CHAPTER II
LITERATURE REVIEWS

2.1 Capsaicin

2.1.1 Structure of Capsaicin

Capsaicin was first identified in a partially purified crystalline form by Christian Friedrich Bucholz in 1816. Subsequently, in 1876, John Clough Thresh succeeded in isolating it in its pure crystalline form and officially named the compound capsaicin. In addition, the physiological effects of capsaicin were first documented by Rudolf Buchheim, who observed that it elicited a burning sensation upon contact with mucous membranes and also stimulated the secretion of gastric juice (Bode & Dong, 2011). Furthermore, capsaicin's chemical structure was discovered in the early twentieth century by scientists L. E. Dawson and E. K. Nelson (Idrees et al., 2020). More specifically, capsaicin (trans-8-methyl-N-vanillyl-6-nonenamide) is the primary bioactive compound found in chili peppers, which belong to the *Capsicum* genus (Mullins et al., 2022). In its pure form, capsaicin is a colorless, odorless solid with a texture ranging from crystalline to waxy, and it is insoluble in water. In terms of chemistry, it is classified as an acid amide, consisting of vanillylamine and a fatty acid moiety containing a carbon chain length between C8 and C13. As shown in Figure 2.1, capsaicin contains a 3-methoxy-4-hydroxybenzylamine (vanilloid) ring and an alkyl side chain, which are essential to its biological activity. In a similar fashion, structurally related analogs are synthesized through similar pathways but have shorter fatty acid chains, which influence their binding affinity and activation potential at the capsaicin (TRPV1) receptor. Of these, capsaicin exhibits the highest binding affinity to the vanilloid receptor, making it the most pungent and pharmacologically potent molecule within the *Capsicum genus*.

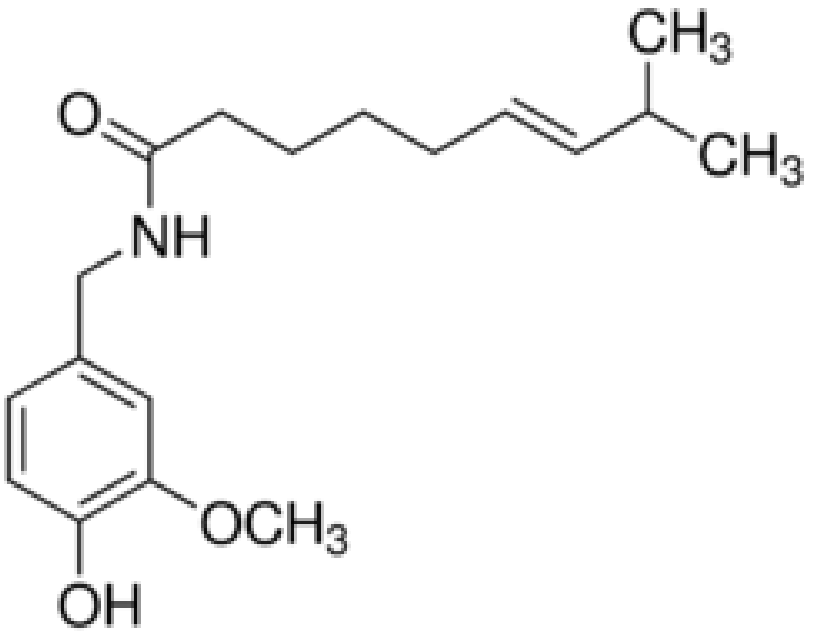

Figure 2.1 Chemical structure depiction the structure of capsaicin

The chemical structure of capsaicin comprises three key components: a vanilloid ring, specifically a 3-methoxy-4-hydroxybenzylamine moiety, and a hydrophobic alkyl side chain. These structural elements are critical to its biological activity, particularly its high-affinity interaction with the TRPV1 receptor ($C_{18}H_{27}NO_3$) (Mullins et al., 2022).

2.1.2 The molecular targets of capsaicin

Over the years, capsaicin has garnered significant attention in the scientific literature as a promising anti-cancer agent, primarily due to its antiproliferative effects. Thus, this review aims to comprehensively examine the principal mechanisms of capsaicin-mediated analgesia reported in contemporary studies and propose an integrated model of its pain-relieving actions. In particular, capsaicin exerts its effects primarily through the transient receptor potential vanilloid 1 (TRPV1) receptor, a ligand-gated, nonselective cation channel predominantly expressed in sensory neurons, particularly those involved in nociception. Currently, both agonists and antagonists targeting TRPV1 are currently undergoing clinical investigation for their analgesic potential. In this context, capsaicin itself acts as a potent TRPV1 agonist, triggering receptor activation that underlies its characteristic effects on pain modulation (Luo et

al., 2011). Additionally, according to (Bode & Dong, 2011), capsaicin exerts its physiological effects on sensory neurons primarily through intracellular binding to the transient receptor potential vanilloid 1 (TRPV1) receptor. Its role was pivotal in the identification and characterization of TRPV1, and the analgesic properties of capsaicin are largely attributed to this receptor interaction. Moreover, in recent years, significant advances have been made in elucidating the molecular mechanisms underlying capsaicin-induced pain relief. Furthermore, beyond its analgesic effects, capsaicin has been shown to modulate key signaling pathways involved in carcinogenesis and tumor progression, suggesting a potential antineoplastic role (Wang et al., 2016). Interestingly, in the majority of cases, capsaicin's effects on cancer cell metabolism appear to occur independently of TRPV1 activation, suggesting alternative molecular targets may be involved in its antineoplastic mechanisms (Rollyson et al., 2014). In line with this, our findings appear to support existing recommendations, indicating that complete. TRPV1 blockade may be a viable strategy for pain relief. Finally, it is noteworthy that capsaicin exhibits a relatively short half-life in systemic circulation. Therefore, to evaluate the oral bioavailability of capsaicin in humans, several pharmacokinetic parameters such as absorption rate, plasma concentration, and elimination half-life have been utilized.

2.1.3 The pharmacokinetics of capsaicin

Capsaicin is a naturally occurring protoalkaloid and the principal pungent compound found in chili peppers (*Capsicum annuum L*.). More specifically, chemically identified as trans-8-methyl-N-vanillyl-6-nonenamide, capsaicin is a crystalline, off-white, lipophilic solid that is both colorless and odorless. It has a melting point of 62–65 °C and is insoluble in water, though it readily dissolves in organic solvents such as ethanol, acetone, and fatty oils (Ilie et al., 2019). Furthermore, capsaicin is well recognized for its effective transdermal absorption. For instance, in a study involving 12 participants who received a 3% capsaicin formulation delivered via three different topical vehicles, capsaicin demonstrated rapid absorption and quickly reached its maximum plasma concentration following application. Additionally, the compound exhibits an approximate elimination half-life of 24 hours, supporting its suitability for sustained topical therapeutic use (Pershing et al., 2004). In another study,

a comprehensive investigation into the tissue distribution, elimination, and metabolism of capsaicin in animal models following oral administration revealed that approximately 94% of the administered dose was absorbed, with peak plasma concentrations occurring within 1 hour. Interestingly, within the same timeframe, 24.4% of the absorbed capsaicin was distributed across the blood, liver, kidneys, and intestines. Nevertheless, tissue levels declined sharply and became undetectable after four days. Moreover, *in vitro* studies using human skin demonstrated that capsaicin undergoes slow biotransformation, with the majority of the compound remaining unchanged. Only a small portion was metabolized into vanillylamine and vanillic acid. Thus, excretion of capsaicin primarily occurs via the renal and gastrointestinal pathways, with the compound eliminated through urine and feces (Reyes-Escogido et al., 2011). Additionally, capsaicin is initially found in plasma 10 minutes after capsicum consumption. Capsaicin had a maximal plasma concentration (C_{max}) of 2.47 $\pm$ 0.13 ng/ml and a T_{max} of 47.08 $\pm$ 1.99 minutes. As a result, the area under the curve (AUC_{0-t}) showed that the amount of capsaicin absorbed into the body was 103.6 $\pm$ 11.3 ng.min/mL. (Chaiyasit et al., 2009; Wang et al., 2017). *In vitro* studies have shown that capsaicin metabolism in human skin proceeds slowly, and the use of topically applied capsaicin patches with an extended elimination half-life reflects a sustained release of the compound at the application site. Collectively, these distinctive pharmacokinetics properties make topical delivery an optimal route for the therapeutic administration of capsaicin in the management of various clinical conditions.

2.1.4 The roles of capsaicin in pain relief

Capsaicin has been used extensively throughout the history of folk medicine. Specifically, this practice relies heavily on the concept of "using like to treat like," which means, for instance, treating a chemical that causes burning pain with another substance that causes burning sensation. For example, in 1850, a recommendation was made to apply an alcoholic extract of hot peppers to any parts of the body that were burning or itching (Turnbull, 1850). Moreover, capsaicin-induced analgesia's underlying processes are increasingly being explored. In particular, receptor

activity is inhibited after long or repetitive exposure to capsaicin, which is known as desensitization in the context of TRPV1.

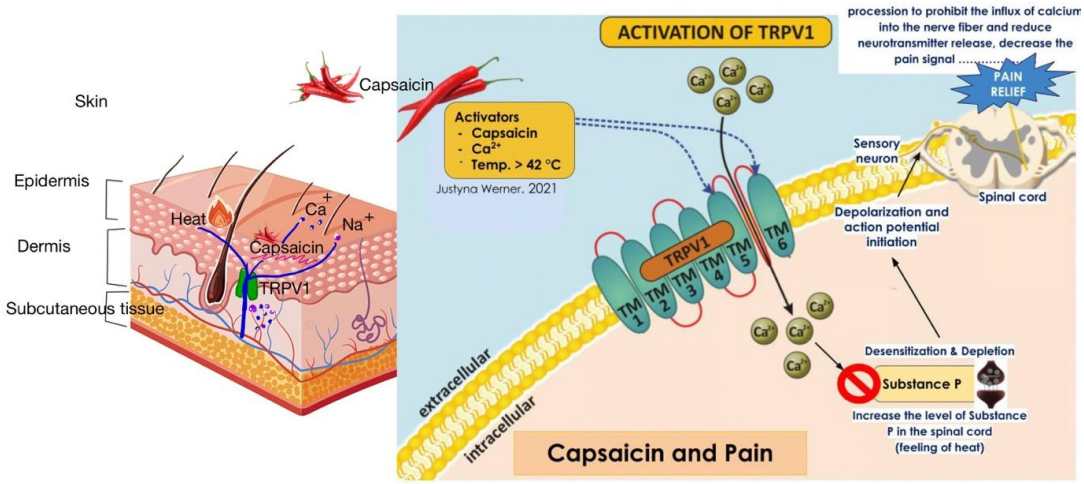

Figure 2.2 The mechanism of capsaicin and pain
(Jeszka-Skowron et al., 2021)

In particular, capsaicin-induced defunctionalization occurs through multiple interrelated mechanisms. For example, one primary pathway involves the direct pharmacological desensitization of TRPV1 receptors on the plasma membrane, as well as the inhibition of voltage-gated sodium (Na^+) channels, both of which contribute to an immediate reduction in neuronal excitability and responsiveness. Moreover, extracellular calcium (Ca^{2+}) influx through TRPV1 channels, coupled with release from intracellular stores, may exceed the cell's buffering capacity, leading to the activation of calcium-dependent proteases and subsequent cytoskeletal degradation. As a result, disruption of microtubule integrity may further impair axonal transport, contributing to functional loss. Furthermore, at supraphysiological concentrations, capsaicin can also induce mitochondrial dysfunction by inhibiting electron transport chain activity, positioning mitochondria as a critical convergence point in the cascade of defunctionalization events. In order to better understand its effects, capsaicin is an agonist following:

1. The transient receptor potential vanilloid 1 (TRPV1) receptor is a transmembrane ion channel complex that is activated by noxious heat (≥ 43 °C), acidic

conditions (pH < 6), and certain endogenous lipid mediators. Exposure to one or more of these stimuli can transiently open the channel, leading to membrane depolarization.

2. A-delta and C fibers express TRPV1 often, so depolarization causes action potentials that convey impulses to the central nervous system.

3. There are several capsaicin effects that come from these electrical impulses. Capsaicin also activates these receptors for a longer period of time than environmental agonists, causing a "defunctionalization" of sensory responses.

4. Over time, capsaicin impairs nociceptor function through disrupting enzymatic and osmotic processes, as well as cytoskeletal and osmotic structure.

5. In addition, TRPV1 activation upon application of Capsaicin, the skin ingests the components and activates the TRPV1 Receptor, which activates the C-Fiber to transmit pain signals and raises the level of Substance P in the spinal cord. This causes the sensation of heat to increase as a result of the increased activity in the pain pathway.

6. Notably, Depletion and desensitization are two related concepts. P - for substance.

Regular applications of capsaicin for 2 to 3 weeks result in desensitization of TRPV1 and Substance P and a reduction in neurotransmitter release, which reduces the pain signal or the sensation of pain.

Moreover, recent studies suggest that capsaicin-induced analgesia may be mediated not only through TRPV1 activation and subsequent desensitization but also by the inhibition of Piezo proteins—a family of mechanically activated cation-selective ion channels in mammals. Activation of TRPV1 by capsaicin triggers calcium-dependent activation of phospholipase Cδ (PLCδ), which in turn leads to the depletion of phosphoinositides. This biochemical change is associated with the suppression of Piezo channel activity, as evidenced by the inhibition of inward ionic currents during mechanical stimulation. Notably, the suppression of Piezo function is reversible upon cytosolic reintroduction of phosphoinositides, as demonstrated in excised inside-out patch clamp experiments. These findings highlight a novel mechanism by which TRPV1 activation indirectly modulates mechanosensation

through Piezo channel regulation (Borbiro et al., 2015). Overall, this study provides insight into the mechanisms underlying capsaicin-induced mechanical analgesia at the local level. Capsaicin has been widely used in the management of muscle pain, joint discomfort, and neuropathic pain, with numerous studies supporting its efficacy and safety. Topical chili-based formulations have demonstrated therapeutic benefits in the treatment of chronic pain conditions such as osteoarthritis, rheumatoid arthritis, diabetic neuropathy, cancer-related pain, postherpetic neuralgia, and psoriasis all of which significantly impair patients' quality of life. In clinical practice, for the treatment of neuropathic and musculoskeletal pain, commercially available topical products typically contain capsaicin concentrations ranging from 0.0125% to 0.075% by weight (Anantaworasakul et al., 2020). In addition to capsaicin-based treatments, drug delivery systems are designed to address limitations associated with certain pharmaceutical agents by enabling controlled release, targeted delivery, and a reduction in adverse effects. Among these, solid lipid nanoparticles (SLNs) and nanostructured lipid carriers (NLCs) represent advanced lipid-based nanocarriers optimized for topical application. These systems offer notable advantages, including sustained drug release, enhanced skin penetration, and diminished side effects associated with high concentrations of active compounds. For example, a prescription-strength capsaicin patch containing 8% capsaicin has demonstrated clinical efficacy in managing severe neuropathic pain, particularly in conditions such as diabetic peripheral neuropathy and postherpetic neuralgia (Anantaworasakul et al., 2020).

2.1.5 The advantages therapy of capsaicin

Capsaicin is known for its selective activation of nociceptive neurons and has been extensively utilized as a tool in the investigation of pain-related mechanisms. This section will explore key aspects of capsaicin's therapeutic potential in pain relief, emphasizing its contributions to the current understanding of neuronal pathways involved in nociception and pain modulation.

In particular, capsaicin has been shown to exhibit a range of pharmacological and physiological effects, most notably its analgesic and anticancer properties (Macho et al., 2003), anti-inflammation, antioxidant, and anti-obesity (Joo et

al., 2010). Moreover, capsaicin demonstrates promising therapeutic potential in pain relief, cancer prevention, and weight management. In addition, it has been shown to exert beneficial effects on the cardiovascular and gastrointestinal systems. Notably, among the various capsaicinoids, capsaicin is the most extensively investigated for its analgesic properties. For instance, studies have indicated that both oral and topical formulations of capsaicin can effectively reduce pain, particularly by attenuating inflammatory heat sensitivity and chemical-induced hyperalgesia (Luo et al., 2011). Additionally, red chili peppers exhibit notable anti-inflammatory effects, primarily attributed to capsaicinoid compounds, which have been shown to possess both anti-inflammatory activity and analgesic properties (Idrees et al., 2020). Furthermore, capsaicin possesses several distinctive properties that render it valuable for applications across the pharmaceutical, food, and agricultural (pesticide) industries. In particular, one of its most prominent characteristics is pungency, which arises from the compound's interaction with sensory receptors in mammals, specifically a family of molecules collectively known as vanilloids. The vanilloid receptor subtype 1 (VR1), now known as TRPV1, is a non-selective cation channel embedded in the plasma membrane, activated by noxious heat and capsaicin, leading to increased sodium and calcium influx. This activation underlies the perception of pungency and, under certain conditions, may contribute to neurogenic inflammation. Moreover, the pungent properties of capsaicin have also been utilized in the development of mammalian deterrents. Importantly, in the medical context, capsaicin's involvement in carcinogenic processes has received growing attention. However, this role remains controversial, as some studies have reported carcinogenic potential, while others provide evidence supporting its antitumor and chemo preventive effects (Díaz et al., 2004). Specifically, the anticarcinogenic effects of capsaicin are thought to result primarily from its capacity to induce apoptosis through the generation of reactive oxygen species (ROS), which are predominantly produced via capsaicin's action on the mitochondrial electron transport chain. Moreover, in addition to its pro-apoptotic properties, capsaicinoids have been identified as potent antioxidants, further supporting their potential therapeutic applications. For example, the use of capsaicin in managing long-term neuropathic pain among cancer patients was first established

by Ellison et al. (1997), marking an important milestone in the clinical application of this compound (Ellison et al., 1997). Looking ahead, future perspectives on capsaicin research will emphasize its therapeutic potential, particularly its clinical applicability in the treatment of pain and related pathophysiological conditions. Indeed, as a potent TRPV1 receptor agonist, capsaicin plays a critical role in modulating oxidative stress, pain perception, and inflammatory responses. Nevertheless, despite certain adverse effects, capsaicin continues to be incorporated as an active pharmaceutical ingredient in various formulations aimed at managing a range of human disorders, underscoring its enduring relevance in medical therapeutics.

2.1.6 Translating *in vitro* to *in vivo* studies into clinical trials

Capsaicin has demonstrated significant anti-proliferative effects against prostate cancer cells in both in vitro and *in vivo* experimental models, highlighting its potential as a therapeutic agent in prostate cancer treatment (Mori et al., 2006). Ellison et al. (1997) reported that capsaicin is an effective therapeutic agent for the treatment of neuropathic conditions such as postherpetic neuralgia and diabetic dysesthesia. Additionally, topical capsaicin formulations have been successfully employed in cancer patients to manage persistent neuropathic pain, particularly that arising post-surgically (Ellison et al., 1997). For instance, according to Nolano et al. (1999), a three-week course of topical capsaicin treatment at a concentration of 0.075% led to approximately 80% reduction in epidermal nerve fiber density, indicating significant epidermal denervation following prolonged exposure (Nolano et al., 1999). In another study, Malmberg et al. (2004) demonstrated that a single 60-minute application of capsaicin could result in up to 60% epidermal denervation, underscoring its potent neurophysiological impact. Specifically, topical capsaicin formulations are generally categorized into low-dose preparations (ranging from 0.025% to 0.075%) and high-dose patches containing 8% capsaicin. High-dose patches are typically applied to the most painful regions of intact skin and left in place for approximately one hour. On the other hand, while low-concentration capsaicin creams have shown moderate clinical efficacy in the treatment of peripheral neuropathic pain (PNP), high-dose formulations offer enhanced therapeutic potential in selected patient populations (Malmberg et al.,

2004). Furthermore, a single 60-minute application of the 8% capsaicin dermal patch has been shown to provide rapid onset and sustained analgesic effects in patients experiencing neuropathic pain (Blair, 2018). Despite their therapeutic potential, low-concentration capsaicin creams require multiple daily applications, and their use is often limited by poor tolerability, particularly due to the initial burning sensation. In response, to overcome these limitations, Qutenza®, a high-dose 8% capsaicin dermal patch, was developed to deliver long-lasting pain relief from a single application. Notably, the capsaicin 8% patch has been approved in the European Union (EU), either as a monotherapy or in combination with other analgesics, for the management of peripheral neuropathic pain (PNP) in adults (Bonezzi et al., 2020). Moreover, topical capsaicin has been proposed as an effective adjunctive therapy for pain management in a variety of conditions, including rheumatoid arthritis, osteoarthritis, neuralgias, and diabetic neuropathy. Additionally, it has shown therapeutic potential in the treatment of neurological dysfunction, inflammatory disorders, and painful or pruritic cutaneous conditions associated with surgical procedures, trauma, or tumor-related complications (Babbar et al., 2009). Importantly, according to evidence-based treatment guidelines, topical capsaicin 8% is recognized as a viable therapeutic option for pain management, with studies such as Laklouk and Baranidharan (2016) supporting its use. In fact, the high-dose capsaicin patch has demonstrated acceptable safety and tolerability profiles, making it particularly beneficial for managing cancer-related and other chronic pain conditions (Laklouk & Baranidharan, 2016). In a recent study, Zis et al. (2016) demonstrated that the 8% capsaicin patch effectively relieves neuropathic pain and enhances quality of life in patients with lumbosacral neuropathic pain. Their study assessed both the safety and efficacy of the capsaicin patch in the treatment of peripheral neuropathic pain (PNP), whether used as a standalone therapy or as an adjunct to existing treatment regimens (Zis et al., 2016). Furthermore, *In vitro* and *in vivo* into the clinical trial studies have explored the antitumor roles of capsaicin in various cancers, such as breast, lung, prostate, and gastric cancers and cholangiocarcinoma, and pain (Zheng et al., 2016). Despite its successes, although capsaicin has been successfully applied in clinical settings for dermatological and pain management purposes, its broader use in pain therapy remains limited. Capsaicin, a

capsaicinoid compound derived from chili peppers, is commonly used to provide temporary relief from musculoskeletal pain associated with conditions such as arthritis. Its mechanism of action involves inducing a burning sensation, which overwhelms nociceptive nerve fibers, thereby inhibiting pain transmission for an extended period.

In order to improve the efficiency of treatment, transdermal therapeutic systems (TTSs) offer a convenient and controlled route of administration. Modified silicone polymer-based matrix diffusion systems have been shown to provide cost-effective and well-regulated drug release. For instance, a study by László S. et al. (2022) evaluated the release kinetics, skin penetration, and analgesic effects of a low-dose capsaicin-loaded TTS. Drug release was assessed using both Franz diffusion cells and continuous flow-through systems, with HPLC and FTIR spectroscopy confirmed that capsaicin penetrated the epidermal and dermal layers of human skin, reaching areas where TRPV1 receptors are expressed. In an in vivo model using male Wistar rats with induced traumatic or inflammatory pain, patches were applied for 6 hours. Capsaicin administration reversed thermal hyperalgesia and increased the mechanical pain threshold in treated animals. The findings suggest that the modified silicone-polymer capsaicin TTS is a promising tool for managing both traumatic and inflammatory pain, offering sustained and targeted analgesic effects through controlled transdermal delivery (László et al., 2022).

Currently, capsaicin is available in the form of creams, gels, and dermal patches containing low doses for topical application. In line with this, previous studies have demonstrated its safety profile in the treatment of pain. Nevertheless, the therapeutic efficacy and target specificity of conventional topical formulations remain limited, primarily due to poor transdermal penetration through the epidermal barrier. This limitation may contribute to adverse effects such as skin atrophy, burning sensations, and systemic absorption, which can negatively impact patient compliance. As a solution, nanotechnology-based drug delivery systems have emerged as promising alternatives. Nano formulations enhance skin permeation, enable targeted delivery, and improve drug release kinetics, thereby offering improved therapeutic outcomes while minimizing unwanted side effects in topical drug administration.

Table 2.1 Therapeutic applications and trials of topical capsaicin for treating pain

Compound	Indicated	Application	Concentration	Route of Administration	Efficacy	References
Capsaicin Gel	Acute Back/Neck Pain	Subjects received one of four topical gels, 2 g twice daily, with 12 ± 4 h between applications: 2% diclofenac+0.075% capsaicin gel, 2% diclofenac, 0.075% capsaicin, and placebo gel	0.075% Capsaicin	Transdermal	Capsaicin, whether used alone or in combination with diclofenac, showed greater pain relief compared to placebo. However, since diclofenac alone was no more effective than placebo, combining it with capsaicin did not enhance analgesic outcomes beyond what capsaicin achieved on its own.	(Iftinca et al., 2021; Predel et al., 2020)
Capsaicin Cream	Diabetic peripheral neuropathy (DPN)	This 12-week, randomized, double-blind, parallel-group experiment compared topical clonidine with capsaicin. Visual analog scale (VAS) pain score of at least 4 treated for 3 months.	0.075% Capsaicin	Topical	In an efficacy analysis involving 69 patients treated with clonidine and 70 with capsaicin, both agents significantly reduced pain over a **12**-week period ($P < 0.01$). However, no statistically significant difference in analgesic efficacy was observed between the two treatments.	(Kiani et al., 2015)
Capsaicin 8% patch (Qutenza™)	Lumbosacral Pain	All selected patients were assessed before and 2 weeks, 8 weeks, and 12 weeks in 60 minutes. VAS was used to measure pain intensity and EQ-5D to measure quality of life.	8% Capsaicin	Transdermal	Application of the 8% capsaicin patch led to notable reductions in pain intensity and enhanced quality of life among patients suffering from lumbosacral neuropathic pain, demonstrating its clinical effectiveness in this population.	(Zis et al., 2016)

Table 2.1 Therapeutic applications and trials of topical capsaicin for treating pain (Continued)

Compound	Indicated	Application	Concentration	Route of Administration	Efficacy	References
Capsaicin hydrogel patch	chronic neck pain	Participants were assigned to wear either capsaicin 0.1% (500 µg) hydrogel patches or placebo hydrogel patches (capsaicin-free) for 12 hours daily over a 4-week period. Outcome assessments were conducted at baseline, 2 weeks after treatment initiation, upon completion of the 4-week intervention, and again at 4 weeks post-treatment using standardized evaluation instruments.	0.1% Capsaicin	Transdermal	Both groups' mean VAS scores reduced at 2, 4, and 8 weeks after intervention. No difference in VAS score or other outcome indicators was seen between the two groups.	(Brodsky et al., 2012)
Capsaicin hydrogel patch	Knee osteoarthritis (OA)	All patients received capsaicin gel or placebo gel three times daily for 4 weeks, then capsaicin gel or placebo gel for another 4 weeks. A blinded examiner did weekly VAS and WOMAC assessments.	0.0125% Capsaicin	Transdermal	VAS and total WOMAC scores were significantly different between the capsaicin and placebo groups (p 0.05). Only burning was recorded. 67% of patients felt a burning sensation during capsaicin therapy, but none withdrew. 0.0125% capsaicin gel helped moderately uncomfortable OA knees.	(Kosuwon et al., 2010)
Capsaicin hydrogel patch	Myofascial Neck Pain	During the 4-week trial, all participants were advised to apply one patch to each side of the neck and shoulder girdle over the region of peak pain for 12 hours daily.	0.1% Capsaicin	Transdermal	Both groups' mean VAS, NDI, and BDI scores dropped 2 and 4 weeks following intervention. No outcome measure differed significantly between the groups.	

2.2 General Pain

2.2.1 The definition of Pain

Historically, pain was defined in 1986 by the International Association for the Study of Pain (IASP) as "an unpleasant sensory and emotional experience associated with actual or potential tissue damage or described in terms of such damage." In addition, the International Association for the Study of Pain (IASP) defines chronic pain as pain that extends beyond the expected period of tissue healing, typically characterized as persisting for more than three months. Furthermore, this standardized definition has played a pivotal role in the establishment and consolidation of pain medicine as a formal medical discipline, building upon the foundational contributions of Dr. John Bonica (Noe, 2020).

More recently, in 2018, Cohen and colleagues proposed an alternative conceptualization of pain, describing it as a shared somatic experience that conveys an individual's perceived threat to their physical or existential well-being (Cohen et al., 2018). However, in a published commentary, Treede critiqued Cohen et al.'s definition for overlooking the multidimensional aspects of pain. Moreover, he also raised concerns about the vague expansion of the concept to include threats to "bodily integrity" and questioned the implication that the recognition of pain necessitates validation by an external observer (Treede, 2018). As a result, the discourse surrounding the optimal definition of pain remains ongoing. Additionally, it is now widely acknowledged that pain including myofascial pain can manifest in the absence of identifiable tissue damage, challenging the outdated structural-pathology model that once equated pain solely with physical injury. This shift in perspective emphasizes that pain is not always a direct indicator of tissue harm (Donnelly et al., 2018). In fact, the IASP definition of pain has been widely accepted by clinicians and researchers in the field and has been officially adopted by numerous professional, governmental, and nongovernmental organizations, including the World Health Organization (WHO). Despite these changes, the accompanying glossary of pain-related terms has undergone multiple revisions over time, the core IASP definition has remained consistent.

IASP definition of pain (1979)

"Pain is an unpleasant sensory and emotional experience associated with actual or potential tissue damage or described in terms of such damage.

Pain is always subjective. Each individual learns the application of the word through experiences related to injury in early life. Biologists recognize that those stimuli which cause pain are liable to damage tissue. Accordingly, pain is that experience which we associate with actual or potential tissue damage. It is unquestionably a sensation in a part or parts of the body but it is also always unpleasant and therefore also an emotional experience. Experiences which resemble pain, eg, pricking, but are not unpleasant, should not be called pain. Unpleasant abnormal experiences (dysaesthesiae) may also be pain but are not necessarily so because, subjectively, they may not have the usual sensory qualities of pain. Many people report pain in the absence of tissue damage or any likely pathophysiological cause; usually this happens for psychological reasons. There is no way to distinguish their experience from that due to tissue damage if we take the subjective report. If they regard their experience as pain and if they report it in the same ways as pain caused by tissue damage, it should be accepted as pain. This definition avoids tying pain to the stimulus. Activity induced in the nociceptor and nociceptive pathways by a noxious stimulus is not pain, which is always a psychological state, even though we may well appreciate that pain most often has a proximate physical cause."

Revised IASP definition of pain (2020):

"Pain is an unpleasant sensory and emotional experience associated with, or resembling that associated with, actual or potential tissue damage. Moreover, pain is always a personal experience that is influenced to varying degrees by biological, psychological, and social factors. Importantly, pain and nociception are different phenomena. Pain cannot be inferred solely from activity in sensory neurons. Through life experiences, individuals learn the concept of pain, and a person's report of an experience as pain should be respected. Although pain usually serves an adaptive role, it may have adverse effects on function and social and psychological well-being. Finally, verbal description is only one of several behaviors to express pain;

thus, inability to communicate does not negate the possibility that a human or a nonhuman animal experiences pain."

2.2.2 Etymology

The Declaration of Montr ´eal, a document developed during the First International Pain Summit on September 3, 2010, states that "**Access to pain management is a fundamental human right**.

The IASP's 1979 definition of pain remains influential in recognizing that pain can occur in the absence of observable tissue damage, highlighting its multidimensional and subjective nature. Its concise and accessible wording has facilitated global consensus among clinicians, researchers, and policymakers, which has significantly shaped health care, pain research, and patient understanding. Pain may be acute, chronic, intermittent, or a combination thereof, and is the most common reason individuals seek medical care.

However, despite its prevalence, pain is often undertreated, largely due to its classification as a symptom rather than a standalone condition. For example, acute pain frequently arises from injury, illness, surgery, or childbirth, and affects 30–80% of patients in clinical settings. If left inadequately managed, it can transition into chronic pain, prolong recovery, and increase both morbidity and hospital stay.

On a global scale, pain affects around 20% of adults, with approximately 10% experiencing persistent pain. Chronic pain, especially-particularly that with neuropathic components, is associated with greater severity and duration, affecting an estimated 6–10% of the population. Furthermore, it significantly diminishes quality of life, contributes to increased mortality risk, and imposes considerable socioeconomic burdens, including reduced work productivity and higher rates of absenteeism. Notably, pain is a common medical problem that is reported in developed countries as well, and it is associated with enormous personal costs and a significant burden on the health care system of the society (Dureja et al., 2017). To better understand the mechanisms and treatment approaches, Table 2.2 outlines the three primary classifications of chronic pain: nociceptive, neuropathic, and nociplastic pain.

Nociceptive pain

Nociceptive pain arises from the activation of neural pathways in response to actual or potentially harmful stimuli affecting body tissues. It is the most prevalent form of chronic pain and typically includes conditions such as arthritis and most types of spinal pain.

Neuropathic pain

According to the IASP, neuropathic pain is defined as pain resulting from injury or disease affecting the somatosensory nervous system (Finnerup et al., 2016). In contrast to nociceptive pain, neuropathic pain is frequently associated with sensory disturbances such as numbness, allodynia, and sudden bursts of intense pain. Additionally, neurological deficits may also be present, depending on the affected nerves (see Table 2.2). While nociceptive pain is often characterized as aching or throbbing, neuropathic pain is commonly described as lancinating or shooting in nature. Common clinical examples include diabetic neuropathy, postherpetic neuralgia, and radiculopathy. Whereas neuropathic pain is estimated to represent approximately 15–25% of all chronic pain cases (Cohen & Mao, 2014). While multiple validated assessment instruments exist for the classification of chronic pain, physician-based clinical evaluation continues to be regarded as the gold standard for accurate diagnosis (Liu et al., 2017). Chronic neuropathic pain, unlike many forms of nociceptive pain or acute nerve injury, is consistently linked to maladaptive behavioral responses. Although the correlation between pain intensity and functional disability is relatively weak, neuropathic pain tends to result in more pronounced impairments in quality of life compared to nociceptive pain of similar severity (Saavedra-Hernández et al., 2012; Spahr et al., 2017).

Nociplastic pain

Nociplastic pain refers to pain arising from altered nociceptive processing without clear evidence of tissue damage or identifiable pathology within the somatosensory system. Notably, previously termed functional pain syndromes, this category includes conditions such as fibromyalgia, irritable bowel syndrome, and potentially non-specific low back pain (see Table 2.2). Moreover, the underlying pathophysiology is thought to involve heightened sensory processing alongside

diminished activity in descending inhibitory pathways. As a result, patients with nociplastic pain often experience less favorable outcomes following interventional procedures—such as joint or epidural steroid injections—compared to those with nociceptive or neuropathic pain, with few exceptions to this trend (Cohen et al., 2021).

Table 2.2 The three main categories of chronic pain: nociceptive, neuro pathic, and nociplastic

	Nociceptive pain	**Neuropathic pain**	**Nociplastic pain**
Causes	Actual or potential damage to bodily tissues	Neurological dysfunction resulting from disease or injury to the nervous system	Maladaptive neurophysiological changes influencing pain processing and regulation, despite the lack of detectable tissue or nerve pathology
Descriptors	Pain descriptors indicative of somatic origin, such as rhythmic throbbing, diffuse aching, or a sensation of internal pressure	Sharp, electric shock-like, transient, or piercing sensations	Visceral pain, such as that experienced in conditions like interstitial cystitis or irritable bowel syndrome, may share similarities with neuropathic pain and is often described using terms such as diffuse, gnawing, aching, or occasionally sharp.
Sensory deficits	Rarely observed, and when present, follows a pattern that does not conform to dermatomal or peripheral nerve distributions	Commonly observed sensory disturbances, including paresthesias such as numbness, tingling, and prickling sensations	Occasionally observed, typically presenting in patterns that do not correspond to dermatomal or specific peripheral nerve distributions
Motor deficits	Weakness	Motor nerve involvement may lead to observable neurological weakness. Movement disorders such as dystonia or spasticity are commonly linked to central nervous system (CNS) lesions but can also occur in certain peripheral nerve conditions.	Generalized fatigue is frequently reported, and any observed weakness is often attributed to physical deconditioning rather than direct neurological impairment.

Table 2.2 The three main categories of chronic pain: nociceptive, neuro pathic, and nociplastic (Continued)

	Nociceptive pain	**Neuropathic pain**	**Nociplastic pain**
Hypersensitivity	Typically rare, except for localized hypersensitivity near the site of an acute injury.	Frequently reported in neuropathic pain, where pain is triggered by normally non-painful stimuli (allodynia) or an exaggerated response to painful stimuli (hyperalgesia)	Common in nociplastic pain, often presenting as diffuse pain, with heightened sensitivity to mechanical stimuli and hyperalgesia being more prevalent than allodynia.
Autonomic signs	Uncommon	Autonomic symptoms, such as alterations in skin color or temperature, swelling, and abnormal sweating (sudomotor activity), are observed in approximately one-third to one-half of affected individuals.	Increased sympathetic nervous system activity is commonly reported in patients with widespread pain syndromes, such as fibromyalgia, as well as in visceral pain conditions like irritable bowel syndrome.
Effective nonopioid pharmacological treatments	Commonly managed with NSAIDs, administered either topically or systemically, and muscle relaxants particularly effective for acute and subacute spinal pain. Additional treatments include serotonin–norepinephrine reuptake inhibitors (SNRIs), tricyclic antidepressants (TCAs), disease-modifying antirheumatic drugs (DMARDs) for inflammatory arthritis, nerve growth factor (NGF) inhibitors, and tramadol.	The typically treated with TCAs, SNRIs, and gabapentinoids. For regional neuropathic pain, high-concentration capsaicin patches and lidocaine patches may be utilized. Tramadol may also be considered.	The management includes TCAs, SNRIs, and gabapentinoids. In select cases, ketamine infusions have shown therapeutic benefit.

Table 2.2 The three main categories of chronic pain: nociceptive, neuro pathic, and nociplastic (Continued)

	Nociceptive pain	Neuropathic pain	Nociplastic pain
Precipitating or relieving factors	Flare-ups are relatively infrequent and typically triggered by physical activity.	Exacerbations occur frequently and are often unpredictable in nature.	Exacerbations are common, frequently correlating with psychosocial stressors.
Examples	Common examples include musculoskeletal and injury-related pain such as back pain, headaches, neck pain, shoulder pain, and pain resulting from burns or physical trauma.	These include pain syndromes associated with nerve damage or dysfunction, such as peripheral neuropathy, diabetic neuropathy, trigeminal neuralgia, complex regional pain syndrome (CRPS), and neuropathic pain following spinal cord injury.	Characterized by altered nociceptive processing without clear tissue or nerve injury, this group includes chronic widespread pain, fibromyalgia, chronic non-specific low back pain, chronic temporomandibular joint (TMJ) disorders, irritable bowel syndrome (IBS), chronic primary bladder pain syndrome, and chronic primary pelvic pain syndromes in both men and women (Fitzcharles et al., 2021)

2.2.3 The mechanism of pain and pain pathways

Pain arises from both physical injury and psychological responses. Specifically, nociceptive signals originate in the periphery and are transmitted to the central nervous system (CNS) for modulation. Within the spinal cord, primary afferent fibers terminate in the dorsal horn, where they synapse with second-order neurons. These neurons project through ascending pathways, including the spinothalamic and spinoreticular tracts, conveying pain signals to higher cortical centers. In addition, descending modulatory pathways—such as those involving the periaqueductal grey and nucleus raphe magnus—play a critical role in regulating pain perception. In neuropathic pain, several mechanisms have been proposed. Among these, both peripheral and central sensitization contribute to abnormal pain processing following nerve injury (Steeds, 2009). Overall, pain pathways converge to form a complex and dynamic system encompassing sensory, cognitive, and behavioral components.

Evolutionarily, this system has developed to detect and integrate harmful stimuli, coordinating protective responses essential for organismal survival (Melzack, 1999). The pain defense system ranges from basic spinal reflexes serving as primary protective mechanisms in simple organisms all the way to highly sophisticated emotional and cognitive responses in humans. Ultimately, the perception of pain results from intricate interactions between the peripheral and central nervous systems, modulated by a balance of excitatory and inhibitory neurotransmitters released in response to noxious stimuli.

The sensation of pain is composed of Figure 2.3)**:**

1. **Transduction**: The nociceptors are responsible for this process, which entails the transformation of noxious stimuli into electrical impulses or action potentials. These action potentials can be triggered by a wide variety of stimuli, including those that are mechanical, thermal, or chemical in nature.

2. **Transmission**: It is a process in which the action potential that is generated at the nociceptor is propagated along the axon of the main afferent neuron. This process takes place in the primary afferent neuron.

3. **Perception**: The somatosensory cortex is primarily involved in processing the sensory-discriminative aspects of nociception, such as intensity and location, whereas the deeper limbic structures are responsible for the affective-motivational (emotional) dimensions of the pain experience.

4. **Modulation**: This refers to a neural mechanism that functions to suppress activity within the pain transmission pathways, thereby diminishing the overall perception of pain.

The process of nociception

Pain perception is mediated by multiple neural signaling pathways. When tissues are exposed to harmful thermal, mechanical, or chemical stimuli, they release various inflammatory mediators such as globulins, proteins, kinases, arachidonic acid, histamine, nerve growth factor (NGF), substance P (SP), and calcitonin gene-related peptide (CGRP). These substances activate transducer ion channels functionally similar to voltage-gated channels thereby initiating receptor potentials (**1. transduction**). These receptor potentials trigger action potentials in sensory neurons.

Peripheral sensitization refers to the heightened responsiveness of nociceptors to noxious stimuli, commonly observed in inflamed tissues. During inflammation, nociceptors located in the skin and deeper tissues exhibit increased sensitivity, thus, reducing the activation threshold. As a result, even normally non-painful or mildly painful stimuli elicit amplified responses. Moreover, this process can also activate previously silent nociceptors, which substantially intensifying the pain experience. To manage this, pharmacological agents such as non-steroidal anti-inflammatory drugs (NSAIDs), opioids, cannabinoids, and TRPV1 receptor antagonists target peripheral nociceptors to modulate pain at the site of injury (Dureja et al., 2017). One key mediator, prostaglandin E2 (PGE2), is produced from arachidonic acid via the enzyme cyclooxygenase-2 (*COX-2*). Traditional and *COX-2*-selective NSAIDs exert their analgesic effects by inhibiting this enzyme, thereby reducing the synthesis of PGE2 and mitigating pain and inflammation.

During pain transmission, afferent signals travel via sensory nerve fibers to the dorsal root ganglia and the dorsal horn of the spinal cord (**2. transmission**). Next, these signals are then relayed upward through the spinal cord to the brainstem and thalamus, where complex processing occurs (**3. modulation**). Finally, the signals reach the somatosensory cortex, enabling conscious awareness of pain (**4. Perception**). In addition, the perception of pain is further shaped by biopsychosocial factors, involving multiple brain regions:

Amygdala: Mediates the emotional and affective aspects of pain and contributes to pain modulation.

Hypothalamus: Regulates the neuroendocrine response to pain, particularly through the corticotropin-releasing pathway.

Periaqueductal gray (PAG): Serves as a central hub for descending pain modulation and is involved in aversive and defensive pain behaviors.

Basal ganglia: Contributes to the cognitive, emotional, and discriminative aspects of pain processing, including the localization and interpretation of sensory input. Stage 4 also activates a wide range of autonomic, emotional, cognitive, and behavioral responses, reflecting the integrative nature of pain perception (Dureja et al., 2017). The cerebral cortex serves as the terminal site for pain perception

and possesses the ability to initiate descending modulatory pathways that regulate nociceptive input, as illustrated in Figure 2.3. Within the spinal cord and dorsal root ganglia, endogenous opioid peptides including endorphins, dynorphins, and enkephalins play a critical role in attenuating pain transmission by modulating nociceptive afferent input. This occurs through activation of descending inhibitory pathways, particularly involving the periaqueductal gray (PAG) in the midbrain. Endogenous opioids exert their effects by binding to mu (μ), kappa (κ), and delta (δ) opioid receptors, resulting in decreased presynaptic calcium influx and reduced release of excitatory neurotransmitters such as glutamate and substance P (SP). Additionally, these mechanisms enhance potassium conductance in dorsal horn neurons, contributing to neuronal hyperpolarization and further inhibition of pain signaling. Other key neurotransmitters involved in descending modulation include norepinephrine (NE), glycine, and gamma-aminobutyric acid (GABA).

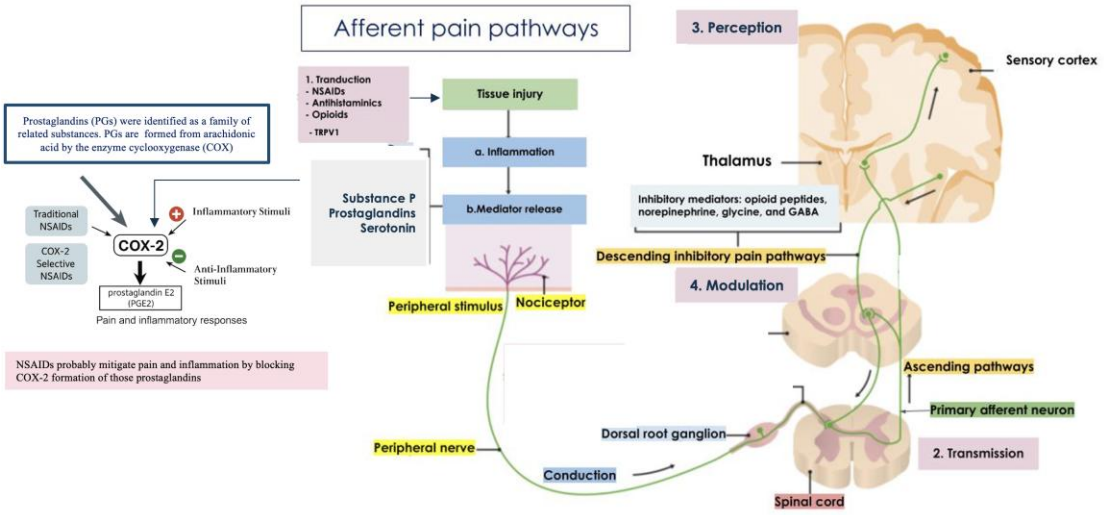

Figure 2.3 The mechanism of pain

(Modified form Lecturio and Dureja et al. (2017))

Afferent pain pathways illustrating the four primary stages of pain processing: (1) Transduction – conversion of noxious stimuli into electrical signals at the site of tissue injury involving inflammatory mediators such as prostaglandins and substance P; (2) Transmission – propagation of electrical impulses via peripheral nerves to the

spinal cord and ascending tracts; (3) Perception – conscious awareness of pain within the sensory cortex; and (4) Modulation – regulation of pain signals by descending inhibitory pathways using neurotransmitters like opioids, GABA, and norepinephrine. The diagram also highlights the role of *COX-2* in prostaglandin synthesis and the action of NSAIDs in reducing pain and inflammation.

2.2.4 Pain management

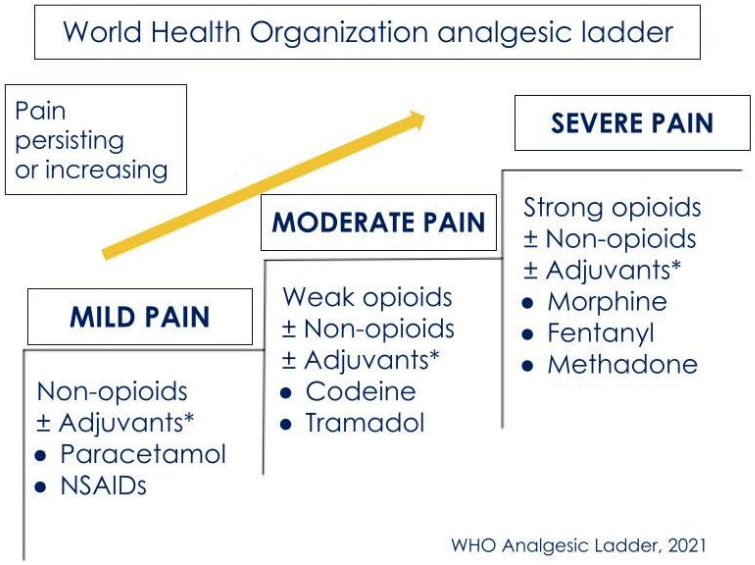

Figure 2.4 The World Health Organization (WHO) pain relief ladder (Anekar & Cascella, 2021)

The first ladder consisted mostly of three steps:

Step 1 – Mild Pain: Management begins with non-opioid analgesics such as nonsteroidal anti-inflammatory drugs (NSAIDs) or acetaminophen, which may be administered alone or in combination with adjuvant therapies.

Step 2 – Moderate Pain: For more pronounced pain, treatment involves weak opioids (e.g., hydrocodone, codeine, tramadol), either alone or in combination with non-opioid analgesics and appropriate adjuvants.

Step 3 – Severe or Chronic Pain: In cases of intense or persistent pain, strong opioids—including morphine, methadone, fentanyl, oxycodone, buprenorphine, tapentadol, hydromorphone, and oxymorphone—are prescribed, with or without concurrent use of non-opioid agents and adjuvants.

Adjuvant analgesics, or co-analgesics, encompass a broad category of medications that are primarily prescribed for non-analgesic indications, yet have demonstrated efficacy in managing various pain conditions. These include tricyclic antidepressants (e.g., amitriptyline, nortriptyline), serotonin–norepinephrine reuptake inhibitors (SNRIs) such as duloxetine and venlafaxine, anticonvulsants like gabapentin and pregabalin, as well as topical agents including lidocaine patches.

Furthermore, multimodal analgesia is widely advocated as an optimal approach for pain management due to the involvement of multiple receptor systems across both peripheral and central nervous pathways. This strategy allows for more effective pain control while minimizing adverse effects. Specifically, pharmacological agents used in multimodal regimens include: Opioids, which modulate afferent pain transmission and activate descending inhibitory pathways at both spinal and supraspinal levels, local anesthetics, which transiently block nociceptive signal conduction, antidepressants (TCAs and SNRIs), which enhance monoaminergic modulation of pain, and NSAIDs, such as ibuprofen, which reduce inflammation and peripheral sensitization. Importantly, successful pain management must also address the psychosocial dimensions of pain, including its impact on mood, anxiety, and overall physical and social functioning. A comprehensive biopsychosocial approach remains essential for achieving effective and sustainable pain relief (Webb & Steeds, 2022). In addition, distinct patterns of sensory neuronal alterations highlight the complexity and variability of pain as a dynamic physiological process. Currently, pharmacological strategies for pain management include analgesics such as acetaminophen, NSAIDs, antidepressants, and various combination therapies. Moreover, advances in molecular neuroscience have reshaped our understanding of nociception by revealing that nociceptors possess receptor-coupled ion channels capable of detecting environmental stimuli, leading to neuronal depolarization and the perception of pain. This discovery has not only deepened insight into pain mechanisms, but also facilitated the development of novel therapeutic agents—particularly in light of Mendelian mutations in these receptor proteins that are directly linked to altered pain sensitivity (Fattori et al., 2016; Wolkerstorfer et al., 2016). The foundational principle of the analgesic ladder is that effective pain management relies on thorough assessment and

understanding of the patient's pain severity to guide appropriate pharmacologic intervention. Given that many patients may require opioid therapy at some stage, it is essential to balance effective dosing with the potential for adverse effects. To address this, opioid rotation may be employed to enhance analgesic outcomes while minimizing side effects.

Additionally, patient education on the appropriate use, therapeutic benefits, and potential risks of medications is essential to prevent misuse and ensure sustained treatment efficacy. Certain chemical families exhibit a wide range of pharmacological properties, including anti-inflammatory, antioxidant, and TNF-α inhibitory effects, making them relevant in the management of conditions such as psoriasis. These compounds may also possess cardiostimulant, antioxidant, and anticancer activities. Methyl salicylate, a derivative of salicylic acid, serves as an analgesic and non-steroidal anti-inflammatory drug (NSAID), and has additionally been identified as a TRPV1 receptor activator (Ohta et al., 2009). Although significant progress has been made in elucidating pain mechanisms through emerging methodologies, experimental models remain indispensable tools in pain research. Notably, capsaicin continues to serve as a widely utilized and valuable experimental agent for investigating nociceptive pathways.

2.3 Capsaicin: Mechanisms of Pain Modulation and Cellular Responses in Human Dermal Fibroblasts

Capsaicin is a pungent vanilloid compound derived from chili peppers (*Capsicum spp.)* and is widely recognized for its potent ability to modulate various types of pain, particularly neuropathic, myofascial, and inflammatory pain. Its primary pharmacological activity is mediated through the activation of the transient receptor potential vanilloid 1 (TRPV1) channel, which is predominantly expressed on peripheral nociceptive neurons. Upon binding to TRPV1, capsaicin induces calcium (Ca^{2+}) and sodium (Na^{+}) influx, leading to neuronal depolarization, neurotransmitter release, and subsequent transmission of pain signals to the central nervous system (CNS). However, with prolonged exposure, capsaicin causes TRPV1 desensitization, which reduces

nociceptive signaling and ultimately produces analgesic effects (Bode & Dong, 2011; Caterina et al., 1997).

Recent evidence has expanded our understanding of capsaicin's mechanism of action, revealing that TRPV1 is also expressed in non-neuronal cells, including human dermal fibroblasts (HDFs). These fibroblasts play crucial roles in skin inflammation, tissue remodeling, and wound healing. Within this context, capsaicin exhibits anti-inflammatory effects by modulating intracellular signaling pathways associated with inflammation. Notably, one key mechanism is the downregulation of cyclooxygenase-2 (*COX-2*) an inducible enzyme responsible for prostaglandin E2 (PGE2) synthesis, which contributes to inflammatory pain sensitization at the tissue level. Under inflammatory stimuli, such as interleukin-1β (IL-1β) or lipopolysaccharide (LPS), *COX-2* expression is significantly upregulated in HDFs, thereby intensifying the pain cascade. Capsaicin, however, can suppress *COX-2* expression at both mRNA and protein levels by inhibiting the NF-**K**B and MAPK signaling pathways (Hudita et al., 2021)

In addition to *COX-2* suppression, capsaicin also reduces oxidative stress in dermal fibroblasts by lowering the production of reactive oxygen species (ROS) and increasing antioxidant defenses such as superoxide dismutase (SOD) and glutathione (GSH). This antioxidant activity plays a significant role in protecting skin cells from inflammation-induced damage and contributes to the attenuation of oxidative inflammatory pain (Cuijpers et al., 2025).

Moreover, capsaicin enhances fibroblast migration, proliferation, and collagen synthesis via activation of PI3K–AKT–mTOR signaling pathways, promoting tissue regeneration and supporting wound healing. These effects are crucial for reducing wound-associated pain, which often arises from chronic inflammation and delayed dermal repair (Lee et al., 2013).

Taken together, these findings highlight the dual action of capsaicin: (1) as an analgesic agent that modulates nociceptive pathways via TRPV1 and (2) *COX-2* suppression, and as a dermal modulator that reduces inflammation and oxidative stress in human dermal fibroblasts. Such properties make capsaicin a strong candidate for transdermal delivery systems targeting pain and inflammatory skin conditions.

2.3.1 Anti-Inflammatory and Effects on Human Dermal Fibroblasts (HDFs)

Human dermal fibroblasts (HDFs) are essential components of the skin's structural and functional integrity, as they play pivotal roles in wound healing, extracellular matrix (ECM) remodeling, and regulation of inflammatory responses. Therefore, understanding the molecular and cellular effects of capsaicin on HDFs is critical for evaluating its therapeutic potential, particularly in the context of transdermal drug delivery. Such insights are especially valuable for developing targeted treatments for inflammatory skin disorders and dermal pain syndromes, where fibroblast-mediated mechanisms are central to both pathophysiology and recovery.

Overview of Inflammatory Processes in HDFs

Human dermal fibroblasts (HDFs) are not merely structural components; rather, they actively participate in immune surveillance and regulation. Under pathological conditions such as tissue injury, microbial infection, or oxidative stress fibroblasts significantly upregulate the expression of several pro-inflammatory mediators, including tumor necrosis factor-alpha (TNF-α), interleukin-6 (IL-6), and cyclooxygenase-2 (*COX-2*). These molecules not only initiate and sustain local inflammation but also facilitate the recruitment of immune cells to the site of injury, thereby amplifying the inflammatory response and contributing to pain perception. Furthermore, in dermal tissues, prolonged or dysregulated inflammation often results in adverse outcomes such as fibrosis, delayed wound healing, or the development of chronic, non-healing wounds particularly in individuals with diabetes or advanced age. Therefore, modulating the inflammatory response in fibroblasts is a key strategy for promoting effective tissue repair and managing inflammation-associated pain.

Capsaicin's Anti-Inflammatory Properties

Capsaicin exerts potent anti-inflammatory effects, primarily through the downregulation of key cytokines and enzymes involved in inflammatory signaling. Specifically in human dermal fibroblasts (HDFs), capsaicin has been shown to suppress the expression of interleukin-6 (IL-6), tumor necrosis factor-alpha (TNF-α), and cyclooxygenase-2 (*COX-2*)—all of which play central roles in mediating pain sensitization and inflammatory cascades. By attenuating these molecular mediators, capsaicin contributes to the modulation of inflammatory responses, ultimately

supporting its therapeutic utility in conditions involving chronic inflammation and associated pain (Iftinca et al., 2021). This suppressive effect appears to be mediated through the inhibition of key intracellular signaling pathways, most notably the nuclear factor kappa B (NF-**K**B) and mitogen-activated protein kinases (MAPKs) pathways. Both pathways are known to be rapidly activated in response to various stress-related stimuli, including microbial components, pro-inflammatory cytokines, and oxidative stress. By blocking these upstream signaling cascades, capsaicin effectively downregulates the transcription of genes involved in inflammatory mediator production, thereby attenuating the cellular inflammatory response (Ilie et al., 2019).

NF-KB Pathway Inhibition

Nuclear factor kappa B (NF-**K**B) is a key transcription factor complex that, once activated, translocates to the nucleus and promotes the transcription of various pro-inflammatory genes. Capsaicin, however, has been demonstrated to inhibit the activation of I**K**B kinase (IKK), which is an upstream regulator of NF-**K**B. By blocking IKK activity, capsaicin prevents the phosphorylation and subsequent degradation of I**K**B**α**, the inhibitory protein that normally sequesters NF-**K**B in the cytoplasm. As a result, NF-**K**B remains in its inactive cytoplasmic state, thereby reducing its nuclear translocation and limiting the transcription of inflammatory mediators such as *COX-2*, IL-6, and TNF-**α**. In a study conducted by Hudita et al. (2021), HDFs treated with capsaicin showed significantly decreased expression of these pro-inflammatory markers following exposure to lipopolysaccharide (LPS), a potent inflammatory stimulus. These findings indicate that capsaicin effectively suppresses NF-**K**B-dependent inflammatory signaling in dermal fibroblasts, reinforcing its therapeutic potential for managing skin inflammation and pain (Hudita et al., 2021).

***COX-2* Expression Suppression**

Cyclooxygenase-2 (*COX-2*) is an inducible enzyme responsible for the conversion of arachidonic acid into prostaglandins, most notably prostaglandin E2 (PGE2). PGE2 plays a central role in vasodilation, edema formation, and pain sensitization during inflammation. Overexpression of *COX-2* in skin tissue has been strongly linked to chronic inflammatory conditions and hyperalgesia.

Importantly, capsaicin has been shown to downregulate *COX-2* gene expression and inhibit PGE2 production in human dermal fibroblasts (HDFs), suggesting a direct mechanism by which capsaicin attenuates peripheral sensitization and inflammatory pain. This effect is particularly relevant in pathological conditions such as myofascial pain syndrome, psoriasis, and burn wounds, where *COX-2*–mediated pathways are known to be upregulated (Chen et al., 2018).

Role in Pain Relief

Pain is not solely a neuronal phenomenon; it is also modulated by non-neuronal cells, particularly fibroblasts, which play a critical role in chronic inflammatory states and wound-related conditions. These cells actively contribute to the production of pro-inflammatory cytokines, regulation of extracellular matrix, and interactions with immune cells, thereby influencing the persistence, intensity, and resolution of pain. Consequently, targeting fibroblast-mediated pathways may offer novel therapeutic strategies in managing inflammatory and neuropathic pain syndromes (Fang et al., 2023). Fibroblasts can secrete nociceptive mediators that amplify pain and modulate nociceptor sensitivity (Pinho-Ribeiro et al., 2017). The ability of capsaicin to suppress both inflammatory mediators and oxidative stress in fibroblasts underscores its potential to alleviate not only primary nociceptive pain but also secondary inflammatory pain that originates from the dermal microenvironment. In addition, capsaicin contributes to tissue repair and regeneration, which in turn supports long-term pain relief by restoring structural integrity and mitigating chronic inflammation. Mechanistically, capsaicin stimulates fibroblast migration, proliferation, and collagen synthesis, particularly of type I and type III collagen, which are essential components for dermal remodeling and wound resolution. Furthermore, activation of PI3K/Akt/mTOR and ERK1/2 signaling pathways has been implicated in mediating these regenerative effects. Taken together, these properties highlight capsaicin's therapeutic potential not only as an anti-inflammatory and analgesic agent but also as a promising candidate for wound-healing applications (Lee et al., 2013).

Several studies have extensively investigated the anti-inflammatory and antioxidant properties of capsaicin, particularly its role in modulating pain-related molecular pathways in both neuronal and non-neuronal cells. Notably, research on

human dermal fibroblasts (HDFs) has demonstrated that capsaicin not only suppresses the expression of pro-inflammatory genes such as cyclooxygenase-2 (*COX-2*), interleukin-6 (IL-6), and tumor necrosis factor-alpha (TNF-α) but also enhances cellular antioxidant defenses.

Specifically, capsaicin has been shown to increase levels of superoxide dismutase (SOD) and glutathione (GSH) while reducing the generation of reactive oxygen species (ROS). These molecular effects collectively contribute to the attenuation of inflammation-induced oxidative stress, thereby leading to pain reduction. This dual modulation of both inflammatory and oxidative stress pathways is particularly relevant in the context of inflammatory skin disorders and dermal pain syndromes, as summarized in Table 2.3.

Table 2.3 Comparisons of Capsaicin Studies on Human Dermal Fibroblasts and Pain

Study	**Cell Model / System**	**Key Findings**	**Implication for Pain Modulation**
(Cuijpers et al., 2025)	HDFs under oxidative stress	Capsaicin decreased ROS and MDA, increased SOD and GSH; antioxidant protection in HDFs.	Protection from oxidative stress supports pain control in inflammatory skin conditions.
(Hudita et al., 2021)	Human Dermal Fibroblasts (HDFs)	Capsaicin reduced *COX-2*, IL-6, MMP-1 expression; anti-inflammatory effects via NF-κB inhibition.	Reduction of inflammatory gene expression may alleviate local inflammatory pain.
(Lee et al., 2013)	Fibroblasts (wound model)	Capsaicin promoted fibroblast migration and proliferation; activated Akt/mTOR signaling.	Enhanced wound healing may reduce wound-associated pain.
(Kim et al., 2004)	LPS-stimulated immune cells	Capsaicin suppressed *COX-2* and PGE2 production; reduced inflammation in macrophages.	Inflammation suppression indicates potential for systemic or local pain relief.

Although capsaicin has been widely recognized for its analgesic effects via the activation of transient receptor potential vanilloid 1 (TRPV1) receptor on sensory neurons, recent evidence suggests that its biological activity extends beyond the nervous system. In particular, its effects on non-neuronal cells, such as human dermal fibroblasts (HDFs), remain inadequately understood. HDFs are not only structural components of the skin but also key regulators of inflammation, oxidative stress, and wound healing. While the anti-inflammatory role of capsaicin has been demonstrated in various immune cell lines, few studies have explored its molecular influence on inflammation-related gene expression particularly the suppression of cyclooxygenase-2 (*COX-2*), a gene closely associated with the pathogenesis of inflammatory pain. In addition, the interaction between capsaicin and oxidative stress pathways in HDFs is poorly characterized, despite growing evidence that reactive oxygen species (ROS) contribute to chronic skin inflammation and fibroblast dysfunction.

Another research gap lies in the lack of studies evaluating capsaicin in the form of transdermal delivery systems, especially electrospun nanofiber patches. While capsaicin's use in topical creams and high-dose patches (e.g., 8%) has been approved for neuropathic pain, its potential for localized, controlled delivery via nanofibers has not been fully investigated. Moreover, studies involving in vitro skin permeation using Strat-M™ synthetic membranes are limited, despite the growing need for ethical and reproducible skin models that replace animal or human tissue. These knowledge gaps limit the understanding of capsaicin's full therapeutic potential, particularly in non-neuropathic pain types such as inflammatory or myofascial pain, which involve both neural and dermal components.

Therefore, this study was undertaken to address these gaps by developing a capsaicin-loaded nanofiber transdermal patch using biocompatible polymers (PVA/PVP) via electrospinning, and to investigate its anti-inflammatory and antioxidant effects on HDFs. Specifically, the study aims to evaluate the expression of *COX-2* and oxidative stress markers following capsaicin exposure, and to assess the release profile and skin permeation using a Strat-M™ membrane model. By elucidating the cellular and molecular mechanisms of capsaicin in human dermal fibroblasts, the

findings are expected to support the development of innovative, non-invasive therapies for pain relief.

2.4 Transdermal drug delivery systems

Oral and parenteral drug delivery remain the two most commonly employed administration routes, with the oral route being the preferred method for small-molecule therapeutics due to its convenience, portability, and ease of self-administration (Anselmo & Mitragotri, 2014; Han & Das, 2015). However, the oral route of drug administration is widely favored due to its convenience, portability, and ease of self-administration with fixed dosing regimens, making it one of the most practical delivery methods. However, oral delivery is unsuitable for many therapeutic peptides and proteins, primarily due to enzymatic degradation in the gastrointestinal tract and limited epithelial permeability for large molecules. Consequently, parenteral administration, particularly injection, is the predominant approach for delivering such macromolecules. Nonetheless, this route presents several challenges, including its invasive nature, the potential to cause pain and discomfort, and reduced patient compliance, as it often requires skilled personnel for proper administration. These limitations highlight the need to re-evaluate and innovate beyond conventional drug delivery strategies (Alkilani et al., 2015). Advanced drug delivery approaches, such as transdermal drug delivery (TDD), offer promising solutions to the limitations associated with conventional administration routes. A drug delivery system (DDS) encompasses a range of physicochemical technologies designed to regulate the transport and controlled release of pharmacologically active agents into targeted cells, tissues, or organs, thereby maximizing therapeutic efficacy and minimizing systemic side effects (Vargason et al., 2021; Vega-Vásquez et al., 2020). In essence, drug delivery systems (DDS) encompass both the formulation strategies and routes of administration designed to optimize therapeutic efficacy while minimizing potential adverse effects. Various administration modalities include oral, transdermal (through the skin), mucosal (e.g., nasal or buccal), pulmonary (inhalation via the lungs), and intravenous delivery, each offering unique advantages based on the drug's physicochemical properties and

clinical application (Jeong et al., 2021). Among these, the transdermal drug delivery system (also known as TDDS) stands out as a potentially useful method.

The transdermal drug delivery system (TDDS), a noninvasive method for administering therapeutics through the skin, has emerged as a widely researched alternative to conventional injection-based delivery. TDDS has demonstrated considerable potential in enhancing the administration of a range of pharmacological agents, with notable applications in pain management, hormonal therapies, and the treatment of cardiovascular and central nervous system (CNS) disorders (Leppert et al., 2018; Peña-Juárez et al., 2022; Roohnikan et al., 2019). One of the key advantages of TDDS is that they bypass the gastrointestinal (GI) tract, thereby avoiding interference from pH fluctuations, digestive enzymes, and intestinal microbiota that can compromise drug stability and absorption. TDDS is also characterized by its high persistence, as it enables controlled and sustained drug release in accordance with therapeutic needs. Furthermore, as a noninvasive and painless method, TDDS offers enhanced patient comfort and compliance, making it especially suitable for use in pediatric and geriatric populations (Jeong et al., 2021). However, the natural barrier properties of the skin, particularly the stratum corneum, pose a significant limitation to the full therapeutic potential of transdermal drug delivery systems. An organ with a multilayered structure, the skin serves as a barrier to protect our bodies from harmful substances such as chemicals and heat (Ali et al., 2015; Wang et al., 2021). The epidermis acts as a major barrier to transdermal drug delivery, while the dermis, containing vasculature and various cell types, presents additional challenges to effective drug penetration. Existing transdermal technologies such as patches, ointments, and creams have primarily enhanced the delivery of low molecular weight, lipophilic drugs that are effective at low doses. Although TDDS have been utilized for decades, current research efforts are focused on improving the cutaneous penetration of larger, hydrophilic molecules and macromolecular therapeutics for applications in both disease treatment and vaccination. Nanocarrier systems, composed of lipids, metals, or polymers, have shown promising results in enhancing transdermal drug penetration, enabling controlled drug release, and facilitating site-specific drug targeting within the skin. These advances hold the potential to significantly expand the

therapeutic scope of TDDS; nevertheless, further investigation is needed to fully establish the safety and biocompatibility of nanocarrier-based formulations. This study provides an overview of the current landscape of nanoparticle-mediated skin delivery systems, with an emphasis on their application in the treatment of dermatological disorders. Historically, the first FDA-approved transdermal patch, containing scopolamine for motion sickness, was introduced in 1979, marking a milestone in the field of transdermal therapeutics (Palmer & DeLouise, 2016). The TDD system has since been used to formulate other drugs, such as nicotine, fentanyl, estrogen, and testosterone. Technology is currently being used to improve transdermal drug systems.

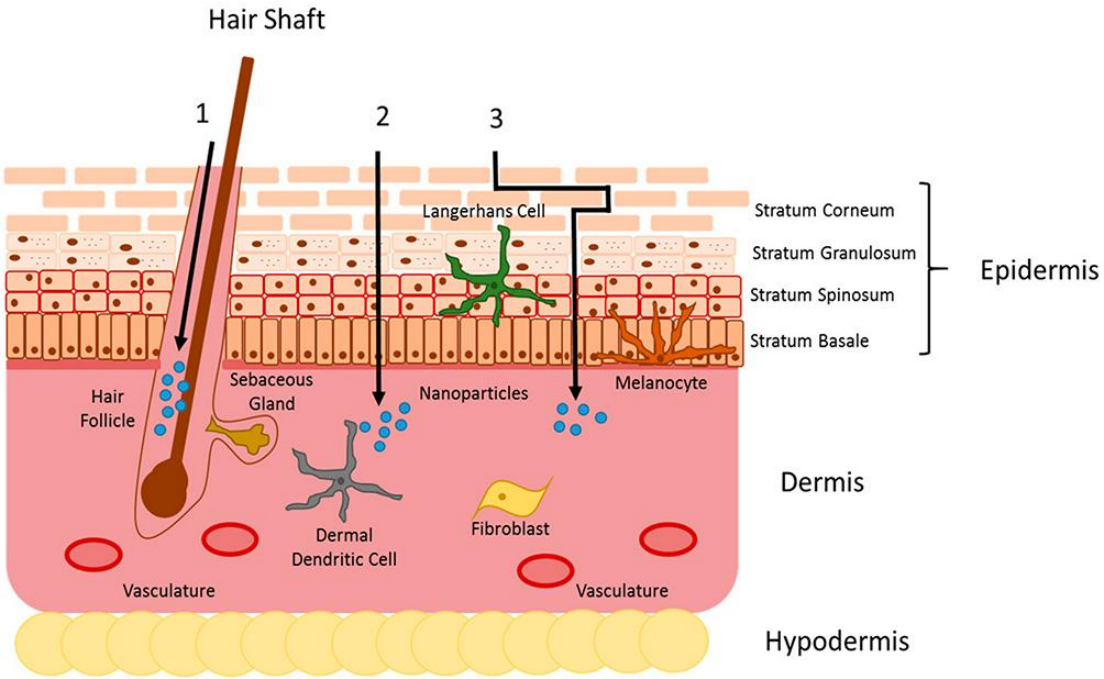

Figure 2.5 Nanoparticle skin penetration illustration

Topically applied nanoparticles can traverse the skin through three primary pathways: (1) the appendageal route, (2) the intracellular route, and (3) the intercellular route. The appendageal pathway involves nanoparticle entry through skin structures such as hair follicles, sweat glands, or cutaneous furrows, facilitating drug retention or enhanced dermal penetration. The intracellular route allows for direct translocation across the cell membranes of the epidermal layers, while the intercellular route involves diffusion through the extracellular lipid matrix between

adjacent skin cells, following a more tortuous path. The physicochemical properties of the nanoparticles namely size, surface charge, shape, and composition play a critical role in determining the preferred penetration pathway (Palmer & DeLouise, 2016).

2.4.1 A Brief Review of Skin Structure

The epidermis and the dermis are the two layers that make up the stratified structure that is the skin (Palmer & DeLouise, 2016; Wysocki, 1999). The skin, often recognized as the largest organ of the human body, comprises multiple layers, each serving distinct yet complementary roles. Among its key physiological functions are thermoregulation, ultraviolet (UV) protection, immune defense, and maintenance of water homeostasis. The epidermis, the outermost layer, plays a pivotal role in acting as a physical barrier, protecting the body from external insults such as pathogens, particulate matter, and large or hydrophilic molecules. In addition, it is critical for preventing transepidermal water loss, thereby contributing to overall skin integrity and systemic hydration (Brandner, 2009; Matsui & Amagai, 2015). The epidermis is primarily composed of keratinocytes, melanocytes, and Langerhans cells, each contributing to distinct physiological roles. Keratinocytes, which constitute the majority of epidermal cells, are central to the formation of the skin's physical barrier. These cells undergo a process of terminal differentiation, progressing from the stratum basale—the innermost layer—to the stratum corneum, the outermost layer of the epidermis. Upon reaching the stratum corneum, keratinocytes lose their nuclei and organelles, becoming corneocytes, which are essentially non-viable, flattened cells. These corneocytes are embedded within a protein-rich matrix composed of keratin, filaggrin, and loricrin, and are enveloped by a lipid envelope primarily consisting of ceramides, free fatty acids, and cholesterol, which together contribute to the skin's barrier function and water retention (van Smeden et al., 2014). The stratum corneum, along with tight junctions in the stratum granulosum, forms a highly selective barrier to water and solutes, effectively restricting the penetration of most hydrophilic drug molecules larger than 500 kDa. In addition, epidermal melanocytes produce melanin, which is transferred to keratinocytes to absorb ultraviolet (UV) radiation and protect against DNA damage, thereby contributing to the skin's defense mechanisms (Bos & Meinardi,

2000; Tolleson, 2005). Langerhans cells in the epidermis, along with macrophages and dermal dendritic cells in the dermis, serve as key components of the skin's immune surveillance system. These immune cells detect xenobiotics and pathogens and can either act locally or migrate to regional lymph nodes to initiate an adaptive immune response through B and T lymphocyte activation (Clausen & Stoitzner, 2015; Haniffa et al., 2015). Due to the skin's natural barrier—particularly the stratum corneum of the epidermis it is challenging to design drug formulations and nanocarriers capable of effective penetration. However, once drugs or nanocarriers traverse the viable epidermis, they can interact with living keratinocytes and immune cells, enabling potential transport to draining lymph nodes. Unlike the avascular epidermis, the dermis is highly vascularized, containing extensive blood and lymphatic vessels, which facilitates systemic drug absorption. The dermis consists of three layers: the papillary dermis (the most superficial), the reticular dermis, and the hypodermis. The upper dermis is rich in collagen and extracellular matrix proteins, synthesized primarily by fibroblasts, providing structural integrity and support (Amano, 2016; Lawlor & Kaur, 2015). The subcutaneous fat is located in the lowest layer of the skin, which is called the hypodermis (Lawlor & Kaur, 2015). The dermis also comprises numerous specialized structures, including sweat glands, hair follicles, nerve fibers, and vascular and lymphatic vessels. These appendages play vital roles in maintaining physiological homeostasis. Specifically, sweat glands and hair follicles, embedded within the dermal layer, are essential for thermoregulation, helping to control body temperature through sweat secretion and modulation of blood flow (Tansey & Johnson, 2015). Secondary structures in skin and the papillary dermis create furrows and invaginations in the skin. These furrows and invaginations have the potential to trap topically applied drugs or nanocarriers (German et al., 2012; Lawlor & Kaur, 2015). These structures may allow for increased drug penetration due to a decreased distance between the stratum corneum and the dermis in these areas (Gupta et al., 2012; Patzelt & Lademann, 2013). In fact, transdermal drug delivery systems (TDDS) are primarily designed to enable drugs to reach the dermis, thereby facilitating systemic absorption. The dermis, richly supplied with blood vessels and lymphatics, supports not only efficient drug uptake into the circulation but also hosts a diverse population of immune cells including

macrophages, T cells, mast cells, and dendritic cells that may interact with administered compounds. Additionally, skin appendages such as hair follicles and sweat glands may act as drug reservoirs, allowing for sustained release while simultaneously enhancing dermal penetration. An example of this systemic approach is seen in the transdermal administration of scopolamine (Graybiel et al., 1976). In transdermal drug delivery (TDD) research, significant efforts are directed toward enhancing drug penetration and retention within the skin by leveraging natural skin structures such as sweat glands, hair follicles, and skin furrows. Strategies include the use of chemical penetration enhancers and physical methods like abrasion to temporarily disrupt the stratum corneum, thereby facilitating improved nanocarrier permeability.

Furthermore, nanocarrier design is optimized based on the stratum corneum's architecture, with a preference for nanoparticles under 100 nm in size that possess surface charges or lipid coatings to promote either deeper flux or retention in the lipid-rich layers of the skin (Abdel-Mottaleb et al., 2012; Lee et al., 2013). Nanoparticle skin penetration is dependent on a number of factors, including size, charge, morphology, and material composition. These factors are discussed in the following section.

Nanocarrier Skin Penetration: In recent decades, there has been a growing incorporation of nanoparticles into various consumer products. Since the 1990s, nano-sized titanium dioxide and zinc oxide have been widely employed in sunscreens and cosmetic formulations for their ability to protect the skin against harmful ultraviolet (UV) radiation. Their nanoscale size enhances product transparency while maintaining effective UV-blocking properties, thereby improving both aesthetic and functional aspects of topical applications (Suzuki, 1987). More recently, silica nanoparticles and fullerenes have been incorporated into cosmetic formulations to enhance product functionality. Silica nanoparticles serve as desiccants, helping to absorb moisture and improve texture, while fullerenes function as free radical scavengers, offering antioxidant protection to mitigate oxidative stress on the skin. These applications highlight the expanding role of nanotechnology in enhancing the efficacy and performance of topical products (Contado, 2015; Xiao et al., 2006).

Together, these developments highlight the expanding role of nanotechnology in enhancing the functionality of topical formulations. Although most nanoparticles used in consumer products are not intended to penetrate the skin, their increasing prevalence has stimulated research into both their therapeutic potential and safety concerns, particularly in the context of transdermal drug delivery (TDD) systems. Early investigations focused on *ex vivo* and *in vivo* skin penetration models, as well as *in vitro* cytotoxicity assays in skin cells. Traditionally, it was believed that intact skin posed an impenetrable barrier to nanoparticles. However, accumulating evidence now challenges this notion. Recent studies have shown that nanoparticles can indeed penetrate the skin, and this capability is strongly influenced by several key factors, including particle size, surface charge, morphology, and chemical composition (Fernandes et al., 2015). Nanoparticle skin penetration is influenced by several physicochemical and biological factors. Beyond size, surface charge, and material composition, additional key determinants include the administered dose, morphological characteristics (such as shape and surface roughness), and biological adhesiveness, which affects their interaction with skin components. Furthermore, the *in vivo* dissociation behavior of nanoparticleshow they disassemble or release their payload also plays a critical role in modulating both penetration efficiency and biological activity within the skin. These variables collectively determine the effectiveness and safety profile of nanoparticles in transdermal drug delivery systems.

2.4.2 Drug absorption via the skin

As illustrated in Figure 2.6 (Ramadon et al., 2022), drug absorption through the stratum corneum (SC) occurs via two principal pathways: transepidermal and transappendageal routes. The transepidermal route considered the primary mechanism of absorption leverages the SC's extensive surface area to enable drug penetration. Within this route, substances may pass directly through the keratinocytes (transcellular pathway) or navigate the intercellular spaces between cells (intercellular pathway). Both modes support diffusion of therapeutic agents from transdermal systems into deeper skin layers, ultimately reaching systemic circulation (Barbero & Frasch, 2006; Haque & Talukder, 2018). The transepidermal route is subdivided into

two distinct pathways: the transcellular and intercellular routes. In the transcellular pathway, drugs penetrate directly through the corneocytes of the stratum corneum (SC), requiring traversal across multiple lipid bilayers of cell membranes. Due to the hydrophobic nature of these lipid domains, this route is generally more favorable for lipophilic (hydrophobic) compounds. Conversely, in the intercellular pathway, drugs must diffuse through the extracellular lipid matrix that surrounds the keratinocytes. This route offers a more tortuous path but serves as a primary route for many substances due to the layered lipid structure of the SC (Zhang et al., 2017). The intercellular route, the most prevalent pathway for transdermal drug absorption, facilitates the movement of hydrophilic compounds and small molecules toward the dermal vascular capillaries. Effective absorption via this route depends on the drug's amphiphilic properties, meaning it must possess both lipid and water solubility to navigate the lipid-rich extracellular matrix. The transappendageal route, the secondary pathway, involves drug transport through hair follicles and sweat glands. This route is particularly important for delivering polar, ionizable compounds and large macromolecules that face challenges in permeating the compact structure of the epidermis due to their size and solubility limitations (Ramadon et al., 2022). Despite the potential advantages of the transappendageal route, its use is limited by the small surface area it occupiesapproximately 0.1% of total skin area, compared to the more dominant transepidermal route. To overcome the barrier posed by the stratum corneum (SC) and enhance drug permeability, researchers have developed a variety of strategies aimed at modifying the SC’s structure. These include chemical enhancers, physical techniques, and combinatory approaches, all designed to facilitate more efficient drug transport across the skin. The subsequent sections explore the progress in transdermal product development and detail the innovative technologies that have been employed to improve cutaneous drug absorption (Alkilani et al., 2015).

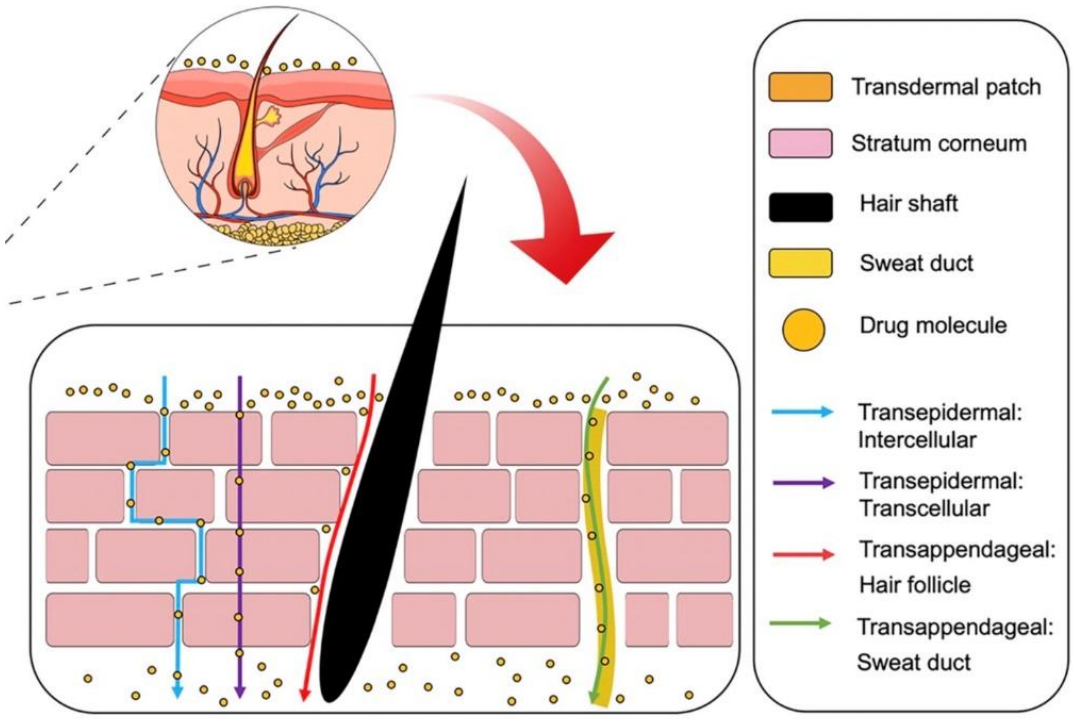

Figure 2.6 Mechanisms for transdermal medication administration

2.4.3 *In vitro* skin permeation by Strat-M^{TM} membranes

The transdermal route is increasingly utilized for systemic drug delivery due to several advantages, including bypassing hepatic first-pass metabolism, ease of administration, prolonged drug release compared to oral routes, and improved patient adherence. However, a major limitation is the low permeability of the stratum corneum (SC), the skin's outermost layer, which serves as a formidable barrier to most compounds. To overcome this challenge, both chemical and physical enhancement techniques are employed to facilitate drug permeation. In a study conducted by Kouchak and Handali (2013), *in vitro* percutaneous absorption using Franz diffusion cells and snakeskin demonstrated that various chemical enhancers significantly increased drug permeability. Specifically, increasing the concentration of lauric acid enhanced drug diffusion, while higher levels of sodium tauroglycocholate (STGC) showed a diminishing effect on enhancement. These findings underscore the importance of optimizing both the type and concentration of penetration enhancers to maximize transdermal drug delivery efficacy (Kouchak & Handali, 2014). However, the skin acts as a highly effective physical barrier that protects the internal environment

from external stressors. This barrier function is largely attributed to the stratum corneum (SC), which presents the primary obstacle to cutaneous drug delivery. The SC's structural composition significantly limits the permeation of molecules, especially those with physicochemical properties that are not inherently favorable for skin absorption. As a result, the delivery of therapeutics through the skin remains a challenge when dealing with compounds that lack optimal solubility, size, or lipophilicity for transdermal penetration (Basto et al., 2021). Permeation enhancers temporarily modify the skin's structure to facilitate drug penetration. Ideal enhancers should be reversible, non-toxic, non-irritating, non-allergenic, and compatible with both drugs and excipients. However, many chemical enhancers carry local or systemic side effects. Consequently, safer and more effective alternatives are being explored, with natural essential oils emerging as promising candidates due to their favorable safety profiles and proven ability to enhance skin penetration in transdermal drug delivery (Nawaz et al., 2022). Transdermal patches offer a promising approach for systemic drug delivery by enabling passive diffusion of therapeutic agents through the skin. This method facilitates direct entry of the drug into systemic circulation, bypassing the gastrointestinal tract and first-pass metabolism, making it a convenient and non-invasive option for managing various systemic conditions (Sabbagh & Kim, 2022). Permeation enhancers are utilized in transdermal drug delivery systems to improve skin permeability by temporarily disrupting or modifying the structural integrity of the stratum corneum. This disruption enhances the diffusion of therapeutic agents across the skin barrier, thereby facilitating the attainment of effective plasma drug concentrations. The use of permeation enhancers is critical in optimizing drug bioavailability in transdermal applications, particularly for compounds with limited intrinsic skin permeability (Latif et al., 2022). Similarly, the study by Klebeko J. et al. (2021) evaluated how modifications to both the drug structure and formulation vehicle influence the skin permeation and accumulation of ibuprofen (IBU). Using human abdominal skin and Strat-M™ membranes in in vitro Franz diffusion experiments, the researchers compared hydrogels containing IBU and its derivatives with commercial ibuprofen gels. Quantitative analysis was conducted via HPLC. The findings demonstrated that the Celugel® formulation significantly enhanced IBU skin

penetration, delivering over three times the cumulative amount through human skin after 24 hours compared to the commercial product. These results highlight the potential of Celugel® as an effective transdermal delivery system when paired with structurally modified drugs. Furthermore, the study supports the utility of Strat-M™ membranes as a reliable substitute for human skin in evaluating transdermal drug permeation and accumulation (Klebeko et al., 2021). Transdermal drug delivery has become increasingly prominent due to its advantages over traditional oral and injectable routes, such as bypassing hepatic first-pass metabolism, protecting drugs from degradation in the gastrointestinal tract, enabling sustained drug release, and enhancing patient adherence. During pharmaceutical development, *ex vivo* permeation testing plays a crucial role in evaluating the quality and performance of transdermal systems. These studies typically utilize excised human skin from the intended application site or appropriate animal skin models to assess drug permeation characteristics under controlled conditions (Neupane et al., 2020). However, the limited availability of human skin and ethical concerns associated with animal use have reduced the appeal of these models in permeation studies. Despite this, permeation studies remain essential for demonstrating the suitability and efficacy of transdermal drug delivery systems, as they provide critical insights into the system's ability to deliver therapeutic agents across the skin barrier.

Franz diffusion cells are commonly used to assess drug permeation through the skin, and the permeation study across the dermatome of human skin explants is regarded as the gold standard for evaluating drug delivery via a transdermal system. Nevertheless, ethical and economic concerns limit the availability and use of human skin. As a result, isolated skin from inbred animals—such as porcine, primates, rodents (guinea pig, rat, and mouse), rabbit, and shed snake skin—has been routinely considered as an alternative to human skin, since it is easier to obtain, can be excised fresh prior to skin permeation studies while maintaining viability and enzymatic activity, and exhibits less variability (Kerimoğlu & Şahbaz, 2018; Todo, 2017).

2.4.4 Strat-M™ membranes

Strat-M™ is a synthetic membrane developed as an alternative to animal or human skin in permeation studies. Specifically, it replicates essential structural and chemical properties of human skin, offering a multilayer design. Its uppermost layer is tightly compacted and coated with a lipid composition that closely resembles the lipid matrix of the human stratum corneum (SC), while its underlying porous layers mimic the structural characteristics of the viable epidermis and dermis. This design enables Strat-M™ to simulate both the barrier and diffusion properties of real skin, making it a valuable tool in evaluating transdermal drug delivery systems (Haq et al., 2018). Moreover, the Strat-M™ membrane demonstrates permeability characteristics that are closely equivalent to those of human skin, particularly in drug diffusion studies. It is therefore designed to offer superior correlation with human skin compared to other biological membranes, and serves as a reliable and reproducible model for transdermal drug evaluation—without the ethical and variability concerns associated with human or animal tissue (Arce et al., 2020).

A previous study conducted in 2015 utilized infinite dose models to evaluate the permeation of various chemicals dissolved in phosphate-buffered saline (PBS, pH 7.4) through the Strat-M™ membrane. The findings revealed that there was a strong correlation between the permeation profiles observed in Strat-M™ and those seen in both rat and human skin, thus supporting the membrane's validity as a surrogate for biological tissues in transdermal drug delivery studies (Uchida et al., 2015). Furthermore, a recent investigation into nicotine permeation from formulations using binary solvents (comprising water and chemical penetration enhancers) applied at a high finite dose (200 µL/0.64 cm^2) demonstrated strong correlation between Strat-M™ and human skin in terms of permeation behavior. This consistency further reinforces Strat-M™ 's relevance as a surrogate model. As a result, multiple studies have recommended the use of Strat-M™ as an alternative membrane in cosmetic development, formulation optimization, regulatory evaluations, and safety testing. To validate its suitability, it is essential to characterize and compare membrane parameters such as permeability coefficient, flux, and penetrant distribution between Strat-M™ and porcine skin, thereby establishing their equivalency for active ingredient

assessment (Arce et al., 2020). For ex *vivo* permeation testing, the use of excised human or animal skin from relevant anatomical sites is traditionally recommended. However, limitations such as the limited availability of human skin and ethical issues associated with animal use have reduced the appeal of these biological models. In response to these limitations, significant progress over the past three decades has led to the development of artificial membranes, including the Strat-M™ model, as viable alternatives. Indeed, Strat-M™ has been shown to effectively mimic human skin in terms of permeability characteristics and is now widely recognized as a suitable substitute for assessing drug permeation and accumulation in transdermal drug delivery research.

Electrospinning was employed to fabricate both drug-free and diclofenac sodium (DS)-loaded polyvinyl alcohol (PVA) patches. Scanning electron microscopy (SEM) confirmed successful integration and structural compatibility between the electrospun PVA nanofiber and the PVA cryogel matrix, with DS uniformly incorporated. Moreover, Fourier-transform infrared spectroscopy (FTIR) analysis revealed no chemical interaction between DS and PVA, as evidenced by the presence of DS-specific peaks in all medicated dual-layer patches. Higher cross-linking density and the presence of DS led to reduced swelling capacity, attributed to diminished water uptake after 24 hours in phosphate-buffered saline (PBS). *In vitro* drug release studies using Franz diffusion cells with a cellulose nitrate membrane (as a skin model) demonstrated that patches containing 2% w/v DS achieved sustained drug release for up to 24 hours (Sa'adon et al., 2021). These studies support the development of cost-effective and environmentally sustainable nanofiber patch technologies that avoid the use of toxic components. The novel capsaicin-loaded nanofiber patches show potential as transdermal drug delivery systems, offering a means to reduce the gastrointestinal side effects commonly associated with nonsteroidal anti-inflammatory drugs (NSAIDs) while meeting growing demands in pharmaceutical and biomedical fields. A 2014 study optimized a capsaicin nanoemulsion formulation for topical use, demonstrating its successful permeation through the Strat-M™ membrane in a Franz diffusion cell system. The nanoemulsions, with particle sizes between 20 and 62 nm, effectively penetrated the membrane layers, indicating their suitability for transdermal

application (Kim et al., 2014). The Strat-M™ membrane has been validated as a reliable substitute for human skin in drug permeation and accumulation studies, offering a consistent and ethical alternative for *in vitro* testing. Moreover, prior literature reviews support the effectiveness of artificial skin models in evaluating skin permeability from transdermal patches.

Therefore, these findings contribute to the advancement of knowledge and technological innovation in the development of capsaicin-loaded transdermal nanofiber patches. The study lays a foundation for improving transdermal drug delivery systems through the fabrication and optimization of capsaicin nanofiber formulations.

2.4.5 Advantages of Transdermal drug delivery system (TDDS)

Transdermal drug delivery systems (TDDS) offer a compelling alternative to oral and injectable routes because they circumvent key limitations such as first-pass hepatic metabolism, gastrointestinal degradation, as well as issues related to gastric emptying and pH variability. Unlike oral medications, TDDS can be administered to unconscious or nauseated patients, and unlike injectable routes, they avoid pain, bruising, bleeding, and needle-associated risks—such as infections, accidental injury, and sharps waste. Therefore, these attributes contribute to enhanced patient compliance and treatment safety, while also supporting cost-effectiveness in healthcare delivery. TDDS systems offer controlled, prolonged drug release, which help to reduce peak plasma concentrations, and minimize the risk of systemic toxicity. Their ease of application and removal enhances administrative flexibility, making them particularly suitable for self-administration. Furthermore, they are effective in targeting localized dermatological conditions, thereby improving therapeutic outcomes while limiting systemic side effects. However, TDDS are not without drawbacks. The most common concerns involve skin irritation and sensitization. Components such as adhesives, excipients, or the active pharmaceutical ingredient may provoke irritant contact dermatitis (ICD) or allergic contact dermatitis (ACD). ICD results from chemical or physical irritants triggering an innate immune response, characterized by erythematous, pruritic, or painful patches or plaques, without the formation of antigen-specific memory T cells. In contrast, ACD is a type IV delayed hypersensitivity reaction

mediated by T cells. It involves a two-phase immune response induction followed by elicitation which explains the delayed onset of symptoms following repeated allergen exposure. Transdermal drug delivery systems (TDDS) serve as a viable alternative to traditional routes of administration, including oral, intravenous, subcutaneous, and transmucosal methods. The advantages of TDDS include the avoidance of first-pass hepatic metabolism, improved patient adherence, reduced systemic drug interactions, the potential for on-demand dose adjustments, elimination of the need for healthcare-assisted administration, sustained drug release, and enhanced therapeutic efficacy. Transdermal patches were among the earliest TDDS technologies introduced and are based on relatively simple engineering principles. The first FDA-approved transdermal patch, Transderm-Scop®, was developed for the prevention of motion sickness using scopolamine. The success of this system led to the subsequent approval of transdermal delivery platforms for drugs such as nitroglycerin, fentanyl, estradiol, nicotine, and testosterone. These advancements paved the way for innovative therapeutic strategies, offering alternative delivery options for existing medications while potentially reducing adverse effects. For instance, estradiol patches, which are widely used by millions of patients annually, avoid the hepatic complications associated with oral estrogen therapies, thus representing a safer and more efficient method for hormone replacement therapy (Cramer & Saks, 1994). Transdermal nicotine delivery systems represent a significant advancement in medical therapy, having assisted millions of individuals in smoking cessation. Their widespread use has not only improved public health outcomes but has also likely contributed to increased life expectancy among former smokers by reducing the risks associated with long-term tobacco use (Murthy, 2012). Over the past three decades, the U.S. Food and Drug Administration (FDA) has approved more than 35 transdermal patch products across a wide range of therapeutic categories. Among these is the capsaicin 179 mg (8% w/w) cutaneous patch, commonly referred to as the capsaicin 8% patch, which delivers localized treatment for peripheral neuropathic pain. Notably, this formulation has demonstrated the ability to provide significant and sustained pain relief following a single application, making it a valuable option in the management of chronic neuropathic conditions (van Nooten et al., 2017). Topical formulations of capsaicin are

commonly utilized for pain management. According to meta-analyses of multiple studies, low-concentration capsaicin preparations are generally safe but exhibit limited efficacy, often requiring frequent daily self-application to achieve therapeutic benefits. To address these limitations, a high-concentration capsaicin 8% patch (Qutenza™) has been developed and has received regulatory approval in both the European Union and the United States for the treatment of peripheral neuropathic pain, offering a more convenient and effective alternative through single-application therapy (Anand & Bley, 2011). In recent years, innovative drug delivery systems have garnered significant research attention due to their potential to overcome limitations of conventional pharmacotherapy. These systems aim to address issues such as drug instability, high systemic toxicity, unfavorable pharmacokinetics, low cellular uptake, and the emergence of drug resistance. By enhancing the precision, efficiency, and safety of therapeutic agents, novel delivery strategies offer promising avenues for improving treatment outcomes across a range of medical conditions (Bibi et al., 2017). Nanostructure-based drug delivery systems offer significant advantages over conventional therapeutic approaches by enhancing drug safety, efficacy, and patient adherence. Their ability to control the spatial and temporal release of pharmacological agents allows for targeted and sustained delivery, thereby improving therapeutic outcomes. Owing to these benefits, the development and commercialization of such nanocarrier systems have rapidly progressed, positioning them as a promising advancement in modern drug delivery strategies.

2.4.6 Disadvantages of Transdermal drug delivery system (TDDS)

A significant limitation in transdermal drug delivery is that many drugs, especially hydrophilic compounds, may exhibit insufficient skin penetration rates, thus preventing them from reaching therapeutic concentrations. Furthermore, local adverse reactions such as erythema, pruritus, and edema may arise due to the active pharmaceutical ingredient, the adhesive, or other excipients within the patch formulation. In addition, the barrier function of the skin is not uniform, as it can vary significantly between anatomical sites, among individuals, and is also influenced by age-related factors.

1. One possible side effect of TDDS is contact dermatitis.

2. The natural constraints of drug penetration mean that transdermal patches can only be used with very effective medications.

3. Some medications, such as the scopolamine transdermal patch put behind the ear, cause discomfort to the patient.

4. Long-term patch adhesion is a problem with TDDS.

5. High manufacturing costs of transdermal patch as comparison to conventional dose form.

6. TDDS does not have the ability to administer ionic medicines via the skin.

7. TDDS is unable to achieve high blood/plasma concentrations of drugs.

8. There is no way to build TDDS for big molecules.

9. If a medicine or formulation irritates the skin, TDDS cannot develop.

2.4.7 Limitation of Transdermal drug delivery system (TDDS)

1. There must be some physicochemical qualities for medication penetration via the skin, and transdermal distribution is extremely challenging if the drug dose is greater than 10-25mg/day. Less than 5mg/day was preferred for the daily dose of medication.

2. Itching, erythema, and local edema may be brought on by the medicine itself or by the excipients used in the formulations.

3. A transdermal product's clinical requirement must also be thoroughly assessed before a choice is made.

4. The components of the system cause contact dermatitis in certain patients.

5. The skin's barrier function varies depending on the location, the individual, and the passage of time.

6. There are only a limited number of medications that may be administered in this technique because of poor skin permeability.

7. Drugs that cause tolerance or those that need to be administered at specific times (e.g. hormones) are not acceptable candidates.

TDDS is an alternative route of drug administration for medications with low efficacy when administered orally, topically, intravenously, or intramuscularly. Recent breakthroughs in TDDS involve the use of nanoparticles (NPs), which have the potential to improve medication absorption over the skin. NPs can also enable controlled release, the capacity to administer both hydrophilic and hydrophobic medications, minimize side effects, and are non-invasive when utilized in a TDDS manner. Transdermal patches are another emerging TDDS technology. TDDS using a minimally invasive technique in which micron-sized pores are formed in the epidermis to allow medication delivery to blood vessels in the dermal layer of the skin. New studies have concentrated on integrating various TDDS methodologies to overcome past limitations of drug delivery using conventional methods. Recent advances in nanotechnology have had an impact on all areas of basic and applied research. The emergence of nanostructured systems offering multiple advantages has significantly heightened scientific interest in the application of nanotechnology within transdermal drug delivery systems (TDDS). In recent decades, it has become increasingly evident that the route of administration plays a critical role in determining a drug's therapeutic efficacy by modulating its pharmacokinetics profile, biodistribution, pharmacodynamics, metabolism, and toxicity. The advent of various nanotechnologies including nanoparticles, nanofibers, nanogels, micelles, and microspheres has paralleled the advancement of innovative drug delivery strategies, establishing these nanocarriers as promising tools in the pharmaceutical and biomedical fields (Pant et al., 2019). By using passive or active targeting strategies based on the final formulation, nanocarriers can be employed to wrap and distribute medications that are too poisonous, insoluble, rapidly removed, or unstable as free molecules. Electrospinning is a cost-effective, easy, and adaptable method for producing polymer nanofibers (Luraghi et al., 2021). Electrospinning is a technique that utilizes a high-voltage electric field to generate fibers from a polymer solution extruded through a needle. The resulting electrospun fibers can be tailored in terms of porosity, morphology, and surface area by modifying both processing parameters and ambient conditions,

allowing for precise control to suit specific drug delivery applications. Drug incorporation into these fibers can be achieved either through direct blending of the drug with the polymer solution or via surface immobilization post-spinning, each offering distinct drug release profiles. A wide range of therapeutics including small molecules, proteins, and nucleic acids can be successfully encapsulated. Advanced electrospinning systems enable the co-delivery of multiple agents for synergistic effects, or allow for stimuli-responsive release, thereby enhancing the therapeutic potential of nanofiber-based drug delivery systems.

2.5. Electrospinning

Electrospinning, also referred to as electrostatic spinning, is a well-established technique for producing micro- and nanofibers from polymer solutions or melts. Although its modern application in material science is relatively recent, the underlying principle—the influence of electrical charges on liquid droplets—has been recognized for centuries. For instance, as early as the 17th century, William Gilbert observed that a droplet of water placed on a dry surface could be drawn into a conical shape when exposed to a charged object, such as a piece of rubbed amber held nearby. This foundational observation laid the groundwork for the development of electrohydrodynamic technologies like electrospinning (Asmatulu & Khan, 2018). Electrospinning is a versatile and relatively simple technique that utilizes a high-voltage electric field to draw micro- and nanofibers from polymer solutions or melts. The process enables fine control over fiber morphology by adjusting both electrospinning parameters (such as voltage, tip-to-collector distance, and flow rate) and solution properties (such as viscosity, conductivity, and surface tension). These adjustments allow for the modulation of key fiber characteristics including diameter, length, surface roughness, porosity, pore interconnectivity, and alignment, as well as the presence or absence of structural defects such as beads (Vong et al., 2021). A core element of the process is the formation of a Taylor cone at the tip of the spinneret under a high-voltage field. Once the electrostatic force overcomes surface tension, nanofibers are ejected and deposited onto a collector, forming nonwoven mats or aligned structures, depending on the setup. Electrospinning also supports the fabrication of ceramic,

composite, and functionalized fibers, thus making it a highly adaptable technique. Because of its tunability and scalability, it has gained widespread attention across diverse domains, such as biomedicine (e.g., wound dressings, transdermal drug delivery systems, scaffolds for tissue engineering), biosensing and diagnostics, cosmetics and personal care products, protective clothing and filtration materials, as well as catalysis and adsorption applications (e.g., dye removal, chromatography), and energy storage (e.g., electrodes for batteries and fuel cells). The combination of precise structural control, broad material compatibility, and functional flexibility makes electrospinning an attractive technology for next-generation material design and industrial innovation (Vong et al., 2018). Electrospinning is an innovative fiber fabrication technique that employs a high-voltage electrostatic field to generate ultrafine fibers from polymer solutions or melts. The method has garnered significant attention within the scientific community due to its ability to produce nanofibers with controlled dimensions and diverse functional properties. Electrospun fibers are characterized by a high surface area-to-volume ratio, excellent porosity, and structural flexibility, making them suitable for various biomedical, environmental, and industrial applications. The process involves dispensing a polymer solution through a syringe equipped with a metallic spinneret, where a high-voltage DC power source is applied. This causes the polymer droplet at the spinneret tip to elongate under the influence of electrostatic forces, forming a structure known as a Taylor cone. Once the electrostatic repulsion surpasses the surface tension, a fine jet is ejected from the cone and travels toward a grounded collector, solidifying into continuous nanofibers. Electrospinning offers a relatively simple, cost-effective, and scalable route for producing fibers with diameters ranging from a few nanometers to several micrometers. Its broad applicability and tunable processing parameters make it a valuable tool in advancing nanotechnology-driven innovations across scientific and industrial sectors (Asmatulu & Khan, 2018).

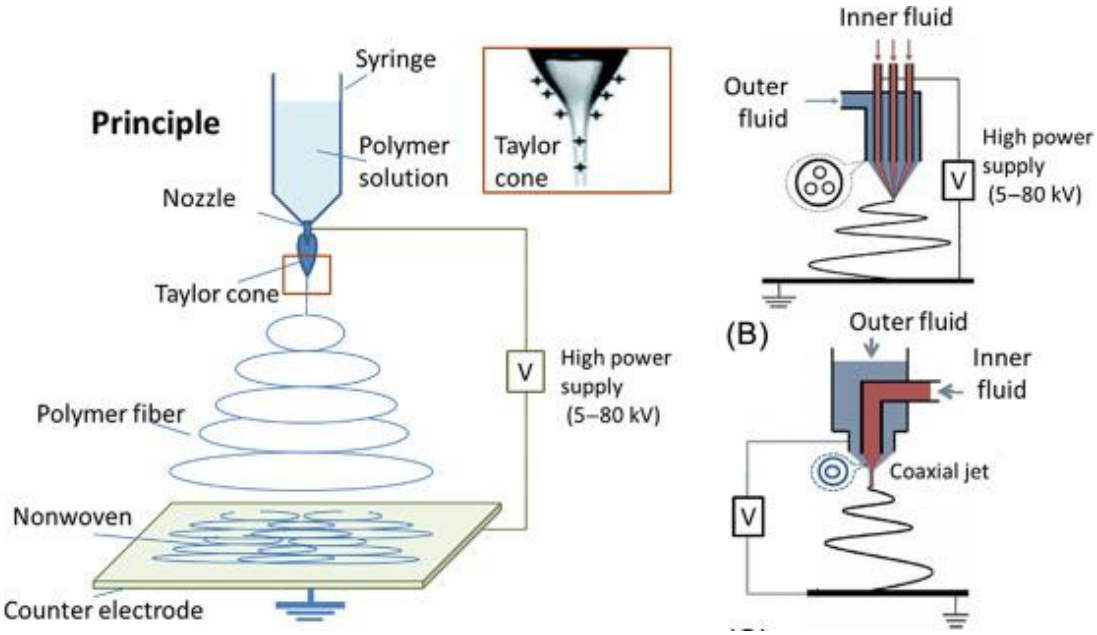

Figure 2.7 Nanofibers electrospinning process

(Zheng, 2019)

2.5.1 Nanofibers Patch

Synthetic and natural polymer-based nanoparticles represent a promising alternative for therapeutic applications owing to their favorable properties, including biocompatibility, non-immunogenicity, non-toxicity, and biodegradability. These characteristics enable their safe interaction with biological systems, thus making them ideal carriers for targeted drug delivery, controlled release, and enhanced therapeutic efficacy, while minimizing adverse effects. Moreover, their versatility in formulation also allows for the encapsulation of a wide range of therapeutic agents, thereby contributing to their growing significance in biomedical and pharmaceutical research (Crucho & Barros, 2017). The advantageous properties of polymer-based nanoparticles stem from their ability to be tailored using both synthetic and natural polymers. For example, synthetic polyester polymers such as polycaprolactone (PCL) and polylactic acid (PLA), along with their monomers, are often employed to reduce immunogenicity and toxicity. In contrast, natural polymers including chitosan, gelatin, albumin, and alginate further enhance biocompatibility and are generally more effective at minimizing toxicity, thereby improving the therapeutic efficacy of encapsulated agents compared to conventional drug delivery systems. Polymeric

nanoparticles are typically structured as matrix systems in which the active therapeutic agents are either uniformly distributed or encapsulated, depending on the nanoparticle design. When the drug is uniformly dispersed within the polymer matrix, the structure is termed a nanosphere. Conversely, when the drug is enclosed within a polymer shell, forming a core–shell configuration, it is referred to as a nano capsule. This structural versatility allows for controlled release, improved stability, and targeted delivery of various pharmaceutical agents (Letchford et al., 2009). Polymeric nanoparticles offer customizable platforms for the controlled release of therapeutic agents, thereby enabling targeted delivery with elevated drug concentrations at the desired site. Their surfaces can be readily modified or functionalized with specific recognition ligands, thus enhancing tissue-specific targeting and minimizing off-target effects. In particular, polymers such as polyacrylic acid (PAA), polyvinyl chloride (PVC), polyurethane (PU), polyvinyl alcohol (PVA), and polyvinylpyrrolidone (PVP) have demonstrated significant potential in electrospinning applications. These materials facilitate the fabrication of nanofiber-based carriers, providing promising avenues for the development of advanced drug delivery systems with improved therapeutic efficacy (Rahmani et al., 2021). Polyvinylpyrrolidone (PVP) is an amorphous synthetic polymer characterized by its high-water affinity and excellent adhesive properties. Due to its low chemical toxicity, biocompatibility, and ease of formulation, PVP has become a crucial material in biomedical and pharmaceutical applications. It is widely utilized in drug delivery systems, wound dressings, and nanofiber fabrication owing to its ability to enhance solubility, stabilize active pharmaceutical ingredients, and promote controlled drug release (Wang et al., 2015). Polyvinylpyrrolidone (PVP) films exhibit promising potential as advanced wound-dressing materials due to their ability to maintain a moist wound environment, which prevents dehydration and scab formation—crucial factors in promoting optimal healing. Electrospun PVP nanofibers are frequently employed as polymeric carriers in drug delivery systems, particularly for water-soluble drugs. Their high porosity, large surface-to-volume ratio, and excellent solubility in water enable the rapid release of encapsulated therapeutics. Similarly, electrospun polyvinyl alcohol (PVA) has gained attention as a biocompatible and biodegradable polymer with broad applications in biomedical fields. PVA nanofibers are well-suited for rapid

drug delivery, as demonstrated in Li., et al's study, where complete release of caffeine and partial release of riboflavin occurred within 60 seconds. These properties underscore the suitability of both PVP and PVA nanofibers for fast-acting transdermal and topical drug delivery applications (Li et al., 2013).

Incorporating hydrophobic polymers into polyvinyl alcohol (PVA) matrices can enhance drug release properties, particularly for hydrophobic compounds. A study investigating the impact of increasing PVA content in PVA/PVP composite nanofibers revealed that such composites could achieve sustained release profiles for ciprofloxacin hydrochloride, a commonly used antibiotic. The results demonstrated that the addition of PVP to PVA not only contributed to the controlled release of the drug but also improved the mechanical properties of the nanofiber membranes, notably increasing the ultimate yield strength. This suggests that PVA/PVP composite nanofibers offer a robust and effective platform for sustained drug delivery applications (Rahmani et al., 2021). Additionally, the high fluid absorption capacity and slow degradation rate of these membranes confirm their ability to maintain a moist wound environment, which is essential for promoting optimal healing conditions and tissue regeneration.

2.5.2 Advantages of nanotechnology

The integration of nanotechnology into medical science offers promising advancements, particularly through the use of nanoparticles, which serve as fundamental units in this field. Recent innovations have highlighted the potential of nanoparticles in therapeutic applications, especially for targeted and controlled delivery of both small and large molecules. Their versatility in size and shape, high drug-loading capacity, and ability to encapsulate both hydrophilic and hydrophobic compounds make them ideal candidates for precision medicine. Additionally, nanoparticles can establish stable interactions with ligands, enhancing tissue-specific delivery. However, despite their advantages, concerns regarding their toxicity and possible side effects persist, necessitating careful evaluation prior to clinical application. Understanding the physicochemical properties of nanoparticles and the strategies employed for their effective delivery is crucial to ensuring their safe and

efficient therapeutic use (Yetisgin et al., 2020). Advancements in nanotechnology engineering have significantly integrated multidisciplinary fields such as materials science, chemical engineering, tissue engineering, and nanomedicine. These innovations are inherently tied to the unique properties and functionalities of materials at the nanometer scale, where quantum and surface phenomena enable novel applications and enhanced performance across biomedical and pharmaceutical domains (Chauhan et al., 2020). Nanostructured materials offer distinct advantages over conventional therapeutic approaches by addressing key limitations, including poor target tissue specificity, uncontrolled drug release rates, and rapid biodegradation of bioactive agents. These materials can effectively encapsulate both hydrophilic and hydrophobic compounds, enhancing drug stability and bioavailability while minimizing systemic side effects. As versatile carriers, nanostructures contribute to more precise, sustained, and safer drug delivery (Karimi et al., 2017). Moreover, advanced strategies such as surface functionalization, passivation, and co-loading of multiple therapeutic agents within a single nanocarrier have significantly enhanced the efficacy of nanomedicines. These approaches contribute to improved pharmacokinetics, targeted delivery, and controlled release, thereby ensuring more uniform and predictable biological responses compared to conventional drug delivery systems (Charelli et al., 2022). This section provides a comprehensive overview of the unique properties of nanoparticles within biological systems, highlighting their clinical applications and therapeutic specificity. Emphasis is placed on both the types of nanoparticles currently employed in clinical practice and the targeted delivery strategies developed for various diseases, including cancer, infectious diseases, autoimmune disorders, cardiovascular conditions, neurodegenerative diseases, ocular pathologies, and pulmonary illnesses. A deeper understanding of nanoparticle biological system interactions will pave the way for the development of novel diagnostic, therapeutic, and preventive approaches, especially for diseases that remain challenging or incurable with current medical interventions.

2.5.3 Nanotechnology as therapeutic agents

The primary objective of nanomedicine is to harness nanotechnology for enhancing the efficacy and safety of pharmaceutical agents. This is often achieved by incorporating uncoated drugs into biocompatible nanocarriers, including nanoparticles, liposomes, micelles, and dendrimers. Nanoparticulate drug delivery systems (NDDSs) are engineered with adjustable parameters such as particle size, morphology, surface charge, and drug loading capacity to enable extended systemic circulation and facilitate precise targeting of tissues or even specific subcellular compartments (Almeida et al., 2011; Blanco et al., 2015). As illustrated in the accompanying figure, nanoparticulate drug delivery systems (NDDSs) can be engineered with surface modifications, such as cell-penetrating peptides or target-specific ligands, to enable drug transport across the blood–brain barrier, thereby facilitating central nervous system (CNS) delivery. By precisely controlling spatial localization, reducing required dosages, and minimizing adverse effects, NDDSs significantly enhance therapeutic efficacy in targeted medical applications (Bhansali et al., 2021). Despite the many advantages offered by nanomaterials, their limited success in alleviating chronic pain underscores the pressing need for more effective therapeutic strategies. Given the heterogeneous etiology of chronic pain, both the type of drug and the optimal dosage must be tailored to the underlying condition. One promising approach to enhancing treatment efficacy while minimizing systemic side effects is to increase drug concentration at the specific site of action. This targeted delivery can be achieved by functionalizing nanomaterials with targeting ligands, such as peptides or antibodies. Additionally, the route of administration plays a crucial role in optimizing therapeutic outcomes. For instance, topical formulations, such as anti-inflammatory creams or sprays, are suitable for cutaneous or localized neuropathic pain, whereas spinal injections are more appropriate for chronic spinal nerve pain. Other conditions involving internal organs or systemic pain may benefit from oral, intranasal, intramuscular, or intravenous administration. The following section reviews recent advancements and targeted strategies in the use of nanomaterials for chronic pain management.

In a recent study, Joshi et al. employed a nanofibrous transdermal delivery system (TDS) characterized by controlled drug release and enhanced mucoadhesive properties for the treatment of periodontitis (Joshi et al., 2015). This approach demonstrated improved therapeutic selectivity and reduced side effects. Among emerging delivery vehicles, nano-sized drug capsules are garnering increased attention due to their distinct advantages over conventional formulations. Compared to traditional capsules, nano capsules possess a substantially larger surface area, enabling accelerated drug degradation and absorption rates, even at equivalent drug masses. Additionally, electrospun nanofibers represent another advanced nanomaterial-based drug carrier, offering sustained drug release and enhanced bioavailability, particularly for compounds with poor solubility. These nanoscale carriers facilitate the gradual and efficient absorption of therapeutics into the body, thereby improving pharmacological outcomes. When constructed from biodegradable polymers, such carriers degrade into non-toxic byproducts that can be safely metabolized or excreted, further enhancing their biocompatibility. The electrospinning process used to fabricate these nanofibers involves the application of a high-voltage electric field to a polymer melt or solution, resulting in the formation of a Taylor cone at the needle tip. The solution is subsequently stretched into a liquid jet, which solidifies into nanofibers upon reaching the collector surface yielding fibrous structures with nanometric diameter and high surface-area-to-volume ratio, ideal for drug delivery applications.

Table 2.4 Electrospinning Nanofibers for transdermal drug delivery

Treatment	Materials	Drugs	Hight Voltage	Results	References
Cancer	Polyvinyl alcohol, polyethylene oxide and polyvinylpyrrolidone	Proteins (e.g., zein, gelatine, and silk) and polysaccharides (e.g., chitosan, cellulose, and sodium alginate)	10 kV–30 kV	Encapsulating drugs in coaxial electrospun nanofibers offers an effective approach for achieving controlled and prolonged drug release.	(Li et al., 2022)
Antibacterial	Polyvinyl alcohol	L-lysine/ PEO solution/ (ibuprofen (IBP)	0–30 kV	The findings indicate that PVA-Lysine (PVA Lys) electrospun membranes incorporating ibuprofen (IBP) or linalool (LO) effectively promote wound healing. Specifically, the PVA Lys LO membranes demonstrated strong antibacterial activity. These results support the potential use of PVA Lys electrospun membranes as effective wound dressing materials.	(Sequeira et al., 2019)
Fabrication, antibacterial and cytocompatibility evaluation and in vitro healing assay	Polyvinyl alcohol	chitosan	25 kv.	To validate the potential of the nanofibrous mats for wound applications.	(Adeli et al., 2019)
Gingivitis	Polyvinylpyrrolidone	ornidazole	-	The findings indicate that ornidazole-loaded electrospun fibers hold potential as an effective drug delivery system for managing gingivitis, offering localized and sustained therapeutic action at the site of inflammation.	(Tort et al., 2019)

Table 2.4 Electrospinning Nanofibers for transdermal drug delivery (Continued)

Treatment	Materials	Drugs	Hight Voltage	Results	References
Psoriasis	polymethyl vinyl ether-alt-maleic acid	salicylic acid, methyl salicylate, and capsaicin	13 kV	GC-MS analysis demonstrated that most encapsulated compounds remained stable over 15 days, with the exception of methyl salicylate. The encapsulated drugs preserved or enhanced their activation of the TRPV1 channel, which is associated with psoriasis treatment.	(Martínez-Ortega et al., 2019)
	Polyvinylpyrrolidone	Ibuprofen	15 kV	*In vitro* dissolution tests showed fiber mats dissolved in 10 s via a polymer-controlled mechanism.	(Yu et al., 2009)
Pain Management	Polyvinylpyrrolidone Polyvinyl alcohol	Buprenorphine	0–30 kV	The buprenorphine-loaded PVP/PVA nanofiber system demonstrates superior physicochemical properties compared to PVP-only formulations. The integration of PVA enhances fiber strength and stability, while cross-linking within the nanofiber matrix enables sustained drug release. This formulation strategy improves drug retention and prolongs therapeutic effects, supporting its potential application as an effective transdermal patch for pain management.	(Rahmani et al., 2021)
Comprehensive wound care	Polyvinyl alcohol	Diclofenac sodium Capsaicin Gentamicin	20 kV 15 kV 20 kV	Multilayer dressing sped rat wound healing from 21 to 7 days (including Diclofenac sodium, capsaicin and gentamicin). Histopathology showed intact epidermis in treated samples.	(Nada et al., 2020)

Table 2.4 Electrospinning Nanofibers for transdermal drug delivery (Continued)

Treatment	Materials	Drugs	Hight Voltage	Results	References
Patches Loaded with a Long-Acting Pharmacological	Polyvinylpyrrolidone Polyvinyl alcohol	Diclofenac Sodium Salt (DS), Gentamicin	20 kV	The study revealed that the release rate of Cucurbitacin (CC) from CC-loaded electrospun polyvinyl alcohol (PVA) mats was significantly higher than that from corresponding as-cast PVA films, with the rate increasing proportionally to CC concentration. These findings highlight the potential of electrospun PVA mats as effective transdermal delivery systems for medicinal applications of CC.	(Hindi et al., 2021)
Topical skin treatment	Polyvinyl alcohol	Capsaicin	15kV,17.5kV	Capsaicin derived from chili extract (CE)-loaded electrospun polyvinyl alcohol (PVA) mats demonstrated enhanced release rates and improved skin permeation compared to non-electrospun formulations. These results support the potential application of CE-loaded electrospun PVA mats as effective transdermal therapeutic delivery systems.	(Sa'adon et al., 2019)
A fast-dissolving loratadine	Polyvinylpyrrolidone	Loratadine	10 kV, 20 kV	Diameter and loratadine amount affected nanofiber drug release and disappearance time. Nanofiber solubility and release time increase with fiber diameter and medication quantity. Electrospinning can produce fast-dissolving loratadine nanofibers.	(Akhgari et al., 2016)

2.6 Related work and study summary

The effectiveness of transdermal drug delivery systems (TDDS) lies in their ability to deliver drug molecules efficiently to targeted cells, tissues, or organs at a precise therapeutic concentration for a specified duration and at a controlled rate. Additionally, the use of suitable drug carriers within TDDS facilitates sustained drug release, ensuring consistent pharmacological activity.

2.6.1 Clinical trials

Transdermal NSAIDs: Nonsteroidal anti-inflammatory drugs (NSAIDs) exert their anti-inflammatory and analgesic effects primarily by inhibiting cyclooxygenase (*COX*) enzymes, thereby reducing prostaglandin synthesis and mitigating chronic hyperalgesia. When administered topically, NSAIDs can achieve therapeutic drug concentrations directly at the site of inflammation or pain with minimal systemic absorption, potentially minimizing adverse systemic effects. The clinical efficacy of topical NSAIDs is contingent upon their ability to penetrate the skin and reach the target site. Various NSAIDs exhibit differing rates of dermal permeation and are utilized in managing conditions such as acute musculoskeletal injuries, back pain, chronic musculoskeletal disorders, and neuropathic pain. Topical formulations include ointments, gels, pastes, and transdermal patches. Among these, patches demonstrate superior skin permeation and adherence compared to gels and ointments. Nonetheless, topical NSAIDs can still lead to local and systemic adverse effects. Approximately 1–2% of users may experience dermatological reactions such as rashes, pruritus, burning sensations, or contact dermatitis, although these are generally mild and resolve upon cessation of therapy.

Transdermal opioids: In China, the most commonly utilized opioid transdermal patches are those containing fentanyl and buprenorphine. In contrast, capsaicin has gained increasing attention due to its promising preclinical, clinical, and pharmacological applications. Clinical evidence suggests that repeated topical applications (three to five times daily for two to six weeks) of low-concentration capsaicin formulations yield modest pain relief in conditions such as post-herpetic neuralgia, diabetic neuropathy, and chronic musculoskeletal pain. A high-concentration

capsaicin patch (8%) is approved in both Europe and the United States (specifically for post-herpetic neuralgia) and is also used for HIV-related neuropathy and other neuropathic pain syndromes. This formulation ensures rapid transdermal delivery of capsaicin with minimal systemic exposure. Clinical studies, including one conducted in Scotland and another across 22 countries involving 629 participants, have demonstrated that the 8% capsaicin patch has comparable efficacy to pregabalin, with no significant difference in time to therapeutic response, highlighting its potential as a reliable alternative in neuropathic pain management. The single 30- to 60-minute application under medical supervision minimizes variability in administration and enhances patient adherence while reducing environmental exposure. Oral formulations of capsaicin, often delivered in chili pepper capsules, are commercially available; however, an official therapeutic dose has not been established. The recommended daily intake ranges from 1350 to 4000 mg of capsicum containing approximately 0.25% capsaicin. Doses ranging from as low as 0.4–2 mg to as high as 135–150 mg have shown benefits in promoting thermogenesis, increasing fat oxidation, and suppressing appetite.

Additional pharmacological formulations include capsaicin-containing nasal sprays and homeopathic preparations, which have demonstrated efficacy in managing nonallergic rhinitis. One prior study reported therapeutic benefit using capsicum nasal sprays (4 g/puff) administered thrice daily over three consecutive days in patients with nonallergic, non-infectious perennial rhinitis (Fattori et al., 2016).

2.6.2 Related work and study summary

The Qutenza® capsaicin 8% dermal patch, containing synthetic capsaicin at 8% w/w, is designed for localized delivery to pain-affected areas. It has received regulatory approval in the European Union for the management of peripheral neuropathic pain (PNP) in adults, either as monotherapy or in combination with other analgesics. Clinical studies have demonstrated that a single 30-minute application can provide up to 12 weeks of sustained pain relief and improved sleep quality when compared to placebo. Extended use over 52 weeks, in conjunction with standard care, has also shown durable analgesic effects without evidence of neurological toxicity. In

individuals with non-diabetic PNP, the patch was associated with a faster onset of action and greater patient satisfaction. Similarly, patients with postherpetic neuralgia experienced both rapid and prolonged pain relief. Results in HIV-associated neuropathy were mixed, with one study showing significant benefit and another reporting no effect. The most frequently observed adverse events were transient, localized skin reactions at the site of application. Overall, the capsaicin 8% dermal patch represents an effective and well-tolerated adjunct in the treatment regimen for various forms of peripheral neuropathic pain (Blair, 2018).

In a 2022 retrospective post-authorization study, María Dolores Ausín-Crespo et al. evaluated the efficacy and tolerability of the capsaicin 8% dermal patch for the treatment of peripheral neuropathic pain within a specialized pain unit. The diagnosis of neuropathic pain was confirmed using the DN4 questionnaire, and treatment outcomes were assessed using visual analog scale (VAS) scores for pain intensity and the EQ-5D instrument for health-related quality of life. A total of 66 patients, most of whom suffered from iatrogenic neuropathic pain (47%) and reported severe baseline pain, participated in the study. Over the course of three months, the mean VAS pain score decreased significantly from 7.20 (±1.95 SD) to 6.02 (±2.77 SD), representing a mean reduction of 1.19 points (95% CI: 0.59–1.78; $p < 0.001$; Cohen's d = 0.49), indicating a moderate effect size. Additionally, the mean pain area significantly reduced from 169.5 cm^2 to 121.2 cm^2 ($p < 0.001$). Improvements were also observed across multiple EQ-5D quality-of-life dimensions, particularly in usual activities, pain/discomfort, and anxiety/depression. The capsaicin patch was well tolerated, with adverse events consistent with known application-site reactions. These findings support the use of the capsaicin 8% dermal patch as a viable treatment option for managing peripheral neuropathic pain in clinical pain management settings (Ausín-Crespo et al., 2022).

In a 2022 clinical trial, Valéria Romero et al. investigated the analgesic efficacy of an 8% capsaicin cream in patients diagnosed with myofascial pain syndrome (MPS). The study employed a double-blind, randomized design involving 40 participants, who were assigned to receive either a capsaicin 8% (CPS) or placebo (PLA) cream. Prior to the application, all participants received local anesthetic pretreatment

for 50 minutes. Subsequently, 10 grams of the test cream were applied for 30 minutes over the trigger point within a standardized 24 mm diameter area. Pain intensity was assessed using a verbal numerical scale (0–10) at multiple time points: baseline, during application, and at 1 hour, 7 days, 30 days, and 60 days post-treatment. While none of the PLA group experienced skin irritation, 85% of patients in the CPS group reported transient hyperemia and a burning sensation at the application site within 15 minutes, which resolved within 24 hours. Over time, the CPS group demonstrated a significant and sustained reduction in pain scores, with statistical significance maintained through Day 60 ($p < 0.0001$). The findings confirm that 8% capsaicin cream is effective, safe, and well-tolerated in MPS patients, with no observed short- or long-term dermatologic adverse effect (Romero et al., 2019).

Transdermal drug delivery systems (TDDSs) have become a prominent focus in pharmaceutical technology and are widely produced globally due to their potential to overcome the limitations associated with conventional administration routes such as oral or parenteral delivery. Notably, TDDSs offer several advantages, including bypassing hepatic first-pass metabolism and enabling convenient self-administration. However, the stratum corneum (SC) with its tightly packed, hydrophobic structure poses a significant barrier, rendering many drugs unsuitable for standard transdermal application. Multiple factors affect cutaneous drug absorption, with skin physiology playing a critical role. For instance, SC thickness and lipid content, which vary by anatomical location, can markedly influence the rate and extent of transdermal permeation. In recent years, nanoparticle-based drug carriers have shown considerable promise for enhancing transdermal drug delivery, offering unique advantages such as improved permeation and targeted release. Importantly, some nanoparticle formulations have advanced to the stage of clinical trials, highlighting their translational potential in therapeutic applications (Jiang et al., 2022; Mitchell et al., 2021). While nanoparticles (NPs) can be administered orally or intravenously, transdermal delivery using microneedles (MNs) has garnered significant research interest due to its potential to enhance drug bioavailability while avoiding the pain and invasiveness associated with hypodermic injections. This review highlights the types of nanoparticles currently

utilized in drug delivery and explores strategies developed to improve the transdermal transport of nanoparticle-loaded therapeutics.

A transdermal patch, or medicated skin patch, offers a controlled method for delivering active pharmaceutical ingredients into systemic circulation through the skin and is increasingly viewed as a promising alternative to oral drug administration. Capsaicin, a bioactive compound derived from chili peppers, is well known for inducing both pain and thermal sensations, which has made it a valuable tool in pain research. By selectively activating nociceptive neurons, capsaicin has been widely applied in the investigation of pain mechanisms. Clinically, its most common therapeutic use is in the management of pain, with low-concentration capsaicin formulations (0.025–0.1% w/w) having been available in many countries since the early 1980s for routine topical application (Anand & Bley, 2011). Topical analgesics are frequently self-administered, with clinical studies showing that three to five applications per day over a period of two to six weeks can yield modest therapeutic benefits in managing various pain conditions, including postherpetic neuralgia, diabetic neuropathy, and chronic musculoskeletal pain.

Recent advances in nanotechnology have highlighted the unique structural and functional properties of nanomaterials, positioning them as promising candidates for the development of innovative therapeutic platforms. In particular, nanofibers characterized by distinctive physicochemical and biological attributes—are increasingly explored in biomedical research for their potential in sustained and controlled drug delivery. These fibers can be fabricated from diverse polymeric materials using electrospinning, a scalable and versatile technique that enables the production of nanofibers with varied morphologies. Nanoparticles, defined as solid particles ranging from 0.1 to 100 nanometers in diameter, exhibit high permeability and a large surface area-to-volume ratio, making them especially suitable for transdermal drug delivery systems. Among the materials investigated for this purpose, polyvinyl alcohol (PVA) has emerged as a favorable carrier due to its non-toxic, biocompatible, and biodegradable nature, as well as its hydrogel-forming and electrospinning capabilities, which enhance drug encapsulation and skin permeation efficiency.

Previous research has demonstrated that capsaicin at a concentration of 0.1% is a promising therapeutic option for pain management, offering favorable safety and tolerability profiles. Concurrently, the electrospinning technique has gained considerable attention for fabricating nanoscale polymer-based drug delivery systems, particularly nanofibers. In this context, Franz diffusion cells are widely employed to evaluate transdermal drug permeation, and studies using Strat-M™ membranes have shown that drug-loaded PVP/PVA nanofibers possess superior physicochemical properties compared to nanofibers composed of either polymer alone. Moreover, skin permeation studies using human skin explants across the dermatome are recognized as the gold standard for assessing transdermal delivery efficacy. However, due to ethical concerns regarding the use of human and animal tissues, the Strat-M™ synthetic membrane has emerged as a reliable in vitro alternative. Accordingly, the objective of this study is to develop and optimize a capsaicin-loaded transdermal nanofiber patch, fabricated from a polyvinyl alcohol/polyvinylpyrrolidone (CAP/PVA/PVP) polymer matrix using the electrospinning process. This work further aims to investigate the drug release mechanism and transdermal permeation behavior of the nanofiber patch through the Strat-M™ membrane.