Design of a Microneedle System for Cell Delivery
A novel repigmentation therapeutic for extensive burns

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Specific Aims

Current commercial skin burn therapies re-establish the barrier function of healthy skin, but fail to address a key issue after burn wound stabilization: irregular pigmentation resulting from the destruction of melanocytes. These therapies fail to address the negative psychosocial effects or higher susceptibility to UV-damage that follow hypopigmentation. A combination of medical needling and the transplantation of non-cultured skin cells in people has resulted in statistically significant improvements in melanin production after burn wounds[1]. We can improve these outcomes through established techniques in material fabrication, cell culture, animal models, and tissue analysis. **Here we propose to build a microdelivery system that administers keratinocytes/melanocytes for use as a repigmentation therapy.** After cell replacement, ultraviolet (UV) light and melanocyte-stimulating hormone (MSH) will stimulate the production of melanin. We will achieve these goals through two specific aims.

Aim 1: Build cultured keratinocyte/melanocyte microneedle delivery system to induce melanin production in simulated skin. Keratinocyte/melanocyte reintroduction in deeply damaged tissue has been shown to reestablish pigmentation and reintegrate the newly formed tissue into the surrounding environment[1]. Thus, we will test the *working hypothesis* that keratinocyte/melanocyte delivery via microneedles, in conjunction with MSH and UV light therapy, will reintroduce photoprotective properties and restore pigment homogeneity after moderate-to-severe skin burns. This microneedle technology will be applied to reintroduce native cells expanded *ex vivo* to induce repigmentation. Healing of moderate-to-severe burns can result in a variety of outcomes, even after restoration of both epidermal and dermal tissue. **As an alternative, reintroduction of lost cell types, in conjunction with UV and MSH stimulation, represents a more inclusive treatment strategy.** The stratum corneum of the newly healed epidermis is a relatively impermeable layer for any therapeutic intervention, preventing the entry of therapeutics past the epidermal layer[2].

This microneedle system will be precisely fabricated from photolithography and micro injection molding (Aim 1.1)[2]. Cells will be tested to assess melanin production *in vitro* and efficacy in a simulated skin model as (Aim 1.2 and 1.3). Microscopy will confirm geometric uniformity in the microneedle patch system. We expect proper microneedle geometry and mechanics as shown by fluidic and structural analysis. Cellular melanin production will be quantified by spectral analysis; cell health and morphology will be qualified by immunofluorescence[3]. Melanin benchmarks have been observed in previously published therapeutic and cosmetic work, and will be achieved in our preliminary experiments with model cell lines and conditions[5]. We will then prove that we can source donor cells by either harvesting dissociated tissue during enzymatic debridement or from a healthy donor site. Once cells are harvested and expanded, the microneedle system will deliver them into a simulated skin layer. Melanin production and the health of our cells will be assessed using metabolic indicators and immunoblotting to ensure the delivery process does not impede their functionality.

Aim 2: Assess efficacy of microdelivery system construct *in vivo*. The microdelivery system will then be tested for efficacy *in vivo* in red Duroc pigs. Porcine skin is similar to human skin in terms of general structure, thickness, hair follicle content, pigmentation, collagen, lipid composition, and epithelialization, making it a good candidate for *in vivo* studies of a repigmentation therapeutic[3]. Keratinocytes/melanocytes will be isolated from the pigs' chemically debrided wounds within the first 24 hours after burn induction, and consequently as needed for the next 2 weeks as additional necrotic tissue develops. Enzymatic debridement will occur using a collagenase/hyaluronidase solution[6]. After the tissue is digested, the dissociated cells will be cultured. The cells will then be applied by microdelivery to the pig model, as demonstrated in Cuttle et al[4]. The site will then be carefully stimulated with UV and MSH to increase melanin production[7]. Pigmentation, cell viability, and functional tissue structure will be quantified by spectral analysis for melanin density of skin biopsies. We expect restoration of pigmentation, tissue morphology, and photoprotection as seen in healthy skin.

If successful, this research endeavor will serve as a first stride in treating burn consequences oftentimes neglected during conventional treatments. We would like to expand this system to treat other long-term outcomes of burns such as the restoration of hair and sweat glands. In future work, this delivery system will be adjusted to treat pigmentation abnormalities originated from causes other than burns, such as diabetic ulcers, melanoma and piebaldism, where the system will be scaled to meet the needs of the damaged tissue and specific condition[8]. This research is expected to combine the uses of microdelivery and hormone therapy by tackling the neglected aspects of burn recovery.

Significance

Every 5 seconds someone is severely burned[9]. Worldwide, an estimated 6 million people seek medical help each year for burns[9]. Burns are amongst the most devastating injuries which leave physical, psychosocial, and emotional damage[9]. In the United States, current annual estimates show that more than $18 billion is spent on specialized care of patients with major burn injuries[9]. Burn care and research has made great strides in the past couple of decades to reduce morbidity and mortality in patients, but has failed to address long term outcomes. The variety of products developed for burns like Apligraf® and Integra® are tailored to restore the barrier function of skin. Overall human skin substitutes are partial solutions to the larger problem of complete skin function restoration. Scarring, discoloration, and photosensitivity are continuously overlooked in the care of these patients. Current burn research is focused on reducing the 4% of deaths related to sepsis from burn wound infection or other infection complications[10].The primary objective of grafts and engineered skin substitutes is simply to close the wound. To achieve this, split-thickness skin autografts are the gold standard of care[11]. After grafting occurs, the wound must be carefully observed to watch for excess bleeding, infection, and/or rejection. If integration is unsuccessful, the entire process is repeated as necessary. Autografting poses it own set of problems because it generates painful donor sites, requires additional care to heal, and are prone to infection. Typically, most adult wounds heal by fibrosis which promotes scarring[2]. Burn survivors are left with major cosmetic and functional impairments[12].Years after a burn, irregular pigmentation is reported in up to 89% of patients[12]. One study reported that 44% of patients noted that they continued to receive stare years after their injury[12]. The standard of care to resolve psychosocial issues due to appearance struggles is simply therapy and counseling. The American Society for Dermatologic Surgery currently recommends tattooing to correct scar discoloration[13]. However, there are consequences associated with tattooing procedures, such as infection, allergy, inflammation, additional scar formation, and removal complications. Plastic surgeons recommend regrafting with partial thickness grafts to correct scars and overall appearance. Unfortunately, the causes of hyper- and hypopigmentation are not well studied in burns. Thus, remains significant room for innovative treatments to restore sun protection and overall appearance of burn injuries.

Innovation

Current modern burn treatments center around wound closure, infection prevention, and an expedited recovery in order to help partially reconstruct the barrier that healthy skin grants[14]. This is accomplished by debridement, or the removal of necrotic tissue, and sterilization with agents such as silver sulfadiazine, before grafting commences. Commercially available composite skin substitutes typically only use two cell types: keratinocytes and fibroblasts. As a result, they typically can only partially replace a specific feature of the skin, and cannot fully mimic all of the functions that healthy skin exhibits due to a lack of innervation, immune cells, sweat glands, hair follicles, and melanocytes[15]. Healing and scarring burn wounds are characterized by poor collagen turnover, excess connective tissue, and fibroblastic scarring, which leave the area aesthetically displeasing and less responsive to future treatment. Not only do burn victims have to deal with the immense physical pain from the burn, they also have to cope with the psychological stress of poor skin tone homogeneity and being scrutinized in public. For this reason, we decided to address the long-term problem of inadequate pigmentation and thus the lack of the photoprotective properties of melanin. Such deficiencies result in increased sensitivity to sun damage. We hypothesize that the microneedle delivery of keratinocytes/melanocytes will restore both pigmentation and photoprotection following initial burn healing for moderate-to-severe burns. Needling, or causing micropunctures to the skin, has been accepted for breaking up connective tissue build-up underneath healed scars, reducing the size of wrinkles, and acne scar softening in a process called percutaneous collagen induction. Microneedling has also been an avid area of research for the painless intradermal delivery of various pharmaceuticals, such as H1N1 or flu vaccines, and insulin for type 1 diabetics[16,17]. Microneedle technology has not yet been applied to cellular delivery. Our design utilizes the patient's own cells upon reintroduction during the burn healing timeline, to minimize the risk of scarring and immunological rejection, while improving skin texture, homogeneity, and repigmentation.

Approach

Burn healing is a time-sensitive process involving complex cellular processes and pathways that are not fully addressed in commercial products. Due to its innate biology, the skin is its own obstacle when designing new dermal therapies. Delivery of therapeutics has just begun to bypass the structural barrier of the epidermis. Microneedle penetration into the skin facilitates proper therapy delivery and helps promote melanin production. We present the use of a microneedle array to insert cells beneath the skin's surface and restore lost functions of the skin. UV and MSH will stimulate melanin generation both *in vitro* and *in vivo*.

We propose to build a microneedle delivery system that administers cultured keratinocytes/melanocytes, for its use as a repigmentation therapy in previously burned skin. We will achieve these goals by building a microneedle delivery system to stimulate pigmentation (Aim 1), and then assess the system's efficacy *in vivo* in a red Duroc porcine model (Aim 2).

Aim 1: Build cultured keratinocyte/melanocyte microneedle delivery system to induce melanin production in simulated skin. Microneedling and microneedle patches have been effectively used to surpass the barrier function of the stratum corneum in order to deliver drugs and vaccines with minimal pain. Microneedles are adaptable depending on their application. In this aim, we will test the hypothesis that cultured keratinocytes/melanocytes delivered through a microneedle system will induce repigmentation in previously burned skin. This hypothesis is supported by preliminary data, described below.

Preliminary Data 1: As seen in various microneedle drug delivery studies, uniform microneedle patches are paramount for consistent flow rate. This can be confirmed by scanning electron microscopy, light microscopy, and syringe testing [18-20]. **Figure 1**, displays an image used for microscopic characterization which confirms uniform microneedle geometry [18,21-23]. Additionally, compression strength and constant flow rate experiments with dyed water were evaluated. A custom fluidic system was used to evaluate the maximum uniform flow rate[22]. The maximum driving rate was determined to be approximately 0.347mL/s as to not damage the array during administration of the dye. Compression and fracture testing has also been conducted with a load cell at a controlled rate of 0.001N/s. Additionally, force displacement curves, with forces ranging from 0.1-15N, were delivered to the microarray to evaluate the point at which deformation occurs[22].

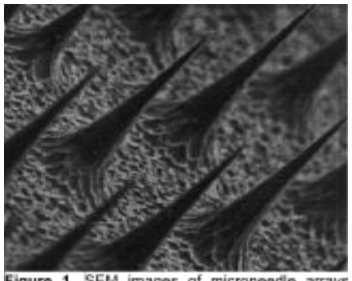

Figure 1. SEM images of microneedle arrays created in Kaushik et al 2001. A full-view micrograph of microneedle geometry [Kaushik].

Approach for Aim 1. Based on the data below, we will build a microneedle system that will deliver cultured keratinocytes/melanocytes into simulated skin.

1.1 Fabricate microneedle delivery system. To achieve successful delivery of the different cell types used in this system, a hollow microneedle array will be created with a plastic backing, creating a microneedle patch similar to the current solutions used for drug and vaccine delivery[24]. Patch geometry will include an 85x54mm^2 array with 800µm-long microneedles with a 100µm taper from base to tip. Hollow microneedle patch fabrication will be accomplished using photolithography and microinjection molding [22, 25, 26]. A polydimethylsiloxane (PDMS) mold with the proper geometry will be microinjection molded and oxygen plasma-treated to provide further encapsulation of the microneedle trenches. A photoresist polymer such as epoxy (SU-8-2025) will be heated and cast to obtain proper geometric thickness. Backside vacuuming for 3 hours will remove any entrapped bubbles and maintain mechanical integrity. The two components will be soft baked and treated with UV to properly define the hollow microneedle geometry. The epoxy will be demolded and developed in propylene glycol methyl ether acetate developer[22]. Scanning electron and light microscopy will confirm proper geometry after fabrication. Compression, fracture, and penetration testing of the microneedles will take place after successful fabrication and geometric characterization[21, 27]. Water and dye will then be used to evaluate the flow rate efficacy at controlled delivery rates[18].

1.2 Assay for production and production of melanin in vitro. The general mechanism for mammalian skin pigmentation is well established. The distribution of melanin-loaded melanosomes from melanocytes to surrounding keratinocytes grants the photoprotective properties and overall tone found in natural skin[28]. The objective of repigmentation in our approach is two-fold. First, melanocyte viability must be sustained both for *ex*

vivo culture and later during long-term patient pigmentation. Second, stimulation of melanosome production must be tunable. Harvested human melanocyte *ex vivo* co-culture with keratinocytes is not only shown to be possible but also necessary for melanocyte viability[29]. We plan to co-culture existing human keratinocyte/melanocyte cell lines and replicate the ideal environmental conditions for growth on plastic tissue culture dishes. Many studies confirm that UV and MSH exposure stimulates melanin production. We plan to test the tunability of these stimulants by observing the effect of varying UV and MSH exposures on our co-cultures. Human studies of UV have shown the minimal required dose for initial inflammation to be around 1700 J/m^2 for the average white adult male, so we expect the optimal UV exposure for our cultures to be much less than that[30]. UV will be applied to the microneedle co-seeded cells using SUP-UVASUN Mutzhas (Munich, Germany) emitting between 340-425nm with a peak at 360nm. A Schott WG 305 cut off filter is added to reduce the spectra below 305nm. UV will be applied in physiologically relevant doses of 6 and 10J/cm^2 [31]. We will assess cell growth and melanosome production over days: 0, 3, 7, 14, 21. We will use the same techniques to quantify melanocyte growth, anatomy, and expression levels after UV exposure: a comprehensive study which has not been previously done. We plan to use a spectrum of MSH concentrations for stimulation, centered around 100nM, which previous studies have found to be the minimum dose for melanosome stimulation[29]. We plan to use immunofluorescence and histological imaging to visualize cellular anatomy, and colorimetric assays to quantify melanocyte growth. Additionally, Western blots (specific for melanosome markers PMEL17 and DCT) will be used to quantify basal expression levels of melanin, its precursors, and melanin-related regulatory enzymes [32]. We expect to find sustained melanocyte viability because it has been achieved in previous studies.

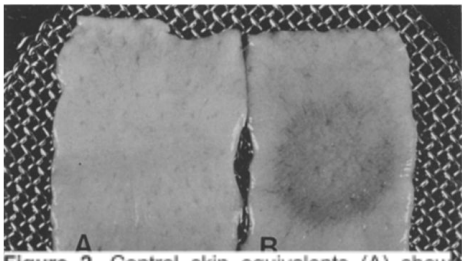

Figure 2. Control skin equivalents (A) show pigmentation which is increased following UV irradiation (B). Circular pigmentation reflects the area within the ring into which melanocyte/keratinocyte co-cultures were seeded in skin equivalent preparation (*Pigmented area,* 1 cm diameter) [Todd]

Preliminary Data 2: Previous studies have established *ex vivo* keratinocyte/melanocyte cultures that have sustained viability and react to UV irradiation. **Figure 2** shows the resulting change in pigmentation in such a culture seeding onto a simulated skin graft, indicating the proof-of-concept of UV-stimulated melanin production in keratinocyte/melanocyte culture. However, there is be a fundamental difference in the results between UV and MSH. UV is genotoxic at high exposures, whereas MSH drives melanocyte division and proliferation as well as melanosome production without risk. In fact, studies tend to preferably stimulate melanocyte growth through MSH than UV[33].

1.3 Validate microneedle cell delivery in vitro. The overall plan is to source patient's cells following enzymatic wound debridement. To mimic this, we will dissociate cadaveric dermal grafts using collagenase and hyaluronidase to extract the cells[5, 34]. Collagenase has a high affinity for cleaving peptide bonds associated with type I collagen[34]. This is favorable because of its selectivity for specifically digesting necrotic debris while avoiding the degradation of other important proteins (e.g. growth factors) and healthy collagen, which is protected by its mucopolysaccharide casings[34]. By cleaving collagen fibrils at the base of the wound that anchor the eschar in place, collagenase severs the necrotic debris from the healthy tissue, and allows for easier removal during subsequent cleansing and dressing changes[34]. We will select for our target cells (keratinocytes/melanocytes) from the various mixture of cells found in the tissue. Using markers for keratinocytes/melanocytes, fluorescence activated cell sorting (FACS) will be used to positively select for our target cells. The cells will be expanded in culture. Prior to loading the microneedle we will prepare a suspension of the cells at a ratio of 1:40 (melanocyte:keratinocyte) in phosphate-buffered saline (PBS). The suspension will be transferred to the microneedle system to seed a density of 50,000 cells/cm^2, conserving the ratio of the two cell types. The average thickness of normal skin ranges from 0.5-4mm. However, hypertrophic scarring that develops in burns and traumatic skin damage can increase the thickness of tissue. Multi-well plates with a collagen gel will mimic the dermal thickness we expect our microneedle to penetrate. Type 1 collagen gels of different thicknesses will be formed into gels in the plates to simulate varying depth[35]. Depth of the gels will be ranged from 0.5-1cm. Radiant exposure will be repeated daily followed by assessment of cell growth and metabolism. The cells will be lysed and prepared for Western blot analysis. We will probe for PMEL17 and DCT which identify both stages I & II and III & IV melanosomes, respectively[32]. Cells will be counted to track growth and

assayed for viability using metabolism indicators. Finally, photos with be taken of the cells in the gel with an inverted microscope to confirm that the cells were seeded in an even grid-like pattern as depicted in **Figure 3.**

Expected outcomes: Through mechanic, fluidic, and geometric characterizations of the microneedle arrays, the establishment of correct properties will be determined. It is expected that the delivery system will be able to be inserted with only a gentle push (approximately 10N) with a constant fluid flow rate. The microneedle delivery system will co-seed the cells at acceptable density, depth and viability.
Post-microneedling, our quantified exposure values of UV and MSH will result in strong insight into their dose-dependent effects on this co-culture.

Figure 3 Graphic representation of microneedle seeded cells

We expect melanocyte proliferation, melanosome production, and proper transfer to keratinocytes without detrimental oxidative stress or significant changes in overall cell morphology. We expect to see a correlation between melanosome production and cell viability due to the UV protective nature of melanin.

Anticipated challenges and alternative solutions for Aim 1: Should geometry of the microneedle patch not be optimal for fluidic delivery or mechanical strength (e.g. breaks, bends, and/or occlusion), additional microelectromechanical system fabrication techniques will be addressed and implemented. Such techniques include laser micromachining, deep reactive ion etching, wet chemical etching or assembly from commercially available hypodermic needles[27,36-39]. Due to microneedle fabrication techniques being highly economical, high-throughput, and tunable, adjustments to the microneedle array in terms of geometry will be financially feasible and easily achieved. An inadequate number of cells may be harvested during enzymatic wound debridement. An alternative solution would be to culture the cells for a longer duration. Otherwise, we could also harvest a small full-thickness graft from the patient. Previous studies in needle-based delivery of cells have shown that shear stress exerted on the cell during delivery may damage the cells and trigger apoptotic responses[40]. If this proves to be substantial, we may need to dampen the stress on the cells by immersing the cells in a biocompatible hydrogel[41]. Many recent publications suggest additional cellular carriers, such as crosslinked alginate hydrogels, which appear to exert a protective carrier effect on cells during needle flow [41].

TABLE 1. Comparison between [SUMMERFIELD]

Criteria	Guinea Pig	Human	Mouse	Pig	Rat
Skin Attachment	Loose-attached	Firmly attached	Loose	Firmly attached	Loose
Hair coat	Sparse or dense	Sparse	Dense (except some breeds)	Sparse	Dense (except some breeds)
Epidermis	Thick	Thick	Thin	Thick	Thin
Dermis	Thick	Thick	Thin	Thick	Thin
Panniculus carnosus	Present	Absent	Present	Absent	Present
Healing mechanism	Contraction	Re-epithelization	Contraction	Re-epithelialization	Contraction

Aim 2: Assess efficacy of microdelivery system construct *in vivo.* After successfully fabricating the microdelivery system and testing it *in vitro*, the next objective is to assess its efficacy *in vivo* in a red Duroc porcine model. Although mice and rat models are cheaper and more plentiful, too many parameters differ from those of human skin for a model to be scientifically, financially, and ethically beneficial (**Table I**). A porcine model, as opposed to a murine model, was chosen based on its striking similarity to human skin in terms of general structure, thickness, hair follicle content, collagen, lipid composition, and pigmentation[3].

The healing process in larger mammals, namely pigs and humans, commonly involves epithelialization, as opposed to wound contraction that is demonstrated in smaller mammals with thinner skin (e.g. mice, rats, guinea pigs) (**Table I**). Like humans, pigs can sustain sunburns and rely on fat as opposed to fur for insulation. The epidermis of both contains Langerhans cells and elastic fibers, and the dermis collagenous tissue networks are very similar. Previous studies have shown red Duroc pigs to be the “gold standard” for approximating hypertrophic scarring after burn wounding[42]. For this *in vivo* study, red Duroc pigs will be anesthetized and a burn will be induced via a reproducible deep dermal burn injury model used by Cuttle et al[4]. The thermal injury device, as shown in **Figure 4,** will consist of a Pyrex laboratory Schott Duran bottle filled with approximately 300mL of sterile water and heated in a microwave to the 92°C[4]. The device will then be placed directly on the pigs’ dorsal flank and held for 15 seconds in order to create an adequate and consistent burn.
Enzymatic debridement via collagenase and hyaluronidase will then occur 24 hours after the initial burn instance[34]. Iterative debridement via the aforementioned enzymes will continue as needed for the next two weeks, as additional necrotic tissue

will need to be removed. This is turn will allow for expedited progression from the inflammation stage, to granulation tissue formation, and epithelialization[34]. A section from the periphery of the debrided skin will be placed and dissociated in serum-free modified IMEM supplemented with 300U/ml collagenase type 3, 100U/ml hyaluronidase, 2% bovine serum albumin fraction V, and 5μg/ml recombinant human insulin at 37°C, 5% CO_2 with gentle agitation for 10 hours until a majority of the tissue was digested [5]. The dissociated cells will then be spun and plated into modified IMEM supplemented with 10% FBS, 5μg/ml recombinant human insulin, and 50μg/ml gentamicin. The keratinocytes/melanocytes will then be grown and passed continuously by detachment with 0.05% trypsin and 0.53mM EDTA. Based on a study by Tang et al., we expect the cells to proliferate most rapidly by days 5-6 and enter the logarithmic growth phase, then asymptote off around day 10[43]. We will then use FACS to positively select for our target cells and treat the burn wound site on the Duroc pig model with the proposed microneedle system. From there, the site will be stimulated with the aforementioned UV and MSH treatments as described in Aim 1.2. This will not only add more melanocytes to the site, but also increase the expression levels of each cell via MSH. We will include a control group with an induced burn and sham treatment of the microneedle system and only inject PBS.

After treatment with UV and MSH at 1, 2, 4, and 8 weeks, we will use biopsy samples from the burn wound sites. The samples will be analyzed for their melanin content using diffuse reflectance spectroscopy and densitometric analysis of Fontana-Masson staining of the samples, following modern standards for analyzing melanin content within human skin[44]. This spectral analysis technique, which has been successfully used to quantify melanin density in relation to skin tone, will allow us to compare melanin distribution in untreated samples versus treated samples. Duroc pigs have dark pigmentation so we expect similar melanin distribution to the top right image in **Figure 6**. The staining and imaging protocol will allow us to see the cellular morphology and development of melanosomes in comparison to basal levels. We expect moderate to full regeneration of normal melanocyte distribution consistent with microneedle placement, because our delivery mechanism should supplement the population of melanocytes that were lost during a burn. The cells within the biopsy samples will also be tested by a hydrogen peroxide oxidative stress assay and imaged using immunofluorescence markers specific for melanosomes, similar to that of the assays used in Aim 1.2. We will also use immunofluorescence markers specific for DNA damage, specifically cyclobutane pyrimidine dimers, which form due to UV exposure[44]. In **Figure 6**, this is visualized with a green fluorescent stain. This allows us to quantify the cytotoxicity of UV exposure on the cells in the scar environment. Overall, we can find a balance between increased melanin density and minimal cellular damage. The assays we propose will allow for quantifiable observation of UV and MSH stimulation on native scar tissue. Additionally, the effect of implanting co-cultured cells and their stimulation within the skin environment will be evaluated. We also expect overall restoration of sustained pigmentation when comparing treated to untreated biopsy samples with minimal adverse effects.

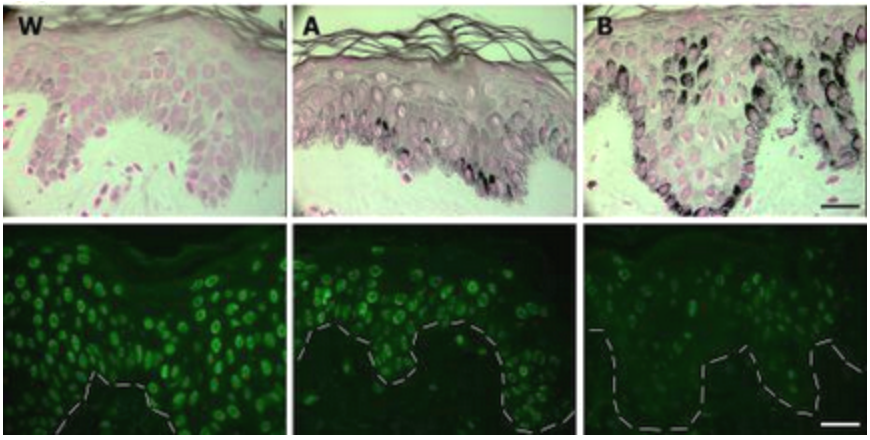

Figure 6. Upper: Examples of Fontana Masson-stained skin showing melanin in the epidermis of "White" (W), "Asian" (A), and "Black/African American" (B) subjects. Lower: Examples of cyclobutane pyrimidine dimers abundance and distribution as revealed by immunohistochemistry in biopsies taken immediately (7 min) after exposure to 1 minimal erythmal dose in the same subjects as indicated above. Dashed lines indicate the border between the epidermis and dermis. Scale bar = 50 μm.[44]

At this point in our study, we can confidently say that our delivery system will greatly improve the patient's quality of life by restoring their skin's natural pigment and photoprotective functions, thus creating a niche in the dermal therapeutic industry. This will propel our proposed research into a multitude of therapeutic disciplines, allowing us to address both the physical and psychological effects of pigmentation abnormalities.

Anticipated challenges and alternative solutions for Aim 2: Due to the novelty and uniqueness of our microneedle system, potential complications might arise. Firstly, keratinocytes/melanocytes are terminally differentiated cells with a limited ability to reproduce. Should they undergo excessive stress, be too damaged, old, or unhealthy to survive once delivered, we propose the use of dermal precursor cells as an alternative. Likewise, if there is insufficient melanocyte quantity and viability, an autograft could be taken from a healthy section of the pig's body. Penultimately, if overexposure to UV results in adverse effects, such as inflammation or sun burns in the porcine model, UV stimulation will have to be used even more conservatively. MSH could also be utilized more heavily instead of UV to mitigate this complication. Furthermore, we acknowledge that the shape of the burn and the microneedle patch will not be an exact fit in this porcine model. The burn will be circular and the patch will apply cells in a rectangular array. Thus, there is a small amount of burn tissue in the treated group that will not receive cells, but will still receive UV and MSH stimulation. To address this, we plan to tailor the shape of our patch in order to match the wound's unique geometry. Lastly, to address potential hyperpigmentation, a study by Choi et. al demonstrated that aloesin, a compound isolated from the aloe plant, could be used to dose-dependently inhibit melanin formation induced by UV radiation, and is thus a promising pigmentation-altering agent for cosmetic and therapeutic applications[45].

Future Work: Once we have proved adequate efficacy in our porcine model, we will then move to phase I clinical trials to assess the microneedle system's viability in humans. Hopefully, the eventual FDA approval of our device will open doors for other treatments and uses, such as autologous cell transplantation, as well as integrated and improved cell, drug, and vaccine delivery. After the implementation of this technology in burn healing and proper repigmentation, additional factors such as burn geometry can be addressed to further develop personalized medicine and care. Therefore, the next steps of our development will focus on increasing the size of our microneedle system and improving its flexibility for patient-to-patient variability . In the future, we would like to expand this system to treat other unaddressed long-term consequences of burns, such as the restoration of hair follicles and sweat glands through the delivery of their respective components.

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