2. Literature Review

2.1. Rheumatoid Arthritis

"Rheumatoid arthritis (RA) can be defined as a lasting systemic autoimmune diorder which majorly involves the lining of synovial joints and can be seen as the cause of advanced disability, untimely death, and along with this brings socioeconomic burdens." This medical condition is reported more commonly in females as compared to males and is also reported in high numbers in the geriatric population.(1, 2) RA majorly attacks the joints but it can be thought as a group of disorders which involves extra-articular expressions, such as pulmonary involvement, rheumatoid nodules, or vasculitis, along with systemic co-morbidities, which majorly affect the vasculature and metabolism.(3)

2.1.1. Etiology

Till date the precise reason of RA is not known, however, it has been observed hormones, genes and some factors present in our surroundings contribute in the development of RA. There are some factors noted to increase the risk of RA, for eg. It is seen more in geriatric population, females, people who smoke, obesity, females having history of live births, and the patients who are reported to be seropositive for etc.(3-5) The risk of RA increases in Patient who is seropositive for anti-citrullinated protein antibodies (ACPAs) or rheumatoid factors (RFs). It has been reported that mothers who have breast fed their children are at low risk from getting RA.(3, 5, 6)

2.1.2. Pathophysiology

Inflammation in joints is noticed in RA which can be attributed to swelling in synovial membrane and further chemokine and cytokine also play a part in this. Interleukin-6 (IL-6), tumor necrosis factor (TNF) and granulocyte-macrophage colony-stimulating factor are present in the inflamed area. The endothelial cells are activated and along with that the cells of the immune system accumulate in the synovial compartment, this leads to exaggerated inflammation response. The generation of osteoclast is triggered because of the activation of nuclear factor

kappa-B ligand due to the stimulation of monocytes, T cells, macrophages, fibroblasts and B. Furthermore, metalloproteinases and other enzymes is reposnsible for the degradation of cartilage matrix in joints. (3, 7)

2.1.3. Prevalence

In 2005, it was reported that 1.3 million of adult population in the United States was suffering from RA, and the figure went up to 1.5 million adults within 2 years. The statistics of RA in advanced territories is reported to be in the range of 0.5% to 1% wherein 0.6% is in the U.S. and 1% is worldwide. Women are two-to-three fold more vulnerable when compared to men in developing RA. Onset of RA is generally reported when a person is in his late adulthood, and it is more observed in the geriatric population and has also been reported in the pediatric population.(3, 5, 7)

2.1.4. Signs and Symptoms

Below mentioned conditions comprise of the symptoms of RA(5, 8):

- Joint pain
- Rigidity in joints
- Soreness or inflammation in the joints
- Symmetry in symptoms such as both knees or hands
- Reduction in body weight
- Pyrexia
- Weariness and weakness

However with RA, “Flares is the term given when the symptoms get worse” and “Remission is the term used when the symptoms get better.”

2.1.5. Treatment

Once RA is diagnosed, main aim in the treatment of RA, is the reversal of inflammation. However, several other aspects to consider during the treatment of RA are to decrease or stop further joint damage, to reduce pain and improve physical function. Various treatment options available for RA are 1). Lifestyle

modification which include exercise and joint care, 2). Surgery includes repairing, replacement and fusion of joints which may help in certain situations and 3). Medications include use of various drugs to control RA.(3, 8-10)

Drugs used for the treatment are divided into the below mentioned categories:(8, 10):

A. Disease modifying antirheumatic drugs
   - Immunosuppressants: Sirolimus, Azathioprine, Methotrexate, Cyclosporine. Tacrolimus, Everolimus etc.
   - Sulfasalazine
   - Hydroxychloroquine or Chloroquine
   - d-Penicillamine
   - Leflunomide
   - Gold sodium thiomalate, Auranfin

B. Biological Response Modifiers
   - TNF-α inhibitors: Infliximab, Etanercept, Adalimumab,
   - IL-1 antagonist: Anakinra

C. Adjuvant Drugs
   - Corticosteroids: Dexamethasone, Prednisolone, Methylprednisolone, etc.

Symptomatic relief is attained by the administration of analgesic, antipyretics NSAIDS such as diclofenac, meloxicam, ibuprofen, and selective COX-2 inhibitor such as celecoxib, rofecoxib, valdecoxib etc, apart from the drugs mentioned in the above list.(8-10)

2.1.6. Immunosuppressants

Immunosuppressants are generally prescribed in treatment of RA in order to slow down the development of RA and enhance the superiority of life of patients as RA is an autoimmune disease. These drugs act by inhibiting the activation of T-cell because high activity of immune system is the primary cause

for this autoimmune disease. Some of the drugs from this class also exhibit some extent of anti-inflammatory activity.(3)

Immunosuppressants can be classified according to the location of their action into the following classes: as drugs which inhibit the differentiation (15-deoxyspergualin), drugs which inhibit nucleotide synthesis (mizoribine, mycophenolate mofetil, azathioprine, leflunomide), drugs which inhibit transcription (TAC and cyclosporine) and drugs that inhibit growth factor signal transduction (sirolimus, leflunomide). (11)

Therapeutic agents targeting T cell function can be classified in the following categories such as those which inhibit signal 1 (a T cell receptor [TCR] complex interact with the antigen-presenting cell [APC]) and its resulting intracellular signaling and those that block pathway of signal 2 (T cell/APC interaction generates costimulatory signal for complete activation of a T cell) (fig 2.1). Moreover, there are agents which block signal-3 (proliferation and downstream activation of T cell).(12)

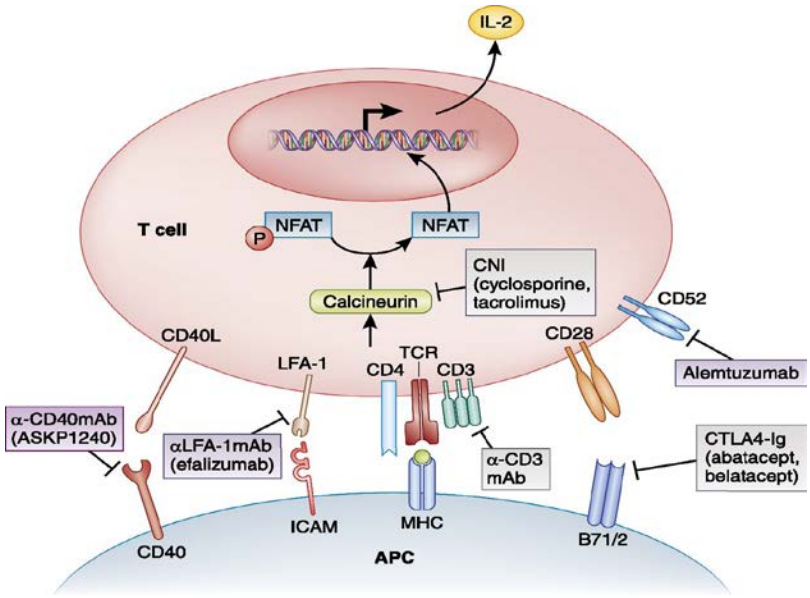


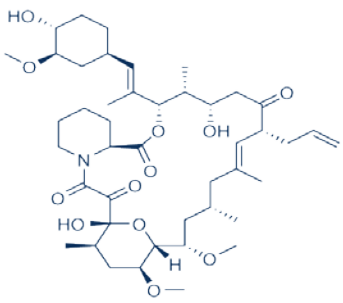
Figure 2.1: Immunosuppressive agents targeting T cell/antigen-presenting cell interaction and early T cell activation. This depicts agents that inhibit signal 1 and signal 2 in T cell activation. APC, antigen-presenting cell; CNI, calcineurin inhibitor;

CTLA4, cytotoxic T lymphocyte–associated protein 4; ICAM, intracellular adhesion molecule 1; LFA-1, lymphocyte function–associated antigen-1; NFAT, nuclear factor of activated T cells; TCR, T cell receptor.(12)

Methotrexate is one of the drugs that is widely prescribed by rheumatologists, but its use is controversial as many adverse events are reported with the use of methotrexate, such as leukopenia, blood in urine or stools, jaundice, signs of liver problems, anemia etc.(13) Daniel E Furst reported successful use of TAC by studying the use of TAC in patients.(14)

2.1.7. Tacrolimus

TAC is categorized as an immunosuppressive drug which is which is marketed with the trade names **Seegraf** and **Tacroz Forte** alongside other brands. TAC is indicated to decrease the threat of organ rejection, after allogeneic organ transplant wherein there is a possibility of organ rejection. Additionally, TAC is administered for the management of other autoimmune disorders like eczema and psoriasis in the form of ointment. However, there are reports indicating its therapeutic benefits in RA and it has a better adverse effect profile when compared to other immunosuppressants. Thus, TAC is selected for the present project. Various marketed dosage forms of TAC include tablet (Crolim-Ranbaxy Laboratories), capsule (Olmis Cap: Unichem Laboratories, Tacloram: Wockhardt (Biotech). Currently, it is administered as an oral dosage form i.e. tablet for indicated for organ rejection and as an ointment in the treatment of psoriasis. Tablets (1 mg, 2 mg and 5 mg) are administered as and when required by the patients. Ointment (Olmis: Unichem Laboratories) is but this type of dosage form faces the issue of incomplete absorption.



2.1.7.1. Tacrolimus Profile

Synonyms: FK-506, Fujimycin

Molecular Formula: $C_{44}H_{69}NO_{12}$

Molecular Weight: 804.031 $g.mol^{-1}$

Description:

In the year 1987, discovery of TAC was made.(15, 16) A soil bacterium, *Streptomyces tsukubaensis* produces TAC. "The naming of TAC was taken from "Tsukuba macrolide immunosuppressant".(17) Initial approval for TAC was received from USFDA in 1994 and it was approved for use in liver transplant. Later, TAC was approved for other indications like bone marrow, skin, lung, trachea pancreas transplants.(18) In 2017, many generics received approval from USFDA for TAC for the US market.(19)

Pharmacokinetic:

The oral bioavailability of TAC is 24%, Protein binding is ≥98.8%, and biological half-life is 11.3 hours when given in patient who have undergone a transplant (range 3.5-40.6 hr) while 43 hr for a normal human being. TAC is primarily degraded by the liver microsomal enzyme and mostly eliminated in feces. Resulting metabolites formed are reported to be inactive. CY3PA present in the intestinal wall is mainly responsible for the degradation of Tacrolimus. TAC exhibits food drug interaction and generally a decrease in the oral bioavailability is observed when it is taken with food which has high fat content. For the treatment of eczema when TAC is applied topically, a little to no bioavailability is observed.(20)

Mechanism of Action:

TAC acts by activating T-cell which is responsible for the high activity of immune system. In T-cell activation, early T cell receptor binding activates calcineurin-dependent signal pathway which initiate T cell gene transcription required for further activation. TAC functions by inhibiting this calcineurin and thus T-cell gene transcription. (12)

Clinical Indication:

Organ Transplantation: TAC exhibits comparable function to cyclospirin when we talk about suppression of the immune system but it is more efficient.

One study has reported that when compared with cyclosporine, when treatment with TAC was given the cases of acute rejection were less i.e. 30.7% vs. 46.4%. (21) During the course of first year of liver transplant, it was observed that better results were attained using TAC against the treatment with cyclosporin. TAC is generally given in a combination regimen after the transplant is performed along includes steroids, IL-2 receptor inhibitor like basiliximab, and mycophenolate. Dose titration is done for attaining proper level of drug in the systemic circulation.(22, 23)

Ulcerative Colitis: TAC is used for suppressing the inflammation which is caused by ulcerative colitis. However, TAC is mostly used clinical trials only, it is found to be significantly effective in flares caused due to ulcerative colitis.(24)

Skin: Tacrolimus, when formulated as an ointment, is indicated for the management of atopic dermatitis and eczema, to be specific. It acts in the same manner like any other steroid to suppress inflammation. A vital merit of TAC is the absence of atrophy and any other adverse events related with other A vital benefit that is associated with TAC is the absence of atrophy and various other adverse events related with steroids.(25)

Lupus nephritis: TAC is found to be very effective in lupus nephritis and also enhances renal remission.(26)

Rheumatoid Arthritis: Daniel E. Furst el al. studied the therapeutic efficacy of **TAC** in the subjects who did not find any relief when treated with methotrexate and reported that TAC enhanced the disease activity in subjects who were not responding to the treatment with methotrexate. Optimal dose for RA that is suggested by Furst et al. is > 1 mg but ≤ 3 mg/day.(14)

Drug Interaction:

CYP450 enzyme present in the liver is majorly responsible for the degradation of Tacrolimus. TAC exhibits food interactions like when

administered along with grapefruit increase in the plasma concentration of TAC is observed. TAC also exhibits drug-drug interactions like when administered with antifungals like voriconazole or fluconazole and Macrolide antibiotics such as clarithromycin or erythromycin, elevation in TAC plasma levels is observed as these drugs compete for cytochrome enzymes with TAC.(27)

Safety/Toxicity:

Adverse effects that are associated with TAC, may be serious such as hypertension, hepatotoxicity and nephrotoxicity, increase in glucose and potassium levels and decrease in magnesium levels, itching, infection, lung damage, cardiac damage, blurred vision, itching, and numerous neuropsychiatric disorders like anorexia, depression, bad dreams, catatonia, digestive disturbances, insomnia, posterior reversible encephalopathy, cramps, confusion and tremors.

Dosage:

Maximum daily dose of TAC is 5 mg to be administered once a day. However, they are also given in a dosage regimen of 0.5, 1, 2 and 3 mg/day.

2.1.7.2. Problem associated with oral delivery of Tacrolimus

TAC has also been reported to cause gastrointestinal disturbances such as diarrhea, nausea, stomach pain, vomiting etc. which in many cases became severe and resulted in the withdrawal of therapy.(28-30) TAC when administered through the oral have limitation such as poor oral bioavailability(30), food dependent absorption(31), gut-wall metabolism(32) and gastrointestinal disturbances(29). A review of work done on improvement of TAC delivery is mentioned in Table 2.1.

Table 2.1: Review of work done on Tacrolimus

Author	Formulation (Route)	Technique	Polymer/Excipient used	Conclusion
Pranav Patel et al.(33)	Liquid/solid SMEDDS (oral)	Prepared by dissolving TAC in the mixture of oil, surfactant and co-surfactant at 40 °C in water bath	Lauroglycol FCC, Cremophor RH, and PEG 400	Liquid SMEDDS and solid SMEDDS exhibited an enhanced in-vitro drug release profile when analyzed against to pure API and the marketed product, moreover it also enhanced the absorption of TAC through oral route.
Vedrana Savic et al.(34)	Microemulsion (transdermal)	-	Lecithin, Propylene glycol, caprylic/ capric triglyceride, propylene glycol monocaprylate	Enhanced in-vitro drug release and ex-vivo permeation compare to pure drug
Shivani Sahu et al.(35)	Nanoemulsion (topical)	Prepared by spontaneous emulsification method	Kalonji oil, Cremophor RH 40, polyethylene glycol 400	It was established that the prepared formulation is efficacious when compared to marketed formulation as a major decrease was observed in serum cytokines and betterment was reported in psoriatic condition in vivo.
T Uno et al.(36)	W/O/W type double emulsion (oral)	Water in oil emulsion was prepared. Then, this emulsion was then added in isotonic sodium chloride solution	Soyabean oil, lecithin, glycerol fatty acid ester	W/O/W emulsion showed decreased levels of TAC in kidney and brain and increased levels in

		containing 3% pluronic F68		liver and spleen which are the targeted organ.
Yingli Wang et al.(37)	Nanosuspension	CO_2 assisted in situ nanoamorphization		The oral bioavailability of the nanosuspension was observed to be higher when compared to Prograf as indicated by the *in-vivo* PK paramaters.
M Erdogan et al.(38)	Liposome (Topical)	Modified solvent evaporation technique	Phospholipon 90-H, Loralan CH-cholesterol, and talc	They achieve higher skin concentrations of TAC than systemic, so it was proved as a suitable delivery method for TAC for the treatment of psoriasis.
Weidong Zeng et al.(39)	Hyaluronic acid coated Niosome (ocular delivery)	Soybean phosphatidylcholine, cholesterol, poloxamer 188 were mixed in ethanol, then drug was added in it. Then, phosphate buffer saline (pH 7.4) was added into it.	Soybean phosphatidylcholine, cholesterol, poloxamer 188	They obtained 2.3 fold increase in ocular bioavailability using HA-coated niosome compare to drug suspension
Wei Lei et al.(39)	Transfersomes (transdermal)	Thin film dispersion-hydration method	Lipoid E80, vitamin E, tween 80, span 80, sodium cholate	They achieved 3.8-4.2 times enhancement in TAC concentration at epidermis and dermis layer of skin compare to marketed TAC ointment
Guiling	Ethosomes	Lipoid S 100, TAC was	Lipoid S 100, Ethanol	They enhanced the

Li et al.(40)	(transdermal)	dissolved in ethanol and then to this solution water was added dropwise		epidermis concentration of TAC compared to conventional liposome
Nishita Mistry et al.(28)	Nanoparticles (oral)	Modified ultrasonic emulsification and solvent evaporation technique	Galactosylated PLGA, poloxamer 188	They obtained enhanced liver uptake of TAC loaded galactosylated PLGA nanoparticles than plain drug suspension and PLGA nanoparticles of TAC which suggests liver targeting of TAC loaded galactosylated PLGA nanoparticles
Ji-Hyun Kang et al.(41)	Solid lipid nanoparticle (transdermal)	Prepared by using modified emulsification and low temperature solidification technique	Cocoglyceride, lecithin, poloxamer 188, PEG-40 sterate, Brij 58, PEG-1000	They obtained deeper penetration of drug using TAC loaded SLN, among which TCR-SLN-1 provide most prominent permeation than other prepared batches of Tacrolimus
Vedrana Savic et al.(42)	Nanostructured lipid carriers (transdermal)	Prepared using hot high pressure homogenization method	Lecithin, glyceryl palmitostearate, propylene glycol monocaprylate	Obtained higher dermal concentration of TAC using SLN compared to nanoemulsion and marketed ointment

2.2. Gouty Arthritis

Gout is a disease which is well known since the Hippocrates in the fifth century BC, and at that time it was referred to as 'the un-walkable disease'. He

linked gout to lifestyle. Previously, the term “gout” used to encompass all types of arthritis popularly known as “disease of kings”.(43, 44)

In ancient Greek medicine, the term “gout” has been derived from the name “podagra”.(45) Historically, macroscopic deposits of MSU or topihi was described by the Greek physician Galen for the first time and he recognized a hereditary trait in gout.(46)

“Gout can be defined as a diorder which is a result of the over accumulation of MSU crystals in tissues such as synovial membranes, bone, cartilage and skin.”(47, 48) Gout involves both articular and extra articular structures of musculoskeletal system which leads to arthritis, tendinitis and bursitis. Deposition of MSU crystals occurs when serum UA levels exceed the saturation points for MSU crystal formation, a condition referred to as hyperuricemia.(48)

2.2.1. Classification of gout

Gout can be classified according to the natural history into four phases on the basis of on signs and symptoms.(43, 48)

2.2.1.1. Asymptomatic Hyperuricemia

“Asymptomatic hyperuricemia can be defined as unusually high serum UA with the absence of gout or nephrolithiasis.” This can be categorized as the first stage of the disease. In this stage patient does not experiences any inflammation or pain, and the serum uric acid levels go up to >6 mg/dl for women and >7 mg/dl for male. With the increase in concentration of serum uric acid, the possibility of formation of uric acid crystals also increases.(43, 49)

2.2.1.2. Acute Gouty Arthritis

Majorly observed manifestation of gout in this stage is acute inflammation which is due to the deposition of MSU crystal within the joint or soft tissue.(48) Initiation of the attacks starts generally from the lower limbs. In 50% of the cases, it is observed that the first attack of gout involves

metatarsophalangeal joint and may last from several hours to one week. Pain commences rapidly and is especially reported at night and can be excruciating. As a result of this, involved joints rapidly become red, hot and inflamed with glossy covering skin and are very painful. The attack can be linked to systematic manifestations of fever, leukocytosis and elevation of ESR.(48, 50) Patients may experience two to three attacks in a year, in early stages of the disease. The frequency of attack may go up to twelve attacks a year with the progression of the disease. Repetition of attach for period of many years may lead to permanent restriction in joint movement. Further, as the disease progresses patients experience chronic gouty arthritis phase which involves multiple joints.(43)

2.2.1.3. Intercritical Gout

"Intercritical or interval gout can be defined as a relief phase for patients because this period is painless and occurs between two attacks (acute gout and asymptomatic phase) and this period and occur for a span of a year to 10 years.(43, 51)

2.2.1.4. Chronic Tophaceous Gout

This stage of gout predominantly comes after many years (10-20 years) of the occurance of acute gouty arthritis. Generally, many attacks of acute gouty arthritis are observed before the occurance of chronic tophaceous gout, which results in the development of nodules of MSU crystals. The nodules which are formed are known as tophi and can be found in tendons, cartilage and bones. (52, 53) The characterization of this stage is the incidence of evident tophi, destructive arthritis of the affected joints and bony erosions (polyarticular in distribution).(54, 55) Tophi can found in many locations such as olecranon bursa of the elbow, extensor surfaces of knees, feet, hands, cartilages of ear and nose and achilles tendon.(43, 54)

2.2.2. Etiology

The leading cause of gout is reported to be hyperuricemia.(43, 49, 56) It has been observed that population with increased amount of uric acid in blood are more prone to gout flare-ups and over the course of time these people are more vulnerable to repetition of these flare ups. A study was conducted in the geriatric population of 2000 subjects diagnosed with gout, it was reported that the subjects who had more than 9 mg/dl of uric acid were 3X more prone to flare-ups when compared to the subjects having uric acid level less than 6 mg/dl over a period of year.(57, 58) However, it has been observed, it is not necessary that people with increased uric acid levels will be diagnosed with gout in future. Geriatric population, obesity, genetics, comorbid disorders, drugs and purine diet considered as other factors which contribute to hyperuricemia and/or gout. Pyrizinamide, aspirin, diuretics, ethambutol, and cyclosporine are the medications which were used for gout in the past.(56, 58) It has been found according to Genome-wide association studies (GWAS), many genes have been found which have a connection with gout i.e. SLC2A9, GCKR, ABCG2, PDZK1 and SLC22A12.(59) There are various food sources which can lead to increased uric acid levels and contribute to gout like seafood, read meat and some drinks like alcohol, sodas, etc.

2.2.3. Pathophysiology

2.2.3.1. Hyperuricemia

Increase in the uric acid level in blood is a major contributor in the progression of gout as the rise in uric acid leads to nucleation and growth of the urate crystal by decreasing the solubility of urate. Endogenous and exogenous purine breakdown is responsible for the accumulation of uric acid in blood this is eliminated by the kidneys. Xanthine Oxidase present in blood plasma is responsible for the breakdown of these purines. Due to the excess production alone or in combination of underneath removal of uric acid from blood, an uric acid level in blood serum is elevated.(46, 58, 60)

2.2.3.2. Inflammatory Response

Macrophages are responsible for the phagocytosis of urate crystals and this leads to the activation and of cytosolic protein complexes (NLRP2 inflammasome), further leading to inflammation. The complexes which are formed then employ caspase-1 which is responsible for the activation of pro-IL-1beta to IL-1beta. IL-1beta is crucially involved in the inflammation which is reported in gout. Further IL-1beta secretion may also lead to breaking of cartilage and bone. Other cytokines, that are involved in the inflammatory response are TNF-1, CXCL8, IL-6, COX-2 and IL-6.(61, 62)

2.2.4. Prevalence

Prevalence of gout varies based on the originating nation, gender, and age. Gout is reported in approximately 1 to 6.8% of the world wide population. Globally, it has been seen that older age people and males are at a higher risk. In developed countries, the incidence of gout in males (3 to 6%) is reported to be more when compared to females by 2 to 6 folds. (1 to 2%). It has been noted that with increasing age, a person is more susceptible to suffer from gout but plateaus after the age of 70. From 2007 to 2008, 3.9 million of the US adults were diagnosed with gout. Other risk factors for gout include, chronic diseases such as increased blood pressure, chronic kidney disease, increase of glucose levels in blood, obesity, CHF, and heart failure.(48, 63)

2.2.5. Signs and Symptoms

Onset of flares in gout is sudden and the duration can range from days to weeks. Gout flares start suddenly and can last days or weeks. After the first flare long periods of remission is reported which may range from weeks or months, or years wherein the patient does not show any signs of flare prior to the beginning of another flare. Initiation of gout is generally found in the toe and affects a single joint. Further, other than the big toe, it may also involve ankle, toe joints and knee. Indications for gout can include: (10, 48, 60, 64)

- **Joint pain:** Joint pain experienced in gout is quite intense. During the first 12 hrs after the beginning of symptoms, pain is the most severe.
- **Inflammation and redness:** Redness, swelling and pain is there in the joints involved.
- **Decreased mobility:** They motility of the patient is also affected with the course of time after getting diagnosed with gout.
- **General discomfort:** Even when the pain caused by the attack is relieved, there may tenderness for many days to weeks.
- **"Tophi", term given to the nodules under the skin**

2.2.6. Treatment

The main aim in the treatment of gouty arthritis is to manage pain, reduce joint damage and enhance the life being led by the patient. According to the American College of Rheumatology, below mentioned options for the treatment of gouty arthritis (65):

- Medications
- Non-pharmacologic therapies (Proper diet)
- Physiotherapy sessions
- Splints or joint assistive aids
- Surgery - joint replacement and joint operation.

Medication

Below mentioned class of drugs are prescribed in the treatment of gouty arthritis: (10, 48, 66, 67):

- **Analgesics** e.g. Acetaminophen, Tramadol and Narcotics comprising of Hydrocodone and Oxycodone.
- **Non-steroidal anti-inflammatory drugs (NSAIDs)** e.g. Peroxicam, indomethacin, Naproxen Sodium, Meloxicam, Celecoxib, Ibuprofen etc.
- **Corticosteroids** e.g. Prednisone, Cortisone, Dexamethasone etc.
- **Colchicine**

- **Xanthine oxidase inhibitors** (Drugs responsible for the inhibition of uric acid formation) e.g. Allopurinol, Febuxostat
- **Uricosurics** (drugs responsible for elimination of uric acid) e.g. Probenecid

2.2.7. Xanthine Oxidase Inhibitors

The class of drugs which are **xanthine oxidase inhibitors** are responsible for the inhibition of an enzyme responsible for the metabolism of purine called "xanthine oxidase". If this enzyme could be inhibited in the human body, formation of uric acid will be reduced. Therefore, drugs which are responsible for the inhibition of gout are prescribed for the treatment of gout and other like disorders. Thus, drugs inhibiting the enzyme xanthine oxidase (like Allopurinol and FBX) are potential candidates for the treatment chronic gout conditions.(10, 48) The merit of FBX is capable of inhibiting both reduced and oxidized forms of the enzyme xanthine oxidase over Allopurinol (blocks only oxidized form). Additionally, unlike Allopurinol, FBX also prevents the enzyme turnover.(68) Thus for the treatment of gout, FBX is preferred over Allopurinol.

2.2.8. Febuxostat

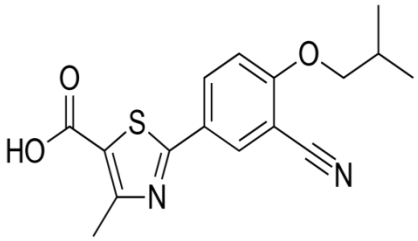
"FBX is a potent, novel, non-purine selective xanthine oxidase inhibitor. It blocks both oxidized and reduced forms of xanthine oxidase." It has been observed that (FBX) is relatively more effective in reducing the levels of urate in serum as compared to Allopurinol. Patients who experience hypersensitive reaction on being treated with Allopurinol, can be safely treated with FBX. FBX is marketed in the form of tablet in market in the strength of 40, 80 and 120 mg to be administered once a day. FBX tablets are marketed under the names of Fabulas, Feboxa, Febuget, Febucip etc. Oral bioavailability of FBX is 49% and is affected by the presence of food due to food interaction. Oral bioavailability of FBX can be attributed to its minimized solubility in aqueous phase i.e. less than 15 μg/ml and high degaradation by liver and intestinal enzymes. Additionally FBX shows food- drug interaction and due to this the Cmax is decreased by 38-49% (69)

2.2.8.1. Febuxostat Profile

Synonyms: Uloric, Adenuric, TEI-6720

Molecular Formula: $C_{16}H_{16}N_2O_3S$

Molecular Weight: 316.38 $g.mol^{-1}$



Description:

In 1998, scientists of a Japanese pharmaceutical company named "Teijin" discovered FBX. (70) **FBX** is marketed with the trade names Alxo, Ebuxo and various others and it is indicated for prolonged treatment of gout which is a result of increased uric acid levels.(71) USFDA approved FBX in 2008 and EMEA in 2009.(72) First generic received approval from the USFDA in 2019.(72) FBX is highly soluble in dimethylformamide (DMF), soluble in DMSO, minimized solubilize in ethanol, in methanol and acetonitrile it is somewhat soluble and insoluble in water. 205°C to 208°C was observed as the melting range for FBX.(70, 73)

Pharmacokinetics:

After administering FBX through the oral route, absorption of radiolabeled FBX was assessed at around a minimum of 49%. After around 1 to 1.5 hours of administrating FBX, maximum concentration of the drug was reported in plasma. Multiple oral doses (40 mg and 80 mg) of FBX was administered once a day and it was reported that Cmax achieved with 40 mg oral dose was 1.6 ± 0.6 mcg/mL and Cmax after administration of 80 mg oral dose was 2.6 ± 1.7 mcg/mL. When multiple oral 80 mg doses of FBX were given once day along with a meal which is rich in fat, it was observed that there was an 18% decrease in AUC and 49% decrease in Cmax. But, it was noticed that a concentration of uric acid in serum remained unchanged. There is extensive metabolism of FBX and it is done by two pathways i.e. conjugation via UGT (uridine diphosphate glucuronosyltransferase) enzymes which comprise of UGT1A9, UGT1A1, UGT2B7, UGT1A3 and oxidation via cytochrome P450 (CYP) enzymes which comprise of 2C8 and 2C9, CYP1A2 and non-P450

enzymes. Both hepatic and renal pathways are responsible for the elimination of FBX. 80 mg radio labelled FBX with 14C was administered orally and 49% of unchanged FBX was excreated in urine. Half-life ($t_{1/2}$) of FBX is reported to be around five to eight hours.(74)

Mechanism of Action:

FBX functions by inhibiting the enzyme known as xanthine oxidase. The active site of xanthine oxidase enzyme is the molybdenum pterin center and FBX functions by binding to this center and blocking it. Due to this binding of FBX to pterin site, it inhibits both reduced and oxidized forms of the enzyme. When FBX is blocking this enzyme, xanthine oxidase is no more free to convert hypoxanthine and xanthine to uric acid. Therefore, this leads to the reduction of uric acid levels in plama.(75)

Clinical Indication:

Hyperuricemia: FBX is given for the prolonged management of increase in uric acid levels. FBX is prescribed to the patients who fail to be treated with Allopurinol.(73, 74)

Drug Interaction:

FBX exhibits drug interaction with 6-mercaptopurine, azathioprine, and theophylline. This drug interaction is the result of metabolism of the latter drugs by Xanthine oxidase. (74)

Safety/Toxicity:

Usual side effects associated with the use of FBX which may even lead to termination of the treatment are mentioned below:

- Hepato-toxicity
- Nausea,
- Pain in a joint
- Light-headedness

- Cutaneous condition

There are some rare adverse events associated with the use of FBX are mentioned below:

- **Blood and lymphatic system disorders**: anemia, thrombocytopenia, idiopathic thrombocytopenic purpura, low WBC count, low neutrophil count, pancytopenia, enlarged spleen,;
- **Cardiac disorders**: Palpitations, Tachycardia, angina pectoris, a-fib, heart murmur, abnormal ECG, sinus bradycardia;
- **Gastrointestinal disorder**: Constipation, loose motion, Gas, abdominal distention and pain, dry mouth, indigestion, gastritis, GERD, ulcers in mouth, gastrointestinal discomfort, gingival pain, blood vomiting, chlorhydria, presence of blood in stool, peptic ulcer, vomiting; etc. (74)

Dosage:

ULORIC which is a brand name of FBX is recommended at a dose of 40 or 80 mg once a day for the management of hyperuricemia. Commencing dose for ULORIC is considered as 40 mg once a day. When 40 mg of FBX is administered for two weeks and even then serum level of uric acid does not come down to 6 mg per dL, then only 80 mg of FBX is administered.(74)

2.2.8.2. Problems associated with oral delivery of FBX

FBX is also responsible for causing gastrointestinal disturbances such as flatulence, dry mouth, abdominal distention and pain, constipation, dry mouth, Indigestion, loose motions, hematochezia, gingival pain, gastritis, GERD, gastrointestinal discomfort, haematemesis, hyperchlorhydria, ulcers in mouth, pancreatitis, peptic ulcer, vomiting etc. which in some cases becomes severe and results in the withdrawal of therapy.(74) Additionally, FBX has a limitation of poor oral bioavailability, first-pass metabolism(76) and gastrointestinal disturbances(74, 77). A review of work done on improvement of FBX delivery is mentioned in Table 2.2.

Table 2.2: Review of work done on FBX

Author	Formulation (Route)	Technique	Polymer/Excipient	Conclusion
Singh Sanju et al.**(78)**	Niosomal Gel (Transdermal)	Prepared by thin film hydration	Tween 20, Span 60, Cholesterol	They obtained good ex-vivo permeation (80.37 ±0.2 %) of FBX compare to plain drug using rabbit skin. Moreover, in-vivo result also indicates better efficacy of niosomal gel compare to plain drug.
Nabil A. Alhakamy et al.(76)	SNEDDs loaded transdermal film (transdermal)	Drug, tween 20 was added in lemon oil and then this mixture was added in water containing PEG-400 with continuous stirring	Tween 20, lemon oil, PEG-400	In vivo plasma data showed improved PK parameters and FBX plasma levels when it was compared to raw FBX loaded film.
Pralhad K. Kanke et al.(79)	Nanoemulsion (transdermal)	Drug was dissolved in Capmul MCM, then Smix of tween 80 and transcutol P was added into it. Then water was added in this mixture dropwise with ultrasonication	Capmul MCM, tween 80, transcutol P	Prepared formulation exhibited improved transdermal flux compared to drug dispersion in phosphate buffer pH 7.4
Ahmed A El-Shenawy et al.(80)	Ethosomal Gel (transdermal)	Prepared by cold method	Propylene glycol, ethanol, cholesterol, phospholipid	They obtained higher C_{max} and T_{max} using ethosomal gel of FBX (transdermal) than oral administration FBX

Bhupesh K Ahuja et al.(81)	Nanosuspnsion (oral)	Prepared by wet media milling	Hydroxypropyl methylcellulose (HPMC E3) and D-a-tocopherol polyethylene glycol 1000 succinate (TPGS)	They obtained enhanced C_{max}, AUC and relative bioavailability (221.6%) compare to plain drug
Amin M. Vohra et al.(69)	Solid SMEDDs (oral)	Prepared by dissolving drug in oil phase and S_{mix}	FBX, aceclofenac, Caprol MPGO, kolliphor KL, transcutol HP	They obtained enhanced relative bioavailability of FBX (187.2 %) and Aceclofenac (418.98 %) compared to marketed product of same
Yasir A. Al-Amodi et al.(82)	Self-nanoemulsifying lyophilized tablets (oral)	First self-nanoemulsifying system of FBX was prepared and this emulsion was mixed with fumed silica, xylitol, mannitol and lactose to convert it in to tablet	Castor oil, polyethylene glycol 40 stearate, transcutol, fumed silica, xylitol, mannitol, lactose	They increased Cmax, AUC, and oral relative bioavailability (146.4 %) and reduce Tmax compare to the marketed tablet of FBX

2.3. Transdermal Route of Drug Delivery

As mentioned earlier, there are numerous shortcomings associated with the oral administration of the TAC and FBX which arises the need of the utilization a different and better route of drug delivery which can provide a better sustained plasma level of the drug throughout the duration of therapy. In this context, the consideration has been given to non-invasive, user-friendly transdermal route which is reported to have the potential of avoiding gut-wall metabolism, gastrointestinal disturbances, food dependent bioavailability; achieving infusion like zero order drug delivery profile, avoiding the trauma associated with parenteral therapy, improving patient compliance due ease of application(83, 84). Moreover, bioavailability of these drugs can also be improved

using the transdermal route of drug delivery. Additional advantage of a transdermal route is that it gives the healthcare professionals an opportunity to cease absorption by removing the patch in the event of an overdose or other problems.

2.3.1. Structure of Skin

"Human skin can be defined as a stratified epithelium wherein every individual tissue layer comprises of various types of cells that have different purposes." This layer can be classified in the following: the overlying epidermis, dermis and underlying hypodermis (or subcutis) (fig. 1). Further, subdivision of the epidermis can be done when considered from outside to inside. Epidermis comprises of the stratum corneum, stratum granulosum, stratum spinosum and stratum basale. A stratum corneum is 10–20 μm thick and comprises of corneocytes which is roughly in 10-15 layers. The morphological structure of corneocytes is flat and elongated and has a thickness of 0.2 μm and a width of 40–60 μm. The rest of the epidermis other than stratum corneum comprises of nucleated and so as a whole it is known as viable epidermis. The thickness of this viable epidermis is 50-100 μm and does not contain blood vessels or nerves. The elasticity and strength of the skin comes from the dermis and this also forms the bulk. The dermis has a thickness of less than 1 mm. This layer mainly comprises of fibroblasts in a matrix of protein and even has immune cells such as elastin and collagen. Innermost layer of the skin is characterized as the hypodermis. The composition of hypodermis is mainly subcutaneous fat. This layers also comprises of blood capillaries and lymphatic cells.(85, 86)

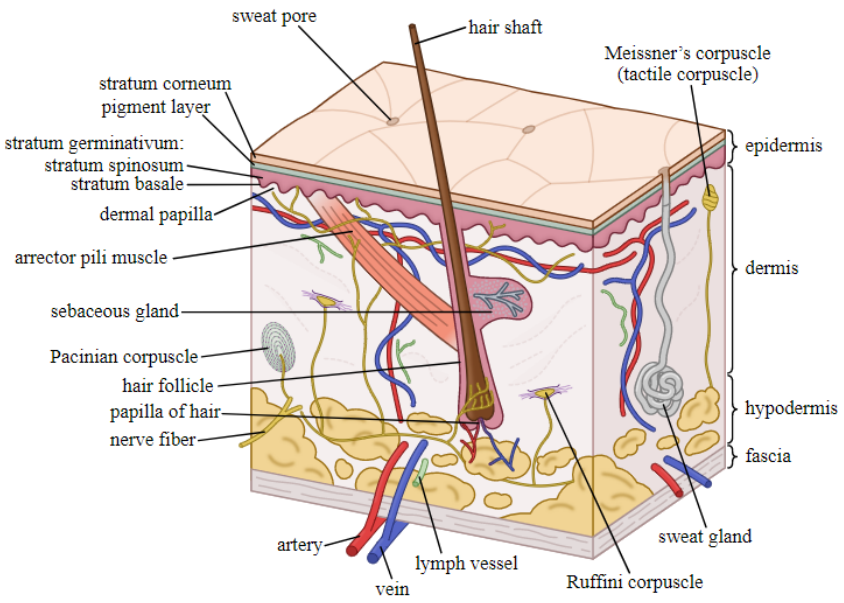


Figure 2.2: Transverse Section of Human Skin(87)

2.3.2. Breaching of the Stratum Corneum Barrier

Though, the transdermal route offers numerous benefits as mentioned previously, it is not much preferred as the delivery of drug by this route is limited by the presence of outer-most non-viable layer of epidermis, stratum corneum.(88, 89) Due the presence of keratinocytes bordered by the lipid matrix in stratum corneum, it forms a packed structure termed as brick-and-mortar system which makes penetration of drugs difficult. This layer can be considered as the major hindrance for the permeation of drugs and almost hinders 90% of drugs administered by the transdermal route. Though, almost all drugs can infiltrate this layer to a certain extent but nearly none of them are able to permeate a layer to the extent where the drug can reach systemic circulation. After the stratum corneum, comes the epidermis and this layer offers better permeation for the drugs given to the higher degree of hydration Below this is the dermis layer which is responsible for helping the drugs to reach the systemic circulation because of the presence of blood vessels. This layer is also referred to as the "skin's microcirculation" as it is responsible for regulating skin's temperature. Therefore, if we can work out a way for the drug to reach the epidermis and

dermis layer, the drug will be available in the systemic circulation. Various mechanical or chemical approaches are employed to deliver a drug molecules fruitfully through the skin and overcome the problems associated with transdermal route of delivery as mentioned above.(85, 88-90) Below-mentioned are some of these approaches(91):

A. Use of chemical penetration enhancers-surfactants, co-solvents eg. Dimethysulphoxide (DMSO), Ethanol, Decanol, Propylene Glycol, Azones (e.g. laurocapram), Sodium Lauryl Sulphate

B. Novel drug delivery systems such as Pharmacosomes, Liposomes, Ethosomes, Niosomes, Ultra Deformable Vesicles, Emulsomes, Nanoparticles etc.

C. Mechanical approach for drug delivery

- Micro needles injection techniques
- Iontophoresis
- Sonophoresis
- Laser radiation
- Suction ablation
- Radio waves
- Magnetophoresis
- Stretching of skin
- Thermophoresis
- Application of pressure
- Chemical modification
- Skin abrasion

With the prolonged use of chemical penetration enhancers, it is reported that it can cause skin toxicity and irritation. Thus, for the present project, various nanocarriers would be employed for enhancement of transdermal permeation. For example, Wei Lei et al. for the improvement of the penetration of drug and enhancement in in-vivo therapeutic efficacy, prepared TAC loaded transferosomes and reported that as compared to conventional gels and ointments

transferosomes-gel loaded TAC had enhanced retention of TAC in dermis and epidermis layers of the skin.(92) Sanju Singh et al. prepared the niosomal gel of FBX and proved that the developed formulation had transdermal potential and anti-gout efficacy.(78)

Many a times, it has been observed that use of such nanocarriers is not sufficient enough to enhance the transdermal permeation and achieve desired plasma concentration. To overcome this limitation, use of some physical penetration enhancement techniques is necessary. Eneko Larraneta et al. suggested that nanomedicine and microneedle technology strategies can be combined given to the development of new technologies. Few nanomedicine technologies like lipidic vesicle, lipid nanoparticles, polymeric nanoparticle and microparticle, microemulsion, metallic and mineral nanoparticles etc are delivered with the help of microneedles.(93) Among the various mechanical techniques employed to evade stratum corneum , application of microneedles seems to be advantageous as it facilitates the permeation across the toughest barrier, stratum corneum. Another advantage of microneedles is that given to the short length and narrow diameter, they are painless.(90)

2.4. Novel Drug Delivery Systems-Nanocarriers

Drugs can be incorporated into nanocarriers that easily deposit and continuously release the drug in the vicinity of papillary area having rich capillary network may ensure better systemic availability of the drugs. For achieving this aim, cubosomes was selected as nanocarriers given to its great potential for transdermal delivery.

2.4.1. Cubosomes

Cubosomal formulations are generally prepared with the help of lipid, stabilizer and co-solvent. The phases formed by the lipids are due to their structure and the interactions that they have in an aqueous environment. Broadly, lipids are classified in two major classes i.e., lamellar and non-lamellar lipids. “Lamellar lipids can be understood as the ones which facilitate the formation of planar lipid bilayers whilst the non-lamellar lipids can result in the formation of

phases such as the hexagonal and bicontinuous cubic phases." Many factors are there which influence the structure and stability of the cubosomes such as temperature, lipid composition, pressure and hydration. It has been reported that, there are three types of cubosomes exist in the lipid membrane namely, primitive, gyroid and double diamond. Till date, cubosomal structures that are reported are mostly possess either double diamond or primitive.(94)

When lipid mixture is combined with a stabilizer, it automatically assembles itself into the structure of a cubosome. Phytantriol and Monolein are most popularly used lipids for the preparation cubosomes. These lipids when kept under excess water condition and exposed to a temperature 80°C for Monolein and 43°C for Phytantriol they form Pn3m cubic phase morphology. These above mentioned lipids have received approval for in-vivo use.(94, 95)

Table 2.3: Literature Review on Cubosomes for Transdermal Delivery

Author	API used	Material used	Observations
Xinsheng Peng et al.(96)	Capsaicin	Phytantriol or glycerol monooleate, poloxamer 407	They reported that the capsaicin present in the cubosomal formulation has a comparable permeation rate (0.32±0.05 $\mu g \cdot cm^{-2} \cdot hour^{-1}$) when seen against that of a cream (0.28±0.15 $\mu g \cdot cm^{-2} \cdot hour^{-1}$)
Mohamed Nasr et al.(97)	Colchicine	Glyceryl monooleate, poloxamer 407	They reported that the absorption of transdermal COL gel by the transdermal route is much more than the oral COL solution. This was proved as the relative bioavailability of the transdermal gel was 4.6 times than that of oral COL solution.
Salwa Salah et al.	Etodolac	Poloxamer 407, monoolein	They performed pharmacokinetic study in human volunteer and reported that the bioavailability of etodolac-loaded cubosomes was higher as compared to the bioavailability of the oral capsules (266.11%). There was proof of longer half-life and higher Mean Residence Time (MRT) that reached 18.86 and 29.55 h, respectively.(98)
Radhakrishna Nithya et	Dapsone	Glyceryl monooleate and	They obtained high transdermal flux for cubosomes of dapsone (71.28 ± 4.65 $\mu g/cm^2/h$) in compare to

al.(99)		poloxamer 407	the dosage form present in market (55.28 ± 2.13 µg/cm^2/h) and dapsone-PBS (45.44 ± 3.09 µg/cm^2/h).
Hadel Abo El-Enin et al.(100)	Clonazepam	Glycerol monooleate, pluronic-F127, PVA (polyvinyl alcohol)	They found out transdermal flux of cubosomes of clonazepam (0.5547 µg/cm^2/h) was higher than pure drug solution (3.357 µg/cm^2/h).
Salma M. Mohyeldin et al.(101)	Progesterone	Glyceryl monooleate, pluronic-F127,	They found that cumulative amount of drug permeated from each developed nanocarrier was 6.08 ± 0.49, 9.05 ± 0.47, 12.09 ± 1.60 and 18.07 ± 2.58 µg/cm^2 for nanoliposomes, polymeric nanomicelles, nanoemulsion and cubosomes, respectively which was much higher that pure drug suspension (≤3 µg/cm^2).

2.5. Nanocarriers Loaded Microneedles

Literature suggests that, to administer the drug and achieve skin microporation in a single step can be done by incorporating these nanocarriers in dissolving microneedle patch (MNP). There are many advantages of incorporating nanocarriers in microneedle patch like, delivering a constant and calculated fraction of drug each time, providing occlusive condition to prolong the time for which the pore remains opened, better handling and storage of the formulation, providing environmental protection to the ingredients and avoiding any microbial invasion through such pores. It was reported by Eneko Larraneta et al. that the combination of nanomedicine and microneedle technology strategies has been possible because of new technologies. Currently, there are few nanomedicines that are being delivered by microneedles such as lipidic vesicle, lipid nanoparticles, polymeric nanoparticle and microparticle, microemulsion, metallic and mineral nanoparticles etc. Various advantages of microneedles are: lack of pain, delivery of large molecules efficiently, patient compliance, avoidance of first pass metabolism, target based delivery, minimal potential for tissue trauma

from an injection. A literature review for delivering of drug loaded nanocarriers using biodegradable microneedles is given in table 2.4.

Table 2.4: Literature review on nanocarriers loaded microneedles for transdermal permeation

Author	API	Material used	Finding and discussion
Qiu Y. et al.(102)	Docetaxel	Phosphatidylcholine, sodium cholate	From their finding it can be concluded that permeation of docetaxel can be improved through skin by combine approach of microneedle and elastic liposomes.
Gui Chen et al.(103)	Triptolide	Egg lecithin, cholesterol	They have improved the permeation of triptolide across the skin by combining liposomes with hydrogel microneedles and overcome the disadvantage associated with drug like first-pass metabolism and digestive toxicities.
Yahua Cui et al.(104)	Paeoniflorin	Phospholipid, cholesterol, ethanol,	They have improved the permeation of Paeoniflorin across the skin with cumulative amount of drug permeation of 307.17±26.36 μg/cm²
Hetal P. Thakkar et al.(105)	Raloxifene HCl		A permeation of ethosomes of Raloxifene HCl was increased through microporated pig ear skin which transdermal flux of 4.621 μg/cm2/h for ethosomes while 6.194 μg/cm2/h through microporated skin.
Juha Monkare et al.(106)	Oval albumin	PLGA, PVA, Hyaluronan	They obtained enhanced immune response with PLGA nanoparticle loaded microneedle compare to the hollow microneedle.
Zhilin Li et al.(107)	Oval albumin, CpG oligodeoxynucleotides	Chitosan, polyvinylpyrrolidone (PVP) K29/32,	The antibody dose–response curve demonstrated that immunization by microneedle was comparable to conventional subcutaneous injections in a more convenient and less invasive way.
Liang-Cheng Su et al.(108)	Coumarin 6	PLGA, PVA, PVP	In *in-vivo* transdermal delivery study they proved that approx. 90% of the loaded PLGA NPs were delivered to the viable epidermis and dermis, whereas only <2% of topically applied PLGA NPs were detected in the skin after being treated with a commercial 3M™ MN product.
E Ramadan	Lamivudine	PLGA	Polymeric lamivudine-loaded NPs could serve as a potential NDDS for the sustained transdermal delivery.

et al.(109)			The steady state flux could be enhanced by more than two folds using the microneedle-mediated transport

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