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Capsaicin Hydrogel Skin Patch: Development, Characterization, and Safety Evaluation of Cytotoxicity, Anti-Inflammatory Effects, and Pain-Relief Applications

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Abstract

Background: Capsaicin, the primary pungent component of *Capsicum annuum*, exhibits significant anti-inflammatory and analgesic properties by acting on TRPV1 receptors and modulating COX-2 expression. However, its therapeutic use is limited by poor solubility, instability, and potential skin irritation. Myofascial Pain Syndrome (MPS), a prevalent musculoskeletal disorder, presents a need for safer and more effective topical treatments. This study aimed to develop and evaluate capsaicin loaded hydrogel patches for cytotoxic effects, anti-inflammatory properties, and pain relief.

Methods and Results: A hydrogel patch containing 0.1% capsaicin was fabricated using polyvinyl alcohol (PVA), gelatin, glycerol, Tween 80, triethanolamine through the freeze–thaw technique. Structural analysis by GC-MS revealed bioactive constituents, predominantly dodecyl acrylate (45.78%), oleic acid (25.06%), and trans-oleic acid (7.52%), all known for their anti-inflammatory and skin-permeation-enhancing properties. FTIR confirmed the successful incorporation of capsaicin into the hydrogel matrix with characteristic shifts in N–H, C=O, and C–N functional groups, while UV-Vis spectroscopy supported capsaicin release over time. Cytotoxicity testing on human skin fibroblast (HSF) cells demonstrated high cell viability (>90%) at concentrations below 1 mg/mL, with an IC_{50} of approximately 20 mg/mL, indicating low toxicity at therapeutic doses. The hydrogel exhibited dose-dependent anti-inflammatory activity. Notably, the anti-inflammatory efficacy was statistically comparable to diclofenac ($P = 0.183$).

Conclusion: The capsaicin-loaded hydrogel patch showed excellent physicochemical characteristics, structural stability, and biocompatibility. Its anti-inflammatory efficacy was on a par with standard diclofenac, supporting its potential as a safe and effective treatment for localized pain and inflammation management with MPS.(**International Journal of Biomedicine. 2025;15(3):572-582.**)

Keywords: myofascial pain • anti-inflammatory activity • Capsaicin • cytotoxicity

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Abbreviations

BSA, bovine serum albumin; **CHP**, Capsaicin hydrogel patch; **DW**, distilled water; **FTIR**, Fourier transform infrared spectroscopy; **MPS**, Myofascial Pain Syndrome; **NRS**, Numeric Rating Scale; **NSAIDs**, nonsteroidal anti-inflammatory drugs; **VAS**, Visual Analog Scale; **UV-vis**, ultraviolet-visible; **w/v**, weight/volume.

Introduction

Capsicum annuum, a widely cultivated member of the Solanaceae family, is known for its use in culinary, industrial, and medicinal applications.[1] This plant exhibits considerable diversity in fruit morphology and pungency, contributing to its global significance as both a spice and a functional food. The fruit is rich in phytochemicals, notably capsaicinoids and carotenoids, responsible for its spiciness and coloration. Capsaicin and dihydrocapsaicin dominate the capsaicinoid profile, while xanthophylls and carotenes account for the vibrant pigmentation. Additionally, chili peppers contain volatile compounds, fatty acids, phenolics, vitamins, and essential minerals, contributing to their reported bioactivities, including antimicrobial effects. Despite these findings, further investigation is needed to elucidate the bioactive compounds responsible for these therapeutic properties fully.[2]

Capsaicin, the active component in chili peppers, is well-known for its anti-inflammatory properties. Capsaicin (8-methyl-N-vanillyl-6-nonenamide), a naturally occurring protoalkaloid and a neuropeptide-active agent that affects the transient receptor potential vanilloid-1 receptor (TRPV1), is used to treat pain disorders, particularly myofascial pain syndrome,[3] as well as an anti-inflammatory agent.[4] These effects are primarily mediated through its action on the TRPV1 receptor, which is involved in pain and inflammation pathways. When capsaicin binds to TRPV1 receptors, it initially causes an influx of calcium ions into the nerve cells, leading to a burning sensation. However, with repeated application, capsaicin depletes substance P, a neuropeptide associated with inflammation and pain transmission. This depletion reduces the sensitivity of nerve fibers to pain and decreases inflammation.[5,6] Marked mechanism is particularly beneficial in conditions associated with chronic pain, such as neuropathic pain and arthritis.[6] Topical capsaicin in low concentrations (0.025–0.1%) is recognized for its moderate efficacy in treating certain types of pain, particularly neuropathic and musculoskeletal pain, as well as conditions like arthritis. Capsaicin targets the TRPV1 receptors on pain nerve fibers,[7] which transmit pain sensations. Capsaicin 0.1% is minimally absorbed systemically, which reduces the risk of systemic side effects, making it a safer alternative to oral pain medications, especially in chronic pain conditions. Its minimal absorption is part of the reason why it is generally well-tolerated. Overall, capsaicin 0.1% is safe when used as directed.[6]

Furthermore, capsaicin's role in reducing oxidative stress contributes to its anti-inflammatory and analgesic effects; capsaicin mitigates oxidative damage, a key driver of chronic inflammation and pain.[8] The mechanism of capsaicin antioxidant action has been studied, and in particular, the ability of its phenolic structure to provide hydrogen to peroxyl and alkoxy radicals has been revealed.[9]

This interaction forms a complex with reduced transition metals, while the C7-benzyl carbon and methoxy group enhance its potent antioxidant and free radical scavenging properties.[10] This antioxidant activity reduces inflammation and helps prevent the sensitization of pain pathways. Capsaicin also modulates the expression of cyclooxygenase-2 (COX-2),[11] an enzyme that plays a significant role in the inflammatory process and pain signaling. By downregulating COX-2 expression, capsaicin can decrease both inflammation and pain.

Myofascial pain syndrome (MPS) is a chronic soft tissue pain disorder characterized by local and referred musculoskeletal pain originating from trigger points (TrPs) within the fascia surrounding skeletal muscles. It is a prevalent cause of both acute and chronic pain. MPS can manifest independently, termed primary myofascial pain syndrome. Traditional treatments include physical therapy, medications, and trigger point injections.[12] However, recent research is increasingly focused on capsaicin, the active component of chili peppers, as a potential alternative or adjunctive therapy, investigating its mechanisms of action and clinical effectiveness.[13] Myofascial pain syndrome is characterized by muscle pain originating from a trigger point. Despite extensive research, the pathophysiology of MPS remains incompletely understood,[12] leading to the exploration of various treatment modalities. Physical treatments include techniques such as stretch and spray, massage, ischemic compression therapy, and local heat. Needle-based interventions encompass dry needling, trigger point injections with local anesthetics, and botulinum toxin injections. Transcutaneous electric nerve stimulation (TENS) is also utilized for MPS management.[14] Supplementary treatments often involve nonsteroidal anti-inflammatory drugs (NSAIDs), though their efficacy, especially topically, in treating MPS is unclear. There is no clear evidence of the effectiveness or relative efficacy of one treatment compared to another.[14] Topical capsaicin is another option, potentially serving as an adjuvant treatment. The etiology of trigger points and myofascial pain remains incompletely elucidated. Nevertheless, studies have demonstrated that capsaicin can elicit pain and hyperalgesia in human tendon tissues. Furthermore, it has been observed to enhance trigger point sensitivity in individuals while concurrently mitigating associated pain.[15] The treatment of MPS should be multimodal, with the main purpose of managing the underlying disease-causing pain. Although nonsteroidal anti-inflammatory drugs (NSAIDs) are commonly used for pain relief, their application in the treatment of chronic pain disorders is restricted due to the potential for adverse effects on the gastrointestinal (GI) tract and the kidneys (GI symptoms, renal injury, nausea and vomiting, dizziness), and depression.[12] Less invasive options have also been proposed to improve the MPS treatment.

Given capsaicin's analgesic properties, a comprehensive understanding of its physicochemical properties is necessary for the development of drug delivery systems that provide

a safer and more effective treatment option. In this study, a hydrogel delivery system was designed to enhance the sustained release of capsaicin, improve its efficacy, and reduce side effects, with a focus on creating a pain relief patch that enhances performance. Hydrogels are three-dimensional crosslinked polymeric matrices characterized by properties such as swelling and deswelling behaviors, shock absorption, and low friction coefficients, as documented in the literature.[16] Hydrogels have garnered considerable attention and are utilized in various fields, including effluent management, tissue engineering, drug delivery systems, and biomolecular filtration.[17] Their hydrophilic nature, biodegradability, and biocompatibility make them valuable synthetic polymers in the biomedical sector.[18] Furthermore, capsaicin has been shown to inhibit the production of pro-inflammatory cytokines, further contributing to its anti-inflammatory properties. Using capsaicin in topical formulations, such as creams and patches, provides a targeted approach to managing localized inflammation and pain, making it a valuable tool in treating inflammatory conditions. With the limited stability of certain patch types, there is a growing interest in developing novel hydrogel-based patches that incorporate capsaicin to enhance pain relief. This research, based on laboratory tests, aims to inform practical applications for patients in the future. The study evaluates both the safety and efficacy of the transdermal delivery system to advance its clinical use.

This study aimed to develop and evaluate capsaicin loaded hydrogel patches for cytotoxic effects, anti-inflammatory properties, and pain relief.

Materials and Methods

Preparation of Capsaicin Hydrogel Skin Patch

Polyvinyl alcohol (PVA) (CAS 9002-89-5) 1% w/v (Molecular weight 13,000-23,000) from Sigma-Aldrich solution at a concentration of 1% (w/v) by weight and gelatin (CAS Numbers, 9000-70-8) at 10% w/v were dissolved in boiling water at 90 °C under magnetic stirring for approximately 1-2 hours[19] until a clear and transparent solution was obtained. 10 ml of glycerol 99.5% (CAS Number: 56-81-5), 1.25 ml of Tween 80 (CAS Number: 9005-65-6), and 4 drops of triethanolamine (CAS Number: 102-71-6) in combination play a significant role in hydrogel preparation, functioning as a pH adjuster, stabilizer, and emulsifier. The above components and capsaicin (CAS number: 404-86-4) at a concentration of 0.1% were added to the prepared solution. The solution was stirred at room temperature for 45-60 minutes until complete dissolution and uniform consistency were observed. Once the solution had achieved uniform consistency, it was left to cool down gradually. Subsequently, 10 ml of the solution was prepared and poured into a Petri dish. The solution-filled Petri dish was then subjected to the freeze-thaw process by alternately freezing at -20 °C for 48 hours and thawing at room temperature (25 °C) for 24 hours, resulting in a well-consolidated hydrogel sheet. The obtained hydrogel sheet underwent sterilization and examination for the persistence of capsaicin (Figure 1).

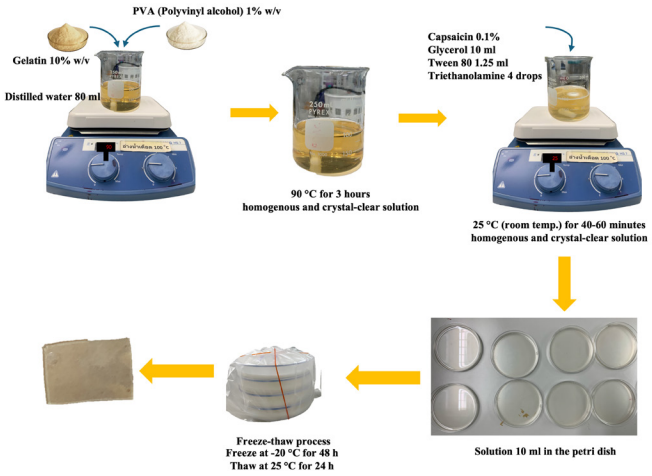

Fig. 1. *A schematic diagram displays the preparation steps of the capsaicin hydrogel patch.*

Capsaicin Hydrogel Patch Characterization

Gas Chromatography-Mass Spectrometry (GC-MS) Analysis

Capsaicin was prepared at an appropriate concentration and injected into the GC-MS system using an autosampler in liquid injection mode with an injection volume of 1 µL.[2] The sample components were separated using an HP-5 capillary column under predefined conditions. The separated compounds were then introduced into the mass spectrometer (MS), where electron ionization (EI) at 70 eV was applied to generate molecular ions. Mass scanning was conducted over a range of 35–550 m/z to identify the chemical constituents. The resulting spectra were analyzed by comparison with the NIST MS Search 2.0 database to confirm compound identification.

Investigation of the Physical Properties of Hydrogel Patch Characterization by UV-Visible Spectroscopy

The selected hydrogel sheets containing capsaicin were incubated at either 4°C to facilitate slow and controlled gelation, allowing the gradual formation of the hydrogel network, or 50°C to promote crosslinking of the hydrogel structure, thereby enhancing its mechanical strength and stability with 75% relative humidity for 6 weeks. Physical characteristics and biocompatibility adherence abilities were evaluated before and after the testing period, following the methodology by Simone et al.[20] UV-visible spectroscopy, using a T80+ model from PG Instruments (Spectrum mode) (Serial Number: 23-1885-01-0190), was conducted in the laboratory. A sample was employed to assess the drug release from the hydrogel sheets containing the drug in distilled water. Quartz cuvettes of 4 ml capacity were utilized for spectroscopic measurements within the wavelength range of 200-700 nm, with data recorded at 30 or 60-minute intervals throughout the entire experiment, repeated at least three times.

Investigation of the Physical Properties of Hydrogel Patch Characterization by Fourier Transform Infrared Spectroscopy

The procedure for studying room temperature hydrogel patch stability for 30 days was conducted following the method by Ilie et al.,[21] and the morphology of the samples was characterized using Fourier Transform Infrared Spectroscopy (FTIR) Vertex 70, Bruker Switzerland, (Serial Number: 3099/HYP.1097) with a resolution of 4, Sample Scan Time: 64, Background Scan Time: 64. Analysis was repeated at least 3 times using OPUS Software, with the test results recorded and subjected to further analysis. The physical characteristics of the prepared patches were evaluated through visual examination and tactile perception, such as the smoothness of the material and clarity, to select patch materials with favorable physical attributes.

Cytotoxicity Assays

Cytotoxicity Testing on Human Skin Fibroblast (HSF) Cells Using the MTT Assay

HSF cells were seeded at a density of 10,000 cells/well in a 96-well plate using Dulbecco's modified Eagle medium (DMEM) supplemented with 1% FBS and 1% penicillin/streptomycin. The cells were incubated for 24 hours in a CO_2 incubator maintained at 37 °C with 5% CO_2. Test samples were prepared at various concentrations by diluting them in DMEM. Each concentration (100 µL) was added to the respective wells, and the cells were further incubated for 24 hours. After 24 hours, 100 µL of MTT solution (0.5 mg/mL) was added to each well, and the plate was incubated in the dark for 2 hours. The supernatant was then carefully removed, and the resulting formazan crystals were dissolved with 100 µL of dimethyl sulfoxide (DMSO). Absorbance was measured at 570 nm using a microplate reader. The percentage of cell viability (mg/mL) was calculated using the following formula: [22]

$$\%\ \text{Cell viability} = \frac{\text{Absorbance of treated cell}}{\text{Absorbance of control cell}} \times 100\%$$

Anti-Inflammatory Activity

The anti-inflammatory properties were evaluated using a modified version of the protocol.[23] One milliliter of the test compounds or diclofenac sodium (positive control) at varying concentrations 25, 50, 100, 200, 500, and 1000 µg/mL (conducted in triplicate) was added to 1 mL of an aqueous solution containing 5% bovine serum albumin (BSA) (CAS Numbers, 9048-46-8) and incubated for 15 minutes at 27°C.

A mixture of distilled water (DW) and BSA was the control. The protein denaturation process was initiated by heating the mixture for 10 minutes at 70 °C.

After cooling at room temperature, the absorbance of each sample was measured at 660 nm by VICTOR® Nivo™ Multimode Plate Readers (Serial Number: HH35L2020289). The percentage inhibition was calculated from optical density (OD) values by following the formula:[24]

$$\%\ \text{Inhibition} = \frac{\text{Absorbance of control} - \text{Absorbance of sample}}{\text{Absorbance of control}} \times 100\%$$

Efficacy of Capsaicin Hydrogel Patch for Pain Relief

The research design included a pilot study methodology to ascertain the adequacy of sample group sizes, as outlined.[25,26] The determination of sample group size was calculated with a confidence level of 0.7 and a probability of 0.3, following the methodology.[26] The computation resulted in an estimated sample group size of 3.4 individuals to mitigate potential dropouts; therefore, it was adjusted to 5 participants. Participants were aged equal to or greater than 18 years. This pilot study recorded visual analogue scale (VAS) scores equal to or greater than 5 out of a maximum of 10 for neck pain duration and clinical manifestation of MPS.

Pain Reduction Assessment

Participants applied adhesive patches to the affected shoulder or back for 2 hours per session, twice daily (morning and evening) for 3 days. Thereafter, volunteers recorded pain scores using the Visual Analog Scale (VAS) and Numeric Rating Scale (NRS) (0-10 points) before and after patch application (a total of 6 times) and noted any adverse skin reactions that may occur, such as redness, swelling, clear vesicles, and itching. Volunteers assessed and recorded their discomfort with the continuous use of the patches. In addition, skin reactions were assessed before and after patch use, assessing redness (0-3 points), swelling (0-4 points), the presence of papules, the presence of wheals, and the presence of large, clear vesicles.[27]

Statistical analysis

Statistical analysis was performed using SPSS Statistics version 29.0 (SPSS Inc., Chicago, IL). The Wilcoxon signed-rank test was used to compare the differences between the two dependent groups (for non-parametric data). The probability value of $P \leq 0.05$ was considered statistically significant.

Results

Capsaicin Hydrogel Skin Patch

A capsaicin hydrogel skin patch containing 0.1% capsaicin was developed in the experimentation, as shown in Figure 1. Capsaicin at a concentration of 0.1% is widely applied topically for pain relief in various conditions. In this study, a skin patch was designed with a thickness of 0.5 cm and dimensions of 4×5 cm². The capsaicin hydrogel patch, including its physical characteristics and optimized concentration of 0.1% capsaicin, is illustrated. The active compound remained encapsulated within the hydrogel patch (Figure 1).

Characterization of Capsaicin Hydrogel Skin Patch

Gas Chromatography–Mass Spectrometry (GC-MS) Analysis

Gas chromatography-mass spectrometry (GC-MS) was employed to characterize the chemical composition of the capsaicin. 17 peaks were identified, corresponding to various fatty acids, esters, alcohols, and hydrocarbons. Compound identification was performed by matching mass spectral data with the NIST MS Search 2.0 database. The predominant

constituent was dodecyl acrylate (RT: 24.096 min), accounting for 45.78% of the total peak area, followed by oleic acid (RT: 30.558 min; 25.06%) and trans-oleic acid (RT: 35.612 min; 7.52%). Additional major components included 1-dodecanol (RT: 20.836 min; 6.49%), propanoic acid, decyl ester (RT: 24.178 min; 4.55%), and n-hexadecanoic acid (RT: 27.830 min; 3.21%). Minor components such as nonanamide, elaidic acid ethyl ester, and octadecanoic acid were also detected, contributing to the overall chemical complexity of the capsaicin. Several of these compounds, particularly long-chain fatty acids and their esters, are recognized for their bioactive properties, including anti-inflammatory, emollient, and skin-permeation enhancing effects. The presence of these compounds suggests that the capsaicin contains multiple bioactive molecules that may synergistically contribute to the analgesic and anti-inflammatory activity of the formulated hydrogel patch, as shown in Table 1.

Table 1.

Chemical constituents identified in the capsaicin crude extract using GC-MS.

RT	Area	Area Sum %	Name
5.037	28839	0.20	2-Propenoic acid
5.618	35676	0.25	Silane, triethylfluoro-
9.421	19960	0.14	4,4-dimethyl-1,3-Dioxane
14.199	15724	0.11	Nonanal
18.392	34202	0.24	n-Decanoic acid
20.836	930588	6.49	1-Dodecanol
21.499	48734	0.34	Nonanamide
24.096	6569297	45.78	Dodecyl acrylate
24.178	653059	4.55	Propanoic acid, decyl ester
27.406	149881	1.04	3-Chloropropionic acid, dodecyl ester
27.830	460111	3.21	n-Hexadecanoic acid
28.315	94546	0.66	Hexadecanoic acid, ethyl ester
30.558	3595726	25.06	Oleic Acid
30.857	177826	1.24	Octadecanoic acid
30.940	388904	2.71	Elaidic acid, ethyl ester
35.612	1079067	7.52	Trans-oleic acid
36.435	66942	0.47	Diisooctyl phthalate
Total	14349082	100	

Chemical constituents identified in the capsaicin crude extract using GC-MS. Each compound was identified based on retention time (RT) and mass spectral comparison with the NIST MS database. The relative abundance of each component is expressed as the percentage of total peak area (Area%).

UV-Visible Spectrophotometry

The capsaicin hydrogel patches were loaded into cuvettes, and testing began at minute 0 without changing the wavelength. Changes began at 30, 60, 90, 120, 150, and 180 minutes. Optimal light absorption measurements of capsaicin dilution were identified through UV-Visible scan spectrum readings at 278, 281, 280, 284, 283, and 283 nanometers, respectively (Figure 2). The absorbance of capsaicin was measured spectrophotometrically at a wavelength, demonstrating the peak of the capsaicin spectra.

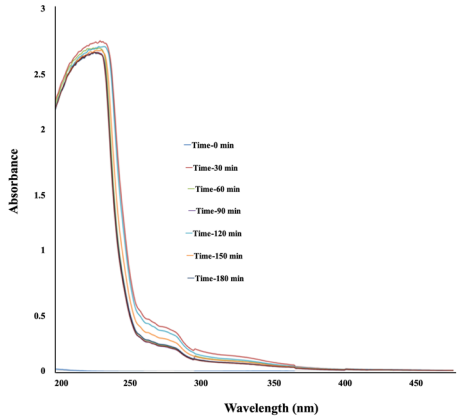

Fig. 2 *The UV-Vis absorption spectrum of capsaicin.*

Fourier Transform Infrared Spectroscopy (FTIR)

The stability of the substance at ambient temperature was evaluated over a 30-day period following the protocol described by Ilie et al.,[21] and its presence was confirmed using a Vertex 70 FTIR spectrometer in the laboratory. Analysis of data from hydrogel patch samples and standard capsaicin samples revealed that FTIR measurement aimed to identify possible biomolecular substances of capsaicin. The FTIR spectrum of capsaicin displayed prominent absorption bands at 3295, 2925, 1633, 1457, 1,360, 1244, 1148, 1078, 994, 930, 847, 758, 572, and 520 $cm.^{-1}$.

Clear peaks were observed when testing the FTIR spectrum of hydrogel patches containing capsaicin. These peak values slightly shifted to 3303, 1637, 1475, 1244, 1111, 1043, and 992 cm^{-1} in the FTIR spectrum of the hydrogel patches mixed with capsaicin. The FTIR spectrum of capsaicin displays peaks corresponding to its characteristic functional groups. Capsaicin, with the molecular formula C18H27NO3, is systematically named 8-methyl-N-vanillyl-6-nonenamide. Its molecular structure comprises key functional groups, including an amide group, an aromatic vanillyl ring, and a long hydrocarbon chain. These groups play a significant role in its chemical and physical properties, which are reflected in its FTIR absorption spectrum. Characteristics of the standard capsaicin peaks reveal N-H stretch (3295 cm^{-1}), aliphatic C-H stretch (2925 cm^{-1}), C=O stretch (1633 cm^{-1}), and C-N stretch (Amide II) (1148 cm^{-1}). Upon comparison with hydrogel skin patch samples containing capsaicin, the peak characteristics in the FTIR spectrum include N-H stretch (3303 cm^{-1}), C=O stretch (1637 cm^{-1}), and C-N stretch (Amide II) (1111 cm^{-1}). While the aliphatic C-H group may exhibit less distinct peaks, minor variations in peak intensity are noted compared to

standard capsaicin. These alterations confirm the presence of capsaicin peaks in the hydrogel patch samples (Figure 3).

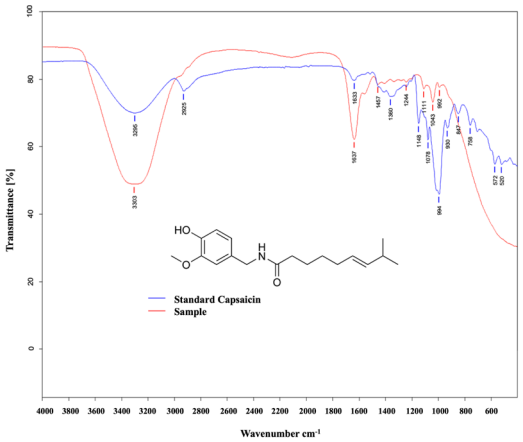

Fig 3. *FT-IR spectrum and chemical formula of capsaicin hydrogel. (Red line - capsaicin hydrogel skin patch, blue line - standard capsaicin).*

Cytotoxicity Assays

The cytotoxic effects of capsaicin solution on human skin fibroblast (HSF) cells were evaluated using the MTT assay. Figure 4(A) shows cell viability percentage following capsaicin treatment at various concentrations (0.0001–10 mg/mL) for 24 hours. The results are presented as mean ± standard deviation (SD). Capsaicin at 0.0001 to 1 mg/mL concentrations showed no significant cytotoxic effects, with cell viability remaining above 90%. However, at 10 mg/mL, a statistically significant reduction in cell viability ($P< 0.01$) was observed, indicating cytotoxicity at high concentration. Representative microscopic images showing morphological changes in HSF cells treated with capsaicin at selected concentrations are presented in Figure 4(B). Control: Cells exhibit normal spindle-shaped morphology with high confluence. Capsaicin 10 mg/mL: Marked cell shrinkage and reduced density observed, consistent with significant cytotoxicity. Capsaicin 0.1 mg/mL: Minor morphological changes with slightly reduced cell density, indicating mild cytotoxicity. Capsaicin 0.001 mg/mL: Morphology similar to control, suggesting negligible cytotoxic effect at this concentration, and morphological changes were captured under an inverted microscope at 200 µm scale.

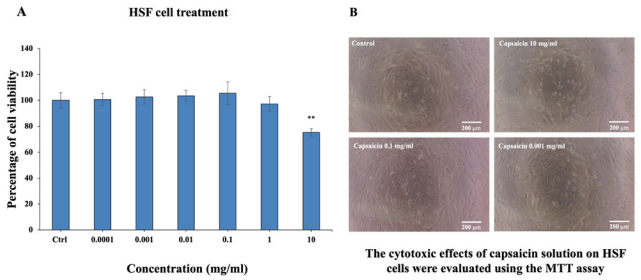

Fig. 4. *Cytotoxic effects of capsaicin on human skin fibroblast (HSF) cells assessed by MTT assay.*
(A) Percentage of cell viability following treatment with capsaicin at various concentrations (0.0001–10 mg/mL) for 24 hours. (B) Representative microscopic images showing morphological changes in HSF cells treated with capsaicin at selected concentrations.

These findings confirm that capsaicin induces dose-dependent cytotoxicity in HSF cells, with higher concentrations compromising cell viability and morphology. The half-maximal inhibitory concentration (IC_{50}) was approximately 20 mg/mL, indicating that capsaicin exhibits cytotoxicity at elevated concentrations. Morphologically, treated cells maintained normal spindle-shaped appearances at non-cytotoxic concentrations, while cell shrinkage and reduced confluence were evident at higher doses, suggesting compromised cell health.

Anti-Inflammatory Activity

The assessment of anti-inflammatory properties demonstrated that the capsaicin hydrogel patch exhibited increased efficacy at all concentrations, with the highest percentage of inhibition observed at concentrations ranging from 25 µL to 1000 µL (100%). Figure 5 illustrates the anti-inflammatory effects of the capsaicin hydrogel patch at various concentrations compared to the standard diclofenac. A comparison between diclofenac and the capsaicin hydrogel patch revealed no statistically significant difference in anti-inflammatory efficacy (P=0.183).

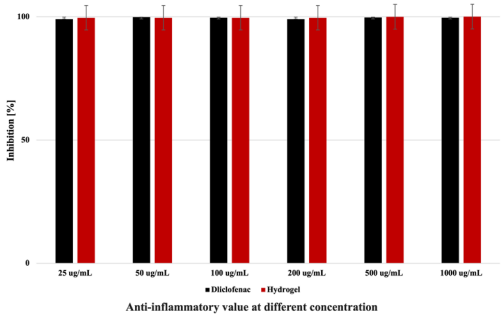

Fig. 5. *Anti-inflammatory activity of diclofenac and capsaicin hydrogel patch at various concentrations.*

Efficacy

The anti-inflammatory activity of the capsaicin hydrogel patch was evaluated to assess its potential therapeutic efficacy. The baseline characteristics of the participants are presented in Table 2.

Five participants without underlying medical conditions were included in the study. Participants applied the patch for 60 minutes both day and night at the site of pain. Pretest and post-test scores were analyzed for mean differences using a non-parametric test. The results indicated that the mean VAS score decreased after three days, but this change was not statistically significant. In contrast, the analysis of pre-test and post-test NRS scores using the Wilcoxon Signed-Rank Test revealed a statistically significant difference (P = 0.05) as shown in Table 3.

Adverse Events

Table 4 presents the adverse events caused by using the capsaicin hydrogel patch. These events were transient, resolving within a short duration. Notably, no severe adverse events were reported.

Table 2.

Main characteristics of the study participants (mean age of 26.02±4.39 years).

Variable		n	%
Sex	Male	3	60.00
	Female	2	40.00
Occupations	Students	2	40.00
	Professional Nurses	1	20.00
	Owner Business	1	20.00
	University Staff	1	20.00
Duration of computer usage for work	1-5 hours	2	40.00
	6-10 hours	2	40.00
	> 11 hours	1	20.00
Common symptoms experienced during prolonged sitting	Neck, shoulder, and back pain	2	40.00
	Numbness, Sweating	2	40.00
	Blurred vision	1	20.00

Table 3.

Wilcoxon signed ranks test results.

	Pre-test – Post-test	n	Mean rank	Sum of ranks	Z	*P*-value
VAS	Negative Ranks	2	1.50	3.00	-1.342	1.180
	Positive Ranks	0	0.00	0.00		
	Tiles	3				
	Total	5				
NRS	Negative Ranks	4	2.50	10.00	-1.890	0.050
	Positive Ranks	0	0.00	0.00		
	Tiles	1				
	Total	5				

*NRS, Numeric Rating Scale; VAS, Visual Analog Scale***;** *Z = Asymp. Sig. (2-tailed).*

Table 4.

Adverse events in volunteers caused by the capsaicin hydrogel patch (n=5).

Side effects	Day 1		Day 2		Day 3	
	n	%	n	%	n	%
Rash	1	20.00	0	0.00	0	0.00
Pruritus	2	40.00	2	40.00	1	20.00
Asymptomatic	2	40.00	3	60.00	4	80.00

Discussion

This study explored the development and characterization of a capsaicin hydrogel patch, with particular focus on its physicochemical properties and therapeutic effects. The structural and compositional attributes were analyzed through GC-MS, UV-vis spectroscopy, and FTIR, offering insights into the patch's molecular composition and light absorption behavior. Capsaicin below 1 mg/mL showed no cytotoxicity on HSF cells, maintaining viability above 90%, while higher concentrations caused dose-dependent cytotoxicity, with an IC_{50} of approximately 20 mg/mL. The patch's anti-inflammatory activity was clinically evaluated through a pre- and post-test design involving five patients suffering from muscle pain. Pain levels were measured using the Visual Analog Scale (VAS) and Numerical Rating Scale (NRS). Statistical analysis, performed using the Wilcoxon Signed-Rank test, revealed a significant reduction in pain scores post-application, demonstrating the potential efficacy of the capsaicin hydrogel patch in providing symptomatic relief.

The GC-MS analysis of the capsaicin crude extract in this study revealed a distinct profile composed primarily of dodecyl acrylate (45.78%), oleic acid (25.06%), and trans-oleic acid (7.52%), with additional contributions from 1-dodecanol, propanoic acid decyl ester, and hexadecanoic acid derivatives. These findings demonstrate a chemical fingerprint dominated by fatty acids, fatty acid esters, and long-chain alcohols. Notably, dodecyl acrylate was the most abundant compound, accounting for nearly half of the total peak area, indicating its significant presence in the bioactive matrix.

When compared with the results of Ahmad et al.,[2] who characterized the volatile composition of 27 different Capsicum annuum cultivars, several key differences and similarities emerge. Their study reported a predominance of alcohols (26.13%), hydrocarbons (18.82%), and esters (14.97%), with compounds like 1-decanol and docosanoic acid, docosyl ester as major constituents. While both studies identified esters and alcohols as major components, the proportion and dominant compounds differ notably. The present extract exhibited higher concentrations of fatty acid esters such as oleic acid ethyl ester and propanoic acid decyl ester. In contrast, Ahmad et al.[2] reported broader chemical diversity across several classes, including aldehydes, ketones, and pyrazines.

The discrepancies can likely be attributed to differences in extraction techniques, geographical source, capsicum variety, and maturity stage, as highlighted by Ahmad et al.,[2] who observed variation in volatile composition based on cultivar and origin. Furthermore, while their study emphasized volatile oils contributing to aroma and antimicrobial activity, capsaicin focuses more on fatty acid-based constituents, which are known to play roles in skin permeability enhancement, anti-inflammatory activity, and drug delivery efficiency.

This focused chemical profile may correlate with the intended functional role of capsaicin, specifically, enhancing the anti-inflammatory and analgesic properties of a capsaicin hydrogel patch. Compounds such as oleic acid and n-hexadecanoic acid have been independently reported to possess anti-inflammatory properties, suggesting a potential synergistic effect with capsaicin in the transdermal formulation.

In the present study, the capsaicin patches were subjected to an examination of their physical properties and adherence to biocompatibility both before and after testing. UV-Vis analysis was conducted at specific time intervals to assess the light absorption characteristics of capsaicin dilution. The absence of data at 0 minutes indicated that no chemical change occurred at the initial time point. However, changes began manifesting at 30, 60, 90, 120, 150, and 180 minutes. The UV-Visible scan spectrum revealed absorbance values suitable for capsaicin dilution at wavelengths of 278, 281, 280, 284, 283, and 283 nanometers, respectively. Prior research demonstrated that standard capsaicin exhibits light absorption at 279 nanometers when exposed to UV radiation.[28] Similarly, the previous report shows capsaicin's absorption peak at 281 nanometers.[29] This study demonstrated that the UV spectrophotometric method provides clear advantages in terms of simplicity, accuracy, and sensitivity for analyzing the complex structure of capsaicin. The standard curve of capsaicin in various solutions displayed a distinct peak corresponding to capsaicin.

The FTIR spectrum exhibited distinctive peaks corresponding to capsaicin (trans-8-methyl-N-vanillyl-6-nonenamide) chemical formula (C18H27NO3), indicative of typical capsaicin characteristics. These findings affirm the encapsulation of capsaicin within the hydrogel patch, aligning with previous research that explored FTIR analysis of silver nanoparticles. The FTIR spectrum of capsaicin displayed prominent absorption bands (Fig. 1). Clear peaks were observed when testing the FTIR spectrum of hydrogel patches containing capsaicin. These peak values slightly shifted to 3303, 1637, 1475, 1244, 1111, 1043, and 992 cm^{-1} in the FTIR spectrum of the hydrogel patches mixed with capsaicin. In the case of capsaicin-coated silver nanoparticles, lower maximum absorption points were identified at 1,653.96 and 1,027.44 cm^{-1}. The FTIR analysis revealed peak shifts, likely attributed to variations in peak maxima and interface features associated with the silver metal surface, as recent documents show.[30]

The peaks mentioned align well with FTIR spectra associated with capsaicin, a compound found in 2917–2846 cm^{-1}, corresponding to the aliphatic C–H stretching vibrations, typically seen in CH_2 and CH_3 groups. A peak at 1733 cm^{-1} indicates C=O stretching, which is often seen in ester or carbonyl groups; a peak at 1460 cm^{-1} represents aromatic C–C stretching, commonly observed in aromatic rings; a peak at 1165 cm^{-1} refers to C–O–C stretching, which could suggest ether linkages or similar oxygen containing groups; a peak at 720 cm^{-1} is characteristic of C–H and C–C vibrations related to the aromatic phenyl ring, confirming the aromatic nature of capsaicin. These FTIR data help establish the structure of capsaicin in various applications, such as studying its role in biological systems or determining its presence in natural products.[31] The variation in FTIR absorption between pure Capsaicin and Capsaicin blended in hydrogel sheets arises from molecular interactions between capsaicin and the hydrogel patch. The peak intensities differ slightly between pure capsaicin and the hydrogel patch containing capsaicin. For instance, the aliphatic C-H group exhibits reduced peak clarity, suggesting changes in the local environment of the capsaicin molecules due to their incorporation into the hydrogel network. These interactions likely restrict the mobility of capsaicin, leading to alterations in vibrational energy levels and, consequently, the infrared (IR) absorption. Despite these variations, the IR spectrum of the hydrogel patch retains the characteristic peaks of capsaicin, confirming its presence. Thus, the IR absorption variations can be attributed to the molecular interactions between capsaicin and the hydrogel patch.

The present study evaluated the cytotoxicity of capsaicin on human skin fibroblast (HSF) cells using the MTT assay, revealing that concentrations below 1 mg/mL preserved cell viability above 90%. In contrast, higher concentrations led to a dose-dependent decline in viability, with an IC_{50} of approximately 20 mg/mL. These findings are consistent with earlier research demonstrating that capsaicin exhibits a concentration-dependent cytotoxic effect on various cell types. For instance, Sancho et al.[32] reported that capsaicin concentrations above 100 µM (~30 µg/mL) induced apoptotic cell death in glioma cells, whereas lower concentrations had minimal impact on cell viability. Notably, the current findings highlight that HSF cells tolerate capsaicin well at low concentrations, as evidenced by the retention of normal spindle-shaped morphology and high confluence, supporting the potential use of capsaicin in topical applications when used at sub-cytotoxic levels. Morphological alterations observed at higher doses, including cell shrinkage and reduced confluence, align with previous reports indicating that high-dose capsaicin can disrupt cytoskeletal integrity and induce oxidative stress.[33] These observations reinforce the importance of dose optimization in developing capsaicin-based formulations to ensure both efficacy and safety.

Overall, the results contribute to the growing body of evidence supporting the biocompatibility of capsaicin at low concentrations and underscore the need for careful dose selection in biomedical applications.

The study demonstrated that the capsaicin hydrogel patch exhibited enhanced anti-inflammatory efficacy across all tested concentrations, with maximum inhibition (100%) observed between 25 µL and 1000 µL. Comparative analysis with diclofenac showed no statistically significant difference in anti-inflammatory effects between diclofenac and the capsaicin hydrogel patch (P>0.05). This study demonstrates the effectiveness of using low concentrations of capsaicin, showing significant anti-inflammatory properties. These findings are consistent with previous studies, which reported that induced inflammation in treated animals was associated with a corresponding rise in C-reactive protein levels, pointing to the inflammation-inducing properties of egg albumin. The results indicated that capsaicin in both forms of Capsicum frutescens Linn. [Solanaceae] (CPF) and capsaicin (Fluka Biotechnika) (CFE) demonstrated anti-inflammatory effects comparable to diclofenac in the experimental rat model (P< 0.05). It can be concluded that capsaicin possesses both analgesic and anti-inflammatory properties. The objective of the study was to determine whether pre-treatment with CPF and CFE could produce a reduction in inflammation and inhibition of egg albumin-induced edema similar to diclofenac, a standard non-steroidal anti-inflammatory agent.[34] Its anti-

inflammatory efficacy was on par with standard diclofenac, supporting its potential as a safe and effective treatment for localized pain and inflammation management.

Our study showed a significant reduction in pain after three days according to NRS scores when using a capsaicin hydrogel patch for 60 minutes twice a day. The topically applied capsaicin is acknowledged for its analgesic effects. While studies on its efficacy in MPS presented conflicting outcomes, there is a notable absence of research examining the application of capsaicin in elevated concentrations, specifically in treating peripheral neuropathic pain. Despite the established association of clinically utilized capsaicin concentrations with transient pain alleviation, sustained usage is hindered by adverse effects, notably a burning sensation post-application, leading to poor adherence. The efficacy of capsaicin has been linked to concentration-dependent outcomes. Consequently, individuals with MPS may experience prolonged and enhanced pain relief by applying concentrated capsaicin.[14,35] The safety of capsaicin patches has been reported, with findings regarding their pharmacokinetics.[36] Notably, an assessment of 8% capsaicin patches revealed minimal systemic absorption.[37] Therefore, it is inferred that the 0.1% patch is likely safe concerning local skin irritation, systemic absorption, and epidermal nerve fiber degeneration.[38] We conducted a small pilot study using topical capsaicin to treat MPS. Capsaicin selectively stimulates nociceptive neurons and has been widely used to study pain-related events. Capsaicin exerts multiple pharmacological and physiological effects, including analgesia,[39] anti-inflammation, and antioxidant effects.[40] Therefore, capsaicin may be valuable for pain relief, cancer prevention, and weight loss in clinical settings.

In addition, capsaicin also has benefits for the cardiovascular and gastrointestinal systems. The most studied capsaicinoid for pain relief is capsaicin. It has been shown that capsaicin was used to reduce the inflammatory heat and noxious chemical hyperalgesia or pain.[41] Despite the acknowledged potential for a burning sensation with capsaicin, treatment utilizing the 0.1% patch was as effective. Nevertheless, this study showed that the mean VAS decreased after three days and was not statistically significant. However, the NRS scores decreased statistically significantly. This is consistent with previous investigations.[42] No serious adverse events were reported. This aligns with the previous study,[43] which investigated the efficacy of a 0.1% capsaicin hydrogel patch for myofascial neck pain in a double-blind randomized trial. The study developed a hydrogel patch, where participants were randomly assigned to receive either a 0.1% capsaicin hydrogel patch or a control hydrogel patch without capsaicin. All participants were instructed to apply one patch to each side of the neck and shoulder, directly over the areas of greatest pain, for 12 hours per day over a 4-week period. The results showed that among the 57 patients, there was a significant reduction in mean VAS, NDI, and BDI scores at 2 and 4 weeks. No significant differences were found between the two groups in the outcome measures. In conclusion, the treatment was found to be effective.[43] Capsaicin 0.1% is widely applied topically for pain relief in osteoarthritis, neuropathic, and muscle pain.[44] Its mechanism of action involves desensitizing TRPV1 pain receptors and reducing the levels of substance P, a neuropeptide that transmits pain signals to the brain. Capsaicin's primary clinical use is for pain management.[45] Since the early 1980s, low-concentration capsaicin formulations (0.025%–0.1%) in creams, lotions, and patches have been available globally for daily application.[21] Notably, topical capsaicin exhibits minimal drug interactions and can be safely used alongside other analgesics, with generally mild adverse effects. Studies demonstrate its safety in pain management.

Conclusion

This study characterized a capsaicin-loaded hydrogel patch for transdermal application to manage myofascial pain syndrome (MPS). The hydrogel, composed of polyvinyl alcohol and gelatin, demonstrated excellent physicochemical stability and biocompatibility. GC-MS analysis revealed the presence of several bioactive compounds, including dodecyl acrylate and oleic acid, known for their anti-inflammatory and permeation-enhancing properties. FTIR and UV-Vis spectroscopy confirmed the successful incorporation and sustained release of capsaicin within the hydrogel matrix. Cytotoxicity evaluation using the MTT assay on human skin fibroblasts indicated low toxicity at therapeutic concentrations, with an IC_{50} of approximately 20 mg/mL. The hydrogel also exhibited potent anti-inflammatory activity, achieving complete inhibition of protein denaturation across tested concentrations, with efficacy statistically comparable to the standard drug diclofenac (P=0.183). In a pilot clinical trial, participants reported a significant reduction in pain intensity as measured by the Numeric Rating Scale (P=0.05), with no severe adverse effects observed.

Additionally, these findings support the capsaicin hydrogel patch as a promising, safe, and effective treatment for localized pain and inflammation, offering a potential alternative to conventional pharmacological interventions in treating MPS.

Ethical Considerations

The study protocol was reviewed and approved by the Human Research Ethics Committee, Suranaree University of Technology, Nakhon Ratchasima, Thailand (Reference letter No. EC-64-152 dated 25-02-2022). Written informed consent was obtained from all the participants.

Competing Interests

The authors declare that they have no competing interests.

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