Effect of Capsaicin on Proliferation and Wound Healing of Dental Pulp Cells In Vitro

Chonniphat Charoenwongsawad[1], Pornpoj Fuangtharnthip[1]*, Tasanee Tengrungsun[1], Theeralaksna Suddhasthira[2], Yukihiko Tamura[3]

1. Department of Advanced General Dentistry, Faculty of Dentistry, Mahidol University, Bangkok, Thailand.
2. Department of Oral and Maxillofacial Surgery, Faculty of Dentistry, Mahidol University, Bangkok, Thailand.
3. Division of Bio-Matrix, Department of Pharmacology, Graduate School of Medical and Dental Sciences, Tokyo Medical and Dental University, Tokyo, Japan.

Abstract

Aim of the study was to evaluate the effect of capsaicin on the proliferation and wound healing in dental pulp cells. A clonal cell line derived from dental pulp of the rat incisor was treated with capsaicin solutions of 1 nM - 10 µM, prepared from capsaicin gel. Cell proliferation and cytotoxity were evaluated by cell counting and using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide colorimeter assay. Areas of cell migration towards the wound scratch were measured to determine its capability on wound healing. While the 10 µM capsaicin solution expressed very high cytotoxicity, the lower concentrations (1 nM - 0.1 µM) showed a significant increase in cell viability on days 2 -7 ($P < 0.05$).

Dental pulp cells treated with 0.01 µM capsaicin solution migrated towards the scratched wound more widely ($P = 0.03$), compared to the control. Capsaicin of lower than 1 µM is non-toxic to dental pulp cells and able to stimulate proliferation and migration of the dental pulp cells, suggesting its benefit on healing dental pulp injury.

Introduction

Capsaicin (8 – methyl - N - vanillyl – 6 - nonenamide) is an active component of chili peppers, belonging to the genus Capsicum, which are widely used in many cuisines especially in Asian countries. Besides its nutritional values, capsaicin has been claimed for its pharmacological properties, such as analgesia, anti-inflammation, antimicrobial effects and anticancer effects.[1]

For decades, the use of capsaicin, as an analgesic agent in the treatment of painful disorders ranging from viral and diabetic neuropathies to rheumatoid arthritis, has been widely accepted.[2] Capsaicin selectively stimulates nociceptive sensory neurons and has been used to study pain mechanisms extensively. It has been shown that capsaicin modulates pain through the capsaicin receptor, which is known as transient receptor potential vanilloid subfamily member 1 (TRPV1).[3] Apart from capsaicin, TRPV1, expressed in nociceptive neurons, responds to noxious heat (> 43°C) and acidic conditions (pH < 6.6).[4] However, upon prolonged exposure to capsaicin, TRPV1 activity seems to decrease, resulting in desensitization of TRPV1 that is thought to cause the paradoxical analgesic effect of capsaicin.

Regarding anti-inflammatory effects, capsaicin has been shown to increase heme oxygenase-1 protein expression, inhibiting nitric oxide production and inducible nitric oxide synthase protein expression in macrophages.[5] In an animal experiment, applying capsaicin cream, containing *Capsicum annuum L.* extract, was able to significantly decrease inflammatory swelling and reduce white blood cell count. It was explained that anti-inflammatory mechanisms of capsaicin may have come from increasing local microcirculation and decreasing porosity of blood vessels.[6]

A variety of chili pepper extracts have been proved for their antimicrobial properties on gram-positive and gram-negative pathogens.[7] An in vitro study demonstrated antimicrobial and

Corresponding author:
Dr. Pornpoj Fuangtharnthip,
Faculty of Dentistry, Mahidol University,
Bangkok, Thailand. 10400
E-mail: pornpoj.fun@mahidol.ac.th

anti-virulence activities of capsaicin against *Streptococcus pyogenes* (Group A streptococci), a major human pathogen, likely through inhibition of cell invasion and haemolytic activity.[8] The antimicrobial properties of capsaicin are well considered very beneficial for medical purpose.

Capsaicin has an in vitro antiproliferative effect on prostate,[9] colon,[10] gastric,[11] hepatic,[12] lung,[13] breast[14] and leukemic cancer cells,[15] while leaving normal cells unharmed.[16] The suppression in growth of these various cancer cells occur via induction of cycle arrest, apoptosis, autophagy, and/or via the inhibition of cellular metabolic activation.[14,17,18] Moreover, capsaicin is shown to inhibit migration of melanoma and breast cancer cells.[14,19]

Despite the antiproliferative effect on cancer cells, capsaicin seems to have different effects on cell viability depending on cell types. Bone mesenchymal stem cells after exposure to capsaicin showed reduction in cell viability and proliferation via 2 pathways; capsaicin induced cell cycle arrest and increased apoptosis.[20] Hepatic stellate cells, the major cell type involved in formation of scar tissue in response to liver damage, were decreased in number, when treated with capsaicin.[21] On the contrary, capsaicin potentially increased airway smooth muscle cells (ASMCs) proliferation through stimulating TRPV1 activity and inhibiting apoptosis.[22] The proliferation of gingival epithelial cells (GECs) treated with capsaicin was also increased, with a conclusion that functional TRPV1 expressed by GECs was involved in the regulation of cell proliferation.[23]

Dental pulp is mainly composed of undifferentiated mesenchymal cells, fibroblasts, and odontoblasts, which have their healing capability. Once dental pulp is injured, treatment on the dental pulp becomes necessary, in order to eradicate the infection and heal the remaining one. Researchers have been searching for any supplement that could well promote dental pulp healing.[24] With its pharmaceutical properties, capsaicin may be beneficial during the process of dental pulp healing. Previously, in vivo study revealed, the anti-inflammation effect of capsaicin in animal dental pulp.[6,25] Nonetheless, there were few evidences on cytotoxicity and wound healing activity of capsaicin on dental pulp cells. Our study was aimed to assess in vitro the toxicity of capsaicin to dental pulp cells as well as its effect on dental pulp wound healing, if any.

Materials and methods

Preparation of capsaicin solution

A local commercial product, capsaicin gel (Government Pharmaceutical Organization, Bangkok, Thailand) was used in this study. Capsaicin gel contains 0.25 g of capsaicin in 100 g of base gel. The 1.4 g of capsaicin gel was dissolved with a volume of 10 ml Dulbecco's modified Eagle's medium (DMEM) and filtered by 0.02 μm syringe filter to obtain 100 μM capsaicin solution. The 100 μM capsaicin solution was diluted with DMEM to make different concentrations of 1 nM, 0.01 μM, 0.1 μM, 1 μM and 10 μM capsaicin (w/v), before being used.

Rat dental pulp cell culture and treatment

RPC-C2A cells, a clonal cell line derived from dental pulp of the rat incisor, were used in the study.[26] RPC-C2A cells were cultured in DMEM, supplemented with 10% fetal bovine serum, $NaHCO_3$ and penicillin at 1×10^4 units/ml, and incubated at 37°C in humidified atmosphere of 95% air and 5% CO_2. The culture medium was changed every 2 days. In the treatment groups, RPC-C2A cells were treated with 1 nM, 0.01 μM, 0.1 μM, 1 μM and 10 μM capsaicin solutions for 24 hours, while cells in the control group were treated in the same way with DMEM containing no capsaicin. The cells were further investigated for cell proliferation, cytotoxicity. Except for wound healing assay, the treatment with capsaicin occurred after the scratched wound of cultured cells had been formed.

Cell proliferation

Cell proliferation was evaluated by cell counting. The seeding density was 9×10^3 cells per well in a 24-well plate containing 500 μl of culture medium. On days 1, 2, 4 and 7, the cells from each well were trysinized with 500 μl of 0.25% trypsin for 2 minutes. After removal of trypsin, the cell suspension was made by adding 500 μl of culture medium, and pipetted thoroughly before 10μl of the cell suspension was transferred to Eppendorf micro-tube. The 10 μl of 0.4% trypan blue solution was added and well mixed. Then, 10μl of the mixed suspension was drawn and applied to the counting chamber of hemocytometer for cell counting.

Cytotoxicity: MTT assay

Cytotoxicity test was assessed by using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay (Sigma,

USA). To evaluate the number of viable cells, the seeding density was 1.6×10^3 cells per well in a 96-well plate containing 100 µl of culture medium. On days 1, 2, 4 and 7 after the treatment with capsaicin solutions, the cells were tested with MTT assay. Briefly, 50µl of MTT solution was added into each well and incubated for 2 hours after rinsing with 100µl of PBS. Then, 100µl of isopropanol was added into each well and placed on a shaker for 30 minutes. The absorbance was measured at 570 nm by microplate reader.

Wound healing assay

Cell migration was observed under the wound healing model on monolayer culture. A confluent monolayer of RPC-C2A cells was cultured on 24-well plates at an initial seeding density of 5×10^4 cells in 500 µl of medium per well. Cells were incubated for 24 hours. Then, a wound was created in the culture cells by gently scraping with a 1,000 µl-sized sterile pipette tip. The scratched wound areas were observed under a converted microscope and taken pictures at the beginning of time zero (T0). The width of wound areas was measured with Image Pro-Plus software (Media Cybernetics), and accepted when it was 1.2 mm ± 50µm wide.

After the wound was created, the cells were treated with capsaicin solutions (concentrations of 1 nM, 0.01 µM, 0.1 µM and 1 µM (w/v)): the 10 µM capsaicin solution was skipped due to its extensive cell toxicity, previously found in the cytotoxicity study. Cells were incubated for 8 hours, in order to allow the remaining cells to migrate towards the scratched wound area. When migration time elapsed, the whole cells on the wells were fixed with methanol solution and stained with 2% Giemsa solution (Merck, Germany). The scratched wound areas in the middle of the plate with the length of 2.74 mm were photographed and analyzed by measuring the area that the cells had migrated beyond the beginning scratch line at T0, using Image Pro-Plus software. Each experiment was performed in triplicate.

Statistical analysis

Statistical analyses were performed by using SPSS software (Version 18.0, Standard Software Package Inc., U.S.A.). Data of cell proliferation, cytotoxicity and migration areas in wound healing assay were presented as the mean ± SD. Kolmogorov Smirnov test was used to find out the normality of the data, and differences of the means between the control and capsaicin-treated groups were assessed with the Student's t-test at a significant level of 0.05.

Results

Cell proliferation

No viable cells were detected in the 10 µM group throughout all periods of observation. The results of cell proliferation after treated with capsaicin solutions (1 nM - 1 µM), evaluated by cell counting, are shown as in Figure 1. On days 1 and 2, all capsaicin-treated groups except the 10 µM showed similar numbers in cell counting, compared to the control group. On days 4 and 7, cell numbers in the 1 µM group were apparently decreased compared to the control with statistical difference (P = 0.004). However, cell numbers of the 1 nM group were found significantly higher (P = 0.000) on day 7.
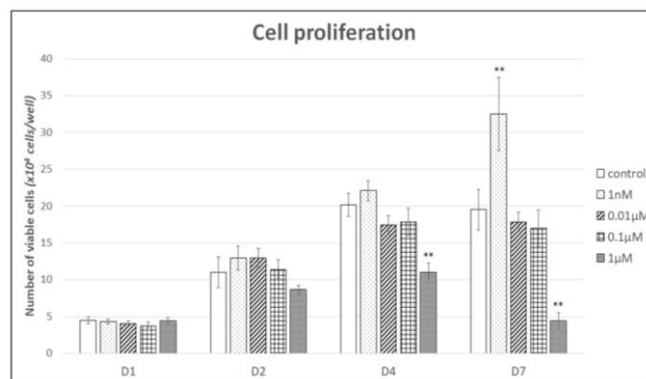

Figure 1. Cell proliferation shown as numbers of RPC-C2A cells after treated with capsaicin solutions (1 nM, 0.01 µM, 0.1 µM and 1 µM) during days 1-7. The cell number in the 1 nM group is higher on day 7, while those of the 1 µM are lower on days 4 and 7, when compared to the control. ** P< 0.01 vs. control.

Cytotoxicity

The cytotoxicity of capsaicin to the RPC-C2A cells, using MTT assay was shown in Figure 2. Along the observation periods, the 10 µM group exhibited strongly cytotoxic effect on the RPC-C2A cells, whereas the lower concentrations of capsaicin solutions were non-toxic. On day 1, MTT assay showed similar mean values of optical density absorbance for all groups. On days 2 and 4, significant increase in absorbance values of the 0.1 µM, 0.01 µM and 1 nM capsaicin groups were found (P < 0.05),

while the 1 µM group had lower value than the control without statistical significance. The result of day 7 showed the similar values for all groups, except for the 10 µM group.
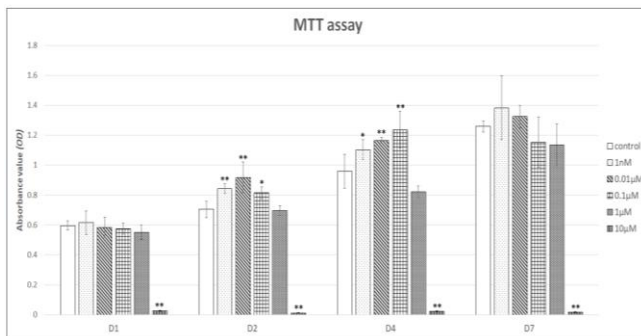
Figure 2. Mean values of absorbance of the MTT assay show cytotoxicity of capsaicin after RPC-C2A cells were treated with capsaicin solutions (1 nM - 10 µM) during days 1-7. The values of the 10 µM group are very low, showing the cytoxicity, while those of the 1 nM – 0.1 µM are higher than the control on days 2 and 4.
*$P < 0.05$; **$P < 0.01$ vs. control.

|	Migration area (mm^2) Mean ± SD	P-value
Control	1.45 ± 0.26	
Capsaicin 1 nM	1.08 ± 0.15	0.11
Capsaicin 0.01 µM	2.12 ± 0.25	0.03*
Capsaicin 0.1 µM	1.28 ± 0.15	0.40
Capsaicin 1 µM	0.82 ± 0.35	0.95

Table 1. Measurement of the migration areas after 8 hours towards the scratch zones.
SD: standard deviation.
*Significant difference at p<0.05 when compared with the control.

Wound healing assay

Wound healing was assessed by measuring the areas in which cell migration had occurred. After treated with capsaicin solutions for 8 hours, the migration of RPC-C2A cells towards the scratched wound area could be observed obviously under a microscope in every treatment group, as shown in Figure 3. The measurement of the migration areas, shown in Table 1, revealed that the 0.01 µM group had a wider migration area (2.12 ± 0.25 mm^2) with a statistical significance ($P = 0.03$). However, other capsaicin groups did not exhibit a significant difference, compared to the control.
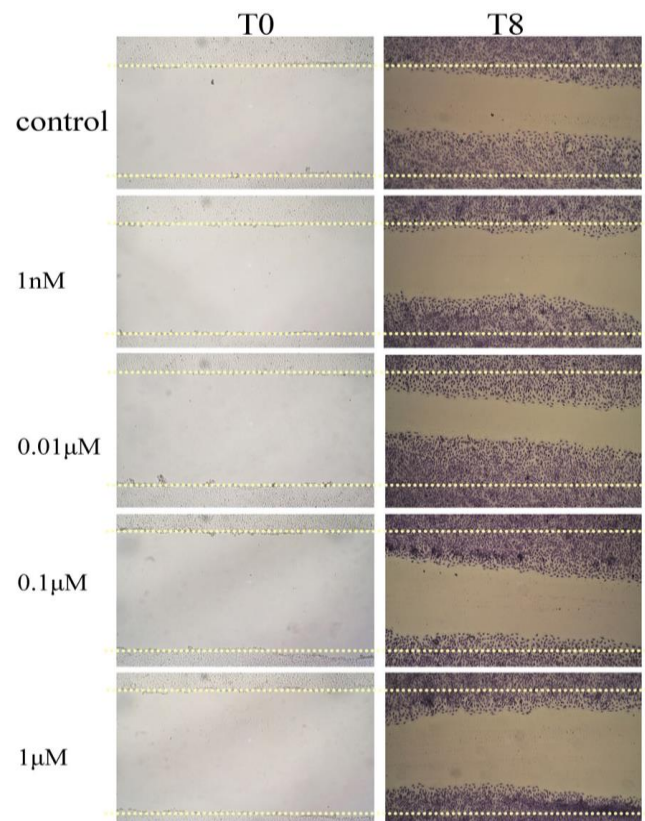
Figure 3. Micrographs of the RPC-C2A cell migration towards the scratched wound at 8 hours (T8) after treated with capsaicin solutions (1 nM - 1 µM). All groups show cell migration from the beginning time (T0) towards the scratched wound and the wound areas are decreased at T8. Dotted lines indicate the beginning scratch line at T0.

Discussion

The application of capsaicin on oral health has increased recently. Due to its analgesic effect, capsaicin is used to treat neuropathic pain in trigeminal neuralgia and temporomandibular disorders.[27,28] Ingestion of capsaicin is suggested to stimulate salivary secretion and enhance swallowing reflex in dysphagia patients.[29,30] However, its use on dental pulp treatment remains very rare due to lack of scientific evidence on the effect of capsaicin on dental pulp cells. In our present study, we investigated cell proliferation by counting the viable cells on the plate after capsaicin treatment and its cytotoxicity using the MTT assay, in order to find out the effect of capsaicin on dental pulp cells and its appropriate concentrations to be tested on wound healing assay.

The capsaicin concentration of 10 µM, the highest in our study, caused total cell death at all time of observation, while other lower concentrations (≤1 µM) could keep normal cell viability with the increased cell numbers at specific times (Fig. 1 and 2). These suggest strong cytotoxicity of 10 µM capsaicin to dental pulp cells, when lower concentrations (1 nM, 0.01 µM and 0.1 µM) of capsaicin are likely to be non-cytotoxic and somehow promote cell viability that was observed on days 2 and 4. Our results on cell proliferation and cytotoxicity seem to agree with previous studies of ASMCs and GECs that low concentration (1 µM) of capsaicin was non-cytotoxic and significantly increased cell proliferation.[22,23] Nevertheless, our RPC-C2A cells are likely a bit more sensitive to capsaicin than these cells, giving some decreased cell numbers when treated with 1 µM capsaicin on days 4 and 7 (Fig.1). This can happen due to difference in cell characteristics and cell type. Known as a capsaicin receptor, it was explained that functional TRPV1 expressed by ASMCs and GECs may contribute to the regulation of cell proliferation by capsaicin.[22,23] Some have demonstrated that human dental pulp cells including odontoblasts also expressed heat/mechanosensitive TRP channels, including TRPV1,[31-33] leading to the possibility that it plays the same role in regulating cell proliferation of dental pulp cells when treated with capsaicin.

Interestingly, it has been evidenced that activation of TRPV1 in sensory neurons can evoke the release of neuropeptides, including substance P and calcitonin gene-related neuropeptide (CGRP), which are major initiators of neurogenic inflammation[34,35] and likely contribute to peripheral sensitization in the trigeminal sensory system and dental pain.[36,37] This brings us a question of whether capsaicin can modulate inflammation of the dental pulp cells via TRPV1 activation and the release of proinflammatory mediators.

There are several reports on its anti-cancer effect both in vitro and in vivo. High concentrations of capsaicin solution (100-350 µM) showed cytotoxicity to many kinds of cancer cells, such as tongue,[38] prostate,[9] colon,[13] gastric[11] and breast cancer cells.[14] Furthermore, even low concentrations of capsaicin (0.1-10 µM) were shown to prevent formation of pre-cancerous lesions in mammary organ cultures.[14] The anti-cancer effect was explained to occur via induction of cell cycle arrest, apoptosis, autophagy, and/or via the inhibition of cellular metabolic activation.[17,13,14,38] However, despite the anti-proliferative effect, Lui et al. demonstrated that low concentrations of capsaicin (0.1-10 µM) enhanced cell proliferation of HCT116 human colon carcinoma cells by up-regulating tumor-associated NADH oxidase.[39] Data from these studies suggest that capsaicin may exert dual effects – anti-proliferative or proliferation promoting - depending on the cellular targets of capsaicin and the molecular mechanisms induced by capsaicin.

Regarding wound healing assay, we chose to examine cell migration over the wound area using two-dimensional mono-layer model as described by Shin et al. and Takahashi et al., due to its simplicity and reliability.[19,23] In our study, capsaicin did not inhibit cells migrating towards the wound area in every treated group (1 nM-1 µM). Interestingly, 0.01 µM capsaicin treated group was very likely to have a stimulating effect of migration on RPC-C2A cells, while others could maintain their cell migration covering over the wound area. At first, we also concerned about the influence of cell proliferation overcoming our cell migration results. But considering the fact that none of the capsaicin-treated groups (1 nM-1 µM) showed different numbers in cell counting and absorbance value of the MTT assay at 24 hours, it is unlikely that, at the observation time of cell migration (8 hours), any enhanced proliferation of the cells could overcome the results of cells migrating towards the scratched wound.

Promotion of cell migration induced by capsaicin has been also reported in human hepatoblastoma (HepG2) cells. Low concentrations (≤0.1 µM) of capsaicin showed stimulatory cell migration of HepG2 cells, while migration was not affected with some higher concentrations of capsaicin (≥1 µM).[40] A moderate activation of TRPV1 channels were explained to stimulate cell migration via modification of the microtubule dynamics of the cells, whereas disruption of the microtubule network initiated by higher concentrations of capsaicin may cause the inhibition of cell migration. Others studies expressed the inhibition of cell migration in melanoma cells and normal hepatic stellate cells induced with high concentrations (100 -200 µM) of capsaicin.[19,21]

Cell proliferation and migration play an

important role in several physiological processes including wound healing. Our results on stimulatory effects of capsaicin on proliferation and migration of dental pulp cells have provided the possibility of clinical use of capsaicin on vital dental pulp therapy.

Conclusions

In our study, it is demonstrated that higher concentration (10 µM) of capsaicin is toxic to dental pulp cells, while lower concentrations (<1 µM) of capsaicin are non-toxic and able to stimulate proliferation and migration of the dental pulp cells. This suggests that capsaicin is likely to be a beneficial effect on dental pulp wound healing.

Acknowledgments

Declaration of Interest

The authors report no conflict of interest.

References

1. Luo XJ, Peng J, Li YJ. Recent advances in the study on capsaicinoids and capsinoids. Eur J Pharmacol 2011;650(1):1-7.
2. Campbell E, Bevan S, Dray A. Clinical applications of capsaicin and its analogues. in: Wood J, Capsaicin in the study of pain. London: Academic Press, 1993. p. 255–72.
3. Caterina MJ, Schumacher MA, Tominaga M, Rosen TA, Levine JD, Julius D. The capsaicin receptor: a heat-activated ion channel in the pain pathway. Nature 1997;389(6653):816-24.
4. Dhaka A, Viswanath V, Patapoutian A. TRP ion channels and temperature sensation. Annu Rev Neurosci 2006;29:135-61.
5. Kim Y, Lee J. Anti-inflammatory activity of capsaicin and dihydrocapsaicin through heme oxygenase-1 induction in Raw264.7 macrophages. J Food Biochem 2014;38(4):381-7.
6. Boiko YA, Kravchenko I, Mohammad A, Shandra A. Anti-inflammatory effect of semisolid dosage forms containing capsaicin. Sud J Med Sc 2016;11(4):151-7
7. Omolo MA, Wong ZZ, Mergen K, et al. Antimicrobial properties of chili peppers. J Infect Dis 2014;2(4):145.
8. Marini E, Magi G, Mingoia M, Pugnaloni A, Facinelli B. Antimicrobial and anti-virulence activity of capsaicin against erythromycin-resistant, cell-invasive group a streptococci. Front Microbiol 2015;6:1281.
9. Mori A, Lehmann S, O'Kelly J, et al. Capsaicin, a component of red peppers, inhibits the growth of androgen-independent, p53 mutant prostate cancer cells. Cancer Res 2006;66(6):3222–9.
10. Kim CS, Park WH, Park JY, et al. Capsaicin, a spicy component of hot pepper, induces apoptosis by activation of the peroxisome proliferator-activated receptor gamma in HT-29 human colon cancer cells. J Med Food 2004;**7**(3):267–73.
11. Lo YC, Yang YC, Wu IC, et al. Capsaicin-induced cell death in a human gastric adenocarcinoma cell line. World J Gastroenterol 2005;11(40):6254–7.
12. Jung MY, Kang HJ, Moon A. Capsaicin-induced apoptosis in SK-Hep-1 hepatocarcinoma cells involves Bcl-2 downregulation and capsase-3 activation. Cancer Lett 2001;165(2):139–45.
13. Choi CH, Jung YK, Oh SH. Selective induction of catalase-mediated autophagy by dihydrocapsaicin in lung cell lines. Free Radical Bio Med 2010;49(2):245-57.
14. Thoennissen NH, O'kelly J, Lu D, et al. Capsaicin causes cell-cycle arrest and apoptosis in ER-positive and-negative breast cancer cells by modulating the EGFR/HER-2 pathway. Oncogene 2010;29(2):285-96.
15. Zhang J, Nagasaki M, Tanaka Y, Morikawa S. Capsaicin inhibits growth of adult T-cell leukemia cells. Leukemia Res 2003;27(3):275-83.
16. Ito K, Nakazato T, Yamato K, et al. Induction of apoptosis in leukemic cells by homovanillic acid derivative, capsaicin, through oxidative stress: implication of phosphorylation of p53 at Ser-15 residue by reactive oxygen species. Cancer Res 2004;64(3):1071–8.
17. Ghosh AK, Basu S. Fas-associated factor 1 is a negative regulator in capsaicin induced cancer cell apoptosis. Cancer Lett 2010;287(2):142-9.
18. Choi CH, Jung YK, Oh SH. Autophagy induction by capsaicin in malignant human breast cells is modulated by p38 and extracellular signal-regulated mitogen-activated protein kinases and retards cell death by suppressing endoplasmic reticulum stress-mediated apoptosis. Mol Pharmacol 2010;78(1):114-25.
19. Shin DH, Kim OH, Jun HS, Kang MK. Inhibitory effect of capsaicin on B16-F10 melanoma cell migration via the phosphatidylinositol 3-kinase/Akt/Rac1 signal pathway. Exp Mol Med 2008;40(5):486.
20. Ibrahim M, Jang M, Park M, et al. Capsaicin inhibits the adipogenic differentiation of bone marrow mesenchymal stem cells by regulating cell proliferation, apoptosis, oxidative and nitrosative stress. Food Funct 2015;6(7):2165-78.
21. Bitencourt S, Mesquita F, Basso B, et al. Capsaicin modulates proliferation, migration, and activation of hepatic stellate cells. Cell Biochem Biophys 2014;68(2):387-96.
22. Zhao LM, Kuang HY, Zhang LX, et al. Effect of TRPV1 channel on proliferation and apoptosis of airway smooth muscle cells of rats. J Huazhong U Sci-Med 2014;34(4):504-9.
23. Takahashi N, Matsuda Y, Yamada H, et al. Epithelial TRPV1 signaling accelerates gingival epithelial cell proliferation. J Dent Res 2014;93(11):1141-7.
24. Illmilda, Asrianti D, Margono A, Julianto I, Wardoyo MP. Advanced platelet rich fibrin (A-PRF) supplemented culture medium for human dental pulp stem cell proliferation. J Int Dent and Med Res 2019;12(2):396-400.
25. Caviedes-Bucheli J, Azuero-Holguin MM, Munoz HR. The effect of capsaicin on substance P expression in pulp tissue inflammation. Int Endod J 2005;38(1):30-3.
26. Kasugai S, Adachi M, Ogura H. Establishment and characterization of a clonal cell line (RPC-C2A) from dental pulp of the rat incisor. Arch Oral Biol 1988;33(12):887-91
27. Epstein JB, Marcoe JH. Topical application of capsaicin for treatment of oral neuropathic pain and trigeminal neuralgia. Oral Surg Oral Med Oral Pathol 1994;77(2):135-40.
28. Hersh EV, Balasubramaniam R, Pinto A. Pharmacologic management of temporomandibular disorders. Oral Maxillofacial Surg Clin N Am 2008;20(2):197–210
29. Kono Y, Kubota A, Taira M, Katsuyama N, Sugimoto K. Effects of oral stimulation with capsaicin on salivary secretion and neural activities in the autonomic system and the brain. J Dent Sci 2018;13(2):116-23
30. Ebihara T, Takahashi H, Ebihara S, et al. Capsaicin troche for swallowing dysfunction in older people. J Am Geriatr Soc 2005;53(5):824-8.

31. Wen W, Que K, Zang C, et al. Expression and distribution of three transient receptor potential vanilloid (TRPV) channel proteins in human odontoblast-like cells. J Mol Histol 2007;48(5-6): 367–77.
32. Son AR, Yang YM, Hong JH, Lee SI, Shibukawa Y, Shin DM. Odontoblast TRP channels and thermo/mechanical transmission. J Dent Res 2009;88(11):1014-9.
33. Miyamoto R, Tokuda M, Sakuta T, Nagaoka S, Torii M. Expression and characterization of vanilloid receptor subtype 1 in human dental pulp cell cultures. J Endod 2005;31(9):652-8.
34. Richardson JD, Vasko MR. Cellular mechanisms of neurogenic inflammation. J Pharmacol Exp Ther 2002;302(3):839-45.
35. Li D, Ren Y, Xu X, Zou X, Fang L, Lin Q. Sensitization of primary afferent nociceptors induced by intradermal capsaicin involves the peripheral release of calcitonin gene-related peptide driven by dorsal root reflexes. J Pain 2008;9(12):1155-68.
36. Cady RJ, Glenn JR, Smith KM, Durham PL. Calcitonin gene-related peptide promotes cellular changes in trigeminal neurons and glia implicated in peripheral and central sensitization. Mol Pain 2011;7:94.
37. Hossain MZ, Bakri MM, Yahya F, Ando H, Unno S, Kitagawa J. The role of transient receptor potential (TRP) channels in the transduction of dental pain. Int J Mol Sci 2019; 20(3):526.
38. Ip SW, Lan SH, Huang AC, et al. Capsaicin induces apoptosis in SCC-4 human tongue cancer cells through mitochondria dependent and independent pathways. Environ Toxicol 2012;27(6):332-41.
39. Liu NC, Hsieh PF, Hsieh MK, et al. Capsaicin-mediated tNOX (ENOX2) up-regulation enhances cell proliferation and migration in vitro and in vivo. J Agr Food Chem 2012;60(10):2758-65.
40. Waning J, Vriens J, Owsianik G, et al. A novel function of capsaicin-sensitive TRPV1 channels: involvement in cell migration. Cell Calcium 2007;42(1):17-25.