Dermo-Metabolomics

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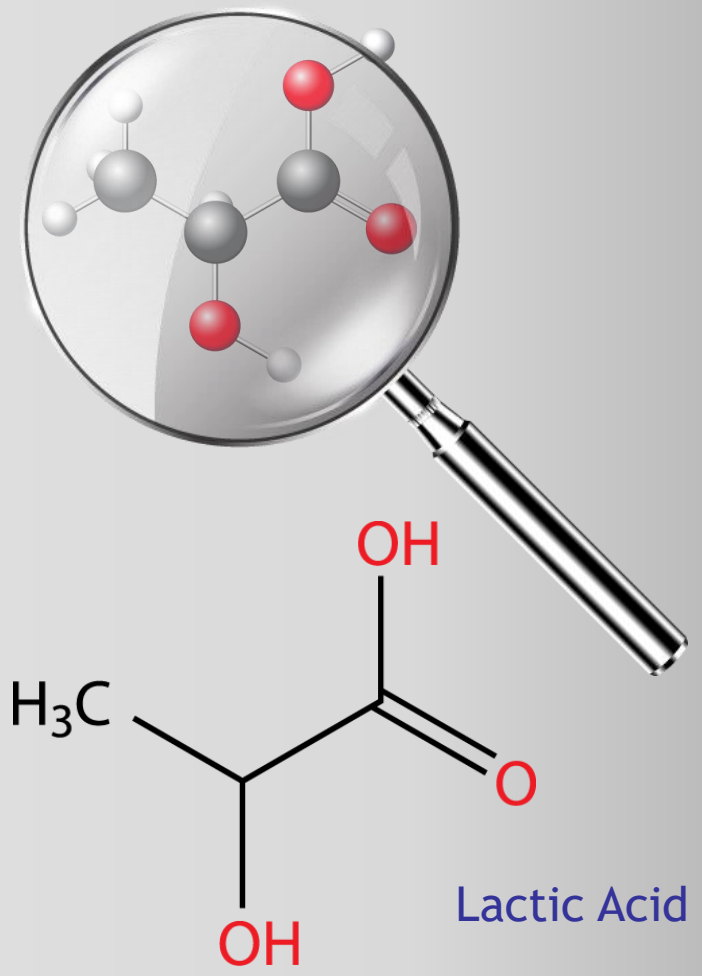
Dermo-Metabolomics

- The skin is a **doorway** into the human body and our first line of defense.
- Healthy, good-looking skin is also a measure of our **well being**.
- Skin is the site of **Vit D synthesis**, contains 5% of our blood supply and removes nitrogenous wastes.
- **Dermo-metabolomics** is a process whereby we measure metabolites and lipids in the epidermis and dermis, and measuring metabolites in cosmetic and beauty products both pre and post marketing.
- Dermo-metabolomics can be applied anywhere along the value chain and be informative for both the cosmetic industry and health industry.
- Metabolomic profiles provide information on **probiotics** (bacterial metabolites, hydroxy acids), on **cosmetic products** (antioxidants, emollients) and **health industry** (inflammatory pathways, cellular stress).



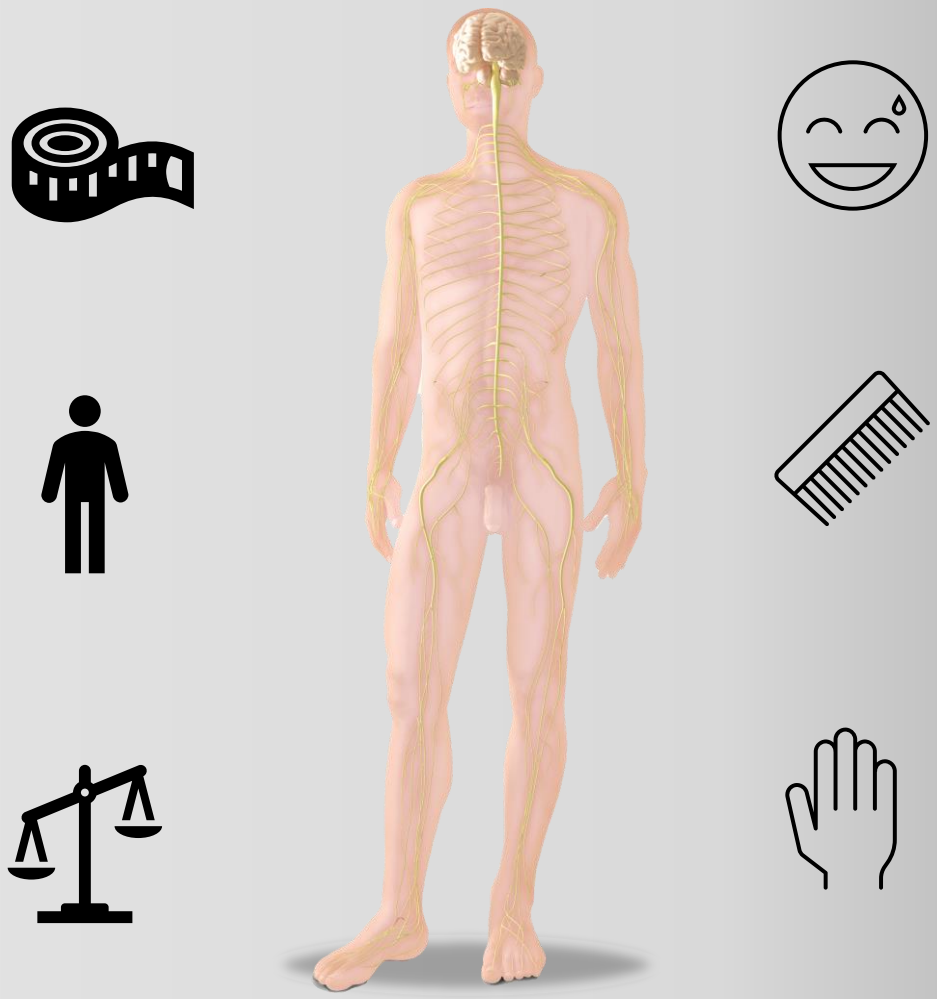
Contents

A. Integumentary System – Structure and Function of Healthy Epidermis and Dermis

Skin is one part of a large system

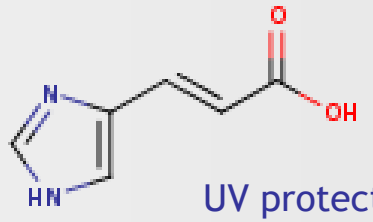
- The **Integumentary system** consists of our skin, hair, nails, sebaceous glands and sweat glands.

- The average person has 6 pounds of skin, covering more than 3,000 square inches, making it the **largest organ of our body**.

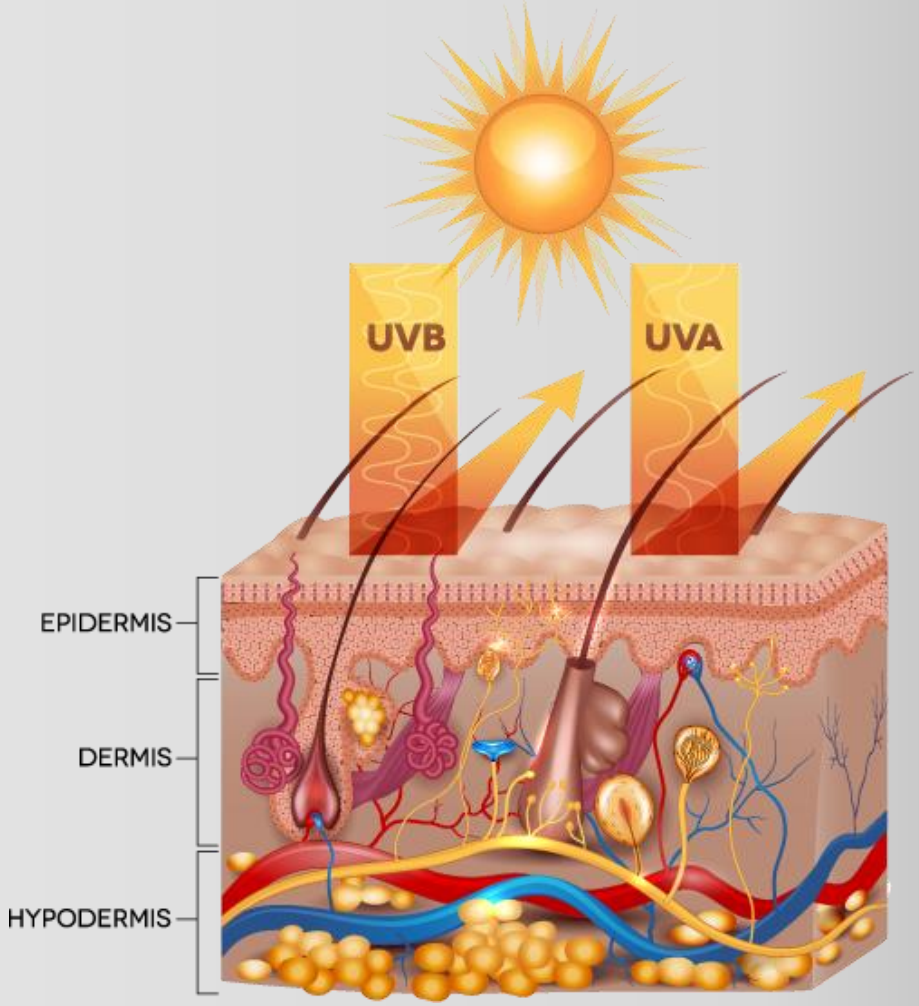


Skin Function

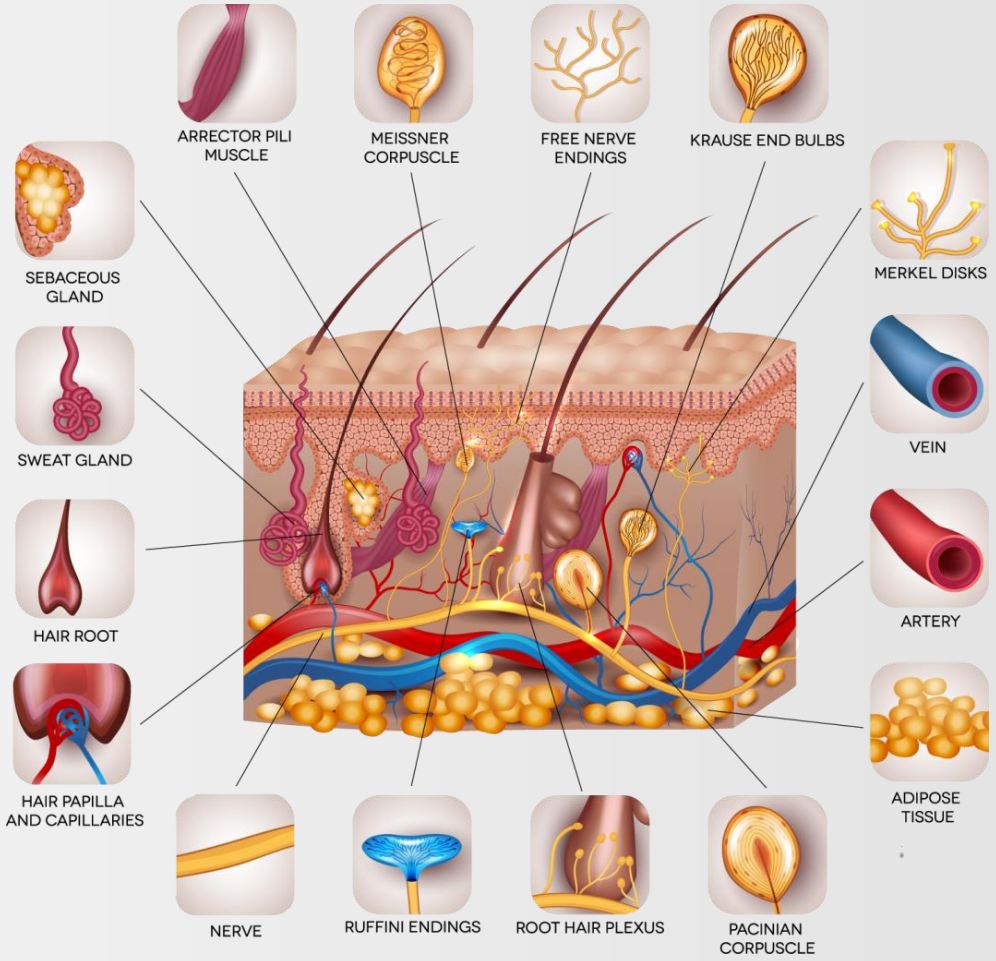
- One of the main functions of the skin is to maintain a barrier to **protect our organs**.
- Skin is not like a sponge that absorbs everything you put on it.
- In fact, the skin is such **a great barrier** that it's actually quite difficult to get anything through it without disturbing the lipid layer of the stratum corneum.
- Skin **provides protection** from the environment, but also inhibits excessive loss of water and electrolytes. **Pigmentation** protects us from sun damage and provides our Vit D.
- Skin also regulates our **body temperature**.



UV protectant – Urocanic acid



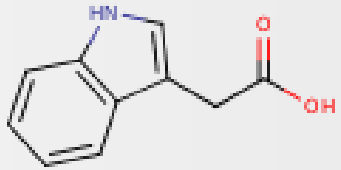
Skin Structure



- Skin provides **physical sensation** containing millions of nerve endings that are receptors for pain, heat, cold, and pressure.
- Skin has **three main layers** – epidermis and dermis and strata within. Also, the subcutaneous layer called the hypodermis or adipose layer.
- The stratum outer most layer is mostly dead cells and an outer layer of protective protein called **keratin**.

The Microbiome

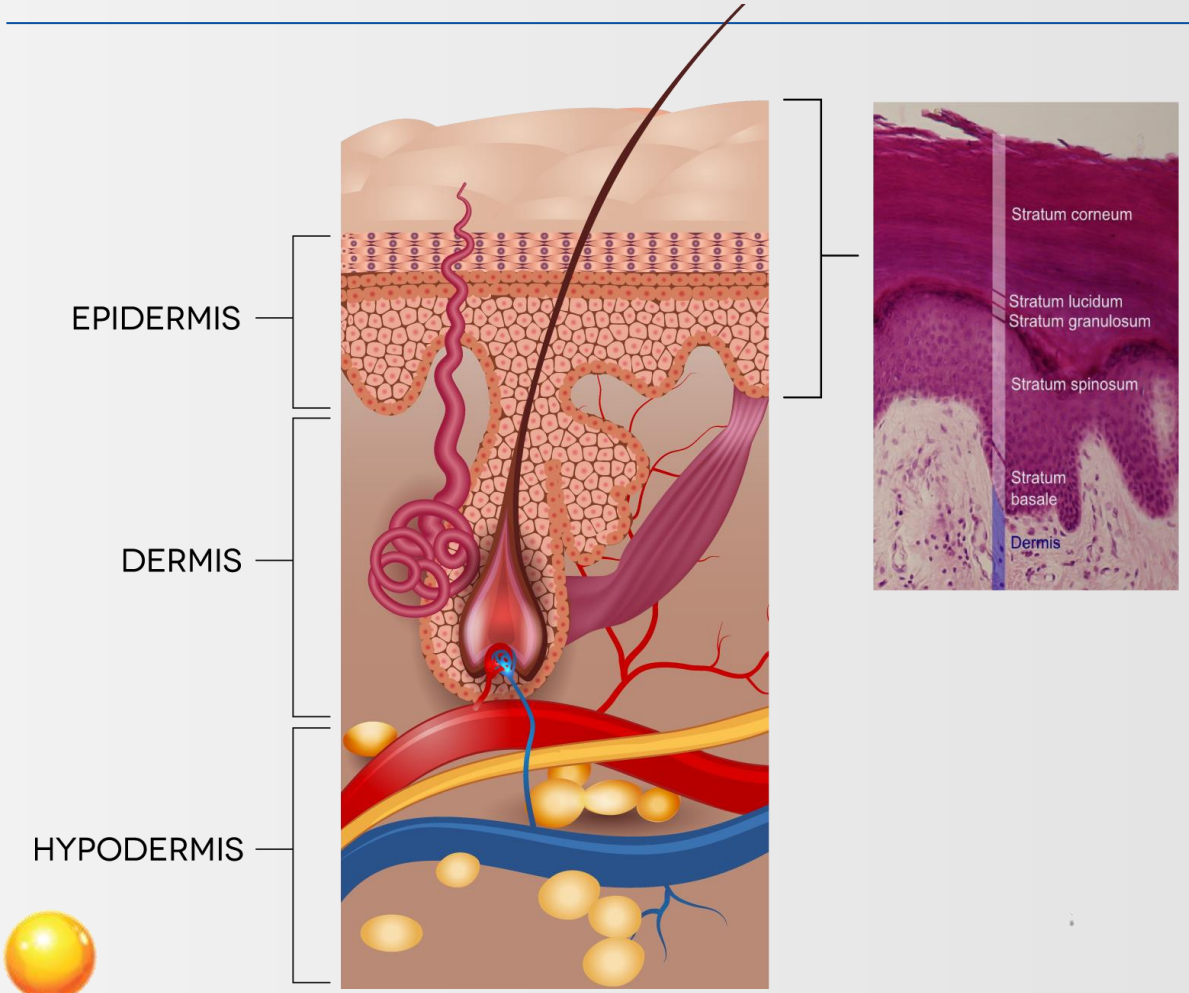
- Our skin is also host to a thriving ecosystem of microbes that are part of the **human microbiome**. They help control the “bad guys” living on our skin



Indoleacetic acid (IAA)

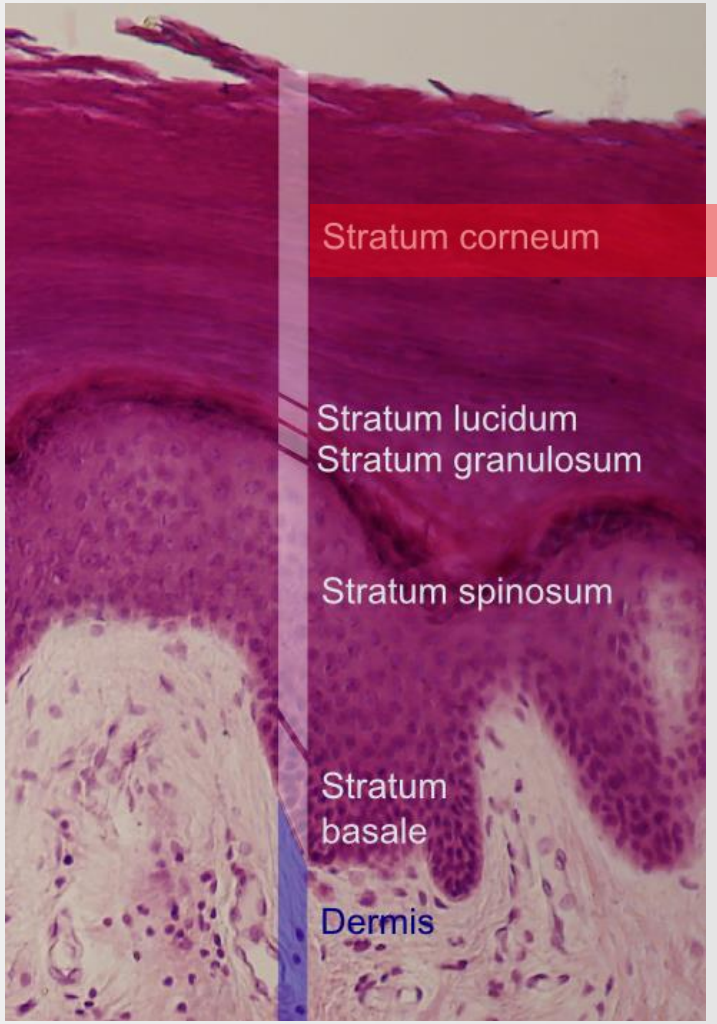
High levels of IAA can inhibit the growth and survival of the beneficial *Lactobacillus* species, which converts sugars such as glucose and fructose to lactic acid, which may inhibit the growth of some harmful bacteria. High levels of IAA also have been associates with poor kidney and heart health.

Skin is Dynamic



- The epidermis is around 0.1 mm thick, contains no blood vessels and is **replaced** every 30 days.
- Skin cells called **keratinocytes** are synthesized at the bottom layer of the epidermis. Through a process called **keratinization**, these skin cells will rise from the bottom layer to the top where their cytoplasmis organelles are replaced with keratin.
- These keratin filled cells are called corneocytes. **Corneocytes** are skin cells which have no nuclei or other organelles, thus they are often referred to as "dead" cells. **Keratin** are filament-like proteins which adhere these cells together and enhance the integrity of the skin.
- The skin will shed the top layers of corneocytes as keratinocytes go through cornification. The shedding of these layers is known as **desquamation**.

Stratum Corneum



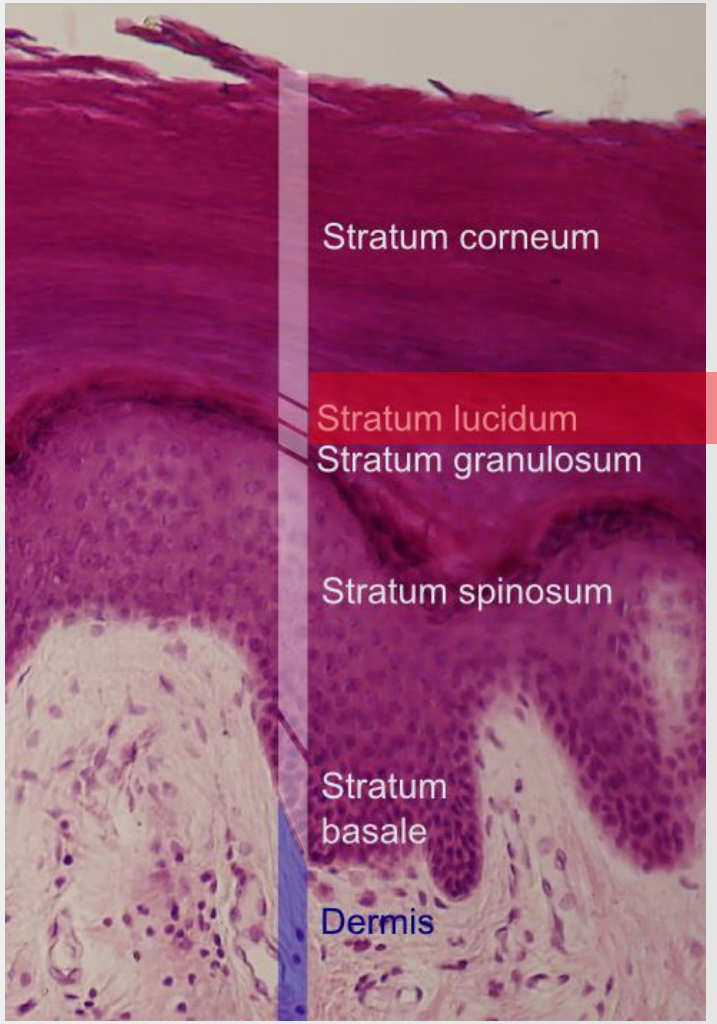
Stratum Corneum

- Corneocytes lay in a bed of lipids including ceramides, cholesterol and fatty acids in around 25 – 30 layers.
- The integrity of the skin cells due to keratin as well as the lipid layer of the stratum corneum helps to protect the skin from infection, dehydration and the environment.

Sebaceous gland

- These lipids are secreted onto the surface by the **sebaceous gland**. This layer has a high amount of dead cells and is constantly turning over.
- This layer is most often examined by tape patches or strips.

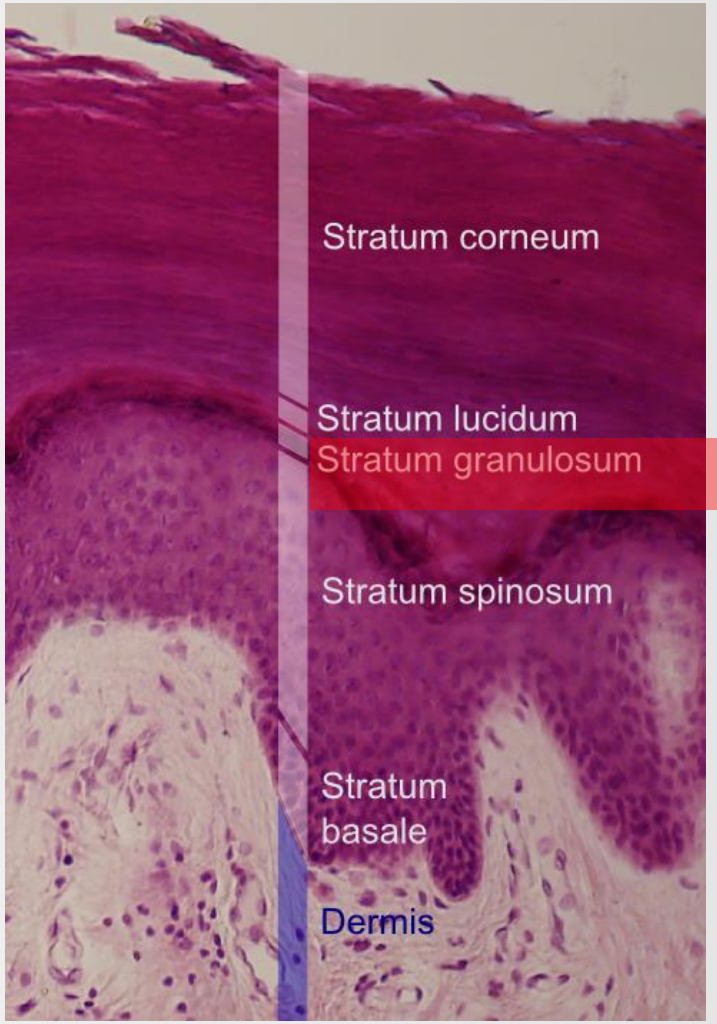
Stratum Lucidum



Stratum Lucidum

- **Stratum Lucidum** is only 3-5 layers of mostly keratin and dead cells, and largest on the bottom of feet and on the palms of hands.

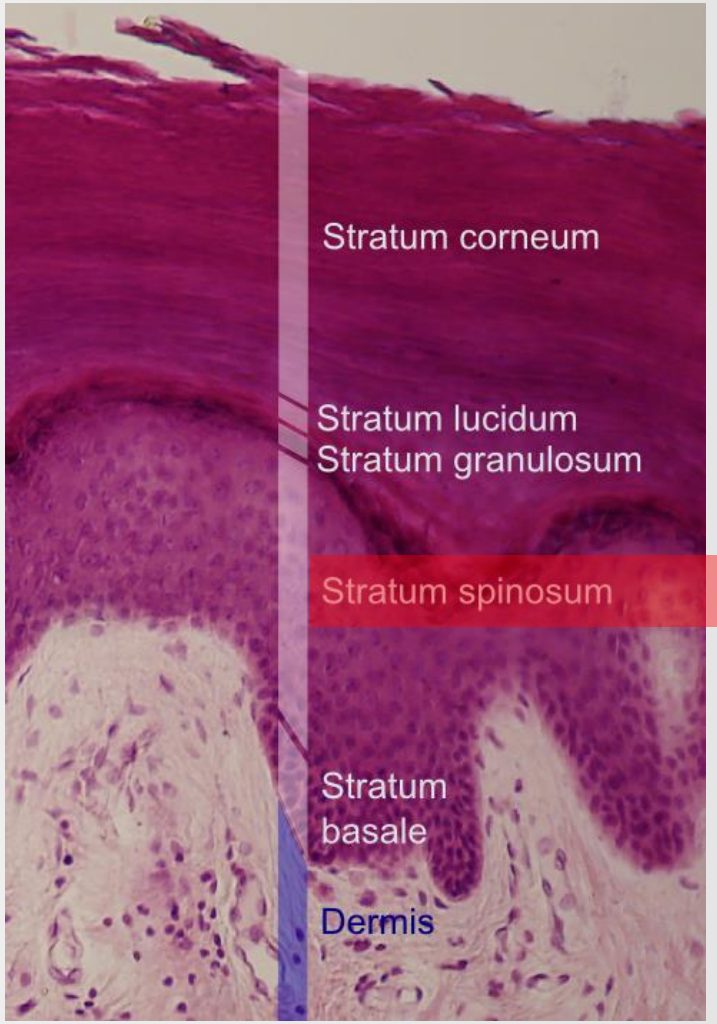
Stratum Granulosum



Stratum Granulosum

- **Stratum Granulosum** is only 3 – 5 layers thick. At this level of the epidermis, keratinocytes release lipid-containing organelles called **lamellar bodies**. Their function is to fuse with the cell membrane and release its lipids onto the stratum corneum, contributing to the formation of the skin's lipid layer.

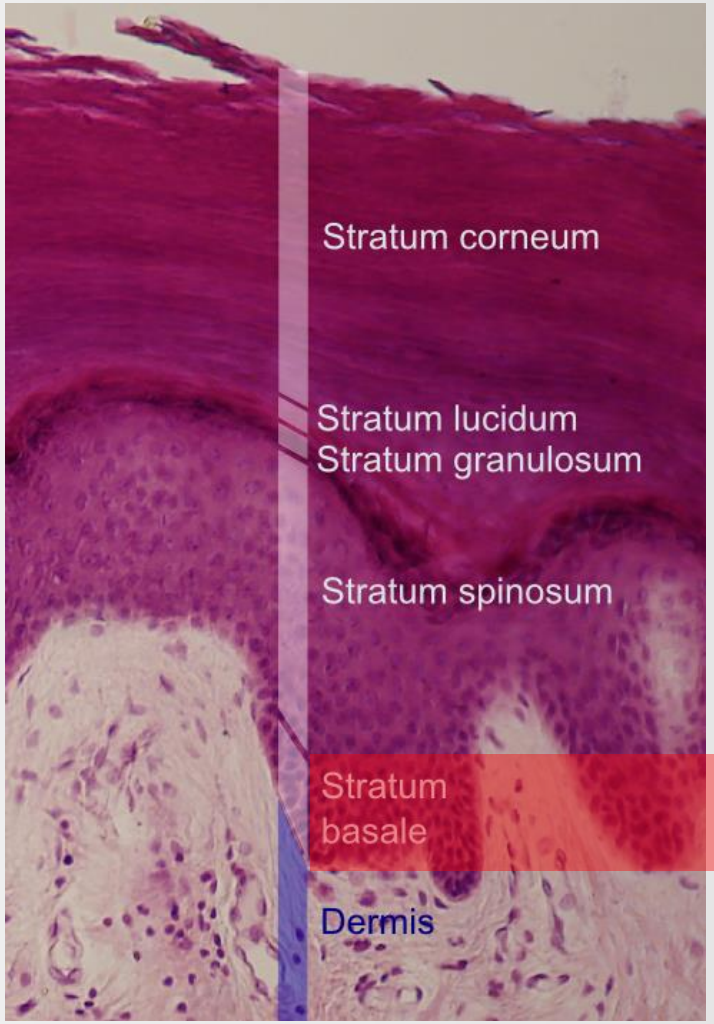
Stratum Spinosum



Stratum Spinosum

- **Stratum Spinosum** is a bit thicker at 8 to 10 layers. **Desmosomes** are formed to adhere the keratinocytes together. It is at this level the skin cells will start to produce keratin.

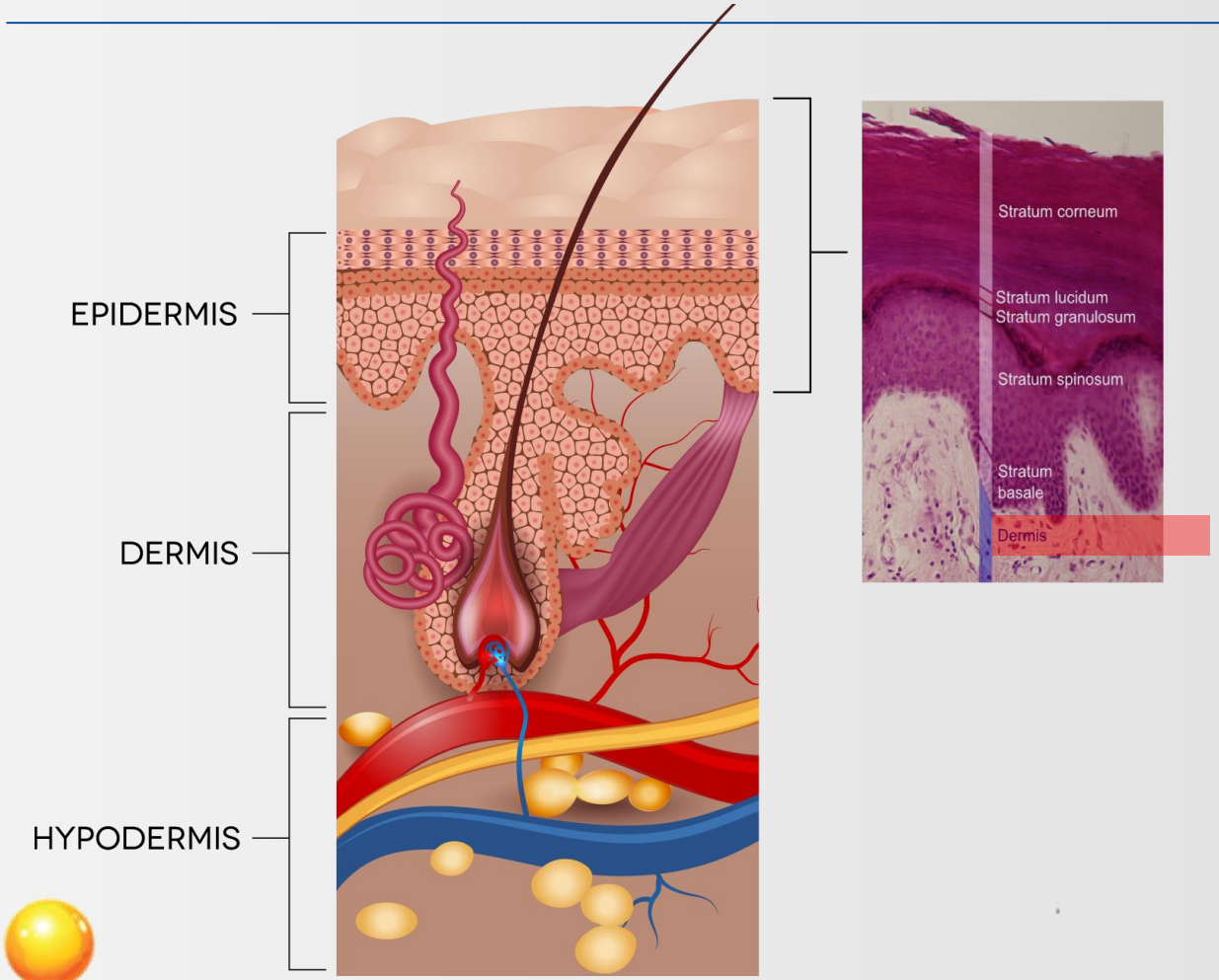
Stratum Basale



Stratum Basale

- This layer contains the "stem cells" of the epidermis--the basal keratinocytes. These basal keratinocytes will eventually make their way to the stratum corneum and become keratin-filled cells which protect our body from harm. This level is also contains cells called **melanocytes** which produce **Melanin,** a pigment in our skin.

Dermis and Subcutaneous Layer



Dermis

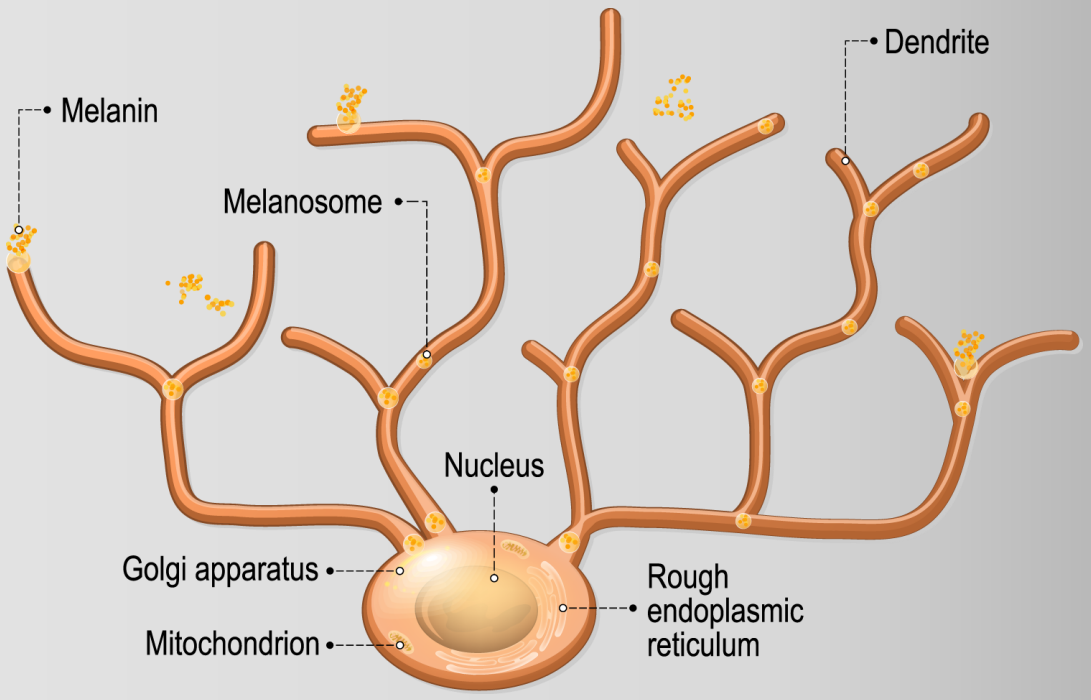
- The dermis contains two layers, the papillary dermis and the reticular dermis. The papillary dermis contains some loosely coiled collagen and is the layer that is in contact with the stratum basale layer of the epidermis. Also contains nerves and blood carrying capillaries.
- The lower reticular dermis is the primary location for collagen and elastin, the elastic fibers which give our skin its youthful, wrinkle-free structure. As we age and our elastic fibers get damaged by the environment and other intrinsic factors, our skin loses its structural integrity and we start to see the formation of wrinkles.

Subcutaneous Layer (Hypodermis)

- Below the dermis is our subcutaneous layer, which contains our fat cells (adipocytes). Cells called fibroblasts also exist in this layer. They are responsible for producing collagen as well as the extracellular matrix which supports our cells.

Upper layer skin cells

- The epidermis consists of two kinds of cells:
- **Keratinocytes** that migrate upward, forming the outermost layer. These eventually die and slough off. The color of skin depends on pigment packages called melanosomes, in these cells.
- **Melanocytes** that produce the pigment melanin (there are two major kinds) in packages called melanosomes, which are transferred to the keratinocytes.

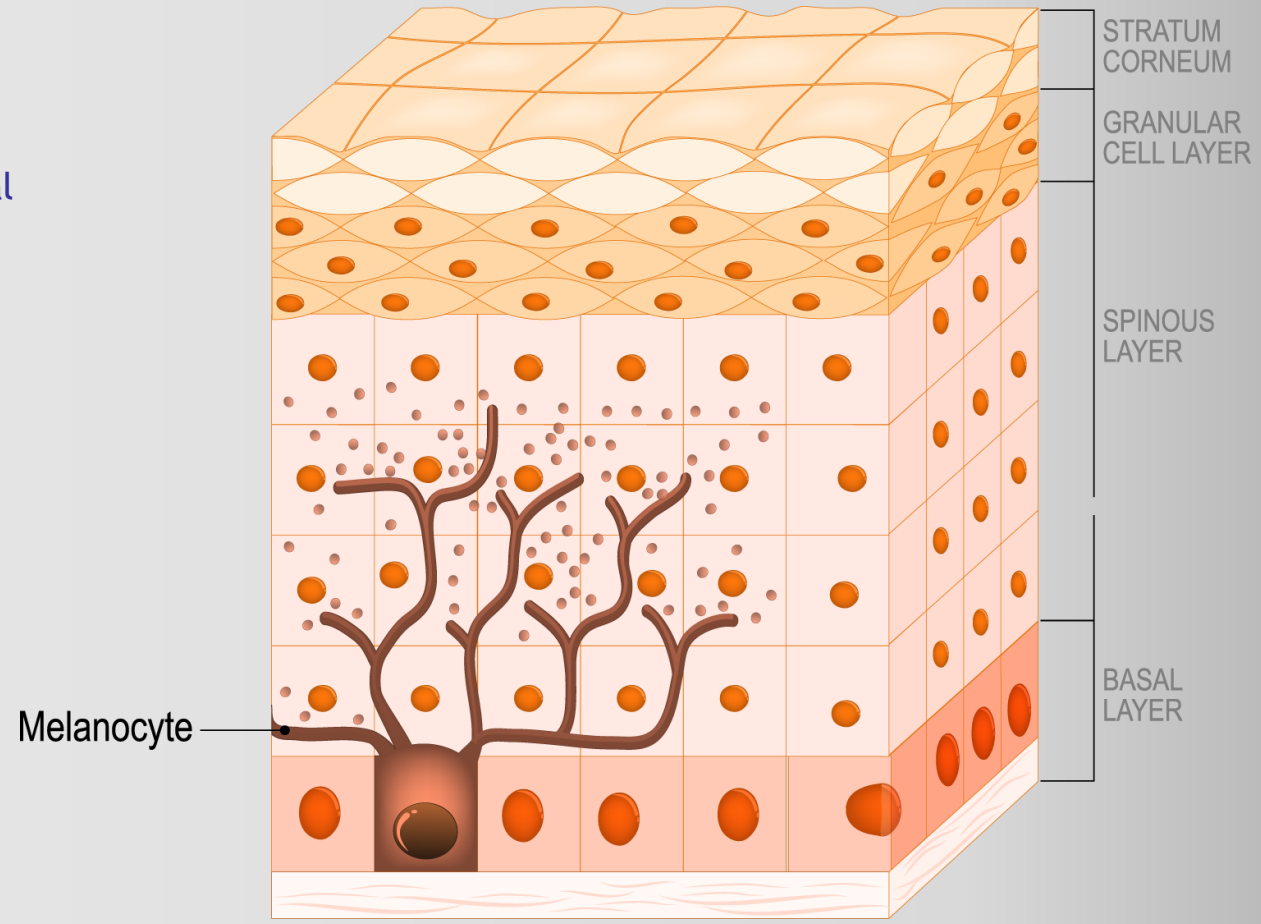


Melanocyte

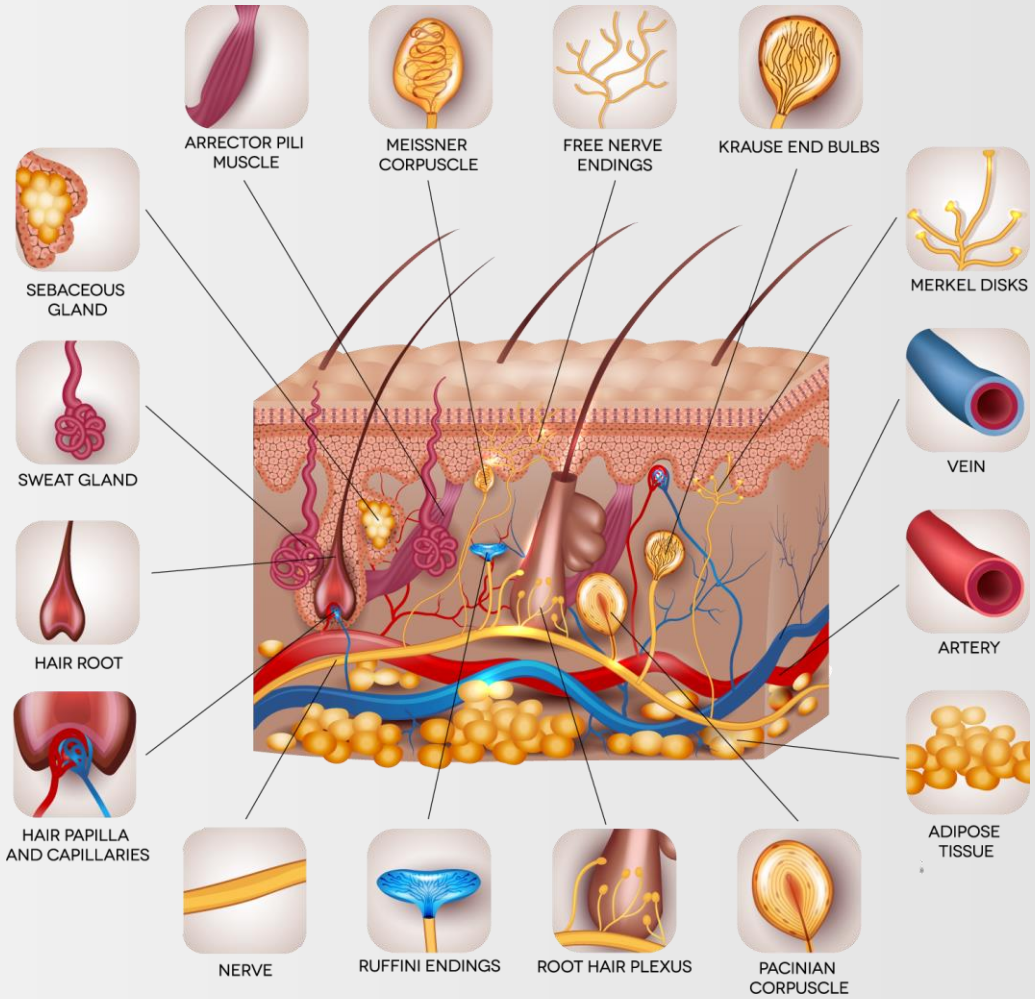
Keratinocytes and Melanocytes Key Features

- Melanocytes make and transport **melanosomes** for several "client" keratinocytes. They have long skinny "arms" to accomplish this.

- The **Keratinocytes** have variable numbers and sizes of melanosomes that cluster around the nucleus.



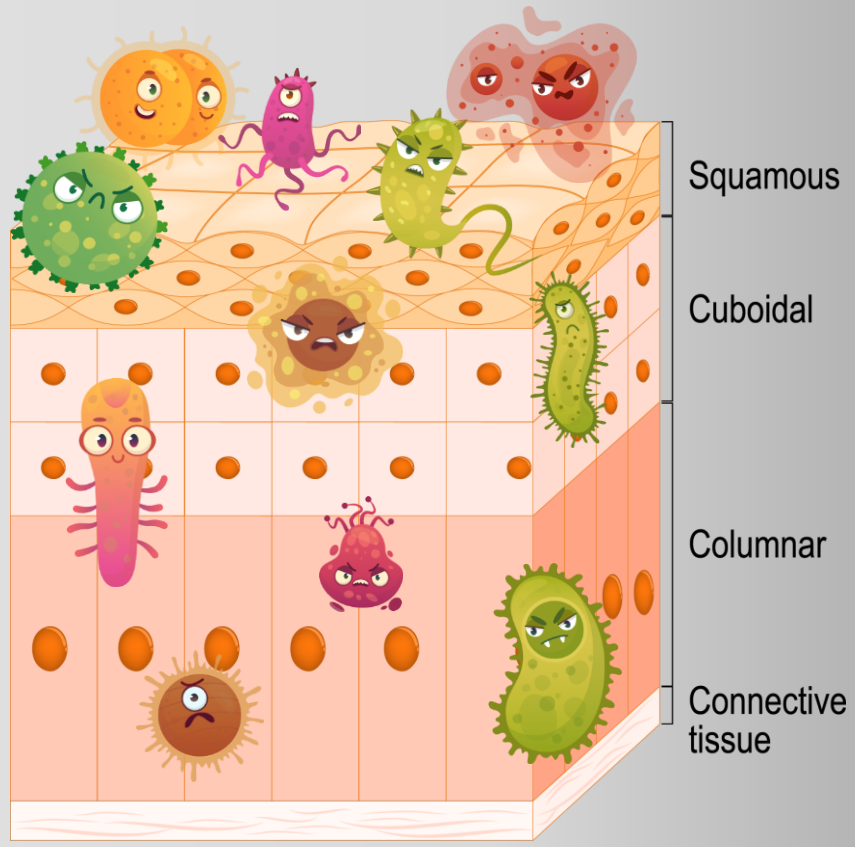
A Very Busy 1 Square inch of skin



- **65** hairs
- **100** sebaceous (oil) glands
- **650** sweat glands
- **78** heat sensors
- **13** cold receptors
- **1300** nerve endings (70 meters)
- **19,500** sensory nerve fibers
- **160** pressure apparatus to tactile stimuli
- **17** meters of blood vessels
- **9.5** million cells

Surface layers - bacteria, damaged cells

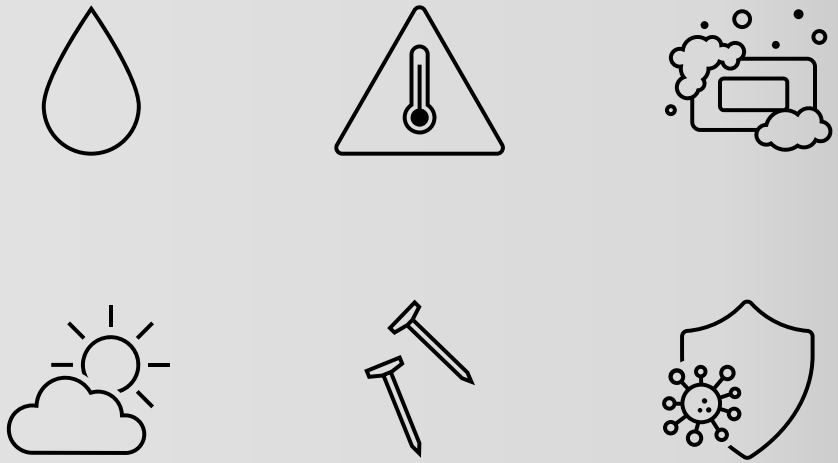
- The skin is the human body's largest organ, colonized by a diverse milieu of **microorganisms**, most of which are harmless or even beneficial to their host.
- Colonization is driven by the ecology of the skin surface, which is highly variable depending on topographical location, endogenous host factors and exogenous environmental factors. The cutaneous innate and adaptive immune responses can modulate the skin microbiota, but the microbiota also functions in **educating the immune system**.
- The development of molecular methods to identify microorganisms (16S mRNA, WGS) has led to an emerging view of the resident skin bacteria as **highly diverse and variable**.
- An enhanced understanding of the skin microbiome is necessary to gain insight into microbial involvement in human skin disorders and to enable novel **promicrobial and antimicrobial** therapeutic approaches for their treatment.



Epithelial Cells

Skin is Multifunctional and Dynamic

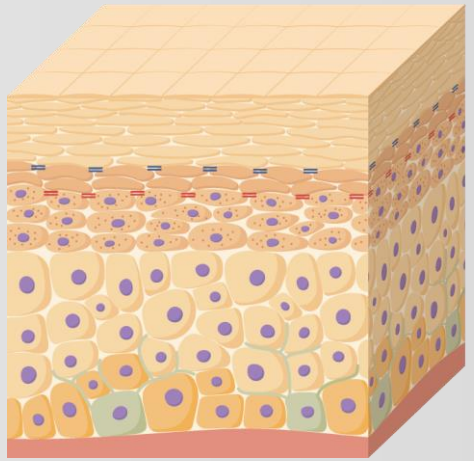
- Secretion of oils referred to as “**sebum**” for cleaning and moisturizing.
- **Heat regulation** to maintain our internal body temperature.
- **Absorption** of drugs, cosmetics, bacterial metabolites, and contact with environmental contaminates.
- **Protection** against sun, punctures, bacteria infections, with immune healing, formation of calluses and scabs, and acid secretions can also inhibit some bacterial growth.
- **Sensation** of hot, cold, pressure and pain.
- **Excretion** of salts, excess water, oils, CO_2 and urea.



B. Aging and Damaged Skin

Disease and Aging

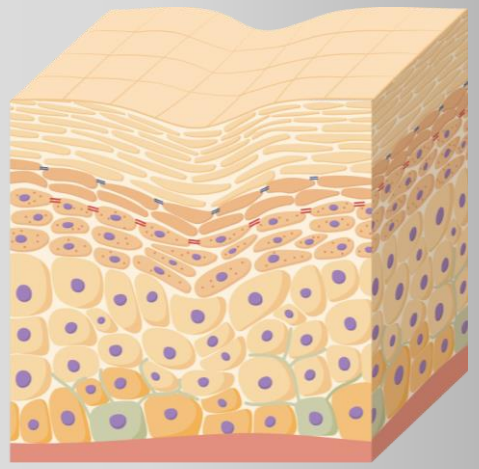
- With advancing age, these functions just described are increasingly **impaired**. Changes occur in the epidermis, dermis and hypodermis leading to thin, dry and sagging skin, while loss of structure and integrity diminishes the skin's ability to protect the body and detect changes in temperature and pressure. **Aging skin is more prone to infection, trauma, tears and pressure ulcers.**



Youthful skin

- Intrinsic skin aging results from the passage of time and is mainly due to the action of reactive oxygen species (ROS). It occurs within the skin itself due to reductions in dermal mast cells, fibroblasts and collagen production, and a flattening of the junction between the epidermis and dermis. Intrinsically aged skin is unblemished, smooth, pale, dry and less elastic with fine wrinkles.

Aged skin

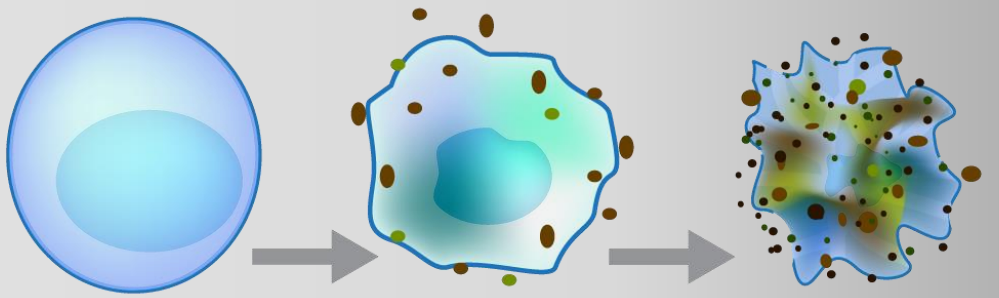


Oxidative Damage

- **Reactive oxygen species** (ROS) are molecules generated by the skin during normal cellular metabolism that destroy skin cell membranes, DNA and enzymes.

- ROS cause the activation of **collagenases** and enzymes that degrade collagen, and also other proteins that make up the extracellular matrix, thereby impairing the structural integrity of the skin.

- With advancing age, the number of ROS increases and the ability of the body's **antioxidant defense** system to get rid of them declines. (See HMTs' ebook on vitamins and anti-oxidants).



Oxidative Stress

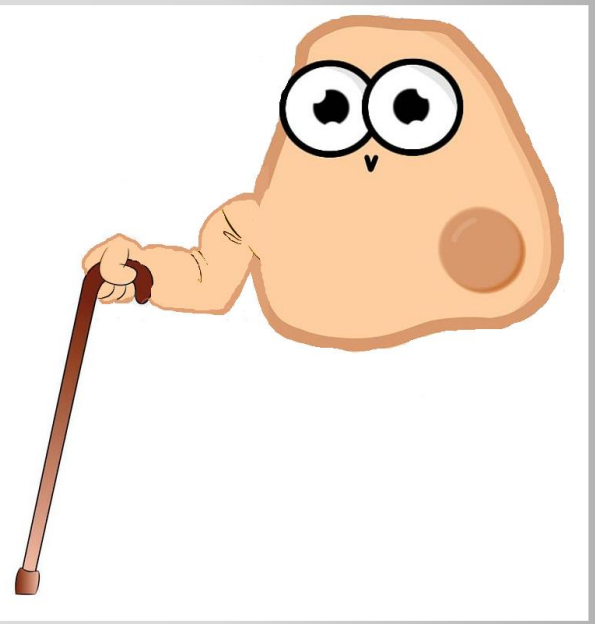
Extrinsic Aging

- The greatest source of **extrinsic aging** is accumulated, unprotected exposure to UV radiation. Sun radiation exposure affects the epidermis, causing irreparable damage to cellular DNA, and induces the generation of ROS. UV exposure also disrupts collagen synthesis, leading to acute collagen loss.

- Exposure to **UV radiation** also increases skin pigmentation and stimulates melanocyte proliferation. Melanin helps protect against the cumulative damaging effects of UV radiation.

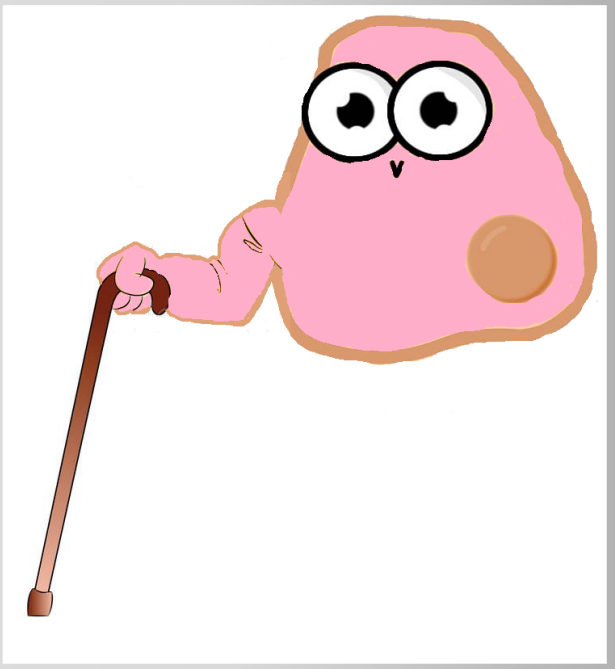
The Aging Epidermis

- **Keratinocytes and melanocytes** are found in the epidermis. Also present are Langerhans cells and dendritic cells that guard the 'front door' of the immune system, preventing unwanted foreign micro-organisms from entering the body via the skin. With age, there is a substantial loss of melanocytes and Langerhans cells.
- Having **no blood supply**, the epidermis gains its nutrition through contact with the dermis. A major cutaneous change in intrinsic skin ageing is a reduction in the surface contact between the epidermis and dermis. There is a loss of rete ridges which negatively affects the capillary-rich dermal papillae, resulting in a reduced supply of nutrients, metabolites and oxygen to the epidermis.
- The stratum corneum is not replaced as quickly, so skin is increasingly rough and dry. Extreme skin dryness **(xerosis)** can be seen in ageing skin, and this brings about an increased susceptibility to irritant dermatitis.
- With advancing age, there is a **reduction in the hormones and chemical signals** that are important for skin growth and repair, as well as a decline in the receptors that detect them; as an example, the number of vitamin D receptors in epidermal keratinocytes declines with age.



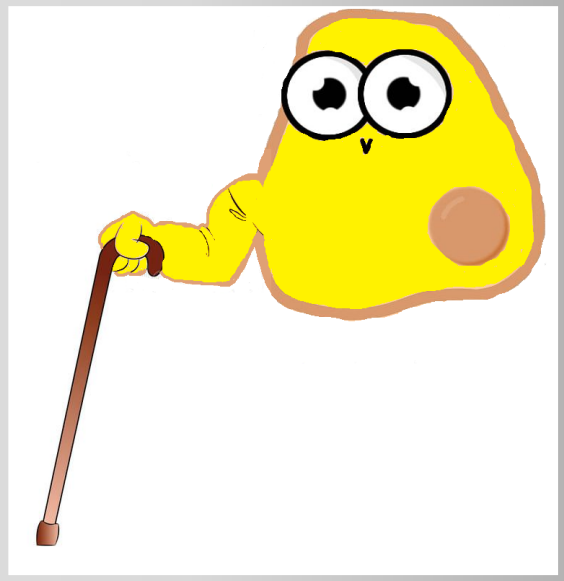
Aging Dermis

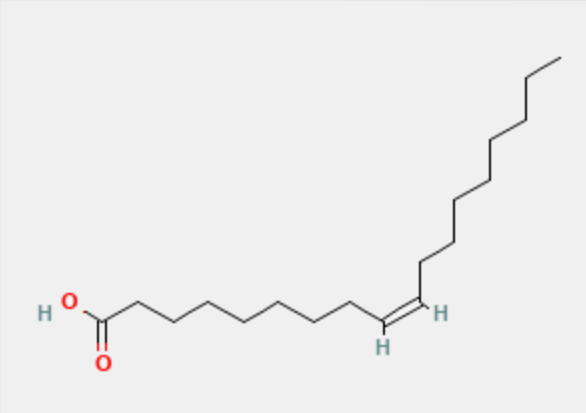
- Key cells in the dermis are: **Fibroblasts** – these synthesize collagen, elastin and the other structural molecules of the matrix and Mast cells – these are immune cells that produce histamine.
- With increasing age there is a 50% decrease in the number of **mast cells** and a 60% decrease in blood flow.
- The **collagen** content of the dermis decreases by 1% per year throughout adult life.
- Fibroblast activity **decreases with age**.



Aging Hypodermis

- The hypodermis consists mainly of **fat,** and acts as an insulator and shock absorber.
- With age, there is a loss of subcutaneous fat and the skin becomes thinner and **less resistant to trauma**.
- The **distribution** of subcutaneous fat changes too: it decreases in parts of the face and hands, but increases in the thighs and abdomen.

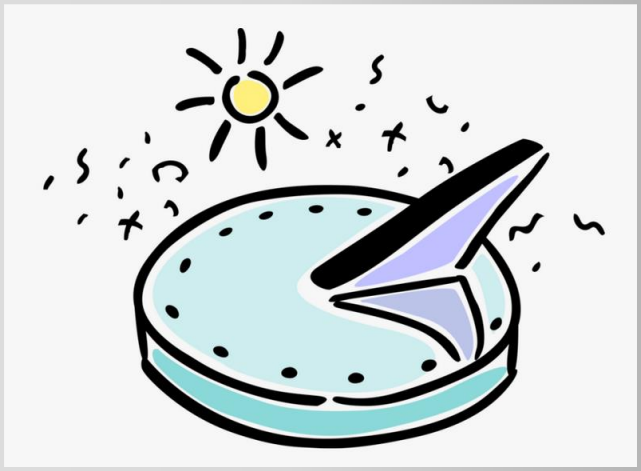




Oleic Acid

UV vs Chronological Aging

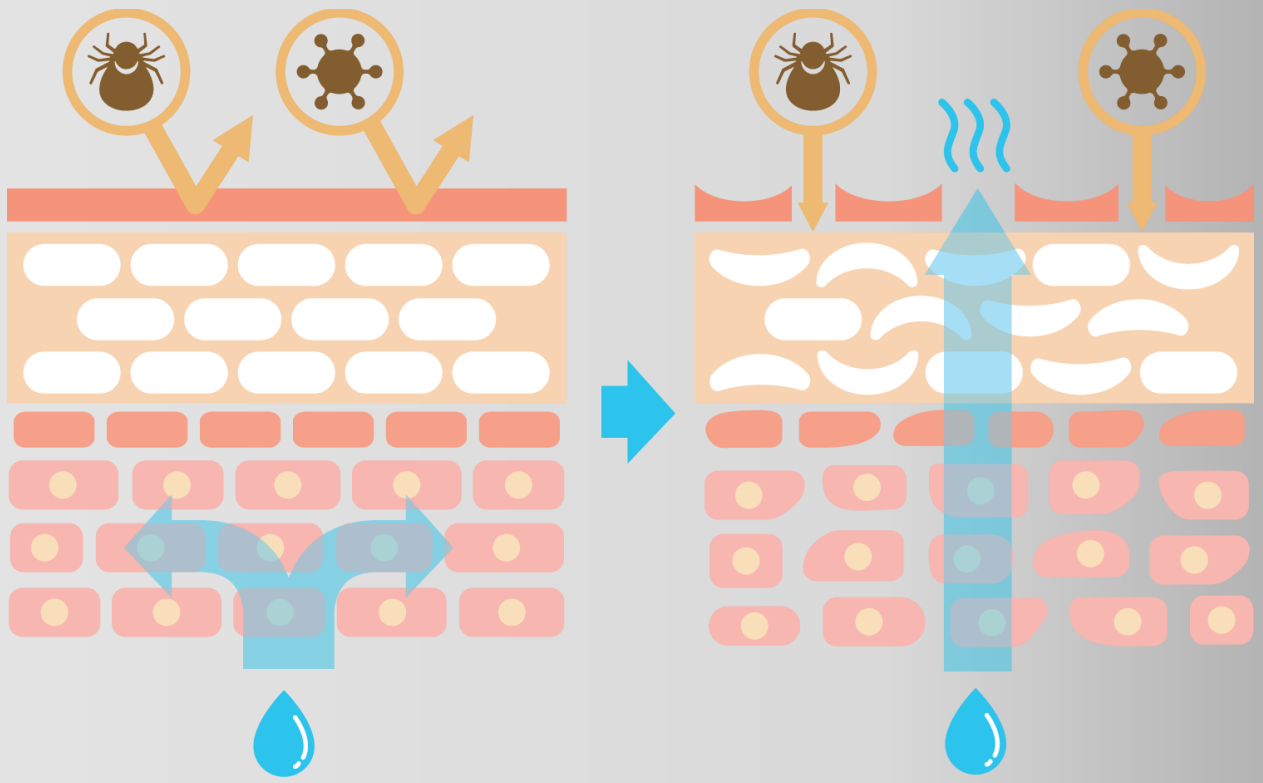
- Skin aging can be divided into **photoaging and chronological aging**.
- Skin aging alters epidermal lipids and free **fatty acid composition**.
- Palmitic acid (C16:0), stearic acid (C18:0), palmitoleic acid (C16:1), oleic acid (C18:1), linoleic acid (C18:2), and (all-cis)-11,14,17-eicosatrienoic acid (ETA, C20:3n-3) are major fatty acid components in the human epidermis.
- Palmitic acid, stearic acid, linoleic acid and ETA decrease in aged skin.
- Palmitoleic acid and oleic acid increase in aged skin.
- In contrast, linoleic acid and ETA increase with UV damage.
- Hence chronological aging and accumulated UV damage from the sun have a direct and complex impact on the **lipid composition in the epidermis**.
- These lipids are linked to cellular lipid membranes, cellular strength, inflammatory and immune response.



Landscape of Epidermis and Dermis with Age

- Harmful bacteria, ROS damage, and protein degradation leads to **molecular changes** in epidermis and dermis.

- Damaged Barriers allow **allergens and bacteria** under the surface and moisture out.

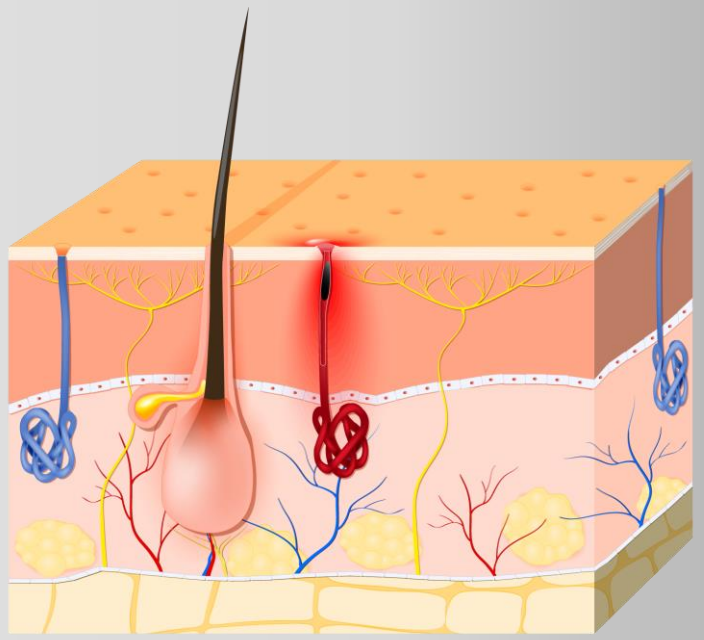


Diseases of skin

- Age-related skin problems can **cause distress** and decrease quality of life.

- Skin conditions include eczema, psoriasis, infections and pruritus, many of which are associated with **dry skin and itching**. Decreased sebaceous secretions, loss of oil glands and circulatory changes contribute to dry and scaly skin in the lower extremities, and skin becomes more susceptible to inflammation, infection and rashes.

- **Pruritus** may be caused by dryness, irritation or infection. It is also linked to diabetes, kidney disease and anemia. Pruritis can substantially decrease quality of life, especially if it leads to sleep deprivation.



C. Cosmetic and Beauty Industry & Use of Probiotics

Restoration and Healing

- According to the FDA, **cosmetics** only alter the appearance of our skin or act on the top layer (such as moisturizing it).
- If a product has any kind of biological function (i.e., reducing wrinkles), they are considered a drug. For example, **Botox** is a drug, but moisturizers are not.
- **Emollients, moisturizers, humectants** have crucial roles to play in skin health for dry and itchy skin; these **increase the amount of water** held in the stratum corneum, either by drawing it from the dermis or by trapping it and preventing its evaporation. Many effectively alleviate skin dryness. They are often used alongside other treatments, such as steroids, for conditions like psoriasis or eczema.
- As skin ages, it becomes thinner, more transparent and more easily bruised. Medications such as **corticosteroids** help with repair, however, can make the skin even more fragile.
- The **Cosmetic, Beauty and Probiotic industries** serve general public by providing contact solutions to aging and damaged skin and also help protect against disease.

Cosmetic and Beauty Industry

- Globally, the industry is **strong** and only getting stronger.
- Up from $483B in 2020 to $511B in 2021 — and with an annual compounded growth rate of 4.75% worldwide — it's **predicted to exceed $716B by 2025**. And $784.6B by 2027.
- Rapid expansion is from increased marketing through digital channels and the attraction of more customers willing to pay higher prices for higher quality.
- International companies are also entering the field with high quality products.
- Currently, Asia Pacific and North America dominate, accounting for more than 60% of the total sales.
- Across product categories, skincare made up 40% of the global cosmetic market in 2019.

Growth in Number of Players

- Aside from **superstores** like Walmart and Target, the biggest vertical-specific retailers are Ulta and Sephora.
- L'Oréal, Unilever, Procter & Gamble, and Estée Lauder Companies made up 81.7% of worldwide revenue in 2019.
- However, many **boutique** smaller companies are presenting unique formulations and probiotics that are entering the market.

How do we define these new products?

- Because of rising incomes, consumers increasingly consider **effectiveness and ingredients** over price.
- To compete, marketing uses three powerful words: ***natural, organic, and/or clean***.
- **"clean beauty" and "organic makeup"** are more than just buzzwords. The global value for natural cosmetics is expected to reach **$54.5 billion by 2027.**
- How do we **define** clean and organic? Is there a molecular basis for this presentation?

Other Key Words: Natural and Essential

- As consumers become increasingly wary of potential toxicity, safe and fragrance-free, products made using **natural ingredients and essential oils** are likely to record strong growth in the future.

- What are **“natural”** ingredients and what are the “essential” oils? Does business have a molecular basis for these definitions?

What are the issues with growing field?

- **Greenwashing** occurs when a company focuses marketing their brand and products as environmentally friendly and safe rather than doing the science to actually make or demonstrate their brand and products meet these qualifications.

- Chemical ridden products, vague ingredient labels, and hidden chemicals are three of the major problems with the beauty industry. Knowing more of what is in product and presentation of ingredients (**open disclosure**) can do a lot to promote product.

- Women may apply over **150 different chemicals** to their body in a day. Now that is something quite alarming because our skin can absorb most any chemical into our bloodstream. Hence, knowledge of product ingredients and definition of natural and organic will help promote products of quality.

Education

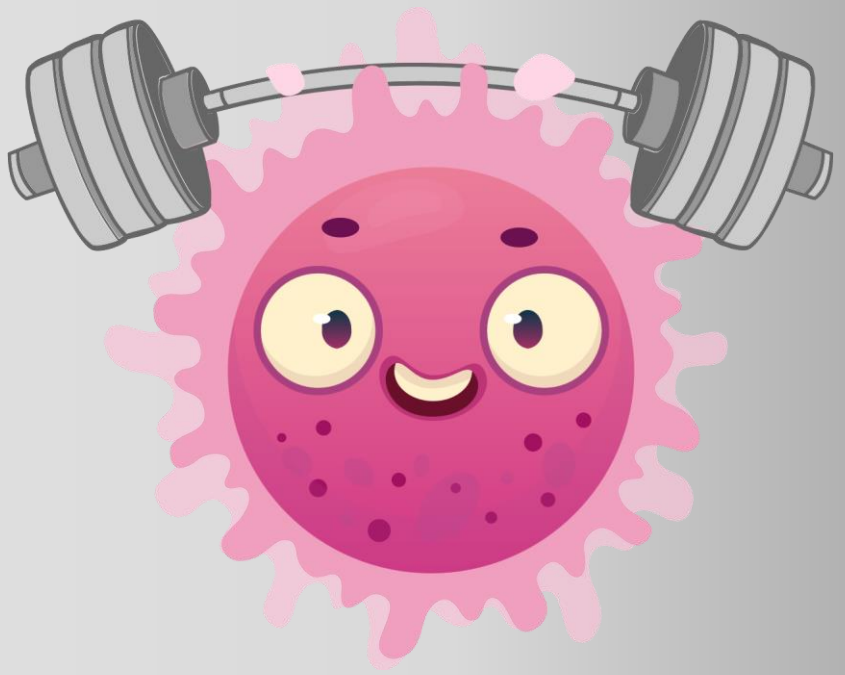
- Learning about the **ingredients** in cosmetic, beauty and probiotic products is not clear to the consumer, as much of the trustworthy data is within scientific publications, unvalidated or lack credible sources.

- Research involves deep dives on search engines and lots of clicking to find trustworthy sources.

- **Metabolomic research** and measurements will help provide significant and trustworthy data for the industry and consumers, and help to define product ingredients classifications as natural, essential and establish product ingredients functionalities as immunoreactive, anti bacterial, anti oxidative.

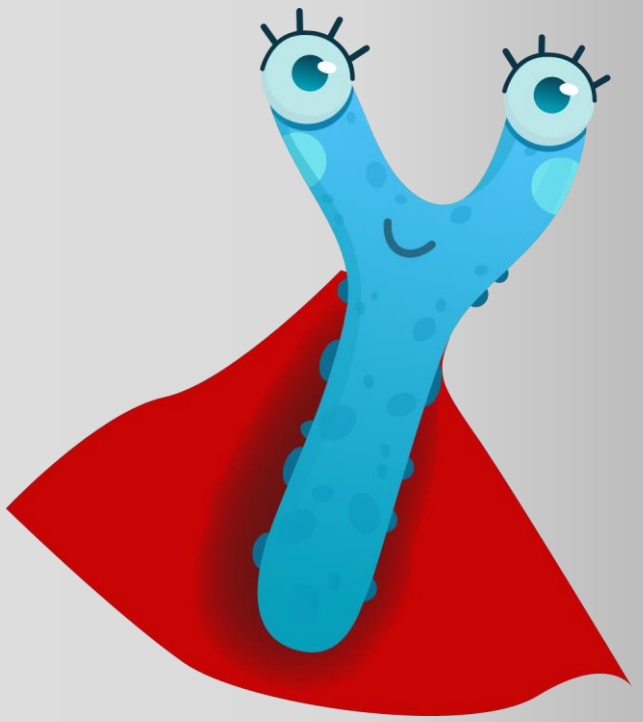
Probiotics Rising

- There is much research linking **microbiome** imbalances in the skin and gut to inflammatory skin conditions such as acne, rosacea, and eczema.
- Research suggests that taking **probiotics** can replenish your body's own healthy gut flora, improve your body's immune system function, repair your digestive tract after illness or injury, fight off "unfriendly" microorganisms such as bacteria and viruses and improve your body's ability to use nutrients from food by improving digestive function.
- Studies suggest that applying probiotic skin care products may **reduce acne** outbreaks and manage dry skin and eczema . Several small but promising studies also suggest that probiotics can help battle skin aging and even skin cancer.
- *Lactobacillus Acidophilus* is one of the best-known probiotics strains. This strain has been tested and found to be beneficial in helping reduce acne. Another beneficial strain of probiotics for treating acne is called *Lactobacillus Bifidobacterium*.



Probiotics - Adding more to the Product

- **Probiotic skin care** has the potential to change the entire skin-care game — just not in the way we thought.
- In the future, we could have a bacteria that live on our faces that **secrete UV protectants.**
- Probiotics also have the potential to deliver ceramides, alpha and beta hydroxy acids, antioxidants and other **nutrients at beneficial levels** without the need for constant reapplication.
- All of this potential adds to the molecular complexity of our skin and the need for **metabolomic profiling** to determine the polar and lipid skin metabolome.



D. Health providers, standard care and testing

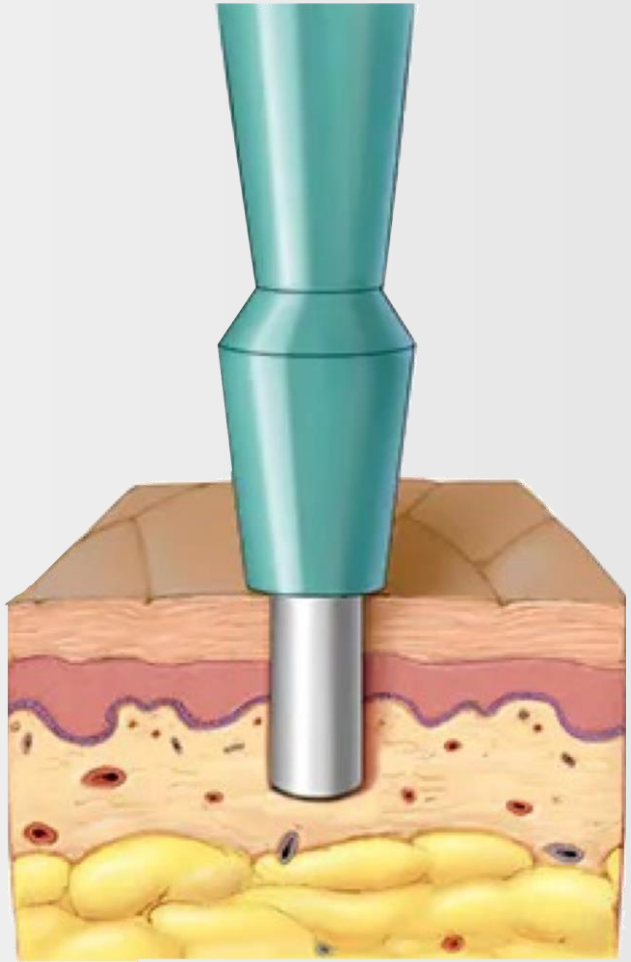
Health Care

- Doctors **diagnose** some skin problems by how they look. For other problems, they will use skin tests.

- **Skin tests** can help to diagnose allergies, infections, and other problems affecting the skin. They are also used to tell the difference between malignant cells and benign cells.

- The most common skin tests include **Patch testing**: Patch tests are used to diagnose skin allergies. Allergens are applied to the skin on the back with adhesive patches and left for a period of time. The skin is then examined for any reaction.

Skin Tests



- **Skin biopsy**: Skin biopsies are used to diagnose skin cancer or benign skin disorders. Skin may be removed with a scalpel, razor blade, or a cylindrical punch biopsy tool.
- **Cell Culture**: A culture is done to identify the microorganisms that may be causing an infection. Skin scrapings, biopsies, contents of pus, hair or nails may be cultured.
- **Dermoscopy**: confocal imagers, and tape stripping are other diagnostic techniques that may be used to look at genetic material.

What conditions may be diagnosed?

The Most **Common Skin Conditions**:

1. Acne (Acne vulgaris), the most common skin disorder in the U.S.
2. Atopic dermatitis (Eczema)
3. Shingles (Herpes Zoster)
4. Hives (Urticaria)
5. Severe Sunburn
6. Contact Dermatitis
7. Rosacea

Diagnosis may include infections

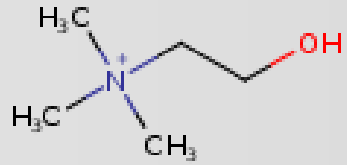
Common skin infections include:

- **Cellulitis:** *Streptococcus or Staphylococcus*
- **Erysipelas:** group A *streptococcal bacteria, Streptococcus pyogenes*
- **Impetigo:** group A *Streptococcus and Staphylococcus aureus.*
- **Folliculitis:** infection of hair follicles with *Staphylococcus aureus*
- **Furuncles (Boils):** usually caused by the bacteria *Staphylococcus aureus*

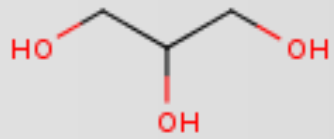
Limitations

- Current **clinical testing** does little to understand the molecular basis of diseases, impact of treatments, pre and post beauty treatments, and definitions of product effectiveness.

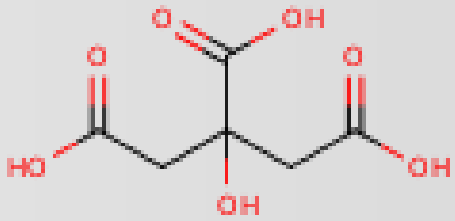
- **Metabolomics** provides a deeper molecular report of the metabolites and lipids within the top layers of the skin for both health and beauty industries.



Choline



Glycerol



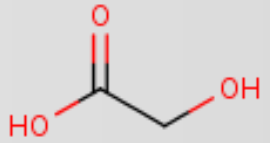
Citric Acid

E. Dermo-metabolomics

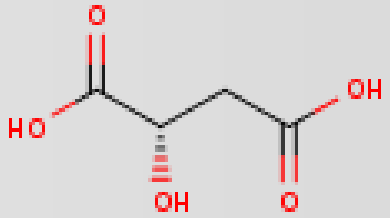
Dermo-Metabolomics

- **Skin metabolites** are low-molecular-weight compounds found in different layers of the skin.
- The field of skin metabolomics relies on the availability of methods for efficient **skin sampling** and analysis of metabolites in low-volume specimens (e.g., sweat, biopsies).
- Skin metabolomics (**Dermo-metabolomics**) is an emerging tool for discovery and medicine.
- Skin metabolomics seeks out metabolic differences with the use of cosmetics, pro-biotics, and standard care dermatology by measuring changes in emollients, humectants, bacterial metabolites, inflammatory and anti-inflammatory lipids, and levels of anti oxidants providing a **molecular picture of change**.

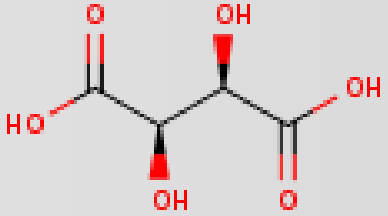
Alpha-Hydroxy-Acids



Glycolic acid



Malic acid



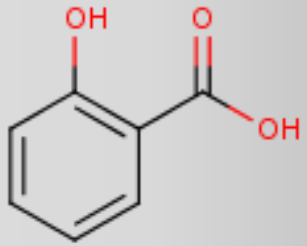
Tartaric acid

Skin Care Ingredients

- **Hydrators**
  - Niacinamide (form of Vit B3) – antioxidant and anti inflammatory
  - Hyaluronic acid (combination of glucuronic acid and N-Acetylglucosamine) – hydrator and humectant
  - Polyglutamic acid, also a humectant
  - Ceramides, along with oils, help to stabilize skin cells, part of extracellular matrix
  - Essential oils – mostly monounsaturated long chain fatty acids that help to add tone and repair damaged epidermis and dermis
- **Anti-aging or Anti-Oxidants**
  - Vit C, Vit E, Ferulic Acid, Resveratrol, Ubiquinone – anti-oxidants, increase collagen, and reduce melanin
  - Retinol (Vit A), Bakuchiol, Collagen, and Collagen peptides
- **Exfoliators**
  - Alpha hydroxy acids
  - Beta hydroxy acids
  - Polyhydroxy acids

Metabolome-Insights of change

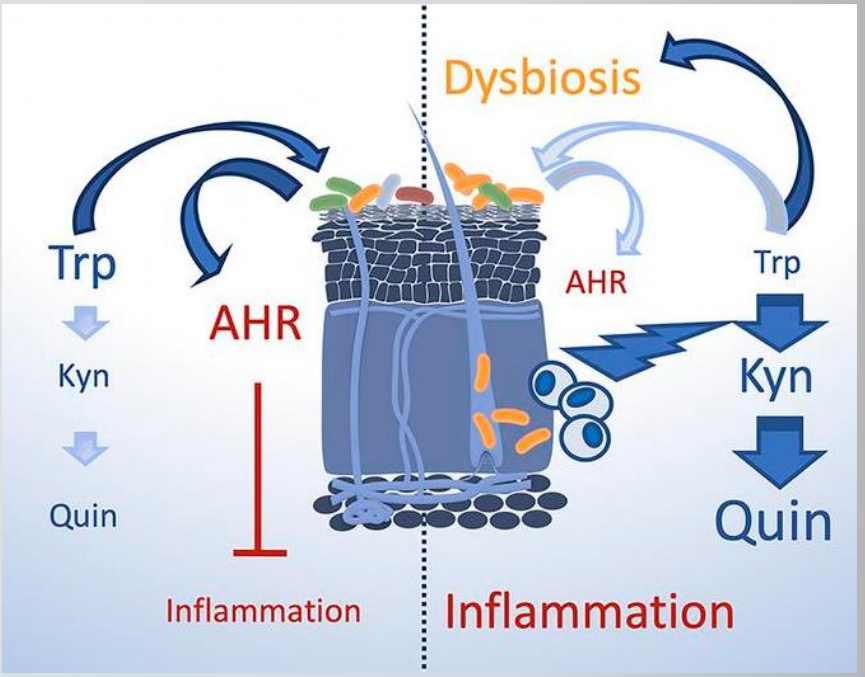
- The metabolome of the **skin reflects dynamic changes** that occur in relation to the macro and microenvironment, intrinsic factors, and in relation to skin and systemic disease.
- **Aging** is one intrinsic factor that influences the skin metabolome such as Coenzyme Q10, an anti-aging metabolite and DHEA-sulfate.
- **UV light exposure** upregulates lactate, a natural moisturizing factor.
- ***Staphylococcus aureus*** can be identified by changes in the metabolome.
- Metabolites indicate the progression of **wound healing** in the skin, where linolenic acid is initially increased followed by an increase in adenosine.
- The skin metabolome gives insights into the physiology of wound healing, **topical diseases** and their link to systemic comorbidities, cosmetic aspects and aging related topics.



Salicylic acid

How to sample skin?

- The skin surface can be sampled to detect and quantify **skin metabolites**.
- To characterize a broad spectrum of skin metabolites, specimens are collected with one of several available methods, and the processed specimens are analyzed by **mass spectrometry**.
- **Diseases** for which skin-related biomarkers have been found include cystic fibrosis (CF), psoriasis, Parkinson's disease (PD), and lung cancer.
- To increase the clinical significance of skin metabolomics, it is desirable to verify **correlations** between metabolite levels in skin and other biological tissues/matrices.

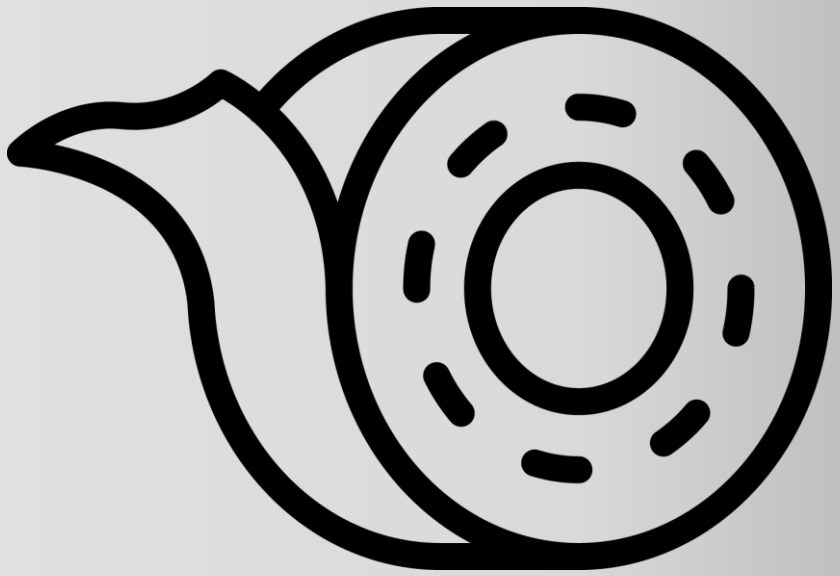


Tape Strips

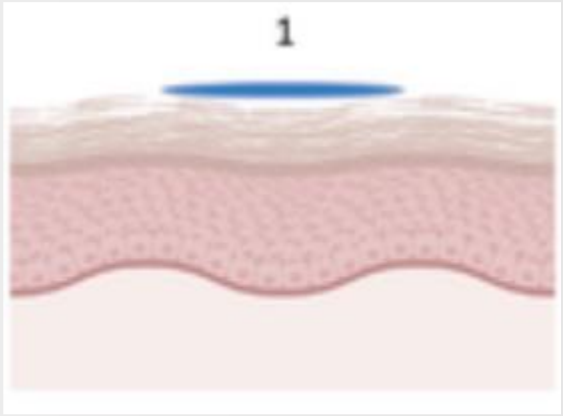
- **Tape strips** have been used widely in dermatology research as a minimally invasive method to sample the epidermis, avoiding the need for skin biopsies.

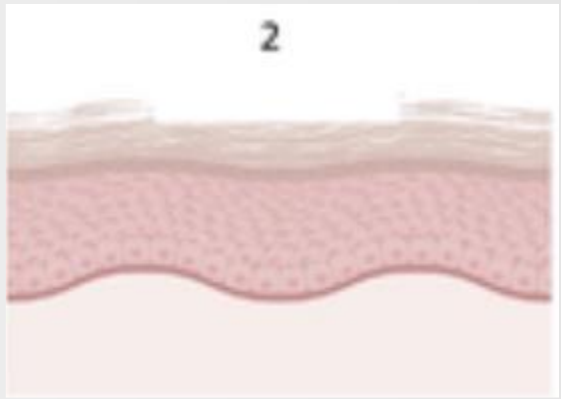
- Tape stripping is a less invasive method to **obtain skin samples**.

- Tape strips are plastic discs or patches with an adhesive side. The adhesive **sticks to the skin surface** and components of the epidermis adhere to the plastic when removed. Sequential application allows deeper levels of the stratum corneum (SC) to be accessed with each strip.



Sequential removal skin layers

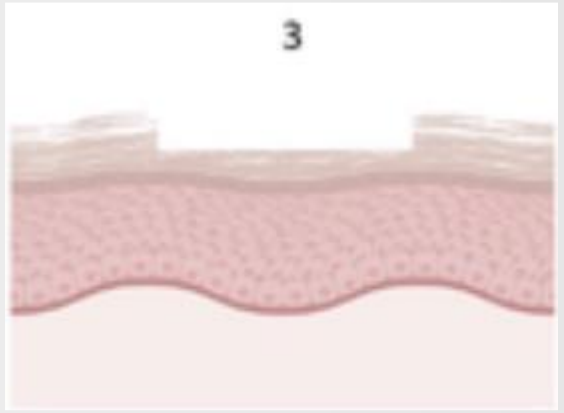




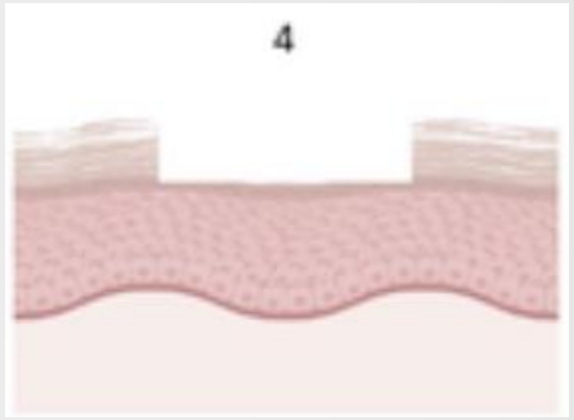
1. The stratum corneum is intact when the **tape strip (TS) is placed**.

2. Superficial components of the epidermis **adhere to the first TS** and are removed with it.

Sequential removal skin layers



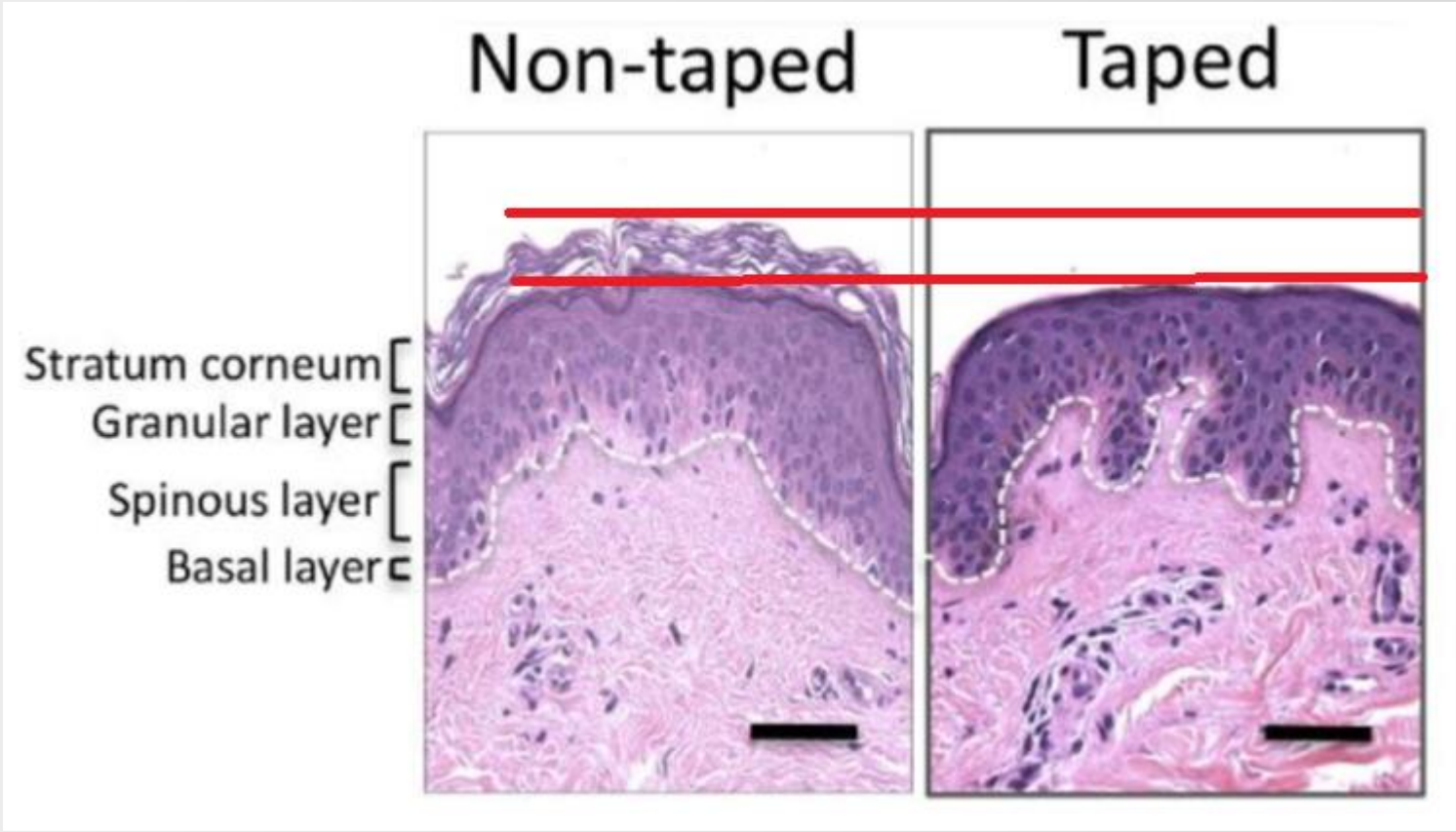
3. **Additional tape strips** can be used to detect lipids/ceramides and superficially expressed proteins and peptides, such as natural moisturizing factor, proteases and some cytokines.



4. After approximately **20 tape strips the stratum corneum** has been stripped and the granular layer is accessed.
    - The mass of stratum corneum removed declines exponentially with each TS and stratum corneum thickness varies between.

Intact cells, mRNA granular layer

- About 20 strips are required to remove the stratum corneum as seen here.



Dermatoses studies with skin tape

Atopic eczema	Squamous cell carcinoma	Dermatophytoses	Basal cell carcinoma
Psoriasis	Cutaneous T-cell lymphoma	Candida balanoposthitis	Rosacea
Contact dermatitis	Pityriasis versicolor	Scabies	Acne vulgaris
Photodamage	Seborrhoeic dermatitis	Cutaneous leishmaniasis	Lichen planus
Actinic keratosis	Perioral dermatitis	Melanoma	Genodermatoses*

*(Netherton syndrome, ichthyosis vulgaris, peeling skin syndrome type B, X-linked recessive ichthyosis)

F. Odor and Breath Analysis

Breath Analysis

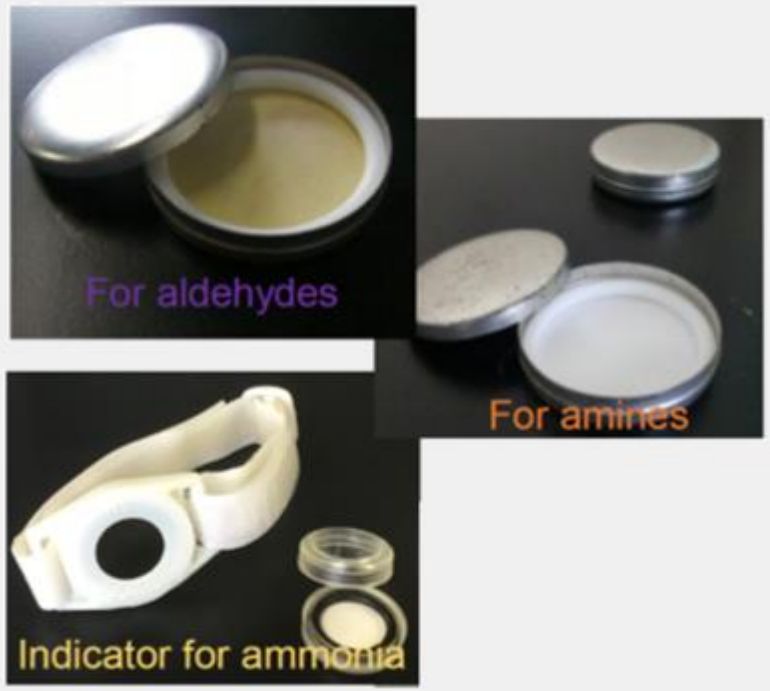
BioVOC by Markes

Markes' BioVOC-2™ breath sampler is a simple-to-use device designed for non-invasive monitoring of breath VOCs. BioVOC-2 collects the last 129 mL of expired breath, largely alveolar air, for transfer to a sorbent tube, and subsequent analysis by HMT for molecular identification and quantitation.

Non-invasive and easy to use, removing the need for medically qualified staff, the device can be used multiple times for one participant within a single sample sitting. A disposable mouthpiece is used to avoid contact with the BioVOC-2 body.

HMT will arrange for shipping of the BioVOC-2, which upon administration, would be sent back to HMT for molecular analysis using GC-MS and the HMT library.

Body Odor Analysis



Human body odor consists of various kinds of volatile organic compounds (VOCs) that reflect skin health, skin conditioning and skin age.

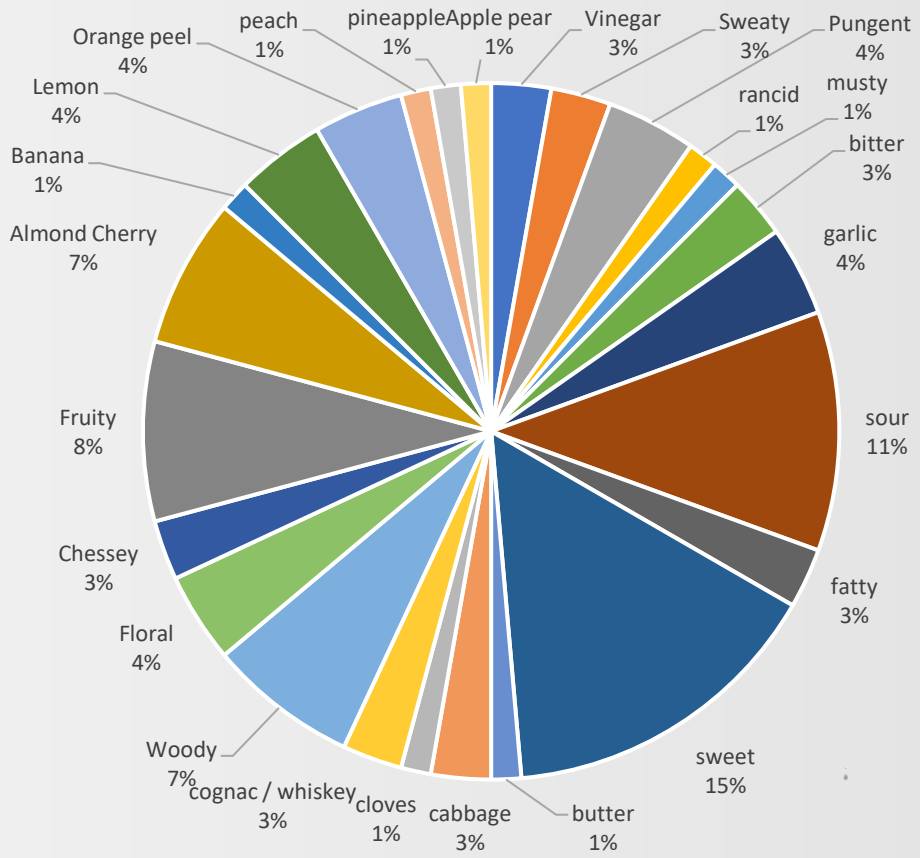
Using a unique Passive Flux Sample (PFS) developed by Sekine et. al., odor is trapped from forearms, arm pits or back of the neck.

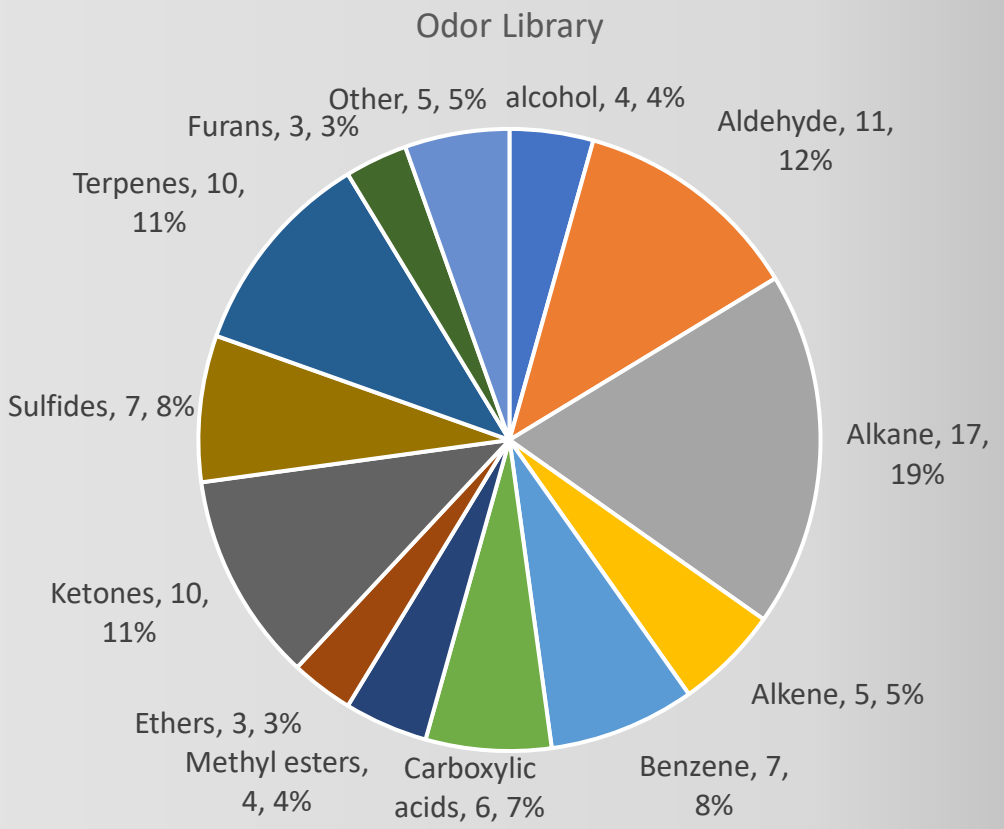
The PFS is comprised of a MonoTrap DCC18 filter that absorbs VOCs that can be removed and analyzed by HMT's GCMS system.

The HMT library of VOCs is categorized by high and medium notes, types of odors and fragrances (apple, peach, woody, pine, garlic, sour, fruity for example).

Sekine Y.,et al., *J. Chromatogr. B.*, 1028, 181-185 (2016)

HMT GC-MS analysis of VOCs





F. HMT Solutions & Testing

Tape Strips - Microbiome

- Tape strips have been used to **sample cutaneous microbes**.
- Lange-Asschenfeldt *et al*. showed that bacteria were found on all 15 sequential strips and that 85% of bacteria resided in the first six tape strips .
- Unlike biopsies, differential tape stripping allows the **identification of microorganisms** within specific stratum corneum layers.
- **Skin swabbing**, however, detects only surface bacteria. Ogai *et al*. found that, while microbiome bacterial composition was equivalent between Tape strips and skin swabs using next-generation sequencing or anaerobic culture, there was greater bacterial diversity in Tape strips samples using aerobic culture. Similarly, Chang *et al*. compared the use of tape strips to swabs and cup scrubs in patients with AE and found a higher intra-patient concordance of microorganisms detected using tape strips.

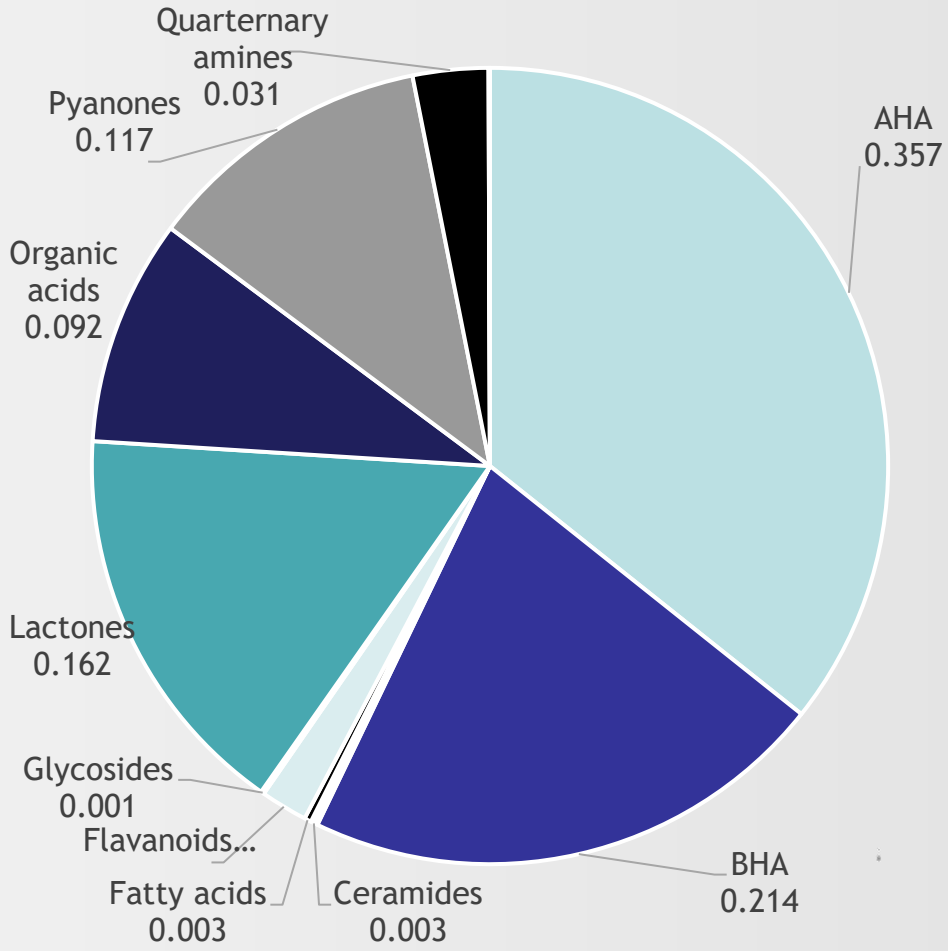
How Many Strips?

- The number of tape strips used for lipid and metabolite analysis varies.
- Chiba *et al*. detected a difference in trihydroxy-linoleic acid levels from only one tape strip.
- Leung *et al*. compared tape strips from levels 5 and 6 with those from levels 15 and 16. This study found no difference in ceramide abundance at TS levels 5-6 between patient groups, but a substantial difference at levels 15-16.The authors hypothesized that environmental oxidation of lipids affects the results of tape strips levels 5-6 but not levels 15-16.
- **HMT has been successful just combining strips 1 and 2 (first strip is often discarded).**
- The number of strips may be linked to goal of study – disease, bacterial infection, effects of skin applications of cosmetics, beauty treatments and/or probiotics.

Factors that affect success

Factor	Comment
Anatomical site	The forearm is commonly used
Pressure of application	Pressure application can be standardized by using a pressure instrument
Duration of pressure applied	The time that pressure is applied should be standardized
Contaminants on the skin surface	Strips number 1–2 are typically discarded,-although bacteria have been shown to be present up to number 15
Stretching the skin during application	A decision must be made about whether to stretch the skin or not
Speed of removal	The speed of removal should be considered
Brand of adhesive tape	The same brand must be used throughout the study
Hydration of the skin	Emollients have been shown to alter gene expression
Activity of skin disease	Studies should distinguish between samples from lesional and nonlesional skin
Season	Exposed sites should be avoided if studies are longer than one season

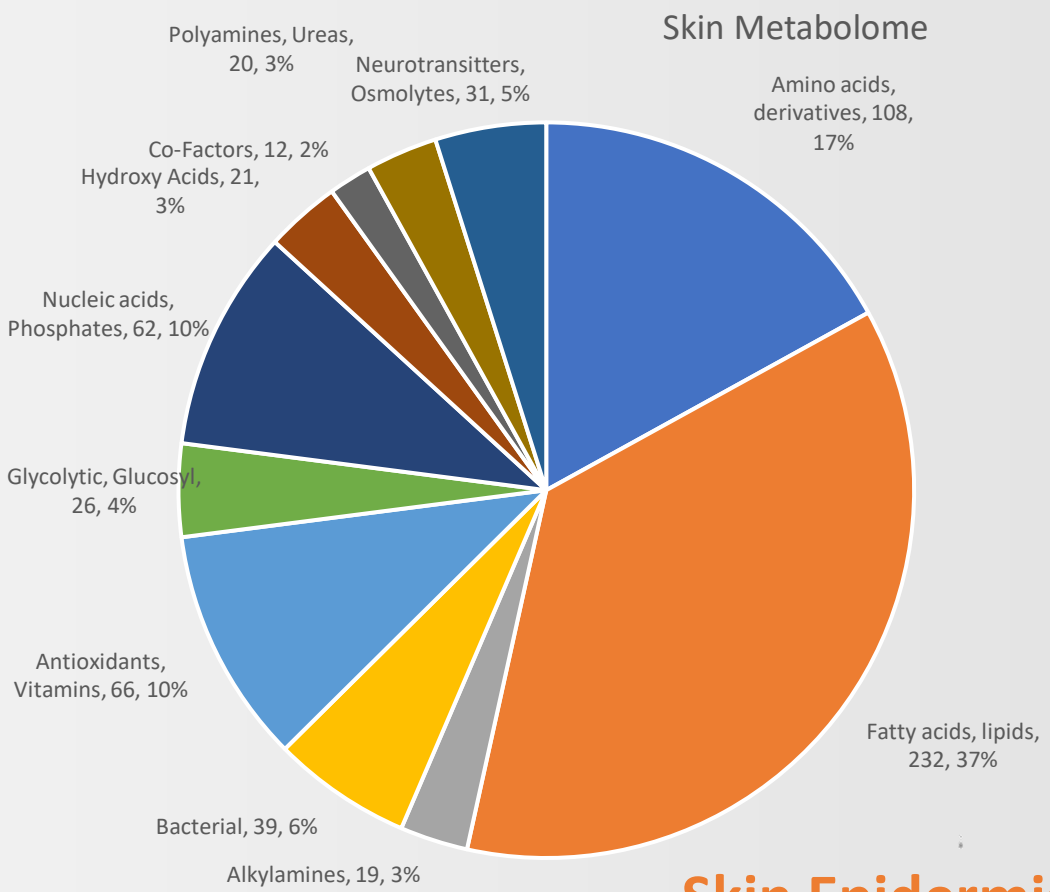
HMT Library from skin testing



- Metabolomics can be applied to **product development pipeline**.
- **Pro-biotic formulations** may be screened for metabolic composition against known active ingredients and bacterial related metabolites.
- AHA (Alpha-hydroxy acids).
- BHA (Beta-hydroxy acids).

Pre product formulation

HMT Library from skin testing

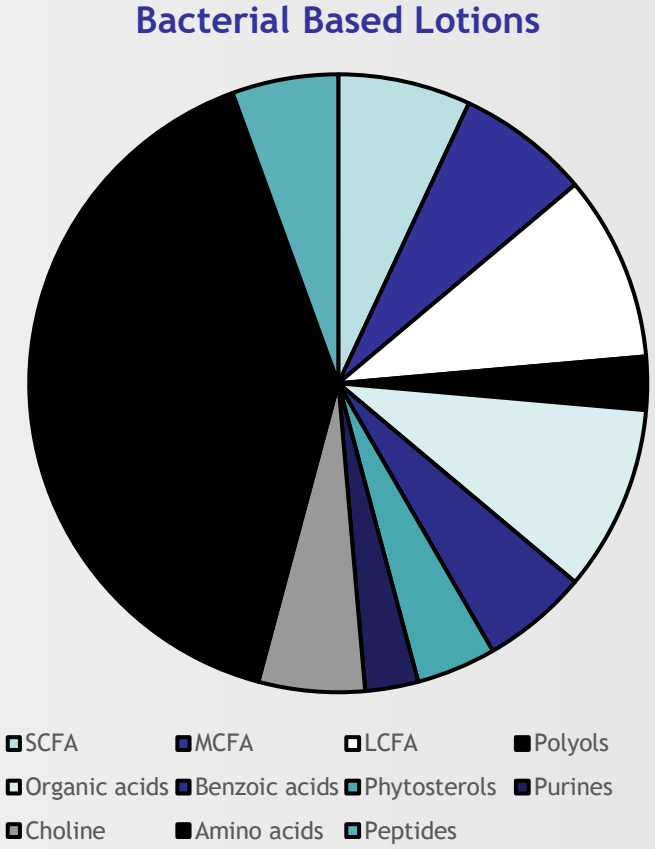


Skin Epidermis

- Tape strips can be used **pre-marketing,** as well as, in clinical settings to measure changes in metabolic profile due to disease, treatments, beauty applications, probiotics etc.

- Skin tissues contain large percent of free fatty acids, amino acids and their derivatives, anti oxidants and vitamins that can be **measured by dermo-metabolomics**.

HMT Library from skin testing



- **Skin Care Products** can also be tested.
- The compositions are dependent upon the type and use of product.
- **Probiotic formulations** are expected and observed to contain a large number of bacterial metabolites.
- These metabolites can be linked in many cases to a functional role in **health and cell renewal**.

HMT Dual Scan

Alpha hydroxy acids	MCFA
Amino acids	MUFA
Bacterial	Nucleic acids
Benzoic Acids	Peptides
Beta hydroxy acids	Phosphates
Bile Acids	Phytosterols
Catechols	Polyols
Collagen amino acids	PUFA
Complex lipids	Quarternary amines
Flavanoids, Cartonoids	Saturated fatty acids
Glucolytic acids	SCFA
Glycosides	Sphingosine
Lactones	Steroid Hormones
LCFA	Vitamins

- **HMT's "Dual Scan"** approach measures both polar and lipid metabolites in pre-formulation products, liquid products, skin biopsies and skin tapes.
- Over **30 biochemical classes** are found in product and skin (left).
- Over **14 different active or skin functional ingredient categories** (Right).

Anti Aging	Exfoliator
Anti inflammatories	Floral scents
Anti microbials	Humectant
Anti oxidants	Lubricant
Cell renewal	Moisturizing
Emoilluents	Osmoprotectant
Emulsifier	Ph buffering

Not all AHA's and BHA's are alike

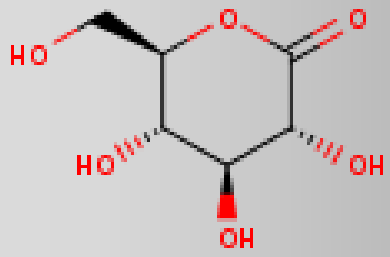
AHA

Glycolic Acid is the smallest, most effective AHA with the deepest penetration into dermis, best for oily skin, shrinks pores, helps melasma.

Lactic Acid reduces age spots, reduces hyperpigmentation, more hydrating than other AHAs.

Mandelic Acid is mild, less irritating than other AHAs.

Other common AHAs include: Tartaric Acid, Citric Acid and Malic Acid.



Gluconolactone

BHA are more oil soluble than AHAs and can get deeper penetration into skin.

Salicylic Acid (the most common BHA) is an acne fighter, dissolves sebum and acts as anti-inflammatory.

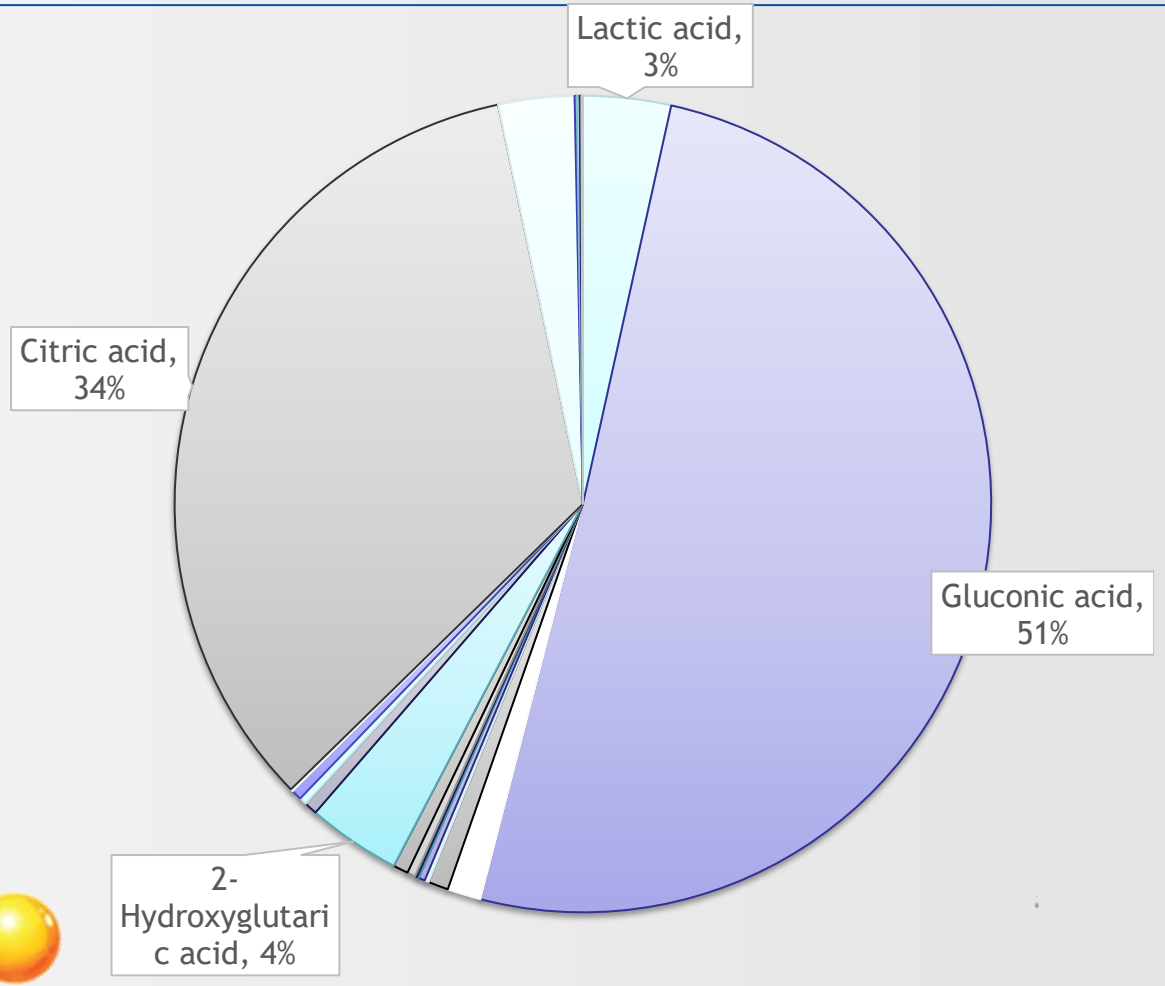
Polyhydroxy Acids (PHA)

Gluconolactone is a humectant and exfoliant, lesser irritating than other hydroxy acids.

Other common PHAs include: Galactose and Lactobionic acid (a gluconic acid and galactose polymer).

Often AHAs and BHAs are combined with Vit B5, Vit E, and Vit C to reduce sun sensitivity, itching, and skin irritations.

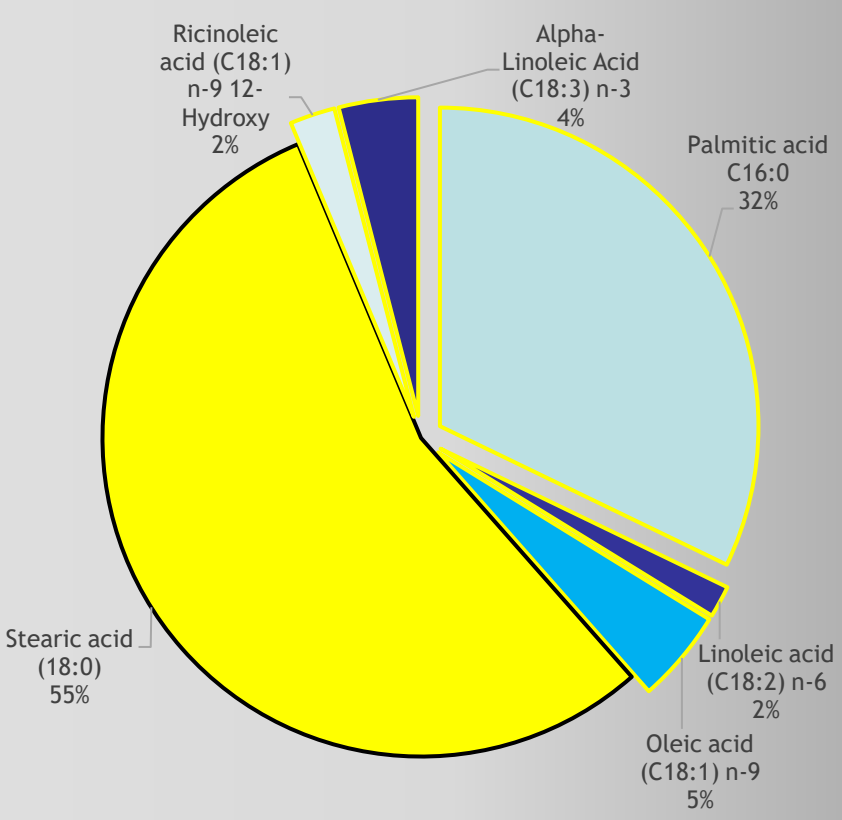
Know your AHA - BHAs



- Alpha Hydroxy Acids (AHA), Beta Hydroxy Acids (BHA) and polyhydroxy acids are considered **EXFOLIATORS**, helping to rid the skin of dead cells and stimulate growth of new cells to replenish epidermis.
- Cell growth also stimulates **fibroblasts** to produce collagen adding to firmness and tone.
- In addition, they can help against acne, sebum and inflammation.
- Overall, hydroxy acids are an **essential part of skin health**.

Know your fatty acids

Caproic acid (C6:0)	Cleansing, emulsifying, masking, surfactant, and perfuming
Caprylic Acid (C8:0)	Antibacterial, antifungal, and anti-inflammatory
Palmitic Acid (C16:0)	Emollient
Stearic acid (C18:0)	Emulsifier, emollient, and lubricant
Linoleic acid (18:2 (n-6)	An oil cleanser unclogs pores, strengthens the skin barrier, and even soothes dermatitis and eczema, and helps promote healthy cell activity. Anti-inflammatory and helps stimulate cell regeneration.
Oleic Acid (C18:1) n-9	Penetrates easily and deeply into the skin's surface, replenishing lost moisture that naturally comes with age. An anti-inflammatory substance that stimulates wound healing.
a-Linoleic acid (C18:3) n-3	Strong antioxidant, antiwrinkle, UV protection
Ricinoleic acid (C18:1) n-9 12-Hydroxy	An effective moisturizer, easily penetrating the skin to soothe and soften dry, rough patches while stimulating elastin and collagen production—thereby diminishing the signs of aging.



Common oils for fatty acids

Common Name	Structure	Jojoba	Rose Hip	Sesame	Arsan	Olive	Marula	Rosemary
	10:0							0.5
	12:0							0.5
Myristic	14:0			0.2				
Palmitic	16:0	0.3	3.8	9.2	14	15	12	40
Palmitoleic	16:1	0.3		0.2				1.8
	17:0							0.2
Stearic	18:0	0.2	12	6.2	7	3.5	5	6.2
Oleic	18:1	9.3	21	41	45	65	75	50
Linoleic	18:2		51.2	42	34	15	5	0.2
Linolenic	18:3		12	0.33		1.5	3	
Arachidonic	20:0			0.67				0.4
Gadoleic	20:1	76.7		0.2				
Behemic	22:0			0.2				0.2
	22:1	12.1						
	24:0	0.1						
	24:1	1						

Oils act as **hydratons and protectants**. The most common are MUFAs, monounsaturated fatty acids.

Often the extracted oils contain fat soluble vitamins like Vit A, Vit D and Vit E.

** Values are % averages as specific amounts can vary depending upon source of oil, extraction process and extent of measurements.*

Contact & References

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