Freie Universität Berlin

Dermal delivery of active agents with polyglycerol-based nanocarriers

DISSERTATION

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by
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from Salams, Iran

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Declaration of honesty

Hereby, I declare and confirm the authenticity of my PhD thesis. It is the result of my own research work and also collaboration with other research groups; any other sources than those cited have not been used.

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Fatemeh Zabihi

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List of abbreviations

hPG	Hyperbranched polyglycerol
m-THPC	Temoporfin
TAC	Tacrolimus
SC	Stratum corneum
AD	Atopic dermatitis
PTDs	Protein transduction domains
SLN	Solid lipid nanoparticle
NLC	Nanostructured lipid carrier
TPGS	D-α-Tocopheryl polyethylene glycol 1000 succinate
MPEG-dihex-PLA	Methoxypoly(ethylene glycol)-dihexyl-substituted polylactide
ODMSs	Oligodimethylsiloxanes
G	Generation
PAMAM	Polyamidoamine
5FU	5-Fluorouracil
Cat	Catalyst
CMS	Core-multishell
PEG	Polyethylene glycol
PNIPAM	Poly(N-isopropylacrylamide)
LCST	Lower critical solution temperature
NHK	Normal human keratinocytes
MPEG-dihex-PLA	Aneuploid immortal keratinocyte cell line from adult human skin
mPEG	Methyl poly(ethylenglycol)
$Sn(Oct)_2$	Tin(II) 2-ethylhexanoate

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1 Introduction

Biomedical applications of polymers including drug delivery,[1] gene delivery,[2] bioimaging,[3] stem-cell differentiation,[4] tissue engineering,[5] regenerative medicine,[6] and antimicrobial activity[7] became very popular due to their versatility and unique physicochemical and biological properties. Drug delivery systems, however, have been at the forefront of almost every biomedical application of polymers. Type and structure of drug delivery systems significantly depend on the targeted organ. Skin is the heaviest single organ of the body, and in combination with the mucosal linings of the respiratory, digestive, and urinogenital tracts forms a protective structure to conserve the internal parts of body against the external environment. Furthermore, skin is an alternative route for drug administration, when it is the target organ or a route to target other organs inside the body.[8] Accordingly, transdermal and topical drug delivery systems have attracted a high amount of attention in the last several decades. Transdermal delivery is the diffusion of an agent through different layers of skin into the systemic circulation, for a special therapeutic effect,[9] e.g., treatment of withdrawal symptoms by administration of nicotine.[10] Dermal or topical delivery, on the other hand, targets a pathological site inside the skin by therapeutic agents with a minimum of side effects,.[11] The main goal of dermal delivery is localization of drug molecules in the targeted site to improve their therapeutic efficiency and treatment variety of skin diseases such as skin cancer,[12] psoriasis,[13] eczema,[14] and microbial infections.[15] Skin penetration of drugs strongly depends on their molecular weight and hydrophobicity. Therapeutic agents with molecular weights more than 500 Da and high hydrophobicity do not efficiently overcome the skin barrier. For example, tacrolimus with 804 g/mol molecular weight and high hydrophobicity shows a very low penetration into the skin barrier, stratum corneum.[16] In addition, localization of drugs in the therapeutic level in the target site depends on the efficiency of dermal delivery system. There are several roles for dermal delivery systems including: (i) penetration enhancer,[17] (ii) stimuli[18] or controlled release[19] of cargo, (iii) targeting a special site of skin,[13b], and (iv) therapeutic effect[20] in addition to delivery.

A variety of polymers have been used as dermal delivery systems in the past two decades and their efficiency to improve the therapeutic efficiency of different drugs is being investigated. Polymers are interesting for dermal delivery because they show a high versatility in terms of structure, which in turn affect their diffusion into the skin, loading capacity, immunogenicity, biocompatibility, and degradability. Hyperbranched polyglycerol (hPG) is polyether polyol, which is especially good for topical drug delivery due to its hydrophilicity, biocompatibility, and high functionality.[21] Different derivatives of polyglycerol in the form of

core-multishell nanocarriers,[22] nanogels,[23] and stimuli-responsive carriers have been used for intradermal drug delivery and promising results in each case have been obtained. However, non-specific loading of therapeutic agents by hPG derivatives and non-biodegradability of hPG nanocarriers are two challenging issues that hamper their further development as dermal delivery systems. Therefore, the main goal of this research work was to synthesize new biodegradable hPG derivatives with the ability of specific and efficient loading of two skin-related therapeutic agents including temoporfin (*m*-THPC) and tacrolimus (TAC). Other useful characteristics of polyglycerol such as biocompatibility, hydrophilicity, low toxicity, and small sizes should be improved or preserved. Investigation of the efficiency of the synthesized hPG derivatives for the dermal delivery of these therapeutic agents was another topic of the present study.

1.1 Skin structure

Skin is the largest organ in body in terms of surface area and weight. In an adult male the average surface area of the skin is almost 2 m^2.[24] It covers the entire body and consists of three main layers, which are called epidermis, dermis, and hypodermis (Figure 1). Each layer has its own metabolism and a lot of functions for body. Epidermis is the outermost layer of the skin and subdivided to two different layers, stratum corneum and viable epidermis. The stratum corneum is the main barrier of skin and is the most difficult part for penetration of foreign objects. It is composed of dead keratinocytes within a heavily packed lipid matrix. Below the stratum corneum is viable epidermis. Viable epidermis, based on keratinocyte differentiation steps, is divided into three different sublayers: *stratum basale, stratum spinisum,* and *stratum granulusom*. The thickness of viable epidermis is much higher than the stratum corneum. Thus, diffusion of therapeutic agents in this part is more efficient than stratum corneum, which is due to its higher degree of hydration.

Directly beneath the epidermis is the dermis layer of skin. This layer has many biological functions such as producing sweat and oil, growing hair, feeling, and regulating the body's temperature. There are blood vessels, nerve endings, oil and sweat glands, as well as hair follicles. The dermis layer also plays a critical role in the firmness and strength of skin due to its containing collagen and elastic tissue. Under the dermis layer is a hypodermis layer, which provides a fat storage for the body.[25]

1.1.1 Epidermis

Epidermis is made of several keratinocyte-corneocyte cell layers with different functions and structures. The outermost layer of epidermis is called the stratum corneum. It is composed of dead corneocytes that are fully keratinized and assemble in 10-25 layers with 0.01-0.02 mm thickness.[25] Dead corneocytes are surrounded by extracellular lipids similar to bricks that are built up by mortar.[26] It is worth noting that the lipids of extracellular matrix of the stratum corneum are not similar or the same as those in other biological membranes. Lipid matrix of SC consist of ceramides, free fatty acids, and cholesterol.[27] The ceramide macromolecules are composed of fatty acids linked to sphingoid bases by amide bonds.

The structure and thickness of the outer part of the epidermis, the size of corneocytes, and the composition of lipids have a high effect on the barrier function and less importantly on regenerative properties of the skin, which in turn contribute to a variety of dermatological diseases and the healing process of skin. Organs that are more exposed to external forces and medium are surrounded by a thicker epidermis layer.[28] Organs with a thin protective outer layer of epidermis, for example, the face are highly susceptible to external factors and could be damaged by different elements.[28b]

Intercellular lipids of the stratum corneum are produced by changing their precursors and provided by multilamellar Odland corpuscles of the granular layer.[29] Tightly packed lamellas are in the same direction with epidermal cells produced by hydrolysis of glycolipids ceramides, metabolizing phospholipids in fatty acids, as well as hydrolyzing polar lipids to non-polar analogs.[26, 30] A variety of intercellular lipids including ceramides, cholesterol, fatty acids, and lipids with alkyl chains can be sub-classified into other categories.[29, 31] For example, ceramides are classified in twelve subcategories marked 1 to 12 depending on the chemical structure of tails or main chains.[32] The most important ceramides and fatty acids are those with C24-C26 and C22-C24 alkyl chains, respectively. Cholesterol sulfate is another part of this matrix, which inhibits enzymatic degradation of connections between the epidermal cells.[31]

Other sublayers of the epidermis include the *stratum granulosum*, *stratum spinosum,* and *stratum basal*. The combination of these sublayers with a thickness of around 0.05–0.1 mm is called viable epidermis. The viable epidermis contains various types of enzymes such as esterases, phosphatases, proteases, and lipases, which show a high metabolic activity and impact the bioavailability of delivered therapeutic agents.

The barrier function and biological properties of epidermis as the protective layer of skin depend on the (i) composition and (ii) integrity of this layer.

One of the most important compositions of the epidermis layer that regulates diffusion of foreign materials from the skin's surface is the lipid construction of this layer.[25]

1.1.2 Dermis

The next layer after the epidermis is the dermis which is mainly composed from a thick layer of flexible fibrous proteins including fibrillin, collagen, and a small but important component of elastin[33]. The dermis improves the mechanical properties of skin and causes a high flexibility and strength.[34] It contains blood vessels, sweat glands, oil (sebaceous) glands, *arrector pili* muscle, pacinian corpuscles, Meissner's corpuscles, hair follicles, and nerve endings[35] (Figure 1). Due to the presence of the above-mentioned systems, the dermis is an active part of the skin and involved in the immune response, sensing, healing, thermal regulation, excretion, antibacterial activity, and many other functions.[36] The nerve endings are responsible for sensing different forces and factors including pain, touch, pressure, and temperature.[37] Depending on the organ and function of skin, some areas of the skin have more nerve endings than other parts. For example, the fingertips and toes contain a large number of nerve endings and are very sensitive to external factors.[38] The sweat glands could be stimulated by external factors such as temperature and stress. Stimulation of sweat glands by these factors result in perspiration through which water, salt, and other chemicals can be exerted from the body. Evaporation of sweat acts as a cooler and decrease the body temperature.[39] Some of the sweat glands, especially in organs such as armpits and the genital region, produce an oily compound, which causes the body odor upon digestion by bacteria of the skin in those areas. Sebum, which is an oil and keeps skin soft and wet, is secreted by sebaceous glands into hair follicles. Sebum protects skin from bacterial and fungal infections. The hair follicles are responsible for the production of hair on the surface of skin. It is also a place that stem cells can be found.[40] Hair helps to regulate the temperature and improve the mechanical properties and sensation of skin.[41] The main function of blood vessels in the dermis layer is to provide nutrients and regulate the body temperature.[42] Different parts of the dermis obtain their nutrients and energy from the blood stream inside the skin blood vessels. These blood vessels can also enlarge and increase circulation blood close to the surface of skin for a heat transfer or they can contract to keep the heat inside the body.

1.1.3 Subcutaneous fat

The deeper layer of skin is subcutaneous, which lies under the dermis,[43] and contains a layer of fat which mainly functions as insulation of the body from heat and cold.[44] Therefore, the subcutaneous layer has a crucial role in energy and heat storage for the body. The thickness of this layer varies from less than an inch up to several inches depending on the area and function of the covered organ.[45]

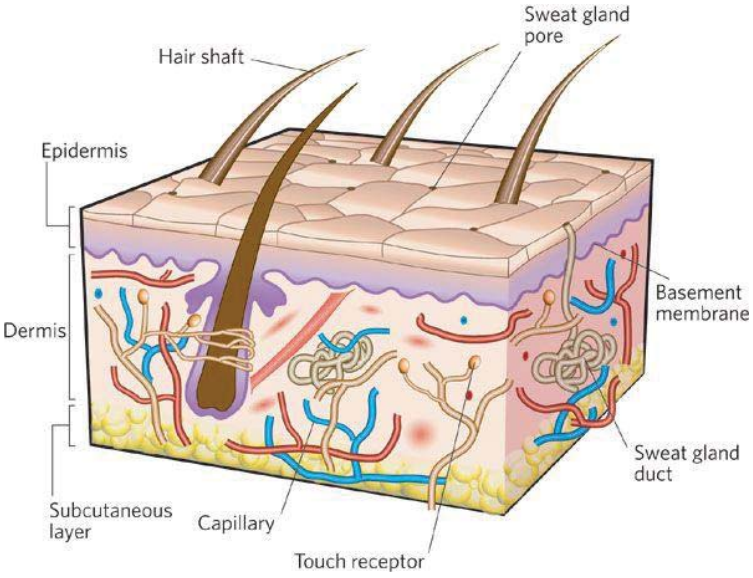


Figure 1. Skin consists of two different layers, epidermis and dermis. A fatty layer underlying the dermis acts as a foundation for this layer. The structure of the dermal layer of skin includes blood vessels, sweat glands, oil (sebaceous) glands, *arrector pili* muscle, pacinian corpuscles, Meissner's corpuscles, hair follicles, and nerve endings. Reprinted with the permission of ref [35].

1.2 Skin function and physiological properties

The most important functions of skin include (a) a barrier of body against toxic materials, (b) protection of body against external forces, (c) sensation by transmitting information of environment to the brain, (d) regulation of the temperature of body, (e) excretion of wastes such as sweat from body, (f) function as a part of the immune system, and (g) endocrine function. Some of these functions will be discussed below.

1.2.1 Barrier of body against different objects

The most outer layer of skin, epidermis, is the protective part of skin with defensive functions. The most important part of skin to regulate these functions is the stratum corneum.[46] The stratum corneum (SC) regulates the permeability of skin and retards transcutaneous evaporative water loss. The main components of SC are nucleate corneocytes that are embedded in multiple planar lamellae sheets and are enriched in ceramides, cholesterol, and free fatty acids.[47] The

hydrophobicity of these components in extracellular matrix of SC enables this layer to inhibit outward leaking of water. This part of skin regulates the digestion of corneodemosomes and transfers intracellular junctions, which are degraded and allow corneocytes to shed at the skin's surface.[48]

Antimicrobial peptides in this part are responsible to deactivate microbes, which diffuse into the skin.[49] The barrier function of skin depends on the integrity of epidermis, and the defective permeability of this part leads to different diseases such as atopic dermatitis (AD).[50]

1.2.2 Excretion of some materials from body

As the body's largest waste removal system, skin is the main way of excreting most of toxins and waste materials through its sweat glands and pores. It is estimated that three to four million eccrine sweat glands exist in the human skin and their weight is almost the same as the kidney (around 100 g). This large amount of sweat glands enable us to perspire several liters per hour through which many waste chemicals can be excreted from the body.[51] Water soluble chemical such as drugs,[52] metals,[51] cytokines,[53] and steroids[54] can be eliminated from the body with sweat. Interestingly, some of xenobiotics, which can rarely be excreted by kidney without being metabolized, can be eliminated in sweat.[55] For example, an excess of nicotinamide cannot be eliminated through urine but can be efficiently eliminated through the sweat glands [56]

1.3 Topical drug delivery

In addition to all skin's properties and functions, skin offers a convenient site for administrating therapeutic agents. The topical delivery is a method in which formulations are applied to the superficial areas including skin, eyes, nose, and vagina for the treatment of local diseases.[57] The advantages of the topical drug delivery are listed below[58]:

i) Compliance and acceptance by patients

ii) Application of formulations in topical delivery is easy and convenient

iii) It is painless and noninvasive

iv) It improves the bioavailability of therapeutic agents

v) Immunogenicity of this method is lower than for other methods

vi) Side effects and toxicity of this method are relatively low.

If drugs and other reagents transport through the skin to the circulation system of the body, this process is called “transdermal drug delivery.” Targeting a site of skin for a special therapy by different therapeutic agent is called “dermal drug delivery.” However, diffusion of most of drugs with high molecular weights (> 500 Da) and high hydrophobicity into the skin is not sufficient and an efficient therapeutic effect cannot be observed. Over the past 30 years, many research groups have tried to overcome challenges associated with the skin delivery. However, the growth of technologies for practical administrations has been relatively slow. One of the main procedures to overcome these problems is to use topical delivery systems to increase the skin penetration of therapeutic agents, which lead to more effective therapies.[59] A topical delivery system is defined as an object that carries a therapeutic agent into contact with and through the skin.

There are three main pathways for topical delivery of therapeutic agents: (i) appendageal, (ii) intracellular and (iii) intercellular routes.[60] (Figure 2) The appendageal route includes hair follicles, sweat glands, and skin furrows. This pathway makes continuous channels for the penetration of therapeutic agents but it is hindered by hair follicles and sweat ducts. The intracellular route is penetration through the cell membranes, and intercellular pathway involves a path between the epidermal cells. The intracellular pathway is a direct route to the dermis and it occurs through the keratinocytes and lipids. The intercellular route is the most common way for skin penetration of therapeutic agents and in this pathways drugs remain in the lipid interfaces. The hydrophobicity of lipids helps to solubilize drugs and improve penetration.[61]

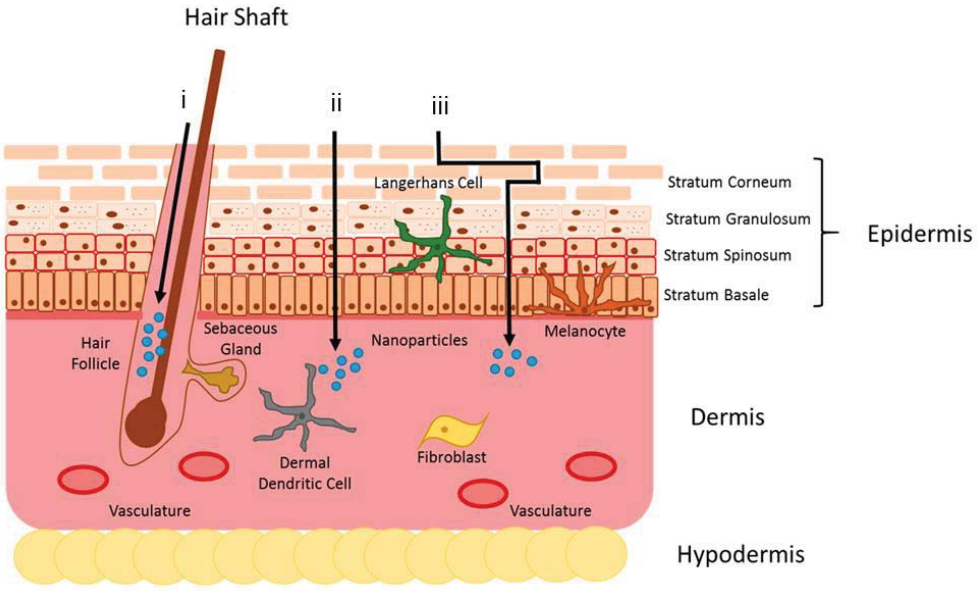


Figure 2. Different pathways for skin penetration of therapeutic agents (i) appendageal, (ii) intracellular and (iii) intercellular routes. Reprinted with the permission of ref [60].

1.3.1 Dermal delivery systems

Disorders of skin affect millions of people around the world daily. These diseases can be categorized into bacterial (impetigo, cellulitis), fungal (sporotrichosis, chronomychosis, blastomycosis), viral (herpes simplex virus, eczema), and autoimmune (scleroderma, psoriasis).[62] Since one of the functions of skin is to be a barrier against external forces, skin plays a key role in protecting body against foreign agents. Therefore it hampers or at least limits penetration of drugs into and through this organ.[63] For therapeutic agents that passively deliver to the skin should have molecular weights lower than 500 Da and adequate lipophilicity.[64] Different strategies are used to improve the delivery into or through the skin, which can be classified into "passive" and "active" methods.[63] Active methods are usually physical or mechanical procedures to enhance transferring a drug to the target site, while the passive diffusion of agents across the skin is possible with optimal physical and chemical properties. Many research reports have presented different chemical and physical procedures to enhance the skin penetration of therapeutic agents.[65] The chemical penetration enhancers include solvents, terpenes, fatty acids, surfactants and polymers, which affect the lipids and proteins inside the skin.[17a, 63] A carrier should be able to load drug, protect it against different destructive systems and enzymes inside the skin, and also increase the diffusion of the skin for a better penetration.

There are many systems that have been used a dermal delivery systems which has been used to transport therapeutic agents passively.[66] In recent years, many systems have been evaluated as carriers to transport drugs into the skin. The most prominent of these carriers are shown in Table 1. The size of carriers, their loading capacity, rate of release of cargo, mechanism of penetration into the skin, biocompatibility, toxicity, and biodegradability are issues that should be optimized to use them for topical drug delivery.[67]

Table 1. Different types of carriers for topical drug delivery. The most usual application and advantage of each carrier is explained. Reprinted with the permission of ref.[62]

Carrier	Application	Advantage
Creams	Improving the mechanical properties and penetration of skin. Suitable for people with a dry or sensitive skin	Consistent with many hydrophobic and hydrophilic drugs
Foams	Improving the skin properties and increasing penetration of a carrier. Some side effects have been observed	It can be easily applied and cost is low.
Proteosomes	It can be used as adjuvant or protein carrier	Defined catalytic activity

Virosomes	They can be used as immunological adjuvants	High ability to target a special site.
Vesosomes	To transport hydrophobic drugs with a high loading capacity. They are in early experimental phases.	They can protect cargo against biosystems
Gels	To encapsulate and transport both hydrophilic and hydrophobic drugs	High content of water and high loading capacity
Ointments	It can be used for very dry skin and to transport drugs in areas where skin is thick	Insoluble in water
Lotions	Can be used for all types of skin	The most versatile group
Novasomes	They are based on amphiphilic polyethylene glycol and cholesterol and can be used for deep skin penetration	They penetrate into the skin deeply
Photosomes	They are liposomes that can be stimulated by light for controlled release of cargo. They are in early experimental phases.	Light is a clean and easy available stimuli factor for controlled release of drug.
Shampoos	They can be used for short contact skin treatments. Administration could be performed several times per day.	Cheap and low cost and low side effects
Genosomes	They are liposomes made of cationic lipids and can be used for gene delivery	They show a high biodegradability
Ethosomes	They can be used for target delivery in deep layers of skin	They are not complex and consist of phospholipids
Solutions	They can be spread with low residues	The cost is low but they can cause irritation
Sprays	They can be used for treatment a large area of skin in a short time	Easy available and applicable

The mechanism of penetration of a dermal delivery system is important because it helps find out the interaction between biosystems with the administrated delivery system. Such information results in a better penetration and targeting with a high therapeutic efficiency and low side effect.[68]

A dermal drug delivery system should be biocompatible with a very low toxicity at high dosages. The conventional penetration enhancers are small amphiphilic compounds. The amphiphilicity of penetration enhancers is crucial, which leads to small (nano-size) aggregations with a hydrophobic inner part for the efficient loading of hydrophobic drugs and strong interactions with the hydrophilic and hydrophobic parts of skin as well as the membrane of skin cells. However, conventional penetration enhancers show not only a low ability to load and transport high molecular weight hydrophobic drug molecules but also in the most cases are not biocompatible and show a local irritation. Therefore, there is a high demand for the dermal delivery systems with high loading capacity, for a broad range of therapeutic agents, and in particular those with high molecular weights and skin penetration but low toxicity and high biocompatibility. Biomaterials are of high interest to be used as penetration enhancers, because

in the most cases they show a high biocompatibility, low toxicity, and immunogenicity, as well as a high loading capacity for hydrophobic therapeutic agents and suitable skin penetration.[69] Among the different types of biomaterials that have been used for topical drug delivery, protein transduction domains (PTDs) are the most promising candidates and have gained much interest to be used in both cosmetically and pharmaceutical research.[70] PDTS consist of amino acids with positive charge such as arginine and lysine.[71] Such amino acids usually induce a positive surface charge for PTDS, leading to a high interaction with the negatively charged extracellular matrix and cell membranes. Therapeutic agents can be covalently conjugated to the functional groups of PTDS and then be released at the target site by a stimulus factor at such as pH. Figure 3 shows the dermal delivery of different therapeutic agents by PDTS schematically.

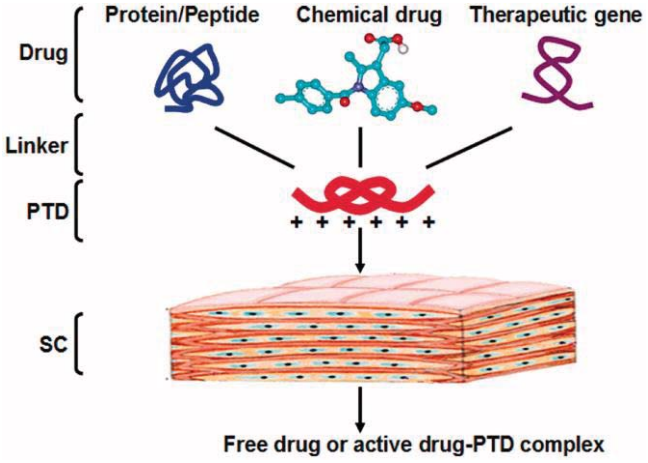


Figure 3. Schematic representation of the application of PTDS as a dermal delivery system for different therapeutic agents such as protein/peptide, drug and gene. Reprinted with the permission of ref [69].

As it can be seen in Table 1, many systems are investigated for the dermal delivery of therapeutic agents. In order to simplify the study of these systems, they have been categorized in six main classes and several subclasses in literature[62] (Figure 4). Although other types of delivery systems such as metal nanoparticles are not integrated and some of the subclasses overlap in this classification, it is useful for differentiating these systems based on their composition.

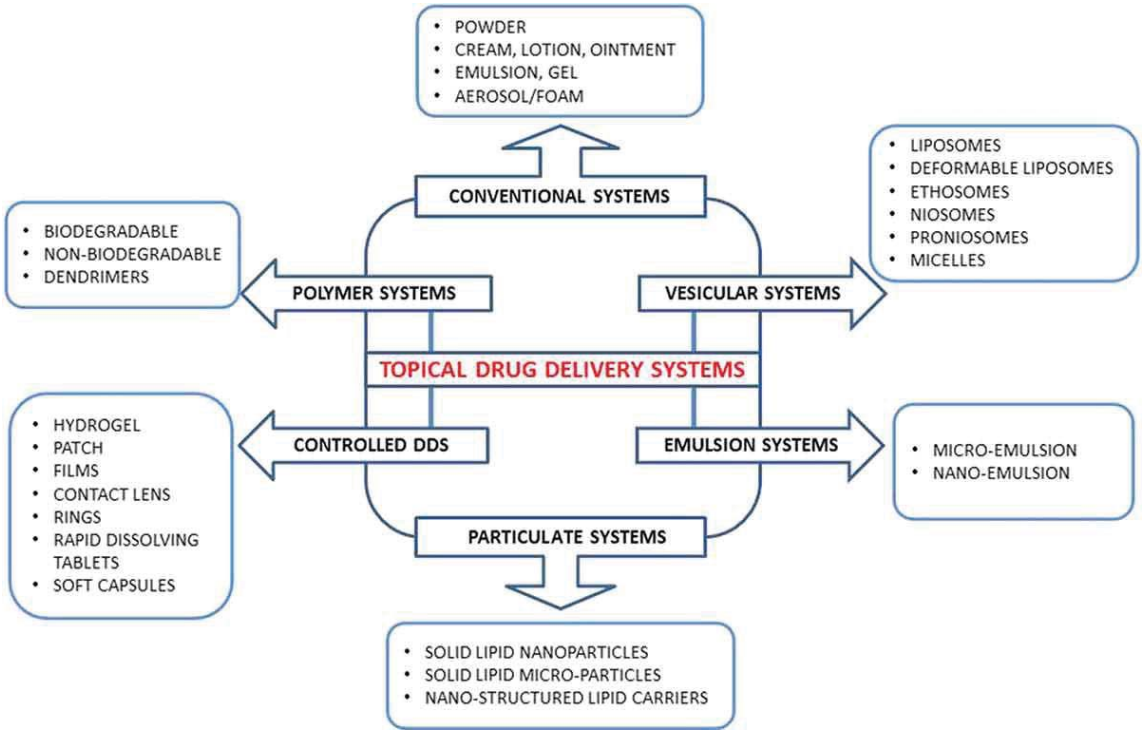


Figure 4. Various types of topical drug delivery systems that have been investigated in recent decades. Reprinted with the permission of ref [62].

1.3.1.1 Conventional dermal delivery systems

The category of conventional dermal delivery systems contains the most common delivery systems, including creams, ointments, and lotions. Different formulations of this class of materials are used to improve the skin penetration of a wide range of therapeutic agents.[72] Both commercially available and synthetic gels are used for dermal delivery, and their efficiency has been studied by means of different spectroscopy and microscopy methods. Two main strategies are used to improve the water content of SC, covering the surface of skin and introduction of humectants. Cream, ointments, and lotions are able to form a greasy layer and reduce water loss of the SC layer.[73]

1.3.1.2 Emulsion dermal delivery systems

Emulsions are abundantly applied as cosmetic and pharmaceutical formulations because they show excellent solubilizing capacities for different hydrophobic and hydrophilic therapeutic agents. Several factors including, type of emulsion (w/o vs. o/w emulsion), the size of droplets, the emollient, the emulsifier, and the aggregation pattern of surfactants (micelles, lyotropic liquid crystals) affect the cutaneous and percutaneous absorption.[74]

In some classifications, emulsions range from liquid formulations (lotions) to semi-solid formulations (creams), meaning that the conventional dermal delivery systems take place in this category. Emulsions are a broad range of different systems comprising two or more immiscible liquid phases in which one liquid (dispersion) is dispersed in the other liquid(s) (continuous phase) phase. Oil-in-water (o/w) emulsions can be obtained by dispersing the oil phase in the water phase. When water phase is dispersed in an oily continuous phase, a water-in-oil (w/o) emulsion is formed. The type of emulsion, which is defined as the hydrophilic-hydrophobic balance, strongly depends on the type of emulsifiers. The hydrophilic-hydrophobic balance is scaled from 1 to 20 so that the higher amounts show the more hydrophilic character for the surfactant. Based on the Bancroft rule, the continuous phase is able to dissolve the emulsifier better than others. This definition was modified by Harusawa et al. who supposed an external phase in which the surfactant forms micelles.[75] More complex emulsions can be formed by mixing multiple phases. Two example of complex emulsions are (i) oil globules, which are dispersed in a water globules and the whole system is dispersed in an oily continuous phase (o/w/o), and (ii) water globules, which are dispersed in oil globules and this system is in a continuous water phase (w/o/w). Emulsions in contrast to micro-emulsions are less thermodynamically stable and they required emulsifiers for a high stability. Emulsifiers are therefore one of the most important components that effect the efficiency of emulsion formulations.[76] Many types of emulsifying agents including surfactants, copolymers, and polymers; proteins are accessible and they can be used for dermal delivery systems. However, a common aspect for all emulsifying agents is an amphiphilic structure (a polar head and nonpolar tail) with the ability of stabilization of dispersed phase droplets. The mechanism of stabilization depends on the structure of emulsifying agents and mostly varies by reduction of interfacial tension and therefore reduced tendency for coalescence. This induces a steric hindrance by making a film at the oil/water interface, which causes an electrostatic repulsion between droplets and therefore increases the viscosity of the continuous phase. In some cases, a mixture of emulsifying agents improve the stability and applications of emulsions, e.g., ionic, non-ionic surfactants, and fatty based amphiphilic compounds can be mixed or separately added to a continues phases, which produces emulsions for pharmaceutical applications.[76] Some examples of the mixture of emulsifying agents are shown in Table 2.

Table 2. Some examples for combinations of emulsifying agents that are used in dermal delivery systems or pharmaceutical applications.[74]

Formulations (mixture of emulsifying agents)
Mixture of phospholipids including phosphatidylcholine, phosphatidylethanolamine, phosphatidylinositol, which is called lecithin[77]
Polysorbate 60/sorbitan monostearate[78]
Cetrimide/cetostearyl alcohol[79]
Steareth-2/steareth-21[80]
Cetostearyl alcohol/sodium lauryl sulphate (emulsifying wax BP)[81]
Isostearic acid/triethanolamine[82]
Cetylstearyl alcohol/cetylstearyl alcohol sulphate (emulsifying wax DAB 8)[83]
Cetearyl glucoside/cetearyl alcohol[84]
Synperonic PE/F127 (block copolymer of ethylene oxide and propylene oxide)/hypermer A60 (modified polyester)[85]
Cetostearyl alcohol/polyoxyethylene alkyl ether[86]
Sucrose cocoate/sorbitan stearate[84b]

Penetration of therapeutic agents into the skin can be either promoted or delayed, when they are added to a continuous phase of an emulsion. For example, the dermal delivery of the main component of sunscreen agent, which is called ethylhexyl methoxycinnamate, could be enhanced by w/o emulsion and this is higher than when o/w is used.[87] Conversely, skin penetration of some therapeutic agents enhances by o/w emulsion in comparison with w/o emulsion.[88] Interestingly, there is a big difference between the effect of emulsions on the transdermal and dermal delivery of therapeutic agents. While the transdermal delivery of a wide range of materials accompanied by o/w and w/o emulsions has been similar, the dermal delivery has been higher when therapeutic agent is incorporated in the dispersed phase.

1.3.1.3 Vesicular dermal delivery systems

Vesicular dermal delivery systems are different materials with the ability of aggregation in the form of vesicles and increased penetration of therapeutic agents into different layers of skin.

The liposomes are artificially created vesicles which are made of amphiphilic materials with lipid tails. Liposomes and their next generations are frequently used both in pharmaceuticals and cosmetics for controlled delivery of therapeutic agents into particular layers of skin (Table 3). Liposomes are able to encapsulate hydrophobic therapeutic agents in their lipid bilayers and hydrophilic agents in their aqueous core. This property enables

liposomes to carry two types of therapeutic agents into particular regions of skin. Conventional liposomes have been modified thus creating the next generation, so-called "transfersomes," with the ability of high skin permeation. Transfersomes are very elastic, ultra-flexible vesicle delivery systems that are capable to greatly deform. Insertion of surfactants such as span, tween, sodium cholate, sodium deoxycholate, and potassium glycyrrhizinate in the structure of liposomes as an edge activator destabilize the produced vesicle and impart the flexibility and deformability in its structure.[88-89]

Another type of vesicular dermal delivery systems is niosomes, which are vesicular alternatives to liposomes without phospholipids in their structure. Usually, their components are nonionic surfactants. Self-assembly of non-ionic amphiphilic materials in aqueous solutions results in bilayer vesicles with the ability of loading hydrophobic and hydrophilic therapeutic agents. Loading capacities of liposomes and niosomes strongly depend on their compositions including surfactant and lipid contents as well as the particle size.[90]

Vesicular drug delivery systems can be prepared by hydration, homogenization, and sonication. Their size depends on the preparation method and is in the range of 50–800 nm.[91] Smaller sizes are more appreciated and show higher diffusion but with the cost of lower loading capacity and stability. The type of surfactant affects the morphology and shape of liposomes and niosomes.[92] Vesicular dermal delivery systems that have been prepared by different methods are used for the skin penetration of many therapeutic agents.[93]

Table 3. Some dermal delivery systems based on liposomes and their next generations. Reprinted with the permission of ref [94].

Vesicular lipid carrier	Therapeutic agent	Application	References
Liposomes	Curcumin	Anti-inflammatory, enhanced skin permeation	[95]
Liposomes	siRNA	To treat melanoma, enhanced skin permeation	[96]
Liposomes	Loperamide hydrochloride	Analgesic, anti-inflammatory, enhanced skin permeation	[97]
Ultradeformable liposomes	Diclofenac	Analgesic, enhanced skin permeation	[98]
Ultradeformable liposomes	Quercetin	Antioxidant and anti-inflammatory, enhanced skin permeation	[99]

Ultradeformable liposomes	Amphotericin	Antifungal, enhanced skin permeation	[100]
Ultradeformable liposomes	Itraconazole	Antifungal, enhanced skin permeation	[101]
Ultradeformable liposomes	Papaverine hydrochloride	Erectile dysfunction, enhanced skin permeation	[102]
Ultradeformable liposomes	Insulin	Antidiabetic, enhanced skin permeation	[103]
Ethosomes	Ammonium glycyrrhizinate	Anti-inflammatory, enhanced skin permeation	[104]
Ethosomes	Hyaluronic acid	Wound healing and anti-aging, enhanced skin permeation	[105]
Ethosomes	Diclofenac	Anti-inflammatory, enhanced skin permeation	[106]
Ethosomes	Testosterone	Androgen hormone, enhanced skin permeation	[107]
Ethosomes	Buspirone	To treat menopausal syndromes, enhanced skin permeation	[108]

Vesicular dermal delivery systems enhance skin penetration of therapeutic agents through a complex mechanism including distortion in intercellular lipid construction of SC, adsorption, and fusion with the SC layer, as well as transappendageal penetration, and intact vesicular skin penetration and finally thermodynamic activity enhancement effects.[109] Recently, other mechanisms have been suggested for skin permeation of liposomes based on which liposome interacts with the skin lipid components thereby inducing partial fluidization of the lipid bilayer to carry the cargo in the deeper layers of skin.[55]

1.3.1.4 Particulate dermal delivery systems

Particulate dermal delivery systems can be categorized into two classes, hard and soft nanoparticles. The skin penetration of hard nanoparticles including metal nanoparticles, e.g., gold nanoparticles,[110] gold nanorods,[111] and silver nanoparticles and iron nanoparticles[112] has been investigated. In some cases, hard nanoparticles have been used for treatment of skin disease through their intrinsic biological, physicochemical, and optoelectronic properties. For example, application of silver nanoparticles as topical antimicrobial agents to inhibit proliferation of pathogens is supposed to be a new way for the burn wound care therapy.[113] Penetration of metal nanoparticles into the skin depends on their formulations, functionality, surface charge, morphology, and shapes.[113-114] Titanium dioxide (TiO_2) or zinc oxide (ZnO)

nanoparticles are widely used in sunscreens.[115] It is reported that TiO_2 nanoparticles with 10-50 nm average size are able to penetrate to the SC to the dermis after several applications.[116] Gold nanoparticles are able to penetrate into the deep layers (epidermis and dermis) layers of rat and human skin.[117] They interact with the lipid layer of skin and after disruption penetrate into the deeper layers.[118] Skin penetration of metal nanoparticles are relevant in both toxicology (health risk) and therapy aspects.[119]

Soft nanoparticles are another class of particulate dermal delivery systems with soft skeleton consisting polymer or lipids. Lipid nanoparticles and lipospheres are the most important particulate lipid carriers and are of great interest for the dermal delivery applications due to their versatility and biocompatibility.[120] Lipid nanoparticles can be further classified into solid lipid nanoparticles (SLN) and nanostructured lipid carriers (NLC). The lipid phase in SLNs consists of both liquid and solid lipid phases. NLCs are a new and modified version of SLNs with improved loading capacity and stability.[91] Lipid carriers improve the therapeutic efficiency because they prevent the degradation, hydrolysis, and oxidation of encapsulated drugs.[91, 121]

lipospheres are soft core shell structure with a solid hydrophobic lipid core and a phospholipid shell. The phospholipid layer improves the water dispensability of particles. Lipospheres can be prepared by a variety of techniques including melt dispersion, solvent emulsification, solvent emulsification evaporation, high pressure homogenization, and ultrasonication, and their size is in the range of 0.2–500 mm.[91] The particle size and their loading capacity affect their efficiency dramatically.[122]

NLCs show advantages over the SLNs such as overcoming the gelation, particle aggregation and uncontrolled drug release. Preparation of NLCs is very similar to the construction methods of SLNs. They can be formed by adding a mixture of solid and liquid lipids with 7/3 to 9/1 ratios in an aqueous solution.[123]

The mechanism of the skin penetration of NLCs is similar to SLNs. Because of their small sizes and high surface area, lipid particles strongly interact with SC layer of skin and adhere to it. Because of their high loading capacity, improved skin hydration, release of drugs in a controlled manner, and increased drug stability, NLCs are good candidates for dermal delivery applications.[124]

1.3.1.5 Polymeric dermal delivery systems

Polymers have been widely used in dermal delivery for the past few decades.[125] Different classes of polymers improve the versatility of the skin route for the administration of therapeutic

agents.[126] Polymers can be used for dermal delivery in two forms.[127] They are either directly used as penetration enhancers or building blocks to make supramolecular assemblies and load therapeutic agents, which are applied to the skin.[128]

Three different classes of polymers including linear polymers, dendrimers, and hyperbranched polymers are used for the dermal delivery of therapeutic agents. Each class can be divided into several subclasses. For example, these polymers are changed to amphiphilic structures, gels, and nanoparticles and they have been used for dermal delivery.[129]

i) *Linear polymers:* Linear polymers and their derivatives have been used for dermal delivery abundantly. Polyethylene glycol is the most useful for dermal delivery due to its biocompatibility, water solubility, and low immunogenicity.[130] Amphiphilic derivatives of polyethylene glycols have been also used for the efficient loading and transportation therapeutic agents in the form of supramolecular nanosystems. D-α-tocopheryl polyethylene glycol 1000 succinate (TPGS) is used to load griseofulvin, which is an antifungal therapeutic agent that is usually administrated orally. However, griseofulvin is incompletely absorbed in the gastrointestinal tract in humans. The conventional oral therapy of this drug has clinically failed, because it is poorly soluble in biological media and its bioavailability is therefore too low. Formulations of this drug incorporated in TPGS are applied to the mice skin and its effect against *Microsporum gypseum* and *Microsporum canis* has been evaluated. An efficient enhancement of the drug penetration and retention in the skin has been observed.[131] Amphiphilic block copolymers and their supramolecular structures have shown high potential for the dermal delivery of different therapeutic agents. Methoxypoly(ethylene glycol)-dihexyl substituted polylactide (MPEG-dihex-PLA) is a biocompatible and biodegradable diblock copolymer and creates micelles in aqueous solutions through which TAC can be efficiently transported to the epidermis and upper dermis layers.[128] MPEG-dihexPLA has been also used for the systemic and ocular administrations.[132] It has been used for the dermal delivery of econazole nitrate, and results have been compared with the commercially available liposome-based delivery system (Pevaryl). It has been shown that MPEG-dihexPLA is more efficient than Pevaryl to improve the cutaneous bioavailability of this therapeutic agent.[133]

The mechanism of enhancing dermal delivery by polymeric micelles is not well understood, because there are few studies on such nanomaterials. Some researchers suggest polymeric micelles can penetrate while carrying their cargo the skin intact.[134] Others argue that polymeric micelles disassemble upon interaction with the skin and the individual polymer chains transfer therapeutic agents.[135] MPEG-dihexPLA is more efficient than marketed

formulation based on liposomes (Pevaryl). This is attributed to the smaller sizes of micelles and higher contact area with the skin.[133]

Chitosan is a biodegradable and biocompatible polymer that is used for abundant dermal delivery.[69] A challenge for using chitosan in dermal delivery is its poor solubility in aqueous solutions at neutral and alkali media. In these pHs, amino groups of chitosan are not protonated and the water solubility of chitosan is low (Figure 5).[136] Conjugation of alkyl groups to the amino groups of chitosan improves its solubility and its effect as a permeation enhancer in alkali media.[137] Other parameters including degree of deacetylation and degree of quaternization affect the activity of chitosan as a penetration enhancer.[138]

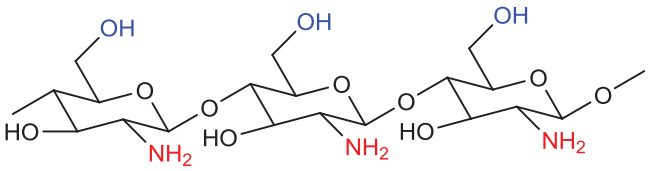


Figure 5. The chemical formula of chitosan.

Chitosan is a high molecular weight natural polymer with low solubility. To overcome these problems and enhance its efficiency as a dermal delivery system, low molecular weight chitosan polymers are synthesized and their ability to enhance the penetration of baicalin has been investigated.[139] It has been shown that the deacetylation of the amino functional group has a huge effect on the ability of low molecular-weight chitosan as a penetration enhancer. Chitosan derivatives such as N-arginine chitosan are used and their potential as dermal delivery is investigated. It was found that both degree of arginine substituents and the molecular weight of chitosan affect the ability of this derivative as a penetration enhancer.[140] The best results were obtained with a 10 kDa chitosan and 6% arginine substituents at pH 7.

Oligodimethylsiloxanes (ODMSs) are another class of linear polymers that hardly permeate into the skin due to their high molecular weight and high hydrophobicity.[69] However, they can be modified for dermal delivery applications.[141] OMDSs with the end pyridine,[142] quaternary amino,[143] and carboxyl ionic end groups have shown a high potential for the topical delivery applications. Polydimethylsiloxane-*b*-polyethylene glycol copolymers (PEG-PDMS) are synthesized and used as amphiphilic agents for the topical delivery of both hydrophilic and hydrophobic drugs. The lengths of both PEG and PDMS blocks significantly affected the permeation of their cargo and the optimum case was a balance between these two blocks.[144] ODMS with a conjugated sugar segment were investigated as topical delivery systems. Cellobiosyl-terminated ODMS (Cell-ODMS), glucopiranosyl-terminated ODMS (GluO-

ODMS) and its thioether analog (GlcS-ODMS) were synthesized and applied for the loading and delivery of antipyrine. While GluO-ODMS and GlcS-ODMS improved the permeation of this therapeutic agent, Cell-ODMS did not show a significant effect. It was found that Cell-ODMS is efficient for the hydrophilic drugs more than hydrophobic agents and the short ODMS showed better results than longer analogs.[145] In order to investigate the effect of hydrophobicity of ODMS with a end sugar groups, a series of these materials with different alkyl chains has been synthesized and used to enhance the permeation of both hydrophilic and hydrophobic drugs. It has been shown that a balance between the hydrophilic and hydrophobic blocks has the best permeation activity and optimal results were obtained by octyl and decyl chains.[141]

ii) Dendrimers: The tree-like structure of dendrimers results in a large number of peripherial functional groups which are both important for conjugation and loading of therapeutic agents (Figure 6). Due to such properties, their small sizes, and well-defined structures, they have been extensively used as drug and gene delivery systems.[146] Owing to their minimal skin irritation and a high loading capacity, amphiphilic dendrimers are of great interest for topical drug delivery.

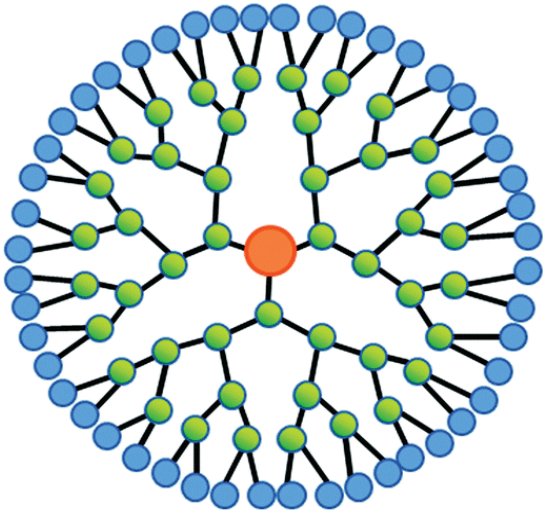


Figure 6. A typical structure for a dendrimer. Blue spherical objects in the periphery show the functional groups to which different therapeutic agents could be conjugated. A dendrimer is also able to host therapeutic agents with the right size and structure in its skeleton. Reprinted with the permission of ref [147].

Therefore dendrimers are widely used as skin permeation enhancers. Interesting results are obtained for different therapeutic agents, which are administrated together with dendrimers to penetrate the skin (Table 4).

Table 4. Different formulations of dendrimers for topical delivery of therapeutic agents. Reprinted with the permission of ref [148].

Application category	Type of dendrimer	Application	Strategy
Topical (skin surface)	Higher generation dendrimers (> G6)	Carrier system for cosmetic agents including sunscreens	Skin permeation retarding agent
Topical (intradermal)	Lower generation dendrimers (G2–G5)	To target skin diseases localized within viable epidermis	Smaller size leads to better penetration across the SC
	Neutral dendrimers	To release the drug within the epidermal interstitium	Neutral dendrimers partition well through SC membrane
	Cationic dendrimers	To release the drug intracellularly	Cationic dendrimers can effectively get internalized into cells
Transdermal	Lower generation dendrimers (G1–G4)	To deliver and release the drug molecule in systemic circulation	Cationic dendrimers in combination with physical permeation enhancement strategies

Several parameters including molecular weight, surface charge, and type of functional groups affect the penetration of dendrimers into the skin and their efficiency as penetration enhancers. Different studies have shown that cationic dendrimers (dendrimer with positive surface charge) penetrate into the deeper layers of skin further than anionic dendrimers (dendrimers with anionic surface charge) due to their higher interactions with the negatively charge skin surface. It is found that cationic dendrimers interact with the lipid components of skin and then penetrate into the SC layer.[149] Appendages in the skin including hair follicles, sweat glands, sebaceous glands, and pilosebaceous glands provide a possibility for the penetration of bigger particles but their total contribution in the skin surface is too low,[150] Low generation of dendrimers (G < 4) have a diameter less than 5 nm due to their tree-like structure. Therefore, they are able to interact with hydrophilic conduits of intercellular lipid matrix with a width up to 10 nm and penetrate into the skin efficiently.[150] Considering all structural parameters, smaller dendrimers with positive and neutral surface charges are able to penetrate into the skin better that those with negative surface charge and bigger sizes. These possible penetration pathways and effect of the structural parameter of dendrimers on their penetration pathways are shown in Figure 7. One of the main pathways for the penetration of dendrimers

into the skin is the skin pore. This is due to the low size hydrophilic nature, in the most cases, of dendrimers. Because it is proposed that hydrophilic and other small molecules are able to penetrate into the skin through the pores with a size range 1.5 - 2.5 nm.[151] The influence of the dendrimers' size on their skin penetration is investigated by evaluating the ability of different generations of polyamidoamine (PAMAM) dendrimers on the skin penetration of 5-fluorouracil (5FU).[152] It is shown that there is a linear correlation between the skin penetration of 5FU and size of dendrimers with the highest effect for the lower generations. It has been also proven that interactions between the skin and dendrimers decrease with increased size of dendrimers. Smaller dendrimers interact with the skin more efficiently and change the lipid structure of SC as the main barrier of skin. Perturbation of the lipid structure of skin decreases the transepidermal electrical resistance of skin and results in higher penetration of therapeutic agents. Generations three and four of PAMAM dendrimers are also used to transport 8-methoxypsoralen into the skin. It is shown that generation three increases the skin penetration of this therapeutic agents better than generation four.[153] Surface functionality and charge of dendrimers are another factor that influences their interactions with skin. It is shown that PAMAM dendrimers with amino functional groups (positive surface charge) improve the skin penetration of indomethacin almost two times compared to the same dendrimer with carboxyl functional groups.[154] Neutral dendrimers did not show a significant effect on the penetration of this therapeutic agent.

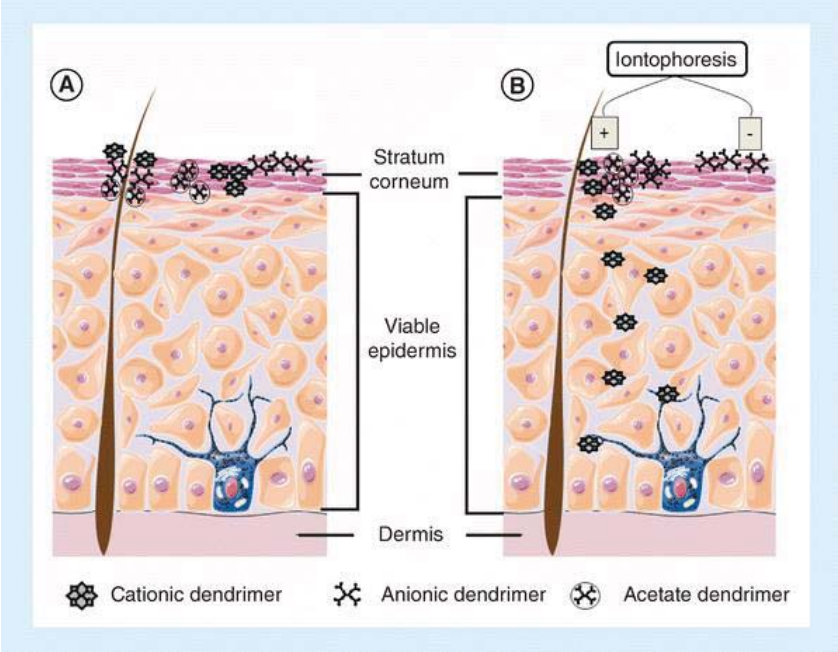


Figure 7. Different pathways for the penetration of dendrimers into the skin after passive (A) and iontophoretic (B) applications. Reprinted with the permission of ref [148].

The higher penetration effect for cationic dendrimers (PAMAM-NH_2) than the anionic analogs (PAMAM-COOH) is due to the electrostatic interactions between the cationic dendrimers with the negative surface charge of skin.[149] Conjugation of the acetate groups to PAMAM-NH_2 improves their penetration into the skin. It is proposed that conjugation of acetate groups decreases the positive charge of dendrimer. Decreasing the positive surface charge of dendrimer diminishes the interaction between dendrimer and surface of skin and results in higher penetration into the skin.[155] It has been shown than even negatively charged PAMAM (PAMAM-COOH) demonstrates a higher penetration because of less interactions with the negatively charged skin surface. These experiments prove that surface charge and size significantly dominate the interactions between skin and dendrimers. Both properties affect skin penetration as well as the penetration-enhancing effect of these macromolecules (Figure 8).

The partition coefficient is one of the important factors that affects the skin penetration of therapeutic agents. Usually a therapeutic agent with an optimum-partition coefficient shows a high penetration into the SC with lipophilic features and viable epidermis and dermis with hydrophilic components. For example, conjugation of oleic acid to the amino functional groups of PAMAM increases the partition coefficient of these macromolecules. While this value is negative for dendrimers with amino, carboxyl, and acetate functional groups, it is positive for PAMAM with oleic acid groups. These results indicate the hydrophobic nature of oleic acid-functionalized PAMAM dendrimers result in a higher absorption into the skin.

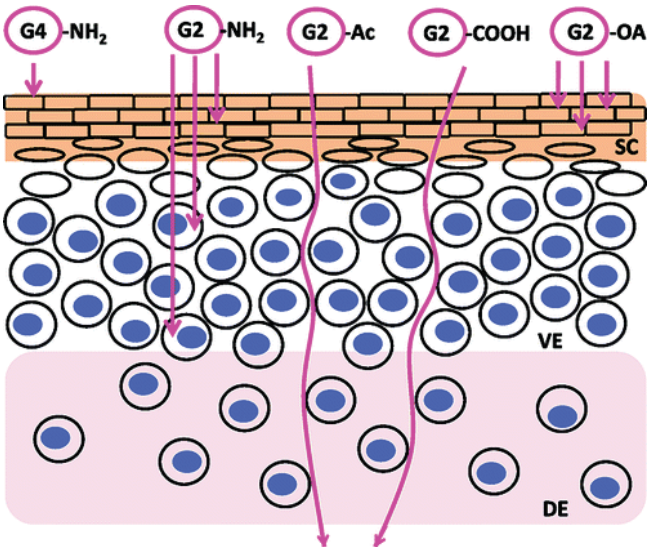


Figure 8. The size, surface charge, and hydrophobicity of dendrimers dictate their penetration pathways. Skin is treated with generation 2 [G2] and generation 4 [G4] of PAMAM with different functional groups including amino (NH_2), carboxylic acid (COOH), acetate (Ac), and oleic acid (OA). The penetration of these macromolecules into the skin was investigated by different methods. Reprinted with the permission of ref [155].

ii) *Hyperbranched polymers:* This class of polymers, which has the same characteristics of dendrimers but less defined structures, is very interesting for drug delivery applications.[156] Synthesis of hyperbranched polymers is a one-step process and more feasible than dendrimers. They can be produced on a large scale with lower cost, thus, closer to marketability. This class of polymers has also been investigated for different biomedical applications.[157]

Hyperbranched polyglycerol (hPG) is a polyether polyol with a high number of hydroxyl functional groups and degree of branching between 40%-65%.[158] It can be synthesized via cationic and anionic ring-opening polymerization of glycidol[159] (Figure 9). Owing to its high water solubility, low nonspecific interaction with biosystems (low protein interaction), high biocompatibility, and low toxicity, polyglycerol is very interesting for many biomedical applications.[21, 160]

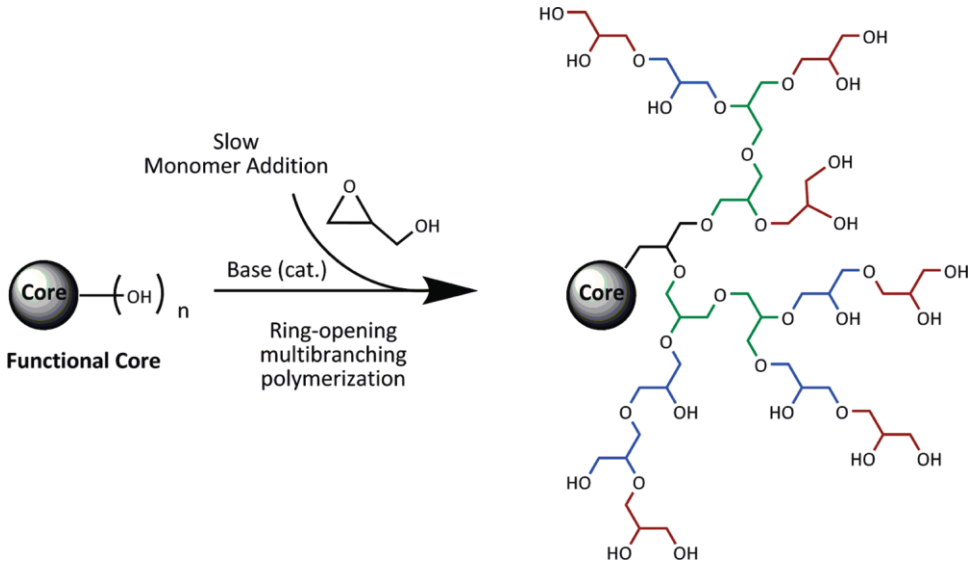


Figure 9. Synthesis of hyperbranched polyglycerol by anionic ring-opening polymerization of glycidol. The color indicate dendritic (green), linear (blue), terminal (red) unites in the scaffold. Reprinted with the permission of ref [159].

The potential application of hyperbranched polymers for topical delivery has been investigated for several years now.[161] Since hyperbranched polyglycerol does not show a high loading capacity for therapeutic agents, it is modified to different nanoarchitectures including core-multishell architectures, nanogels, and new hyperbranched structures. Its ability as a dermal drug delivery system is being investigated.

1.3.1.6 Core-multishell polyglycerol architectures (CMS)

Conjugation of low molecular amphiphilic polymers e.g., PEG-di or fatty acids to the end hydroxyl functional groups of polyglycerol results in amphiphilic structures with a polyglycerol

core and an amphiphilic shell, which are called Core-Multishell architectures (CMS).[162] Due to their amphiphilic structure, CMS are able to load hydrophobic drugs in the form of monomolecular micelles or aggregates in aqueous solutions.[163]

The effect of the alkyl chain on the drug loading capacity, skin penetration, and biocompatibility of CMS has been investigated.[1a] Among three different C12, C15, and C18 alkyl chains, CMS with C15 alkyl chains have effectively loaded and transported dexamethasone through the skin. Furthermore, they have been more biocompatible than two other analogs and their formulation with the loaded dexamethasone has shown the highest anti-inflammatory effect.[164]

HPG-amid-C18-mPEG core-multishell nanocarriers have been investigated for the topical drug delivery. They accumulate in the stratum corneum without penetration into deeper viable epidermal layers not only in the normal skin but atopic dermatitis mice. These results show that alteration in the skin barriers did not affect the skin penetration of CMS.[165] The hydrophobic block of CMS can be low molecular weight polylactide or polycaprolactone that is conjugated to polyglycerol by "grafting to" or "grafting from" methods. For example, polymerization of lactide monomers by polyglycerol, as a macroinitiator, resulted in polyglycerol-*b*-polylactide core-shell structure with the ability of loading small molecules.[22b] Loading capacity of polyglycerol-*b*-polylactide core-shell structures for Congo red was increased with an increase in the length and number of polylactide arms.

Conjugation of polycaprolactone-*b*-polyethylene glycol block copolymers to the polyglycerol core by the "grafting to" method resulted in CMS that was suitable for the dermal delivery applications.[22a] While CMS were accumulated in the stratum corneum layer, they were able to increase the skin penetration of Nile red 7-fold, compared to the conventional cream formulation[162c].

1.3.1.7 Polyglycerol nanogels

Polyglycerol nanogels are polymeric networks composed of hPG building blocks crosslinked by small or long chain linkers. They are hydrophilic nano-objects and attractive candidates for loading and transport therapeutic agents.[166] However, they do not show affinity for the hydrophobic drugs due to their hydrophilic nature. In order to improve their capability for the encapsulation of such agents, polyglycerol nanogels should be modified with hydrophobic segments. Crosslinking of hPGs by oligo ethylene glycol derivatives resulted in thermosensitive polyglycerol nanogels with the tunable hydrophobicity.[167] Such nanogels are able to penetrate into the skin, accumulate in the stratum corneum and show a potential for

dermal delivery applications. Crosslinking of hPG units by poly(N-isopropylacrylamide) (PNIPAM) led to thermosensitive poly(N-isopropylacrylamide)-polyglycerol-based nanogels that exhibited a lower critical solution temperature (LCST) > 35 ^{0}C.[23] These nanogels were in the extended conformation below the LCST and due to the opened pores were able to load such big macromolecules as transglutaminase 1. Higher LCST nanogels collapsed and released the loaded proteins more efficiently (more than 9%), resulting in a restoration of skin barrier function. The thermoresponsive behavior of such nanogels was matched with the temperature gradient of skin (Figure 10).

The toxicity and cellular uptake of thermoresponsive nanogels, both with oligoethylene glycol derivatives and PNIPAM chains, were investigated against primary normal human keratinocytes (NHK) and in spontaneously transformed aneuploid immortal keratinocyte cell line from adult human skin (HaCaT). It was found that thermoresponsive nanogels were taken up by these cells and accumulated in the lysosomal compartments. Different assays showed that such nanogels were highly biocompatible and did not show any adverse effects of the examined cells. Also, no eye irritation potential was observed for these nanogels. These results show that such nanogels are promising candidates for future dermal delivery of a wide range of therapeutic agents.[21]

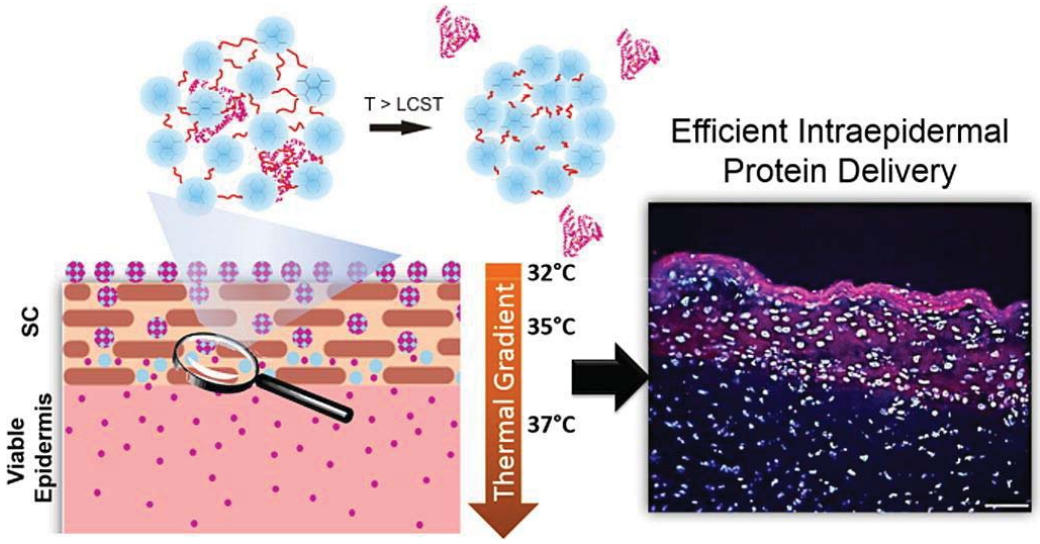


Figure 10. Thermoresponsive poly(N-isopropylacrylamide)-polyglycerol-based nanogels are able to load proteins at lower than LCST and release it at higher than LCST (top left image). This behavior makes such nanogels attractive for dermal delivery due to the temperature gradient of skin (lower left). They are able to release most of the loaded proteins in the intraepidermal layer (lower right). Reprinted with the permission of ref [23].

In addition to their application for the loading and dermal delivery of therapeutic agents, polyglycerol nanogels are used to investigate the pH gradient of skin. Conjugation of a pH

sensitive dye to the functional groups of nanogels and administration through the hair follicular pathway showed that the pH of pig hair follicles increased from 6.5 from the surface of the skin to 7.4 in the deeper layers.[168].

1.3.1.8 New architectures of hyperbranched polyglycerols

In spite of many advantages including straightforward production, water solubility, high functionality, biocompatibility, low toxicity, and low immunogenicity, there are some disadvantages that hinder future biomedical applications of this polymer. For example, polyglycerols are not biodegradable due to their polyether backbone and accumulate in some organs after administration.[169] Therefore their backbone should be modified by inserting biodegradable segments such as esters, ketals, and disulfides into it.[170] Incorporation of oligoester biodegradable hydrophobic segments in the backbone of polymers not only improves their loading capacity for hydrophobic drugs but also their biodegradation in the biological media. Glycerol-based hyperbranched polymers have been synthesized by a reaction between octadecen-1-yl succinic anhydride and methyl poly(ethylenglycol) ($mPEG_{500}$). Such polymers have encapsulated dexamethasone and finasteride, which makes them suitable candidates for dermal delivery applications.[171] Biodegradable poly(lactic acid)-hyperbranched polyglycerol have been synthesized; their ability to penetrate into the skin and to transport drugs though the skin is still being investigated. These polymers have shown epidermal penetration to the dermal-epidermal junction and they have been accumulated in hair follicles significantly.[172]

2 Scientific Goals

Polyglycerol derivatives are excellent candidates for dermal delivery of therapeutic agents. However, some challenges such as non-biodegradability, non-specific interactions with the therapeutic agents, and low affinity for the encapsulation of hydrophobic drugs remain unsolved problems, which should be overcome before developing these fascinating avenues into future biomedical applications. Furthermore, most of the synthetic routes for the preparation of biodegradable polyglycerols and in particular their nanogels are milligram-scale protocols, which hinders their development for the biomedical applications. Therefore, such protocols should be scaled up and polyglycerol derivatives should be produced on a gram-scale to extend further their biomedical applications.

The aim of this study is to synthesize new biodegradable polyglycerol-based nanocarriers for dermal delivery applications. Such nanocarriers should be biodegradable in the skin and they should show high loading capacity and specific interactions with hydrophobic drugs. Another objective of this study is to design new synthetic protocols for the production of biodegradable polyglycerol nanogels on a gram scale.

Specific interactions between nanocarriers such as nanogels and therapeutic agents not only improve their loading capacity but also their efficiency to deliver target therapeutic agents. Decreasing nonspecific interactions between nanogels and different systems improves their bioavailability and the therapeutic efficacy of their cargo. In this study, the target drug for specific interactions with nanogels is *m*-THPC, which is a very hydrophobic drug and is being used as a photosensitizer for the photodynamic therapy of skin cancer.[173] Conjugation of a peptide, which was selected for combinatorial means, to polyglycerol nanogels was the planned strategy for improving specific interactions between nanogels and this drug. The human skin will be treated with the polyglycerol nanogel-peptide conjugates loaded with *m*-THPC and their penetration into the skin will be evaluated.

The second challenge was the gram-scale synthesis of biodegradable polyglycerols for the efficient loading and transport of drugs across the skin layers. One-pot copolymerization of glycidol with hydrophobic cyclic monomers including lactide and ε-caprolactone was our strategy to incorporate ester segments in the polyglycerol backbone. Such ester segments should not only improve the loading capacity of polyglycerols toward hydrophobic drugs but also their biodegradability. Tacrolimus (TAC) was the targeted therapeutic agent to be loaded and delivered into the skin by these copolymers. TAC is a macrolide immunosuppressant therapeutic agent, which is usually applied for treating atopic dermatitis. TAC hampers the

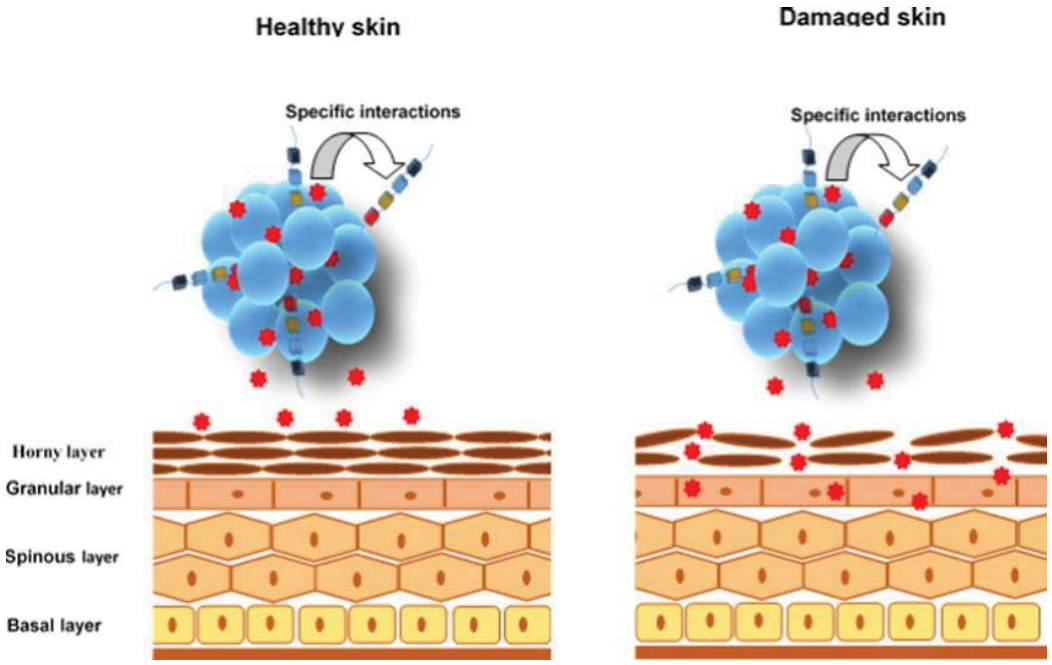
expression of cytokines from the keratinocytes, basophils, and mast cells and decreases the number of inflammatory dendritic epidermal cells in inflamed skin layers.

The third goal of this Ph.D. thesis was a large scale production of biodegradable polyglycerols with a high loading capacity for the dermal delivery of tacrolimus and *m*-THPC. Novozyme was chosen as the main reagent to catalyze the ring-opening polymerization of a glycidol and succinic anhydride mixture as well as crosslinking the resulting copolymers by esterification reactions. Hydrophobic succinic segments should improve the loading capacity of the obtained copolymers as well as their biodegradability in the skin media.

3 Publications and Manuscripts

In the following section, the scientific outcomes of this PhD thesis are listed and the contributions of the authors are specified.

3.1 Intradermal Drug Delivery by Nanogel-Peptide Conjugates; Specific and Efficient Transport of Temoporfin



Fatemeh

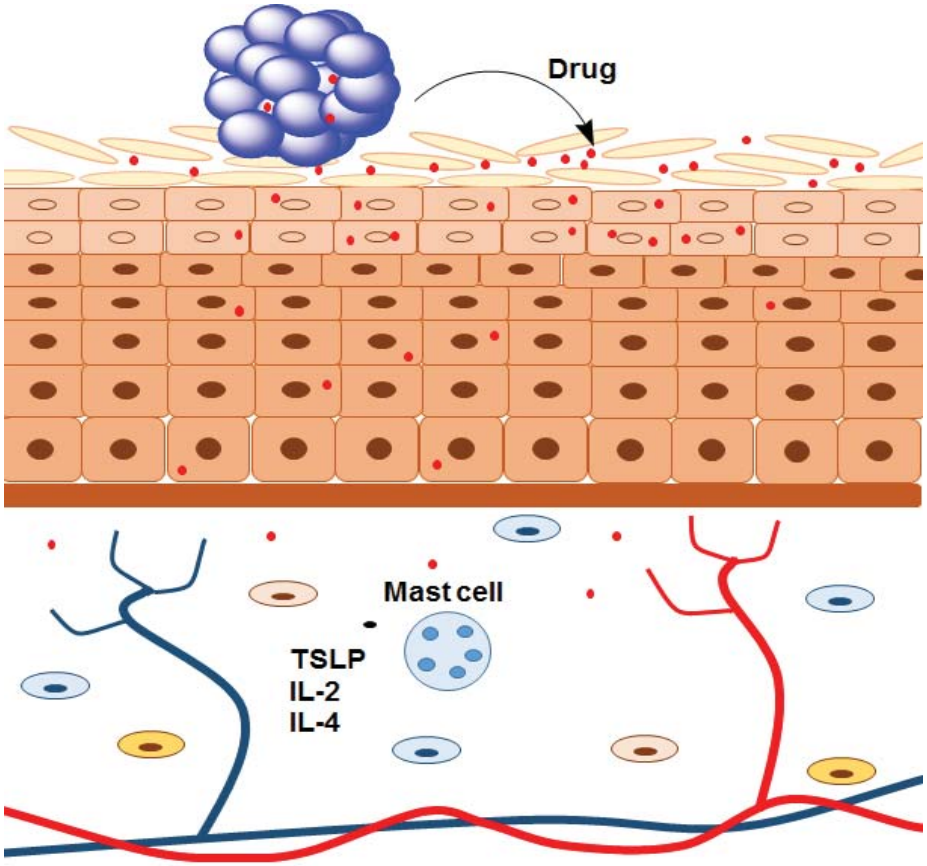
Zabihi, Sebastian Wieczorek, Mathias Dimde, Sarah Hedtrich, Hans G. Börner and Rainer Haag

https://doi.org/10.1016/j.jconrel.2016.07.033

Author contributions

Fatemeh Zabihi performed the main experiments, and wrote the manuscript. Sebastian Wieczorek synthesized the peptide segments. Mathias Dimde collaborated in nanogels synthesis. Hans G. Börner supervised synthesis of the peptide segments.
Sarah Hedtrich and Rainer Haag conceptualized the project and corrected the manuscript.

3.2 Synthesis of Poly(lactide-co-glycerol) as a Biodegradable and Biocompatible Polymer with High Loading Capacity for Dermal Drug Delivery



Fatemeh Zabihi, Patrick Graff, Fabian Schumacher, Burkhard Kleuser, Sarah Hedtrich and Rainer Haag

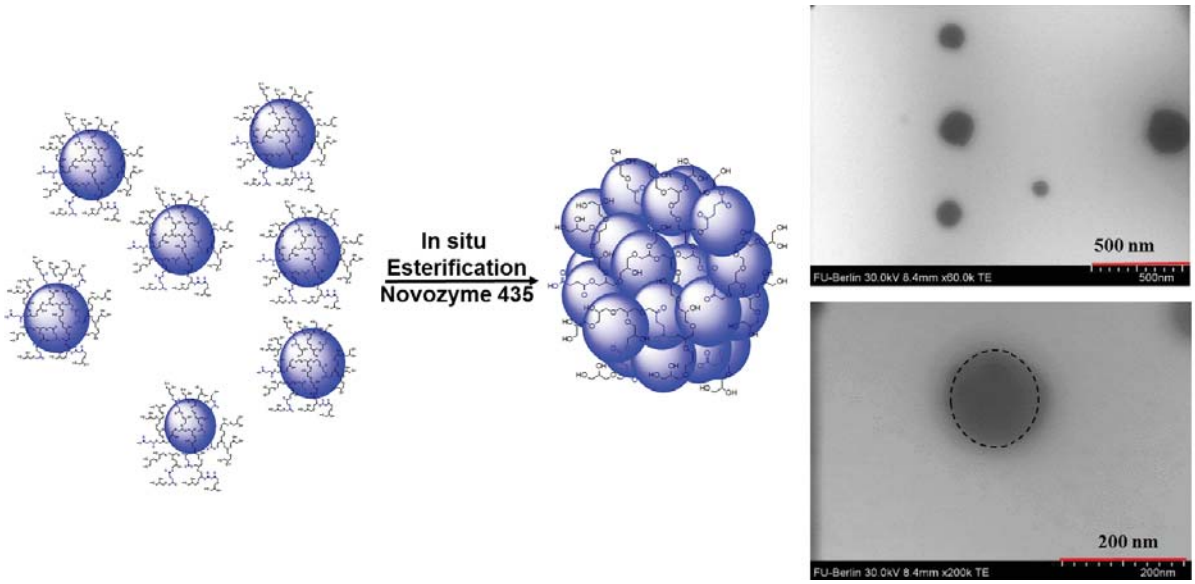
https://doi.org/10.1039/C8NR05536J

Author contributions

Fatemeh Zabihi performed the main experiments, and wrote the manuscript. Patrick Graff buil t the *in*

vitro inflammatory skin models and performed efficacy experiments. Fabian Schumacher collaborated in determining concentration of tacrolimus in nanocarriers and different layers of skin. Burkhard Kleuser supervised determination of concentration of tacrolimus. Sarah Hedtrich and Rainer Haag discussed the project and corrected the manuscript

3.3 One-Pot Synthesis of Poly(glycerol-succinic anhydride) Nanogels for Dermal Delivery



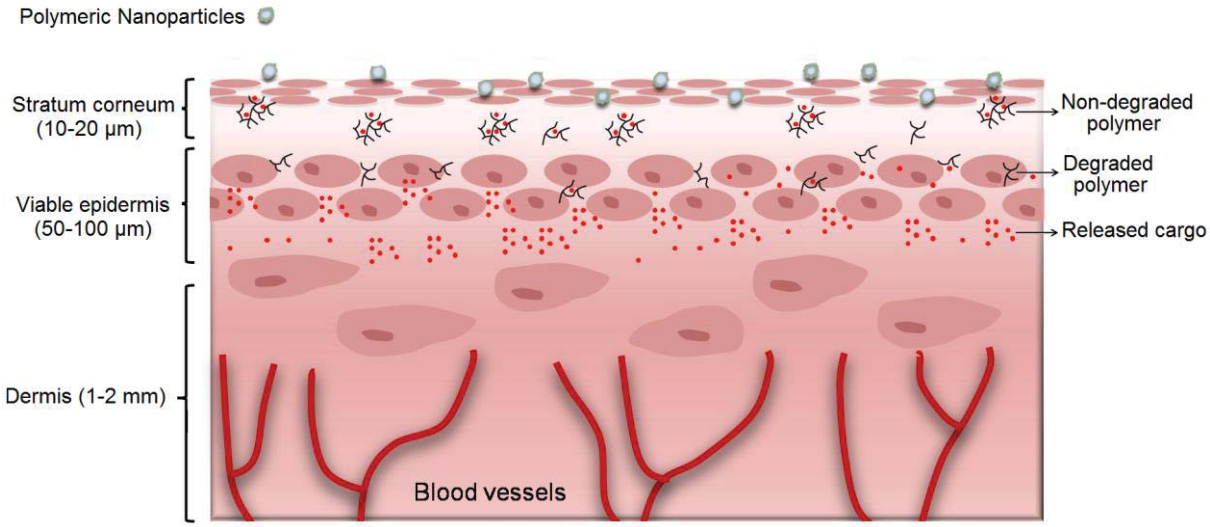
Fatemeh Zabihi, Hanna Koeppe, Katharina Achazi, Sarah Hedtrich, and Rainer Haag

https://doi.org/10.1021/acs.biomac.8b01741

Author contributions

Fatemeh Zabihi performed the main experiments and wrote the manuscript. Hanna Koeppe helped with the synthesis of copolymers and nanogels. Katharina Achazi performed the toxicity and cellular uptake study. Sarah Hedtrich and Rainer Haag discussed the project and corrected the manuscript.

3.4 One-pot and Gram-scale Synthesis of Biodegradable Polyglycerol at Ambient conditions; Nanocarriers for Intradermal Drug Delivery



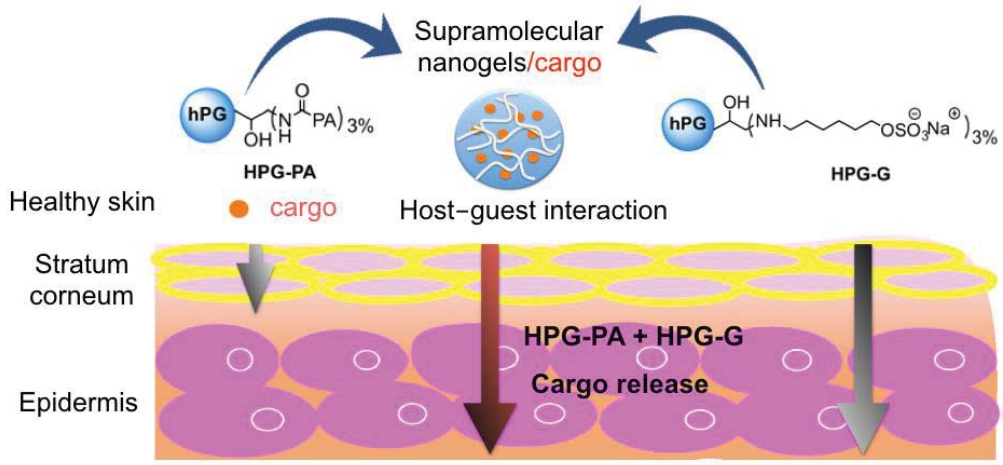
Ehsan Mohammadifar, **Fatemeh Zabihi**, Zhaozu Tu, Sarah Hedtrich, Ali Nemati Kharat, Mohsen Adeli, and Rainer Haag

https://doi.org/10.1039/C7PY01470H

Author contributions

Ehsan Mohammadifar performed the synthesis of materials and wrote the manuscript. Fatemeh Zabihi performed the skin experiments and helped with the writing of the manuscript. Zhaozu Tu helped with the cellular uptake studies. Sarah Hedtrich, Ali Nemati Kharat, Mohsen Adeli, and Riner Haag conceptualized the project and corrected the manuscript.

3.5 Supramolecular Nanogels Fabricated via Host–guest Molecular Recognition as Penetration Enhancer for Dermal Drug Delivery



Lingyan Gao, **Fatemeh Zabihi**, Svenja Ehrmann, Sarah Hedtrich, Rainer Haag

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Author contributions

Lingyan Gao performed the synthesis of materials and wrote the manuscript. Fatemeh Zabihi performed the skin experiments and helped with the writing of manuscript. Svenja Ehrmann recorded TEM images and performed the data analysis. Sarah Hedtrich and Rainer Haag discussed the project and corrected the manuscript.

4 Summary and Outlook

Non-biodegradability, low loading capacity, non-specific interaction with drugs, and low yield synthetic protocols are four main challenges that prevent many biomedical applications of polyglycerol derivatives. In this study we tried to address and solve these problems. We also showed the advantages of the synthesized materials for the biomedical applications exemplified by dermal delivery of known therapeutic agents.

In the first project, new nanogel-peptide conjugates for the specific interactions with *m*-THPC, as targeted therapeutic agent, were synthesized and their ability to delivery this drug through the skin was evaluated. Loading capacity of nanogels was improved 16-fold compared to the nanogels without peptide segments. Furthermore, nanogel-peptide conjugates specifically loaded the targeted drug in a complex media. Our results show that peptide segments, which were synthesized and selected because they combined well, are very effective bioligands for specific interaction with the target molecules. Conjugation of these bioligands to the surface of nanocarriers, in this case, polyglycerol nanogels improved their specific biointeraction, loading capacity, and bioavailability.

In the second project, short poly(lactide) segments were incorporated in the backbone of polyglycerol to obtain biodegradable polyglycerol derivatives. Due to the one-pot and straightforward synthesis, poly(lactide-co-glycerol) was synthesized on a gram scale. This copolymer was freely soluble in water and showed loading capacity as high as 14.5% w/w for tacrolimus (TAC), because of the hydrophobic poly(lactide) segments in its backbone.

Skin penetration tests on human skin showed that the synthesized copolymer was able to efficiently deposit Nile red and TAC into the stratum corneum and viable epidermis. The cutaneous biodistribution profile of cargo of poly(lactide-co-glycerol), TAC, showed that 80%, 16%, and 4% of the cutaneous drug level was deposited in the stratum corneum, viable epidermis, and upper dermis, respectively. The delivered TAC efficiently suppressed the IL-2 and TSLP expressions in human skin models. One of the main goals of this project was to synthesize a biodegradable polyglycerol derivative. In order to investigate the biodegradability of the synthesized copolymer, it was incubated with skin lysates. It was found that poly(lactide-co-glycerol) efficiently is broken down to small segments. Results of this project showed that a short-chain polylactides could be incorporated in the backbone of polyglycerol; the reaction can be performed in one step and on gram scale. In spite of considerable molar ratio of poly(lactide) segments, poly(lactide-co-glycerol) are highly soluble in aqueous solutions. This is due to the fact that poly(lactide) segments are incorporated in the backbone of copolymer and they are surrounded by polyglycerol segments. Increasing the hydrophobicity of polyglycerol

backbone resulted in the highest known loading capacity for a very hydrophobic drug, i.e., TAC. Biodegradability of poly(lactide-co-glycerol) in a skin-mimicked media decreased the risk of toxicity, which is very promising for future dermal delivery.

In the third project, succinic acid was copolymerized with glycidol using two different catalysts, $Sn(Oct)_2$ and novozyme. It was found that both catalysts were able to copolymerize these monomers. Interestingly, copolymerization in the presence of novozyme resulted in nanogels, because this enzyme showed a dual role including activation of monomers and catalyzing esterification of the produced copolymers. Succinic anhydride is a cheap and commercially available compound and the one-pot copolymerization with glycidol resulted in nanogels in gram quantities. To the best of our knowledge, this is the first protocol for a gram-scale synthesis of polyglycerol nanogels. The synthesized nanogels showed a high ability for loading of hydrophobic drugs such as *m*-THPC and TAC. Different experiments showed that poly(glycerol-succinic anhydride) nanogels were able to efficiently improve the skin penetration of their cargos. Straightforward and gram-scale synthesis, high loading capacity, skin penetration, and biodegradability of poly(glycerol-succinic anhydride) nanogels make them fascinating candidates for dermal delivery application with a minimal health risk.

In the fourth project, similar to the second project, glycidol was copolymerized ε-caprolactone and biodegradable poly(glycerol-caprolactone) with the ability of loading and transport TAC through the skin. Poly(glycerol-caprolactone) was degraded by skin lysate efficiently, but it showed much lower loading capacities for TAC in comparison with poly(lactide-co-glycerol). The lower loading capacity for poly(glycerol-caprolactone) could be due to the flexibility of poly(caprolactone) segments. High biocompatibility, water solubility, biodegradability, and the ability to improve the skin penetration of its cargo show that this copolymer is a promising candidate for topical drug delivery applications.

In the final project, supramolecular nanogels compromised of pillar[5]arene and guest alkyl chains were produced and used for the dermal delivery of dexamethasone. Owing to their supramolecular nature, such nanogels were able to efficiently diffuse into skin and improve the skin penetration of their cargo. This project showed that supramolecular systems are very efficient alternatives for the dermal delivery of therapeutic agents. They could be more promising when both host and guest segments are natural compounds.

In summary, incorporation of ester linkages in the backbone of polyglycerols by gram scale one-pot copolymerization of glycidol with the known cyclic monomers resulted in biodegradable polyglycerols or nanogels with a high ability to load and transfer therapeutic agents into the skin.

5 Kurzzusammenfassung

Drei Hauptprobleme behindern, die viele biomedizinische Anwendungen von Polyglycerin-Derivaten. Die nicht vorhandene biologische Abbaubarkeit, die geringe Beladungskapazität und die schwache Wechselwirkungen mit hydrophobenWirkstoffen. In dieser Arbeit wurden, die oben genannten Probleme adressiert. Die Vorteile der synthetisierten Materialien für die biomedizinischen Anwendungen wurden am Beispiel der dermalen Wirkstoffabgabe bekannter Therapeutika untersucht.

Im ersten Projekt wurden neue Nanogel-Peptidkonjugate für die spezifischen Wechselwirkungen mit Temoporfin (m-THPC) als zielgerichtetem Therapeutikum synthetisiert und ihre Fähigkeit, dieses Medikament über die Haut zu verabreichen, untersucht. Die Beladungs-Kapazität von Nanogelen mit Peptidsegmenten wurde im Vergleich zu den Nanogelen ohne Peptidsegmente um den Faktor 16 verbessert. Weiterhin konnte gezeigt werden, dass die Nanogel-Peptidkonjugate dieses gezielte Medikament in einem komplexen Medium spezifisch einschliessen. Die ausgewählten Peptidsegmente weisen sehr effektive Bioliganden für die spezifischen Wechselwirkungen mit den verwendeten Wirkstoffen auf. Die Konjugation dieser Bioliganden auf Nanotransporter, in diesem Fall Polyglycerin-Nanogelen, verbesserte deren spezifische Biointeraktionen, Beladungskapazität, sowie deren Bioverfügbarkeit.

Im zweiten Projekt wurden kurze Poly(lactid)-Segmente in das Rückgrat von Polyglycerin integriert, um biologisch abbaubare Polyglycerin-Derivate zu erhalten. In der Eintopfreaktion wurde Poly(lactid-co-glycerin) im Gramm-Maßstab synthetisiert. Dieses Copolymer war wasserlöslich und zeigte aufgrund der hydrophoben Poly(lactid)-Segmente eine erhöhte Ladungskapazität von Tacrolimus (TAC) von bis zu 14,5 Gewichtsprozent.

Hautpenetrationstests an der menschlichen Haut zeigten, dass das synthetisierte Copolymer in der Lage ist, Nil Rot und TAC in das Stratum corneum und in die Epidermis zu transportieren. Das kutane Biodistributionsprofil der Poly(lactide-co-glycerol) beladen mit TAC zeigte, dass 80%, des kutanen Medikamentenspiegels im Stratum corneum, 16% der Epidermis und 4% der oberen Dermis deponiert wurden. Das zum Wirkort transportierte TAC unterdrückte effizient die IL-2 und TSLP-Expressionen in menschlichen Hautmodellen. Eines der Hauptziele dieses Projekts war die Synthese von biologisch abbaubaren Polyglycerin-Derivaten. Um die biologische Abbaubarkeit des synthetisierten Copolymers zu untersuchen, wurde es mit Hautlysaten inkubiert. Hier wurde festgestellt, dass sich Poly(lactide-co-glycerol) effizient zu kleinen Segmenten abbaut. Die Ergebnisse dieses Projekts zeigten, dass kurzkettiges Polylactide in das Polyglycerin Rückgrat eingebaut werden können und die

Eintopfreaktion des Copolymers im Gramm-Maßstab möglich ist. Trotz des beträchtlichen Molverhältnisses von Poly(lactid)-Segmenten sind Poly(lactide-co-glycerine) gut wasserlöslich. Dies ist darauf zurückzuführen, dass Poly(lactid)-Segmente in das Rückgrat des Copolymers integriert und von Polyglycerin-Segmenten umgeben sind. Die Erhöhung der Hydrophobie des Polyglycerin-Grundgerüsts führte zur erhöhten Beladungskapazität für sehr hydrophobe Wirkstoffe, wie TAC.

Die biologische Abbaubarkeit von Poly(lactide-co-glycerin) in den Hautmedien vermindert das Toxizität-Risiko des Transporters und ist vielversprechend für zukünftige therapeutische Anwendungen in der Haut.

Im dritten Projekt wurde Bernsteinsäure mit Glycidol unter Verwendung von Zinn(II)-2-ethylhexanoat und Novozym als Katalysator copolymerisiert. Beide Katalysatoren sind in der Lage, diese Monomere zu copolymerisieren. Interessanterweise führte die Copolymerisation in Gegenwart von Novozym zu Nanogelen, da dieses Enzym zum einen die Monomeren aktiviert und die Veresterung der hergestellten Copolymere katalysiert. Bernsteinsäureanhydrid ist eine preiswerte und kommerziell erhältliche Substanz und führt in einer Eintopfreaktion im Gramm-Maßstab zur Copolymerisation mit Glycidol zu Nanogelen. Nach unserem Kenntnisstand ist dies das erste Reaktions-Protokoll für die Gramm-Synthese von Polyglycerin-Nanogelen. Die synthetisierten Nanogele weisen eine hohe Beladungs-Kapazität für hydrophoben Wirkstoffe wie m-THPC und TAC auf. Verschiedene Experimente zeigten, dass Poly(glycerin-bernsteinsäureanhydrid)-Nanogele in der Lage sind, die Hautpenetration der beladenen Wirkstoffe zu verbessern. Die einfache Synthese im Gramm-Maßstab, eine hohe Beladungskapazität, eine hohe Hautpenetration und biologische Abbaubarkeit der Poly(glycerin-bernsteinsäureanhydrid)-Nanogelen machen diese zu erfolgsversprechenden Transportern für die dermale Applikation.

Im vierten Projekt wurde, ähnlich wie im zweiten Projekt, Glycidol mit ε-Caprolacton zu einem biologisch abbaubares Poly(glycerin-caprolacton) copolymersiert. Der resultierende Nanotransporter hat die Fähigkeit, TAC aufzunehmen und durch die Haut zu transportieren. Poly(glycerin-caprolacton) wurde im Hautlysat effizient abgebaut, zeigte aber im Vergleich zu Poly(lactide-co-glycerin) eine wesentlich geringere Beladungseffizienz mit TAC. Dies könnte auf die Flexibilität von den Poly(caprolacton)-Segmenten zurückzuführen sein. Hohe Biokompatibilität, Wasserlöslichkeit, biologische Abbaubarkeit und die Fähigkeit, die Hautpenetration des zu transportierenden Wirkstoffes zu verbessern, zeigen, dass dieses Copolymer ein vielversprechender Kandidat für die topische Anwendung ist.

In fünften Projekt wurden supramolekulare Nanogele bestehend aus Pillar[5]arenen und Gast-Alkylketten hergestellt und für den dermale Transport von Dexamethason verwendet. Aufgrund ihrer supramolekularen Natur konnten die Nanogele effizient in die Haut diffundieren und die Hautpenetration des geladenen Wirkstoffes verbessern. Dieses Projekt zeigte, dass supramolekulare Systeme sehr effiziente Alternativen für den dermale Transport von therapeutischen Wirkstoffen darstellen. Diese könnten durch Verwendung von natürlichen vorkommenden Wirt- und Gast-Segmenten noch vielversprechender für die therapeutische Anwendung sein.

Zusammengefasst führt der Einbau von Esterbindungen in das Polymerrückgrat durch eine Eintopfreaktion von Glycidol mit den oben genannten cyclischen Monomeren zu biologisch abbaubaren Polyglycerinen oder Nanogelen im Gramm Maßstab. Die hergestellten Nanotransporter weisen eine hohe Beladungskapazität für hydrophobe, therapeutische Wirkstoffe auf und können diese effizient in die Haut transportieren.

6 References

[1] (a) S. Kumar, N. Alnasif, E. Fleige, I. Kurniasih, V. Kral, A. Haase, A. Luch, G. Weindl, R. Haag, M. Schäfer-Korting, S. Hedtrich, *European Journal of Pharmaceutics and Biopharmaceutics* **2014**, *88*, 625-634; (b) K. E. Uhrich, S. M. Cannizzaro, R. S. Langer, K. M. Shakesheff, *Chemical Reviews* **1999**, *99*, 3181-3198.

[2] (a) D. W. Pack, A. S. Hoffman, S. Pun, P. S. Stayton, *Nature Reviews Drug Discovery* **2005**, *4*, 581; (b) D. Putnam, *Nature Materials* **2006**, *5*, 439; (c) B. Shi, M. Zheng, W. Tao, R. Chung, D. Jin, D. Ghaffari, O. C. Farokhzad, *Biomacromolecules* **2017**, *18*, 2231-2246.

[3] (a) O. S. Wolfbeis, *Chemical Society Reviews* **2015**, *44*, 4743-4768; (b) A. Reisch, A. S. Klymchenko, *Small* **2016**, *12*, 1968-1992.

[4] (a) A. Higuchi, Q.-D. Ling, S.-T. Hsu, A. Umezawa, *Chemical Reviews* **2012**, *112*, 4507-4540; (b) B. B. Shotorbani, E. Alizadeh, R. Salehi, A. Barzegar, *Materials Science and Engineering: C* **2017**, *71*, 1192-1200.

[5] T. Nezakati, A. Seifalian, A. Tan, A. M. Seifalian, *Chemical Reviews* **2018**, *118*, 6766-6843.

[6] C. A. García-González, A. Concheiro, C. Alvarez-Lorenzo, *Bioconjugate Chemistry* **2015**, *26*, 1159-1171.

[7] A. Dong, Y.-J. Wang, Y. Gao, T. Gao, G. Gao, *Chemical Reviews* **2017**, *117*, 4806-4862.

[8] F. Rancan, M. Giulbudagian, J. Jurisch, U. Blume-Peytavi, M. Calderón, A. Vogt, *European Journal of Pharmaceutics and Biopharmaceutics* **2017**, *116*, 4-11.

[9] S. Abdus, U. Zabih, I. A. Mohammad, W. Mohd, S. Mohammad Shabaz, *Recent Patents on Drug Delivery & Formulation* **2009**, *3*, 143-152.

[10] J. Wu, K. S. Paudel, C. Strasinger, D. Hammell, A. L. Stinchcomb, B. J. Hinds, *Proceedings of the National Academy of Sciences of the United States of America* **2010**, *107*, 11698-11702.

[11] K. S. Paudel, M. Milewski, C. L. Swadley, N. K. Brogden, P. Ghosh, A. L. Stinchcomb, *Therapeutic delivery* **2010**, *1*, 109-131.

[12] L. B. Naves, C. Dhand, J. R. Venugopal, L. Rajamani, S. Ramakrishna, L. Almeida, *Progress in Biomaterials* **2017**, *6*, 13-26.

[13] (a) A. Mitra, Y. Wu, *Expert Opinion on Drug Delivery* **2010**, *7*, 977-992; (b) B. C. Palmer, L. A. DeLouise, *Molecules (Basel, Switzerland)* **2016**, *21*, E1719.

[14] T.-K. Lin, L. Zhong, J. L. Santiago, *International Journal of Molecular Sciences* **2018**, *19*, 70.

[15] (a) A. Pfalzgraff, K. Brandenburg, G. Weindl, *Frontiers in Pharmacology* **2018**, *9*, 281; (b) M. A. Dos Santos Ramos, P. B. Da Silva, L. Spósito, L. G. De Toledo, B. V. Bonifácio, C. F. Rodero, K. C. Dos Santos, M. Chorilli, T. M. Bauab, *International Journal of Nanomedicine* **2018**, *13*, 1179-1213.

[16] A. S. B. Goebel, R. H. H. Neubert, J. Wohlrab, *International Journal of Pharmaceutics* **2011**, *404*, 159-168.

[17] (a) Y. Chen, P. Quan, X. Liu, M. Wang, L. Fang, *Asian Journal of Pharmaceutical Sciences* **2014**, *9*, 51-64; (b) P. Karande, A. Jain, K. Ergun, V. Kispersky, S. Mitragotri, *Proceedings of the National Academy of Sciences of the United States of America* **2005**, *102*, 4688-4693.

[18] C. S. Linsley, B. M. Wu, *Therapeutic Delivery* **2017**, *8*, 89-107.

[19] A. Zaid Alkilani, M. T. C. McCrudden, R. F. Donnelly, *Pharmaceutics* **2015**, *7*, 438-470.

[20] (a) A. T. Haine, Y. Koga, Y. Hashimoto, T. Higashi, K. Motoyama, H. Arima, T. Niidome, *European Journal of Pharmaceutics and Biopharmaceutics* **2017**, *119*, 91-95; (b) T. Niidome, *Oleoscience* **2014**, *14*, 17-21.
[21] C. Gerecke, A. Edlich, M. Giulbudagian, F. Schumacher, N. Zhang, A. Said, G. Yealland, S. B. Lohan, F. Neumann, M. C. Meinke, N. Ma, M. Calderón, S. Hedtrich, M. Schäfer-Korting, B. Kleuser, *Nanotoxicology* **2017**, *11*, 267-277.
[22] (a) F. Du, S. Hönzke, F. Neumann, J. Keilitz, W. Chen, N. Ma, S. Hedtrich, R. Haag, *Journal of Controlled Release* **2016**, *242*, 42-49; (b) M. Adeli, H. Namazi, F. Du, S. Hönzke, S. Hedtrich, J. Keilitz, R. Haag, *RSC Advances* **2015**, *5*, 14958-14966.
[23] M. Witting, M. Molina, K. Obst, R. Plank, K. M. Eckl, H. C. Hennies, M. Calderón, W. Frieß, S. Hedtrich, *Nanomedicine: Nanotechnology, Biology and Medicine* **2015**, *11*, 1179-1187.
[24] F. Erdő, N. Hashimoto, G. Karvaly, N. Nakamichi, Y. Kato, *Journal of Controlled Release* **2016**, *233*, 147-161.
[25] M. Boer, E. Duchnik, R. Maleszka, M. Marchlewicz, *Advances in Dermatology and Allergology/Postępy Dermatologii i Alergologii* **2016**, *33*, 1-5.
[26] (a) G. M. El Maghraby, B. W. Barry, A. C. Williams, *European Journal of Pharmaceutical Sciences* **2008**, *34*, 203-222; (b) C. Pailler-Mattei, S. Nicoli, F. Pirot, R. Vargiolu, H. Zahouani, *Colloids and Surfaces B: Biointerfaces* **2009**, *68*, 200-206.
[27] P. W. Wertz, *Skin Pharmacology and Physiology* **2013**, *26*, 217-226.
[28] (a) H. Tagami, *International Journal of Cosmetic Science* **2008**, *30*, 413-434; (b) J. W. Fluhr, H. Dickel, O. Kuss, I. Weyher, T. L. Diepgen, E. Berardesca, *British Journal of Dermatology* **2002**, *146*, 770-776.
[29] S. Verdier-Sévrain, F. Bonté, *Journal of Cosmetic Dermatology* **2007**, *6*, 75-82.
[30] (a) J. A. Bouwstra, P. L. Honeywell-Nguyen, G. S. Gooris, M. Ponec, *Progress in Lipid Research* **2003**, *42*, 1-36; (b) M. Stawczyk-Macieja, A. Szczerkowska-Dobosz, K. Rębała, D. Purzycka-Bohdan, *Advances in Dermatology and Allergology/Postępy Dermatologii i Alergologii* **2015**, *32*, 123-126.
[31] I. Plasencia, L. Norlén, L. A. Bagatolli, *Biophysical Journal* **2007**, *93*, 3142-3155.
[32] (a) J. A. Bouwstra, M. Ponec, *Biochimica et Biophysica Acta (BBA) - Biomembranes* **2006**, *1758*, 2080-2095; (b) K. Feingold, P. Elias, *Biochimica et Biophysica Acta (BBA) - Molecular and Cell Biology of Lipids* **2014**, *1841*, 279.
[33] T. Krieg, M. Aumailley, *Experimental Dermatology* **2011**, *20*, 689-695.
[34] (a) M. Pawlaczyk, M. Lelonkiewicz, M. Wieczorowski, *Advances in Dermatology and Allergology/Postępy Dermatologii i Alergologii* **2013**, *30*, 302-306; (b) H. Joodaki, M. B. Panzer, *Proceedings of the Institution of Mechanical Engineers, Part H: Journal of Engineering in Medicine* **2018**, *232*, 323-343.
[35] S. MacNeil, *Nature* **2007**, *445*, 874.
[36] R. R. Wickett, M. O. Visscher, *American Journal of Infection Control* **2006**, *34*, S98-S110.
[37] G. WEDDELL, E. PALMER, W. PALLIE, *Biological Reviews* **1955**, *30*, 159-195.
[38] L. Johannsen, S. R. L. Coward, G. R. Martin, A. M. Wing, A. v. Casteren, W. I. Sellers, A. R. Ennos, R. H. Crompton, S. K. S. Thorpe, *Scientific Reports* **2017**, *7*, 1135.
[39] A. A. Romanovsky, *Acta physiologica (Oxford, England)* **2014**, *210*, 498-507.
[40] M. E. Balañá, H. E. Charreau, G. J. Leirós, *World Journal of Stem Cells* **2015**, *7*, 711-727.
[41] C.-C. Yang, G. Cotsarelis, *Journal of dermatological science* **2010**, *57*, 2.
[42] T. J. Ryan, *Journal of Investigative Dermatology* **1976**, *67*, 110-118.
[43] A. Sbarbati, D. Accorsi, D. Benati, L. Marchetti, G. Orsini, G. Rigotti, P. Panettiere, *European Journal of Histochemistry : EJH* **2010**, *54*, e48.
[44] W. F. Richter, B. Jacobsen, *Drug Metabolism and Disposition* **2014**, *42*, 1881-1889.

[45] T. Ishida, K. Takeuchi, S. Hayashi, S. Kawata, N. Hatayama, N. Qu, M. Shibata, M. Itoh, *Okajimas Folia Anatomica Japonica* **2015**, *92*, 1-6.
[46] P. M. Elias, *Journal of Investigative Dermatology* **2005**, *125*, 183-200.
[47] P. M. Elias, G. K. Menon, in *Advances in Lipid Research, Vol. 24* (Ed.: P. M. Elias), Elsevier, **1991**, pp. 1-26.
[48] M. Brattsand, K. Stefansson, C. Lundh, Y. Haasum, T. Egelrud, *Journal of Investigative Dermatology* **2005**, *124*, 198-203.
[49] (a) M. H. Braff, A. D. Nardo, R. L. Gallo, *Journal of Investigative Dermatology* **2005**, *124*, 394-400; (b) K. M. Aberg, K. A. Radek, E.-H. Choi, D.-K. Kim, M. Demerjian, M. Hupe, J. Kerbleski, R. L. Gallo, T. Ganz, T. Mauro, K. R. Feingold, P. M. Elias, *The Journal of Clinical Investigation* **2007**, *117*, 3339-3349.
[50] J. L. Sugarman, J. W. Fluhr, A. J. Fowler, T. Bruckner, T. L. Diepgen, M. L. Williams, *Archives of Dermatology* **2003**, *139*, 1417-1422.
[51] K. Sato, in *Reviews of Physiology, Biochemistry and Pharmacology, Volume 79*, Springer Berlin Heidelberg, Berlin, Heidelberg, **1977**, pp. 51-131.
[52] H. L. Johnson, H. I. Maibach, *Journal of Investigative Dermatology* **1971**, *56*, 182-188.
[53] G. Cizza, A. H. Marques, F. Eskandari, I. C. Christie, S. Torvik, M. N. Silverman, T. M. Phillips, E. M. Sternberg, *Biological Psychiatry* **2008**, *64*, 907-911.
[54] T. TAKEMURA, P. W. WERTZ, K. SATO, *British Journal of Dermatology* **1989**, *120*, 43-47.
[55] S. J. Genuis, D. Birkholz, I. Rodushkin, S. Beesoon, *Archives of Environmental Contamination and Toxicology* **2011**, *61*, 344-357.
[56] S.-S. Zhou, D. Li, W.-P. Sun, M. Guo, Y.-Z. Lun, Y.-M. Zhou, F.-C. Xiao, L.-X. Jing, S.-X. Sun, L.-B. Zhang, N. Luo, F.-N. Bian, W. Zou, L.-B. Dong, Z.-G. Zhao, S.-F. Li, X.-J. Gong, Z.-G. Yu, C.-B. Sun, C.-L. Zheng, D.-J. Jiang, Z.-N. Li, *World Journal of Gastroenterology : WJG* **2009**, *15*, 5674-5684.
[57] H.-Y. Chen, J.-Y. Fang, *Expert Opinion on Therapeutic Patents* **2000**, *10*, 1035-1043.
[58] M. Joshi, B. S. Butola, K. Saha, *Journal of Nanoscience and Nanotechnology* **2014**, *14*, 853-867.
[59] (a) A. C. Williams, B. W. Barry, *Advanced Drug Delivery Reviews* **2004**, *56*, 603-618; (b) D. Chantasart, S. K. Li, *Pharmaceutics* **2012**, *4*, 71-92.
[60] B. Palmer, L. DeLouise, *Molecules* **2016**, *21*, 1719.
[61] (a) N. Parnami, T. Garg, G. Rath, A. K. Goyal, *Artificial Cells, Nanomedicine, and Biotechnology* **2014**, *42*, 406-412; (b) R. Ghaffarian, S. Muro, *Journal of visualized experiments : JoVE* **2013**, e50638-e50638.
[62] D. Singh Malik, N. Mital, G. Kaur, *Expert Opinion on Therapeutic Patents* **2016**, *26*, 213-228.
[63] M. B. Brown, G. P. Martin, S. A. Jones, F. K. Akomeah, *Drug Delivery* **2006**, *13*, 175-187.
[64] B. J. Bruno, G. D. Miller, C. S. Lim, *Therapeutic Delivery* **2013**, *4*, 1443-1467.
[65] K. T. Kim, J. S. Kim, M.-H. Kim, J.-H. Park, J.-Y. Lee, W. Lee, K. K. Min, M. G. Song, C.-Y. Choi, W.-S. Kim, H. K. Oh, D.-D. Kim, *Biomolecules & Therapeutics* **2017**, *25*, 434-440.
[66] X. Tan, S. R. Feldman, J. Chang, R. Balkrishnan, *Expert Opinion on Drug Delivery* **2012**, *9*, 1263-1271.
[67] (a) J. Pham, A. Nayel, C. Hoang, T. Elbayoumi, *Drug Delivery* **2016**, *23*, 1514-1524; (b) M. Chen, S. Kumar, A. C. Anselmo, V. Gupta, D. H. Slee, J. A. Muraski, S. Mitragotri, *Journal of Controlled Release* **2015**, *199*, 190-197; (c) D. Cosco, D. Paolino, J. Maiuolo, L. Di Marzio, M. Carafa, C. A. Ventura, M. Fresta, *International Journal of Pharmaceutics* **2015**, *489*, 1-10; (d) Y. T. Zhang, L. I. N. Shen, J. H. Zhao, N. P. Feng, *International Journal of Nanomedicine* **2014**, *9*, 669-678; (e) M. L. Bikkad, A.

H. Nathani, S. K. Mandlik, S. N. Shrotriya, N. S. Ranpise, *Journal of Liposome Research* **2014**, *24*, 113-123; (f) K. Frederiksen, R. H. Guy, K. Petersson, *European Journal of Pharmaceutics and Biopharmaceutics* **2015**, *91*, 9-15.
[68] K. Sugibayashi, S. Nakayama, T. Seki, K.-i. Hosoya, Y. Morimoto, *Journal of Pharmaceutical Sciences* **1992**, *81*, 58-64.
[69] Y. Chen, M. Wang, L. Fang, *Drug Delivery* **2013**, *20*, 199-209.
[70] S. A. Nasrollahi, C. Taghibiglou, E. Azizi, E. S. Farboud, *Chemical Biology & Drug Design* **2012**, *80*, 639-646.
[71] J. L. Zaro, W. C. Shen, *Biochemical and Biophysical Research Communications* **2003**, *307*, 241-247.
[72] P. Thitilertdecha, M. G. Rowan, R. H. Guy, *International Journal of Pharmaceutics* **2015**, *478*, 39-45.
[73] B. A. Brod, *Dermatitis* **1993**, *4*, 124.
[74] A. Otto, J. Du Plessis, J. W. Wiechers, *International Journal of Cosmetic Science* **2009**, *31*, 1-19.
[75] F. Harusawa, T. Saito, H. Nakajima, S. Fukushima, *Journal of Colloid and Interface Science* **1980**, *74*, 435-440.
[76] G. M. Eccleston, *Colloids and Surfaces A: Physicochemical and Engineering Aspects* **1997**, *123-124*, 169-182.
[77] P. Aikens, S. E. Friberg, *Current Opinion in Colloid & Interface Science* **1996**, *1*, 672-676.
[78] J. L. Chollet, M. J. Jozwiakowski, K. R. Phares, M. J. Reiter, P. J. Roddy, H. J. Schultz, Q. V. Ta, M. A. Tomai, *Pharmaceutical Development and Technology* **1999**, *4*, 35-43.
[79] (a) B. W. Barry, G. M. Saunders, *Journal of Colloid and Interface Science* **1970**, *34*, 300-315; (b) H. K. Patel, R. C. Rowe, J. McMahon, R. F. Stewart, *International Journal of Pharmaceutics* **1985**, *25*, 13-25.
[80] A. Otto, J. W. Wiechers, C. L. Kelly, J. C. Dederen, J. Hadgraft, J. du Plessis, *Skin Pharmacology and Physiology* **2010**, *23*, 273-282.
[81] P. L. Goggin, R. He, D. Q. M. Craig, D. P. Gregory, *Journal of Pharmaceutical Sciences* **1998**, *87*, 559-564.
[82] S. E. Friberg, *The Canadian Journal of Chemical Engineering* **2007**, *85*, 602-608.
[83] B. Santini, I. Zanoni, R. Marzi, C. Cigni, M. Bedoni, F. Gramatica, L. Palugan, F. Corsi, F. Granucci, M. Colombo, *PLoS ONE* **2015**, *10*, e0126366.
[84] (a) S. Savic, G. Vuleta, R. Daniels, C. C. Müller-Goymann, *Colloid and Polymer Science* **2005**, *283*, 439-451; (b) N. Vučinić-Milanković, S. Savić, G. Vuleta, S. Vučinić, *Drug Development and Industrial Pharmacy* **2007**, *33*, 221-234.
[85] L. A. M. Ferreira, J. Doucet, M. Seiller, J. L. Grossiord, J. P. Marty, J. Wepierre, *International Journal of Pharmaceutics* **1995**, *121*, 169-179.
[86] G. M. Eccleston, L. Beattie, *Drug Development and Industrial Pharmacy* **1988**, *14*, 2499-2518.
[87] M. M. Jiménez, J. Pelletier, M. F. Bobin, M. C. Martini, *International Journal of Pharmaceutics* **2004**, *272*, 45-55.
[88] A. D. POZZO, N. PASTORI, *International Journal of Cosmetic Science* **1996**, *18*, 57-66.
[89] G. M. M. El Maghraby, A. C. Williams, B. W. Barry, *International Journal of Pharmaceutics* **2000**, *204*, 159-169.
[90] J. A. Bouwstra, D. A. van Hal, H. E. J. Hofland, H. E. Junginger, *Colloids and Surfaces A: Physicochemical and Engineering Aspects* **1997**, *123-124*, 71-80.
[91] S. Jain, N. Patel, M. K. Shah, P. Khatri, N. Vora, *Journal of Pharmaceutical Sciences* **2017**, *106*, 423-445.

[92] I. F. UCHEGBU, A. SCHÄTZLEIN, G. VANLERBERGHE, N. MORGATINI, A. T. FLORENCE, *Journal of Pharmacy and Pharmacology* **1997**, *49*, 606-610.
[93] S. Hua, *Frontiers in Pharmacology* **2015**, *6*, 219.
[94] B. Iqbal, J. Ali, S. Baboota, *International Journal of Dermatology* **2018**, *57*, 646-660.
[95] H. E. J. HOFLAND, J. A. BOUWSTRA, H. E. BODDÉ, F. SPIES, H. E. JUNGINGER, *British Journal of Dermatology* **1995**, *132*, 853-866.
[96] M. Dorrani, O. B. Garbuzenko, T. Minko, B. Michniak-Kohn, *Journal of Controlled Release* **2016**, *228*, 150-158.
[97] S. Hua, T. H. Dias, D.-G. Pepperall, Y. Yang, *Frontiers in Pharmacology* **2017**, *8*.
[98] G. M. El Zaafarany, G. A. S. Awad, S. M. Holayel, N. D. Mortada, *International Journal of Pharmaceutics* **2010**, *397*, 164-172.
[99] D. Liu, H. Hu, Z. Lin, D. Chen, Y. Zhu, S. Hou, X. Shi, *Journal of Photochemistry and Photobiology B: Biology* **2013**, *127*, 8-17.
[100] A. P. Perez, M. J. Altube, P. Schilrreff, G. Apezteguia, F. S. Celes, S. Zacchino, C. I. de Oliveira, E. L. Romero, M. J. Morilla, *Colloids and Surfaces B: Biointerfaces* **2016**, *139*, 190-198.
[101] F. Wang, X. Bao, A. Fang, H. Li, Y. Zhou, Y. Liu, C. Jiang, J. Wu, X. Song, *Frontiers in Pharmacology* **2018**, *9*, 91.
[102] M. F. M. Ali, H. F. Salem, H. F. Abdelmohsen, S. K. Attia, *Drug Design, Development and Therapy* **2015**, *9*, 2431-2447.
[103] J. Malakar, S. O. Sen, A. K. Nayak, K. K. Sen, *Saudi Pharmaceutical Journal : SPJ* **2012**, *20*, 355-363.
[104] D. Paolino, G. Lucania, D. Mardente, F. Alhaique, M. Fresta, *Journal of Controlled Release* **2005**, *106*, 99-110.
[105] C. Kasetvatin, S. Rujivipat, W. Tiyaboonchai, *Colloids and Surfaces B: Biointerfaces* **2015**, *135*, 458-464.
[106] S. Jain, N. Patel, P. Madan, S. Lin, *Pharmaceutical Development and Technology* **2015**, *20*, 473-489.
[107] D. Ainbinder, E. Touitou, *Drug Delivery* **2005**, *12*, 297-303.
[108] M. Shumilov, E. Touitou, *International Journal of Pharmaceutics* **2010**, *387*, 26-33.
[109] J. Torin Huzil, S. Sivaloganathan, M. Kohandel, M. Foldvari, *Wiley Interdisciplinary Reviews: Nanomedicine and Nanobiotechnology* **2011**, *3*, 449-462.
[110] R. Gupta, B. Rai, *Scientific Reports* **2017**, *7*, 45292.
[111] O. Lee, S. H. Jeong, W. U. Shin, G. Lee, C. Oh, S. W. Son, *Skin Research and Technology* **2013**, *19*, e390-e396.
[112] U. M. Musazzi, B. Santini, F. Selmin, V. Marini, F. Corsi, R. Allevi, A. M. Ferretti, D. Prosperi, F. Cilurzo, M. Colombo, P. Minghetti, *Journal of Nanobiotechnology* **2017**, *15*, 14.
[113] Y. K. Tak, S. Pal, P. K. Naoghare, S. Rangasamy, J. M. Song, *Scientific Reports* **2015**, *5*, 16908.
[114] (a) J. Lademann, H. J. Weigmann, C. Rickmeyer, H. Barthelmes, H. Schaefer, G. Mueller, W. Sterry, *Skin Pharmacology and Physiology* **1999**, *12*, 247-256; (b) J. Schulz, H. Hohenberg, F. Pflücker, E. Gärtner, T. Will, S. Pfeiffer, R. Wepf, V. Wendel, H. Gers-Barlag, K. P. Wittern, *Advanced Drug Delivery Reviews* **2002**, *54*, S157-S163; (c) F. Rancan, Q. Gao, C. Graf, S. Troppens, S. Hadam, S. Hackbarth, C. Kembuan, U. Blume-Peytavi, E. Rühl, J. Lademann, A. Vogt, *ACS Nano* **2012**, *6*, 6829-6842.
[115] (a) G. P. Dransfield, *Radiation Protection Dosimetry* **2000**, *91*, 271-273; (b) C. Antoniou, M. Kosmadaki, A. Stratigos, A. Katsambas, *Journal of the European Academy of Dermatology and Venereology* **2008**, *22*, 1110-1119.
[116] M.-H. Tan, C. A. Commens, L. Burnett, P. J. Snitch, *Australasian Journal of Dermatology* **1996**, *37*, 185-187.

[117] G. Raju, N. Katiyar, S. Vadukumpully, S. A. Shankarappa, *Journal of Dermatological Science* **2018**, *89*, 146-154.
[118] R. Gupta, B. Rai, *The Journal of Physical Chemistry B* **2016**, *120*, 7133-7142.
[119] (a) F. Larese Filon, M. Crosera, G. Adami, M. Bovenzi, F. Rossi, G. Maina, *Nanotoxicology* **2011**, *5*, 493-501; (b) D. Pissuwan, T. Niidome, in *Functional Nanoparticles for Bioanalysis, Nanomedicine, and Bioelectronic Devices Volume 2, Vol. 1113*, American Chemical Society, **2012**, pp. 69-80.
[120] (a) F. S. Abdel-Salam, S. A. Elkheshen, A. A. Mahmoud, H. O. Ammar, *Bulletin of Faculty of Pharmacy, Cairo University* **2016**, *54*, 1-7; (b) S. Mukherjee, S. Ray, R. S. Thakur, *Indian Journal of Pharmaceutical Sciences* **2009**, *71*, 349-358.
[121] R. H. Müller, K. Mäder, S. Gohla, *European Journal of Pharmaceutics and Biopharmaceutics* **2000**, *50*, 161-177.
[122] T. B. Dudala, P. R. Yalavarthi, H. C. Vadlamudi, J. Thanniru, G. Yaga, N. L. Mudumala, V. K. Pasupati, *International Journal of Pharmaceutical Investigation* **2014**, *4*, 149-155.
[123] F. Chia-Lang, A. A.-S. Saleh, F. Jia-You, *Recent Patents on Nanotechnology* **2013**, *7*, 41-55.
[124] (a) H. Fan, G. Liu, Y. Huang, Y. Li, Q. Xia, *Applied Surface Science* **2014**, *288*, 193-200; (b) B. Li, Z.-Q. Ge, *AAPS PharmSciTech* **2012**, *13*, 276-283.
[125] C. Valenta, B. G. Auner, *European Journal of Pharmaceutics and Biopharmaceutics* **2004**, *58*, 279-289.
[126] K. Sugibayashi, Y. Morimoto, *Journal of Controlled Release* **1994**, *29*, 177-185.
[127] (a) J. K. Oh, *Molecular Pharmaceutics* **2017**, *14*, 2459-2459; (b) M. W. Tibbitt, J. E. Dahlman, R. Langer, *Journal of the American Chemical Society* **2016**, *138*, 704-717.
[128] M. Lapteva, K. Mondon, M. Möller, R. Gurny, Y. N. Kalia, *Molecular Pharmaceutics* **2014**, *11*, 2989-3001.
[129] J. Suksaeree, W. Pichayakorn, C. Monton, A. Sakunpak, T. Chusut, W. Saingam, *Industrial & Engineering Chemistry Research* **2014**, *53*, 507-513.
[130] A. A. D'souza, R. Shegokar, *Expert Opinion on Drug Delivery* **2016**, *13*, 1257-1275.
[131] N. Aggarwal, S. Goindi, S. D. Mehta, *AAPS PharmSciTech* **2012**, *13*, 67-74.
[132] (a) K. Mondon, M. Zeisser-Labouèbe, R. Gurny, M. Möller, *Photochemistry and Photobiology* **2011**, *87*, 399-407; (b) C. Di Tommaso, F. Behar-Cohen, R. Gurny, M. Möller, *Annales Pharmaceutiques Françaises* **2011**, *69*, 116-123.
[133] Y. G. Bachhav, K. Mondon, Y. N. Kalia, R. Gurny, M. Möller, *Journal of Controlled Release* **2011**, *153*, 126-132.
[134] J. Liaw, Y. Lin, *J Control Release* **2000**, *68*, 273-282.
[135] P. Loan Honeywell-Nguyen, A. M. de Graaff, H. W. Wouter Groenink, J. A. Bouwstra, *Biochimica et Biophysica Acta (BBA) - General Subjects* **2002**, *1573*, 130-140.
[136] M. Thanou, J. C. Verhoef, H. E. Junginger, *Advanced Drug Delivery Reviews* **2001**, *50*, S91-S101.
[137] M. M. Thanou, A. F. Kotzé, T. Scharringhausen, H. L. Lueßen, A. G. de Boer, J. C. Verhoef, H. E. Junginger, *Journal of Controlled Release* **2000**, *64*, 15-25.
[138] (a) C. Jonker, J. H. Hamman, A. F. Kotzé, *International Journal of Pharmaceutics* **2002**, *238*, 205-213; (b) G. Di Colo, S. Burgalassi, Y. Zambito, D. Monti, P. Chetoni, *Journal of Pharmaceutical Sciences* **2004**, *93*, 2851-2862.
[139] X. Zhou, D. Liu, H. Liu, Q. Yang, K. Yao, X. Wang, L. Wang, X. Yang, *Journal of Pharmaceutical Sciences* **2010**, *99*, 2991-2998.
[140] H.-X. Lv, Z.-H. Zhang, X.-P. Wang, Q.-Q. Cheng, W. Wang, X.-H. Huang, J.-P. Zhou, Q. Zhang, L.-L. Hou, W. Huo, *Molecules* **2011**, *16*, 6778.
[141] T. Akimoto, Y. Nagase, *Journal of Controlled Release* **2003**, *88*, 243-252.

[142] (a) T. Aoyagi, T. Nakamura, Y. Yabuchi, Y. Nagase, *Polymer Journal* **1992**, *24*, 545; (b) T. Aoyagi, Y. Takamura, T. Nakamura, Y. Yabuchi, Y. Nagase, *Polymer* **1992**, *33*, 2203-2207.
[143] T. Aoyagi, T. Akimoto, Y. Nagase, *Die Makromolekulare Chemie* **1992**, *193*, 2821-2828.
[144] T. Akimoto, T. Aoyagi, J.-i. Minoshima, Y. Nagase, *Journal of Controlled Release* **1997**, *49*, 229-241.
[145] T. Akimoto, K. Kawahara, Y. Nagase, T. Aoyagi, *Macromolecular Chemistry and Physics* **2000**, *201*, 2729-2734.
[146] (a) J. Yang, Q. Zhang, H. Chang, Y. Cheng, *Chemical Reviews* **2015**, *115*, 5274-5300; (b) S. H. Medina, M. E. H. El-Sayed, *Chemical Reviews* **2009**, *109*, 3141-3157.
[147] M. Sowinska, Z. Urbanczyk-Lipkowska, *New Journal of Chemistry* **2014**, *38*, 2168-2203.
[148] K. Dave, V. V. K. Venuganti, *Therapeutic Delivery* **2017**, *8*, 1077-1096.
[149] (a) V. V. K. Venuganti, O. P. Perumal, *Journal of Pharmaceutical Sciences* **2009**, *98*, 2345-2356; (b) V. V. Venuganti, P. Sahdev, M. Hildreth, X. Guan, O. Perumal, *Pharmaceutical Research* **2011**, *28*, 2246.
[150] G. Cevc, U. Vierl, *Journal of Controlled Release* **2010**, *141*, 277-299.
[151] (a) K. D. Peck, A.-H. Ghanem, W. I. Higuchi, *Pharmaceutical Research* **1994**, *11*, 1306-1314; (b) V. Aguilella, K. Kontturi, L. Murtomäki, P. Ramírez, *Journal of Controlled Release* **1994**, *32*, 249-257; (c) T. Inamori, A.-H. Ghanem, W. I. Higuchi, V. Srinivasan, *International Journal of Pharmaceutics* **1994**, *105*, 113-123; (d) M. Bragagni, N. Mennini, F. Maestrelli, M. Cirri, P. Mura, *Drug Delivery* **2012**, *19*, 354-361.
[152] V. V. K. Venuganti, O. P. Perumal, *International Journal of Pharmaceutics* **2008**, *361*, 230-238.
[153] K. Borowska, S. Wołowiec, A. Rubaj, K. Głowniak, E. Sieniawska, S. Radej, *International Journal of Pharmaceutics* **2012**, *426*, 280-283.
[154] A. S. Chauhan, S. Sridevi, K. B. Chalasani, A. K. Jain, S. K. Jain, N. K. Jain, P. V. Diwan, *Journal of Controlled Release* **2003**, *90*, 335-343.
[155] Y. Yang, S. Sunoqrot, C. Stowell, J. Ji, C.-W. Lee, J. W. Kim, S. A. Khan, S. Hong, *Biomacromolecules* **2012**, *13*, 2154-2162.
[156] in *Biomedical Applications of Nanotechnology*.
[157] U. Agrawal, N. K. Mehra, U. Gupta, N. K. Jain, *Journal of Drug Targeting* **2013**, *21*, 497-506.
[158] S. Abbina, S. Vappala, P. Kumar, E. M. J. Siren, C. C. La, U. Abbasi, D. E. Brooks, J. N. Kizhakkedathu, *Journal of Materials Chemistry B* **2017**, *5*, 9249-9277.
[159] D. Wilms, S.-E. Stiriba, H. Frey, *Accounts of Chemical Research* **2010**, *43*, 129-141.
[160] (a) M. Calderón, M. A. Quadir, S. K. Sharma, R. Haag, *Advanced Materials* **2010**, *22*, 190-218; (b) R. Rai, M. Tallawi, A. Grigore, A. R. Boccaccini, *Progress in Polymer Science* **2012**, *37*, 1051-1078.
[161] E. A. T. Vargas, N. C. do Vale Baracho, J. de Brito, A. A. A. de Queiroz, *Acta Biomaterialia* **2010**, *6*, 1069-1078.
[162] (a) E. Mohammadifar, M. Adeli, A. N. Kharat, H. Namazi, R. Haag, *Macromolecular Chemistry and Physics* **2017**, *218*, 1600525; (b) A. Boreham, M. Pfaff, E. Fleige, R. Haag, U. Alexiev, *Langmuir* **2014**, *30*, 1686-1695; (c) S. Küchler, M. Abdel-Mottaleb, A. Lamprecht, M. R. Radowski, R. Haag, M. Schäfer-Korting, *International journal of pharmaceutics* **2009**, *377*, 169-172; (d) J. Keilitz, M. R. Radowski, J.-D. Marty, R. Haag, F. Gauffre, C. Mingotaud, *Chemistry of Materials* **2008**, *20*, 2423-2425.
[163] M. Unbehauen, E. Fleige, F. Paulus, B. Schemmer, S. Mecking, S. Moré, R. Haag, *Polymers* **2017**, *9*, 316.

[164] S. Hönzke, C. Gerecke, A. Elpelt, N. Zhang, M. Unbehauen, V. Kral, E. Fleige, F. Paulus, R. Haag, M. Schäfer-Korting, B. Kleuser, S. Hedtrich, *Journal of Controlled Release* **2016**, *242*, 50-63.
[165] M. Radbruch, H. Pischon, A. Ostrowski, P. Volz, R. Brodwolf, F. Neumann, M. Unbehauen, B. Kleuser, R. Haag, N. Ma, U. Alexiev, L. Mundhenk, A. D. Gruber, *Nanoscale Research Letters* **2017**, *12*, 64.
[166] M. Asadian-Birjand, A. Sousa-Herves, D. Steinhilber, J. C. Cuggino, M. Calderon, *Current Medicinal Chemistry* **2012**, *19*, 5029-5043.
[167] (a) F. Rancan, M. Asadian-Birjand, S. Dogan, C. Graf, L. Cuellar, S. Lommatzsch, U. Blume-Peytavi, M. Calderón, A. Vogt, *Journal of Controlled Release* **2016**, *228*, 159-169; (b) M. Asadian-Birjand, J. Bergueiro, F. Rancan, J. C. Cuggino, R. C. Mutihac, K. Achazi, J. Dernedde, U. Blume-Peytayi, A. Vogt, M. Calderón, *Polymer Chemistry* **2015**, *6*, 5827-5831.
[168] M. Dimde, F. F. Sahle, V. Wycisk, D. Steinhilber, L. C. Camacho, K. Licha, J. Lademann, R. Haag, *Macromolecular Bioscience* **2017**, *17*, 1600505.
[169] R. K. Kainthan, D. E. Brooks, *Biomaterials* **2007**, *28*, 4779-4787.
[170] (a) E. Mohammadifar, A. Bodaghi, A. Dadkhahtehrani, A. Nemati Kharat, M. Adeli, R. Haag, *ACS Macro Letters* **2017**, *6*, 35-40; (b) R. A. Shenoi, J. K. Narayanannair, J. L. Hamilton, B. F. L. Lai, S. Horte, R. K. Kainthan, J. P. Varghese, K. G. Rajeev, M. Manoharan, J. N. Kizhakkedathu, *Journal of the American Chemical Society* **2012**, *134*, 14945-14957; (c) S. Son, E. Shin, B.-S. Kim, *Macromolecules* **2015**, *48*, 600-609; (d) R. A. Shenoi, I. Chafeeva, B. F. L. Lai, S. Horte, J. N. Kizhakkedathu, *Journal of Polymer Science Part A: Polymer Chemistry* **2015**, *53*, 2104-2115; (e) M. Hu, M. Chen, G. Li, Y. Pang, D. Wang, J. Wu, F. Qiu, X. Zhu, J. Sun, *Biomacromolecules* **2012**, *13*, 3552-3561.
[171] S. Stefani, S. Hönzke, J. L. Cuellar Camacho, F. Neumann, A. K. Prasad, S. Hedtrich, R. Haag, P. Servin, *Polymer* **2016**, *96*, 156-166.
[172] E. S. Yin, J. Lewis, H. Suh, W. M. Saltzman, M. Girardi, *Journal of Investigative Dermatology* **2017**, *137*, S115.
[173] V. Reshetov, H.-P. Lassalle, A. François, D. Dumas, S. Hupont, S. Gräfe, V. Filipe, W. Jiskoot, F. Guillemin, V. Zorin, L. Bezdetnaya, *International Journal of Nanomedicine* **2013**, *8*, 3817-3831.

7 Appentix

7.1 Publication and conference contributions

Publications:

1. M. Adeli, N. Mirab, **F. Zabihi**, Nanocapsules Based on Carbon Nanotubes-graft-polyglycerol Hybrid Materials, *Nanotechnology* **2009**, *20*, 485603.

2. M. Adeli, B. Rasoulian, F. Saadatmehr, **F. Zabihi**, Hyperbranched Poly(citric acid) and its Application as Anticancer Drug Delivery System, *Journal of Applied Polymer Science* **2013**, *129*, 3665.

3. M. Adeli, H. Hosainzadegan, I. Pakzad, **F. Zabihi**, M. Alizadeh, F. Karimi, Preparing Starchy Foods Containing Silver Nanoparticles and Evaluating Antimicrobial Activity, *Jundishapur Journal of Microbiology* **2013**, *6*, 5075.

4. **F. Zabihi**, S. Wieczorek, M. Dimde, S. Hedtrich, H. G. Börner, R. Haag, Intradermal Drug Delivery by Nanogel-Peptide Conjugates; Specific and Efficient Transport of Temoporfin, *J. Control. Release* **2016**, *242*, 35-41.

5. **F. Zabihi**, P. Graff, F. Schumacher, B. Kleuser, S. Hedtrich, R. Haag, Synthesis of Poly(lactide-co-glycerol) as a Biodegradable and Biocompatible Polymer with High Loading Capacity for Dermal Drug Delivery, *Nanoscale* **2018**, *10*, 16848-16856.

6. E. Mohammadifar, **F. Zabihi**, Z. Tu, S. Hedtrich, A. Nemati Kharat, M. Adeli, R. Haag, One-pot and Gram-scale Synthesis of Biodegradable Polyglycerol at Ambient Conditions; Nanocarriers for Intradermal Drug Delivery, *Polym. Chem.* **2017**, *8*, 7375-7383.

7. L. Gao, **F. Zabihi,** S. Ehrmann, S. Hedtrich, R. Haag, Supramolecular Nanogels Fabricated via Host–guest Molecular Recognition as Penetration Enhancer for Dermal Drug Delivery, (Submitted 2018)

8. **F. Zabihi**, H. Koeppe, K. Achazi, S. Hedtrich, R. Haag, One-Pot Synthesis of Poly(glycerol-co-succinic acid) Nanogels for Dermal Delivery. (Submitted 2018)

Books:

M. Adeli, N. Mirab, **F. Zabihi**, Novel Polymers a Gatway to Nanoscience and Nanotechnology: Novel Polymers and Nanoscience,Transworld Research Network, 2008.

Patents:

E. Mohamadifar, M. Ferraro, **F. Zabihi**, M. Adeli, R. Haag, Synthesis of Biodegradable Polyglycerol Sulfates as Extracellular Matrix Mimic. 2018, WO 2008/015015 A2.

Conference contributions

1. 5th Galinus Workshop: "The Advanced Use of Nanocarriers in Future Skin Drug Delivery," Berlin, Germany, November 16-18, 2016, poster presentation: **Fatemeh Zabihi**, Sebastian Wieczorek, Mathias Dimde, Sarah Hedtrich, Hans G. Börner, and Rainer Haag. Title of poster: "Intradermal Drug Delivery by Nanogel-Peptide Conjugates; Specific and Efficient Transport of Temoporfin".

2. Final Colloquium of the SFB 1112: Freien Universität Berlin, Germany, June 6. 2018, oral presentation: **Fatemeh Zabihi**, "Synthesis and skin penetration of biodegradable polyglycerols".

3. 9th International Seminar on Polymer Science and Technology, Iran Polymer and Petrochemical Institute, Tehran, Iran, October 17-21, 2009, M. Bavadi, M. Kalantari. M. Sagvand, M. Ashiri, **F. Zabihi**, Z. Sobhani, F. Atyabi, M. Adeli.

7.2 Curriculum Vitae

Due to privacy reasons, it has not been included.