An in-depth review on the biological and chemical aspects of hyperpigmentation and contemporary strategies for achieving even skin tone
Melanin

- Pigment that provides color to skin, hair and eyes

- Two different types:
  - Eumelanin: Brownish black pigment
  - Pheomelanin: Reddish yellow pigment

- Determines race, sex and phenotypic appearance

Hyperpigmentation

- Darkening of an area of skin caused by increased melanin.

- Causes:
  - Sun damage
  - Inflammation
  - Poisoning/intoxication
  - Fungal infections
  - Hormonal changes
The variation in skin color

- Occurs at the functional level of the epidermal melanin unit

**Factors involved:**

- Density of melanocyte
- Number, size and dispersion of melanosomes transferred to epidermal keratinocytes
- Nature of the pigment
- Degradation rate
## Biological agents affecting pigmentation

<table>
<thead>
<tr>
<th>Mechanism</th>
<th>Biological effector</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tyrosinase, TRP1, TRP2 inhibition (mostly through Mitf downregulation)</td>
<td>TGF-β1, C2 Ceramides, Vitamin E, Calpain Inhibitors, Lysophosphatidic acid, Sphingosylphorylcholine</td>
</tr>
<tr>
<td>Increased tyrosinase ubiquitination</td>
<td>Phospholipase D2, Fatty Acids</td>
</tr>
<tr>
<td>Inhibition of tyrosinase maturation</td>
<td>Glycosphingolipids</td>
</tr>
<tr>
<td>Decrease of MC1R activity</td>
<td>Agouti protein</td>
</tr>
<tr>
<td>Interference with melanosome maturation &amp; melanosome transfer</td>
<td>TGF-β1, Serine protease inhibitors, Lectins, neoglycoproteins</td>
</tr>
</tbody>
</table>
Tyrosinase and other melanogenic enzymes

Microphthalmia Associated transcription factor (Mitf)

- The most interesting point for this approach

- Transcription factor
  a) Regulates melanocyte proliferation & melanogenesis
  b) Major regulator of tyrosinase related enzymes (TRPs) and melanosome structural proteins (pMel17)
Tyrosinase and other melanogenic enzymes

TGF-β1

- Transforming growth factor
  - Plays inhibitory role in pigment formation
  - Interferes with tyrosinase synthesis and intracellular stability of protein itself
  - Down-regulates Mitf

Unlike other growth factors, it induces a delay in extracellular signal regulated kinase (ERK) activation

Lysophosphatidic acid and C2 Ceramides

- Capable of blocking Mitf expression
  - Mediated by an initial effect on AKT/PKB and ERK
Fatty Acids

- Unsaturated linoleic acid decreases tyrosinase activity

- Saturated palmitic or stearic acids increase it

- Linolenic, linoleic and oleic acids
  - Shown to produce a bleaching effect on guinea pig skin stimulated with UV light
  - Number of melanosomes or levels or tyrosinase mRNA intacts
  - Melanin inhibition is produced by a decrease in amount of active tyrosinase inside melanocyte
  - Stimulation of tyrosinase ubiquitination and degradation by the proteosome
α-Tocopherols

- Shown to provide hypopigmenting activity
  - Not through direct inhibition on tyrosinase activity
  - Block dopaquinone and subsequent chemical oxidations in the polymerization pathway leading to the pigment
Tyrosinase and other melanogenic enzymes

Vitamin E

- Antioxidant properties
- Inhibits lipid peroxidation
- Enhances glutathione synthesis
  - Control apparent hyperpigmentation
  - Derivates dopaquinone to pheomelanin
  - Less eumelanin accumulation
  - Decrease in apparent pigmentation (lighter color of pheomelanin)
Tyrosinase and other melanogenic enzymes

New Approach

Stimulation of DKK1 and calpain inhibitors

1. Protein product of the gene dickopf1 (DKK1)
   - Negative regulator of Wnt signaling pathway
     - Decreases Mitf
     - Reduced melanocyte growth and pigment production

2. Calpain inhibitors
   - Significant decrease in tyrosinase and mRNA levels in B16 cells
Decrease in MC1R activity

Hypopigmentation can result from mutations in Melanocortin Receptor 1 (MC1R) gene, its expression and functionality of its products.

**Agouti signal protein (ASIP)**
- Regulates mammalian pigmentation
  - Antagonizes the binding of αMSH to MC1R
- Effects:
  1. A direct competition at the binding site
  2. Down regulation of the receptor signaling

Peptides that work as analogues to the ASIP
  - Isolated from bacteria strains
Interference with melanosome maturation and transfer

**Centaureidine**
- A flavonoid glucoside isolated from yarrow
  - Reduces dendrites growth and transfer of melanosomes to keratinocytes
  - Diminishes the amount of tyrosinase

**Protease-activated receptor 2 (PAR2)**
- Inhibition of serine proteases
  - Impaired activation of PAR-2 on the keratinocyte
    - Accumulation of melanosomes with the melanocyte
    - Blocks the transfer among cells – pigment dispersion to keratinocytes
Interference with melanososome maturation and transfer

**Lectins and Neoglycoproteins**

- Glycosilated residues on melanocyte and keratinocyte membranes
  - Essential in receptor-mediated endocytosis
  - Facilitate melanosome transfer

Lectins and Neoglycoproteins are capable of inhibiting this transfer
Chemical hypopigmenting agents – Simple phenols

**Arbutin**

- A natural β-glycoside of hydroquinone
  - Inhibits tyrosinase and melanosome maturation
  - Acts on DHICA polymerase activity

- Its mild effect is due to the controlled release of hydroquinone by the in-vivo hydrolysis of the glycosidic bond

- Good photostability

- Decomposes four-times more at basic pH than at acidic pH

α-glucosides of arbutin can be synthesized to increase efficiency
- Easier hydrolyzed to release hydroquinone due to higher availability of α-glycosidases
Chemical hypopigmenting agents – Simple phenols

Deoxyarbutin

- Synthesized by removing every hydroxyl group of arbutin
- Excellent tyrosinase inhibitor (more sustained effect than hydroquinone)
  - Attributed to chemical structure
    - Deoxysugars are characterized by a significant increase of skin penetration ability and binding affinity for tyrosinase
- Lacks skin irritation associated with hydroquinone application
- Reversible skin lightening effect
  - Absence of permanent destruction of melanocytes
Hydroxystilbene derivatives

- High affinity to tyrosinase
  - Good tyrosinase-activity inhibitors

- Resveratrol and other isomers
  - Oxyresveratrol and gnetol are more efficient tyrosinase inhibitors than resveratrol

- Capable of reducing Mitf and tyrosinase promoter activation in B16 cells

- Reversible inhibition of the enzyme
  - Thus in-vivo treatments should maintain high intracellular levels of the hydroxylated stilbene inside melanocytes

Morus alba extract

- Contains 2-oxyresveratrol
- Inhibits tyrosinase activity
- No irritation
- No toxicity
Chemical hypopigmenting agents

Ascorbic acid

- Antioxidant
  - Capacity to reduce back o-dopaquinone to dopa
  - Thus avoiding melanin formation

- Adverse effect: Can induce large increase of free radicals with traces of metal ions by the Fenton reaction

- Alternative - Ascorbate esters
  - Improved stability
  - Improved skin absorption
  - Superior hypopigmenting effect

![Ascorbic Acid](image)
Lipophilic antioxidant compounds

Lipoic acid and dihydrolipoic acid

- Block expression of Mitf
- Inhibit activation of NF-κB transcription factor
- Antioxidant properties
- Dopaquinone trapping
- Modulation of melanogenic enzyme expression

Main group of agents used due to:
- Important involvement of quinones and oxidative reactions in the polymerization of melanogenic intermediates to the final melanin product

Main roles:
- Inhibit chemical reactions leading to melanin formation
- Change type of melanin formed
- Interfere with the distribution of pigment and melanosome transfer
Stimulation of desquamation

Retinoids
- Retinoic acid
- Tretinoin
- B-Carotene

Chemical peels
- Trichloroacetic acid (TCA)
- α-hydroxy acids
  - Lactic acid
  - Glycolic acid
  - Salicylic acid

Retinoids
- Accelerates loss of melanin by the peeling of the *stratum corneum* cells

Chemical peels
- Produce a derangement of the melanosomal structure
- Produce arrest of the melanosome maturation at stages I and II

As a result:
- Induce dispersion of keratinocyte pigment granules
- Interfere with pigment transfer
- Accelerate epidermal turnover
- Reduce cohesiveness of corneocytes
- Induce desquamation
Effective skin lighteners

Successful treatments need combination of two or more agents acting on different mechanisms
- Synergistic hypopigmenting effects

Aspects to consider:
1. Synergistic effects of combined therapies
2. Stability of whitening formulation
3. Toxicity and skin penetration
4. Definition of markers and targets for evaluating depigmenting properties in vitro and in vivo.
Than you!