

STAR PARTICLES FOR ENHANCED CUTANEOUS DELIVERY OF TOPICAL THERAPEUTICS

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To my loving family and trusted friends.

For believing in me.

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LIST OF SYMBOLS AND ABBREVIATIONS

5-FU	5-fluorouracil
AIBN	azobisisobutyronitrile
Al ₂ O ₃	alumina or aluminum oxide
ANOVA	analysis of variance
BLEO	bleomycin
CMC	carboxymethyl cellulose
CO ₂	carbon dioxide
CPE	chemical permeation enhancer
D	dermis
DI	deionized
DNA	deoxyribonucleic acid
DMSO	dimethyl sulfoxide
FITC-Dextran	fluorescein isothiocyanate-dextran
H&E	hematoxylin and eosin
IACUC	Institutional Animal Care and Use Committee
IRB	Institutional Review Board
MN	microneedle
MTX	methotrexate
P	statistical value
PBS	phosphate buffered saline
PDMS	polydimethylsiloxane

PLA	polylactic acid
PLGA	poly(lactic-co-glycolic acid)
PVP	polyvinylpyrrolidone
RNA	ribonucleic acid
SC	stratum corneum
SRB	sulforhodamine B
STAR	skin treatment and rejuvenation
cSTAR	ceramic STAR particles
mSTAR	metal STAR particles
SU8	polymeric photoresist epoxy
VE	viable epidermis

SUMMARY

Skin disorders are among the most prevalent medical indications in the world and are estimated to impact 30-70% of the global population. It is therefore no surprise that the dermatologic and cosmeceutic industries represents a fast growing multi-billion-dollar market segment. Despite the medical need and market opportunity for efficacious skin products, only a small subset of therapies possesses suitable physicochemical properties that allow passive skin absorption: namely, low molecular weight and lipophilic molecules. As a result, there is significant unmet need for innovative solutions to enhance delivery of bioactive agents for treatment of skin conditions.

To that end, STAR particles were developed in this study as a novel technology platform designed to capture the simplicity of formulation-based delivery methods (i.e., skin creams, lotions, gels) and the efficacy of physical permeation enhancers (e.g., microneedles). STAR particles are millimeter-scale particles with micron-scale projections. The projections found on STAR particles are similar in size to those found on conventional microneedle patches (approximately 50-1000 μm). As STAR particles are rubbed onto skin, the microscopic projections painlessly penetrate skin's stratum corneum to thereby enhance skin permeability. STAR particles differentiate themselves from conventional microneedle-based technologies in that they are not bound by a macro-scale substrate (e.g., a patch or roller-style device). Rather, STAR particles can be directly incorporated into topical products and applied to skin in a manner that is similar to conventional skin products (e.g., sunscreen). In this way, STAR particles can be spread across skin surface areas typically inaccessible to microneedle patches. The overall

objectives of this study were to design, fabricate, characterize and assess STAR particles to increase skin permeability in a minimally invasive, user-friendly and flexible manner.

The first objective of this study focused on the design and development of STAR particles made from stainless steel and ceramic materials. Stainless steel and ceramics were chosen for STAR particles due to their historical precedence for safe use in clinical settings (e.g., hypodermic needle injections for metal and orthopedic/dental implants for ceramics), robust mechanical integrity and capacity for high-throughput prototyping using conventional micro-fabrication techniques, such as laser ablation. Additionally, ceramic STAR particles satisfy criteria related to low-environmental burden, low-cost of materials, and cosmetically favorable for use in topical creams (i.e., ceramics can blend in with white skin creams).

STAR particle parameters were characterized for their effects to increase skin permeability by measuring gentian violet staining area, skin electrical resistance and skin delivery of fluorescent model compounds after pre-treatment with STAR particle formulations. Results from these studies demonstrated that STAR particles created microscopic punctures in skin and thereby increased skin permeability.

Next, metal STAR particles were studied on hairless rats (*in vivo*) to determine if STAR particles are well tolerated and efficacious when applied to the skin of live animals.

Assessment of skin following application of STAR particles showed very slight erythema at the site of application with no adverse effects such as bleeding, swelling or bruising. It

was also demonstrated through gentian violet staining, skin electrical resistance and delivery of fluorescent dye that STAR particle application significantly increased skin permeability in hairless rats *in vivo*. Moreover, an increase in skin delivery of fluorescent dye was observed after 15 minutes of topical application relative to 3 hours of fluorescent dye application to intact skin controls. We conclude from these results that STAR particles enabled an increase in topical delivery into live animals with less than one-tenth the topical wear time relative to untreated skin. Additionally, STAR particle delivery enhancement of 6- to 14-fold was demonstrated with biomolecules of clinical significance for treatment of skin disease: 5-fluorouracil, methotrexate and bleomycin.

The next objective of this study focused on characterization of metal and ceramic STAR particles. In these studies, STAR particle parameters (e.g., size, concentration, thickness and geometry) were investigated to determine their effects on skin permeability *ex vivo*. We found that STAR particles could be engineered to increase skin permeability to varying degrees (e.g., by varying the concentration of STAR particles applied to skin). A wide range of delivery enhancement was demonstrated, between 14- to 90-fold increase, after 10 seconds of STAR particle skin application (i.e., by adjusting STAR particle concentration). Greater delivery enhancement was also demonstrated if STAR particles were applied to skin for longer times (i.e., 2 minutes). Additionally, ceramic STAR particles with varying geometries were fabricated to investigate design limitations with current fabrication methods. Ceramic STAR particles were assessed for tip sharpness and applied to excised porcine skin to determine their effects on skin permeability. Additionally, fabrication parameters like sintering temperature were determined to have

significant impact on STAR particle mechanical integrity and subsequent performance to increase skin permeability.

In the final objective, STAR particles were assessed in human volunteers for safety, tolerability, acceptability and efficacy. To accomplish this, stainless steel STAR particles were incorporated into commercial aloe vera gel and applied to the forearms of eleven human volunteers. Human subjects were asked to provide qualitative and quantitative assessment of their experience during the study and skin application sites were scored for adverse events (e.g., erythema, tenderness, swelling). Following STAR particle application, most subjects experienced mild, transient erythema for several hours which completely resolved within a day. Additionally, most study participants described STAR particle applications to be comfortable, similar to conventional skin products and concluded they would feel comfortable in self-applying STAR particles. Gentian violet staining of skin application areas revealed that STAR particles were effective to create microscopic punctures in skin. We conclude from these studies that STAR particles were very well tolerated and well accepted when applied to skin of humans, produced very mild, transient erythema and were efficacious to increase skin permeability.

ABSTRACT

Cutaneous delivery of bioactive agents is important for treatment of many medical indications, especially in dermatology. In this work a novel skin penetration enhancement technology, called STAR particles, was designed, fabricated, developed and characterized *ex vivo* and *in vivo*. STAR particles are millimeter-scale particles with micro-scale projections that painlessly pierce skin to thereby enhance topical drug delivery. STAR particles were fabricated from biocompatible materials (stainless steel and alumina) and applied to skin similarly to conventional formulation-based skin products. STAR particle functionality was demonstrated and characterized through quantification of gentian violet skin staining; skin electrical resistance; and delivery of topically applied compounds (sulforhodamine B, 4 kDa FITC-Dextran, 5-fluorouracil, methotrexate and bleomycin).

After STAR particle pre-treatment, skin electrical resistance decreased by an order of magnitude or greater. Additionally, gentian violet skin staining enabled visualization of the many dozen microscopic skin puncture sites created by STAR particles. STAR particles skin treatment also enhanced cutaneous delivery of molecules by up to 90-fold. The results from these studies demonstrate that STAR particles functioned to increase skin permeability through the formation of microscopic skin punctures. STAR particles were also demonstrated to be safe, tolerable, efficacious and acceptable when applied to skin of healthy human participants. In conclusion, STAR particles can be used as a formulation-based additive in topical therapies to enhance delivery of topically applied

therapies. In this way, STAR particles may potentially improve the clinical potential of topically applied drugs and expand the number of biomolecules that can be administered into skin.

Chapter 1 : Introduction

Dermatologic (e.g., psoriasis, eczema, acne, skin cancers) and cosmeceutic (e.g., skin aging, blemishes, scarring) indications, collectively, comprise the most prevalent medical conditions in the world and cause considerable physical, economic and psychosocial distress to those who are affected [1-3]. Despite the great need for effective therapies, delivery of bioactive agents into skin is severely constrained. This is due to the skin's inherent barrier functionality that is derived primarily from the outermost layer – the stratum corneum (SC). As a result, only small, lipophilic molecules are capable of sufficient skin absorption [4]. To overcome this limitation, several delivery strategies have been developed to increase cutaneous delivery.

Formulation-based strategies (e.g., chemical penetration enhancers, CPE, like dimethyl sulfoxide) to increase skin permeability solubilize the lipid-rich SC barrier and thereby marginally increase skin delivery. However, CPE often result in skin irritation and are generally not broadly useful for delivery of macromolecules. In contrast to CPE, physical-based methods to increase skin permeability (e.g., ultrasound, iontophoresis, electroporation, microneedles) are effective at delivering a broader range of molecules including hydrophilic, small molecule drugs, biologics, vaccines, and nucleic acids. However, many physical-based delivery methods suffer from limitations related to device cost, ease of use, procedural invasiveness and ability to target large skin surface areas (e.g., $> 10 \text{ cm}^2$). However, because many skin conditions can manifest across large,

disseminated body surface areas, formulation-based methods (i.e., not physical-based methods) have made the most impact for treatment of cutaneous disorders.

To overcome the limitations associated with currently available skin delivery methods, we developed a novel technology called STAR particles. STAR particles are millimeter-scale particles with radially emanating, micro-scale projections that can be incorporated into topical formulations to increase skin permeability in a simple to use and minimally invasive manner.

Because STAR particles are not restricted to an underlying macro-scale substrate (e.g., microneedle patch) they can be applied to skin as a formulation (e.g., cream, ointment, gel) which facilitates their application to large skin surface areas. Moreover, since STAR particles are envisioned to be a formulation-based ingredient (i.e., not a separate medical device), their application can be a single-step process which improves patient usability and reduces potential for mishandling. In summary, STAR particles combine the usability, acceptability and flexibility of conventional formulation-based treatments while also leveraging the skin permeability enhancement capabilities of physical-based delivery using microneedle-like projections.

The main objective of this project was to fabricate, characterize and develop STAR particles for improved delivery of topical therapies to skin. This objective can be segmented as follows:

- 1) Design, fabrication and development of metal and ceramic STAR particles to increase skin permeability in excised skin. Assess STAR particle safety, tolerability, acceptability and efficacy in live animals and in humans
- 2) Characterization of metal and ceramic STAR particle parameters and fabrication methods to increase skin permeability

Chapter 2 : Background

2.1 SKIN ANATOMY, PHYSIOLOGY AND FUNCTIONALITY

Human skin is a multi-functional organ system that provides essential roles such as thermoregulation, immunological protection, sensory perception and production of vitamin D3 [5]. The skin is composed of stratified squamous epithelia and is divided into two main layers: the outermost layer (epidermis, 100-150 μm thick) that is cell-rich, and the underlying layer (dermis, 1-2 mm thick) that is largely acellular. The dermis, primarily composed of collagen and elastin fibers in an aqueous extracellular matrix, provides mechanical support for the overlaying epidermis. Given its highly porous, sponge-like structure, dermal tissue houses blood capillaries which form plexus sheets near the epidermal basement membrane in the papillary dermis as well as in the deeper reticular dermis. The epidermis can be divided into five distinct sub-layers: (i) stratum corneum (SC), (ii) stratum lucidum, (iii) stratum granulosum, (iv) stratum spinosum and (v) stratum basale, from most superficial to deepest layers, respectively. Skin epidermis is renewed as stem cells in the basal layer continually divide and migrate outwards. In contrast to the dermis, the epidermis is avascular. Therefore, all components necessary for cellular function (e.g., transport of oxygen to and removal of waste products from cells) must diffuse between the epidermal keratinocytes located above the basement membrane and the nearby capillary bed below in the papillary dermis. In addition to skin keratinocytes, other important cell types reside in the skin's epidermis, such as: melanocytes (pigment producing cells), Merkel cells (mechanosensory cells) and Langerhan cells (immune cells) [6].

Unlike the deeper viable epidermis (VE), the SC is composed of terminally differentiated and denucleated skin keratinocytes (i.e., corneocytes) that are continually shed as skin regenerates. Corneocytes are intracellularly filled with crosslinked keratin, high-molecular-weight lipids, fatty acids and ceramides, and are tightly bound together in a densely packed matrix of intercellular lipids – forming what is commonly described a “brick-and-mortar” structure where the corneocytes are bricks and intercellular fatty acids, ceramides and cholesterol forming the mortar [7]. Although only spanning 15-20 cell layers (10-25 μm thick), the SC forms a highly lipophilic membrane that effectively precludes essentially all hydrophilic ($\log P < 1$) and macromolecular species ($M_w > 500$ Da) from entering the body. This SC function is vastly important to prevent harmful chemical compounds and pathogens from entering the body. However, the SC also severely limits delivery of topically applied therapies into skin [8, 9].

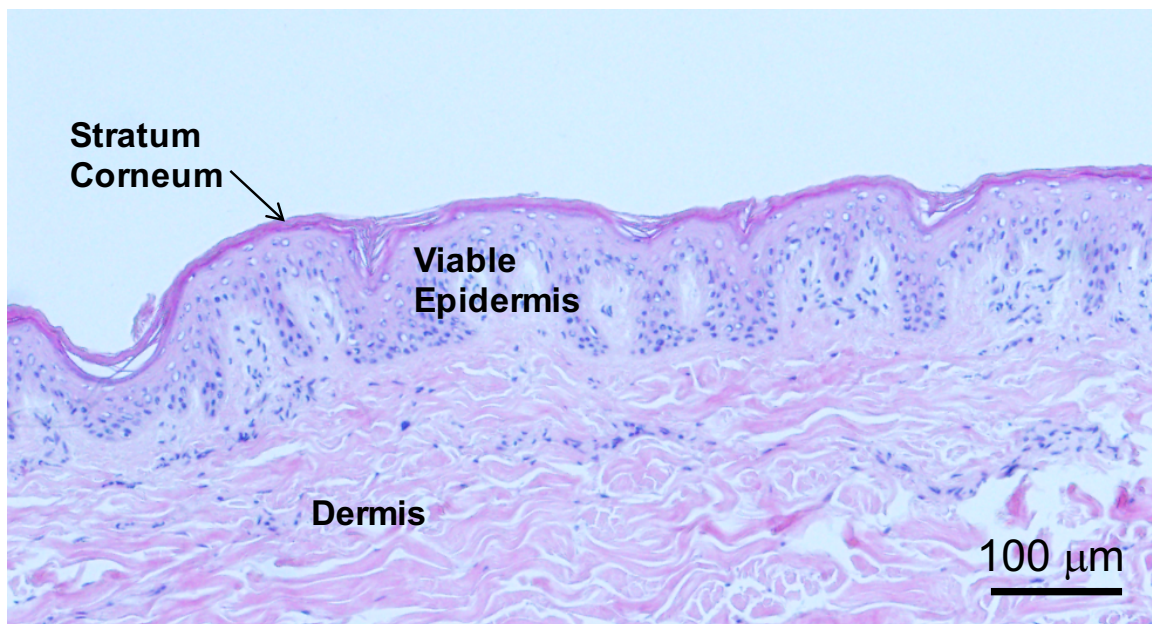


Fig. 2.1: Histological cross section of porcine skin stained with hematoxylin and eosin (H&E) to show skin layers (i.e., stratum corneum, viable epidermis and dermis).
Image credit: Juan L. Mena Lapaix

2.2 CUTANEOUS DISORDERS

Cutaneous disorders are, collectively, the most prevalent group of diseases, estimated to impact 30-70% of all people on the planet [2]. There are approximately 10,000 cutaneous disorders ranging from the relatively benign to highly lethal. What all these skin conditions share is the formidable challenge to develop effective therapies that are simple to use, safe, non-invasive, low-cost and provide rapid restoration of healthy skin physiology. In the following sections, we will outline several skin conditions, their prevalence, pathophysiology and currently available treatments for patients with these disorders.

2.2.1 - Psoriasis

Psoriasis vulgaris is a chronic autoimmune skin disease, affecting 2-3% of the world's population. Psoriasis manifests as hyperkeratotic, scaly skin plaques that can significantly reduce the quality of life for patients with the disease. Pathogenic features of psoriasis include dermal infiltration of T lymphocytes and other leucocytes, and over expression of cutaneous cytokines such as tumor necrosis factor (TNF). Psoriasis can impact patients of all ages; however, it is common for psoriasis to manifest in early adulthood – likely due to genetic factors [10]. Psoriasis vulgaris also has comorbidities for diseases such as psoriatic arthritis. The extent of cutaneous manifestations can either be localized and small, or disseminated across a patient's entire body (**Table 1**).

Table 1: Prevalence of psoriasis severity and body surface area involvement.

Severity	Body surface area affected (%)	Prevalence in patients with psoriasis (%)
Mild	< 3	65-80
Moderate	3-10	20-25
Severe	> 10	5-10

Source: National Psoriasis Foundation.

The extent of disease severity is commonly described through a generalized Psoriasis Area and Severity Index (PASI) score and other clinical assessment tools [11].

Unfortunately, there are limited animal models for psoriasis which makes translational research efforts more difficult.

Due to its high prevalence and chronic, life-long nature, the psoriasis treatment market is estimated to reach \$9B by 2019 and has received a great deal of attention by the pharmacologic and medical research communities [12]. Treatments include small molecule topical agents (e.g., calcitriol, tazarotene), oral systemic drugs (e.g., methotrexate, apremilast) and injectable biologics (e.g., etanercept, adalimumab). Topicals are the most widely used therapies accounting for approximately 80% of treatments for mild, 70% of treatments for moderate and 50% of treatments for severe psoriasis patients. Biologics are used most commonly in patients with severe forms of the disease, or who are unresponsive to other treatments, due to the high cost of biological therapies [13].

2.2.2 - Atopic Dermatitis

Atopic dermatitis, more commonly referred to as atopic eczema (or more simply eczema), is a chronic pruritic skin disease with prevalence of up to 20%. Eczema pathophysiology is not completely understood but is attributable to a heightened immune response to various stimuli (e.g., stress, environmental, diet) that results in excessive skin inflammation, rash and pruritus (itchiness). As a result, patients with eczema experience significant decreases in quality of life, social stigmatization due to visible skin disease, excessive itching, sleep loss and financial burdens [14, 15].

There is currently no known cure for atopic dermatitis. Treatments mainly focus on symptom amelioration such as itch relief, skin repair and reduction of inflammation. Topical emollients are commonly used as a first-line therapy for patients. Topical corticosteroids have also been a mainstay therapy to limit skin inflammation, although corticosteroids can only safely be used for limited periods. Topical calcineurin inhibitors (e.g., cyclosporine, tacrolimus) were more recently introduced and act to limit skin inflammation without many of the side effects experienced with potent steroids (e.g., skin atrophy) [16].

2.2.3 - Acne Vulgaris

Acne vulgaris (i.e., acne) is a common skin disease, estimated to affect nearly 80% of adolescent youth and can extend into adulthood [17]. Acne is the result of hormonal activation of the pilosebaceous unit (i.e., sebaceous gland) and manifests as oily/greasy skin, both non-inflammatory and inflammatory skin lesions, bacterial colonization of hair

follicles, as well as increased risk for skin infections and scar-tissue formation [18]. Unfortunately, acne is most common on visible body regions (e.g., face, neck, upper chest, back) where there is a high density of pilosebaceous units [19]. The prominence of acne on highly visible body regions makes social stigmatization and reduced psychological wellbeing common among individuals with acne [20].

Acne treatments are generally either topical or systemic medications. Topical acne medicines, although sometimes causing minor skin irritation, are well accepted by patients as a first-line therapy for mild acne. Non-prescription topical medications may include active ingredients such as benzoyl peroxide, retinoids (e.g., tretinoin, adapalene, and isotretinoin), antibiotics, salicylic acid and many others. Because many topical agents used have different mechanisms of action, combination therapies are considered more efficacious compared to monotherapy [21].

When topical therapies are insufficiently effective or impractical due to large body surface area involvement, systemic (oral) therapies can be prescribed to patients. Oral therapies such as antibiotics, contraceptives (for women) or isotretinoin are the most common acne medications. Antibiotics (e.g., tetracycline, oxytetracycline, doxycycline, lymecycline) are typically only used for severe cases of acne that are disseminated across large body regions and where topical antibiotics have been ineffective. Contraceptives (e.g., ethinylestradiol, progestogen, levonorgestrel, norethisterone) are commonly prescribed for women with acne as these hormones reduce sebaceous gland activity and reduce adrenal androgens [22]. Finally, isotretinoin has been demonstrated to be highly

efficacious for treatment of moderate to severe acne. Oral isotretinoin is associated with several side effects, most significant of which is teratogenicity. Therefore, isotretinoin is always prescribed along with contraceptives, as well as significant medical guidance and regulation, to women of childbearing age [23].

2.2.4 - Non-Melanoma Skin Cancer

Non-melanoma skin cancer (NMSC) is the most prevalent of all cancers types – constituting more than a third of all new cancer diagnoses in the U.S. [24]. While only a small number of NMSC diagnoses are fatal, patients with NMSC experience significant decreases in their quality of life, disfigurement and high-economic burden [25-27].

NMSC can be subdivided into two primary types which differ in potential for malignancy, clinical manifestation, histological appearance and incidence rate. Basal cell carcinoma (BCC) is the most prevalent form of NMSC and constitutes approximately 80% of NMSC diagnoses. Squamous cell carcinoma (SCC) is less prevalent but has a higher risk of metastasis (between 2-6% [28]) and therefore treated more aggressively. There is increased risk of developing NMSC in fair-skinned populations (i.e., skin types I and II) who sunburn easily and experience frequent sun exposure. In addition to UV exposure, chemical carcinogens (e.g., arsenic, tobacco, coal-tar products) have been shown to increase risk of developing NMSC. Both BCC and SCC are the result of mutated keratinocytes that arise from epidermal basal or squamous cells, respectively [29]. Depending on disease severity (e.g., size, borders, growth rate, depth, histologic features) and anatomical location of NMSC lesions, several treatment options are

available including lesion ablation or excision, photodynamic therapy (PDT) [30] and topical therapies.

The current gold standard for treatment for NMSC is ablative or excisional techniques in which the entire lesion is destroyed or surgically removed, typically in an outpatient setting, by a primary care physician or dermatologist. Superficial NMSC lesions are typically removed via ablative techniques (e.g., electro-desiccation and curettage, cryotherapy) while high-risk NMSC lesions are typically surgically removed (e.g., Mohs micrographic surgery [31], excisional surgery, radiotherapy [32]). In both ablative and excisional techniques, complete removal of all cancerous cells along with a safety margin is critical to minimize potential for future recurrence [33]. Although effective, destructive treatment methods should not be used on immunocompromised or elderly patients as there is increased risk for excessive bleeding and infection. In addition, invasive methods to remove NMSC lesions often produce negative cosmetic outcomes for patients which can reduce quality of life.

In circumstances where excisional techniques are difficult or undesired (e.g., on the face, nose or eyes where cosmetic outcomes have high impact on patient quality of life), non-destructive treatment methods may be utilized. PDT is a form of therapy which involves local delivery of a photosensitizing agent (e.g., 5-aminolaevulinic acid) used in conjunction with an activating light source [34]. In response to photo-activation, the photosensitizer generates reactive oxygen species (ROS) that preferentially accumulate in and locally destroy cancerous causing skin cells. Alternatively, topical agents can be used

to treat NMSC lesions that are superficial and low-risk. Some common drugs that are used for topical therapies are 5-fluorouracil (5-FU) and imiquimod (IMQ) [35]. In recent clinical trials patients with superficial basal cell carcinoma treated with either 5-FU or IMQ experienced reduced recurrence compared to patients treated with PDT. However, in both PDT and topical therapies a significant limitation is poor skin penetration of the topical agents. Therefore, non-destructive treatment methods are only useful for treatment of superficial lesions, which represent approximately 15% of all BCC diagnoses [36].

2.2.5 - Melanoma

Cutaneous melanoma (CM) develops due to an accumulation of DNA damage in the skin's pigment producing cells – melanocytes. Mortality rates for CM have been relatively constant since the 1980s (approximately 2 per 100,000 cases per year) while the incidence of CM diagnoses has increased almost 3-fold from the 1980s to early 2000s (3 cases per 100,000 to 15 cases per 100,000) [37]. There are several genetic mutations that are widely prevalent in CM cells including NRAS and BRAF mutations [38]. Early diagnosis of CM is crucial to prevent disease progression. Diagnoses are typically performed by dermatologists through clinical examination. CM lesions can be identified by clinical features such as lesion asymmetry, border irregularity, color variation, morphogenesis (i.e., rapidly changing size, color or shape) and a diameter greater than 6 mm [39].

Due to its high potential for malignancy, CM are preferentially excised within 4-6 weeks of diagnosis. When lesions are excised early (i.e., tumor depth < 1.5 mm), 10-year

survival rate is greater than 85% [40]. To provide adequate histological analysis and safety margins surrounding the lesion, excisional biopsies are the preferred method to remove cutaneous melanomas. Recommended excision margins are between 1-2 cm and are dependent on the stage of tumor growth, size, thickness and anatomical locations that require favorable cosmetic outcomes (e.g., on the face). In patients where melanoma cells have metastasized, sentinel lymph node biopsies (or excision) may be recommended to assess degree of disease progression. Other types of therapy can be employed alone, or in conjunction with surgical excision, to suppress tumor growth systemically, these include radiotherapy, adjuvant chemotherapy, adjuvant immunotherapy, chemotherapy, chemoimmunotherapy, polychemotherapy, chemoimmunotherapy [41]. In addition to systemic therapies, localized tumor regression can be achieved via intralesional injections of IL-12 [42], DNA vectors [43] as well as others [44, 45].

2.5.6 - Vitiligo

Vitiligo affects 1-4% of the global population and manifests as depigmented macules that range in size from millimeters to centimeters across a patient's body [46]. The pathophysiology of vitiligo is not fully understood but is believed to be an autoimmune response in which melanocyte specific antibodies induce melanocyte death and the elimination of melanin production in skin [47]. Other hypotheses for vitiligo pathogenesis include dysregulation of the melanin production pathway and/or susceptibility of melanocytes to the accumulation of neurochemicals, both of which will result in melanocyte death and loss of skin pigmentation [48]. Histologic features of vitiligo include a lack of both melanin and melanocytes in the epidermis [49]. Most vitiligo

patients are diagnosed before 25 years of age. Previous studies have reported a distribution of 70:15:5 for vitiligo vulgaris, focal vitiligo and segmented vitiligo, respectively [50].

Several methods have been shown to be efficacious in treatment of vitiligo.

Phototherapies are the gold standard of treatment for vitiligo and involve the use of ultraviolet radiation to re-pigment affected skin areas [51]. Phototherapies can be used alone or in conjunction topical formulations (e.g., tacrolimus, tacalcitol) to further enhance re-pigmentation efficacy [52]. Alternatively, topical formulations (e.g., calcipotriol, dihydroxyacetone, corticosteroids, tacrolimus) can be used as a monotherapy treatment of vitiligo [53-56]. Despite the availability of treatment methods for vitiligo patients, there are several limitations associated with ease of phototherapies (which require expensive phototherapy units) and limited bioavailability of topically applied bioactive agents (which limit efficacy).

2.2.7 - Epidermolysis Bullosa

Epidermolysis bullosa (EB) is a group of rare genetic skin disorders that results in chronic formation of skin blisters due to minor trauma (e.g., scratching, abrasion, heat). The most severe form of EB is recessive dystrophic epidermolysis bullosa (RDEB) that results in a mutated type-VII collagen (C7) fibril and total loss of C7 functionality. C7 is a key protein located at the dermal-epidermal junction and acts as an anchor between the basal layer of the epidermis and the papillary dermis. The loss in C7 functionality thereby results in a significantly increased propensity for skin blister formation, complications

from infection (e.g., sepsis), pain, increased likelihood to develop skin cancers, physical deformities like pseudosyndactyly (i.e., fusion of the digits) and an overall reduction in quality and duration of life [57-60].

Treatment options are incredibly limited for RDEB patients and are geared towards palliative care. Although there are no presently efficacious therapies for RDEB, there are several reports of investigative treatments including: allogeneic fibroblast cell implantation [61], induced pluripotent stem cell therapy [62], bone marrow transplants [63], retroviral vectors [64] and injection of recombinant C7 protein [65].

2.2.8 - Skin Aesthetics

Skin appearance has a profound impact on the physical, psychosocial and economic wellbeing of all people [66-68]. Due to strong social pressures for healthy and aesthetically pleasing skin, many people seek cosmetic therapies to improve skin appearance for a wide range of reasons. Some types of aesthetic procedures are accomplished through delivery of bioactive agents (i.e., drugs and/or cosmeceuticals).

Arguably, the most widely used therapies for improvement of skin appearance are topical products that can be purchased without prescription and self-applied by users. The strong preference for topical cosmeceuticals is clearly demonstrated by the growing sales of multi-national corporations that develop products for this market segment (e.g., L'Oréal, Johnson and Johnson, Estee Lauder, Proctor and Gamble) [69]. For example, skin lightening agents (e.g., hydroquinone, azelaic acid, glycolic acid) have found great

popularity for treatment of skin hyperpigmentation from sun damage, trauma, aging, acne and other causes [70]. Certain demographic populations also show preference for light skin tone and therefore utilize skin lightening products [71]. Additional types of topical agents have wide appeal which act to improve skin appearance by reducing wrinkles and stretch marks, improving skin tone and others (e.g., α -, β - and poly-hydroxy acids, salicylic acid, ceramide, vitamin C and E) [72].

In addition to topicals, injectable agents are widely used in cosmetic dermatology. Fillers such as collagen and hyaluronic acid are intradermally injected to reduce wrinkles [73] as well as botulinum toxin can be intramuscularly injected for treatment of glabellar and other facial lines [74].

2.3 DELIVERY OF BIOACTIVE AGENTS INTO SKIN

Given the wide prevalence and negative impact of skin conditions, delivery of bioactive agents into skin is vastly important for efficacious therapies. Several methods to administer bioactive agents into skin exist, each with advantages and limitations.

2.3.1 - Topical Drug Delivery

Topical therapies are highly useful for treatment of skin due to their ease of use, non-invasive nature, capacity to treat flexible size skin surface areas (both large and small), relatively low-cost and direct spatial targeting of skin itself. As a result, topical therapies are widely used within dermatologic and cosmeceutic treatments.

Despite these advantages, topical skin therapies have poor cutaneous bioavailability due to limited skin permeability past the SC barrier layer. Moreover, only a limited number of bioactive compounds, mainly small, lipophilic molecules, are capable of passive-topical absorption into skin. Because of limited skin permeability, as well as other factors, only a limited number of topicals have been approved by the US FDA [75]. Additionally, topical therapies that are currently used to treat skin conditions are restricted to medical indications with only superficial manifestations (e.g., superficial basal cell carcinoma [76]). Topicals therefore have limited efficacy to target deeper lesions (e.g., > 1 mm deep) or in hyperkeratotic skin lesions (e.g., psoriasis, warts). Therefore, although simple and non-invasive, topical therapies are only of limited value in treatment of skin due to poor cutaneous penetration.

2.3.2 - Transdermal Patches

The first transdermal patch was approved by the US FDA for clinical use in 1979 (scopolamine). Since then, a number of drugs have been introduced for delivery through the skin via transdermal patches (e.g., clonidine, fentanyl, lidocaine, nicotine, nitroglycerin, oestradiol, oxybutinin, scopolamine and testosterone) [77]. Like topical formulations, transdermal patches can deliver small (< 500 Da) and lipophilic (i.e., oil soluble) drugs into skin for several days and up to multiple milligrams. The two general types of transdermal patches are reservoir and matrix. Reservoir-style patches incorporate a bioactive agent that is separated from the skin by a semi-permeable membrane. The membrane may act to control the rate of drug diffusion into skin. In contrast, matrix-style patches directly incorporate the bioactive agent along with the supporting structure (e.g.,

polymer) and often the adhesive of the patch. Reservoir-style patches can have greater delivery control relative to matrix-style patches. However, reservoir patches are more complex to design and fabricate which increases cost. Transdermal patches provide an added benefit of more controlled drug delivery into skin when compared to topical formulations. However, because drugs must still cross the SC barrier, transdermal patches are limited to a small number of medical applications in which drugs have sufficient cutaneous bioavailability.

2.3.3 - Intracutaneous Injection of Bioactive Agents

In contrast to the delivery limitations associated with topical creams and transdermal patches, is delivery by intracutaneous injection (i.e., intradermal or intralesional injection via needle and syringe). Intracutaneous injections are performed by superficially inserting a hypodermic needle into skin and depositing essentially any dose or therapeutic molecule directly to the target skin area. Within clinical medicine, intralesional injections have been used to deliver bioactives for treatment of NMSC [78], CM [79], vitiligo [80], warts [81], psoriasis [82], scar tissue [83], skin aging [84] and other indications. Although intracutaneous injections are highly effective for delivery purposes, injections are not feasible in many circumstances due to factors related to patient usability and acceptability. Firstly, intracutaneous injections cannot be performed without a trained healthcare professional and therefore there is little opportunity for patient self-administration. This also makes challenging any treatment schedules that require frequent dosing. Hypodermic needles also produce biohazardous sharps waste and introduce potential for needle-stick injuries or needle reuse. Finally, treatment of large,

disseminated body surfaces is not practical for intracutaneous injections because that would require multiple painful injections to effectively distribute drug across a treatment area. Therefore, although effective at delivering bioactives into the skin, intracutaneous injections do not meet important criteria related to patient usability.

2.3.4 - Systemic Drug Delivery

In contrast to spatially localized delivery methods in skin (i.e., topical, intracutaneous injections), systemic administration can be used to deliver a wide array of bioactive agents with no limitations of skin permeability and favorable patient usability and acceptability. Additionally, systemically administered therapies can easily treat large areas of skin since the bioactive agent is distributed throughout the whole body (including skin). Depending on the bioactive agent, its stability and physiochemical properties, systemic administration can be accomplished through either oral or injectable drug forms.

2.3.4.1 Orally Administered Bioactives

Oral medications are widely used in medicine due to their simplicity in administration relative to other drug administration forms (e.g., injection by needle and syringe). Within treatment of skin, oral medications are used to treat patients with psoriasis [85], atopic dermatitis [14], acne [23], vitiligo [86] and most others indications. Oral therapies are also ideal for treatment of chronic skin conditions because of their simple administration, high efficacy, capacity to deliver a wide array of molecules and ability to distribute bioactive compounds across large skin surface areas. Oral therapies, however, distribute

drug across a patient's whole body which often produces unwanted side effects (e.g., nausea) and thereby reduce patient compliance. Additionally, because oral drugs are non-targeted, most of the bioactive agent is effectively wasted since only a small fraction will reach the site of action in skin.

2.3.4.2 Injectable Agents

Injection-based systemic administration methods (i.e., IV, IM, SC), like oral medications, distribute drug throughout the body. However, unlike oral medications, systemic injections bypass the harsh gastrointestinal tract where many sensitive bioactive agents (e.g., biologics) are degraded. Therefore, injections are necessary for delivery of biomolecules (e.g., proteins, DNA/RNA). Biologics have shown great efficacy over conventional small molecule drugs given their ability to target specific disease markers. The market for biologics to treat psoriasis represent a \$7.5 billion market (2014) [12]. However, like oral therapies, because injected drug forms are usually not spatially targeted to the skin a majority of the (usually very expensive) therapeutic agent is wasted.

2.4 METHODS TO ENHANCE SKIN PERMEABILITY

To overcome delivery limitations associated with the skin, several techniques have been utilized with varying advantages and limitations which are discussed further.

2.4.1 - Chemical Penetration Enhancers

The use of CPE has been widely reported in literature for capability of CPE to increase skin permeability via a formulation-based application method (i.e., non-device based). It

is understood that CPE function to increase skin permeability through several mechanisms including: (i) solubilization of lipophilic components in SC, (ii) denaturing intracellular keratin to increase skin hydration, (iii) reducing adhesion of intercellular desmosomes (iv) increasing drug solubility and (v) improving drug partitioning into skin [87]. CPE have been classified and into numerous categories (e.g., water, urea, sulfoxides, alcohols, pyrrolidones, fatty acids, surfactants, terpenes). In general, CPE are effective at increasing delivery of small molecular entities (e.g., 5-fluorouracil [88]). However, CPE are limited in delivery enhancement potential for larger molecular compounds, especially macromolecules like peptides and proteins. Additionally, many effective CPE (e.g., DMSO) tend to have negative side effects, such as erythema and irritation, when applied to skin [89].

2.4.2 - Skin Abrasion Methods

Dermabrasion involves the removal of the SC barrier layer through forceful application of microscopic, irregularly shaped particles which can be composed of many different biocompatible materials (e.g., polymers, metal oxides, sodium tetraborate decahydrate granules, natural materials like ground fruit pits and ground nut shells) [90]. The microscopic-abrasive particles can be rubbed onto skin via conventional hand application (i.e., like application of other topical agents) or with the assistance of a hand-held microdermabrasion device that projects abrasive particles at the skin's surface. In this way, particles physically abrade/remove skin layers to increase skin permeability. Microdermabrasion methods have been used to treat skin for purposes of increasing skin permeability to topically applied molecules such as 5-FU, clobetasol 17-propionate, and

5-aminolevulinic acid (ALA) [91]. Microdermabrasion methods have also been extensively studied on both animal and human skin to demonstrate safety, tolerability, acceptability and efficacy [92-94].

Microdermabrasion methods have been reported to increase delivery by up to 25-fold when used as a device (i.e., not hand application) [91]. However, microdermabrasion devices are restricted to use only in clinical settings due to device cost and complexity, which limits use. Additionally, if not properly controlled, microdermabrasion devices may remove large amounts of skin tissue and increase risks for infection and scarring.

Exfoliates in topical formulations, which do not involve the use of separate devices, have found broader appeal by consumers in cosmeceutical applications due to the ease of application and ability for patients to self-apply topical formulations in a non-invasive and cost-effective manner [95]. However, application of abrasive agents by hand generally do not function well to significantly increase skin permeability.

2.4.3 - Medical Devices

Several types of medical devices have been employed to overcome skin barrier properties and increase delivery into and through the skin. Medical devices function to impart electrical, sonic, mechanical, thermal or other energy forms onto skin to enable increased penetration of exogenous compounds.

2.4.3.1 Iontophoresis

Transdermal iontophoresis involves the application of a low-voltage, low-current external electric potential to increase delivery of bioactive agents into skin. The increase in skin transport can result from electro-repulsion between current flow and charged drug molecules, electroosmotic flow as solvent convectively draws the bioactive agent into skin along with the solvent's movement [96] or (less commonly) through temporary disruption of skin barrier function [97]. Because transport increase can occur via multiple mechanisms, iontophoretic delivery is not limited to just charged drug compounds.

Several medical applications have found significant value in iontophoretic mediated delivery including: analgesics such as lidocaine [98], steroids and retinoids for acne scar resurfacing [99], tap water for palmar hyperhidrosis [100], pilocarpine for diagnosing cystic fibrosis [101] and others. Iontophoretic mediated skin delivery has been demonstrated to increase delivery by up to several orders of magnitude relative to passive diffusion across the intact skin [102]. Drug delivery can also be modulated (i.e., increased or decreased) by modifying current strengths below 1 mA/cm² to minimize procedural discomfort. There are also accounts of delivering macromolecules such as peptides into skin via iontophoresis [103]. Because of these factors, iontophoresis has received significant attention by clinicians, patients and research communities for its ease of use and potential to increase delivery of small, hydrophilic and charged bioactive agents into skin.

2.4.3.2 Electroporation

Electroporation, like iontophoresis, involves the application of an external electric potential. However, unlike iontophoresis, electroporation in skin utilizes short-duration (up to milliseconds) and high-voltage pulse (up to hundreds of volts) to temporarily perturb the intercellular lipid bilayers of SC. The result of this electrical breakdown is believed to cause an increase (in number and or size) of aqueous pathways in the SC which can enable delivery into skin primarily through diffusion and electrophoretic movement [104]. It should be noted, while iontophoresis acts primarily to increase skin delivery by acting on the drug molecule, electroporation induces a physiological change in skin structure to increase skin delivery. Skin electrical resistance has been measured to dramatically decrease by up to three orders of magnitude and transdermal flux can increase by up to four orders of magnitude immediately after application of electroporation pulses.

Moreover, electroporation has been shown to increase delivery of a wide range of bioactive agents such as application to skin tumors for delivery of chemotherapeutic agents [105]. Effects induced by electroporation on skin are transient, with reports showing up to 90% skin barrier recovery after 30 minutes and greater than 99% recovery after 1-2 hours [106]. Electroporation methods have also been studied in conjunction with other skin penetration enhancement methods to further increase delivery potential [107]. Although effective to increase skin permeability, electroporation has been shown in some settings to cause painful sensations if electrical pulses, duration or voltage are increased beyond certain limits [108].

2.4.3.3 Acoustic Methods

Acoustic methods to increase cutaneous drug delivery have been extensively investigated for several decades. In such methods, ultrasonic waves are directed onto the skin to thereby temporarily permeabilize the SC barrier layer. Both low-frequency ($f < 100$ kHz) and high-frequency ($f > 1$ MHz, therapeutic ultrasound) sound waves have been studied for their skin-penetration enhancement effects. Ultrasound-induced cavitation, where gas bubbles quickly grow and collapse to release shockwaves, is believed to be the primary driver behind low-frequency sonophoresis (LFS). In one study, 20 kHz sonic waves (i.e., low frequency) were applied to excised skin to demonstrate that the mechanism of LFS results in external bubble cavitation on the skin's surface (i.e., not inside the skin) results in a temporarily disrupted skin barrier functionality [109]. High-frequency sonophoresis (HFS) has also been demonstrated to increase skin permeability by focusing energy on the uppermost skin layers. HFS uses high intensity sound waves that are applied to skin for short durations of time (< 20 min). The increase in skin permeability is significantly (up to 1000 times) greater for LFS compared to HFS [110]. Additionally, advancements have enabled shorter application times for LFS skin pre-treatment (between 5-30 minutes) which improves patient usability. The decreased application time has resulted in the use of LFS for applications such as cutaneous delivery analgesics, delivery of insulin for diabetes mellitus, extract interstitial fluid for glucose monitoring as well as others [111].

2.4.4 - Microneedles

Microneedles (MN) were first described in the 1970s but, given limitations associated with micro-fabrication at the time, it wasn't until the turn of the century that MN were reintroduced and became available for investigation. Through the adaptation of microelectronic fabrication methods (e.g., photolithography), MN were developed and utilized to increase skin permeability, as first described by Henry et. al. [112]. MN are needle-like projections, typically 50-1000 μm in length, that are attached to a substrate such as a flat coin-sized patch that is pressed onto skin (i.e., MN Patch). As MN devices are applied to skin they pierce past SC and create transient-aqueous micro-punctures which are large enough to allow delivery of essentially any molecule regardless of molecular size or lipophilicity. Because of their microscopic size, MN penetrate only deep enough to cross the skin barrier but not deeply enough to significantly stimulate nerve endings and cause uncomfortable pain sensations.

In addition, MN are capable of precise spatial drug delivery to the skin itself (e.g., to treat dermatologic conditions [113]) or near dermal capillaries for systemic delivery (e.g., for delivery of vaccines [114] or insulin [115]). MN-based technologies have received significant attention by the business, medical and research communities and multiple ventures have launched to commercialize the technology (e.g., Clearside Biomedical, Micron Biomedical, Corium International, Zosano Pharma). Several generations of MN have been developed for different purposes some of which are discussed further.

2.4.4.1 Silicon and Metal Microneedles

The first MN that were introduced were developed with materials commonly found in the semiconductor industry, such as silicon, and by adapting fabrication techniques such as photolithography and deep reactive chemical etching. Although a strong material that is capable of penetrating into skin, silicon is not widely used in medicine and therefore was not ideal from a regulatory perspective. Moreover, silicon is a relatively expensive material that requires fabrication in a cleanroom environment. As a result of these factors, solid metal MN were developed given the established safety profile for metals in medical practice (e.g., stainless steel hypodermic needles), low commercial cost and relatively simple fabrication.

Solid-metal MN have been fabricated for pre-treatment of skin followed by application of a topical agent. Alternatively, solid-metal MN can be coated with a bioactive molecule in which the coating dissolves once in the skin. In the former, solid-metal MN are pressed onto the skin as a pre-treatment method and a subsequent application of a substance of interest is applied topically to the pre-treated area. This two-step process is often referred to as a “poke-and-patch” method. The advantages of poke-and-patch techniques is the relative simplicity and adaptability to currently used topical therapies. Additionally this method lends well to use of MN in conjunction with other penetration enhancement techniques such as iontophoresis [116], sonophoresis [117] and electroporation [118].

Alternatively, solid-metal microneedles can be coated with a formulation containing a bioactive agent. Coated-metal MN require formulating the drug, vaccine or other

bioactive agent into a viscous solution which can then be dip-coated onto the surface of each MN structure [119]. An important characteristic of coated MN is strong adhesion of the coating formulation to the metal MN and maintaining a sharp tip radius that is capable of skin penetration. Coated MN have been used to deliver a number of bioactive agents into skin for applications such as influenza vaccination via delivery of virus-like particles [120], local anesthesia via delivery of lidocaine [121], treatment of enuresis via delivery of desmopressin [122]. Coated metal MN were also recently demonstrated to be safe and effective in a phase II clinical trial to deliver teriparatide [human PTH 1-34 (TPTD)] for osteoporosis treatment in postmenopausal women [123].

2.4.4.2 Hollow Microneedles

Hollow MN have also been extensively studied in the context of infusing a substance of interest into the skin. Hollow MN have been typically fabricated out of metal or glass to provide mechanical strength during skin penetration and to enable relatively simple fabrication methods such as metal deposition [124] and glass micropipette pulling [125]. Tip geometries have also been extensively studied for their effects on skin insertion and fracture force [126]. A significant benefit of hollow MN is their capacity to quickly deliver relatively large payloads of any bioactive agent directly into the skin at controlled depths [127]. The ability to deliver formulations quickly and without significant reformulation (from the original liquid stock) has enabled hollow MN to be used in multiple applications such as insulin delivery [128], vaccination [129], anesthetics [130] as well as ocular drug delivery to the posterior segment of the eye [131].

2.4.4.3 Polymeric Microneedle Patches

Polymeric MN offer numerous advantages compared to their metal and glass counterparts. Because the MN itself is composed of a biodegradable or water-soluble polymer, the entire MN structure can degrade or dissolve once it penetrates the skin. Moreover, once the MN degrades or dissolves, there is no biohazardous sharps waste and the used MN patch can be disposed of without risk for accidental needle stick injury or needle reuse. Despite these safety and usability benefits, polymeric MN must be properly designed and formulated to achieve a MN tip diameter and compressive strength sufficient to allow for penetration into skin. Additionally, the polymeric material composition should degrade or dissolve quickly once it enters the skin to allow for simple end use. Any polymers used should also be cleared from the skin within a relatively short time frame and not accumulate especially if multiple polymer MN patches are administered to the same skin area (e.g., to treat a chronic skin condition).

The wide diversity of polymeric materials which have been studied for use in drug delivery applications have broadened polymeric MN both in terms of manufacturing methods and functionality. Polymeric MN have been fabricated through techniques such as: solution casting [132], in situ photopolymerization [133], ultrasonic welding of micro-particles [134] and controlled polymer drawing/evaporation [135]. All of these methods lend themselves well to mass production and scalability for mass manufacturing purposes such as in an emergency situation (e.g., pandemic) [136]. In addition, the functionality of polymeric MN can be tuned so delivery rate is controlled based on factors such as polymer degradation time or based on physiochemical triggers (e.g., pH change, glucose

concentration) [137-139]. Therefore, drugs or vaccines can be delivered in a spatially and temporarily controlled manner through proper selection of polymeric materials.

In most circumstances, polymeric MN must be formulated to incorporate various compounds which are necessary to provide functionalities such as increased mechanical strength [140]. The addition of excipients like sugars, carbohydrates and amino acids is essential to limit activity loss of some biomolecules (e.g., vaccines, biologics, polypeptides) during manufacturing and storage of patches [141]. Like with other MN devices, polymeric MN are limited by their small size and can typically only be used to deliver drugs or vaccines which have small dose requirements (i.e., less than a few milligrams).

2.4.4.4 Microneedle Roller Devices

In addition to the MN devices already discussed, MN rollers (e.g., Dermalroller) have also been developed. Rather than a flat patch, which is limited in surface area (e.g., $< 10 \text{ cm}^2$), MN rollers incorporate MN structures onto a cylindrical body that can be rolled onto the skin's surface. In this way, MN rollers can increase skin permeability across large surface areas which are inaccessible to traditional MN patches [142]. The MN roller functions as pre-treatment device where the MN roller is followed by a second-step topical treatment such as a transdermal patch or skin cream. Although the MN roller is useful to target larger skin surface areas, the two-step process is burdensome and may negatively impact patient compliance. There are also safety considerations

associated with device sterility if the MN roller is to be used multiple times (i.e., not a single use, disposable device).

2.4.4.5 Ceramic Microneedles

Due to their high compressive strength, harness and bioinert (or bioactive surface) properties, ceramics have become widely used within orthopedic and dental applications [143]. Numerous materials have been studied including alumina, titanium dioxide, calcium phosphate, zirconium, hydroxyapatite and others. Likewise, ceramics have been applied to the fabrication of MN devices [144]. In contrast to polymeric materials which typically have a Young's Moduli of less than 10 GPa, ceramics have very large Young's Moduli which are generally greater than 100 GPa [145, 146]. The high strength of ceramic materials make them an ideal choice for applications that require robust mechanical properties. Because ceramics are porous materials they can be used to slowly deliver a substance of interest which has been incorporated into the microporous network. Despite these advantages, ceramics are significantly more difficult to process relative to metal, glass and polymer MN due to the need for high-temperature sintering processes which can reach 1600°C for full densification. There are, limited reports of self-setting ceramics, which do not require high temperature sintering cycles to achieve desired strengths but further investigation is required [147].

Chapter 3 : STAR particles for delivery enhancement of topical compounds to skin

3.1 ABSTRACT

Delivery of bioactive compounds to skin is severely limited by the stratum corneum barrier layer. Towards the goal of increasing skin permeability, we developed a novel technology called Skin Treatment and Rejuvenation (STAR) particles. STAR particles incorporate micro-scale projections, similar in size to those found on conventional microneedle patches, onto millimeter-scale particles which painlessly pierce superficial skin layers during their application. As such, STAR particles can be added directly into topical formulations, as an inert mechanical-penetration enhancer, and applied similarly to conventional topical skin products. In this work, STAR particles were designed, fabricated and developed to increase delivery of topically applied compounds. STAR particles fabricated from stainless steel (mSTAR) were demonstrated to increase delivery of sulforhodamine B up to 90-fold. mSTAR particles were demonstrated to be safe and efficacious when applied to skin of hairless rats in vivo. Next, STAR particles were fabricated from alumina (cSTAR), a common material found in conventional topical products, and demonstrated to increase skin delivery for bioactive compounds of clinical significance (e.g., 5-fluorouracil, methotrexate, bleomycin) by up to 6-fold. Finally, mSTAR particles were applied to a small cohort of human participants and demonstrated to be safe, tolerable, acceptable and efficacious. These results demonstrate for the first time that STAR particles significantly increase delivery of topically applied therapeutics into skin.

3.2 INTRODUCTION

Cutaneous disorders are among the most prevalent medical indications in the world, estimated to affect 30 to 70% of the global population, and cause considerable physical, economic and psychosocial distress to those affected [15, 148-152]. Despite the significant clinical need for efficacious skin therapies, only a small subset of bioactive compounds possess the necessary physicochemical properties that allow for passive absorption past the skin's stratum corneum (SC) barrier layer [4, 153]. As a result of the SC barrier layer, most topical therapies have limited bioavailability and therefore poor therapeutic efficacy for treatment of cutaneous disorders [154-156]. In addition, although some dermatoses result in a compromised SC barrier functionality (e.g., psoriasis, warts), skin permeability in diseased skin is still limited due to a variety of factors (e.g., hyperkeratosis) [157].

Due to limited skin permeability, a large number of dermatologic compounds are delivered systemically, either by injection or (more commonly) via oral formulations. Injections are commonly used for systemic delivery of macromolecules which would otherwise degrade or not be sufficiently absorbed in the gastrointestinal tract (e.g., Etanercept, Adalimumab) [158, 159]. Alternatively, oral formulations can be used as a simple, non-invasive method to systemically deliver bioactive compounds (e.g., isotretinoin, methotrexate, cyclosporine) [23, 160]. Although effective for delivery of many dermatologic compounds, systemic administration methods, in general, distribute drugs throughout the body in a non-targeted manner. As a result of poor drug localization to the skin, systemically administered drugs often require large-initial doses, which

increases the overall cost of therapy and potential risk for adverse, off-target side effects (e.g., nausea, hepatic toxicity, teratogenicity) [161-163].

To overcome limitations associated with conventional skin delivery methods, intensive efforts by the pharmaceutical and medical research communities have gone towards increasing skin permeability. Formulation-based approaches (i.e., chemical penetration enhancers, CPE) fluidize the lipid-rich SC to enhance delivery of small, lipophilic molecules by a few fold, thereby marginally increasing the number of drugs that can be administered to skin [164]. Despite their enhancement effects, CPE are associated with adverse side effects (e.g., skin irritation) and are generally not useful for delivery of macromolecules like biologics [165]. Alternatively, intralesional injections have been explored for delivery of dermatologic agents to treat localized, relatively small skin surface areas (e.g., warts, non-melanoma skin cancer) [81, 166].

Additionally, physical-based approaches (e.g., iontophoresis [167], electroporation [104], ultrasound [168]) enable delivery of hydrophilic drugs and macromolecules, such as peptides/proteins and DNA/RNA, but typically involve devices used to treat small areas of skin. Also, many of these devices require complex, costly equipment (e.g., requiring electric power, batteries, etc.) that limits their usability, especially for indications requiring patient self-administration. Transdermal and microneedle (MN) patches [114, 169-171] provide a simple and low-cost means for patients to self-administer therapies. However, patch-like technologies are predominantly used for delivery of systemic drugs and vaccines, and not useful for treatment of large, disseminated body surface areas.

In summary, current skin delivery technologies, although useful for certain medical indications, have not overcome several limitations that would enable their use in treatment of dermatologic disorders as a whole. In envisioning an ideal skin delivery technology, several key criteria must be realized. First, the technology should locally (i.e., non-systemic) increase skin delivery to spatially target the skin and reduce potential adverse, off-target side effects. Second, the technology should increase skin permeability to a wide variety of drug molecules – ranging from small molecules to protein therapeutics. Third, the technology should be safe, tolerable and not cause procedural discomfort for patients. Fourth, because skin diseases can manifest in various ways, the technology should be capable of treating both small and large skin surfaces. Finally, the technology should be simple to use, enable patient self-administration and low-cost so as to not be economically burdensome for those who require chronic treatment.

Herein, we report for the first time the development and characterization of a novel Skin Treatment and Rejuvenation (STAR) particle for increased delivery of bioactive molecules into skin. STAR particles are millimeter-scale particles with micron-scale projections that minimally invasively pierce the skin during their topical application (**Fig. 2.1**). Because of their relatively small size, STAR particles can be directly incorporated, as an inert formulation additive, into topicals and thereby applied to skin in a manner that is indistinguishable from conventional skin products (e.g., sunscreen). As STAR particles are applied to the skin's surface, the microscopic needle-like projections (i.e., “arms”) penetrate the uppermost skin layers and thereby act to increase skin permeability to

topical compounds. In this way, STAR particles take advantage of formulation based delivery methods which can be applied across any size skin surface area – large or small. Additionally, STAR particles can be fabricated from commercially available, low cost, biocompatible and environmentally benign friendly materials. In the following sections, we will explore the use of STAR particles for delivery enhancement of topical therapies to skin.

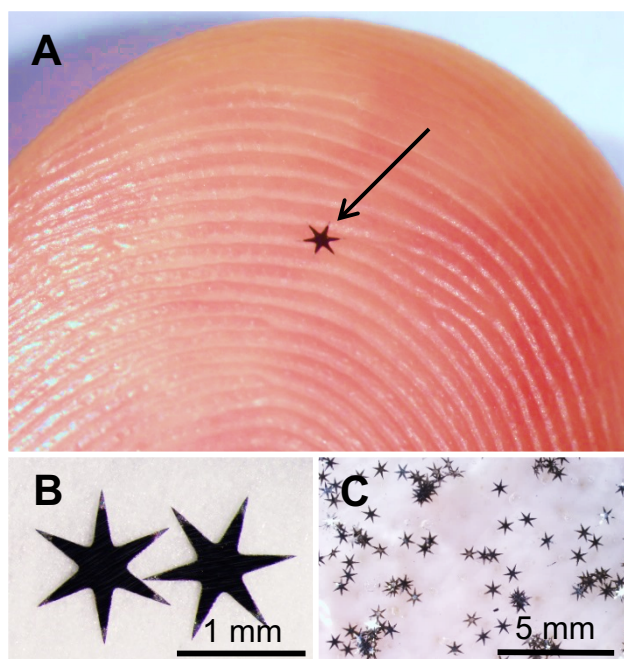


Fig. 3.1: STAR particles are millimeter-scale particles with micro-scale projections. Metal STAR particle(s) containing six arms on a fingertip (A); lying on a flat substrate (B); and applied to skin *ex vivo* (C).

3.3 MATERIALS AND METHODS

3.3.1 - Fabrication of metal STAR particles. mSTAR particles were fabricated via infrared laser ablation (Resonetics Maestro, Nashua, NH) of stainless steel sheets (Trinity Brand Industries, SS 304, 12.5 μm thick; McMaster-Carr, Atlanta, GA). Stainless steel sheets were mounted to a glass substrate using double sided adhesive tape (3M,

Minneapolis, MN). The infrared laser was operated at 1000 Hz, 20 J/cm² energy density, 10 mm/s cutting velocity, 10 mm/s stage velocity and 80% attenuation of laser energy. A single pass was sufficient to cut each STAR particle. STAR particles were fabricated to have two, four or six arms and had a total tip-to-tip length of 1 mm. Desired STAR particle geometries were designed in AutoCAD (Autodesk, Cupertino, CA) and the laser cutting process was automated using the laser programming software. mSTAR particles and adhesive tape were then placed in acetone (Sigma, St. Louis, MO) and vortexed for 1 minute to dissolve away adhesive tape. The acetone wash was repeated until all tape had been removed. mSTAR particles were then mixed aloe vera gel (Fruit of the Earth, Fort Worth, TX) and stored in a closed container at room temperature until subsequent use.

3.3.2 - Fabrication of ceramic STAR particles. cSTAR particles were fabricated via CO₂ laser ablation (VLS3.50, Universal Laser Systems, Scottsdale, AZ) of 150 µm thick alumina-green-ceramic tapes (Maryland Tape Casting, Bel Air, MD). Briefly, green tapes were laser cut with laser settings of 0.1% power, 2% speed, 1000 points-per-inch (PPI) for a total of three passes per STAR particle. Unsintered (green) cSTAR particles were then placed on magnesium oxide trays (Alfa Aesar, Haverhill, MA) and sintered in high-temperature box furnace (Carbolite Gero, RHF 16/3, Hope, UK). The temperature cycle used was as follows: ramp from room temperature to 600°C at 2°C/min, hold at 600°C for 1 hour, ramp to 1600°C at 5°C/min, hold at 1600°C for two hours and finally cool to 30°C at 10°C/min. Following sintering, cSTAR particles were inspected using scanning electron microscopy (Hitachi TM3000, Tokyo, Japan) to measure cSTAR geometrical

features such as tip radius. cSTAR particles were stored at room temperature in a sealed glass container until subsequent use.

3.3.3 - Preparation of excised porcine ear skin. Freshly excised porcine ears (Holifield Farms, Covington, GA) were washed in room-temperature water and frontal ear skin was carefully dissected from underlying cartilage. Subcutaneous fat was removed and excess hair shaved using a disposable razor (Dynarex, Orangeburg, NY). Prepared ear skin was gently cleansed with alcohol wipes (BD, Franklin Lakes, NJ), wrapped in aluminum foil pouches, and frozen at -80°C until ready for use. Prepared ears were stored in deep freeze for a maximum of four months before use in skin permeability studies. Skin samples that showed visible defects (e.g., tears in skin) or had skin-electrical resistance below 15 k Ω were discarded and not used for skin permeability studies.

3.3.4 - Application of STAR particles to porcine skin *ex vivo*. Porcine ears were thawed in room-temperature water before further preparation. For skin pre-treatment studies, either aloe vera gel, abrasive gel (NuPrep, Weaver, Aurora, CO) or formulations of STAR particles suspended in aloe vera gel were applied to the surface of porcine ear skin. Each application consisted of placing skin on a flat surface under slight tension. For topical pre-treatment methods, each of the formulations were gently rubbed onto the skin's surface using light pressure with the index and middle fingers in a preferentially curricular motion for 10 seconds. After application, the topical formulations were wiped away using alcohol wipes.

3.3.5 - Skin thickness measurements. After application of each pretreatment method, skin was cut into circular punches (diameter = 24 mm) to fit in a vertical Franz diffusion cell. The mass of each skin sample was measured and recorded. Skin density was approximated using previously reported literature values ($\rho_{\text{skin}}=1.075 \text{ g/cm}^3$; [172]) and used in conjunction with measured sample surface area (SA) and mass (m) to determine skin thickness (t).

$$t = \frac{m}{SA \times \rho} \quad (1)$$

3.3.6 - Gentian violet staining of penetration sites. Gentian violet (GV) (Humco, Texarkana, TX) was applied to skin samples to determine penetration area for each skin pre-treatment method. GV was topically applied to pretreated skin for 15 minutes to allow sufficient time for staining of existing skin-penetration sites (i.e., non-intact SC). Excess GV stain was removed from skin by cleaning with alcohol wipes until non-adherent residual surface staining was removed.

3.3.7 - Assessment of skin penetration *ex vivo*. Stereoscopic images were taken (Olympus SZX16, Tokyo, Japan) of each pre-treated and stained skin sample. Images were digitally processed in ImageJ (U.S. National Institutes of Health, Bethesda, Maryland) to analyze skin gentian violet staining (i.e., skin penetration sites). Briefly, images were first converted to 8-bit greyscale. Then images were manually set to threshold values that would delineate skin penetration sites. Next, total gentian violet area was determined for each skin sample using the ImageJ analysis software.

3.3.8 - Evaluation of skin permeability *ex vivo*. Skin specimens were first mounted in vertical Franz diffusion cells with skin epidermis facing the donor chamber and dermis facing the receiving chamber. Donor and receiving chambers were then filled with phosphate buffered saline (PBS, Sigma Aldrich, St. Louis, MO) and 10 mM sodium azide (Sigma Aldrich) as a preservative agent. Diffusion cells loaded with skin were stored at 4°C for approximately eight hours to allow for skin hydration prior to placement in a heating block (PermeGear HS-2, Hellertown, PA) set to 37°C. Following skin hydration, skin-electrical resistance was measured while skin was mounted in the diffusion cell using a multimeter (Fluke Model 73-III, Everett, WA) and Ag-AgCl sintered electrodes (E205, In Vivo Metric, Healdsburg, CA).

Following electrical-resistance measurements, the donor chamber was emptied and replaced with 0.5 ml of 10 µM sulforhodamine B (SRB, Sigma Aldrich) or 0.5 ml of 10 µM 4 kDa FITC-dextran (Sigma Aldrich) in PBS solution. The donor chamber was wrapped tightly with parafilm (Bemis, Neenah, WI) and the receiving chamber port capped with a rubber stopper to limit evaporation from the diffusion cell throughout the experiment. Aliquots of 150 µl were collected from, and fresh PBS solution was added to, the receiving chamber periodically throughout the experiment to measure transdermal transport of SRB. Solutions were measured for fluorescence intensity in a Synergy H4 Multi-Mode Plate Reader (Biotek, Winooski, VT) with an excitation/emission of 565 nm/585 nm. Cumulative transport was calculated and plotted as a function of time to determine steady state flux values for each pre-treatment method.

3.3.9 - Delivery of bioactive drugs *ex vivo*. For delivery of bioactive agents (5-fluorouracil (5-FU), methotrexate (MTX) and bleomycin (BLEO) excised skin was prepared as was done with previously described. In these studies, ceramic STAR particles, at a concentration of 10wt% in aloe vera gel, were applied to skin as a pre-treatment. At the start of the study, the donor chamber PBS was emptied and replaced with 150 μ l of 5 mg/ml 5-FU (Sigma Aldrich), 1 mg/ml MTX (TCI, Tokyo, Japan) or 1.5 mg/ml BLEO (TCI, Tokyo, Japan). 5-FU and MTX topicals were prepared as 10 mM aqueous sodium hydroxide (Sigma Aldrich) solutions and BLEO was prepared in deionized water. At one, three and six hours of exposure to bioactive agents, the topical solution was carefully pipetted away and skin's surface was washed three to five times with PBS solution. An 8 mm punch biopsy was then taken from the exposed skin area. The skin biopsy was then placed in a 1 ml of equal parts methanol (Sigma Aldrich, St. Louis, MO) and 10 mM sodium hydroxide in D.I. water and sonicated (Fisher Scientific, FS30H, Hampton, NH) for 30 minutes to extract drug from skin samples. Solutions were then analyzed via high pressure liquid chromatography (HPLC 1200 series, Agilent Technologies, Alpharetta, GA).

HPLC analysis of bioactive agents. HPLC was used to quantify delivery each bioactive agent to skin *ex vivo*. All bioactive compounds were analyzed in a C18 column (Eclipse XDB-C18, 3.5 μ m particle size, 4.6 diameter x 150 mm length). The following operating parameters were used for delivery quantification (Table 2). All mobile phase solvents used were HPLC grade (VWR, Radnor, PA).

Table 2: Summary of HPLC operating parameters for quantification of skin delivery *ex vivo*.

Drug	Mobile Phase			λ (nm)	T (°C)	Retention Time (min)		LOD (ng/ml)
5-FU	Constant (5 min)	1 ml/min (70:30)	0.1% TFA in water MeOH	260	40	1.5	-	98
MTX	Constant (10 min)	1 ml/min (85:15)	0.1% TFA in water ACN	303	30	1.7	4.8	39
BLEO	Gradient (60 min)	1 ml/min (9:1) to (6:4)	0.1% TFA in water MeOH	254	30	24.5	34.5	390

5-fluorouracil (5-FU), methotrexate (MTX), bleomycin (BLEO), trifluoroacetic acid (TFA), methanol (MeOH), acetonitrile (ACN), UV detection wavelength (λ), column temperature (T), drug limit of detection (LOD).

3.3.11 - Cryosectioning and fluorescence imaging. Skin was removed from diffusion cells at 1, 6 and 24 hours to perform histological sectioning to visually assess diffusion of SRB or 4 kDa FITC-dextran dyes in skin samples pre-treated with six-armed mSTAR particles (1000 STAR/cm², 10 seconds application, 12.5µm thickness). At each indicated time point, fluorescent solutions were removed from the donor chamber and skin surface was washed with PBS solution between 3 - 5 times. Skin samples were then removed from vertical diffusion cells and an 8 mm punch biopsy was taken from the center of the exposed tissue sample. Biopsies were embedded in optimal cutting temperature compound (OCT, Tissue-Tek, Torrance, CA) and frozen in liquid nitrogen. Frozen skin samples were stored at -80°C until cryosectioning was performed.

Cryosectioning was performed by dissecting frozen skin specimens into 12 µm thick vertical sections with a Leica 3050S cryostat (Leica Microsystems, Wetzlar, Germany).

Cryostat temperature was held at -20°C during cryosectioning. Sectioned samples were mounted onto glass slides (VWR, Radnor, PA) and stored at -80°C until fluorescence imaging was performed. Fluorescence imaging of sectioned samples was conducted using a stereoscope with a 100W mercury burner (Olympus) to illuminate sectioned tissue specimens. Photographs were taken at a constant exposure of 0.1 s.

3.3.12 - Assessment of metal STAR particles *in vivo*. Metal STAR particles were applied to dorsal skin of female CD hairless rats (6-8 weeks old, Charles River Labs, Wilmington, MA), as approved by the Georgia Tech Institutional Animal Care and Use Committee (IACUC). Hairless rats were anesthetized via inhalation of 1-2% isoflurane delivered in 100% medical grade oxygen. Before pre-treatment, pictures were taken (Canon 60d dSLR digital camera, Canon, Melville, NY) and skin impedance was measured (Prep Check EIM105, General Devices, Ridgefield, NJ) with Ag/AgCl electrodes on each application site. Skin sites were then pre-treated with aloe vera gel or mSTAR particles in gel (6 microneedle arms, 12 μm thickness, 1000 particles/cm², 10 second application). After removal of each pre-treatment method, non-woven cotton gauze pads (Crosstex, Hauppauge NY) were saturated with a 10 mM aqueous solution of SRB and applied to pre-treatment sites. In sites pre-treated with gel the application time of SRB solution was 3 h. mSTAR particle treatment sites were exposed to SRB solution for either 3 h or 15 min. SRB solution was reapplied every 20 min to ensure SRB saturation of gauze pads.

At the completion of the study, animals were euthanized via carbon dioxide inhalation. Skin was then immediately excised, cleansed with DI water and gauze pads to remove residual surface dye and imaged for fluorescence intensity to assess transport of SRB dye. Skin was also assessed for microscopic puncture sites via GV staining.

3.3.13 - mSTAR particle assessment in human participants. mSTAR particles were applied to human subjects with informed consent to assess preliminary end points of safety, tolerability and efficacy as approved by the Georgia Tech Institutional Review Board (IRB). Study participants were screened prior to enrollment and excluded if they had, as assessed by the study investigator, abnormal skin within or near application areas, known health conditions which interfere with pain perception or allergies to materials used in this study. A list of demographic information and inclusion/exclusion criteria can be found in supplemental materials.

Four skin treatments were applied to eleven study volunteers: (1) a 26 gauge hypodermic needle inserted to a depth of 5 mm; (2) aloe vera gel alone; (3) aloe vera gel with 0.5 mm diameter circular micro-disks incorporated (12.5 μm thick, 500 disks per gram of aloe vera gel); or (4) aloe vera gel with mSTAR particles incorporated (12.5 μm thick, 6 arms, 500 STARS per gram of aloe vera gel). All applications were performed blinded to study participants but not blinded to the investigator. All skin treatments were applied by the study investigator. Following applications, skin was assessed and scored for erythema size, erythema intensity, swelling, and tenderness. Skin tolerability was determined through a scoring scale which can be found in supplemental materials (**Table 4**).

Participants were also asked to fill out a short questionnaire to describe their sensations and describe their experiences associated with the skin applications. Study participants returned 24 h after applications for evaluation and to complete a follow-up questionnaire.

3.4 RESULTS

3.4.1 - Design of STAR particles. Design of STAR particles was motivated by design elements from conventional MN patches that penetrate skin in a minimally invasive manner [173, 174]. Although MN with much shorter lengths have been demonstrated for skin penetration (e.g., $< 100\ \mu\text{m}$ length), such devices typically involve use of a high-velocity applicator [175]. To satisfy the criteria of minimally invasive skin puncture and simple application, the arms on STAR particles were designed to be of sufficient length to overcome skin deformation but not so long as to induce uncomfortable sensations during their application. Therefore, STAR particles were designed to be 1 mm in total length (tip-to-tip) with an arm length of approximately $300\ \mu\text{m}$. Additionally, in some forms, STAR particles were designed with multiple arms (e.g., six arms, **Fig. 3.1**) to mitigate safety risks associated with embedding of STAR particles in skin.

Conventional MN patches are aligned and applied to skin with a uniaxial load that aligns MN structures perpendicularly to the skin's surface (i.e., MNs and application force are in plane). In contrast, STAR particles are rubbed onto skin to facilitate their penetration (i.e., STAR particles and application force are potentially out of plane). Due to their mechanically demanding application method, STAR particles require greater mechanical strength relative to conventional MN patches. Therefore, STAR particles were fabricated

out of stainless steel due to their high material strength (bulk modulus = 134-152 GPa), biocompatibility, prior use to fabricate MN patches and precedence for safe use in clinical medicine.

It has also been determined that MN tip interfacial surface area (i.e., contact area between MN tip and skin) influences skin penetration force [126]. Therefore, to produce STAR particles with sharp tips that are capable of skin penetration we utilized laser-microfabrication techniques previously demonstrated to fabricate MN capable of skin puncture [119]. We also selected material thicknesses (12.5 μm) to provide tip sharpness in the z-plane.

3.4.2 - Skin permeabilization by metal STAR particles *ex vivo*. mSTAR particles were fabricated and topically applied to excised porcine ear skin to determine their effects on skin barrier function. Skin permeability was characterized by: (1) staining skin with GV to visualize non-intact SC (i.e., puncture sites); (2) measuring transdermal delivery of the fluorescent model compound SRB; and (3) quantifying skin-electrical resistance to determine skin barrier integrity.

Following application of gel, abrasive particles or mSTAR particles, skin permeability was visually assessed through GV staining. GV is a topical antiseptic that preferentially stains regions of non-intact SC and has previously been used to visualize micron-scale skin puncture sites [176]. Skin pre-treated with aloe vera gel or abrasive particles did not produce significant GV staining (**Fig. 3.2A-B**). In contrast, mSTAR particle pre-

treatment of skin was observed to show a multitude of disseminated GV staining sites distributed across the STAR application area (**Fig. 3.2C**). These observations demonstrate that STAR particles can act to create micro-scale puncture sites in skin which can be visualized by GV staining. Moreover, these results demonstrate that STAR particles are not functionally similar to skin abrasives, but rather STAR particles act to puncture skin at discrete microscopic sites. The distinction in functionality, from conventional abrasives, may reduce the amount of tissue damage required to increase skin permeability.

Next, skin permeability was assessed by measuring transcutaneous delivery of SRB. Following application of each pre-treatment method, skin-electrical resistance and steady state flux values (J) were measured (**Fig. 3.2D-E**).

To begin, intact skin (i.e., untreated skin) was used as a negative control. As expected, intact skin had high skin-electrical resistance and low steady state flux. Next, gel pre-treatment was investigated to determine measurable effects on skin permeability. Gel pre-treated skin showed non-significant differences in skin-electrical resistance and steady state flux relative to intact skin. Next, abrasive particle pre-treatment acted as another control to determine if skin abrasion increases skin permeability. Abrasive particle pre-treatment resulted in a small but statistically significant decrease in electrical resistance relative to intact skin but negligible effect on steady state flux as assessed by Student's t-test. The result for abrasive gel was not unexpected, as abrasive gels are commonly used

to decrease skin resistance (e.g., prior to application skin electrodes for electrocardiograms) but not generally used for skin delivery enhancement.

Steady state flux values of mSTAR particle pre-treated skin significantly increased delivery enhancement relative to intact skin as supported by Student's t-test. Enhanced skin permeability generally increased along with to the number of arms per mSTAR particle and concentration of mSTAR particles applied as supported by two-way ANOVA. The results for skin-electrical resistance and steady state flux are corroborated by our visual observations of GV surface staining following each pre-treatment methods (**Fig. 3.2A-C**). These results provide evidence that STAR particles can be used to increase skin permeability more significantly than other skin treatment methods (e.g., skin abrasion) and thereby enhance delivery of topical compounds. These results also demonstrate that STAR particle geometry and concentration can be used as adjustable parameters to control skin delivery enhancement.

It is envisioned that STAR particles may potentially be used as an additive to topical formulations (i.e., left on the skin after their application). Therefore, we sought to determine if STAR particle application without removal would impact skin permeability. To accomplish this, we applied mSTAR particles to skin but did not wipe them away after application. No significant difference in skin-electrical resistance or steady state flux was measured for skin where mSTAR particles were left on or wiped away (**Fig. S 1**). We conclude that leaving mSTAR particles on skin (i.e., without removal after application) did not significantly impact skin permeability or delivery enhancement.

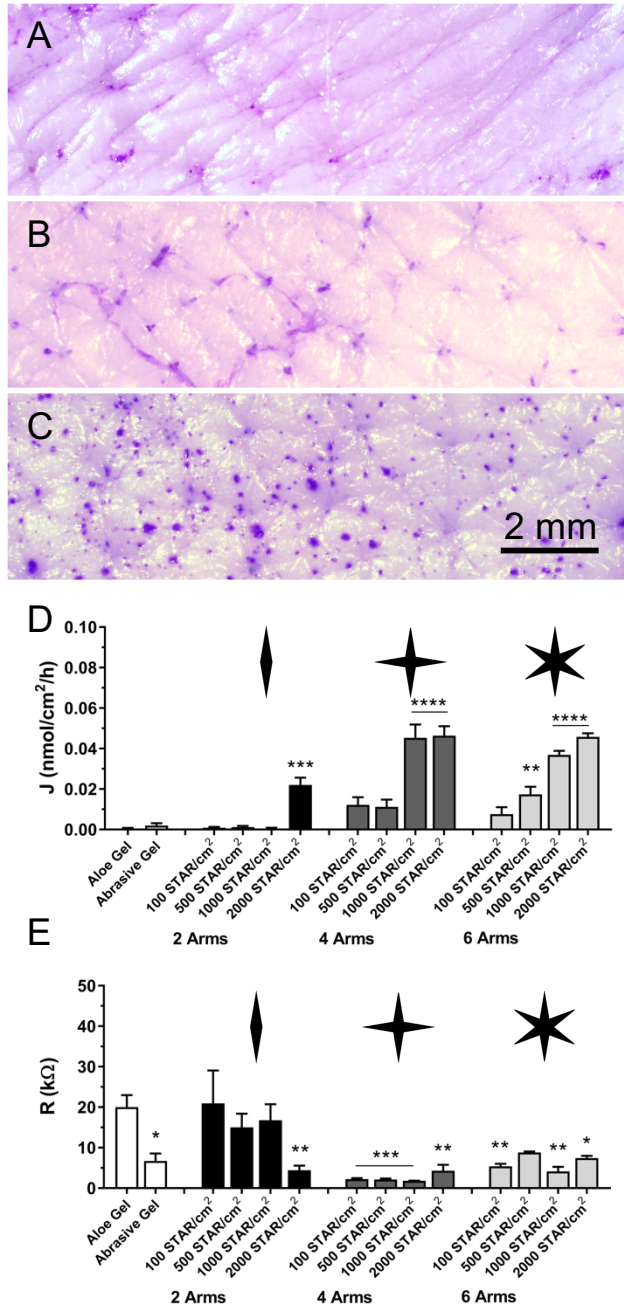


Fig. 3.2: Determination of mSTAR particle effects on skin permeability *ex vivo*. Representative *en face* images of GV-stained skin following pre-treatment with aloe gel (A); abrasive gel (B); or mSTAR particles containing four arms (C). Steady-state flux (J) of SRB across full-thickness skin *ex vivo* for varying STAR particle geometries and topical concentrations (D). Respective skin-electrical resistance measurements for pre-treated porcine cadaver skin (E). Statistical significance is shown for all pre-treatment groups in comparison to aloe gel pre-treatment. Data show averages \pm s.e.m. (n = 4). Symbol key: (*p \leq 0.05); (** p \leq 0.01); (***) p \leq 0.001); (****p \leq 0.0001).

3.4.3 - Imaging of transdermal delivery across skin *ex vivo*. Skin delivery

enhancement was visualized in aloe gel and mSTAR pre-treated skin via histological assessment. Skin pre-treated with gel showed negligible fluorescence across 1, 6 and 24 h of exposure to both SRB and FITC-dextran (**Fig. 3.3A,C**). In contrast, mSTAR particle pre-treated skin was observed to show greater delivery of both SRB and FITC-dextran (**Fig. 3.3B,D**) with the most evidence for delivery enhancement apparent after 6 and 24 h topical exposure. These results visually confirm that mSTAR particles significantly increase skin permeability and enable enhanced delivery of topically applied compounds.

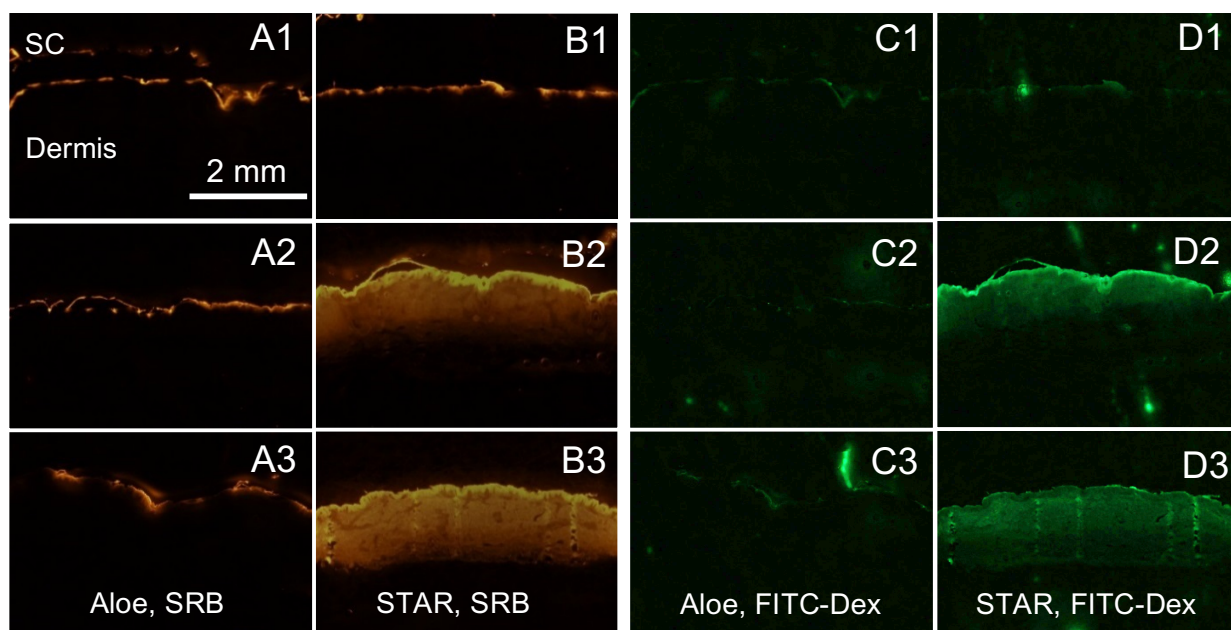


Fig. 3.3: Visualization of enhanced skin delivery with mSTAR particles. Representative images of cryosectioned porcine skin exposed to SRB or 4 kDa FITC-dextran fluorescent model drugs for 1 (A1, B1, C1,D1), 6 (A2, B2, C2, D2) or 24 (A3, B3, C3, D3) h following pre-treatment with gel (A,C) or mSTAR particles *ex vivo* (B,D).

3.4.4 - Skin permeabilization by mSTAR particles in hairless rats *in vivo*. Gel or

mSTAR particles were applied to hairless rat skin to assess skin tolerability and STAR particle efficacy to increase skin permeability *in vivo*. Skin application sites treated with either gel or mSTAR particles showed no significant erythema, irritation, swelling or

bleeding following skin pre-treatments (**Fig. 3.4A**). In addition, skin treated with gel was observed to have minimal GV staining while skin pre-treated with mSTAR particles was observed to have disseminated GV staining in the form of discrete microscopic spots thereby indicating the STAR particles had performed their function to puncture into skin (**Fig. 3.4B,C**). Skin-electrical resistance was also measured and showed significant decrease for skin pre-treated with mSTAR particles relative to gel treated skin (**Fig. S 2**). Collectively these results demonstrate that (1) STAR particles were well tolerated in skin on live animals with no adverse effects observed; and (2) function to increase skin permeability, as was observed in excised tissue, through the creation of microscopic puncture sites in skin.

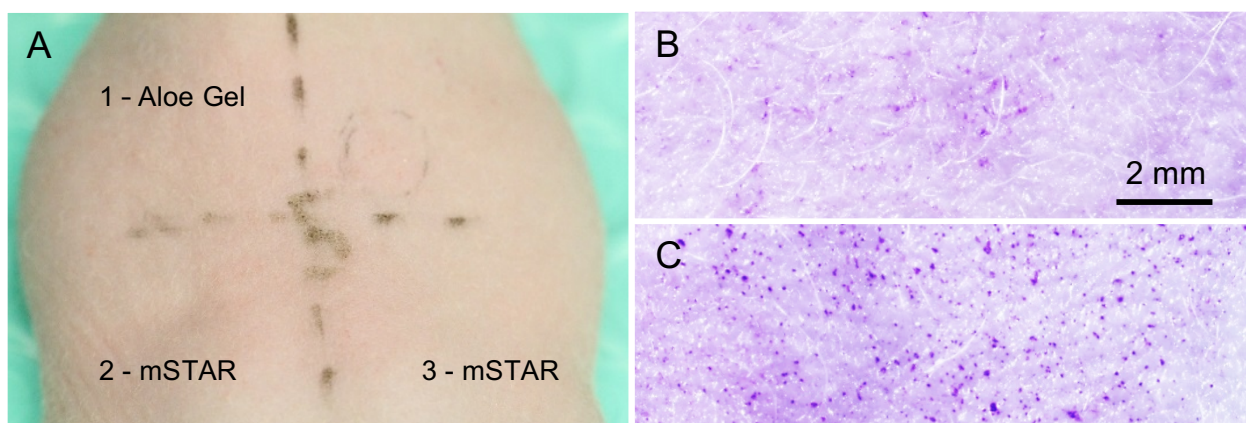


Fig. 3.4: Tolerability of mSTAR particles *in vivo*. Representative image of hairless rat skin *in vivo* following treatment with gel (A1) or mSTAR particles (A2 and A3). Representative *en face* images of GV stained hairless rat skin treated with gel (B) or mSTAR particles (C).

3.4.5 - Enhanced skin delivery using STAR particles *in vivo*. To assess skin delivery *in vivo* we examined and quantified skin fluorescence *en face*. Skin unexposed to SRB showed negligible fluorescence (**Fig. 3.5A**). It was observed that skin treated with gel showed only slight fluorescence (**Fig. 3.5B**). In contrast, mSTAR particle pre-treated skin

was observed to show bright fluorescence intensity after exposure to topical SRB for either 15 min (**Fig. 3.5C**) or 3 h (**Fig. 3.5D**). Surface-fluorescence intensity was quantified for each pre-treatment method and showed a 1.9-fold increase in fluorescence intensity for mSTAR pre-treatment compared to gel treatment alone (**Fig. 3.5E**). These results support previous conclusions that STAR particles increase skin permeability and increase delivery of topical compounds.

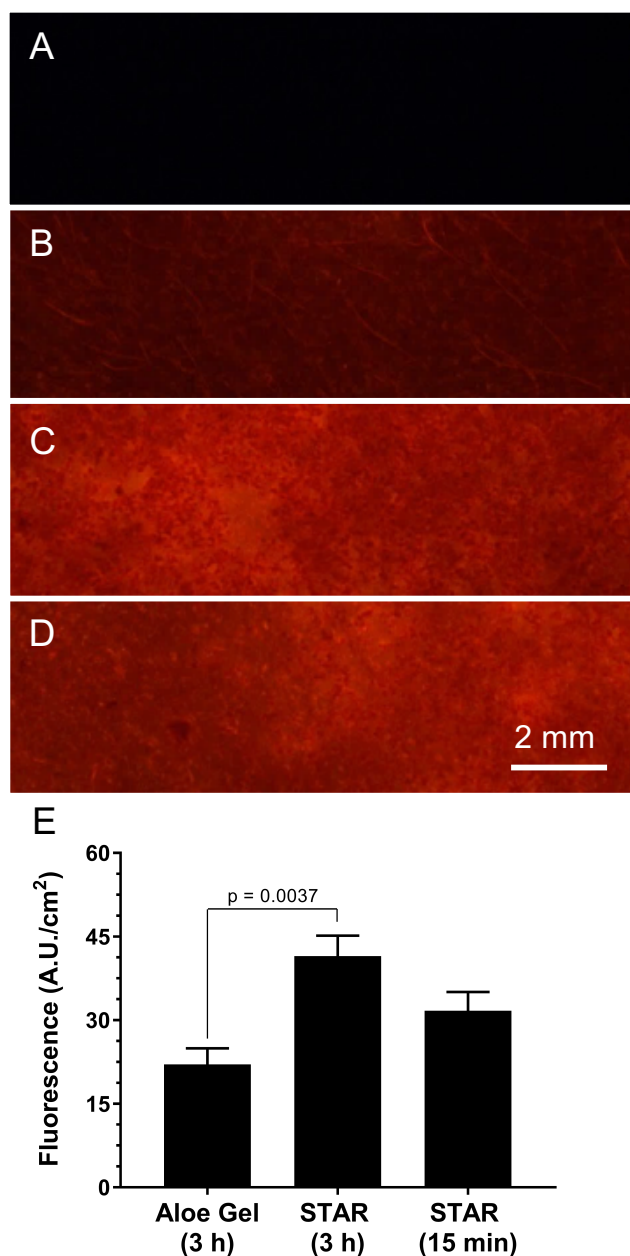


Fig. 3.5: Representative *en face* fluorescence images of hairless rat skin *in vivo* unexposed to SRB (A), pre-treated with gel (B) or mSTAR particles (C, D) and exposed to SRB. Mean fluorescence intensity in arbitrary units per square centimeter (A.U./cm²) of SRB delivered into hairless rat skin for three hours or 15 min *in vivo* following pre-treatment with aloe vera gel or mSTAR particles (E). Data show average \pm s.e.m. (n = 4).

3.4.6 - Drug delivery to skin using ceramic STAR particles *ex vivo*. cSTAR particles were fabricated out of alumina (Al₂O₃) as a means to produce STAR particles out of a biocompatible, low-cost and environmentally friendly material. Moreover, metal oxides

are extensively used in topical formulations within several applications (e.g., titanium dioxide in sunscreen or iron oxides in topical skin abrasives). cSTAR particles were fabricated via laser ablation and high temperature sintering of alumina green tape. Fabricated cSTAR particles with three-arms were measured to have an average tip radius of curvature of approximately 14 μm . Other cSTAR geometries were also fabricated. However, three-armed cSTAR particles were chosen for further investigation due to their relative tip sharpness and shorter manufacturing time (**Fig. 3.6A**).

As was previously demonstrated with metal, cSTAR particles were applied to porcine cadaver skin to demonstrate their effects to increase skin permeability via a simple topical application method. Three drugs, used clinically for dermatologic indications, with diverse physicochemical properties, were selected to demonstrate increased drug delivery into cSTAR particle pre-treated skin: 5-fluorouracil (5-FU; $M_w = 130$ Da; $\log P = -0.9$), methotrexate (MTX; $M_w = 454$ Da; $\log P = -1.8$) and bleomycin (BLEO; $M_w = 1415$ Da; $\log P = -7.5$). cSTAR particle treated skin was then GV stained and skin-electrical resistance measured for assessment of skin barrier integrity. We observed that skin treated with cSTAR particles showed similar disseminated GV staining pattern across the treated skin area (**Fig. 3.6B**). Additionally, skin-electrical resistance after cSTAR application was reduced to approximately 1 $\text{k}\Omega$, which is an order of magnitude in reduction compared to gel treated skin. Also, in all cSTAR particle applications, no mechanical failure was observed (i.e., cSTAR particles looked essentially unchanged before and after topical application). These results demonstrate that cSTAR particles functioned, similarly to mSTAR particles, to increase skin permeability by puncturing

into skin at discrete locations. These observations also provided qualitative evidence that cSTAR particles have sufficient mechanical strength and low probability of breaking during their application to skin.

The total quantity of drug delivered to pre-treated skin was quantified as a function of drug application time (**Fig. 3.6C**). After 6 h of topical application for each drug, delivery enhancement in cSTAR pre-treated skin relative to gel pre-treatment was 5.7-, 4.7 and (approximately) 2-fold increase for 5-FU, MTX and BLEO, respectively. Delivery of BLEO was below the limit of HPLC detection for gel treated skin. Therefore, the fold-delivery enhancement is reported in relation to the limit of detection. These results demonstrate that cSTAR particles enhance delivery of clinically relevant drugs with diverse physiochemical properties. Standard curves for each bioactive molecule are provided in supplementary materials (**Fig. S 6**).

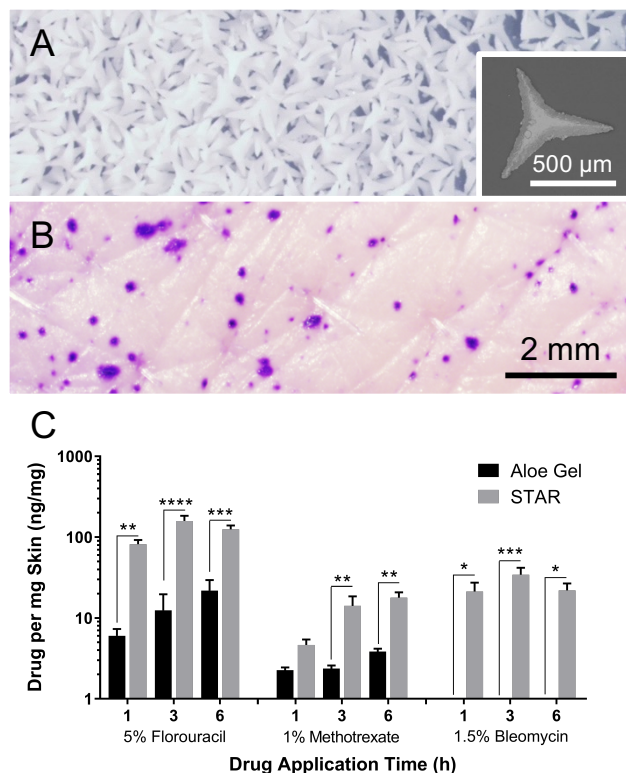


Fig. 3.6: Demonstration of cSTAR particles to deliver clinically relevant drugs *ex vivo*. Representative image showing several hundred cSTAR particles (A) and scanning electron microscopy image showing a single cSTAR particle (A). *En face* image of GV stained porcine cadaver skin following pre-treatment with cSTAR particles (B). Drug delivered into skin pre-treated with gel or three-armed cSTAR particles *ex vivo* (C, n = 3). Data show average \pm s.e.m. Symbol key: (* $p \leq 0.05$); (** $p \leq 0.01$); (***) $p \leq 0.001$); (**** $p \leq 0.0001$). Note: Delivery of BLEO into gel treated skin was below the limit of detection for HPLC.

3.4.7 - Assessment of STAR particle safety, tolerability, efficacy and acceptability in human participants. To assess their safety, tolerability, efficacy and acceptability, mSTAR particles were applied to the forearms of 11 human participants. Following pre-treatment methods, we examined GV stained skin areas under magnification to determine if there existed skin puncture sites. Skin pre-treated with aloe gel showed no GV staining (Fig. 3.7A). In contrast, we observed characteristic, microscopic GV-stained sites indicating skin puncture in mSTAR particle pre-treated skin (Fig. 3.7B). We conclude

from these observations that mSTAR particles were efficacious to puncture into skin of human subjects.

Next, skin application sites were imaged after (**Fig. 3.7C**) treatments and again after 24 hours (**Fig. 3.7D**) to assess for adverse skin reactions such as erythema, bleeding or swelling. mSTAR particles were very well tolerated by study participants. The only notable effect was very slight redness that was barely perceptible at the application site for mSTAR particles. As reported by study participants, any redness present at the application site immediately after application was transient and disappeared within several hours. Moreover, after 24 h, there were no redness or other observations at the mSTAR particle application site.

Tolerability scores to assess erythema size, erythema intensity, tenderness and swelling were quantified on a four-point scale (grade 0 = no observable effect, grade 4 = strongest observable effect) (**Fig. S 3**). Almost all study participants showed at grade 1 erythema size and erythema intensity score for hypodermic needle application. A small number of participants also reported tenderness for hypodermic needle application. Both gel and micro-disk application sites did not induce any erythema, tenderness or swelling. Most mSTAR particle application sites showed grade 0.5 to 1 scores for erythema size and erythema intensity but no other observed effects. In summary, mSTAR particle application was very well tolerated by study participants and only produced very slight transient erythema that was contained to the application area and disappeared shortly after application.

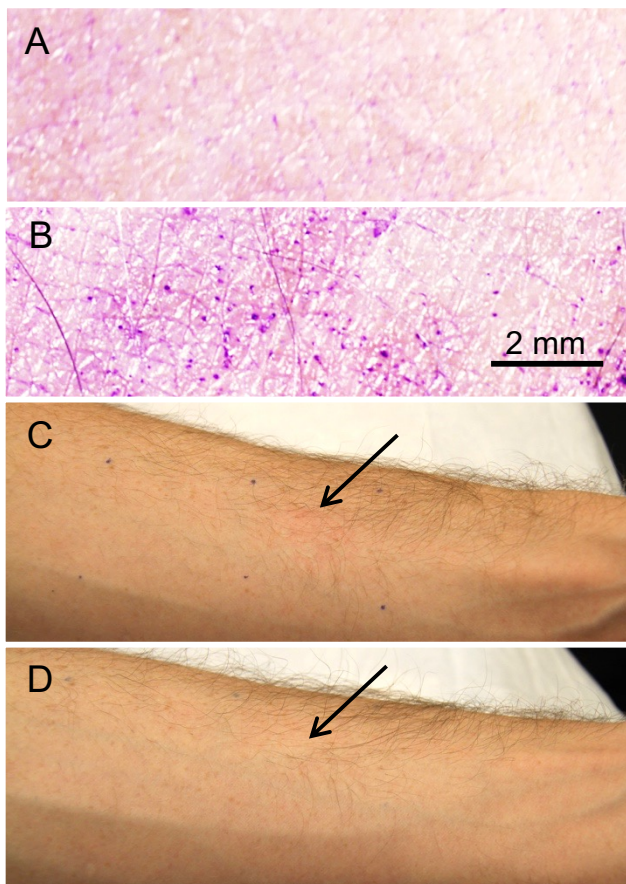


Fig. 3.7: Assessment of mSTAR particles in human subjects. Representative images of GV stained human skin on the forearm pre-treated with aloe gel (A) or mSTAR particles (B). Representative images of skin site immediately (C) and 24 h after (D) application of mSTAR particles. Black arrows indicate STAR particle application area.

Additionally, study participants were asked to fill out a brief questionnaire following skin applications to provide feedback on experienced sensations (**Fig. S 4**). Application of 26-gauge needle was most commonly described with pain (91% of participants) and stinging (73% of participants) sensations. These sensations were also more likely to be described with strong perceptions (i.e., moderate and strong). Gel and micro-disk applications had minimal sensations as reported by study participants. mSTAR particles were most commonly described with tingling (82% of participants) and stinging (64% of

participants) sensations. In addition, the majority (73%) of participants reported only comfortable sensations for mSTAR particle applications. We conclude from these findings that mSTAR particles were well accepted and not associated with significant procedural discomfort when applied to skin of human subjects.

Finally, all study participants noted that they would feel (very) comfortable applying STAR particles alone and the vast majority (91%) described their experience as (very) similar to other skin products (**Fig. S 5**). This feedback suggests that STAR particles are considered to be highly user friendly and similar to conventional topical skin products which are commonly self-applied with no prior training.

3.5 DISCUSSION

This study examined the design, fabrication and development of a novel skin delivery enhancement technology called STAR particles. We designed STAR particles to meet a set of criteria that were motivated by the medical need for safe, tolerable, minimally invasive, simple, low-cost and efficacious skin therapies.

First, STAR particles were designed to increase skin permeability and enhance delivery of topically applied compounds. To accomplish this goal, STAR particles were fabricated to have sharp protrusions (i.e., arms) that, when applied to skin, created microscopic puncture sites and facilitated skin delivery. We were able to visualize a multitude of disseminated microscopic puncture sites across STAR particle treated skin through GV staining. We also demonstrated STAR particle delivery enhancement, with SRB and 4 kDa FITC-dextran, *ex vivo* through measurement of steady state flux and visualization of

frozen skin in cross section. STAR particle parameters (e.g., geometry, concentration) were also modified to alter delivery enhancement up to 90-fold *ex vivo*. Finally, STAR particles created microscopic skin punctures and increased topical delivery in hairless rat skin *in vivo*.

Next, STAR particles were designed to be minimally invasive, safe and tolerable. To satisfy these objectives, STAR particles were fabricated to have arms measuring just several hundred microns in length (approximately 300 μm). This length was chosen based on prior learnings from MN patches which have been designed to overcome skin deformation but not penetrate deeply into skin and cause uncomfortable sensations. In addition, STAR particles were designed to have multiple arms that radially emanate from a central core structure. Their stellate shape prevents the entire STAR particle from fully embedding in skin to minimize potential safety risks (e.g., foreign body reaction). In these studies, STAR particles were demonstrated to be minimally invasive, safe and tolerable in application to animal skin *in vivo* and to skin of human participants. STAR particle skin applications generally produced a very slight, transient erythema that was barely noticeable but no other adverse reactions were observed. Moreover, in human studies, the majority of STAR particle skin applications were described as comfortable and most commonly associated with (very) slight tingling and/or stinging sensations.

A final criterion that we sought to achieve for STAR particles relates to their ease of use and treatment flexibility. Delivery technologies that can easily treat flexible surface areas is crucial for skin disorders which can manifest across large or small, localized or

disseminated surface areas (e.g., psoriasis, vitiligo, warts). Formulation-based strategies are ideal for simple and flexible skin applications. Therefore, to accomplish this goal, we designed STAR particles to be used as an additive ingredient to topical formulations (i.e., incorporated into viscous topical products). The main challenge was to fabricate STAR particles from strong materials to enable their functioning during skin application without breakage. Towards that end, STAR particles were fabricated from biocompatible, low-cost and high-modulus materials such as stainless steel (mSTAR) and alumina (cSTAR). In this way, STAR particles were efficacious to increase skin permeability over flexible size skin surface areas through a simple and intuitive application method.

In comparison to other formulation-based technologies (i.e., CPE and abrasives), STAR particles similarly enable simple and flexible skin application. However, CPE can cause skin irritation and abrasive particles reduce barrier properties (i.e., skin-electrical resistance) without significantly increasing delivery. In contrast, STAR particles were observed to be very well tolerated when applied to animal skin *in vivo* and to skin of human participants. STAR particles were also able to significantly increase skin delivery many fold over skin abrasive agents. There are also a class of physical-based delivery technologies (e.g., iontophoresis, ultrasound, electroporation) which are effective to increase skin delivery without significant adverse skin effects. However, such technologies are not generally useful for application across large skin areas and typically require use of complex, costly and/or bulky equipment. Alternatively, STAR particles can increase skin permeability similarly to physical-based delivery technologies but in a flexible and low-cost platform.

Additionally, STAR particles increased delivery of several clinically relevant bioactive compounds. Although further investigation is required, these results motivate the usefulness of STAR particles to enhance cutaneous delivery and enable more efficacious skin treatment. For example, superficial non-melanoma skin cancers can be treated with topicals such as 5-FU. However, due to limited bioavailability, topicals therapies have low efficacy for non-superficial malignancies [78, 177]. Increased delivery and penetration depth of 5-FU may potentially enable its use to treat thicker, more invasive skin cancers types. Next, MTX is a common oral medication used for treatment of psoriasis. Although efficacious, MTX can be associated with adverse side effects such as hepatic toxicity and nausea. Systemic toxicity may potentially be reduced, and therapeutic efficacy increased, if MTX delivery is increased locally to psoriasis plaques [178, 179]. Finally, BLEO is delivered via injection for several dermatologic indications (e.g., treatment of warts, basal cell carcinoma) [166, 180]. Providing a simple and effective topical application method to deliver BLEO may potentially expand its usefulness and usability for treatment of skin disease. In summary, STAR particles can increase cutaneous bioavailability of bioactive compounds and potentially enable more efficacious, tolerable and user-friendly treatment of skin disease.

Although skin delivery enhancement was reported in this work, the usefulness of STAR particles may potentially be useful in other medical applications. For example, delivery of therapeutic biomolecules to other relatively large, difficult to reach and/or topographically complex biological barriers (e.g., oral cavity [181], nasal cavity [182],

eye [183], GI tract [184] and vasculature [185]) may be investigated in future studies with STAR particle formulations that have been designed for each specific application.

In conclusion, cutaneous drug delivery is limited due to the skin's relatively large surface area and formidable barrier functionality. To overcome these limitations, we designed, fabricated and developed a novel skin delivery platform technology called STAR particles. STAR particles were incorporated, as an inert additive ingredient, into topical formulations and increased skin permeability in a user-friendly, flexible, minimally invasive and low-cost manner. Collectively, these results demonstrate, for the first time, that STAR particles dramatically enhance skin delivery and provide a means to broaden the range of molecules capable of being delivered to the skin.

3.6 ACKNOWLEDGEMENTS

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3.7 CONFLICT OF INTEREST

A. Tadros and M.R. Prausnitz are inventors on a patent and have a financial interest in a company developing STAR particle-based products for cutaneous delivery of bioactive agents (i.e., Microstar Biotech). The potential conflict of interest has been disclosed and is overseen by Georgia Institute of Technology.

Chapter 4 : Fabrication and characterization of metal and ceramic STAR particles

4.1 ABSTRACT

Cutaneous delivery of therapeutic compounds is limited by skin's stratum corneum barrier. Microneedle patches have been shown to increase skin permeability but are limited to small treatment areas. To enable application of microneedles to large areas of skin, we developed particles containing microneedles (aka STAR particles) to microscopically puncture skin while rubbing on topical formulations as a simple-to-use, painless, low-cost and effective means to increase skin permeability. STAR particles were fabricated from biocompatible stainless steel or alumina, and found to increase skin permeability with increased application time, concentration of STAR particles and increased number of their microneedles. Rubbing STAR particles on porcine skin *ex vivo* increased transdermal delivery of sulforhodamine B and 4 kDa FITC-dextran by up to 98-fold and 15-fold, respectively, and reduced skin electrical resistance by at least an order of magnitude. Transport across skin treated with STAR particles was modeled as simple diffusion through micro-pores in skin, and was in general agreement with experimental results. Sintering temperature above 1200°C was needed to produce mechanically robust cSTAR particles capable of skin puncture. We conclude that STAR particles provide a simple, formulation-based method to increase skin permeability to topically applied compounds.

4.2 INTRODUCTION

Delivery of bioactive agents into and through the skin is important for treatment of cutaneous disorders (e.g., psoriasis, eczema, acne) and other medical indications (e.g., birth control, vaccination) [2, 77]. Although the skin is easily accessible relative to other organ systems, delivery is severely limited by the skin's formidable barrier properties [9]. The skin derives its barrier functionality from the outermost stratum corneum (SC) layer [4]. The SC is approximately 10 – 25 μm thick and composed of densely packed, terminally differentiated keratinocytes (i.e., corneocytes) which form a lipophilic membrane, primarily composed of free fatty acids, ceramides and cholesterol, that preclude water-soluble and macromolecular compounds from entering the body [186, 187]. In addition to limited skin permeability, effective delivery of bioactive compounds across large body surface areas can be difficult, which is of particular importance for treatment of dermatologic and cosmeceutic indications that can often manifest across large portions of a patient's body (e.g., > 10% body surface area involvement) [188, 189].

Many drugs are delivered to skin topically [190]. Topicals are simple to apply, non-invasive and generally well accepted by most patient populations. However, as discussed, because of the SC barrier only a small subset of bioactive agents can passively diffuse into skin. This SC barrier also hinders the clinical efficacy of already used topical drugs (e.g., 5-fluorouracil, imiquimod, methyl 5-aminolaevulinate) which have limited therapeutic value for skin indications with non-superficial involvement [177, 191].

When topical agents are ineffective due to limited skin permeability, systemic administration methods can be used. Systemic agents are usually administered orally (e.g., tablet, capsule) or via injection (i.e., by needle and syringe). Systemic agents are highly effective at delivering large doses across large body surface areas without being limited by poor skin penetration. However, systemic agents distribute drugs across the body in a non-spatially-targeted manner which can, in many cases, result in adverse off-target effects that lower patient quality of life (e.g., nausea, hepatic toxicity, birth defects) [161, 192]. Additionally, to achieve therapeutic concentrations within the skin, high initial drug dosing should be administered because only a small fraction of the initial dose reaches the skin. Finally, bioactives which are degraded in the GI tract (e.g., biologics) cannot be orally administered and are therefore injected by needle and syringe (e.g., subcutaneous, intramuscular, intralesional) which causes procedural discomfort for patients which may thereby lead to reduced therapy compliance [193, 194]. Although systemic delivery methods overcome skin permeability limitations, they introduce other limitations associated with adverse side effects, poor-spatial targeting and ease of administration.

To overcome the limitations associated with conventional skin delivery methods, several methods have been developed to increase skin permeability [195]. Chemical penetration enhancers can be added directly into topical formulations to increase delivery of some molecules by several-fold [164]. In addition to their delivery enhancement, chemical penetration enhancers are advantageous because they are easily applied to skin as a formulation based additive which minimizes the need for end-user training. However,

effective chemical penetration enhancers tend to cause skin irritation and chemical penetration enhancers are not broadly useful for many bioactive agents (e.g., macromolecules like proteins, peptides) [196]. Alternatively, physical means to increase skin permeability (e.g., iontophoresis [100], electroporation [104], sonophoresis [197]) can be used to increase skin permeability. Physical penetration enhancement methods function by imparting energy in a spatially targeted manner towards the skin to thereby increase skin permeability to exogenous molecules. Such types of medical devices are also highly effective at delivering a wide class of bioactive agents including water-soluble and macromolecular entities. However, many physical penetration enhancement methods are limited by their complexity, high-cost and difficulty to use across larger skin surface areas.

More recently, microneedle (MN) patches have been used to increase skin permeability and deliver small-molecule drugs, protein therapeutics and vaccines that would otherwise require injection, and do so in a minimally invasive, painless, cost-effective and simple-to-use manner [114, 169, 170, 198]. MN patches contain needle-like projections (approximately 100-1000 μm in length) that are incorporated onto a macro-scale patch. Although MN patches have numerous advantages, because of their small footprint, they cannot easily treat large skin areas (e.g., $> 10 \text{ cm}^2$). A variation on MN patches that has been developed to resolve the skin surface area limitation is the MN roller [142]. The MN roller incorporates MN onto a cylindrical body that can be rolled onto the skin's surface to thereby pre-treat large areas across the body. However, the MN roller procedure is performed as a two-step application process (i.e., first apply the MN roller, then apply a

topical product) which complicates end usability. Additionally, MN rollers are multi-use devices (i.e., not single-use devices) which potentially increases safety risks if not properly sterilized between uses or if the same device is used by multiple individuals. Given the limitations associated with current skin delivery technologies, there is a need for novel technologies that increase skin permeability, especially to hydrophilic molecules and macromolecules, across large skin areas in a simple-to-use and low-cost manner.

To that end, we have introduced STAR particles, which leverage the MN technology platform in a way that enables drug delivery to large areas of skin. Instead of incorporating MNs onto a macro-scale patch, STAR particles incorporate MN onto millimeter-scale particles. Because of their particulate nature, STAR particles can be added into topical formulations and applied to the skin through a simple rubbing motion that is analogous to application of other topical products (e.g., sunscreen). As they are applied to skin, STAR particles are designed to painlessly puncture across SC to create transient, aqueous micro-pores through which topical compounds can more readily be absorbed. Additionally, STAR particles can be spread across any size skin surface area (large or small), which makes them ideal for cutaneous disorders that can have diverse clinical manifestations.

In this present study, we developed two types of STAR particles: metal STAR (mSTAR) particles made of stainless steel and ceramic STAR (cSTAR) particles made of alumina. We present methods to fabricate STAR particles, as well as detailed characterization of

skin puncture and permeability to optimize STAR particle design ex vivo. The objective of this study was to determine how STAR particle parameters such as material of construction, fabrication parameters, geometry, size, thickness, concentration and application time influence STAR particle performance to reduce the skin barrier and thereby increase drug delivery into skin.

4.3 MATERIALS AND METHODS

4.3.1 - Fabrication of mSTAR particles

mSTAR particles were fabricated out of stainless steel by adapting previously described methods to fabricate metal MN [119]. Briefly, stainless steel sheets (SS 304, 12.5 μm and 50 μm thickness, McMaster-Carr, Atlanta, GA) were mounted onto a glass substrate using double-sided adhesive tape (3M, Minneapolis, MN). The glass substrate was then secured on the micro-positioning platform of an infrared laser (Resonetics Maestro, Nashua, NH, USA). The laser was operated at 1000 Hz, 20 J/cm² energy density, 10 mm/s cutting velocity, 10 mm/s stage velocity and 80% energy attenuation. For stainless steel sheets of 12.5 μm thickness, a single laser pass was sufficient for cutting. For sheets of 50 μm thickness, three passes were needed.

mSTAR particles were fabricated to various geometries (i.e., two, four and six arms) and sizes (i.e., 0.5, 1.0 and 2.0 mm). Designs were drawn using AutoCAD software (Autodesk, Cupertino, CA) and the laser ablation process was automated using programming language compatible with the infrared laser operating system. Following the laser ablation process, double-sided adhesive and cut stainless steel sheets were together peeled away from the glass substrate. Acetone (Sigma, St. Louis, MO) was used to

dissolve away the double-sided adhesive, leaving behind the cut mSTAR particles. mSTAR particles were then mixed with aloe vera gel (Fruit of the Earth, Fort Worth, TX) at desired concentrations and placed in a closed container until ready for subsequent analysis.

4.3.2 - Fabrication of cSTAR particles

cSTAR particles were fabricated by laser micro-machining (VLS3.50, Universal Laser Systems, Scottsdale, AZ) 150 μm thick alumina (Al_2O_3) green tape (Maryland Tape Casting, Bel Air, MD). Laser settings of 0.1% power, 2% speed, 1000 PPI for a total of three passes were used to cut cSTAR particles. cSTAR particle designs were drawn with AutoCAD software. Once cut, cSTAR particles were gently washed in a water bath and dried at 70°C for 24 h.

Dried cSTAR particles were placed on magnesium oxide trays (Alfa Aesar, Haverhill, MA) and sintered in a high-temperature box furnace (Carbolite Gero, RHF 16/3, Hope, UK). The heating cycle used to sinter cSTAR particles involved first ramping to 600°C at 2°C/min, holding for 1 h to burn off organic material, then ramping to desired sintering temperature (i.e., 800°C - 1600°C) at a ramp rate of 5°C/min and holding at the desired sintering temperature for 2 h. The furnace was then cooled to 30°C at 10°C/minute.

cSTAR particles were then imaged using scanning electron microscopy (Hitachi TM3000, Tokyo, Japan) to measure geometrical features such as tip radius. cSTAR particles were then incorporated into gel at desired concentrations and placed in a closed container until subsequent skin application.

4.3.3 – Skin treatment with STAR particles

Porcine ear skin was acquired from a local meat processing facility (Holifield Farms, Covington, GA). Porcine ears were prepared by dissecting skin from underlying cartilage and removing subcutaneous fat with a scalpel blade. Porcine ears were stored at -80°C for up to three months prior to use. To study the effects of STAR particles on skin permeability, skin was treated by application of (i) gel (not containing STAR particles), (ii) gel containing abrasive particles (NuPrep, Weaver, Aurora, CO), (iii) a 10x10 MN patch made from non-dissolving polymer (see below) or (iv) STAR particles (metal or ceramic) incorporated into gel. All topical formulations (i.e., gel, abrasive particles and STAR particles) were applied to skin for the same amount of time and using roughly the same application force (i.e., hand application) to facilitate direct comparison between study groups. Following skin treatment, skin surfaces were wiped clean multiple times with alcohol wipes (BD, Franklin Lakes, NJ).

MN patches were used in a poke-and-patch fashion (i.e., as a skin pretreatment to enable subsequent delivery of a topical agent). MN patches were applied using the force of a thumb and pressed onto skin for roughly 10 s before removal. MN patches were fabricated using a melt cast technique. Briefly, polylactic acid (PLA) pellets (Ingeo 3215D, Natureworks, Minnetonka, MN) were melted for 1 h into polydimethylsiloxane (PDMS) MN molds. In these studies, MN on patches were approximately 500 µm in length and conical in shape.

4.3.4 – Effect of STAR particles on skin barrier properties

Several methods were utilized to determine the effects of STAR particles on excised porcine skin and compared to other skin treatment methods (i.e., gel, abrasive particles, MN patch).

4.3.4.1 Gentian violet staining

Gentian violet (GV) (Humco, Texarkana, TX, USA) was applied to pre-treated skin for 10-15 min and then wiped away with alcohol wipes to stain sites of SC puncture.

Microscopic images (Olympus SZX16, Tokyo, Japan) of GV-stained skin were digitally processed using ImageJ software (U.S. National Institutes of Health, Bethesda, MD) to quantify skin penetration area. Briefly, image analysis involved converting original images to 8-bit grayscale, thresholding images to show GV sites and using particle analysis plugin in ImageJ to measure total GV-stained area.

4.3.4.3 Skin thickness

Skin was punched into circular disks with a diameter of 24 mm and massed. Skin thickness was calculated by dividing skin sample mass by sample surface area and density ($\rho = 1.075 \text{ g/cm}^3$; [172]).

4.3.4.4 Skin electrical resistance

After mounting pre-treated skin samples in vertical diffusion cells and hydrating in phosphate-buffered saline (PBS, Sigma Aldrich) for approximately 8-12 h at 4°C, skin

electrical resistance was measured. Ag-AgCl sintered electrodes (E205, In Vivo Metric, Healdsburg, CA) were submerged into donor and receiver chamber PBS solutions (i.e., electrodes were placed into PBS solutions near epidermal or dermal sides of the pre-treated skin sample). Electrodes were connected to an electrical multi-meter (Fluke Model 73-III, Everett, WA) and electrical-resistance measurements were recorded.

4.3.4.5 Transdermal delivery of fluorescent model drugs

Pre-treated skin samples were mounted in vertical diffusion cells that were filled with PBS and 10 mM sodium azide (Sigma Aldrich) as a preservative agent. Skin samples were hydrated at 4°C for 8-12 h and then placed in a heating/mixing block (PermeGear HS-2, Hellertown, PA) at 37°C. PBS was then removed from the donor chamber and replaced with fluorescent model compounds such as sulforhodamine B (SRB) and/or FITC-dextran (Sigma Aldrich). The donor chamber was wrapped tightly with parafilm (Bemis, Neenah, WI) to minimize evaporation during the course of the experiment. Periodically, samples of 150 μ l were taken from the receiver chamber and replaced with fresh PBS solution. Solutions were measured for fluorescence intensity using a Synergy H4 Multi-Mode Plate Reader (Biotek, Winooski, VT) with an excitation/emission of (565/585) nm for SRB and (490/520) nm for FITC-dextran. Fluorescence values were converted to concentration using standard curves, which allowed for calculation of cumulative transport and steady-state flux (J).

4.3.4.6 Histological sectioning to visualize delivery into skin

To visually assess skin delivery of fluorescent model compounds, skin was cryosectioned following topical application of 1 mM SRB solution in PBS. Briefly, skin samples were

pre-treated with gel, a MN patch or STAR particles. Skin was exposed to SRB for 1, 6 or 24 h and then embedded in optimal cutting temperature compound (OCT, Tissue-Tek, Torrance, CA) and frozen in liquid nitrogen. Frozen skin was cut into 12 μm sections on a Leica 3050S cryostat (Leica Microsystems, Wetzlar, Germany) at -20°C and mounted onto glass slides.

4.3.5 - Characterization of alumina material properties based on sintering temperature

cSTAR particles were sintered at 200°C intervals between 800 - 1600°C using a high-temperature box furnace (Carbolite RHF16/3). Sintered cSTAR particles were then imaged using scanning electron microscopy to determine degree of sintering and grain-boundary morphology of alumina particles. cSTAR particles were also applied to excised skin for each sintering temperature using previously described skin treatment methods (e.g., 10 s skin application) and assessment techniques (e.g., GV staining, skin electrical resistance) used to determine their functionality. Following skin application, STAR particles were collected and imaged to assess their structural integrity.

4.4 RESULTS

4.4.1 - STAR particle fabrication

Several examples of STAR particles are shown in **Fig. 4.1**. mSTAR particles were fabricated from stainless steel sheets via IR laser ablation to have varying geometries (two, four and six arms), sizes (0.5, 1.0 and 2.0 mm) and thicknesses (12.5 and 50 μm).

Due to the relatively small laser spot size ($\sim 50\ \mu\text{m}$) and intersection of two cutting vectors, mSTAR particles were able to have sharp tips ($< 10\ \mu\text{m}$ tip radius) which have been shown to be sufficiently sharp to penetrate skin when part of a MN patch [199].

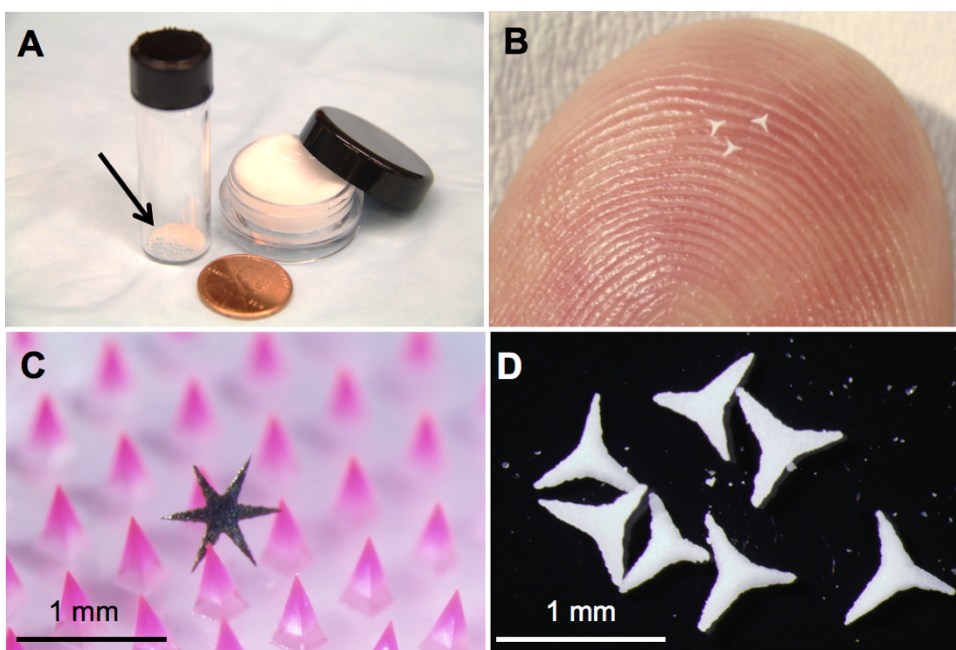


Fig. 4.1: Alumina STAR particles in dry form (black arrow) and incorporated into a white skin cream next to a U.S. penny for scale (A). Three-armed alumina STAR particles on a fingertip (B). A six-armed stainless-steel STAR particle on a pyramidal microneedle patch (C). Magnified image showing three-armed alumina STAR particles (D).

cSTAR particles were fabricated from ceramic (alumina) tapes by CO_2 laser ablation and high-temperature sintering with varying geometries of three, four, six and nine arms (**Fig. 4.2B-E**). Due to the relatively larger laser spot size ($\sim 100\ \mu\text{m}$), it was more difficult to achieve sharp tips. We found an inversely proportional relationship between the number of arms per cSTAR particles and tip radius, where average tip radius for three-armed STAR particles was $14.2\ \mu\text{m}$ compared to $20.5\ \mu\text{m}$ for nine-armed STAR particles (**Fig.**

4.2A). Three-armed cSTAR particles were chosen for further study here due to their increased tip sharpness and shorter fabrication time.

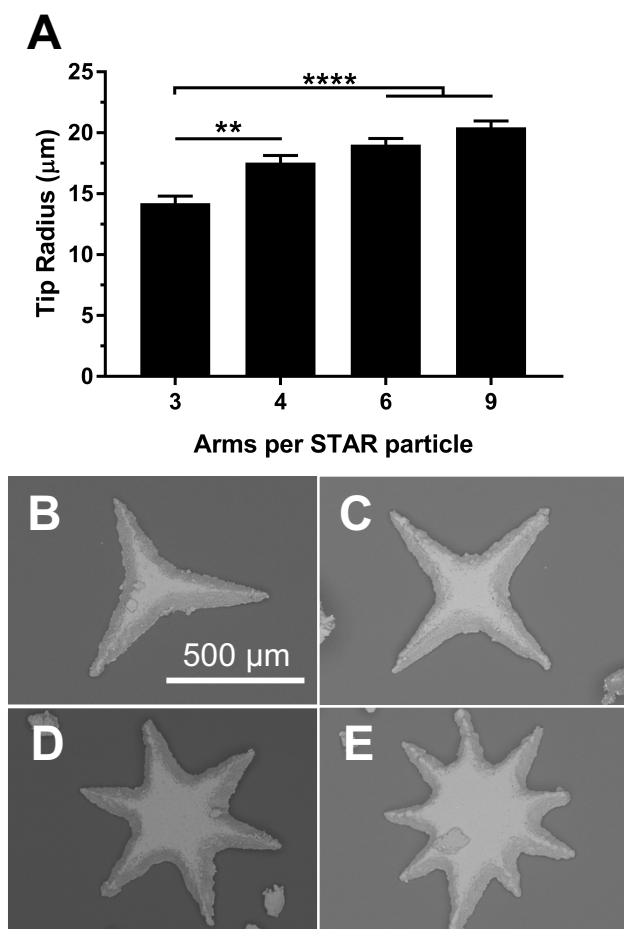


Fig. 4.2: Plot showing tip radius of curvature for fabricated alumina STAR particles with varying geometries (A, $n \geq 63$). Representative SEM images of ceramic STAR particles with three (B), four (C), six (D) and nine (E) arms. Data show averages \pm s.e.m. (** $p \leq 0.01$), (**** $p \leq 0.0001$).

4.4.2 - Imaging of transport into skin following STAR particle treatment

We next assessed the effect of rubbing STAR particles on porcine skin *ex vivo* for 10 s on skin permeability. Treatment of skin with gel (without STAR particles) had little effect on skin permeability, as shown by the lack of dermal penetration of SRB dye after 1, 6 or 24 h (Fig. 4.3A). Skin treated with a MN patch showed rapid delivery of SRB after just 1

h, which steadily increased over the 24 h study. Skin treatment with four-armed mSTAR particles measuring 1.0 mm (**Fig. 4.3C**) or 0.5 mm (**Fig. 4.3D**) tip-to-tip treatment also showed significant skin penetration of SRB, where 0.5 mm mSTAR particles led to less delivery and 1 mm STAR particles led to more delivery compared to the MN patch.

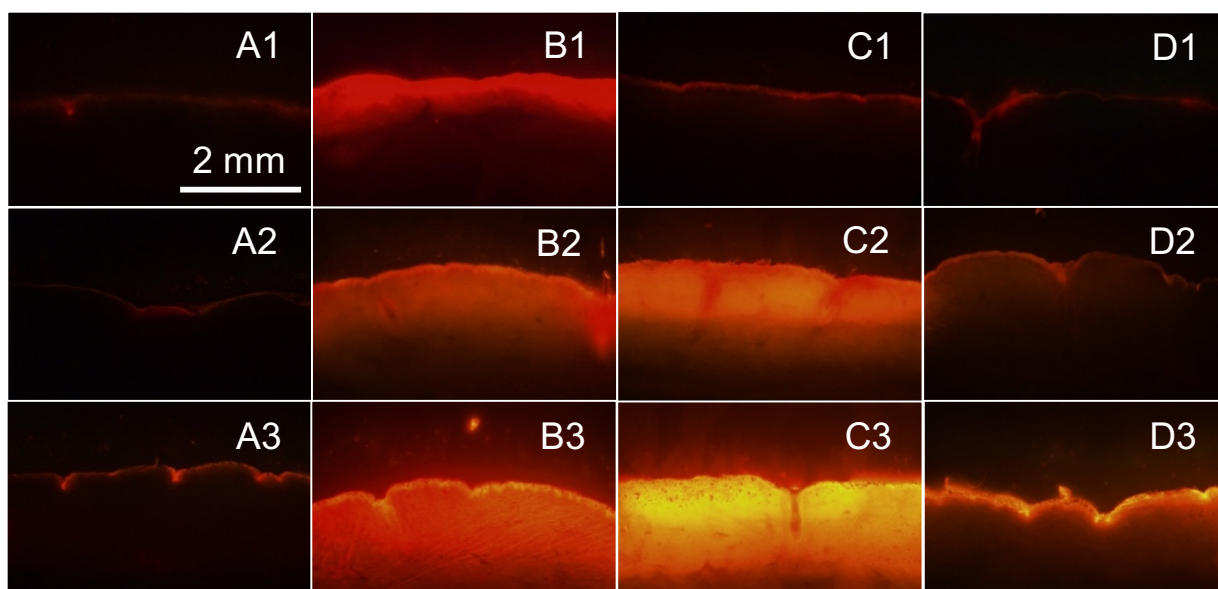


Fig. 4.3: Histological cross sections of excised porcine skin pre-treated with gel (A), MN patch (B), 1.0 mm mSTAR particles (C) or 0.5 mm mSTAR particles (D) with four arms. Skin was topically exposed to SRB for 1 (top row, A1-D1), 6 (middle row, A2-D2) or 24 h (bottom row, A3-D3).

4.4.3 - Quantification of skin permeability following treatment methods *ex vivo*.

Guided by this qualitative imaging, we quantified three different measures of skin barrier function reduction (i.e., skin electrical resistance, GV staining and steady state transdermal flux of SRB) were measured following skin treatment with STAR particles of varying topical concentrations, geometries, application times, thicknesses and sizes (**Fig. 4.4**).

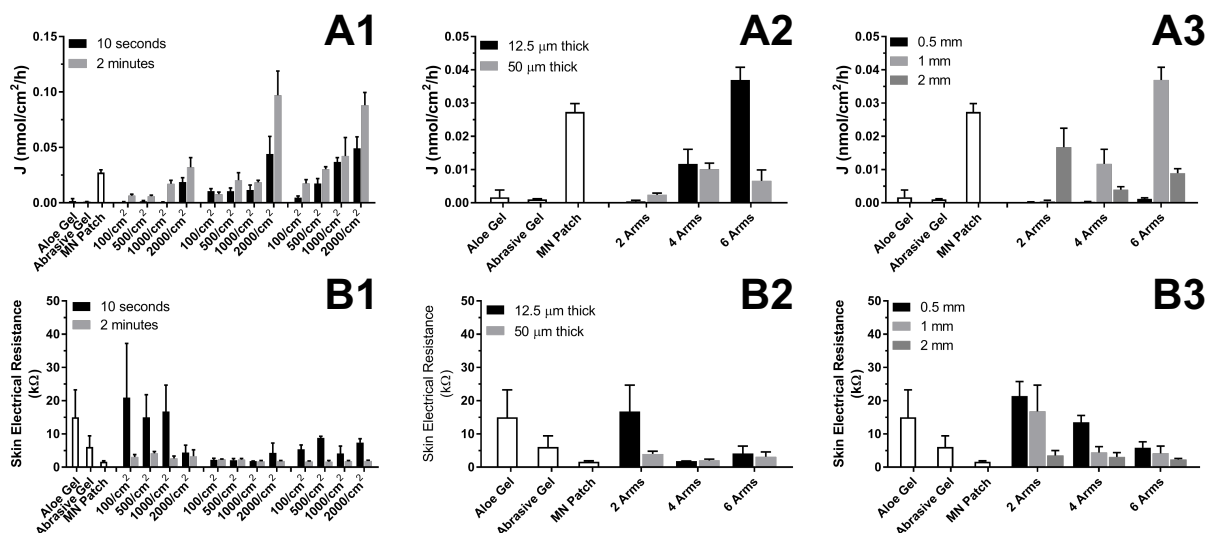


Fig. 4.4: Steady state flux (A) and corresponding skin electrical resistance (B) values across full-thickness, excised porcine skin following treatment with gel, abrasive particles, a MN patch or mSTAR particles. mSTAR thickness is held constant at 12.5 μm while varying geometry, concentration and application time (A1, B1). mSTAR concentration and application time are held constant at 1000 mSTAR/cm² and 10 s, respectively, while varying thickness and geometry (A2, B2). mSTAR thickness, concentration and application time are held constant at 12.5 μm, 1000 mSTAR/cm² and 10 s, respectively, while size and geometry are varied (A3, B3). Data show averages ± s.d. (n ≥ 3).

4.4.3.1 Skin abrasive gels.

As a control for comparison, we found that skin treatment with an abrasive gel did not significantly increase skin permeability based on steady state flux measurements compared to gel treatment ($p > 0.99$). However, abrasive particles did significantly reduce skin electrical resistance compared to gel pretreatment ($p < 0.02$). These different findings may be explained by the much more sensitive measurements of skin electrical resistance, that only needs to pass small ions under an electrophoretic driving force compared to skin permeability that needs to pass much-larger SRB molecules by a purely diffusive driving force. The limited efficacy of skin abrasion to decrease the skin barrier

may be due to this method removing tissue laterally, which requires a lot of tissue to be removed before crossing the SC.

4.4.3.2 Microneedle patch.

As a positive control for comparison, we applied a polymeric (non-dissolving) MN patch to skin (i.e., poke and patch approach). Steady state flux significantly increased and skin electrical resistance significantly decreased for MN treatment compared to gel treatment alone ($p < 0.001$). These results were in agreement with expectations that MN patches can be used to increase skin permeability.

4.4.3.3 STAR particle concentration.

Increased STAR particle concentration correlated with increased skin permeability (**Fig. 4.4A1**). For six-armed STAR particles applied for 10 s, at a concentration of 100 STAR particles/cm² of skin surface area, steady state flux showed no significant increase compared to skin treated only with gel ($p > 0.6$). Increasing STAR particle concentration 10 times higher to 1000 STAR particles/cm² resulted in a flux 68-fold greater than gel-treated skin ($p < 0.0001$). Increasing concentration to 2000 STAR particles/cm² caused steady state flux increased 90-fold over gel-treated skin ($p < 0.0001$). Because 10-fold and 20-fold increases in STAR particle concentration increased flux by just 5-fold and 6-fold, respectively, there may be diminishing advantage to increasing STAR particle concentration at a short application time of 10 s.

4.4.3.4 STAR particle application time.

When STAR particle application time was increased from 10 s to 2 min, for the majority of applied concentrations, there was no significant increase in sulforhodamine flux into

skin or decrease in skin electrical resistance (**Fig. 4.4B1**). This may be because STAR particles became ineffective during use due to particle aggregation, bending or other deformation that caused loss in functionality. However, effectively increasing skin permeability after just 10 s is advantageous for applications that require only briefly rubbing topical formulations on the skin.

4.4.3.5 STAR particle geometry.

Next, we found that STAR particle geometry influenced skin permeability enhancement. STAR particles with two, four and six arms performed differently from one another when other parameters were held constant. A general trend was observed that four and six-armed STAR particles performed more robustly than two-armed STAR particles (i.e., more skin effects were observed for four and six-armed STAR particles at low and high topical mSTAR particle concentrations). This observation may potentially be attributed to the presence of multiple “penetration axes” in STAR particles with greater than two sides (i.e., four and six-armed STAR particles have greater probabilities of being oriented in a direction where their arms are directionally oriented to penetrate into skin).

4.4.3.6 STAR particle thickness.

The effects of STAR particle thickness did not conclusively show general trends across the studied parameter space. There were statistically significant differences in steady state fluxes ($43.38 \pm 10.6 \times 10^{-2}$ and $5.68 \pm 6.81 \times 10^{-2}$ nmol/cm²/h) for six-armed STAR particles with different thicknesses (**Fig. 4.4-A2, $p < 0.0001$**). However, this result was not observed for other STAR geometries and standard deviation was large for these groups. Further investigation could potentially elucidate the effects of STAR particle thickness on skin permeability.

4.4.3.7 STAR particle size.

The effect of STAR particle size on functionality was also investigated. STAR particles between 0.5 and 2.0 mm showed differences in resulting skin permeability enhancement. For both four and six-armed STAR particles 0.5 mm STAR particles showed minimal and non-significant increases in skin permeability compared to gel pre-treated skin. Next, when four and six-armed STAR particle size was increased to 1.0 mm, skin permeability significantly increased ($p < 0.001$). Interestingly, and in contrast to two-armed particles, four and six-armed STAR particles 2.0 mm in size showed significantly lower steady state flux values in comparison to their 1.0 mm counterparts ($p < 0.01$). This unexpected result may potentially be attributed STAR particle bending during skin application. We observed that the arms of 2.0 mm STAR particles, especially four and six-sided geometries which are narrower, bent more easily during skin application than 0.5 and 1.0 mm STAR particles, which may have impacted their functionality.

4.4.4 - Summary of results from skin permeability studies *ex vivo*.

Another key result from this characterization study was the wide range of skin permeability enhancement effects that were produced. It can be reasonably concluded that STAR particle parameters can be altered to adjust their interaction with skin and thereby tune their effects to increase skin permeability in a controlled manner. This tunability enables STAR particles to be used in the delivery of a wide range of bioactive agents which may have physiochemical properties or required doses.

4.4.5 - Cumulative penetration and GV staining area.

In conjunction with steady state flux and skin electrical resistance measurements, cumulative permeation after 24 hours and gentian violet (GV) staining area were determined to provide additional information for STAR particle characterization (**Fig. 4.5**). As expected, results for cumulative permeation were proportional to steady state flux values. Additionally, GV staining generally correlated well with other skin permeability measurements. However, GV staining values were more variable relative to other measurements and did not always precisely predict skin delivery enhancement. Despite a few non-predictive values for GV staining, the trend is valid that increased GV staining area is predictive of increased skin permeability.

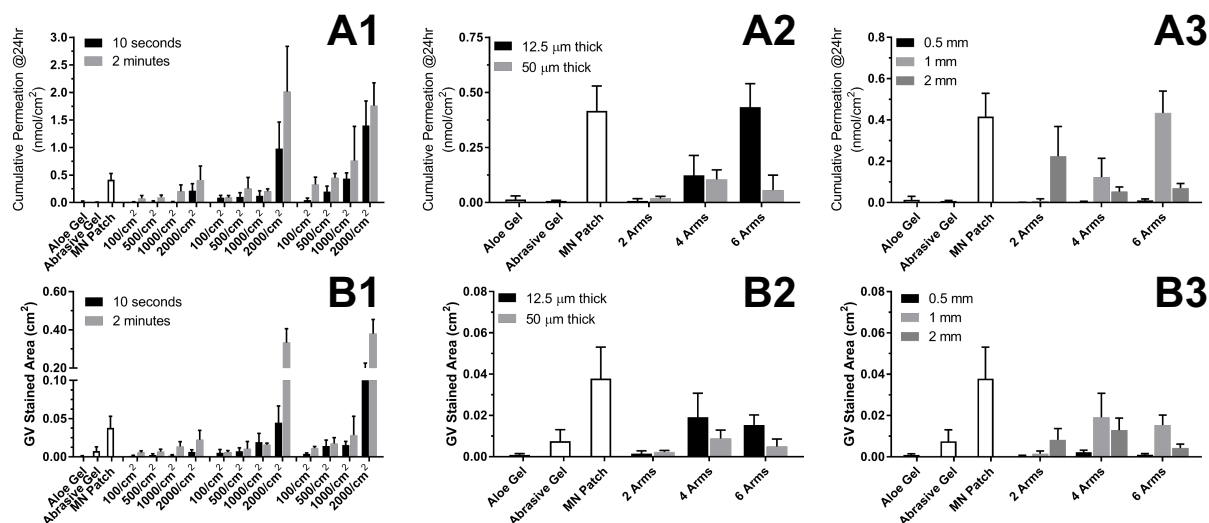


Fig. 4.5: Cumulative permeation after 24 hours (A) and GV staining area (B) for skin treatment with gel, abrasive particles, a MN patch or mSTAR particles. mSTAR thickness is held constant at 12.5 μm while varying geometry, concentration and application time (A1, B1). mSTAR concentration and application time are held constant at 1000 mSTAR/cm² and 10 s, respectively, while varying thickness (A2, B2). mSTAR thickness, concentration and application time are held constant at 12.5 μm , 1000 mSTAR/cm² and 10 s, respectively, while size is varied (A3, B3). Data show averages \pm s.d. (n \geq 3).

4.4.6 - Skin permeability enhancement modeling and correlations.

Next, we correlated measurements obtained from characterization studies to develop an understanding of skin barrier properties and resulting permeability effects following STAR particle treatment (**Fig. 4.6**). Not surprisingly, measurements of skin integrity (i.e., GV staining) correlated well with permeability measurements (i.e., steady state flux) as indicated by the best-fit lines which pass through experimental data in **Fig. 4.6A**. Also, statistically significant correlations were found to exist between steady state flux and skin electrical conductance (**Fig. 4.6B**, $R^2 = 0.23$) as well as conductance and GV staining (**Fig. 4.6C**, $R^2 = 0.49$). We conclude from these results that skin barrier properties and resulting delivery were dependent and predictive of one another.

In addition, we sought to better understand the fundamental mechanisms involved in STAR particle mediated delivery enhancement. We utilized a simplified model of one-dimensional diffusion through aqueous micro-channels in the skin to accomplish this goal,

$$J = fD \frac{C}{l} \quad (1)$$

where f is the fraction of skin punctured with micro-channels (as determined by GV staining), D is aqueous diffusivity of SRB (estimated by Stokes-Einstein equation), C is the topical concentration of SRB (10 μ M) and l is the diffusion distance (estimated to be 50 μ m for epidermal thickness). This model requires no fitted parameters and can be used to make predictions using only parameter values from independent literature.

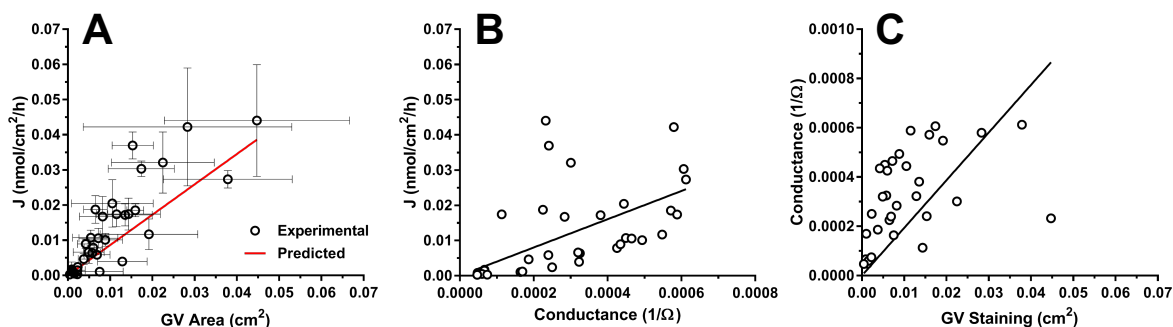


Fig. 4.6: Graphs showing correlations between steady state flux vs. GV staining area – both predicted (red line) and experimental values (open circles) (A); steady state flux vs. skin conductance (B); and conductance vs. GV staining area (C). All experimental data is reproduced Fig. 4.4 and Fig. 4.5 (A-C, $n \geq 3$).

4.4.7 - Characterization of cSTAR particles *ex vivo*.

cSTAR particles were fabricated out of alumina (Al_2O_3) through laser ablation and high-temperature sintering. Ceramic materials have many favorable properties that warrant their use to fabricate STAR particles. Ceramics are biocompatible and have extensively been used in medical applications for dental and orthopedic implants due to their high strength and durability [200]. Also, ceramics have extensively been used in many types of topical skin products (e.g., TiO_2 , ZnO , iron oxides) because of their low-environmental burden, low cost and satisfactory aesthetic properties (i.e., most ceramics are white in color which allow them to blend in with the surrounding topical formulation).

To determine the ability of cSTAR particles to penetrate into and increase skin permeability, we applied cSTAR particles at topical concentrations between 0.1 to 10 wt% to excised porcine skin for 10 s. We then determined skin permeability through measurements such as GV staining area, skin electrical resistance and steady state flux

(**Fig. 4.7**). A dose-dependent response was observed. Greater concentrations of cSTAR particles resulted in greater skin permeability. A significant enhancement in skin delivery was observed when cSTAR particle concentration was increased to 10 wt% for both SRB and 4 kDa FITC-dextran ($p < 0.01$).

Another observation from characterization of cSTAR particles is the presence of relatively large GV stain sites, especially in skin treated with 10 wt% STAR particles (**Fig. 4.7-C2**). A likely hypothesis for this result may be due to cSTAR particle tip sharpness, which is currently about 2-fold less sharp than mSTAR particles (i.e., more blunt STAR particle tips cause more tissue damage during puncture). Additional studies should be conducted with other cSTAR particle concentrations (e.g., between 1 – 10 wt% and greater than 10 wt% if additional delivery is desired).

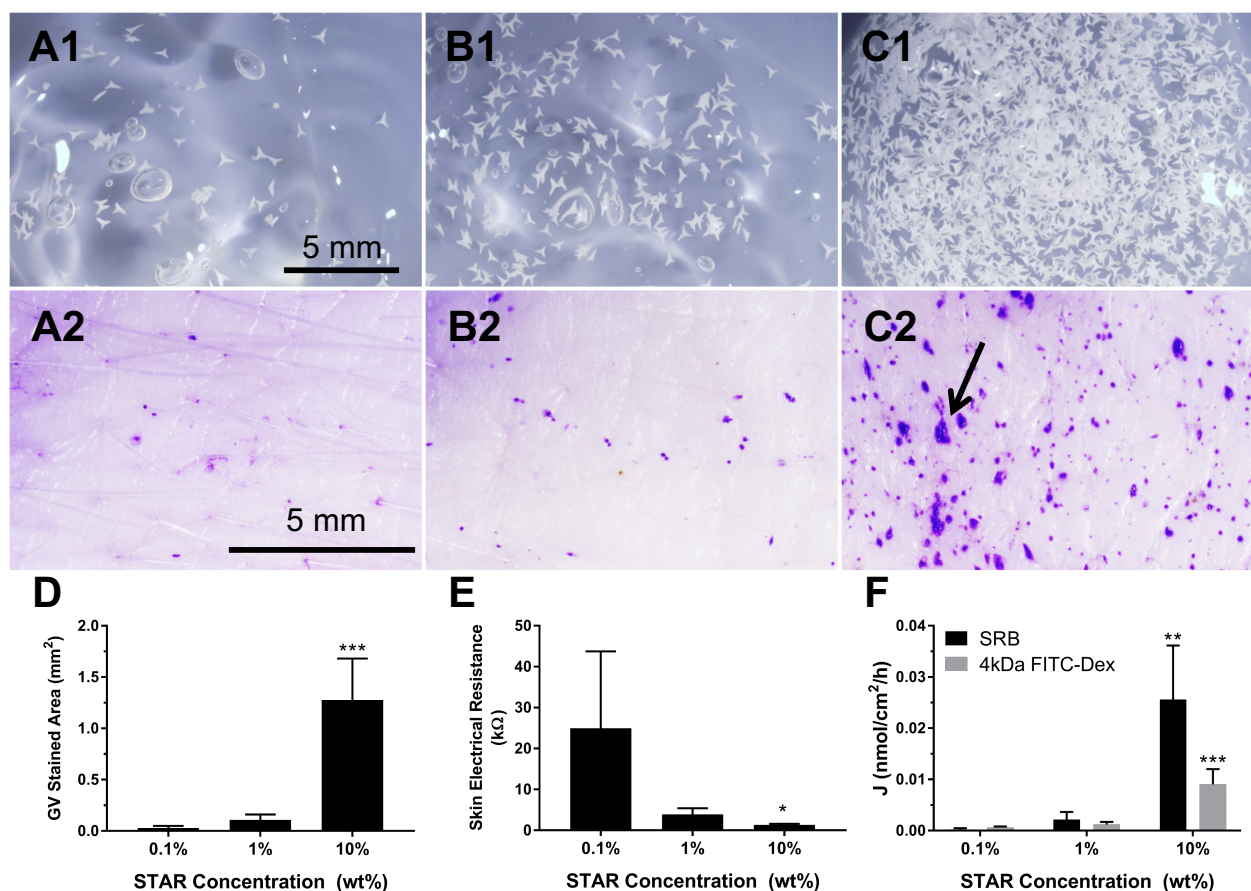


Fig. 4.7: Representative mages showing topical formulations of cSTAR particles incorporated into gel at 0.1 wt% (A1), 1 wt% (B1) and 10 wt% (C1). Representative *en face* images of excised porcine skin which has been GV stained following treatment with 0.1 wt% (A2), 1 wt% (B2) and 10 wt% (C2) cSTAR particles. Graphs showing GV staining area (D), skin electrical resistance (E) and steady state flux of SRB and 4 kDa FITC-dextran (F) following treatment with cSTAR particles. Arrow in C2 points to large penetration site. Data show averages \pm s.d. (n = 4). Statistical significance of difference in values compared to 0.1% STAR particles. Symbol key: (* $p \leq 0.05$); (** $p \leq 0.01$); (***) $p \leq 0.001$).

4.4.8 - STAR particle sintering temperature.

We next sought to investigate the effect of lower sintering temperatures on cSTAR particle functionality for skin application. High-temperature sintering is needed to coalesce ceramic micro-particles into solid or semi-porous structures that can withstand higher mechanical loading. However, the high temperatures that typically needed to

sinter alumina often require specialized furnace ovens which can reach upwards of 1600°C. Such specialized equipment can be costly and thereby increase the final cost to manufacture cSTAR particles at scale. Therefore, sintering temperatures between 800-1600°C were investigated to determine if lower sintering temperatures could produce functional cSTAR particles. As can be seen in **Fig. 4.8**, GV staining patterns began to appear with STAR particles that were sintered above 1200°C (**Fig. 4.8E**, $p < 0.025$). A significant decrease in skin electrical resistance was measured with cSTAR particles sintered at 1200°C or greater (**Fig. 4.8F**, $p < 0.01$). Significant increases in steady state flux for SRB and 4 kDa FITC-dextran were measured for sintering temperatures at or above 1400°C (**Fig. 4.8G**, $p < 0.05$).

STAR particles were also collected following their application to skin to visually observe their structural integrity. cSTAR particles fired below 1200°C were unable to withstand the forces involved during skin application and fractured. STAR particles sintered at or above 1200°C showed superior mechanical properties with minimal or no observed fractures (**Fig. 4.8 C2-D2**). Therefore, we conclude that cSTAR particles can potentially be fired at lower temperatures and maintain their functionality when applied to skin. Additional studies may be conducted to lower sintering temperatures further if sintering time is increased.

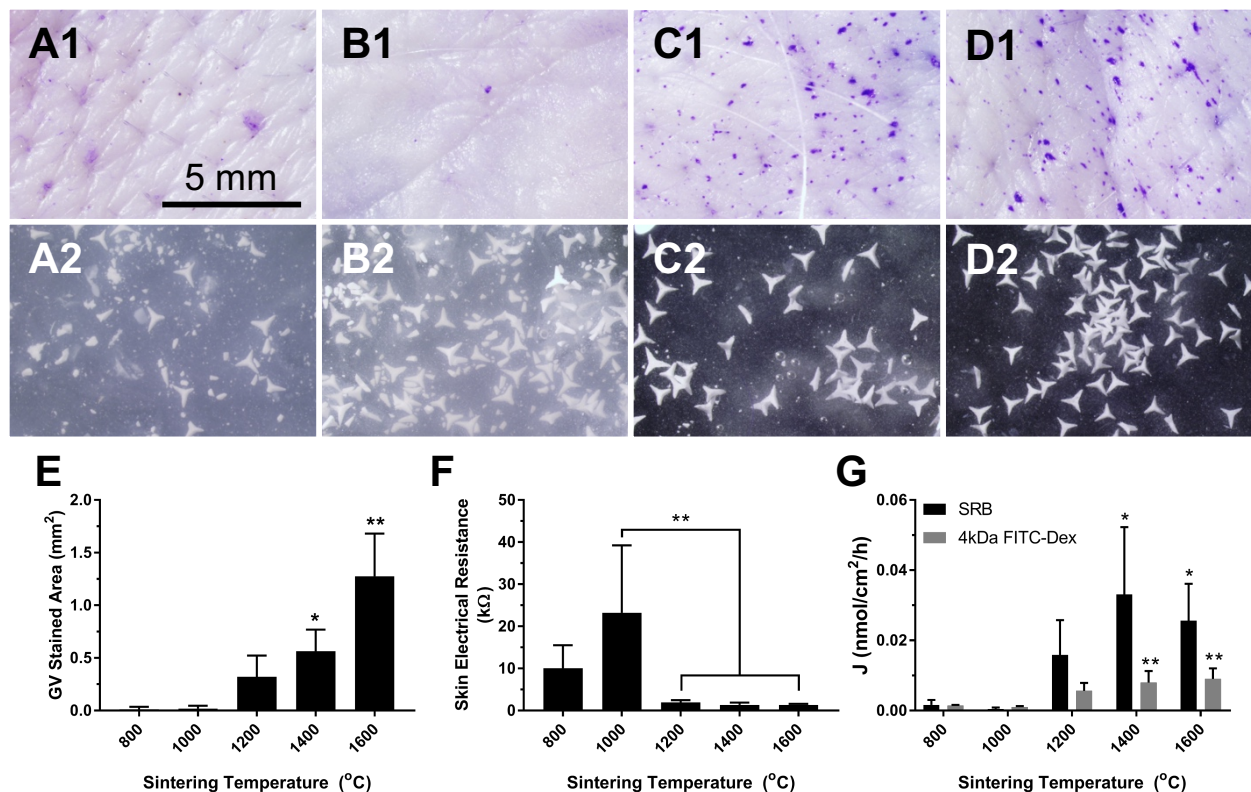


Fig. 4.8: Representative *en face* images showing GV stained skin treated with cSTAR particles at 10wt% concentration which were sintered at 800°C (A1), 1000°C (B1), 1200°C (C1) or 1400°C (D1) for 120 min. Representative images to show structural integrity of cSTAR particles sintered at 800°C (A2), 1000°C (B2), 1200°C (C2) or 1400°C (D2) after application to porcine skin *ex vivo* for 10 s. Graphs of GV stained area (E), skin electrical resistance (F) and steady state flux of SRB and 4 kDa FITC-dextran dyes (G) as a function of sintering temperature. Data show averages \pm s.d. (n = 4). Statistical significance of difference in value compared to 1000 °C: (*p \leq 0.05); (** p \leq 0.01).

4.5 DISCUSSION

Delivery of bioactive compounds into skin is severely limited by the SC barrier layer that precludes hydrophilic and macromolecular compounds from entering the body. Because of poor skin penetration, many bioactive compounds have insufficient cutaneous bioavailability and therefore must be delivered through other, potentially less desired, delivery methods (e.g., injection via needle and syringe). To overcome this limitation,

several skin delivery enhancement technologies have emerged to increase skin permeability (e.g., CPE, iontophoresis, ultrasound, electroporation, MN patches). While such technologies have provided improvements in skin delivery, there are issues related to ease-of-use, cost, presence of side effects, inability to treat large skin surfaces and procedural discomfort.

We therefore sought to develop a novel technology capable of enhancing skin delivery in a simple-to-use, non-invasive, low-cost, safe and flexible manner. To achieve this goal, we developed STAR particles, which uniquely blend the advantages of both formulation-based delivery technologies that can simply and painlessly be applied to large skin surfaces and MN-based technologies that have found broad applicability in delivery of hydrophilic molecules and macromolecules into skin. STAR particles function similarly to MN patches in that they non-invasively penetrate superficial skin layers to thereby increase skin permeability. However, unlike MN patches, STAR particles can be directly incorporated into topical formulations, which reduces the need for end-user training since most people intuitively understand how to apply topical skin products; imparts flexibility to treat large, disseminated skin surface areas; simplifies skin application to a single-step (i.e., not a multi-step “poke and patch” application); and eliminates the need for multiple-use, potentially non-sterile devices (e.g., MN rollers).

The objectives of these studies were to characterize first-generation stainless steel and second-generation alumina STAR particles for their functionality in excised skin models. To achieve this, we fabricated STAR particles with varying geometries, sizes and

thicknesses. We also investigated the effects of end use parameters such as STAR treatment application time and concentration of STAR particles incorporated into applied skin formulations. We then utilized assessment techniques such as GV staining, skin electrical resistance and steady state flux to evaluate the effects of STAR particle parameters on skin permeability. In this way, we characterized STAR particles to better understand how their design can be optimized to meet varied skin delivery needs. Although a wide range of skin delivery enhancement was demonstrated in the present study, other STAR particles could potentially be fabricated to achieve further delivery enhancement, if desired (e.g., by increasing the number of arms per STAR particle).

An important property of STAR particles relates to their mechanistic function of skin penetration. Most types of particles that are added into topical formulations have rough surfaces and thereby function as abrasive agents. As abrasive particles are applied to skin, they shear the skin's surface and remove tissue laterally from the skin's surface during the process. In contrast, STAR particles do not function as abrasive agents because their arms puncture perpendicularly to the skin's surface without removing tissue. This is supported by GV staining patterns that appears as delineated spots, not streaks. To our present understanding, as STAR particles are applied to skin, their arms puncture into superficial skin layers to reduce skin barrier function. Therefore, a key distinction between skin abrasives and STAR particles is their mechanism of action (i.e., abrading vs. puncturing skin). Because of this mechanistic difference, we believe STAR particles are inherently safer relative to skin abrasion methods, which can cause significant damage during tissue abrasion sufficient to increase skin permeability.

Moreover, under presently studied skin application conditions (i.e., the time and force of skin application), skin treated with abrasive particles did not show a significant increase in skin delivery. Therefore, we conclude that STAR particles function to increase skin permeability to a greater extent than abrasive agents.

Another important property of STAR particles is their unique stellate shape which prevents them from becoming fully embedded as they puncture skin. Because of their geometry, it is highly unlikely, and was never observed in these studies, that STAR particles become fully embedded in skin. Their star-like geometry provides an anchor to the skin's surface. Therefore, the shape of STAR particles is a key differentiator in comparison to other forms of particulate structures that penetrate and become embedded in skin [201]. Moreover, because dermatologic therapies can be applied on a frequent basis (e.g., twice daily), foreign materials that are introduced to the body may accumulate in skin and cause complications such as foreign body granulomas [202]. In addition to improved safety, STAR particles not embedding themselves in skin increases the chances that each STAR particle can puncture skin multiple times. Therefore, STAR particles are believed to be safe and efficacious because they do not fully embed themselves in skin.

Certainly, the most important characteristic of STAR particles is their ability to increase skin permeability across large skin surface areas, in contrast to MN patches that increase skin permeability across only smaller areas. Therefore, STAR particles can enhance

delivery to a greater extent than MN patches given their ability to deliver therapies across larger skin surface areas.

4.6 CONCLUSION

This study introduced a novel technology called STAR particles which were shown to dramatically enhance skin delivery of topically applied agents in a simple-to-use, non-invasive and flexible treatment manner. Upon application by simply rubbing on the skin, STAR particle arms puncture into skin similarly to conventional MN-based technologies. STAR particles were fabricated into various geometries, sizes and thicknesses from biocompatible materials such as stainless steel and alumina. STAR particle parameters were modified and investigated for their effects to increase skin permeability. Increases in SRB and 4 kDa FITC-dextran dyes were increased by one to two orders of magnitude by STAR particle treatment. Also, skin permeability was modeled based on a simple diffusion model and found to be in general agreement with predicted values.

Overall, these studies demonstrate the capacity of STAR particles to significantly increase skin delivery. Moreover, these results provide insight into parameters that influence STAR particle functionality for skin application.

4.7 ACKNOWLEDGEMENTS

The authors would like to thank R. Shafer for his training and support of infrared laser operation and D. Bondy for her administrative support.

4.8 CONFLICT OF INTEREST

A. Tadros and M.R. Prausnitz are inventors on a patent and have a financial interest in a company that is developing STAR particles for commercial use (i.e., Microstar Biotech). This potential conflict of interest has been disclosed and is overseen by Georgia Tech.

Chapter 5 : Discussion

In this project, a novel skin delivery enhancement technology, called STAR particles, was designed, fabricated, developed and characterized. STAR particles were inspired by MN-based technologies that minimally invasively puncture superficial skin layers to increase skin permeability. Although mechanistically similar to MN, STAR particles are not restricted by a macro-scale substrate such as a patch or roller which are commonly used to apply MN to skin. This key distinction enables STAR particles to be incorporated into formulation-based vehicles (e.g., creams, ointments, gels) and thereby applied across larger skin surface areas in an intuitive and user-friendly manner.

5.1 CONVENTIONAL METHODS TO DELIVER DRUGS INTO SKIN

Drug delivery to skin is important for many medical applications, especially for treatment of dermatologic (e.g., psoriasis, atopic dermatitis, acne) and cosmeceutic (e.g., blemishes, wrinkles) indications [77]. Despite the clear clinical need, cutaneous delivery of bioactive agents remains a challenge due to the skin's formidable barrier properties [186]. As a result, only a small number of bioactive agents have the necessary physiochemical properties that allow for topical application [4]. Alternatively, systemic administration (i.e., oral or injectable forms) can provide a means to effectively deliver bioactive agents without being limited by skin permeability. However, due to inherently poor drug targeting, systemic agents can often result in adverse side effects (e.g., nausea, hepatic toxicity). Moreover, systemic drugs typically generally require administration of large initial doses since a significant percentage of drug is distributed and metabolized

elsewhere in the body (i.e., not in the skin). Additionally, bioactives which require administration by injection (i.e., via needle and syringe) are limited by safety, usability and acceptability [203].

To overcome these limitations, several skin delivery technologies have been developed to increase skin permeability. When evaluating new skin delivery technologies, it is important to consider several criteria. In the following discussion, these criteria are discussed and STAR particles are contextualized in relation to other skin delivery methods and technologies.

5.2 CRITERIA FOR EVALUATING SKIN DELIVERY METHODS/TECHNOLOGIES

Safety: Most medical technologies inherently have safety risks. However, it is of paramount importance to manage potential safety risks for patients and satisfy FDA regulatory requirements.

Side Effects: Of additional importance for new technologies is to minimize adverse side effects or, if side effects are unavoidable, to provide a measurable clinical benefit relative to incurred side effects. If the method/technology is associated with adverse side effects (e.g., pain, nausea) that lower patient quality of life, then there is reduced likelihood of patient compliance and therefore reduced efficacy.

Delivery Efficacy: The efficacy of the new delivery technologies should be superior, or at least non-inferior, to the current standard of care within each particular medical indication. Additionally, the technology should enhance delivery of many drug compounds (e.g., small molecule, peptides, biologics).

Low cost, Manufacturability: Of particular importance for skin-based delivery technologies is the need for low-cost therapies. Because many dermatologic indications can be chronic in nature, cost effectiveness is essential to provide patients therapies that are not economically burdensome.

Usability and Acceptability: Patient experience with the technology is important to provide a delivery platform that is simple to use, does not require extensive training and is amenable to self-application. If the skin delivery technology does not meet these criteria than patient compliance can be an issue.

Product Life Cycle: The technology should also be safe to dispose of after use and not harm the environment. The technology should also be in compliance with present environmental regulations (e.g., Microbead-Free Waters Act of 2015, H.R.1321).

Treatment Flexibility: Due to the heterogeneity of skin types (e.g., diseased vs. healthy skin, surface area of skin needing treatment, differences between skin anatomical sites, mechanical elasticity/rigidity of skin), there is great importance for the medical technology to perform robustly regardless of the skin to which it is applied.

Skin Targeting: Localized, efficient delivery of bioactive agents directly to the skin is important to reduce both the total required dose (to reduce cost of treatment) and minimize potential off-target, adverse side effects.

5.2.1 - Safety

In these preliminary studies, STAR particles were applied to live animals and to a small cohort of healthy human volunteers. The results from these studies showed that STAR particles induced only very slight, transient skin erythema with no other apparent side effects. Also, STAR particles were never observed to fully embed themselves during skin applications. Because STAR particles have been designed to puncture skin without fully embedding, it is believed their use is potentially safer than skin delivery technologies which can become embedded in skin [201]. However, if STAR particle materials do become embedded in skin (e.g., due to tip breakage during skin application) one of several scenarios can occur. If STAR particle material is deposited inside the epidermis, then the material will likely be sloughed off during the skin's natural development cycle.

Alternatively, if STAR particle material is deposited inside the dermis then a foreign body granuloma (i.e., a skin growth) may occur. Granulomas occur when the body cannot eliminate a foreign substance and therefore the immune system attempts to isolate the foreign material [204]. As a result, foreign-body granulomas need to be surgically removed by a physician. While current STAR particles are mechanically robust, the potential for adverse events occurring due to material deposition can be avoided in future STAR particle designs (e.g., if the STAR particle is composed of water-soluble and/or biodegradable materials).

STAR particle safety can also be compared to the safety profiles of CPE and physical-based delivery technologies. CPE can cause considerable skin irritation due to solubilizing agents (e.g., DMSO) which can partition in skin [205]. Most physical-based delivery technologies have been shown to be safe for use on skin [206].

Given these initial results, we believe STAR particles can be designed to be safe for use during and immediately following a single skin application. However, additional investigation of STAR particles is necessary to provide further evidence of STAR particle safety (e.g., multiple STAR particle applications to the same skin site).

5.2.2 - Side Effects

In a limited number of studies conducted with STAR particles, we have not observed the presence of adverse side effects (in live animals and more in healthy human participants) aside from very slight, transient erythema. STAR particles, in their present configuration,

simply act to puncture into skin (i.e., STAR particles serve purely a mechanical function). Therefore, STAR particles are functionally closer to physical penetration enhancers which themselves disrupt skin barrier functionality and are generally not associated with adverse side effects when used under suitable operating conditions. As already discussed, CPE can have side effects related to skin irritation. Additionally, systemic agents (i.e., oral and injectable drug forms) are commonly associated with adverse side effects due to whole-body drug exposure. In summary, STAR particles in their present configuration are expected to result in minimal adverse side effects. Additional investigation should be conducted if STAR particles are altered beyond their present form (e.g., fabricated from other materials or applied to skin multiple times). In addition, side effects may result indirectly from STAR particle application because larger drug doses are administered, which may increase drug efficacy but may also increase drug side effects.

5.2.3 - Delivery Efficacy

In these studies, STAR particles were shown to be effective in delivery enhancement of topically applied molecules *ex vivo* and *in vivo*. Delivery enhancement was demonstrated for several compounds of clinical interest (e.g., 5-fluorouracil, methotrexate, bleomycin). STAR particles were able to enhance topical delivery in a controlled manner between 14- to 90-fold. Delivery increases could be modified (e.g., by altering concentration of STAR particles applied to skin) to meet the needs of specific clinical applications and bioactive compounds of interest. Compared to abrasive gels or MN patches, STAR particles could be formulated to increase skin permeability more or less than these technologies. Other technologies have extensively been reported for their delivery enhancement potential

(e.g., ultrasound [197], iontophoresis [100], electroporation [104]). These technologies have been demonstrated to significantly enhance the delivery of bioactive compounds to skin including water-soluble and high molecular weight compounds. CPE have also been shown to increase skin permeability. However, CPE are generally ineffective in delivery enhancement of macromolecules. Although the microscopic puncture sites created by STAR particles are sufficiently large to allow delivery of essentially any molecule, a practical limitation exists because STAR particles (in their present form) do not actively deliver drugs. Rather STAR particles rely on molecular diffusion of the topically applied drug through the microscopic puncture sites.

5.2.4 - Low cost, Manufacturability

In their present form, STAR particles are fabricated from low-cost and commercially available materials such as metals (stainless steel) and ceramics (alumina). Moreover, the laser micromachining process used in this study to fabricate STAR particles is almost fully automated. Therefore, we believe this process potentially lends itself for use in production for research and development purposes. However, future developments should be focused to increase production capacity for commercial scale production. In addition, based on estimations for raw materials cost, the cost of a single dose of STAR particles can potentially be very low (approximately \$0.01 materials cost). However, the cost to fabricate STAR particles at significantly larger scales may require sophisticated equipment which may increase fabrication costs. In comparison to physical-based delivery methods, STAR particles are expected to be lower cost and more amenable to mass manufacturing given that these alternative technologies involve more sophisticated

and costly components (e.g., energy sources, mechanical components). STAR particles may potentially be comparable in cost and manufacturability to conventional MN-based technologies in which drugs are not incorporated into the MNs (which adds cost), such as MN patches for skin pretreatment before application of active agents or MN patches for cosmetic skin rejuvenation. However, STAR particles are expected to be more costly to manufacture than conventional skin abrasives as abrasive particles are typically irregular in size/shape.

5.2.5 - Usability and Acceptability

To ensure that medical technologies are used as directed, it is important to provide patients with an experience that is painless, simple and does not create unnecessary burdens. In short, the skin delivery technology should help enable treatment, not limit treatment due to poor patient compliance. In the limited clinical study conducted with STAR particles in healthy human participants, STAR particles produced fewer uncomfortable sensations than application of a hypodermic needle. Additionally, most study participants reported that they would feel (very) comfortable in self-application of STAR particles and an overwhelming majority (91%) indicated that they considered STAR particle formulations to be (very) similar to application of conventional topical products (e.g., body lotion, sunscreen, face wash). These results provide limited, but compelling evidence that STAR particles are user friendly and simple to use for skin treatment.

The usability and acceptability of STAR particles in relation to other skin delivery technologies was not directly investigated in this study, but alternative skin delivery technologies have been reported to provide a minimally invasive, comfortable user experience. However, many of these technologies (e.g., MN-roller, ultrasound) require use of a separate device and/or a multi-step application process, which may diminish the usability for some patients. It can reasonably be concluded that STAR particles may provide a patient friendly experience relative to other skin delivery technologies.

5.2.6 - Product Life Cycle

There is great need for skin delivery technologies which are simple, safe and environmentally benign to dispose of following their use. In prior years, many topical formulations incorporate micro-particles composed of non-biodegradable polymers (e.g., polyolefins) as exfoliants. These micro-particles would then be washed away (i.e., down the sink or shower) and accumulate in waterways, thereby causing harm to the environment. To mitigate this issue, in 2015, U.S. Congress passed a bill banning rinse-off cosmetics containing plastic micro-beads. Therefore, to shift away from use of non-biodegradable polymers, many skin products now incorporate environmentally benign exfoliants (e.g., metal oxides, nut shells, fruit pits).

STAR particles in their ceramic (i.e., metal oxide) form are believed to be simple and environmentally benign to dispose of after their use. Although ceramics are environmentally benign, they are non-biodegradable and will therefore not naturally be degraded in the environment. Therefore, additional work should be conducted to develop

STAR particles that become deactivated (i.e., non-functional) following their use (e.g., mechanical, chemical, breakage of tips, full dissolution) to minimize potential safety risks. In summary, STAR particles are expected to have low-environmental impact but future work is required to ensure that STAR particles are deactivated and thereby increase safety of disposal.

5.2.7 - Treatment Flexibility

There is also great importance for technologies that can enhance skin delivery in a robust and flexible manner regardless of skin type (e.g., healthy vs. diseased skin, surface area, biomechanical environment).

Formulation-based methods (i.e., conventional topicals, CPE, skin abrasives) are less versatile since they have limited skin permeability, especially in hyperkeratotic (thickened) skin (e.g., palms and soles of feet, warts, psoriasis). However, formulation-based strategies offer flexibility in treatment area since they can be spread across the skin regardless of surface area involvement. Physical-based methods are flexible in use since they have been demonstrated to deliver most types of bioactive agents and they can be used in many different skin types but again delivery across large, disseminated surface areas is a limitation for many of these technologies.

STAR particles have presently been demonstrated to increase skin permeability via a formulation-based application method. Their application method enables STAR particles to be applied across large, disseminated body surface areas. To date, STAR particles have

only been investigated on healthy skin so the effects of STAR particles on other types of skin is unknown. However, it is envisioned that STAR particles can enable delivery regardless of skin physiological state because they are mechanistically similar to MN-based delivery technologies which have been demonstrated in a number of different skin disease states [180, 207, 208]. Therefore, we conclude that STAR particles are more versatile compared to conventional formulation-based skin delivery methods and better in treatment of large, disseminated skin surface areas relative to physical-based delivery methods.

5.2.8 - Localized Delivery to the Skin

Assuming the drug site of action is within the skin (i.e., not elsewhere in the body), targeting of the skin itself can be an important criterion to efficiently and safely deliver bioactive compounds. This is especially true if the active compound is costly or is known to cause adverse side effects when delivered systemically. STAR particles, like other formulation-based delivery methods, spatially localize delivery to the skin which should provide for more efficient targeting relative to systemic delivery methods. STAR particles also function similarly to MN-based technologies which directly target delivery to the skin. Alternative skin delivery enhancement methods also function to permeabilize the skin and deliver the bioactive compound directly to skin.

5.2.9 - Overall Analysis and Limitations of STAR Particles

STAR particles offer a number of advantages relative to conventionally used skin delivery technologies. First, STAR particles can be incorporated directly into topical

formulations which enables their intuitive application to flexible sized skin surface areas (i.e., large or small, localized or disseminated). Second, STAR particles have been very well tolerated and, because of their small size, did not induce uncomfortable sensations during skin application. Third, STAR particles can be fabricated out of biocompatible, low-cost and environmentally friendly materials (e.g., alumina) that should be amenable to large-scale manufacturing methods in the future. Fourth, STAR particles enable spatial localization of the skin itself and thereby offer the potential to reduce adverse, off-target side effects associated with systemic delivery methods. Finally, STAR particles can significantly enhance delivery to skin of a broad variety of compounds with a range of different physiochemical properties. Because they function similar to conventional MN-based technologies, STAR particles are expected to be safe, tolerable, acceptable and efficacious across many types of skin and disease states.

STAR particles are similar to many skin delivery technologies in their capacity to increase skin permeability in a minimally invasive, safe and robust manner. However, STAR particles uniquely combine many of the traits that make each skin delivery technology favorable. Mainly, STAR particles combine the ease of use for formulation based-delivery methods and the delivery enhancement capabilities of physical-based delivery technologies.

One particular area in which STAR particles are limited is their capacity to rapidly deliver drugs to the skin. While STAR particles can increase skin permeability, topical delivery requires the passive diffusion of a drug molecule across its concentration

gradient (i.e., from the topical formulation into the skin). Assuming molecular diffusion at zero Reynolds Number (i.e., Stokes-Einstein diffusion), the diffusivity of a molecule is inversely proportional to the cube root of its molecular weight ($D \propto MW^{-1/3}$). In practical terms, this means that many macromolecules will be diffusion limited in their skin delivery potential (e.g., a 500 kDa molecule will diffuse at one-tenth the rate of a 500 Da molecule). This also means that STAR particles in their present form would not be an ideal choice for bioactive compounds that require fast delivery. Finally, the slow diffusion of molecules into skin also means that a significant fraction of the initially applied topical dose will likely not be delivered into the skin and thereby be wasted. Therefore, STAR particles in their present form may not be an ideal candidate if the bioactive compound is costly.

Another current limitation of STAR particles, related to an inherent property of most topical formulations, is related to precise dosing. Unlike delivery methods that precisely deliver an exact dose (e.g., injections, oral tablets), most topical skin products do not have tightly controlled doses due to variability in the quantity of topical applied, patient wear time, environmental factors and others variables [209]. Therefore, STAR particles are expected to be of greatest use for delivery of bioactive compounds with wide a therapeutic index (Therapeutic Index = Toxic Dose in 50% of subjects / Effective Dose in 50% of subjects; $TI = TD_{50}/ED_{50}$). Likewise, STAR particles would not be an ideal delivery technology for drug molecules that have a narrow TI [210].

A third potential limitation for STAR particles at present is their formulation-based application. Because STAR particles are incorporated into topical products (i.e., creams, ointments, gels, etc.), reformulation of the bioactive compound may be required if the drug is not already delivered topically. Reformulation requires that the drug molecule be homogeneously dispersed within the topical and that the drug remain active within the formulation for a sustained period of time that is sufficient for treatment.

To summarize, STAR particles are a unique platform technology which can enable painless, simple to use, flexible, low cost and efficacious skin delivery. However, further characterization and technical developments are essential to ensure that STAR particles reach their clinical and market potential.

Chapter 6 : Conclusions

Delivery of bioactive compounds for treatment of dermatologic (e.g., psoriasis, eczema, skin cancer, acne) and cosmeceutic (e.g., wrinkles, blemishes, skin aging) indications constitute a high-growth, multi-billion-dollar industry that has great impact on improving morbidity, mortality and quality of life. A particularly advantageous method to administer drugs directly to skin is through simple-to-use and non-invasive topical formulations (e.g., creams, ointments, gels). However, only a small number of drugs possess the necessary physiochemical properties that allow for passive topical delivery into skin. As a result, many skin delivery technologies have been developed to increase skin permeability such as CPE, iontophoresis, electroporation, ultrasound and MN-based technologies. Although these technologies have enabled increased delivery of many bioactive compounds (e.g., water-soluble and macromolecular compounds), limitations relating to safety, efficacy and usability still exist.

STAR particles were developed to provide a user friendly, efficacious, safe, minimally invasive and flexible technology platform capable of significantly increasing skin delivery. Although inspired by MN-based technologies, STAR particles fundamentally differ from conventional MN designs which incorporate micro-scale projections onto a macro-scale substrate (e.g., patch or roller). Rather, STAR particles incorporate micro-projections onto particles, which enables their application to large, disseminated body surface areas in a simple-to-use topical formulation.

The goal of this project was to design, fabricate, develop and characterize STAR particles that are capable of increasing skin delivery, via a formulation-based application method, in a simple-use and minimally invasive manner.

6.1 DESIGN, FABRICATION AND DEVELOPMENT OF STAR PARTICLES FOR ENHANCED TOPICAL SKIN DELIVERY

The main goal of this study was to design, fabricate and develop STAR particles for enhanced skin delivery. First, STAR particles should be designed to be minimally invasive and not induce uncomfortable sensations during their application. Next, STAR particles should be fabricated to have sharp tips capable of penetrating into skin. STAR particles must also be composed of materials that are biocompatible, low cost, mechanically robust, environmentally friendly and easily manufactured. Next, STAR particles should enhance delivery of topical compounds through a simple, formulation-based application method that enables their use across large, disseminated body surface areas. The main findings from this project were:

- STAR particles were designed based on learnings from conventional MN-based technologies, which penetrate skin in a minimally invasive manner. Namely, STAR particles were designed to have needle-like projections (i.e., arms) on the length scale of several hundred microns. STAR particle arms of this length could penetrate into skin *ex vivo* and *in vivo*.
- STAR particles were fabricated with sharp tips (tip radius of curvature < 15 μm) from biocompatible materials such as stainless steel (mSTAR) and alumina (cSTAR) via micro-laser ablation. Both stainless steel and alumina are biocompatible, low cost, mechanically strong, easily manufactured and have precedence for use in clinical medicine (e.g., hypodermic needles or orthopedic/dental implants). Moreover, alumina is used in topical skin care products (e.g., as a skin abrasive) and is generally regarded to be environmentally benign.

- First-generation mSTAR particles functioned to increase skin permeability in a manner that was dependent on both geometry and concentration of mSTAR particles applied to skin. mSTAR particles were also incorporated into aloe vera gel to enable their simple, formulation-based application to relatively large skin surface areas (e.g., $> 10 \text{ cm}^2$).
- Next, mSTAR particles reduced skin barrier functionality as measured by skin electrical resistance and gentian violet staining patterns. mSTAR particles also enhanced topical delivery of the fluorescent model compounds sulforhodamine B and 4 kDa FITC-Dextran into excised skin as shown through histological analyses and steady state flux measurements.
- Additionally, mSTAR particles were demonstrated to be very well tolerated, only showing very slight, transient erythema, when applied to hairless rat skin *in vivo*. Topical delivery of sulforhodamine B was also enhanced in skin that had been pre-treated with mSTAR particles relative to skin treated with aloe gel alone.
- Second-generation cSTAR particles were shown to be efficacious in increasing delivery of several clinically relevant drugs with diverse physiochemical properties (i.e., 5-fluorouracil, methotrexate and bleomycin). Delivery for these bioactive compounds was between 2- to 10-fold enhancement after just 10 seconds of cSTAR skin pre-treatment relative to skin treated with aloe vera gel alone.
- Finally, mSTAR particles were topically applied to a small cohort of human participants. Results from this study showed that mSTAR particle skin application was very well tolerated by study participants with only very slight, transient erythema observed. STAR particle applications were also very well accepted and described by study participants as mostly comfortable. STAR particles were also efficacious in creating microscopic skin punctures as shown through gentian violet staining.
- In summary, STAR particles were designed, fabricated and developed to increase skin permeability via a topical-based, minimally invasive, flexible and simple-to-use application method. STAR particles were capable of achieving these criteria as demonstrated in excised skin models, live animal experiments and studies in a limited number of human volunteers. We conclude that STAR particles provide a viable technology platform to improve delivery of existing topical compounds and potentially broaden the range of molecules capable of topical skin delivery. In these present studies, STAR particles were used as a pre-treatment method. However, future efforts should focus on using STAR particles in a single-step application as opposed to a two-step, skin pre-treatment method.

6.2 FABRICATION AND CHARACTERIZATION OF STAR PARTICLES TO ENHANCE TOPICAL SKIN DELIVERY *EX VIVO*

The main goal of this study was to fabricate and characterize STAR particles to determine design (i.e., STAR particle geometry, size and thickness), formulation (i.e., STAR particle concentration) and user (i.e., STAR particle application time) parameters that influence their functionality to increase skin permeability. Briefly, these analyses included measurements of gentian violet (GV) staining area, skin electrical resistance and transdermal delivery of fluorescent model compounds (SRB and 4 kDa FITC-Dextran). Also, the effect of sintering temperature was explored with cSTAR particles to determine the effect of processing parameters on STAR particle mechanical integrity during skin application. The main results from these studies included:

- mSTAR particles were fabricated to have sharp tips independent of their design. Due to their sharp tips and ease of fabrication, we chose mSTAR particles as the primary material for subsequent characterization studies.
- Due to present limitations with laser fabrication (e.g., large laser spot size), cSTAR particle tip sharpness was more dependent on design parameters such as geometry. An inverse relationship between tip sharpness and the number of arms per STAR particle was observed. Within these fabrication studies, three-armed cSTAR particles were measured to have the sharpest tips ($14.2 \pm 0.58 \mu\text{m}$) and nine-armed STAR particles had the least sharp tips ($20.5 \pm 0.52 \mu\text{m}$). Future developments to improve fabrication are expected to resolve this limitation.
- Histological analysis of skin pre-treated with mSTAR particles, and exposed to topical SRB fluorescent dye, showed increased delivery relative to skin pre-treated with aloe gel alone. The increase in skin delivery for mSTAR particle pre-treated skin was visually similar to that of MN patch skin pre-treatment.
- Skin abrasive gel applied for 10 seconds did not significantly increase skin permeability as assessed through gentian violet staining and transdermal flux values.
- Characterization of mSTAR parameters revealed key parameters that influence STAR particle functionality.

- mSTAR particle concentration was directly proportional to skin permeability enhancement. For example, when mSTAR particle concentration (6-arms, 10-second application time) was increased from 100 to 1000 mSTAR particles/cm², transdermal flux of SRB increased 14- and 68-fold, respectively, relative to skin pre-treated with aloe gel alone.
 - mSTAR particle application time marginally increased transdermal delivery when comparing 10-second and 2-minute skin application. We conclude, the majority of delivery enhancement occurred after 10 seconds of mSTAR skin application. It was not clear the exact reason for this effect. However, mSTAR particle arms were observed to bend following their application which may have contributed to their decreased functionality for longer skin application times.
 - mSTAR particle geometry impacted skin permeability enhancement effects. At equivalent concentrations and skin application times, in general, STAR particles with more arms produced greater skin effects. This effect may be attributable to an increased probability of mSTAR particles being spatially oriented to allow for skin puncture.
 - In general, mSTAR particle size influenced skin permeability. For example, when mSTAR particle (tip-to-tip) size was increased from 0.5 to 1.0 mm, steady state flux increased by 38- and 33-fold for four- and six-armed mSTAR particle geometries, respectively.
- General agreement existed between experimental results and predictions, based on a simplified diffusion model, of steady state flux across STAR particle pre-treated skin. Likewise, trends were observed to correlate skin barrier properties such as conductance, steady state flux and gentian violet staining area.
 - Skin permeability enhancement increased in relation to concentration of cSTAR particles applied to skin. Skin delivery enhancement was most significant when concentration of cSTAR particles applied was increased to 10wt%.
 - Sintering temperature influenced mechanical integrity of cSTAR particles and their ability to puncture into skin. cSTAR particles sintered below 1400°C were mechanically fragile, broke during skin application and did not have measurable effects on skin barrier properties.
 - In summary, metal and ceramic STAR particles parameters were characterized for their effects on skin permeability *ex vivo*. Short application times (10 seconds) were sufficient to increase skin permeability. These results demonstrate the capacity of STAR particles to be modified for a wide range of skin delivery enhancement effects. Additional work should seek to understand the practical limitations of STAR particle mediated skin delivery with macromolecular compounds.

Chapter 7 : Future Directions

The STAR particles developed in these studies were demonstrated to reduce skin barrier functionality through a simple and flexible formulation-based application method. STAR particles produced microscopic puncture sites in skin and thereby enhanced delivery of topical compounds *ex vivo* and *in vivo*. Moreover, STAR particles were demonstrated to be safe, tolerable, acceptable and efficacious when applied to skin in a small cohort of human participants. Although these discoveries have provided a solid foundation for the technology, more work is needed to ensure that STAR particles are translated beyond research-stage development. In the following discussion, several areas of research will be described as they relate to future work that would further enable STAR particle mediated skin delivery.

7.1 STAR PARTICLE MATERIALS DEVELOPMENT

In the present study, two different materials were used to fabricate STAR particles – stainless steel and alumina. Both stainless steel and alumina satisfied many key criteria such as biocompatibility, low cost and high strength. However, there are other important criteria that should be considered when selecting next generation STAR particle materials.

Firstly, both stainless steel and alumina are non-biodegradable (i.e., they will not be broken down through natural/biological mechanisms). Although, non-biodegradable materials may not be of concern for many applications, there are some potential safety concerns which could be eliminated if STAR particle materials were biodegradable.

Therefore, to improve their safety profile, STAR particles should be composed of materials that safely degrade in some cases. Potential materials can be biopolymers (e.g., poly [lactic-co-glycolic acid], polylactic acid, polyvinylpyrrolidone), sugars (e.g., sucrose, trehalose), carbohydrates (e.g., carboxymethyl cellulose), waxes and many other natural or synthetic biodegradable materials.

Another material-based development that should be undertaken in future work relates to deactivation of STAR particles following their skin application. In their present form, STAR particles puncture skin and remain intact. Due to their mechanical robustness, STAR particles can repeatedly puncture into skin multiple times to increase their efficacy (i.e., each STAR particle can create multiple puncture sites). However, it may be desired to disable STAR particles after a single or multiple skin punctures (e.g., between 1 to 10 skin punctures per STAR particle arm). Disabling STAR particles after they are applied can provide an additional level of user safety. Disabling STAR particles can potentially be accomplished by fabricating STAR particles (or regions of STAR particles) from materials that can breakdown (e.g., chemical-, photo-, thermal-, mechanical-mediated breakdown). In a particular example, STAR particles could be fabricated from water-soluble materials that dissolve once entering an aqueous environment such as the skin. Such STAR particles could be fabricated through conventional micro-molding based schemes as has been demonstrated with conventional MN patches.

7.2 STAR PARTICLES COMBINED WITH BIOACTIVE COMPOUNDS VIA COATING OR ENCAPSULATION

STAR particles in their present form function similarly to solid, non-dissolving MN in that they increase skin permeability through creation of microscopic puncture sites (i.e., poke and patch approach). Because they serve a purely mechanical function, STAR particles do not directly deliver any drugs to skin which may be limiting in some medical applications. For example, topical delivery of large molecular weight compounds (e.g., biologics) may be diffusion limited. Therefore, enabling STAR particles to actively deliver drugs would potentially increase their usefulness, make drug dosing more reproducible and improve the cutaneous bioavailability for a broader range of bioactive compounds. Several potential strategies may accomplish this goal.

Firstly, currently available STAR particles (mSTAR or cSTAR) could be coated with a water-soluble formulation that has incorporated a bioactive compound. This method has been proven successful with coated MN structures to deliver many types of bioactive compounds [[119](#), [121](#), [122](#), [180](#)]. The advantage of this method is the dual benefit of having a strong material (i.e., metal or ceramic) to provide structural integrity during skin application and a coating that dissolves once skin penetration has occurred. It will be important to find a fabrication method that can evenly deposit the coating formulation onto each STAR particle. It is also important to formulate the coating so it rapidly dissolves during skin penetration. Because STAR particles have not yet been observed to penetrate and remain in skin, it is hypothesized that STAR particle penetration occurs

very quickly (i.e., < 1 second). Therefore, rapid dissolution of the coating formulation is of great importance.

In another strategy, STAR particles could be fabricated to incorporate drugs into a water-soluble matrix such as a polymer or sugar. This fabrication scheme has found usefulness in dissolving MN patches that have been demonstrated to deliver a large number of bioactive compounds [114, 132, 133, 211]. The challenge here will be to adapt these fabrication methods to produce STAR particles with sharp tips, ensure that the resulting structure is mechanically robust and select a material that rapidly dissolves in skin.

7.3 DEVELOPMENT OF HIGH-THROUGHPUT PRODUCTION PROCESS

Current laser-based fabrication of STAR particles has been sufficient to produce quantities useful for early-stage research and development efforts. However, if STAR particles are to be produced at commercial scale, increased throughput is required. Several production methods may potentially be useful to provide greater production capacity of STAR particles. First, STAR particles may be fabricated via a roll-to-roll process where sheets of a desired material are fed through a hot-stamping process to produce the desired STAR geometries. Next a micro-molding process can be used to produce STAR particles (i.e., similar to MN patch based fabrication). Other mass-fabrication strategies may also potentially be explored.

7.4 DETERMINATION OF STAR PARTICLE SKIN PENETRATION DEPTH

To date STAR particle penetration depth has not been directly visualized. Although STAR particles have been designed to have a maximum possible penetration depth (i.e., the total length of each arm), it is hypothesized that this maximum penetration depth is rarely (if ever) actually reached. This is due to the topical application method of STAR particles which makes the likelihood of STAR particles penetrating skin at a non-perpendicular angle more probable. This is in contrast to conventional MN patches which are applied to skin at a perpendicular angle to the skin's surface. Even in the case of MN patches, the full length of the MN structure does not fully insert, likely due to skin deformation. Knowledge and control of skin penetration depth may also have important implications regarding how STAR particles are regulated. Therefore, future studies should focus on elucidating, designing and controlling the depth to which STAR particles puncture skin.

Confocal microscopy may be used to visualize penetration sites created by STAR particles. For example, STAR particles can be applied to excised skin and followed by topical application of a high-molecular-weight fluorescent compound (e.g., 2000 kDa FITC-Dextran). The fluorescent compound would preferentially localize to the microscopic skin puncture sites (i.e., diffusion would be limited outside of the skin puncture sites) and thereby enable their visualization. Appropriate controls would be needed to provide comparisons to known skin penetration depths (e.g., application of a MN patch with a known skin penetration depth).

Another experimental technique that could be used to roughly determine skin penetration depth is heat stripping of skin. In this method, skin epidermis is heat separated from the underlying dermis. In this way, STAR particles could be applied to skin, skin would then be heat separated and the epidermal layer analyzed to determine if STAR particles had fully penetrated past skin epidermis (i.e., through visual examination of the basal layer of the epidermis).

7.5 CLINICAL DEVELOPMENT OF STAR PARTICLES

In present studies, mSTAR particles were demonstrated to be safe, tolerable, acceptable and efficacious in a small cohort of healthy human participants for a single (one time) skin application. While these results were encouraging, additional investigation is needed. In a similar fashion to the first experiment conducted with mSTAR particles, investigation of cSTAR particles should also be conducted in a small number of healthy human participants to demonstrate their safety, tolerability, acceptability and efficacy. In these next set of clinical experiments, it would be preferable to include non-invasive measurements of skin barrier properties following STAR particle application (e.g., skin electrical resistance, transepidermal water loss). Such non-invasive measurements can enable determination of STAR particle efficacy and also provide valuable information on skin repair following STAR particle application [212, 213]. Additional clinical studies should also be conducted with multiple STAR particle applications to the same skin treatment area (e.g., once or twice per day for up to two weeks). Repeated skin applications are important for many dermatologic and cosmeceutic applications.

Therefore, STAR particles should be investigated for their safety, tolerability, acceptability and efficacy during multiple skin applications.

7.6 SUBJECT DEPENDENT VARIABILITY IN SKIN PERMEABILITY

In this present study, STAR particle parameters were characterized for their effects on skin permeability enhancement (e.g., geometry, size, application time, concentration). However, in all of these studies, the application was performed by a single individual. If STAR particle-based therapies are to one day be self-applied, it is important to elucidate the impact of inter-user variability on resulting skin permeability. To accomplish this goal, studies may be conducted both directly in humans and in excised skin models. First, study participants can be enrolled to self-apply STAR particles with a set of written/pictographic instructions. Skin permeability enhancement could then be characterized via non-invasive methods such as gentian violet skin staining, transepidermal water loss and skin electrical resistance measurements. Participants could also be asked to apply STAR particle formulations to excised skin. Skin delivery could then be evaluated through gentian violet staining, skin electrical resistance and diffusion cell measurements of steady state flux. With these studies, user-based variability can be more fully understood and STAR particles and/or their application method can be adapted to reduce such variability.

Chapter 8 : Appendix A - Supplementary figures for chapter 3

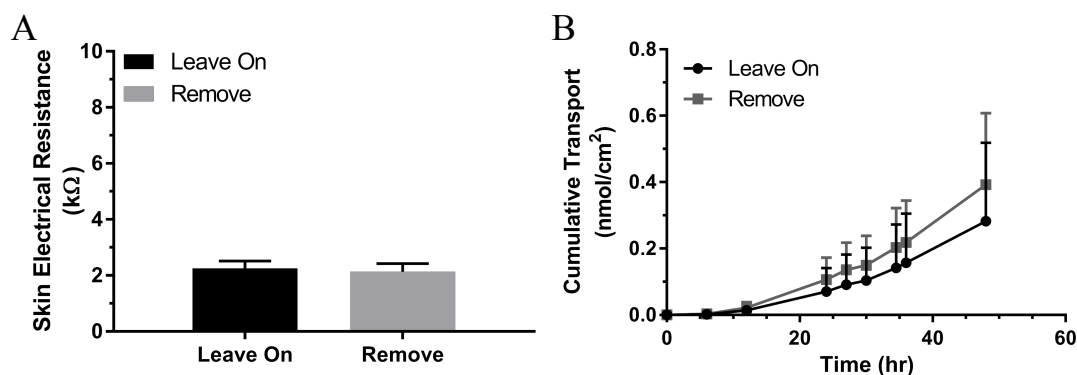


Fig. S 1: Impact of STAR particle removal following application. Skin-electrical resistance (A) and cumulative transport for SRB (B) across porcine cadaver skin pre-treated with STAR particles where STAR particles were left on or removed following their application. (n = 4).

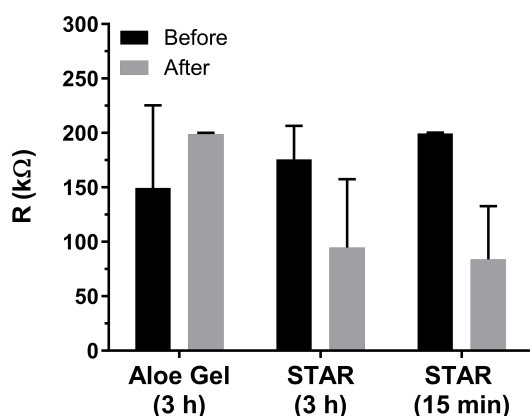


Fig. S 2: Skin-electrical resistance measurements for hairless rat skin pre-treated with either aloe vera gel or mSTAR particles *in vivo* [n=7].

Exclusion/inclusion criteria used for human study. Participants must:

- not have diseased or otherwise abnormal skin at or within 20 cm (approximately 8 inches) of the skin site(s) under study.
- not be using a medicine applied to the skin during or immediately following the study at or within 20 cm (approximately 8 inches) of the skin site(s) under study.
- not have any disease or condition known to affect pain sensation.
- not have a known allergy to the materials used to make the STAR particles or the gels, ointments and/or creams containing STAR particles.
- not have a known allergy to gentian violet.

Table 3: Demographics of participants enrolled in STAR particle application study.

Total number of study participants	11
Male	6
Female	5
Average participant age	29 years
Self-identified race/ethnicity	
Hispanic or Latino	3
White	4
Asian	4

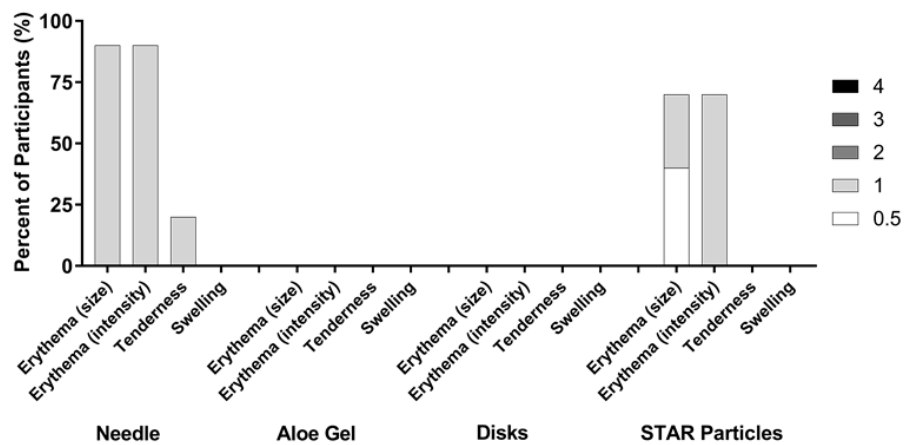


Fig. S 3: Assessment treatment methods on skin tolerability in human participants. Tolerability scores for human skin applications of a 26 gauge hypodermic needle, aloe vera gel, 0.5 mm circular micro-disks in aloe vera gel or mSTAR particles in aloe vera gel.

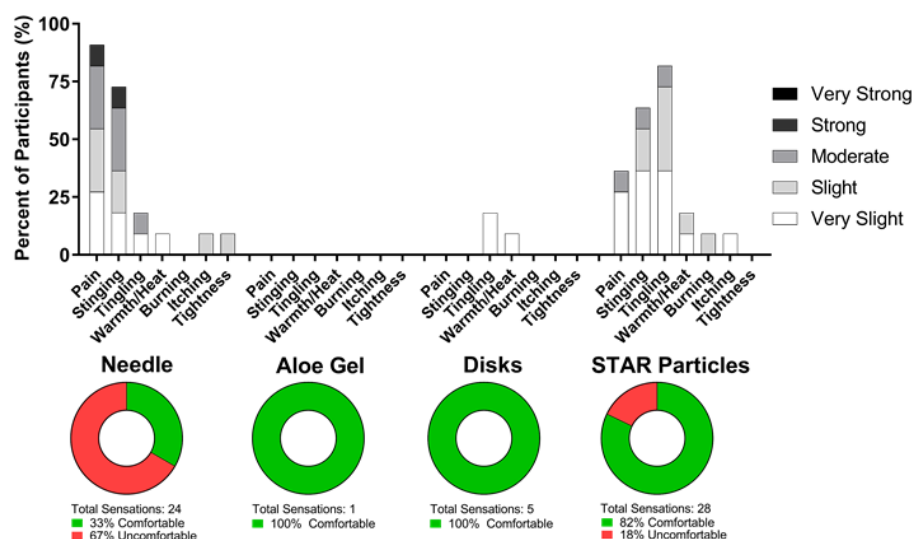
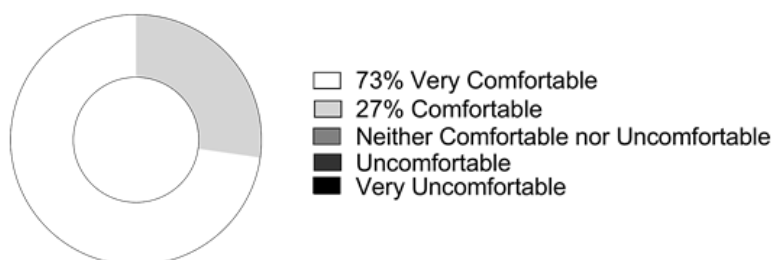


Fig. S 4: Acceptability of skin treatment methods in human participants. Summary of sensations, and sensation comfort, reported by study participants for skin applications of a 26 gauge hypodermic needle, aloe vera gel, 0.5 mm circular disks in aloe vera gel or, six-armed mSTAR particles in aloe vera gel.

How comfortable or uncomfortable would you feel applying these skin products by yourself?



How did the application of today's skin products compare to your usual experiences with other skin products (e.g., body lotion, sunscreen, face wash)?

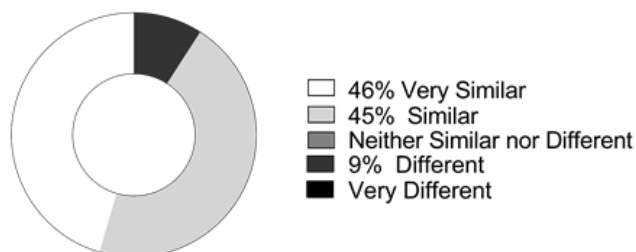


Fig. S 5: Usability of STAR particles relative to conventional skin products. Summary of results reported by study participants related to self-application of STAR particles and similarity of STAR particles to conventional skin products.

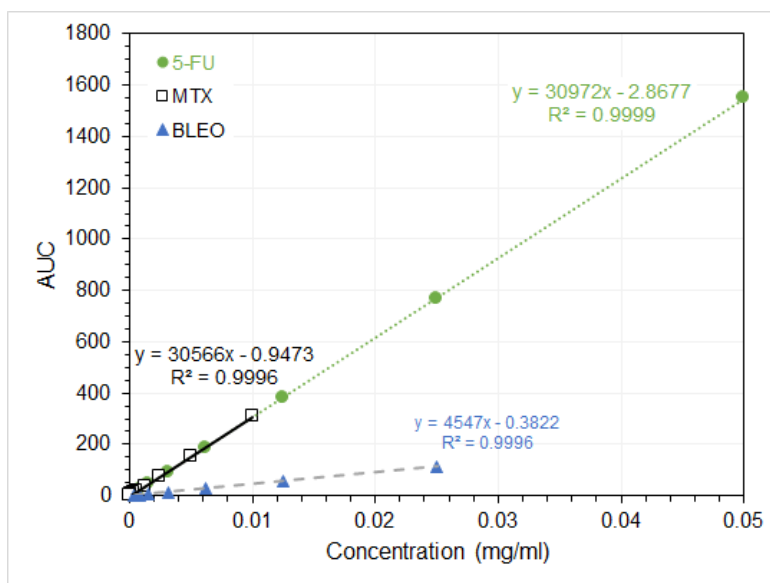


Fig. S 6: Standard curves for 5-fluorouracil (5-FU), methotrexate (MTX) and bleomycin (BLEO) as assessed via high performance liquid chromatography (HPLC).

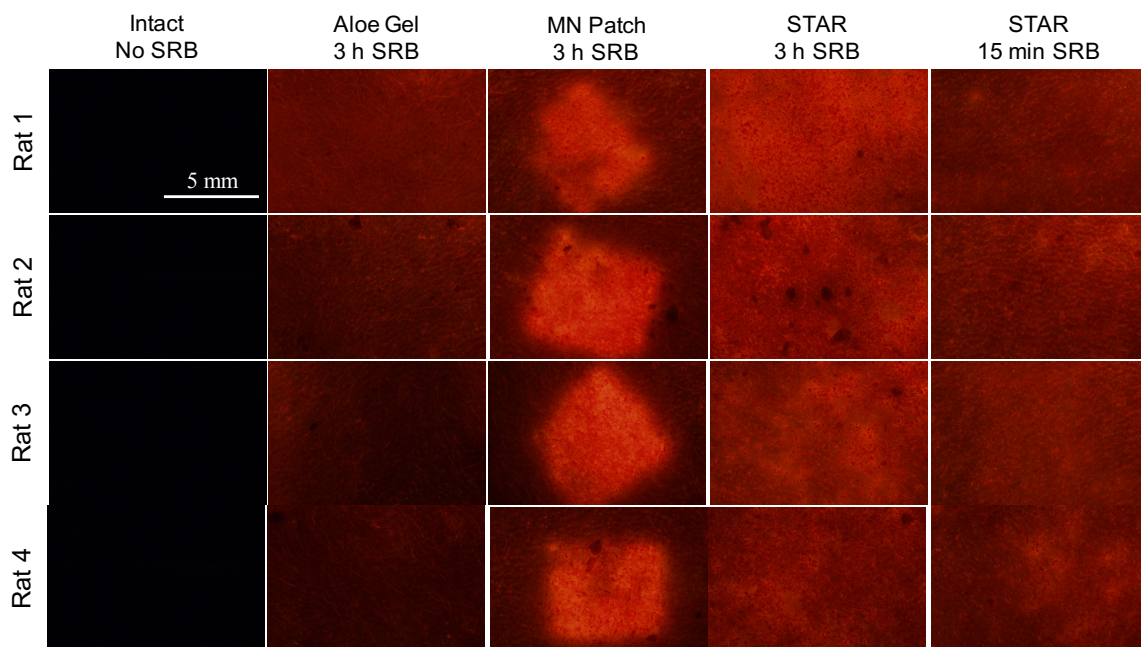


Fig. S 7: Delivery of SRB into hairless rat skin in vivo. (From left to right) - *En face* fluorescence images showing hairless rat dorsal skin which is: intact (i.e., no treatment) and not exposed to topical SRB (first column); pre-treated with aloe gel and exposed to SRB for 3 hours (second column); pre-treated with a MN patch and exposed to SRB for 3

hours (third column); or pre-treated with mSTAR particles and exposed to SRB for 3 hours (fourth column) or 15 minutes (fifth column). All images taken at identical settings (i.e., room darkness, camera exposure).

Chapter 9 : Appendix B - Tolerability Scores for Human Study

Table 4: Tolerability scoring grades used in human study to determine adverse reactions following skin application.

	Grade 0	Grade 0.5*	Grade 1	Grade 2	Grade 3	Grade 4
Tenderness	No discomfort to touch	N/A	Mild discomfort to touch	Discomfort with movement	Significant discomfort at rest	ER visit or hospitalization
Erythema (Size)	0 cm	0.1 – 1 cm [erythema less than or equal to application area]	1.1 – 5 cm [erythema spreading beyond application area]	5.1 – 10 cm	> 10 cm	Necrosis or exfoliative dermatitis
Erythema (Intensity)	No erythema	N/A	Very slight erythema (barely perceptible)	Well-defined erythema	Moderate to severe erythema	Severe erythema (beet redness)
Swelling	0 cm	0.1 – 1 cm [swelling less than or equal to application area]	1.1 – 5 cm and does not interfere with activity [swelling spreading beyond application area]	5.1 – 10 cm or interferes with activity	> 10 cm or prevents daily activity	Necrosis

* 0.5 grade only used only for erythema size and swelling scores

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