>
- Guo, W., Dong, H., & Wang, X. (2024). Emerging roles of hydrogel in promoting periodontal tissue regeneration and repairing bone defect. *Frontiers in Bioengineering and Biotechnology*, 12, Article 1380528. <https://doi.org/10.3389/fbioe.2024.1380528>
- Irimia, T., Dinu-Pîrvu, C.-E., Ghica, M. V., Lupuleasa, D., Muntean, D.-L., Udeanu, D. I., & Popa, L. (2018). Chitosan-based *in situ* gels for ocular delivery of therapeutics: A state-of-the-art review. *Marine Drugs*, 16(10), Article 373. <https://doi.org/10.3390/md16100373>
- Kim, C.-K., Lee, S.-W., Choi, H.-G., Lee, M.-K., Gao, Z.-G., Kim, I.-S., & Park, K.-M. (1998). Trials of in situ-gelling and mucoadhesive acetaminophen liquid suppository in



- human subjects. *International Journal of Pharmaceutics*, 174(1–2), 201–207. [https://doi.org/10.1016/S0378-5173\(98\)00258-0](https://doi.org/10.1016/S0378-5173(98)00258-0)
- Kwon, T., Lamster, I. B., & Levin, L. (2021). Current concepts in the management of periodontitis. *International Dental Journal*, 71(6), 462–476. <https://doi.org/10.1111/idj.12630>
- Lee, Y., Gou, Y., Pan, X., Gu, Z., & Xie, H. (2023). Advances of multifunctional hydrogels for periodontal disease. *Smart Materials in Medicine*, 4, 460–467. <https://doi.org/10.1016/j.smaim.2023.02.001>
- Li, F., Liu, Y., Ding, Y., & Xie, Q. (2014). A new injectable *in situ* forming hydroxyapatite and thermosensitive chitosan gel promoted by Na₂CO₃. *Soft Matter*, 10(13), 2292–2303. <https://doi.org/10.1039/C3SM52508B>
- Li, Z., Huang, Q., Wang, Z., Huang, L., & Gu, L. (2024). Effects of *Porphyromonas gingivalis* and *Aggregatibacter actinomycetemcomitans* on modeling subgingival microbiome and impairment of oral epithelial barrier. *The Journal of Infectious Diseases*, 229(1), 262–272. <https://doi.org/10.1093/infdis/jiad461>
- Pashirova, T. N., Afonso, A. B., Terekhova, N. V., Kamalov, M. I., Masson, P., & Souto, E. B. (2023). Chapter 5 - Nanogels for drug delivery: Physicochemical properties, biological behavior, and *in vivo* applications. In M. H. Santana, E. B. Souto, & R. Shegokar (Eds.), *Nanotechnology and regenerative medicine* (pp. 95–131). Academic Press. <https://doi.org/10.1016/B978-0-323-90471-1.00005-0>
- Peppas, N. A., Slaughter, B. V., & Kanzelberger, M. A. (2012). 9.20 - Hydrogels. In K. Matyjaszewski & M. Möller (Eds.), *Polymer science: A comprehensive reference* (pp. 385–395). Elsevier. <https://doi.org/10.1016/B978-0-444-53349-4.00226-0>
- Piyakhunakorn, P., & Sermsuti-anuwat, N. (2021). The relationship between periodontitis and oral health literacy among the older people in Thailand. *Global Journal of Health Science*, 13(6), 103–109. <https://doi.org/10.5539/gjhs.v13n6p103>
- Rams, T. E., & Slots, J. (2024). Elevated subgingival temperature infers high bacterial pathogen counts in severe periodontitis. *Clinical and Experimental Dental Research*, 10(3), Article e891. <https://doi.org/10.1002/cre2.891>
- Ranjith Raj, V., & Thamaraiselvan, M. (2020). Role of salivary pH on the prevalence of periodontal disease: A cross sectional pilot study. *Journal of Research in Medical and Dental Science*, 8(7), 423–427.
- Sato, S., Fonseca, M. J. V., Ciampo, J. O. D., Jabor, J. R., & Pedrazzi, V. (2008). Metronidazole-containing gel for the treatment of periodontitis: An *in vivo* evaluation. *Brazilian Oral Research*, 22(2), 145–150. <https://doi.org/10.1590/s1806-83242008000200009>
- Sgolastra, F., Gatto, R., Petrucci, A., & Monaco, A. (2012). Effectiveness of systemic amoxicillin/metronidazole as adjunctive therapy to scaling and root planing in the treatment of chronic periodontitis: A systematic review and meta-analysis. *Journal of Periodontology*, 83(10), 1257–1269. <https://doi.org/10.1902/jop.2012.110625>
- Shen, Z., Kuang, S., Zhang, Y., Yang, M., Qin, W., Shi, X., & Lin, Z. (2020). Chitosan hydrogel incorporated with dental pulp stem cell-derived exosomes alleviates periodontitis in mice via a macrophage-dependent mechanism. *Bioactive Materials*, 5(4), 1113–1126. <https://doi.org/10.1016/j.bioactmat.2020.07.002>
- Sherif, S., Bendas, E. R., & Badawy, S. (2014). The clinical efficacy of cosmeceutical application of liquid crystalline nanostructured dispersions of alpha lipoic acid as anti-wrinkle. *European Journal of Pharmaceutics and Biopharmaceutics*, 86(2), 251–259. <https://doi.org/10.1016/j.ejpb.2013.09.008>
- Sund-Levander, M., Forsberg, C., & Wahren, L. K. (2002). Normal oral, rectal, tympanic and axillary body temperature in adult men and women: A systematic literature review. *Scandinavian Journal of Caring Sciences*, 16(2), 122–128. <https://doi.org/10.1046/j.1471-6712.2002.00069.x>
- Teoh, J. H., Abdul Shakoar, F. T., & Wang, C.-H. (2022). 3D printing methyl cellulose hydrogel wound dressings with parameter exploration via computational fluid dynamics simulation. *Pharmaceutical Research*, 39(2), 281–294. <https://doi.org/10.1007/s11095-021-03150-5>
- Urbán-Morlán, Z., Castro-Ríos, R., Chávez-Montes, A., Melgoza-Contreras, L. M., Piñón-Segundo, E., Ganem-Quintanar, A., & Quintanar-Guerrero, D. (2008). Determination of poloxamer 188 and poloxamer 407 using high-performance thin-layer chromatography in pharmaceutical formulations. *Journal of Pharmaceutical and Biomedical Analysis*, 46(4), 799–803. <https://doi.org/10.1016/j.jpba.2007.11.027>
- White, J. M., Garza, A., Griebler, J. J., Bates, F. S., & Calabrese, M. A. (2023). Engineering the structure and rheological properties of P407 hydrogels via reverse poloxamer addition. *Langmuir*, 39(14), 5084–5094. <https://doi.org/10.1021/acs.langmuir.3c00088>
- Xie, Y., Tang, J., Lu, Z., Sun, Z., & An, L. (2013). Effects of poly(propylene oxide)–poly(ethylene oxide)–poly(propylene oxide) triblock copolymer on the gelation of poly(ethylene oxide)–poly(propylene oxide)–poly(ethylene oxide) aqueous solutions. *Journal of Macromolecular Science, Part B*, 52(9), 1183–1197. <https://doi.org/10.1080/00222348.2012.758524>
- Zeqiri, E., da Silva, M. A., Aspinall, S. R., Hoffman, E., Hutter, V., & Cook, M. T. (2024). Thermoreversible gels for the encapsulation of macrophages: Evaluation of polymer type on rheology and cytocompatibility. *RSC Applied Polymers*, 2(1), 32–46. <https://doi.org/10.1039/D3LP00056G>
- Zhang, Y., Jiang, R., Lei, L., Yang, Y., & Hu, T. (2022). Drug delivery systems for oral disease applications. *Journal of Applied Oral Science*, 30, Article e20210349. <https://doi.org/10.1590/1678-7757-2021-0349>