Dexamethasone on loaded transfersomes nanoparticles for skin delivery

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Copyright Statement

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“Dedicated to Amma.”

Abstract

Transdermal drug delivery system (TDDS) serves as excellent route to reach the target site efficiently. However, its use is limited because of the barrier stratum corneum, the top layer of skin. Transfersomes have the ability to evade these barriers because they are highly deformable making them stress-responsive and stress-adaptive complexes. The basic aim of the study was to develop nano-deformable transfersomes (NDTs) nanoparticles for the dermal delivery of Dexamethasone against burn wounds. NDTs were formulated by a modified thin film hydration method. They were composed of phosphatidylcholine and Tween 80, and loaded with anti-inflammatory drug dexamethasone. The physicochemical properties of DM-loaded transfersomes were established in terms of vesicle size and entrapment efficiency. DM-loaded transfersomes were entrapped within Carbopol gel network for the ease of topical application. The in vivo results displayed higher anti-inflammatory activity by efficiently healing burn wounds. Precisely, the outcomes indicated that the efficient delivery of DM could be accomplished by using topically applied transfersomes for the treatment of burn wounds as compared to simple DM gel formulation.

Key Words: Transdermal drug delivery, nano-deformable transfersomes, Dexamethasone, Thin film hydration, anti-inflammatory, Carbopol gel

Table of Contents

List of Abbreviations

DM	Dexamethasone
NDTs	Nano-deformable Transfersomes
DMT	Dexamethasone-loaded Transfersomes
TDDS	Transdermal Drug Delivery System
DMTG	Dexamethasone Transfersomes Gel
DMG	Dexamethasone Gel
SD	Standard Drug
NaOH	Sodium Hydroxide
PBS	Phosphate Buffer Saline
ROS	Reactive Oxygen Species
WHO	World Health Organization
TNF-α	Tumor Necrosis Factor Alpha
TNF-β	Tumor Necrosis Factor Beta
ECM	Extracellular Matrix
SC	Stratum Corneum
GR	Glucocorticoid Receptors
GRE	Glucocorticoid Response Elements
IL	Interleukin
ICAM-1	Intercellular Adhesion Molecule-1
PMN	Polymorphonuclear Neutrophils
ELAM-1	Endothelial Leukocyte Adhesion Molecule-1

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CHAPTER 1

Introduction

As of today, with advances in technology, a drug can be delivered topically at the target site where action is required. Therefore, with skin being a target organ, topical dermatological products are designed to administer drug into the skin directly for the treatment of dermal disorders (Shah, Behl et al. 1992) (Allen, Popovich et al. 2005). Skin is the chief route of topical drug delivery system (TDDS) as it is the one of the most readily available organs of human body for topical delivery. The main hindrance in TDDS is to evade the barrier of skin; the outermost layer of the skin, stratum corneum, is the rate limiting step in topical administration.

Carrier systems like nano-deformabable transfersomes (NDTs) had been extensively studied as for TDDS (Cevc and Blume 2004). They have the ability to enhance drug penetration into the skin. They have the potential to increase therapeutic effectiveness of the drug and decrease its side effects. They can also function as local depots for continuous release of dermally active components (Elsayed, Abdallah et al. 2007).One reason of transfersomes having penetration enhancing effect is may be due to the interactions between the intracellular lipid layer of the skin and the liposomal lipid bilayer. The degree of penetration enhancement depends on the transfersomes size and its lipid composition, the lipophilic nature of the drug and nature of the skin.

Nonetheless, for the desired effects, transfersomes are difficult to administer directly onto the skin. Therefore, these can be incorporated in to gels and can be applied on the skin directly. Transfersomes mixed with gels are found to stable (Jithan and Swathi 2010, Gupta, Aggarwal et al. 2012). Gel formulations for topical application of transfersomes can be used to influence the barrier function of skin and accessibility of drugs to the skin tissues. They are

formulated in such a way that supplies prolonged local contact with minimum absorption in order to serve as a local depot. They are suitable for inflamed and injured skin (Cevc and Blume 2004). Usually, it is difficult to incorporate intact transfersomes in creams/lotions because of the interaction between active surfactants and transfersomal bilayers (Rai, Pandey et al. 2017).

Hydrogels are clinically suitable system for drug transportation because they acquire apt rheological properties and high tissue compatibility (Lee 2018) hence providing long contact time at the target site and continuous drug concentration. They function as localized drug depots for sustained drug delivery. Compared to conventional gels and creams, they are helpful in management of the drug. Moreover, hydrogels such as carbopol gels are anionic hydrogels, having good buffering capacity, helps to maintain the pH of skin. Thus, transfersomes are mixed with carbomer hydrogels in order to obtain semisolid tranfersomal gel formulation to increase drug bioavailability and efficacy (Ibrahim, Nair et al. 2017).

In this study, application of Dexamethasone (DM) using transfersomal gel formulation has been investigated on burn wounds. DM ($C_{22}H_{29}FO_5$) is an effective synthetic (man-made) glucocorticoidal steroid drug. Glucocorticoids suppress the immune system and block the inflammatory response (Coutinho and Chapman 2011). So, it is used in the treatment of various inflammatory and autoimmune diseases e.g. oedema, arthritis, skin conditions, nasal and eye allergies. Glucocorticoids administered orally causes numerous unfavourable and toxic effects such as disturbances in electrolytic balance, stomach upset, negative protein balance, muscle atrophy and increased appetite (Moghadam-Kia and Werth 2010). DM also goes under first pass metabolism which reduces its bioavailability. Therefore, DM administration by other routes is more preferable than oral.

When a burn injury occurs, immune system gets activated and releases monocytes in bloodstream which then differentiate into macrophages. The activated macrophages initiate an inflammatory response by transcription of proinflammatory cytokine genes which are stimulated by NF-kβ (Liu, Zhang et al. 2017). The cytokines increase the expression of neutrophils on endothelial cells, near burned area. Accumulation of neutrophils produce an immense amount of reactive oxygen species (ROS)(Mittal, Siddiqui et al. 2014), leading cells and its cellular components to an oxidative burst that can cause more damage to the skin tissue. So, pharmacological intervention is required in inflammation-induced tissue injury by down regulating proinflammatory cytokines, inhibiting neutrophil infiltration or repressing ROS. Knowing DM can inhibit NF-kβ (Auphan, DiDonato et al. 1995, Crinelli, Antonelli et al. 2000), we studied the effects of DM-NDTs by monitoring the accumulation of neutrophils in burn wounds.

DM gels (Dexagel®) used to treat inflammation are available in the market but DM transfersomal topical gel formulations are not available yet. The topical delivery of DM has been intensively studied by numerous novel delivery methods.

Thus, the present study is aimed to

- Formulate and characterize DM on loaded transfersomes nanoparticles and incorporate them into gel
- Evaluate their anti-inflammatory activity on burn wounds mice model through histology studies and blood tests.
- Assess the delivery efficiency of drug through skin and healing burn wounds.

Literature Review

1.1 Burn Wounds

Burn is any injury to the skin tissue caused by contact with hot materials or chemicals. Other causes may include radiation, friction and electric contacts.

1.1.1 Statistics

World Health Organization (WHO) statistics have shown that approximately 1,80,000 deaths occur every year globally due to burns, mostly occurring in the low-income and middle-income countries. WHO announced in 2004 that nearly 11 million people suffered serious burn wounds that they needed medical attention. Taking into account some more local figures, in Colombia, Egypt, Bangladesh and Pakistan, 17 percent of children suffer from temporary disability and 18 percent of children suffer from permanent disability because of burn injuries. Non-fatal burns are a major cause of morbidity which leads to prolonged disfigurement, disability and hospitalization, often resulting in stigma and dismissal. Most burns are due to flames or hot substances whereas cold and radiation burns are less common ones. According to the report published by Cancer Research UK in January 2014, globally every year more than 305 million traumatic, acute and burn wounds are treated and recorded.

1.1.2 Types of burns

Burns are divided into thermal and non-thermal wounds. Thermal burn wounds are caused by any contact with flames, hot liquids and solids whereas non-thermal burn wounds include any contact with chemical, electrical and radiological burns (Collins 2008). Types of burn are summarized in Table 1 below.

Burn Wounds	Type	Cause
Thermal	Flash	explosives of propane, natural gas, gasoline, or any other inflammable liquids.
	Flame	continuous exposure to severe heat, commonly associated with improper use of combustible liquids, house fires, clothing catch fire by stoves and heaters and automobile accidents
	Scalds	hot liquids e.g. water, grease, oil or tar
	Contact	hot metals, glass, plastics or coals
Non-thermal	Chemical	strong acids or alkali materials
	Electrical	AC or DC current
	Radiological	alpha, beta or gamma radiation

Table 1 Types of burns

1.1.3 Degrees of burn wounds

Burns are differentiated on the basis of the thickness of damage it has caused to the skin(Hettiaratchy and Dziewulski 2004). Burn categorization is summarized in Table 2 below.

Thickness	Degree	Skin Layer Involved	Appearance
Superficial burns	First	Epidermis	Red, painful, dry, and with no blisters. Long-term tissue damage is rare
Superficial Partial burns	Second	Epidermis and part of the dermis	Red, blistered, and may be swollen and painful
Deep Partial burns	Third	Epidermis and dermis (may involve hypodermis)	The burn site may appear white or charred
Full burns	Fourth	All the way down to the underlying muscles, tendons and bones	no sensation in the area as the nerve endings are damaged

Table 2 Categories of burns

1.1.4 Pathophysiology of burns

Skin is the target organ of various burn injuries and manifests many pathophysiological pathways (Dzhokic, Jovchevska et al. 2008). When a burn injury occurs, number of inflammatory cascades gets activated. At first, protein molecules that are present in the burned area are denatured which in turn activates complement system of immune system. Mast cells degranulate and coat the denatured proteins. Complement system attracts the neutrophils, degranulation of which increases the free radical quantity and releases proteases into the burned area. Mast cells then release tumor necrosis factor alpha (TNFα) which

attracts inflammatory cells to the wound site. Release of inflammatory cytokines by the inflammatory cells alters vascular permeability, producing a net flow from intravascular space to the extravascular space. In large burns, this inflammatory phase is prolonged and reduces the cell-mediated immunity in individuals (Schwacha 2003, Dreifke, Jayasuriya et al. 2015)

When burn injury occurs, body activates haemostasis leading towards the clotting of blood over the wound site; initiating wound healing. This process gives immune system enough time to produce a number of essential cytokines and the growth factors to make the wound go through the different phases of healing and gives stability to the primary vasculature. The haemostasis process takes place in the first 2 hours of wound development (Blombäck and Okada 1982, Scheraga 1984, Shah, Omar et al. 2012) (Shah, Omar et al. 2012).

Any damage to cells stimulates the inflammatory response of immune system that marks the first major stage of wound healing. This begins with distribution of different entities carried by blood around the wound site. Mainly, this include blood plasma carrying various components involved in the innate immune system of the body, such as macrophages, antibodies and leukocytes etc. (Majno and Joris 2004, Kumar, Abbas et al. 2017). Inflammatory phase occurs in the next 1-2 days of post burn injury following wound formation and exhibits symptoms of pro-inflammatory phase like pain, redness and swelling etc. (Busti, Hooper et al. 2005). After coagulation of blood, main inflammatory responsive cells such as macrophages, neutrophils and lymphocytes migrate towards the burn wound area and initiate inflammatory phase by releasing a number of cytokines and growth factors (Gosain and DiPietro 2004, Campos, Groth et al. 2008) Proliferative phase starts from 2 days after and end up till around 2 weeks leading to the synthesis of collagen by proliferation of cells and laying new matrix.

Remodeling is the last stage of wound healing process that is achieved after a year of the original wound injury occurred. This final stage is characterized by the reduction and appearance of the wound surface. During this stage new epithelium is constructed as the granulation tissue matures into a scar. The formation of capillaries initiated in the proliferative phase is reduced by combining capillaries to large vessels (Sethi, Yannas et al. 2002, MacKay and Miller 2003).

Intense burn wounds exhibit immunosuppressed mechanism of healing which is frequently characterized by prolonged injury and inflammation, excessive proteolysis and abnormal macrophage role and angiogenic response (Galiano, Tepper et al. 2004, Maruyama, Asai et al. 2007). Macrophages play vital role in the pro-inflammatory phase as they are the major producers of pro-inflammatory entities. Thermal injury causes the hyperactivity of the macrophages which in turn disrupts the production of mediators involved in the pro-inflammatory phase. This causes a prolonged period of inflammation than the normal one. Impaired macrophages activity leads to increase in the release of pro-inflammatory factors which appears to be of primary importance in the formation of the post-burn immune dysfunction especially cytokines. Tumor necrosis factor-β (TNF-β) recruits neutrophils, monocytes and fibroblasts in excessive amount to the wound site for tissue repairment. Neutrophils are known to be able to remove damaged tissue and help in re-epithelialization. However, on the other hand, the uncontrollable capability of neutrophils on endothelial cells contributes to excessive production of reactive oxygen species (ROS) and protease manufacturing can cause damage to tissue due to oxidative stress causing cell and its cellular components to oxidative burst, at the wound site. This is eventually detrimental for wound healing process as it causes the disruption of the extracellular matrix (ECM) and growth factors (Peplow 2005). In addition to this, the abnormal growth of cells within the wound leads to impaired angiogenesis signifying further degradation of deteriorating healing. In

order to overcome this problem, preventing prolonged period of inflammation is the target approach in severe thermal wound treatment. The entire process is summarized in the figures below, adapted from (Dreifke, Jayasuriya et al. 2015).

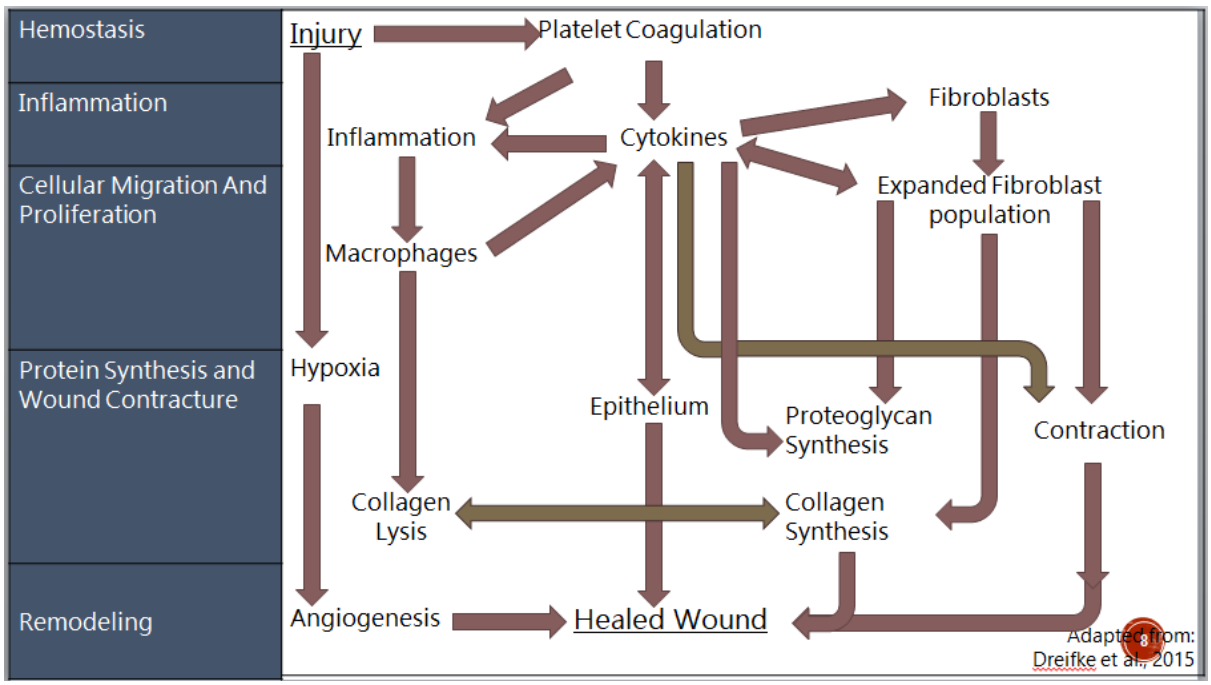

Figure 1.1a Pathway of Normal Wound Healing Mechanism

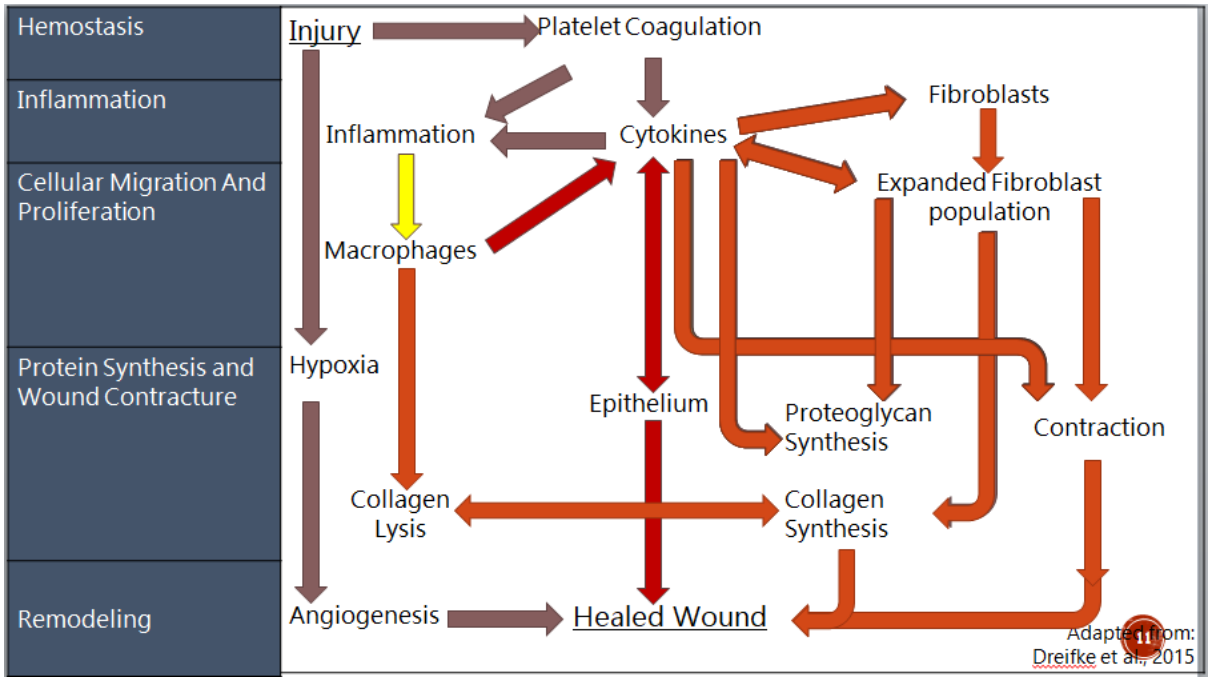

Figure 1.1b Pathway of Impaired Wound Healing Mechanism

1.1.5 Healing process of wounds

All kinds of wounds whether it is burn or incision, follow the same healing processes and pathways. Wound healing has three main phases as described below.

Inflammatory phase was well described above in the pathophysiology of burn wounds. When inflammatory phase is completed, the proliferative phase is initiated which is comprised of fibroblast and keratinocyte activities by cytokines and growth factors, angiogenesis (revascularization) and lastly, wound closure by forming new epithelial layer on the surface of the wound area. This phase of healing usually starts from 3rd day of the injury and lasts till 2-3 weeks, depending on the degree of burn injury. This phase greatly requires vitamin C for collagen synthesis. Type-III collagen is first synthesized which is deposited in asymmetrical patterns.

Remodeling phase starts when enough collagen molecules are deposited and fibroblast proliferation has occurred. This is the third and final stage where wound maturation occurs as collagen and elastin are deposited continuously. Collagen is realigned by replacing type-III molecules with type-I. All the phases are summarized in Table 3.

Along these phases of wound healing, the pH of wound site changes with time. When burn wound occurs, the pH of burnt skin becomes alkaline and as the wound is healing the pH changes from alkaline to neutral to acidic. Wounds with higher pH value tend to have lower rates of recovery (Leveen, Falk et al. 1973) which is why it is necessary to maintain pH value of skin in wound management treatment.

Phase	Characteristics	Key Players
Inflammatory	Vasodilation Fluid extravasation Edema	Neutrophils Monocytes Macrophages
Proliferative	Wound closure Revascularization	Keratinocytes Fibroblasts
Remodeling	Wound maturation Scarring	Collagen Elastin Fibroblasts/myofibroblasts

Table 3 Wound healing phases

1.1.6 Current available treatments for Burn Wounds

Burns are categorized by their size and depth. These features help to identify the treatment and prognosis. It is critical to identify superficial burns from partial thickness burns. Initially burns are cleaned by removing debris or dead tissue. Appropriate dressing is done afterwards. Usually, partial and superficial degree burns heal irrespective of the dressing. But if wound depth increases, chances of infection increases. Hence, managing the burn wounds changes with degree and scarring intensity. Intense burn wounds can be treated with topical solutions or dressings integrated with those solutions. Commonly used topical solutions are cited in Table 4.

Topical Agents	Effect
Polyfax	Antibacterial
1% Silver sulfadiazine cream	Broad-spectrum antibiotic
0.5% Silver nitrate solution	Less effective compared to Silver sulfadiazine, good antimicrobial activity against Pseudomonas *aeruginosa*
Silver sulfadiazine and cerium nitrate	Broad-spectrum antibiotic; boosts cell-mediated immunity.

Table 4 Topical Solutions for Burn Wounds

1.2 Drug Delivery System

Research in recent years is aiming in developing novel drug delivery system in order to achieve maximum therapeutic activity and patient compliance. Such drug delivery systems have been developed with enhanced therapeutic activity but there other complications with systems which are not yet completely resolved (Boateng, Matthews et al. 2008).

Drugs that are administered orally have many severe limitations. First, they have to go through harsh environment of gastrointestinal tract where most of drugs are disintegrated because of the variation in the pH of tract or problem with solubility and lastly, experience first-pass metabolism. Whereas the problem with parenteral drug administration is the inefficiency of drug reversal, risk of infections, hypersensitive reactions and cost. Both of the methods have less patient compliance because some drugs are bitter in taste when taken orally and pain in parenteral delivery (Langer 2004).

Immense attention is given to topical drug delivery due to its several advantages. It is the most favoured route as it provides direct delivery of therapeutic agent at the target site easily and is cost effective. Since it gets to the target site without first pass metabolism optimal concentration of drug is required. It used for the treatment of any infection of vagina, eye, nose and skin. (Singh Malik, Mital et al. 2016)

1.2.1 Transdermal Drug Delivery System (TDDS)

Transdermal drug delivery system (TDDS) uses skin as a route for the delivery of the drugs providing a localized effect. Skin approximately covers $2m^2$ of adult human body and has 3 kg total weight. This transdermal route gives numerous advantages over oral and parenteral delivery system. First and the most important one is high patient compliance. Others are avoiding first pass metabolism, efficacy of short half-life, reducing the side effects, prolonged activity, minimizing the instability of drug levels and increasing pharmacological and physiological response (Barry 2004, Honeywell-Nguyen and Bouwstra 2005).

On the contrary, it has some drawbacks like it can cause irritation, erythema and itching in the target area. A major hindrance to transdermal drug delivery system is the permeability of stratum corneum (SC). This limits the transportation of the drug making it inadequate for medical purpose (Mura, Manconi et al. 2009). SC is basically the outer most layer of the top epidermis acting as a flexible membrane is made up of keratin, formed from epidermal cells, which is not permeable to water.

1.2.2 Vesicular System

Vesicular system is one such novel approach of encapsulating drug which enables a sustained release of drug (Bansal, Kashyap et al. 2012). It is better than the available techniques as it reduces toxicity, increase bioavailability, avoids first pass metabolism, carry both hydrophilic and hydrophobic drugs, enhances stability of drug and patient compliance (Singh, Pradhan et

al. 2015). They are composed of lipid bilayers having both hydrophilic and hydrophobic parts (Jain, Jain et al. 2003).

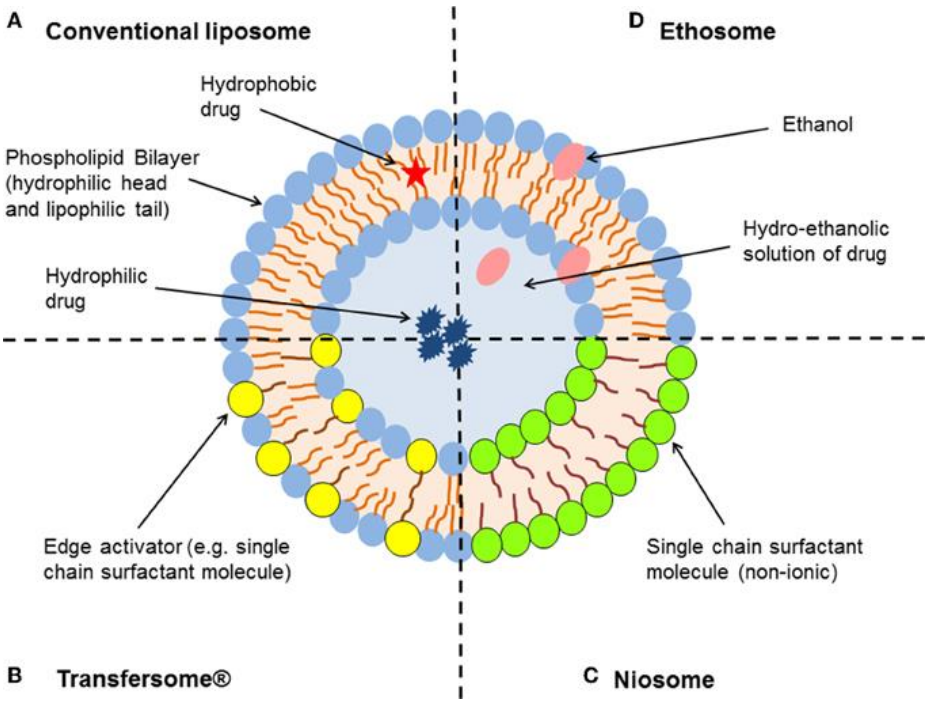

Figure 1.2 Structure of different types of Vesicular System

A number of systems like iontophoresis, electrophoresis, sonophoresis, chemical permeation enhancers, microemulsions, have been examined to elude skin barrier. Vesicular systems have also been vastly studied which includes niosomes, ethosomes, liposome and transfersomes. This is the one of the most effective techniques to deliver drug through skin in a sustained manner (Barry 2001, Hamidi, Azadi et al. 2008, Karande and Mitragotri 2009, Subedi, Oh et al. 2010). A number of vesicular systems are available; a few are discussed in Table 5 with their advantages and disadvantages.

Methods	Advantages	Disadvantages
Penetration enhancers	Increase skin penetration, give local and systemic effect	Skin irritation, carry low molecular weight drugs
Physical methods, e.g., iontophoresis	Increase charged intermediary size molecule penetration	Charged drugs, low transfer efficiency (<10%)
Liposomes	Phospholipids vesicle, biocompatible, biodegradable	Less skin penetration, less flexible, less stable
Proliposome	Phospholipids vesicle, more stable than liposomes	Less penetration, cause aggregation of vesicles
Niosomes	Non-ionic surfactants vesicles, greater stability	Less skin penetration, easy handling
Proniosomes	Convert into niosome in situ, stable	Do not reach deep into skin layer
Transfersomes and protransfersomes	More stable, high penetration, highly deformable, biocompatible, biodegradable, carry both low and high molecular weight for both lipophilic and hydrophilic drugs reach deep into skin layers	None, but for some limitations

Table 5 Advantages and disadvantages of different types of vesicular system

1.2.3 Transfersomes

Among these, considering all the pros and cons, transfersomes appear more effective than the others. Transfersomes were introduced by Gregor Cevc in 1991. IDEA AG (German Company licensed it as a trademark and patented drug delivery technology. It is from a Latin

word ‘transferred’ meaning “carrying body” and Greek word “some” meaning “body”. Transfersomes are biocompatible and biodegradable as they are formulated from natural lipids. They are man-made vesicle which is designed in such a way that it looks a cell involved in exocytosis or they can act as a reservoir for the drug. Thus, this makes it suitable for sustained release of the drug at the target site (Modi and Bharadia 2012). Other than that, they can carry low to high molecular weight drugs which can either be hydrophilic or hydrophobic in nature. Hence, at many times they have suggested for efficient transdermal drug delivery (Cevc, Blume et al. 1997, Jain, Umamaheswari et al. 2003, Vinod, Kumar et al. 2012).

A) Structure

Transfersomes are composed of phospholipids, surfactant and ethanol. They are characterized as deformable, stress responsive and highly adaptable. They are formed of lipid bilayer with an aqueous core inside. They are similar to conventional liposomes but are more deformable due to the presence of surfactant “the edge activator” in their bilayer which gives it the required flexibility (Bhardwaj, Shukla et al. 2010). Ethanol is used for hydration which gives increased permeation through skin.

Phospholipids can be attained from natural sources like phosphatidylcholine is attained from soybeans. They integrate choline as their headgroup. Its structural formula is represented in Figure 1.3.

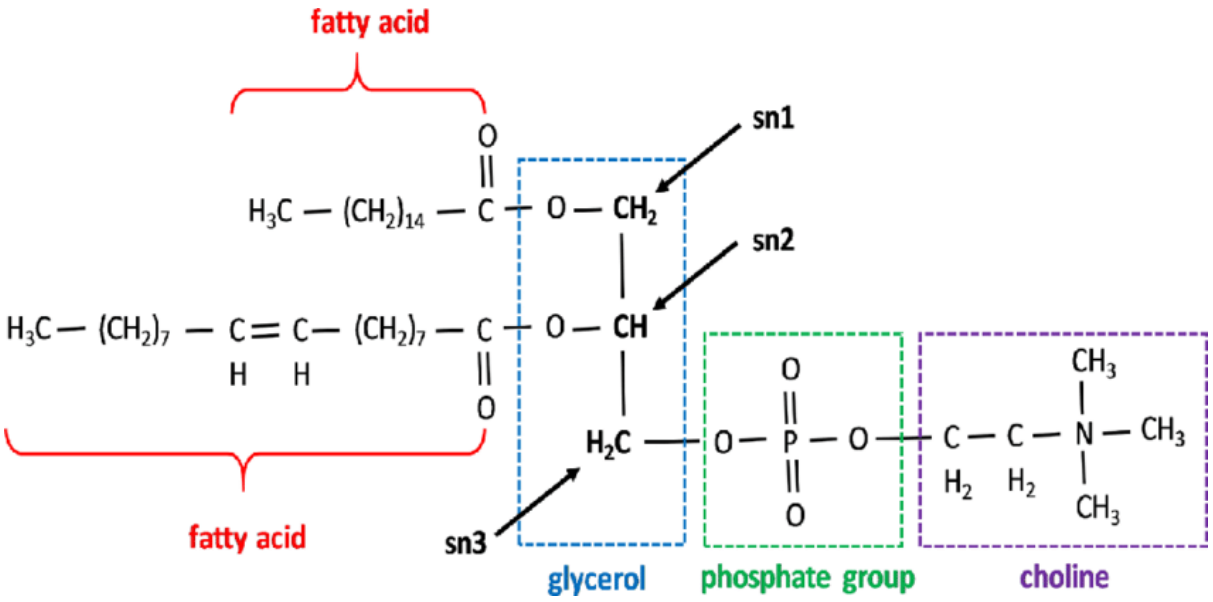

Figure 1.3 Structural formula of Phosphatidylcholine: 1-Oleoyl-2-palmitoyl-phosphatidylcholine

B) Mechanism of action

Transfersome possess ultradeformable property due to they can change their shape from deformation to reformation mechanism. This allows them to easily penetrate in stratum corneum. They can squeeze into to pores 5-6 times lesser than their diameter. They have hydrophilic surface which responds to hydration gradient of skin. This thrust the vesicles to squeeze through pores giving the opportunity to transfersomes to act as non-invasive drug. Due to high elasticity of lipid bilayer membrane, they efficiently respond to hydration and dehydration stress. The hydrostatic pressure difference in skin allows the transfersomes to penetrate stratum corneum through hydrotaxis according to elastomechanics (Ibrahim, Nair et al. 2017).

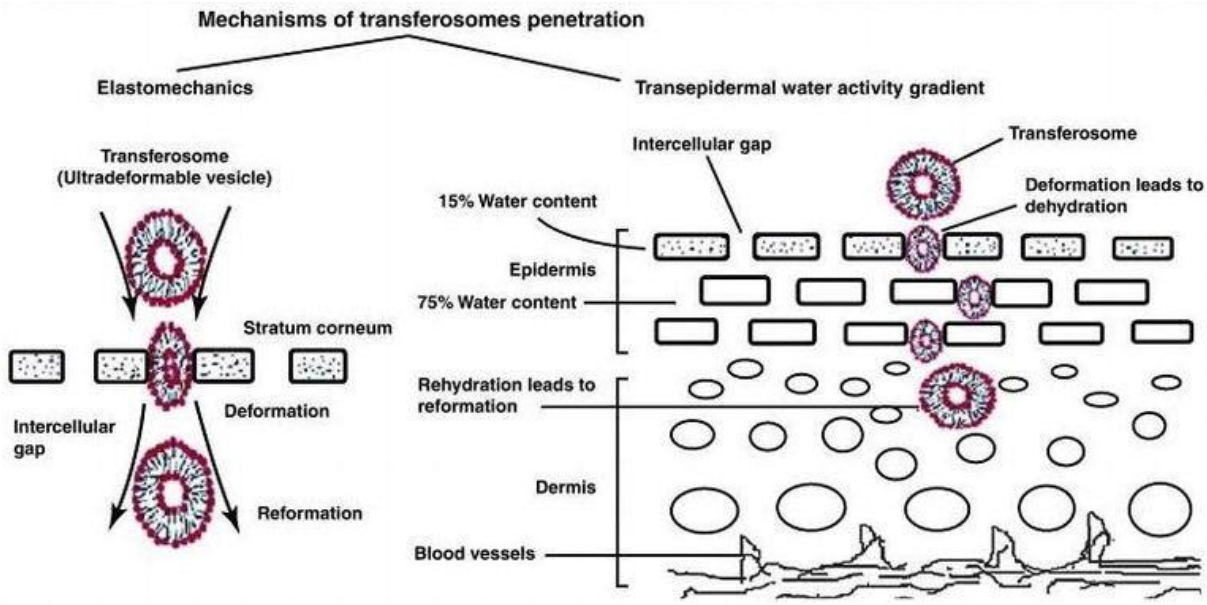

Figure 1.4 Transfersomes Mechanism of Action

1.3 Dexamethasone

Dexamethasone is a known synthetic corticosteroid that suppresses the release of inflammatory substances in the body. It has anti-inflammatory and immunosuppressive activity (Didonato, Saatcioglu et al. 1996) and is about 30 times more potent than cortisol. Since it inhibits inflammatory cells and suppresses the expression of inflammatory mediators, it is commonly used in the treatment of inflammatory and immune-mediated diseases. It is an FDA approved drug. It has many trade names which include Diodex, Maxidex, Decadron, Dexasone, Hexadrol and some others.

1.3.1 Structure

392.47 is the molecular weight this drug. Chemical name of DM is 9-fluoro-11β,17,21trihydroxy-16α-methylpregna-1,4-diene-3,20-dione. Empirical formula is $C_{22}H_{29}FO_5$ and structural formula is as follows:

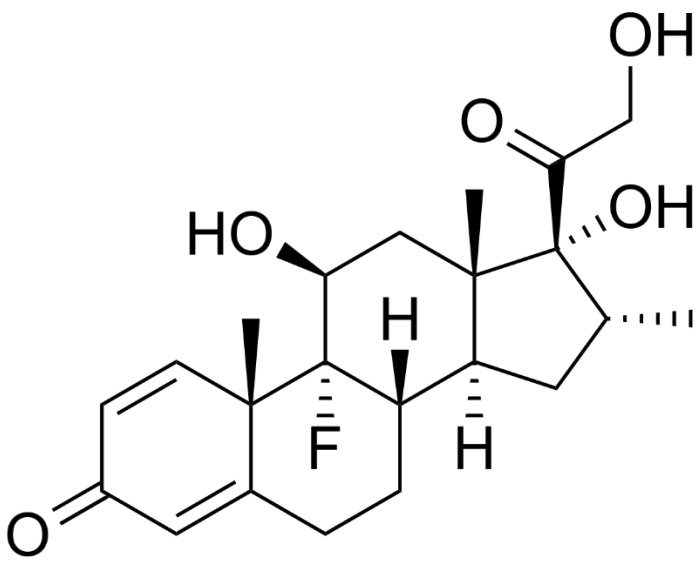

Figure 1.5 Structural formula of Dexamethasone

Dexamethasone is a man-made adrenocortical steroid. It is white and crystalline powder. It is odorless and non-reactive in air. It is not that soluble in water.

1.3.2 Treatment

Dexamethasone is used in the treatment of many diverse inflammatory conditions like allergic disorders, breathing disorders and skin conditions. Having an anti-inflammatory effect, it is used to decrease the swelling in edema and to treat eye inflammation. It has been linked with tumors of brain and brain. Also used in the treatment of some autoimmune diseases. It can also be used as a chemotherapy drug in multiple myeloma leukemia and lymphoma. It is used to improve appetite of cancer patients. Since it is a steroid, it can replace the natural steroid if a person is suffering adrenal insufficiency.

1.3.3 Anti-inflammatory mode of action

Dexamethasone acts as a glucocorticoid agonist when it enters the cell membrane. It simply binds with glucocorticoid receptors (GR) with high affinity. Then, this complex binds to glucocorticoid response elements (GRE) on DNA resulting in modification of protein

synthesis. Normally, activated macrophages modulates the inflammatory response by stimulating NF-κB to initiate the transcription of pro-inflammatory cytokines genes (Chen, Deng et al. 2018). The cytokines mainly tumour necrosis factor-alpha (TNF-α) and interluekin (L-1) increases the expression of cell surface adhesion molecules like intercellular adhesion molecule-1 (ICAM-1) and endothelial leukocyte adhesion molecule-1 (ELAM-1) on the surface of endothelial cells. By this means, polymorphonuclear neutrophils (PMN) adhesion to endothelium increases. Accumulated neutrophils at the infected area produce great amount of reactive oxygen species (ROS) like, O^{2-}, HOCl and H_2O_2, leading to cell death. Dexamethasone induces the transcription of IκBα which is the cytoplasmic inhibitor of NF-κB (Auphan, DiDonato et al. 1995). This will suppress the production of pro-inflammatory cytokines which includes TNF-α IL-1, IL-6 and IL-8 and in turn reducing the expression of cell adhesion molecules (ELAM-1 and ICAM-1) involved in migration of leukocytes. This will reduce the accumulation of neutrophil infiltration at the site of inflammation by inhibiting the production of cytokines and chemokines which attracts neutrophils at inflammatory site. This reduction in neutrophil infiltration will suppress oxidative stress in cells hence avoiding cell burst (Tsurufuji, Sugio et al. 1979).

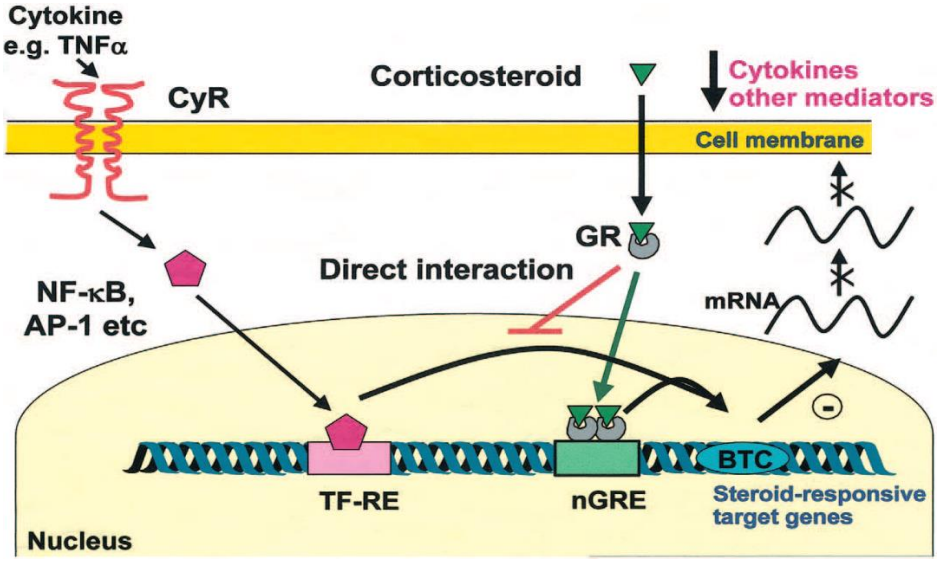

Figure 1.6 Dexamethasone Mechanism of Action

1.4 Carbopol Gel

Gels have two phases of swollen networks which possess are cohesive features of solids and diffusive transport characteristics of liquids. On the contrary, creams and ointments do not release drug immediately as compared to gel. Irrespective of drug soluble in water, it allows slow release of drug. They are biocompatible and have lower risk of causing inflammation and irritation. They can easily be applied on skin. They have many advantages for topical use. They tend to attain thixotropic and emollient property. Gels can spread easily and can be removed conveniently with water (Helal, El-Rhman et al. 2012).

Carbopol gel, a synthetic polymer, is composed of carbomer polymers. These polymers are cross linked together in such a way that they form microgel structure which is very useful in dermatological applications. They have mucoadhesive property (Kumar, Dhawan et al. 2014). Carbomer polymers are anionic in nature, thus neutralization is necessary for the formation of microgel structure. That's why tri ethanolamine or Sodium Hydroxide is used for this purpose. (Shin, Kim et al. 2000).

CHAPTER 2

Materials and Methods

2.1 Materials and Solutions

Dexamethasone was bought from Star Laboratories, Pakistan. Phospholipon 90G was obtained from Lipoid GmbH, Germany. Tween-80 was purchased from Sigma Aldrich, Germany. Chloroform and methanol (Sigma Aldrich, USA) of analytical grade were used for the hydration of lipids. PBS tablet was dissolved in 100ml of distilled water and was used to obtain thin film of lipid. Carbopol 934G was purchased from Avon Chem, UK.

2.2 Methodology Followed

2.2.1 Preparation and purification of DM-NDTs

Dexamethasone-nano deformable transfersomes (NDTs) were prepared by using thin film hydration method according to El Zaafarany et al., 2010 with minute alterations. Phospholipon 90G (lipid) and Tween-80 (surfactant) were used in ratio 85:15 and were dissolved in a mixture of chloroform:methanol (1:1) and sonicate it for 1 minute.. Rotary evaporator under vacuum at 45 ± 1 ºC, rpm 100 was used to evaporate organic solvents and to obtain a clear thin film of lipid. Then, the thin film was hydrated by adding 10ml of phosphate buffered saline (PBS) of pH 7.4 and kept on Rotary evaporator for 1 h at 60 ± 1 ºC. After formulation was prepared, it was extruded manually two to three times through polycarbonate membrane filters (200 nm) and was stored in air tight vial at 4º C for further analysis. Similarly, blank transfersomes vesicles (without dexamethasone) were also prepared.

Following table shows the composition of transfersomes for preparation.

Ingredients				
Dexamethasone	**Phospholipid**	**Tween 80**	**Chloroform**	**Methanol**
5mg	50mg	10mg	5ml	5ml

Table 6 Formulation of Drug loaded Transfersomes

2.3 Characterization

2.3.1 UV-VIS Spectrophotometry

Ultraviolet-Visible absorption spectroscopy is the most commonly used technique that helps to identify presence of components in sample. When a beam of light passes through the sample, it provides the absorption spectra of sample as an output by reflecting the beam.

$$A= -\log(T)$$

UV-Vis spectroscopy obeys the Beer-Lambert law where absorbance of the sample is directly proportional to the molar concentration of the sample present in the cuvette. The molar absorptivity also called absorption value is used to identify entities present in the sample. The equation of Beer-Lambert law is:

$$A = \log (I_0/I) = Ecl$$

UV- Vis spectroscopy is the foremost confirmatory test that is used to verify the synthesis of transfersome nanoparticles. Hence, for this very purpose, an ethanolic suspension of DM-NDTs and DM were prepared. Jasco V-650 spectrophotometer model was used in the range of 200-400 nm and samples were measured. Pure ethanol was used as a relative reference.

2.3.2 Entrapment efficacy

To determine the entrapment efficacy of transfersomes nanoparticles, an indirect method was used. First, standard curve was plotted using different concentration of dexamethasone (1-30µg/ml) by dilution of the stock standard solution in distilled PBS. Then, DM-loaded transfersomes solution was centrifuged at 13,500 rpm at 4°C for 1 hour. The supernatant was extracted and analyzed for the drug concentration after suitable dilution with PBS by measuring absorbance (λmax) at 241 nm using Jasco V-650 UV-Vis Spectrophotometer model. The supernatant was filtered and the entrapment efficiency of the dexamethasone-loaded transfersome were calculated by

$$EE = \frac{Q_t - Q_s}{Q_t} * 100$$

Where;

EE is the entrapment efficacy, Q_t is the total amount of dexamethasone added and Q_s is the amount of dexamethasone detected in the supernatant

2.3.3 Scanning Electron Microscopy (SEM)

Scanning Electron Microscopy (SEM) is a technique that uses electron beam to produce the image of sample and magnify it which helps to determine the morphology of the sample as well as its size and surface topography data. For SEM, samples were diluted in ethanol and were sonicated for 15 minutes till they were completely dispersed. Drops of samples were fixed onto a 1cm×1cm microscopic slide by air drying and were followed by gold coating of samples in order to make the surface conductive by using JFC-1500 sputter coater device. Conductive tape as used to place the coated specimen on the stubs. Specimens were examined using Field Emission-Scanning electron microscope (JEOL JSM-6490LA) at

20kV. Approximately 30 nanoparticles were measured and average particle size was determined by using SEM analysis results and Image J software.

2.3.4 Energy- Dispersive X-ray Spectroscopy (EDXS)

Energy-dispersive X-ray Spectroscopy (EDXS) is an effective technique used to analyze the elements present in the specimen using SEM image. It relies on the interaction of X-ray beam and the specimen. Every element has a specific atomic structure and allows it to emit unique set of peak. On this principle when an X-ray beam hits the specimen, it emits X-rays at different energy levels which help to identify composition and concentration of the specimen. Hence, EDXS was used to characterize blank transfersomes and DM-NDTs.

2.3.5 Fourier Transform InfraRed Analysis (FTIR)

FTIR is a technique that is used to identify the functional groups or bonds present in pure compounds, mixtures or used for their comparison. Every bond between different elements absorbs light energy at different frequencies. This light absorbance is measured by FTIR and produced as a graph identifying the bonds present in sample. The interaction between prepared transfersomes and dexamethasone was studied by using Perkin-Elmer Spectrum-100 spectrocopy at wavelength ranging from 400 cm^{-1} to 4000 cm^{-1} along with blank transfersomes. KBr disc method was used to analyze the samples. KBr was heated at 110^{o}C to evaporate any traces of water. Hydraulic press was used to make pellet of KBr. Samples were loaded in chamber individually and submitted to IR radiation to detect the functional groups. The infrared spectra output was recorded in a graph and interpreted to determine the bond stretching in functional groups by using essential FTIR software.

2.4 In vivo Studies

2.4.1 Animals

Male Balb/c mice (5-6 weeks) were procured from the Laboratory Animal House of Atta-ur-Rahman School of Applied Biosciences (ASAB), National University of Sciences and Technology (NUST) for this study. Animals (n = 35) were housed in standard polypropylene cages and kept under the standard laboratory conditions at constant temperature of 25 ± 2 ºC and 60 ± 5% relative humidity with a 12 hours light/dark cycle and had free access to feed and water.

2.4.2 Development of burn mice model

To develop burn mice model, the protocol outlined by (Pereira, Dos Santos et al. 2012) was followed. For this purpose, shortly after period of weaning, the animals were anesthesized with an intramuscular injection of ketamine hydrochloride (90 mg/kg) and xylazine (10 mg/kg) to all mice. The dorsal sides of the mice were shaved with depilatory cream to achieve bare and clean skin. This shaved area was disinfected with cotton swab. Stainless steel rod was immersed in hot water bath (100°C) to heat it up and was then made in contact with shaved area for 10 seconds. Mice were kept back in their cages and were monitored for 4 hours.

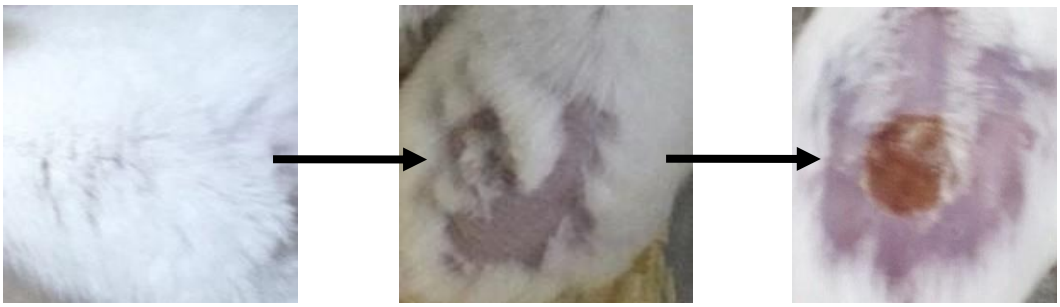

Figure 2.1 Burn Wound Mice Model Steps

2.4.3 Hydrogel preparation

1% of carbopol gel was prepared as a vehicle to incorporate Dexamethasone on loaded transfersomes for transdermal delivery. 1g of carbopol 934G was measured and dissolved in 98ml of distilled water and was kept on constant stirring at 800rpm for 2-3 hours until gel was formed. 10g of NaOH (Sigma Aldrich) was measured and added into 100ml of distilled water to make 10% of NaOH. 2ml of 10% NaOH was added drop wise and was checked with universal pH indicator from Merck Germany to make the pH to 6.5.

2.4.4 Incorporation of DM and DM-NDTs into Gel

Transfersomes containing Dexamethasone were mixed into 1% w/w carbopol hydrogel using an electrical mixture at 25rpm for 5 minutes to get Dexamethasone Transfersomal Gel (DMTG). Similarly, pure dexamethasone drug was mixed into 1% w/w carbopol hydrogel using and electrical mixture at 25 rpm for 5 minutes to get Dexamethasone Gel (DMG) for comparative studies.

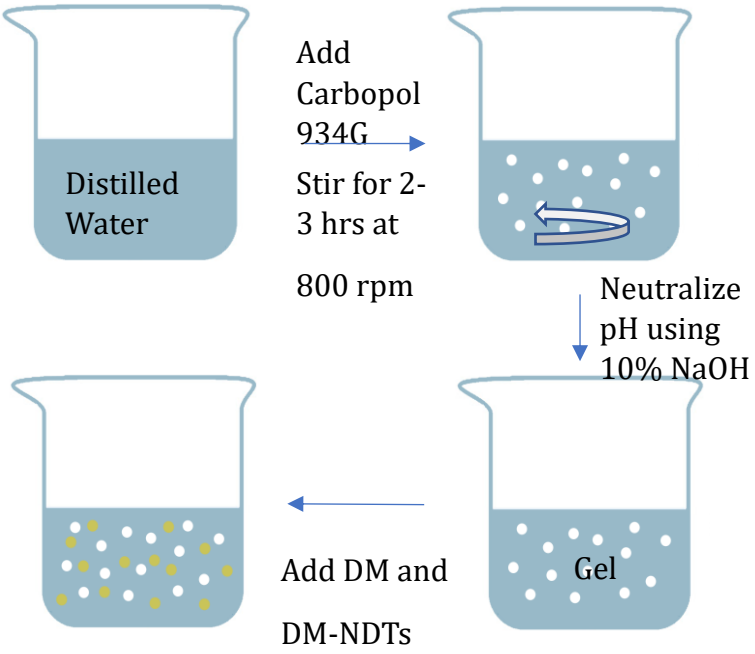

Figure 2.2 Formulation of Carbopol Gel

2.4.5 Gel formulation Treatment

After the development of the burn wound mice model, the animals were divided into four groups, each with 7 animals; control (no burn wounds), negative control (untreated burn wounds), burn wounds treated with DMTG and burn wounds treated with DMG. DM-NDTS (5 mg/kg) dispersed in 1% carbopol gel (Ito, et al., 2008) was applied topically on the wounds to the requisite group 3 and standard DM (5 mg/kg) dispersed in 1% carbopol gel was also applied topically to the required group 4. The treatment spanned a period of 15 days and after which mice were sacrificed.

Figure 2.3 Mice Model Groups

2.4.6 Study Design

Following the methodology detailed by Walter, the burn wound mice model (n = 21) were attained. Gel formulations were applied once daily. Then 3 mice model were randomly selected from each group and sacrificed on fifth day to analyze the pro-inflammatory effect of the drug. The remaining models were sacrificed on 15th day for further assessment.

Figure 2.4 Study Design

2.4.7 Preparation of formalin

10% formalin was prepared for sample storage. 100ml of formaldehyde was added in 900ml distilled water. 4g/L monosodium dihydrogen orthophosphate (NaH2PO4; monobasic) and 6g/L sodium hydrogen phosphate (Na2HP4; dibasic) was added slowly to maintain the pH to neutral.

2.4.8 Sample storage

Chloroform was used to euthanize mice. Collection of blood was done from cardiac perfusion instantly after sacrificing the mice and transmitted into EDTA tubes; to avoid any clotting. Wound skin area was excised from each group and stored in 10% formalin solution and kept at room temperature for histopathology analysis. Whereas, two of the skin samples from each group of day 5 and Day 15 were stored in falcon tubes, each labeled with its defined group name, and was kept at -80 °C in cryo-freeze.

2.5 Pharmacodynamic Studies

2.5.1 Complete blood count (CBC)

Complete blood count (CBC) is test that is used to analyze any fluctuations in blood components which helps to identify diseases and disorders. This test measures the amount of red blood cells, white blood cells, hemoglobin, platelets and many other components present in blood. Hence, blood samples of mice were collected and assessed using MCL-KT-6200 digital hemocytometer analyzer to check the values of granulococytes and monocytes which helps in identifying the inflammation response of body.

2.5.2 Histopathology

The freshly excised wounded skin embedded in 10% formalin for 24 hours. This embedded skin tissue was first dehydrated in alcohol, cleared in xylene and then was fixed in paraffin. Approximately 6 mm sections of wounded skin were cut with a microtome and mounted to a microscopic glass slide. Staining was done with Hematoxylin and Eosin (H&E) in Ali pathology lab, Islamabad and were examined under a Labomed LB-200 Binocular Biological Microscope light microscope in Biochemical lab, SMME, NUST, Islamabad. Images were captured with magnification of 4x and 10x by using Pixel Pro software for a Labomed biological microscope. The histological parameters of skin were determined which included the appearance of unhealed wound, the thickness of epidermis and based on morphological characteristics of cells from H&E staining, inflammatory cell infiltration of neutrophils were determined in the wounded bed. Moreover, the presence of hair follicles and blood vessels in the wound bed were also observed to identify remodeling process.

2.5.3 Wound Healing parameters

Wound healing process was analyzed by observing the physical appearance of the wound like color changing from red to brown, scarring of the wound, re-epithelialization of wounded area, etc.

2.6 Statistical Analysis

The data from CBC were expressed in graphs which were generated using the Prism 5.01 software.

2.7 Ethics Statement

All the experiments carried out in this review were in compliance with the rulings of the Institute of Laboratory Animal Research, Division on Earth and Life Sciences, National Institute of Health, USA (Guide for the Care and Use of Laboratory Animals: Eighth Edition, 2011). This study has the ethical approval of the Internal Review Board (IRB) of ASAB, NUST.

CHAPTER 3

Results and Discussion

3.1 Characterization

3.1.1 UV-Vis Spectrophotometer

UV- Vis spectroscopy was the foremost confirmatory test that was used to verify the synthesis of DM-loaded transfersome nanoparticles. The spectrum range was set UV-Vis 200-400nm where sample gave absorption peak at 258nm. The absorption of dexamethasone occurs at 241 nm (Friedrich, 2009) but due to the presence of transfersomes the peak shifted to the right.

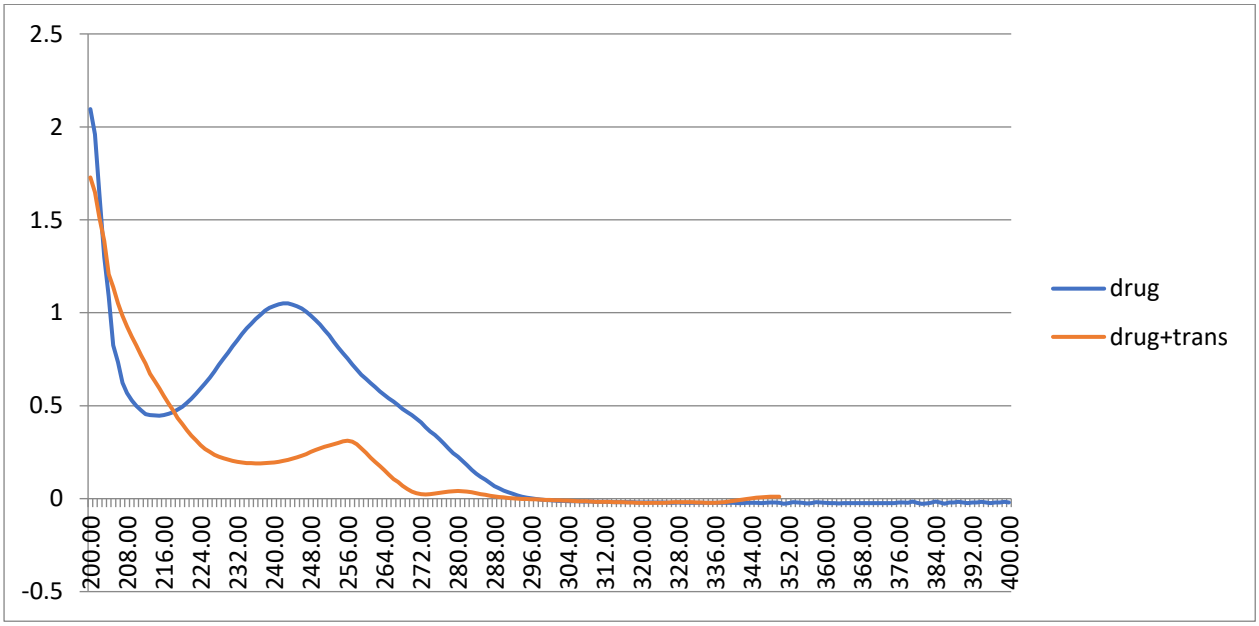

Figure 3.1 UV-Vis absorption spectra of DM (blue) and DM-loaded transfersomes (red)

3.1.2 Entrapment Efficiency

Different concentrations of Dexamethasone were prepared after series of stock solution to plot the standard curve in order to determine concentration of unbound drug in formulation.

Absorbance was recorded at 241nm. The concentrations were plotted against absorbance and the slope was 1.2388.

From this value of slope, using below equation, the concentration of unbound drug present in the supernatant was calculated after centrifuging the DM-loaded transfersomes and observing its absorption.

Lambert-Beer Law equation: A = EcL

Thus, the amount of unbound drug removed from 0.5 mg/ml DM-loaded transfersomes was 0.2351 mg/ml. For calculating the entrapped the equation used was:

$$\textbf{EE (\%)} = \frac{\textbf{Total amount of Drug - amount of Drug in supernatant}}{\textbf{Total amount of Drug}} \times \textbf{100}$$

The amount of DM loaded on transfersomes was calculated out to be 52.96 %.

3.1.3 SEM Analysis

Scanning Electron Microscopy (SEM) is a technique that uses electron beam to produce the image of sample and magnify it which helps to determine the morphology of the sample as well as its size and surface topography data. The size and shape of the nanoparticles were observed. Refer to figure 3.2 below.

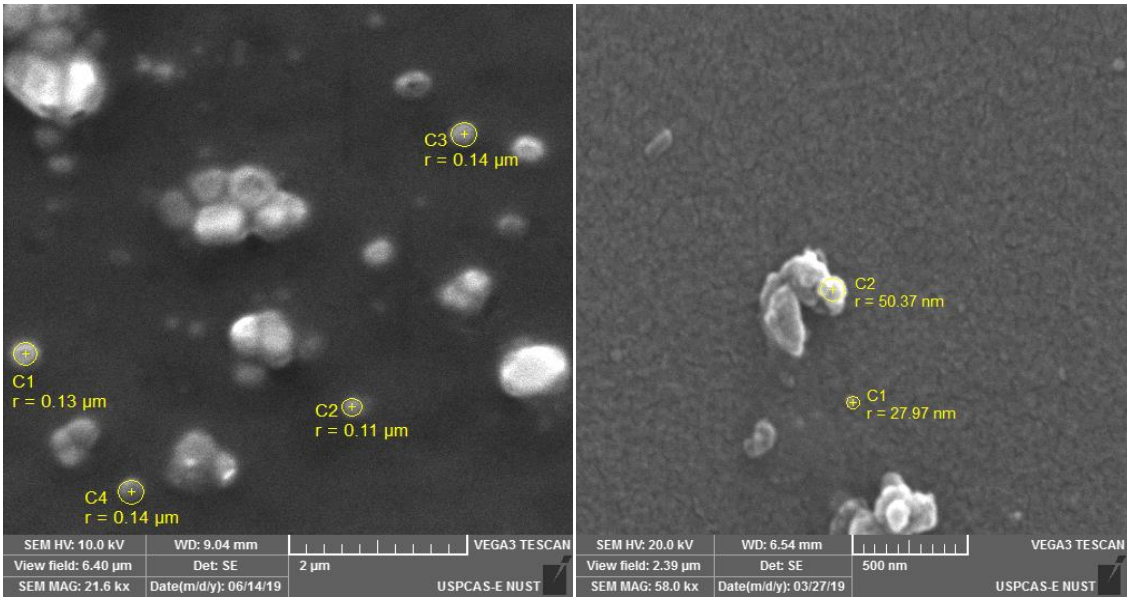

Figure 3.2 SEM Analysis of Blank Transfersomes and DM-loaded Transfersomes

The average size of blank nanoparticles was 260 nm and average size of DM-loaded transfersome nanoparticles was found out to be 78.34 nm. Transfersomes size is usually less than 300 nm (Rai, Pandey et al. 2017). Here the size decreased after loading the drug. This might be because of interdigitation effect of Dexamethasone with transfersomes; means interlocking of drug with vesicle but it does hinder with the structural integrity of vesicles.

3.1.4 EDXS Report

Energy-dispersive X-ray Spectroscopy (EDXS) is an effective technique used to analyze the elements present in the specimen using SEM image. EDXS was used to characterize blank transfersomes and DM-NDTs. Structural formula of DM, transfersomes and Tween 80 are $C_{22}H_{29}FO_5$, $C_{44}H_{84}NO_8P$ and $C_{64}H_{124}O_{26}$ respectively. Figure 3.3a and Figure 3.3b show presence of elements for both blank transfersomes and DM-loaded transfersomes. Whereas, Table 7a and Table 7b show % of elements, respectively.

Element	Weight%	Atomic%
C K	63.77	73.91
O K	23.24	20.22
Na K	2.28	1.38
Si K	1.34	0.67
P K	2.67	1.20
Cl K	6.70	2.63
Totals	100.00	

Table 7a Elements of Blank Transfersomes

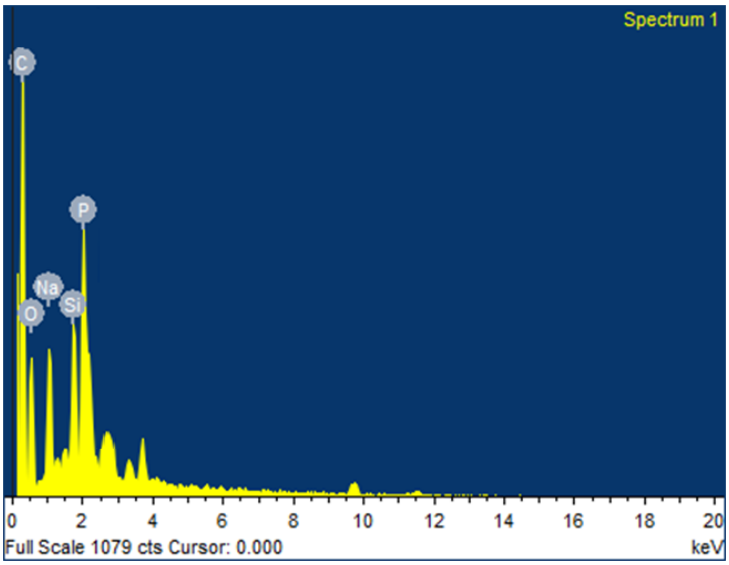

Figure 3.3a EDS of Blank Transfersomes

Element	Weight%	Atomic%
C K	62.03	72.71
O K	17.54	15.43
F K	0.67	0.50
Na K	15.03	9.21
P K	4.73	2.15
Totals	100.00	

Table 7b Elements of DM-loaded Transfersomes

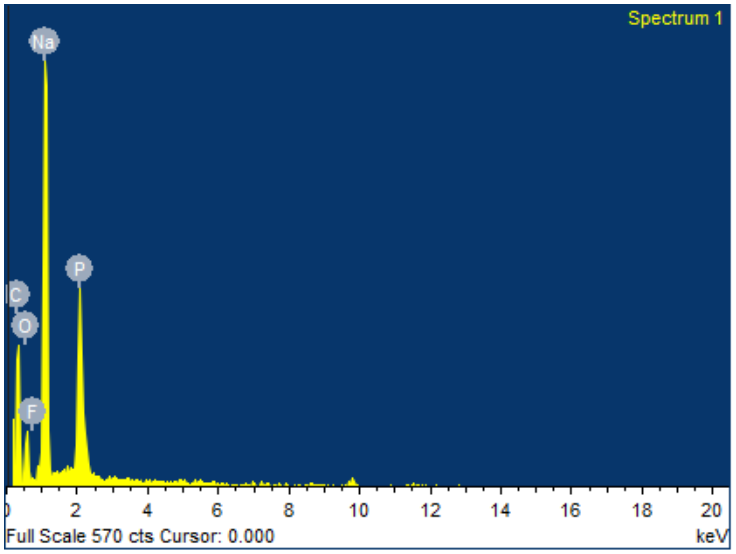

Figure 3.3b EDS of DM-loaded Transfersomes

3.1.5 FTIR Analysis

FTIR is a technique that is used to identify the functional groups or bonds present in pure compounds, mixtures or used for their comparison. The interaction between prepared transfersomes and dexamethasone was studied by using Perkin-Elmer Spectrum-100 spectrocopy at wavelength ranging from 400 cm^{-1} to 4000 cm^{-1} along with blank transfersomes. KBr disc method was used to analyze the samples. The infrared spectra output was recorded in a graph and interpreted to determine the bond stretching in functional groups by using essential FTIR software. Graphs are given in figures 3.4a, 3.4b and 3.4c.

The samples were lyophilized to convert the formulation in powered form and analyzed. Synthesis of transfersomes and successful encapsulation of Dexamethasone on transfersomes was confirmed by FTIR spectra (figure 3.4b). The IR spectra of Dexamethasone showed two characteristic peaks at 1655 cm^{-1}, 858.93 cm^{-1} and 1092.77 cm^{-1} corresponding to C=C, benzene rings and C-F, respectively. This finding is in agreement with previous studies for the IR spectra of DM. It is previously described that Tween 80, which is non-ionic surfactant of ether–ester linkage, is characterized by a sharp spectrum at 1738.17 cm^{-1} (Shan, Chen et al. 2010) which corresponds to surfactant ester groups.

The spectrum of the phospholipid usually showed band at 3428.35 cm^{-1} assigned to OH stretching vibration (Pohle, Gauger et al. 2001). Another characteristic band for the phospholipid at 2355.15 cm^{-1} is because of hydride vibration of phosphine group (P-H) (Coates 2006). After encapsulation of the drug in the transfersome, the characteristic drug C-F bond was only detected in loaded transfersomes. While the hydroxyl was overlapped with the surfactant hydroxyl group at 3428.35 cm^{-1} and ester group at 1738.17cm^{-1}. But hydroxyl (-OH), methyl (-CH3) and carbonyl(C=O) groups peak stretched in loaded transfersomes due to presence of drug. The characteristic long aliphatic chain at 720.21cm^{-1} was detected in all

the transfersomes formulation. Hence, the above finding confirms the encapsulation of the drug by transfersomes. Figure 3.4a and 3.4b show FTIR results and Figure 3.4c shows comparison.

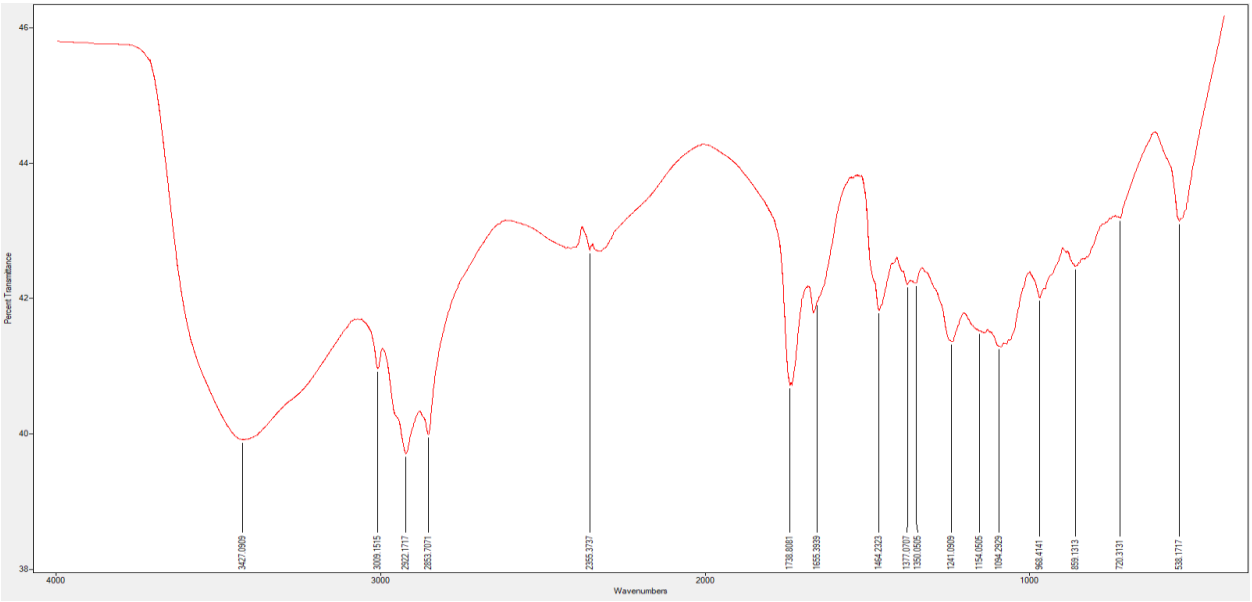

Figure 3.4a FTIR of Blank Transfersomes

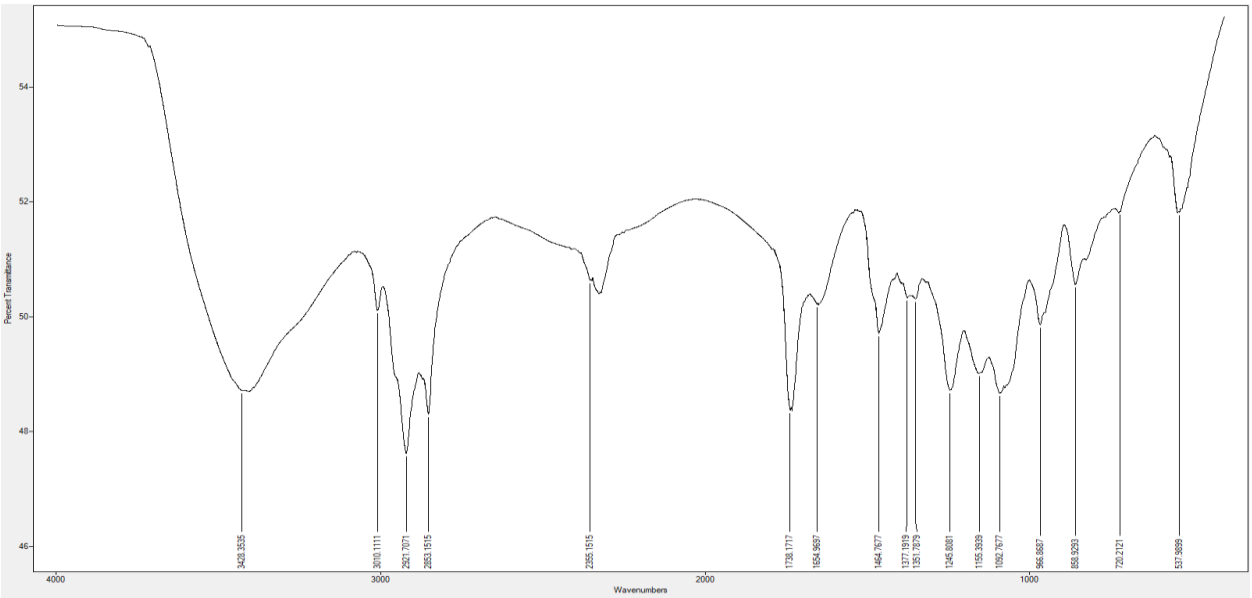

Figure 3.4b FTIR of DM-loaded Transfersomes

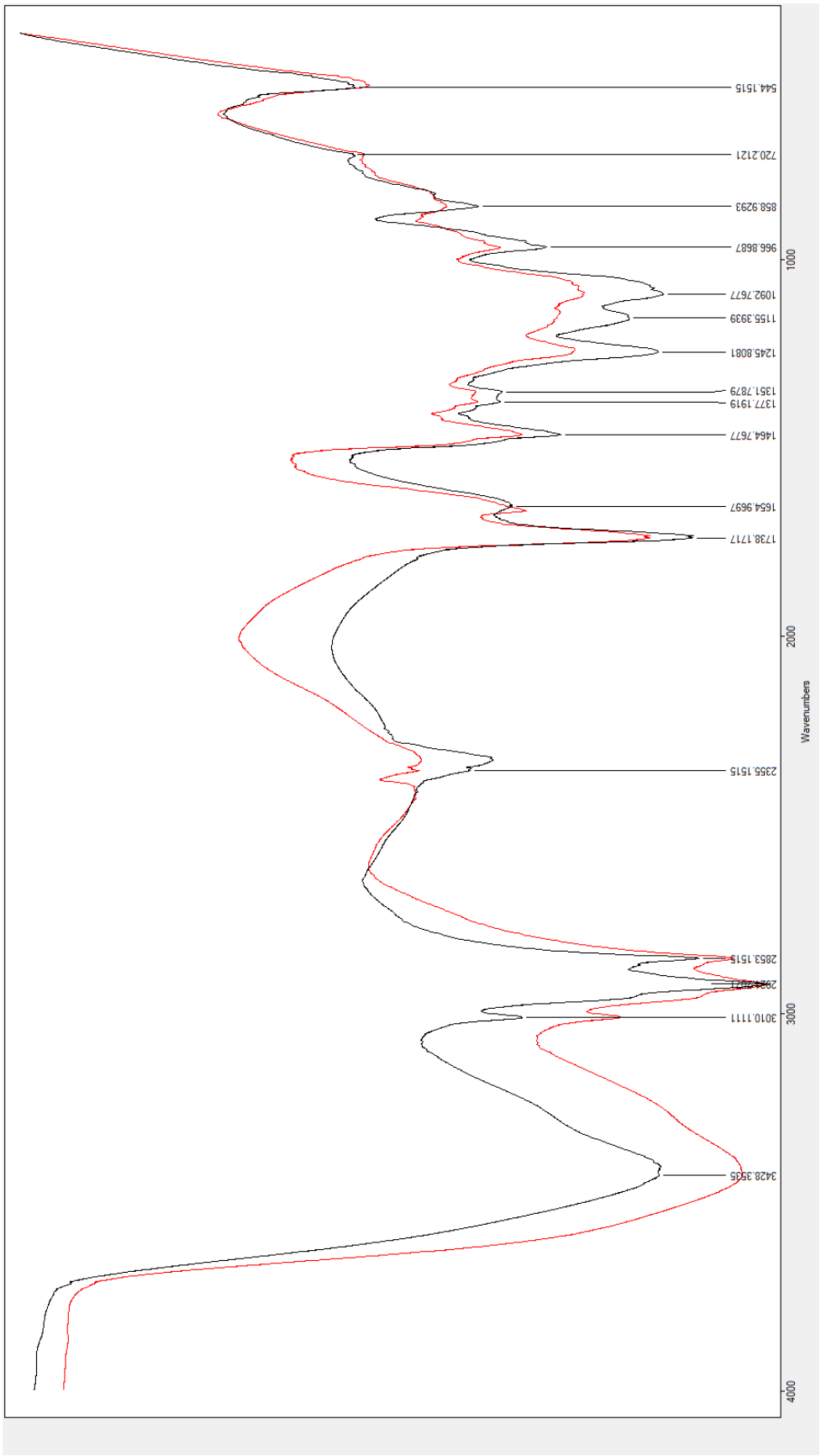

Figure 3.4c Comparison of FTIR Analysis

3.2 Pharmacodynamic Results

3.2.1 DMT reduced number of granulocytes

Complete blood count (CBC) is test that is used to analyze any fluctuations in blood components which helps to identify diseases and disorders. Blood samples of mice were collected and assessed using MCL-KT-6200 digital hemocytometer analyzer to check the values of granulocytes and monocytes which helps in identifying the inflammation response of body. Graphs in Figure 3.5a and Figure 3.5b represent the count of granulocytes and monocytes for 5 days and 15 days respectively.

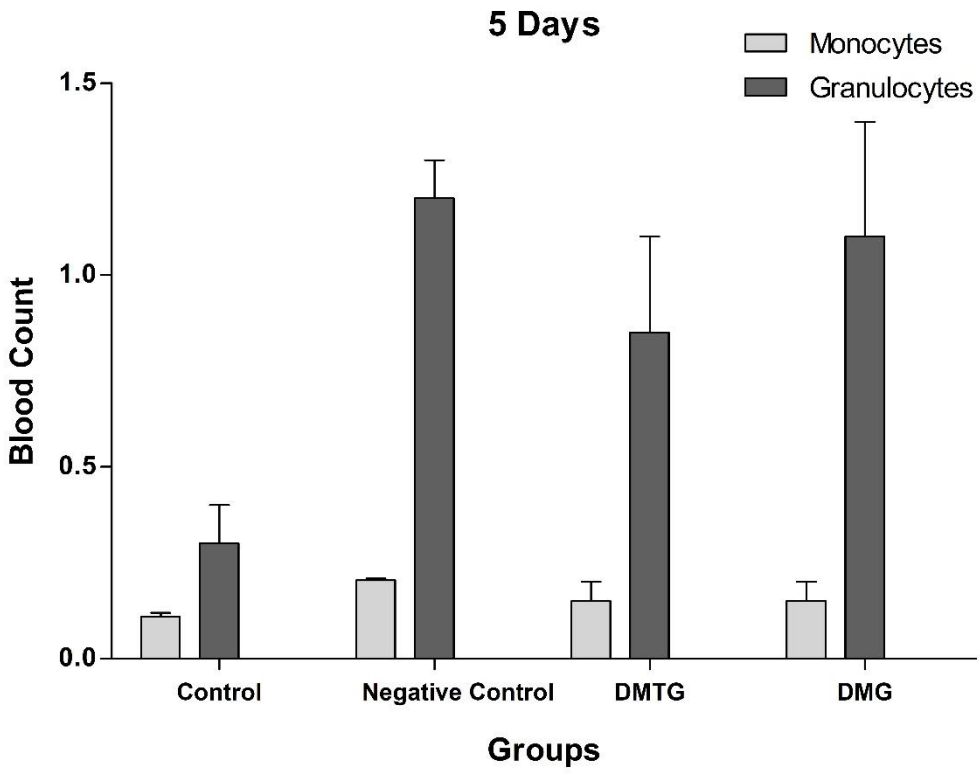

Figure 3.5a CBC Report from 5 Days

Day 5 results showed that the average amount of monocytes i.e. $0.15x10^3$/µl was same in both the treated groups but average amount of granulocytes was different. Group treated with dexamethasone-loaded transfersomes gel (DMTG) had lesser granulocytes ($0.85x10^3$/µl) than group treated with standard dexamethasone gel (DMG) ($1.1x10^3$/µl). Whereas, in negative-

control both the average amount of monocytes (0.2 $x10^3$/µl) and average amount of granulocytes (1.2 $x10^3$/µl) was higher as compared to all the groups. This showed that DMTG was more effective enough to reduce the number of pro-inflammatory granulocytes than the standard dexamethasone gel, as expected. This is due to increased permeability of transfersomes to pass through stratum corneum increasing the bioavailability of the drug.

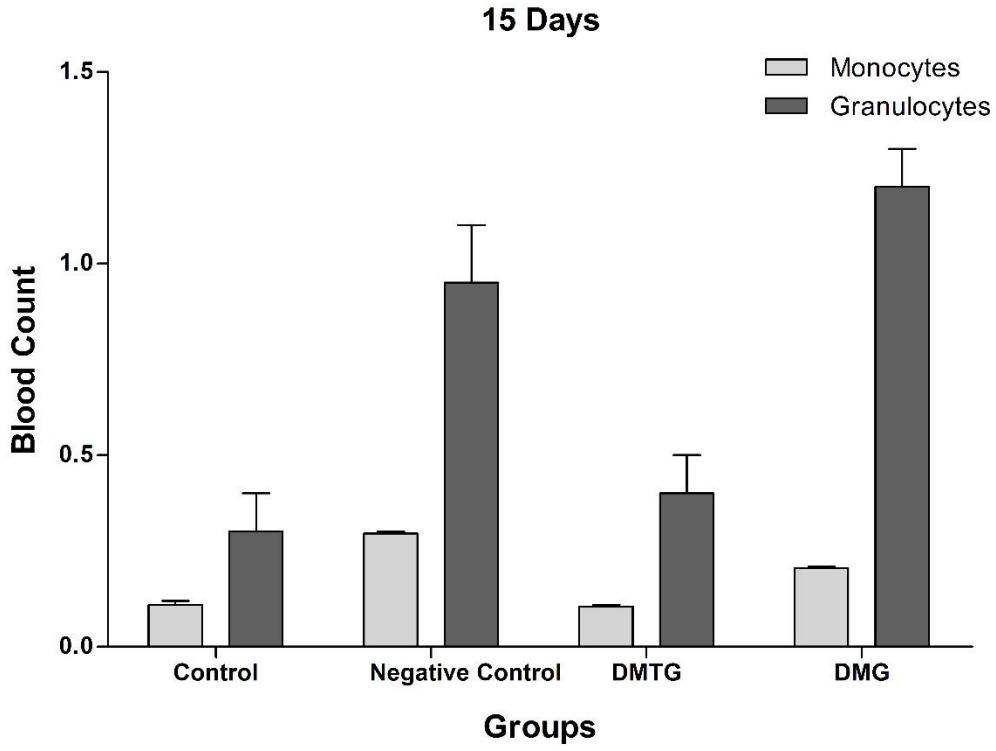

Figure 3.5b CBC Report from 15 Days

Day 15 results showed that the average amount of monocytes and granulocytes of group treated with DMTG was 0.1$x10^3$/µl and 0.4 $x10^3$/µl, respectively whereas group treated with DMT showed 0.2$x10^3$/µl and 1.2 $x10^3$/µl. Group treated with DMTG had lesser number of monocytes and granulocytes than group treated with DMG. But group treated with DMG showed higher amount of granulocytes than the negative-control. This may be due permeability of standard drug to enter the wounded area excessively at a time that instead of suppressing the inflammation phase it blocked it for a moment which let the microbes enter

into blood circulation. This might have increased the infection and released inflammatory cells when drug wasn't available. (Avadhani, Manikkath et al. 2017).

3.2.1 DMT reduced neutrophil infiltration

The freshly excised wounded skin dipped in 10% formalin was prepared and observe under Labomed LB-200 Binocular Biological Microscope light microscope. Images were captured with magnification of 4x by using Pixel Pro software for a Labomed biological microscope. The histological parameters of skin were determined and compared.

Histology results from 5 days showed decreased neutrophil infiltration in Dexamethasone-loaded transfersomes formulated gel as compared to dexamethasone gel. Figure 3.6 shows comparison between histology results of groups. The arrows show the disoriented stratum corneum and epidermis with hypodermis having no vessels due to burn wound and stars represents neutrophil infiltration.

After burn injury, macrophages migrate towards the wounded area and initiate inflammatory phase by releasing a number of cytokines and growth factors (Gosain and DiPietro 2004, Campos, Groth et al. 2008) Since, approximately first 5 days are of inflammatory phase, which are very critical, causes neutrophil accumulation in wounded area for re-epithelialization. Images showed normal number of neutrophils in control with intact stratum corneum and epidermis along with presence of vessels. Whereas, distorted SC and epidermis, and absence of vessels were present in other groups. Excessive neutrophil accumulation was found in negative control. Less amount of neutrophil infiltration was in formulated gel than standard drug treatment.

Histology results from 15 days also showed decreased neutrophil infiltration in Dexmethasone-loaded transfersomes formulated gel as compared to standard dexamethasone gel. Figure 3.6 shows comparison between histology results of groups. The arrows show the

formation stratum corneum and epidermis with hypodermis having vessels in group treated with DMTG and reduced neutrophil infiltration as compared to other groups. In negative control SC isn't fully intact and there still a lot of neutrophil accumulation. In DMT treatment group epidermis is thick showing that it is still in formation whereas DMTG treatment group shows thin epidermis. When and injury occurs, epidermis thickens due to proliferation and comes back to its original thickness when it heals (Kurman and Argyris 1975).

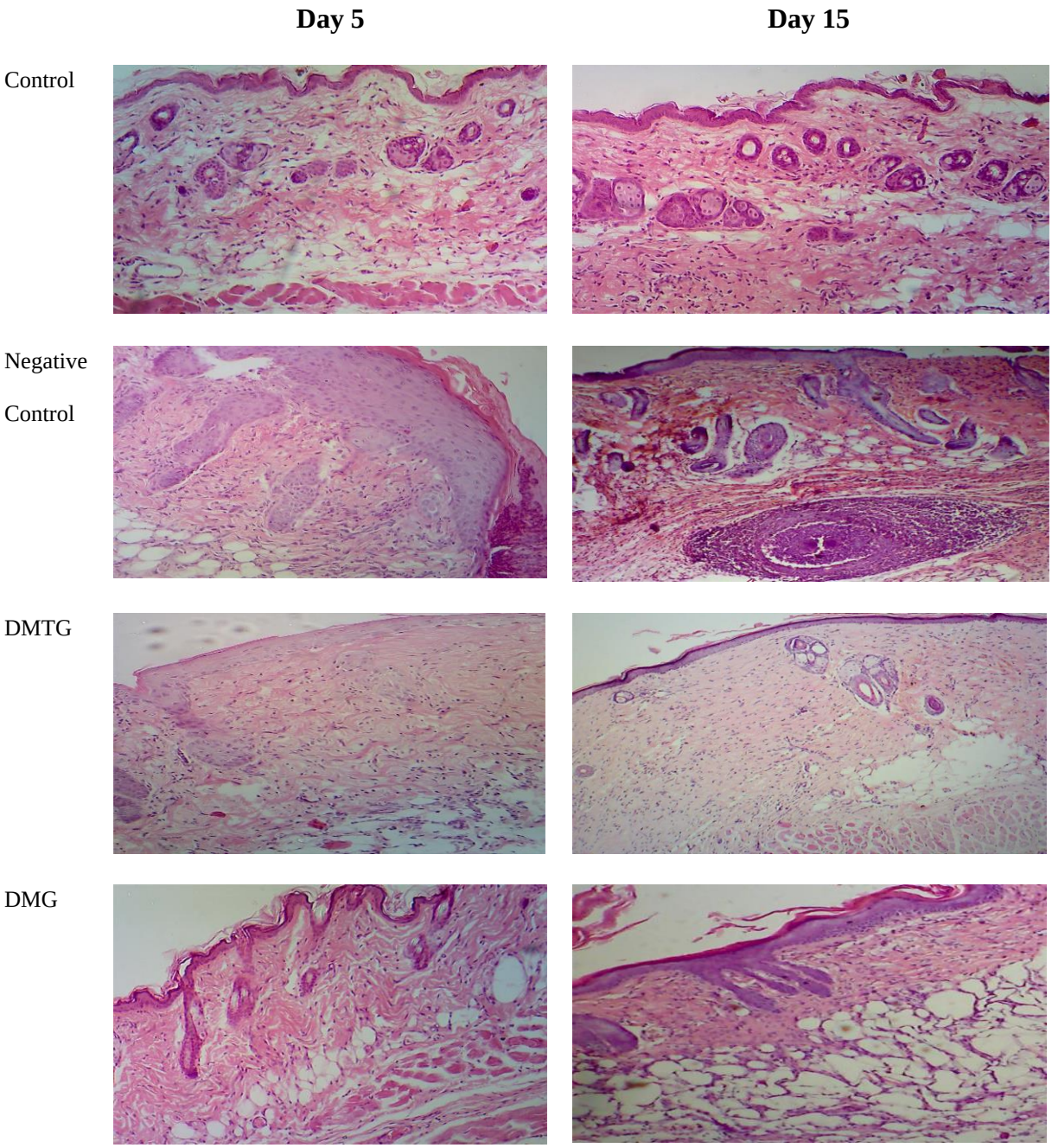

Figure 3.6 Histology of Wounded Skin for Day 5 and Day 15

3.2.3 DMT improved Wound Healing Process

Wound healing process was analyzed by observing the physical appearance of the wound like color changing from red to brown, scarring of the wound, re-epithelialization of wounded area, etc

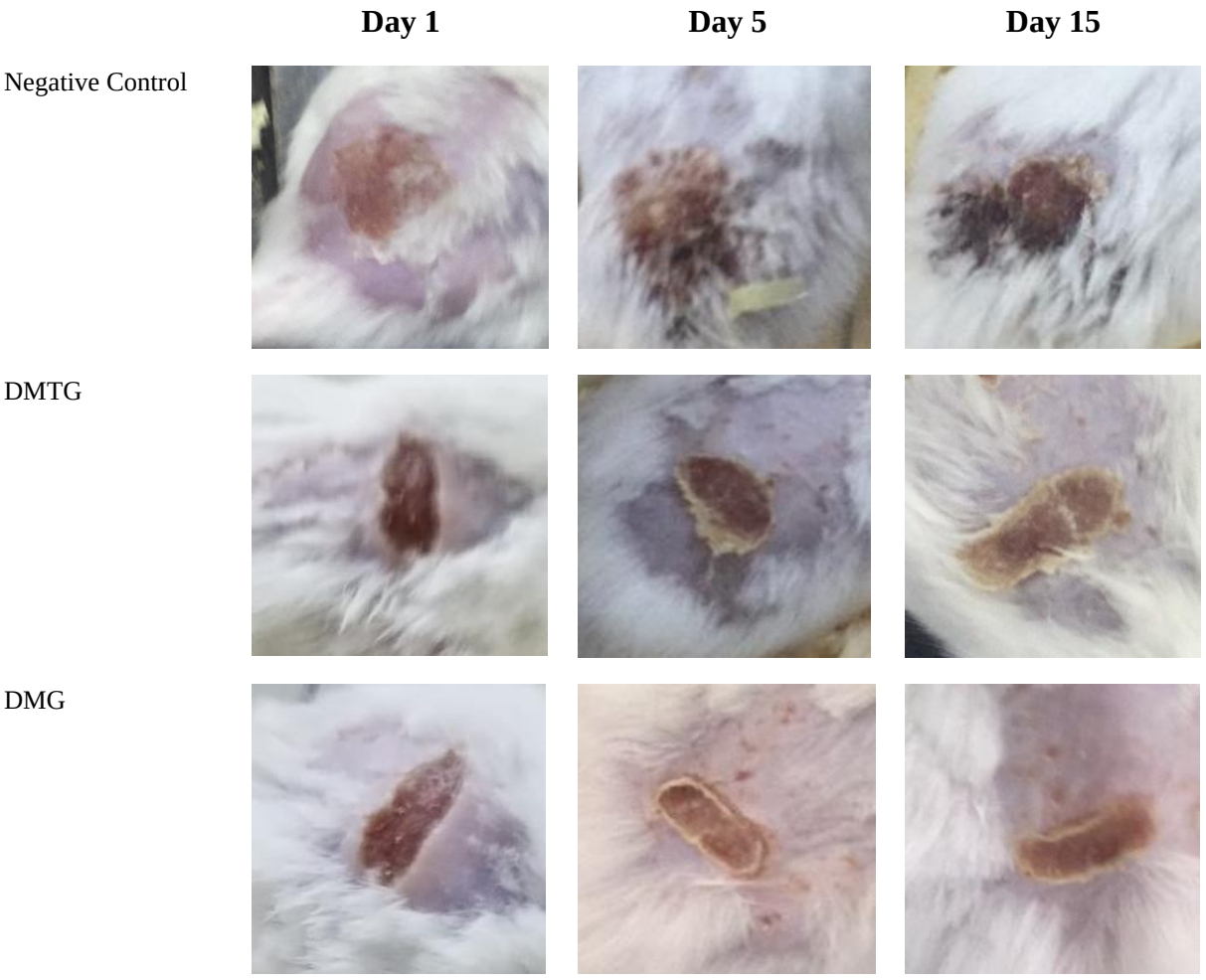

Figure 3.7 Wound Healing Analysis

In negative control, the condition of wound got worse as seen in Figure 3.7 whereas in other treated groups the wound healed to some extent. Group treated with Dexamethasone-loaded transfersomes showed better results than standard drug as there was no redness and scalp was fully developed as compared to Group 4.

3.3 Discussion

In the present study, the anti-inflammatory activity of Dexamethasone was evaluated topically in burn wound mice model as mentioned in the previous section. Suitable transfersomal carrier systems were formulated for transdermal delivery of drug, i.e. Dexamethasone in order to assess the anti-inflammatory efficacy of the prepared test formulation in induced wounded mice. The results of the test formulated gel were compared with the results obtained from control and reference treated groups. In order to attain desired therapeutic efficacy of the model drug with lesser side effects, the transfersomal vesicular system was chosen to design and prepare a test formulation. Owing to both hydrophilic and lipophilic properties, transfersomes possess better skin penetration strength than that of classical liposomes and other nano-vesicular systems (Duangjit, Opanasopit et al. 2011). It is evident from the in vivo study of prepared transfersomes which showed a significant positive results of Dexamethasone-loaded transfersomes when compared with the Dexamethasone gel. This could be attributed due to the presence of phosphatidyl choline (lipid) and tween 80 (surfactant) in the vascular system, which provides sufficient structural flexibility to the transfersomal system. In addition, tween 80 being an edge activating agent destabilizes the lipid bilayers (without affecting the integrity) of the vesicles and hence increases their deformability (Steinberg 2012). Moreover, phosphatidyl choline itself acts as a penetration enhancer which might be due to the fact that it brings some conformational changes like alteration of intracellular lipid lamellae in the stratum corneum layer of skin apart from its moisturizing and soothing properties. In contrast, tween 80 imparting softness and flexibility to the vesicles. At the same time, tween 80 reduces the irritation effect (Fiedler 1996). The thin film hydration technique used in the preparation of lipid vesicles was well optimized for lipid (phosphatidylcholine)–surfactant (tween 80) in the ratio as 5:1, which provided desirable deformability as well as flexibility to the lipid vesicles. In this technique, lipid film

was converted into the spherical vesicles by hydration, which determines the vesicle size and the entrapment efficacy of the formulation. A slight difference in vesicle size between drug-loaded and blank transfersomes was observed, which might be due to the scare step wherein Dexamethasone was encapsulated in vesicles. Dexamethasone having both a polar and a lipophilic part, produces an interdigitation effect, which did not affect the basic structural integrity of vesicles (Caruso, Fedele et al. 2009). This novel elastic carrier is stable without the addition of preservative, which is probably due to the presence of ethanol in the formulation which prevents the microbial growth induced instability (Oh and Marshall 1993). Dexamethasone are also having some antibacterial and antifungal properties which may retard the growth of the microorganisms (Neher, Arnitz et al. 2008). Vesicle size generally affects the penetration property of vesicles across the skin; smaller is the vesicle size, the higher is the cumulative concentration of drug at the site of action, which ultimately brings the desired anti-inflammatory response. In our present investigation, transfersomes prepared were of nano-sized range and possess, therefore, superior penetrating/permeation characteristics over conventional Dexamethasone gel. In test and reference formulations, the therapeutic concentration of dexamethasone was same, which does not raise any question about the therapeutic potential of Dexamethasone. Furthermore, the flexible characteristics of transfersomes (less viscous) allow them to pass easily through the pores of skin, which in turn supports their better anti-inflammatory action than reference gel, a highly viscous system. Burn wounds were induced in all experimental mice and in a control group of animals, which sustained till the end of the study. Burn wounds injured dermal tissue, releasing several inflammatory mediators such as cytokines. It is assumed that reduction in inflammation was brought about after dexamethasone-loaded transfersomes treatment which may be due to inhibition of release of such pro-inflammatory mediators from affected tissues (Abraham, Lawrence et al. 2006). Based on the above facts, transfersomal formulation may be

considered as suitable for the topical delivery of dexamethasone in burn wounds because of its better skin penetrability and efficacy than dexamethasone gel. This must be an approach to improve the patient's compliance of drug delivery system intended for topical administration for the symptomatic relief of inflammation.

3.4 Conclusion

In this study, Dexamethasone-loaded transfersomes nanoparticles were formulated and characterized and its anti-inflammatory activity was evaluated topically on burn wound mice model. The results of the prepared nano deformable transfersomal formulation loaded with Dexamethasone were compared with the Dexamethasone gel formulation containing the same concentration, with the aim to make a comparative assessment of the anti-inflammatory activities of both the formulations. It was revealed that our prepared formulation exhibits better anti-inflammatory activity than using standard Dexamethasone gel formulation. This could probably be due to the increased penetrability of our specially designed vesicular delivery system Dexamethasone transfersomal gel across the skin compared to conventional Dexamethasone gel formulation. Thus, this ultimately leads to the desired concentration of the active drug at the target site and thereby exerting anti-inflammatory efficacy at a satisfactory level which makes it a good candidate for the treatment of burn wounds.

However, further comprehensive biological and clinical studies are needed to be carried out with sizeable sample size in order to validate such novel drug delivery system of Dexamethasone as a potential topical anti-inflammatory therapy.

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