“Aging Skin”
Current and Future Therapeutic Strategies for:

‘Ageless Beauty’

by Linda D. Rhein, Ph.D.
Goals for Today

• My ultimate goal is to inform you of the current fundamental knowledge of aging skin and photodamage

• This will be done in memory of Dr. Albert Kligman, MD known as the ‘pope of dermatology’

• I will also direct you to future strategies to watch in the upcoming years possibly to develop some leads for your research.
Problems of aging skin

• Dryness- Ichthyosis, Winter Xerosis
• Photodamage
  ➢ Fine Lines and Wrinkles
  ➢ Age spots- Hyperpigmentation, Lentigenes
  ➢ Skin Precancers/Cancer-
    ✧ Actinic Keratoses,
    ✧ Squamous Cell and Basal Cell Carcinoma and Melanoma – the deadliest form of skin cancer
Age Spots – Solar Lentigo
Problems of Aging Skin
(a) Type I skin with carcinomas
(b) Type III skin with rhytides
Melanoma

Early melanoma

- **Asymmetry**: Symmetrical vs. Asymmetrical
- **Border**: Even edges vs. Uneven edges
- **Color**: One shade vs. Two or more shades
- **Diameter**: Smaller than 6 mm vs. Larger than 6 mm
Winter Xerosis – ‘dry skin’

Figure 7. Scaling—Grade 10.
Some wrinkles and sags are natural!
Damaging Effects of UV-exposure

- Inflammation - sunburn
- Oxidative damage – ROS, RCS, & RNS
- Cross linking of collagen and elastin
- DNA strand nick or break
- Immunosuppression
- Defective epidermal proliferation and apoptosis
- Photo-carcinogenesis
Aging Effect of Oxidative Stress on the Body

Free Radical Oxidative Stress

- Heart
- Skin
- Kidney
- Joints
- Lung
- Brain
- Immune System
- Blood Vessels
- Multi-organ

Chronic Inflammations, Autoimmune disorders, Lupus, IBD, MS, Cancer

Alzheimer, Parkinson, OCD, ADHD, Autism, Migraine, Stroke, Trauma, Cancer

Restenosis, Atherosclerosis, Endothelial Dysfunction, Hypertension

Diabetes, Ageing, Chronic Fatigue

Macular degeneration, Retinal degeneration, Cataracts

Skin Ageing, Sunburn, Psoriasis, Dermatitis, Melanoma

CHD, Cardiac Fibrosis, Hypertension, Ischemia, Myocardial infarction

Chronic Kidney disease, Renal Graft, Nephritis

Rheumatoid, Osteo-Arthritis, Psoriasis

Asthma, COPD, Allergies, ARDS, Cancer
Environmental Stressors

Free Radicals
- DNA damage
- Lipid peroxidation
- Protein damage

ROS, RNS, RCS
Oxidative Chemical Stressors

- **ROS** - reactive oxygen species are highly reactive due to the presence of unpaired valence shell electrons - \( \text{HO}_2\cdot \text{ or } \cdot\text{O}_2^- \)
- **RNS** – reactive nitrogen species - \( \text{NO}\cdot \)
- **RCS** – reactive carbonyl species are small molecular weight carbonyls that are activated by \( \alpha,\beta\)-unsaturation (such as 4-hydroxynonenal and acrolein), \( \alpha\)-oxo-substitution (such as glyoxal from glycation) and oxidation of lipids (malondialdehyde) - glyoxal
Collagen fibers in dermis – from buttocks skin (b) and sun exposed facial skin (d) of elderly person
Solar Elastosis
Figure 3. Immunohistochemical visualization of type I collagen in facial vs. abdominal skin in biopsies obtained from a 6-year-old female (1st decade) and a 77-year-old male (8th decade). Type I collagen was detected by immunoperoxidase staining with a polyclonal anti-human antibody for type I collagen. (Original magnifications, ×200).
Key Mechanisms in Wrinkle Generation

- Sun
  - AP-1
  - MMPs
  - Collagen Breakdown
  - ROS Generation
  - TGF-b
  - Procollagen Production
  - Imperfect Repair
    - Fibrogenesis
    - Invisible Solar Scar
    - Repeated UV Injury
    - Visible Solar Scar = "Wrinkle of Photoaging"

Aging Skin Treatment Strategies

• Primary strategy is prevention (Sunscreens)
• Secondary strategy is to attenuate or ‘postpone’ the signs/symptoms of photoaging
• Tertiary strategy is to treat existing moderate to severe symptomatic disease
<table>
<thead>
<tr>
<th>Primary</th>
<th>Secondary</th>
<th>Tertiary</th>
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<tbody>
<tr>
<td>Photoprotection</td>
<td>Retinoic Acid</td>
<td>Chemical peels</td>
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<tr>
<td></td>
<td>Antioxidants</td>
<td>Microdermabrasion/Microcoblature</td>
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<tr>
<td></td>
<td>Estrogens</td>
<td>Laser</td>
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<td></td>
<td>Growth factors/cytokines</td>
<td>Botulinum toxins</td>
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Fig 3. Photoaging treatments categorized by prevention strategy. Primary prevention reduces risk factors before disease occurs. Secondary prevention postpones or attenuates the condition. Tertiary prevention treats an existing symptomatic disease process to ameliorate its affects or delay its progress.\textsuperscript{128}
UV Radiation – Depth of Penetration
Broad Spectrum Sunscreens are Best Prevention Strategy
(or just staying out of the sun!!)

- UVB 290 TO 320 nm – several approved sunscreens filters
- UVA II (short) 320 TO 340 nm - numerous filters globally only mexoryl in US
- UVA I (long) 340 TO 390 nm – Avobenzone

Latest technology is TiO$_2$ and ZnO nanoparticles – gives high SPF and high PFA
Latest Technology in Sunscreens – Coated Titanium Dioxide and Zinc Oxide Nanotubes
Think of building a Sunscreen!
Kligman’s Discovery!

Albert Kligman, MD – known as the ‘Pope of Dermatology’, was also a psychiatrist and said -

“Women who have the most beautiful skin in the world are women who reside in mental institutions who never smile and never go out in the sun”!

What a price to pay for ageless beauty!!!

He felt there is a better way!
Retinoic Acid - ‘RENOVA®’

- First FDA approved anti-aging drug; Available by Rx only [patented by Dr. Kligman]
- Indicated as “an adjunctive agent for use in the mitigation of fine wrinkles, mottled hyperpigmentation, and tactile roughness of facial skin”
- Also indicated as a treatment for acne
- Is a nuclear hormone receptor agonist
- Side effects of dryness, redness and irritation [teratogenicity for other retinoids]
Retinoic Acid - ‘RENOVA®’
-about 30% reduction in wrinkle depth

Before

After 48 weeks of 0.05% atRA
| RAR | RXR | VDR | TR | RXR | FXR | GR | AR | PPAR |

[Receptor codes: RAR=retinoic acid, VDR=vitamin D, TR=thyroxine, FXR=farnesol, AR=androgen, GR=glucocorticoid, PPAR=peroxisome proliferator activated receptor, PXR=pregnane]
Mechanisms of UV Damage

- Epidermal transaminase
- Fillagrin
- SMADs
Development of Future Treatment Strategies

‘View Wrinkles as Chronic Wounds’
Flow Diagram for Wound Healing

INJURY

COAGULATION PLATELETS

INFLAMMATION

DEBRIDEMENT, RESISTANCE TO INFECTION

MACROPHAGES GRANULOCYTES

FIBROBLASTS

NEOVASCULAR GROWTH

CONTRACTION

PROTEOGLYCANS SYNTHESIS

EPITHELIUM

COLLAGEN LYYSIS

(COLLAGEN SYNTHESIS)

(REMODELING)

HEALED WOUND

Matrix degradation

Fibrosis
- Temporary ECM with fibronectin & neutrophils degrade damaged tissue

- Fibroblasts convert to myofibroblasts AND as a result - Neocollagen is produced

- Matrix remodeling
Degradation Enzymes
Matrix Metalloproteinases

- MMP-1 (interstitial collagenase) cleaves collagen type I
- MMP-2 degrades elastin as well as basement membrane compounds including collagen type IV and type VII
- MMP-3 has the broadest substrate specificity for proteins such as collagen type IV, proteoglycans, fibronectin, and laminin …..

**Past strategy has been to inhibit these enzymes**
Fibrosis

• In normal skin, the rate of ECM production equals rate of its degradation, resulting in no net accumulation of matrix.

• Downregulation of MMPs by either a reduction in gene expression or an increase in MMP tissue inhibitors (TIMPs) favors the accumulation of abnormal matrix.

• Progressive fibrosis is initiated by TGF-βs that also stimulates increases in TIMP-1 which leads to a net decrease in MMP activity and therefore more unopposed matrix accumulation.
TGF-β’s

• TGF-β is the master switch regulating fibrosis and exists in 3 isoforms TGF-β1, -β2 and -β3.
• TGF-βs are secreted by platelets, fibroblasts and macrophages within the injury. Controlled by SMADs
• TGF-β1 is the lead isoform stimulating fibrosis.
• However persistent presence of TGF-β1 potentiates excessive fibrosis ultimately resulting in scarring of skin or internal organs. Termed – ‘ADHESIONS’
• Scarring of internal organs (e.g., post surgical scars, liver cirrhosis and lung fibrosis) results in a loss of organ function and ultimately death may occur.
• **Scarring due to chronic damage from persistent UV radiation exposure of skin presumably results in fine lines and wrinkles.**
Maturation
# TGF-β Isoform Ratios in Embryonic and Adult Wounds

<table>
<thead>
<tr>
<th>TGF-β isoform</th>
<th>Embryonic scar-free healing</th>
<th>Adult healing with a scar</th>
</tr>
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<tbody>
<tr>
<td>TGF-β1</td>
<td>Low/absent</td>
<td>High</td>
</tr>
<tr>
<td>TGF-β2</td>
<td>Low/absent</td>
<td>High</td>
</tr>
<tr>
<td>TGF-β3</td>
<td>high</td>
<td>Low</td>
</tr>
</tbody>
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*Table 2.2 Summary of TGF-β isoform differences in scar-free embryonic wound healing and scar-forming adult wound healing.*
Future Treatment Strategies
- Balance Matrix Degradation and Synthesis -

Stop or slow down matrix degradation to prevent thinning
- Block MMP catalytic activity - hydroxamates, carboxylates, 4-hydroxypanduratin A
- Induce expression of natural inhibitors of MMPs - called TIMPs, that complex with MMP
- Inhibitors of NFKB action - parthenolide and magnolol

Stop or slow down fibrosis to prevent scar formation
- Antifibrotics that block fibrosis – by inhibiting expression of TGF-β1 and/or increase expression of TGF-β3 - retinoic acid, Relaxin, PPARs α or γ, PAR (protease activated receptor), vitamin D, angiotensin converting enzyme inhibitors

Normalize epidermal proliferation to smooth surface – retinoic acid, PPARs maybe Vitamin D
Kligmanson Pharmacologic Mouse Model for Studying Treatments for Photodamage
Ovariectomy and Cutaneous Aging
‘Wrinkles’ – 3-13 weeks irradiation,
Genji Imokawa, 2004
Ovariectomy and Cutaneous Aging

‘Sagging’
17 β-estradiol

- Upregulates procollagen I and
- Inhibits MMP-1 by stimulating the TGF-β/SMAD signaling pathway

Effective in clinical trials as a topical for treatment of photoaged skin

Unfortunately obsolete because no patent protection
Hot Research!

- Zhu and Li, A combined candidate therapy for the scar-free repair of cleft lip based on inhibitors of TGF-β. Medical Hypotheses, 2010
- Zhang et al., Inhibition of proliferation and TGF-β 3 protein expression by peroxisome proliferators-activated receptor ligands in human uterine leiomyoma cells. Chin Med J 2008;121(2):166-171
Recipe for Ageless Beauty

♥ Use broad spectrum sunscreen daily SPF 15 is ok
SPF 20 or higher for beach all with PFA > 10
♥ Renova® (all trans retinoic acid) every other day -
contribution of my friend and colleague Dr. Albert
Kligman to women for “ageless beauty”
♥ Take 5000 units vitamin D and 1200 mg calcium
daily and antioxidant supplements; consider HRT
♥ Don’t squint or frown, no big smiles, stay out of the
sun!!!
More information can be found in my book on this topic.

**Aging Skin: Current and Future Therapeutic Strategies**

Linda Rhein, PhD

Apart from cosmetic surgery, there are many non-invasive avenues to obtaining beautiful, healthy skin. *Aging Skin: Current and Future Therapeutic Strategies* covers the basics such as physiology and metabolism of the skin, nutrition and vitamins, as well as cutting-edge topics like retinoids, melanogenesis mechanisms, matrix metalloproteinases and antioxidants.