NCL 402 – Review writing

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Mathematical perspective in analyzing the skin as a complex biological system

Summary

Skin is the outermost covering of the human body. It is a complex and interesting biological system playing an important role in protecting the internal organs of the body from the harsh external environmental insults. Skin is primarily made up of three layers – outer epidermal layer, middle dermal layer and the innermost hypodermis. Epidermis contains a pigment producing cell which confers color to the skin. Huge variation in the constitutive skin color of the humans is observed. Skin color varies from pale white as seen in Caucasians to dark black colored Africans. Injury or stress to the skin may alter its color tendering it to hyperpigment or to depigment. Skin reversibly darkens when exposed to sunlight called as tanning. This review summarizes the mechanisms and biology involved in skin pigmentation and also illustrates on the mathematical models build for the skin. We conclude with our proposal of the multiscale model of the skin to understand and lookout for the mechanisms involved in detanning.

Introduction

Skin is a well studied complex biological system. Anatomy of the skin is vastly studied and well documented. Several studies focus on indentifying and characterizing the skin components involved in various skin's functions. In addition to the various experimental studies many groups use mathematical perspective in analyzing skin as a system. Various mathematical methods such as image analysis, regression analysis, reaction kinetics, molecular dynamics and simulations and statistical models are used in analyzing various aspects of skin.

In the following sections we briefly elaborate on the skin biology and the respective mathematical models build on that system. We have clubbed the models on the basis of the part of the skin system that they model and analyze. The review focuses more on the epidermal part of the skin.

Skin layers

Skin is composed of three layers: innermost hypodermis, middle dermal layer and the outermost epidermis. The hypodermal layer is mainly made of adipose tissue which stores fat globules and connects the skin layers with internal bones and organs. It's a house for blood vessels and nerves. The middle dermal layer contains the sebaceous glands, sweat glands and hair follicles. Dermis is mainly made up of fibroblasts, macrophages and adipocytes. The blood vessels in the dermis provide nourishment to the upper epidermal layer. The outermost epidermal layer is made of several layers of continuously differentiating keratinocytes. Immune related Langerhans cells and Merkel cells and pigment producing melanocytes are also present in the epidermal layer.

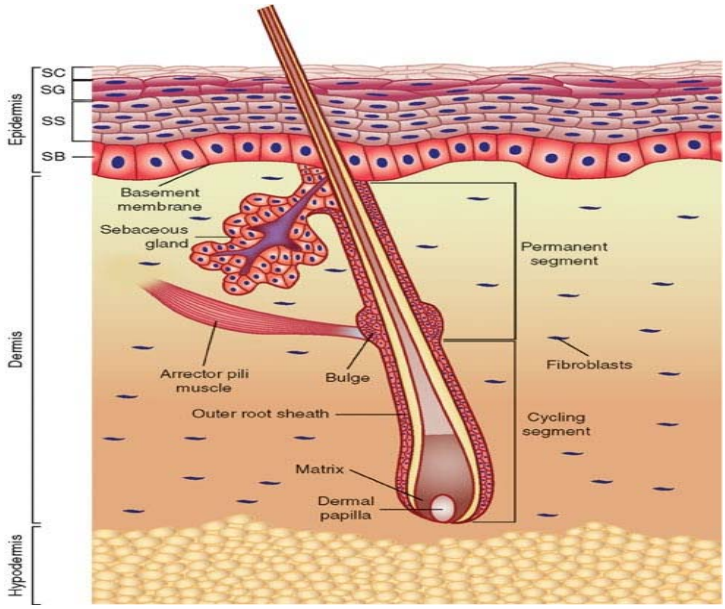

Fig 1: Pictorial representation of different layers of skin [1]

Skin being the outermost part of the body is exposed to sunlight. Diffey used mathematical approach to study the optical properties of the human skin and derive absorption and scattering coefficients for the stratum corneum. The study investigates the effect of variation of thickness and melanin content of the corneum in its role of photoprotection [2].

Optical properties of the tissue are also useful during medical imaging. In the laser based optical techniques, optical photons suffer significant amounts of scattering by different organelles in the skin. This optical data of the skin translates into useful medically significant information [3]. Schmitt et al group has used a diffusion model to describe propagation of photon flux in epidermal, dermal and hypodermal layers of the skin. The model predicts the absorption and scattering coefficients of dermis and subcutis layers [4].

Hair, the visible appendage of the skin is vastly studied. The visible part of the hair is called as shaft while the hair follicle is situated in the dermis. The follicle has stem cells that renew the hair after it falls out. Hair is filamentous and mainly composed of the protein keratin. It consists of three layers namely cuticle, cortex and the medulla. Several groups are working to understand the structural and mechanical hair properties. Akkermans and Warren have carried out molecular dynamics to study hair mechanics [5]. Their statistical mechanical model of hair predicts a linear decrease of the yield stress with temperature. A group in Singapore has constructed a simple physics model to animate human hair by grouping hair strands into strips [6]. Studies are also carried out by keeping in focus the outlook towards hair styling and hair removal. Kolinko and Littler have presented a mathematical model to predict and optimize laser hair removal [7].

Epidermis and its major component keratinocytes

The outer layer of the skin – epidermis itself is multilayered structure. It has 90% of keratinocytes, 5% of melanocytes and rest is Merkel cells and immune related Langerhan's cells.

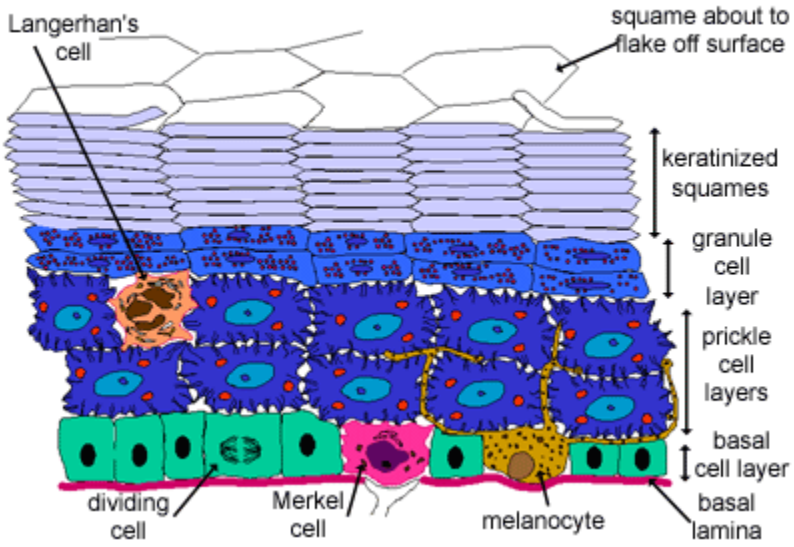

Fig 2: Different layers of epidermis[8]

Epidermis is made up of continuously proliferating basal stem cells and suprabasal differentiating keratinocytes. The basal stem cells rest on a basement membrane (BM) that separates the epidermis from the dermis that lies beneath. Basement membrane plays an important role in keratinocye differentiation [9]. A discrete, off-lattice cell-centre model constructed for the BM for the range of biological epithelia showed that the BM had an important role to maintain homeostasis and stability of the growing epithelia [10].

The basal layer is observed to contains three kinds of keratinocytes: firstly the stem cells (SC) that are self proliferating and multiplying, secondly the transit amplifying (TA) cells and lastly the post mitotic differentiating cells (PMD) [11]. There is just a single layer of basal cells which are proliferating and renewed.

The differentiated basal keratinocytes get detached from the basement membrane and move upwards in the spinous layer. Stratum spinosum is made up of 8-10 layers of cells. These differentiating keratinocytes are connected to each other by lot of desmosomes and hence appear to have spines or prickles and so the name. Stratum spinosum is followed by 3-5 layers of granular cells. These cells contain lipid rich granules which act as water sealant. These cells start to lose their nuclei and are finally differentiate to form corneocytes. Stratum corneum is made up of 15-20 layers of dead cells which are fully keratinized. Also these keratinocytes neither have any cellular organelles nor a nucleus. Corneum of lightly skinned individuals lack melanosomes while darkly skinned individuals might have few intact

melanosomes [12]. In palms and soles, an additional transparent lucidum layer is observed in between stratum granular and stratum corneum.

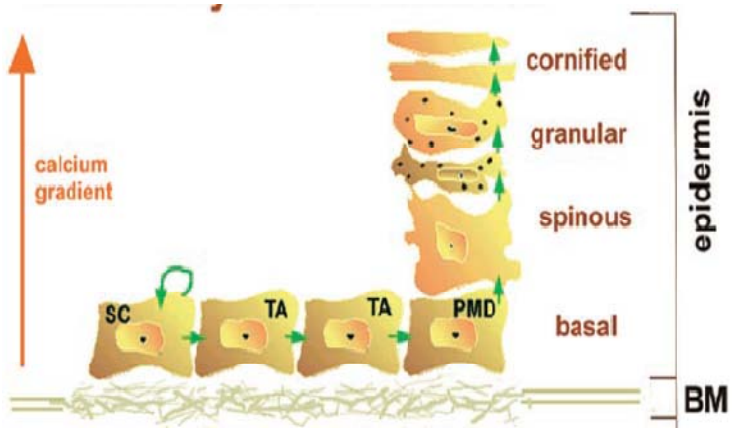

Fig 3: Differentiating keratinocytes as observed in the epidermal layer [11]

Epidermis forms a barrier and blocks easy entry of outside molecules. Permeability of the epidermis is vastly studied area for transdermal drug delivery. Edwards et al constructed a basic model for permeation of solutes through corneal epithelium. They modeled the transcellular movement of large lipophilic molecules through epithelial cell membranes (lateral path) and transverse path for the drug passing through the cytosol of the cell. Hydrophilic and small lipohilic molecules were modeled to pass through intercellular gaps in between the cells (paracellular movement) [13]. Potts and Guy constructed the model to predict the skin permeability by using permeant size and water - octanol partition coefficient [14]. Mitragotri compiled analytical expressions to predict solute permeation in stratum corneum [15].

The whole epidermal layer is renewed every 2-4 weeks. The basal stem cell proliferates and later upon differentiating travels towards the surface from where it is shed off. This regular continuous process of shedding of the corneocytes is termed as desquamation.

Epidermal thickness is observed to be independent of skin type and not seen to vary between individuals. But intra variation in thickness at different body sites of an individual is seen with buttocks having maximum thick epidermis. Thickness of stratum corneum was reported to be positively correlated to pigmentation and negatively to number of years of smoking and to be independent of age and gender. But thickness of viable layers was studied to be positively correlated to blood content and was greater in males than in females [16, 17].

Alike in skin, luminal surface of the gut is also lined by epithelium which is constantly replaced after every 3-5 days in humans. Many groups have constructed mathematical models to study the epithelial homeostasis in the gut. Meineke et al have constructed a spatial model of intestinal crypt. The model considers the proliferation of basal pinned crypt stem cells and their eventual movement towards the gut [18]. A group in Oxford further extended the Meineke model to study the intestinal epithelium homeostasis. Their model considered the round bottom of the crypt and allowed for the lateral

movement of stem cells. Their detailed model considered the temporal and spatial differentiation of epithelium and their shedding of into the intestinal gut. This multiscale model took into account the extracellular Wnt signaling, its effect and further downstream intracellular transcription. Each cell was modeled to have an internal biochemical reaction kinetics based on external signal which affected its cell division time and its location after cell division [19] [20]. Same group earlier had built a discrete and continuous compartmental model explaining the long phase observed in colorectal tumor growth [19].

Melanocytes and melanocyte specific organelle - melanosome

After keratinocytes, pigment producing melanocytes are the second largest population of cells in the epidermis. They form 5% of the total cell population. The melanocytes are functionally attached with the fibroblasts of the dermis and the keratinocytes of the epidermis [21]. Melanocytes lie in the basal epidermal layer. An agent based computational model of cellular interactions in the basal layer of human epidermis was built by a group to study the mechanism that maintains even skin color, to study uniform distribution of melanocytes among the keratinocytes in the basal layer [22].

Neural crest derived precursor cells, melanoblasts proliferate, differentiate and migrate to the skin and are located in stratum basal or the epidermis. Luciani et al constructed a mathematical model to study the main cellular mechanisms involved in melanoblast expansion and its migration to epidermis [23]. Their model predicts the number of founder melanoblasts and studies the gain and loss of function of beta catenin.

Density of melanocytes at particular body site, suppose say at back of the hand, is same in all the individuals. But intra variation in melanocyte density at different body sites of same individual is observed. Constitutive melanocyte density in the skin can be affected by the exposure of skin to harmful ultraviolet light or to toxic chemical compounds [21]. At normal conditions skin melanocytes proliferate slowly and they are resistant to apoptosis because of high expression of BCL2 [24]. But the melanocyte stem cells at the base of hair follicle are observed to proliferate during every hair cycle. What are the different mechanisms involved in case of skin melanocyte and that of hair melanocyte needs to be studied.

Melanocytes are dendritic cells and have their dendrites extending into the spinosum layer. They also contain a lysosomal like organelle melanosomes in which melanin is synthesized. Melanosomes are typically divided in four stages. Stage I premelanosomes lack tyrosinase activity and have no internal structural components, while Stage II melanosomes expresses Tyrosinase and a structural protein Pmel17. Stage III melanosomes are marked by synthesis of melanin and their uniform deposition on internal fibrils. Stage IV melanosomes are the fully melanized and electron – opaque [25].

Stage IV mature melanosomes move away from the perinuclear region of the melanocyte towards their dendritic ends. Melanosomes are transported from the melanocytes to the neighboring keratinocytes from basal and spinosum layer. One melanocyte is believed to pass on the melanosomes to 36 neighboring keratinocytes. Mechanism of transfer of melanosomes from melanocytes to keratinocytes is believed to be either a cytophagocytosis or filipodial mediated melanosomal transfer or discharge of melanosomes into extracellular space and their subsequent uptake by phagocytosis [26, 27].

Melanosomes uptaken by the keratinocytes are observed to cap their nucleus. The distribution of melanosomes in the keratinocytes is believed to be dependent on the size of melanosomes. Large sized melanosomes are singly dispersed in the keratinocytes while the smaller sized melanosomes tend to cluster into aggregates [12, 28].

Skin color

Various skin chromophores observed in humans are: red colored oxyhaemoglobin in dermis, deoxygenated haemoglobin in veins (blue colored), dietary carotenoids (yellow orange), bilirubin (yellow), melanin (brown/black). Skin color renders aid in camouflage, visual communication, prey avoidance, reproductive success and thermal regulation [29]. Humans at birth have a specific basic color called as constitutive pigmentation which is hereditarily passed on and genetically determined. Not all individuals are of same color. Human skin color is observed to vary from whitish pink to dark black. Thomas Fitzpatrick, a Harvard dermatologist classified the human skin into six classes depending on the color and the skin's response to ultraviolet light. Type I class were people with light pale white skin color which never tanned while type VI were deeply pigmented black skinned individuals that tan easily but never have skin burn.

The synthesized melanin is packaged into melanocyte specific lysosome like organelles called melanosomes. The synthesized melanin is of two types: alkali soluble light colored – cystine based polymer – pheomelanin, DHICA containing eumelanin and alkali insoluble dark colored DHI containing eumelanin. Larger sized melanosomes are observed to have darkly colored melanin while smaller melanosomes are lightly pigmented [28].

Constitutive appearance of skin color is effect of total melanin content, melanin composition and the melanosomal size [28]. Surface microtexture and the sebum in the skin affects the reflective property of the skin and hence has an impact on the final appearance of skin color [29].

Various mathematical techniques like regression analysis [30], neural networks [31] and image analysis [32] were developed for human skin to predict the color. Very early in 1952, Turing with his reaction diffusion model explained the color patterns observed in skin of animals [33-35]. Over time various mathematical models for pattern formation questioned the patterning mechanism. One of the questions the model posed was how much disruption could the pattern tolerate before it disintegrated.

Melanin synthesis – at genetic and metabolic level

Melanin synthesized in the melanosomes is result of concerted expression and functioning of pigment related genes. Constitutive pigmentation is result of many internal and external stimulating factors. Following is pictorial representation of some of the signaling pathways from Costin and Hearing's review [25] which activates pigment related genes.

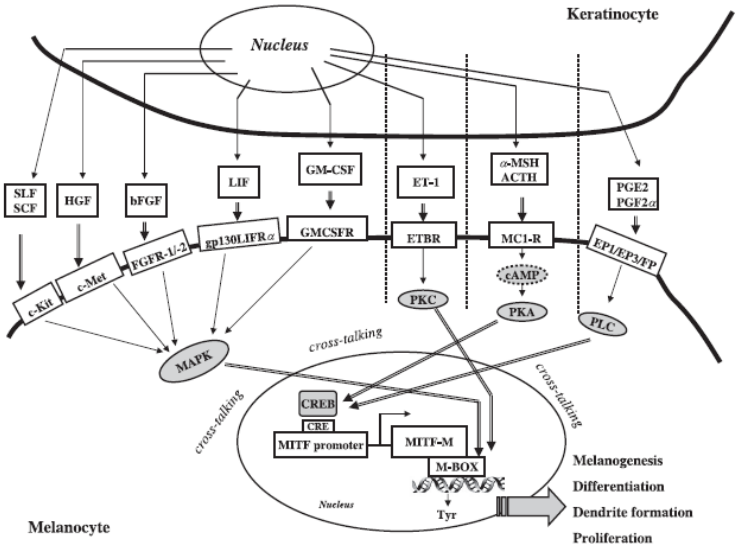

Fig 4: Pictorial representation of few of the signals received by the melanocytes having an effect on pigment related genes.

Micropthalmia associated transcription factor (MITF) is known to be the main regulating gene that activates genes involved in metabolic synthesis of melanin – tyrosinase, TRP2 (DCT) and TRP1. MITF is also known to activate the genes that regulate melanosomes maturation, its structure maintenance and the genes involved in melanocyte dendrite formation. All the various signals binding to their respective receptors on the melanocytes by some way or the other activate MITF. But there are several groups which also state that melanin inhibition takes place in MITF independent way [36] and there are some other unfound signaling networks that downregulate melanin synthesis independent of MITF.

Melanin is metabolically synthesized in melanosomes from its precursor tyrosine. Tyrosine transporter OCA2 on the melanosome membrane transports tyrosine inside the melanosomes. Tyrosine is further catabolized in enzymatically aided steps to synthesize melanin polymer. Type of melanin synthesized is reported to depend upon the signal binding MC1R receptor on melanosome and also on the availability of substrates. Tyrosine is catabolized by tyrosinase in three step process to form Dopaquinone. These steps are common to both eumelanogenesis and pheomelanogenesis. If cysteine is available in the system then it reacts with dopaquinone and form lightly colored cysteine derived pheomelanin. While in absence of cysteine, dopaquinone is spontaneously converted to dopachrome. In presence of two TRP1 and DCT, DHICA containing eumelanin is synthesized; else DHI containing eumelanin is synthesized with the help of tyrosinase.

Oyehaug et al developed a mathematical model to understand the switch between eumelanin and pheomelanin production depending upon an extracellular signal. Their results supported Ito's hypothesis that melanogenic switching is due to covalent binding of the intermediate dopaquinone to the enzyme glutathione reductase. Their results suggested that the melanogenic switching maybe due

to two stable production pattern states because of bifurcation in between the tyrosinase activity levels [37].

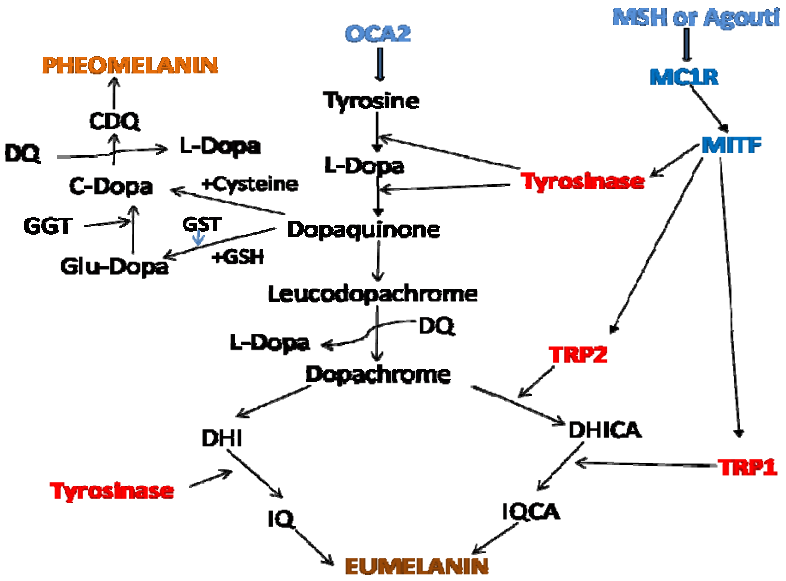

Fig 5: Metabolic synthesis of melanin in the melanosomes *(tyrosine is transported inside melanosomes via its transporter OCA2. MC1R is a receptor on the melanocyte on which either the MSH or agouti binds which decides eumelanogenesis or pheomelanogenesis respectively)*

Rate constants were deterministically calculated for the reaction between cyclodopa and dopaquinone [38]. Emir and Kurnaz modeled the eumelanin synthesis from tyrosine after UV stimulus using a tool GEPASI. Their model considered MITF activation through SCF – ERK/MAPK activity [39]. A kinetic model by Cabanes et al explained the influence of tyrosine and tyrosinase over the lag period during dopachrome accumulation [40]. Rodriguez-Lopez et al in their deterministic kinetic model studied the conversion of tyrosine to dopachrome while considering the mono and di-phenolase activity of tyrosinase [41].

Facultative pigmentation

Over the time color of the human skin is observed to vary from its constitutive color. This additional synthesis of melanin due to effect of external and internal factors on the skin is termed as facultative pigmentation. Darkening of the skin when exposed to ultraviolet (UV) light called as tanning is observed. This temporary reversible hyperpigmentation of the skin is vastly studied area. Hearing et al have in detailed reviewed the short and long term effects of UV radiation on the pigmentation of human skin [42].

Skins' exposure to UV shows 4 distinctive stages in the pigmentation response as reported in [42]:

(1) Immediate pigment darkening (IPD) develops minutes after UV exposure and can remain for several hours. This step is marked by darkening of skin because of oxidation or cyclization of existing melanin.

(2) Persistent pigment darkening (PPD) occurs within hours and remains for several days. During ths phase melanosomes migrate to the upper layers of the epidermis and slight increase in melanin content.
(3) Delayed pigmentation (DP) develops in days and remains for weeks and is marked increase in melanin content due to newly synthesized melanin.
(4) Long-lasting pigmentation (LLP) which remains even for more than 9 months after initial UV exposure.

A non invasive predictive model was developed by Hearing group. The model analyzed for UV sensitivity in skin of subjects from three radically different groups: Asian, African and white [43].

After their melanogenic switch paper, in 2009 Oyehaug et al mathematically conceptualized the skin tanning response. Their model considered a constant external signal which activated melanin synthesis. Melanin as a species, at the constitutive levels, was modeled to be transported only to the basal layer. The model considered melanocyte dendrite lengthening upon UV exposure and the outward movement of the four epidermal layers. And volume of sphere was used to calculate the amount of melanin distributed in lower two epidermal layers by the dendrite after the UV stimulus [44].

Digression from the normal functioning of the pigmentation system – effect of injury, stress and disease on pigmentation

Change in skin color because of sun tanning is temporary and reversible. But many times, some stress to the skin might irreversibly alter the pigmentation as observed in diseased conditions. Vitiligo is one of the skin disease marked by hypopigmentation or whitening of the skin because of loss of melanocytes. Albinism is congenital disorder characterized by complete or partial loss of pigment due to absence or defect in tyrosinase. Psoriasis – an immune mediated lifelong disease is marked by reddening of the skin.

Various mathematical models were developed for cancer. Few of the questions answered by the models were to study mechanisms occurring at the edge of growing tumour or to test the predictions of timing of radiations for cancer [45, 46]. There are several models that talk about aging of the skin, dermal wound repair [47-49]. Image analysis technique was used to predict the coloring of skin after vitiligo treatment [50].

Conclusion and Future Perspectives

A huge variation in skin color among different individuals is observed. The human skin color was classified into 6 classes by Fitzpatrick with class I being the fairest and class VI being the darkest skinned individuals. Outermost epidermal layer of the skin is separated from the middle dermis by a basement membrane. Epidermis is made up of multilayer of differentiating keratinocytes. Melanocytes are present in the lowermost epidermal layer stratum basal and have their dendrites protruding up till the spinosum layer. Melanin is synthesized in melanocyte specific organelle melanosomes. Mature melanosomes are transported to the keratinocytes in the lower two layers of the epidermis. The melanosomes move upwards towards the skin surface along with the movement of differentiating keratinocytes. So in the

skin, at a time, it's the epidermal keratinocytes that are translocated to the skin surface. And melanosomes are initially transferred to the lower two epidermal layers by yet unstudied mechanisms and then move up towards the skin with the differentiated keratinocytes.

Constitutive skin color is function of total melanin content in the epidermis, melanin composition (light or dark melanin) and the size and distribution of melanosomes. Dark skinned individuals have large individually dispersed melanosomes while light skinned individuals have small aggregated melanosomes. The size and distribution of melanosomes along with the skin's texture results in overall color appearance.

Density of melanocytes at particular site is same in all skin types while intra variation in the density is observed at different locations of the same individual. Skin melanocytes are fully differentiated and if at all proliferate very slowly. They are resistant to apoptosis due to higher expression of BCL2 in these cells. But the constitutive melanocyte density might be affected by some external factors which might lead to melanoma. But melanocytes that are present in the hair bulbs strikingly undergo proliferation and divide with every new hair cycle. It would be an interesting study to compare the mechanisms of melanocyte stem cell maintenance and differentiation in case of normal skin melanocytes, melanocytes in hair bulbs and the mutated melanocytes from melanomas.

Skin when exposed to ultraviolet radiation (UV) leads to reversible hyperpigmentation of the skin called as tanning. Darkening of skin initially results from oxidation of existing melanin and, from the upward movement of the melanosomes towards the skin's surface. Persistent exposure results to increase in melanin content due to newly synthesized melanin.

Exposure of skin to UV can negatively affect the skin by causing erythema, photodamage and tanning. UV radiations can cause mutations in DNA and damage the skin cells. Hence the nuclear capping phenomenon of the melanosomes is said to be an important internal protective mechanism of the skin. The melanosomes that migrate to the keratinocytes in the upper epidermal layers, upon UV stimulus, lie above the keratinocytes' nucleus and form a cap around it and protecting it from harmful UV rays. Hence melanin is observed to protect the skin from harmful photodamage. External applications of sunscreen or sunblock also help in preventing damage to the skin. Many pharma companies and research institutes are also investing in various cosmetic applications which either enhance the skin color for hypopigmentary related cases, or for lightening of the skin color. Finding new modes of actions for such cosmetic applications is one of the studied areas.

There are two types of melanin that are synthesized in the melanosomes depending upon the signal that attached to MC1R receptor on the melanocytes. MSH binding to MC1R results in synthesis of eumelanin while pheomelanin is synthesized when agouti binds to MC1R. Yet the pathway resulting to pheomelanogenesis after binding of agouti is yet not completely studies and it is yet not completely known.

Inflammatory component Interferon gamma is not observed in skin at normal physiological conditions but is detected during various stress responses. Interferon gamma levels were observed in hypopigmentary conditions resulting from leprosy, tuberculosis and vitiligo. Also interferon gamma was

observed to play a role in darkening the skin in cases such as melasma, solar lentigo etc. Kameyana and Hearing on their study on melanoma cells had concluded that higher concentrations of Interferon gamma elicited dose-dependent decrease in melanogenic activity while lower Interferon concentrations with MSH synergistically stimulated melanin synthesis [51]. It is interesting to study how the same inflammatory component results into hypopigmentation in some cases while darkening the skin in other cases.

Skin pigmentation is vastly researched area and we have tried to cover at least briefly all the relevant topics in this review. Here we do not cover the diseases that alter skin color or the mechanisms that are involved in inflammation (interferon gamma) related pigmentary changes. We have not included the details of proliferation, differentiation and migration of the embryonic neural crest derived melanoblasts and altogether avoid including melanoma, as it by itself is a deeply studied area.

Our proposed model

Melanin is a complex polymer giving color to the skin. With the enormous research related to skin pigmentation, biologists now know how the pigment is synthesized in melanosomes of melanocytes in basal epidermal layer of the skin. But the question still persists as to how this coloring pigment is catabolized in the system? As to how the tanned skin returns to its basal color? The mechanisms involved in melanin degradation are yet not known. Also the mechanisms for long lasting pigmentation remain unresolved.

We propose to build a multiscale model of pigmentation unit. The model will contain the epidermal layer consisting of keratinocytes at different levels of differentiation and basal melanocytes synthesizing melanin. We will incorporate the upwards movement of keratinocyte layers, melanosome uptake into keratinocytes, migration of melanosomes in upper layers after exposure of UV. We intend to use a freely available tool CHASTE [52, 53] to model the differentiating keratinocytes in the epidermis.

The model will try to answer whether complete epidermal turnover, desquamation (shedding of the stratum corneum) alone can explain detanning i.e returning of the hyperpigmented tanned skin to its basal constitutive level.

Also, interestingly in the case of long lasting pigmentation (LLP), hyperpigmentation is observed months after UV exposure, even when complete epidermal turnover takes place in 35-40 days. The parameter set in the model resulting in continuous higher melanin levels can be analyzed to reason out the persistence of pigmentation in the case of LLP.

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