Efficacy evaluation on whitening cosmetics in Japan

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Abstract

Whitening agents are eagerly demanded especially by oriental women who often suffers from the pigmentary disorders such as melasma and solar lentigines.
As these pigmentary disorders are exacerbated by ultraviolet (UV), the whitening agents could exert its effect not only by inhibiting melanin synthesis but also by inhibiting UV activated signals. Eumelanin protects UV-induced DNA damages so that the chemicals which could reduce UV-induced DNA damages might be the ideal lightening agents. The effect of newly synthesized antioxidants, a-tocopheryl ferulate, on protective effect for UV-induced DNA damages as well as inhibiting melanin synthesis are briefly shown.
For clinical evaluation, our results of the efficacy of lightening agents on treating pigment macules in combination with chemical peeling are shown.
Furthermore, newly developed facial image analyzers to quantitatively evaluate the improvement of pigment macules are introduced.

EDUCATION

1978 Kobe University School of Medicine, Kobe, Japan
1984 Graduate Course (Ph.D.) in Medical Sciences, Kobe University School of Medicine

PROFESSIONAL AND RESEARCH EXPERIENCE

1988 Assistant Professor in Department of Dermatology, Kobe University School of Medicine
1988 Fellow in Department of Dermatology, Osaka Koseinenkin Hospital
1989 Assistant Professor in Department of Dermatology, Kobe University School of Medicine
1989 Postdoctoral Fellow in Department of Dermatology, Yale University School of Medicine, USA
1996 Mombusho Zaigai researcher, Department of Dermatology, Cincinnati University School of Medicine, USA
1996 Associate Professor in Department of Dermatology, Kobe University School of Medicine
2001 Division of Dermatology, Department of Clinical and Molecular Medicine, Kobe University Graduate School of Medicine

MEMBERSHIP

Japanese Society for Pigment Cell Research (Director)
Japanese Society for Investigative Dermatology (Councilor)
Japanese Society for Aesthetic Dermatology(Councilor)
Japanese Society for Photoaging Research (Secretariat General)
Kobe Society for Collagen Disease Research (Councilor)
Hyogo Research Association for Bone Metabolic Disorders (Councilor)
Medical Society Membership:
Society for Investigative Dermatology
American Academy of Dermatology
American Society for Laser Medicine and Surgery
Japanese Society for Dermatology
Japanese Society for Cancer Research
Japanese Society for Photomedicine and Photobiology
Japanese Society for Allergology
Japanese Society for Psoriasis
Japanese Society for Dermatoallergology
Japanese Cosmetic Science Society

국문요약

미백제는 기미와 태양광선노출에 의한 색소침착반과 같은 색소불균형이 주로 나타나는 동양 여성들에게 특히 관심이 많다. 이러한 색소침착의 불균형은 UV에 의해 더 심해지기 때문에 미백제는 멜라닌 합성을 억제시키거나 UV에 의해 활성화된 signal을 억제함으로써 효과가 나타난다.

Eumelanin은 UV에 의해 유도된 DNA 손상을 보호하므로 UV에 의해 유도된 DNA손상을 감소시키는 물질이 이상적인 미백제가 된다.

새롭게 합성된 항산화제인 α-tocophenyl ferulate의 효과는 UV에 의해 손상된 DNA damage에 의한 보호 효과와 멜라닌 합성을 억제시킴으로서 나타난다.

Chemical peeling과 복합적으로 색소 반점을 치료하는 lightning agent의 효과에 대한 결과를 보고하였으며 색소 반점의 개선을 정량적으로 평가하는 새롭게 개발된 facial image analyzer를 소개할 것이다.

Evaluation methods and mechanisms of skin lightening agents

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Evaluation methods of inhibitory effect of skin lightening agents -in vitro test-

1. Inhibitory effect of tyrosinase activity in test tube
2. Inhibition of melanin synthesis in cultured pigment cells (mouse vs human, melanoma vs normal MC)
3. Inhibition of enzyme activities (tyrosinase, TRP-2, TRP-1)
4. Inhibition of protein and gene expression (tyrosinase, TR P-1, 2, pmel 70, etc)
5. Lightening effect of skin color in KC-MC co-culture syste m or 3-D culture (melanin synthesis, paracrine stimulation, melanin transfer)

Evaluation methods of inhibitory effect of skin lightening agents-in vitro test-

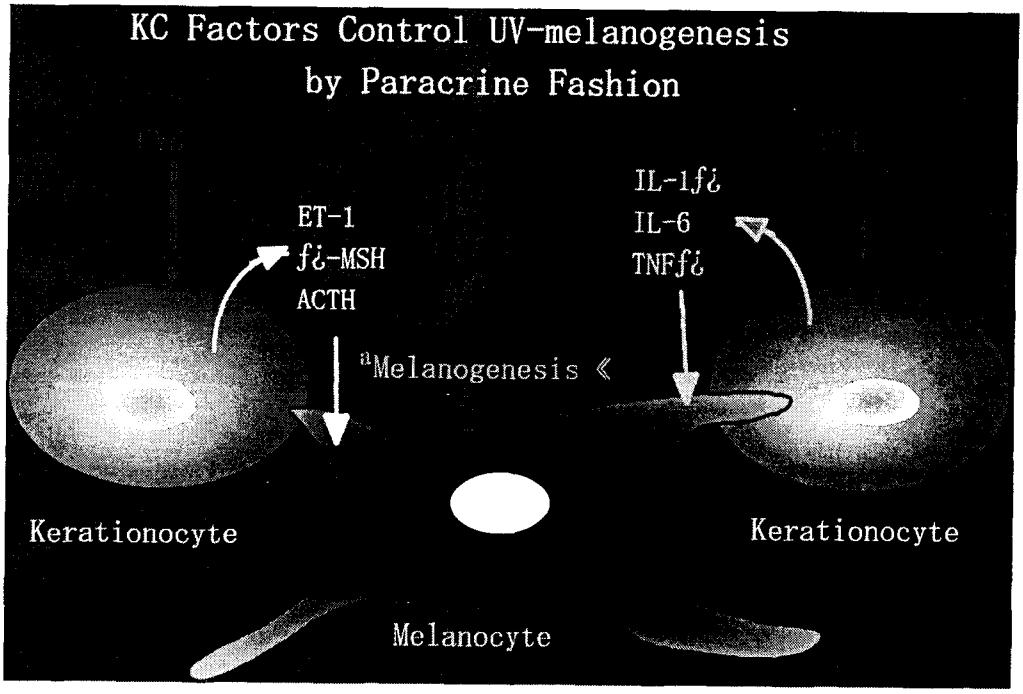
1. The regulation of paracrine factors
   - ä *gene expression in KC
   - ä *protein expression in KC
   - ä *secretion/release from KC
   - ä *the role of oxidative stress/antioxidants in UV signaling
2. The regulation of melanin transfer
   - ä *melanosome specific proteins in MC
   - ä *intracellular trafficking signals in MC
   - ä *protease-activated receptor-2 in KC

The effect of KC-derived factors

KC-derived factors	Effect				
	growth	melanin synthesis	dendrites	migration	survival
bFGF	↑↑				
α-MSH	↑	↑			
ET-1	↑	↑	↑		
GM-CSF	↑	↑			
NGF			↑	↑	↑
PGE2	↑	↑			
LT	↑				
NO		↑			
TRX	↑	↑			
K-CM	↑↑	↑↑	↑↑		

Molecular mechanisms involved in melanocyte gr owth/survival and melanin synthesis

- Growth factors/cytokines
- bFGF, HGF, SCF, ET, MSH/ACTH, GMCSF,
- LT, PG, NGF
- IL1α, IL6, TNFα, GFβ
- Signal transduction
- cAMP, ERK, Raf, cGMP, PKC,
- PI3K, Akt(PKB), $p70^{S6}$ K, Rho
- MITF, TFE3, AP1, NFκB



The effect of oxidative stress/antioxidant system on melanogenesis

$O_2^{\cdot-}$:
Serves as a substrate of tyrosinase (Wood et al. Biochim Biophys Acta 1074:378-385, 1991, Valverde et al. Pigment Cell Res 9:77-84, 1996)
Utilized by TRP2 (Jimenez-Cervantes et al. J Biol Chem 269:17993-18000, 1994)
H_2O_2:
Elevates tyrosinase protein level (Karg et al. J Invest Dermatol 100:209S-213S, 1993)
Not a substrate for tyrosinase (Valverde et al. Pigment Cell Res 9:77-84, 1996)
Competitive inhibitor of tyrosinase (Wood et al. Biochim Biophys Acta 1074:378-385, 1991)
NO:
NO secreted from keratinocytes increases the amount of tyrosinase and TRP1 (Romero-Graillet et al. J Clin Invest 99:635-642, 1997)
Thiol donors
Cystine/GSH:
Extracellular L-cysteine depletion significantly increases tyrosinase activity and promotes eumelanogenesis (del Marmol et al. J Invest Dermatol 107:698-702, 1996)
GSH blocks maturation of tyrosinase (Imokawa et al. J Invest Dermatol 93:100-107, 1989)
GSH blocks exit of tyrosinase from endoplasmic reticulum (Halaban et al. Poc Natl Acad Sci 94:6210-6215, 1997)
Thioredoxin(TRX)
Reduced TRX inhibits tyrosinase 23-fold more than reduced GSH (Wood et al. Biochim Biophys Acta 1074:378-385, 1991)

ADF/TRX(thioredoxin)

Molecular characteristics

***adult T cell leukemia derived factor**

***Mw 13 kD (104AA)**

***possesses consensus sequence -Cys-Gly-Pro-Cys**

***induced by H_2O_2 and UV**

Biological effects

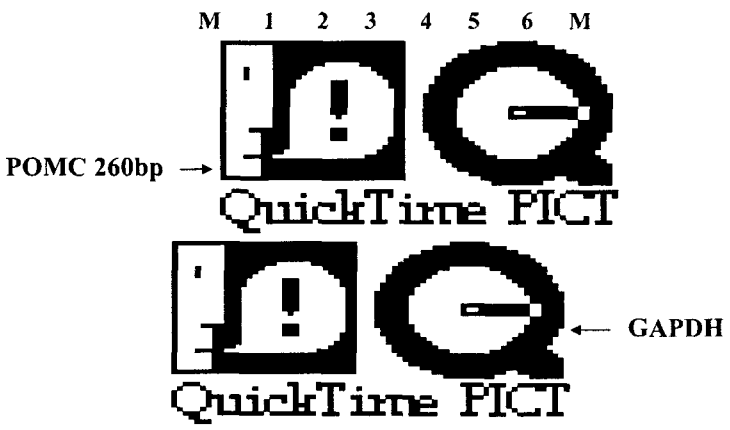
***regulate gene expression (modulate DNA binding of Jun/Fos and NFkB to target DNA)**

***scavenge active oxygen species (H_2O_2 and $\cdot OH$)**

***protect cells from TNF and Fas induced apoptosis**

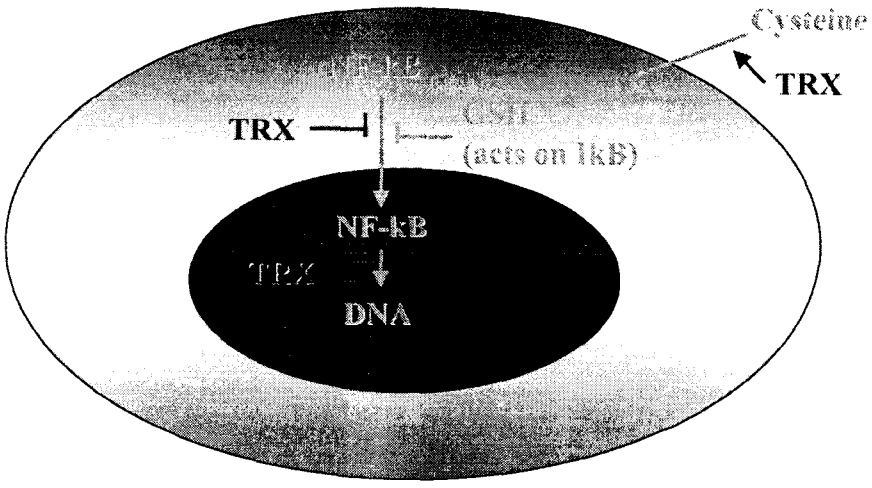
***modulate signal transduction (tyrosine phosphorylation, growth stimulation)**

Detection of POMC mRNA by semiquantitative RT-PCR



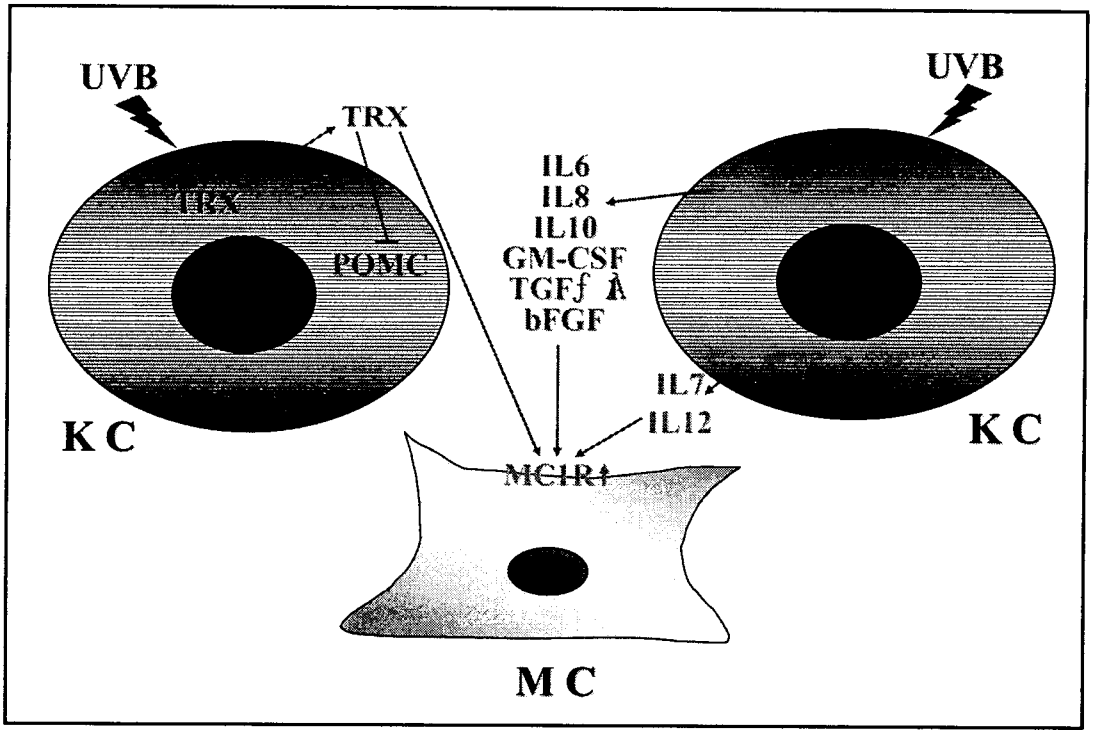
1. Control
2. UVB
3. TRX
4. TRX + UVB
5. NAC
6. NAC + UVB

The action of TRX on activation & suppression of NF-kB



Effect of UVB and Cytokines on
MSH Receptor Activity in Normal Human Melanocytes

Treatment	Cell no. (~10 6 per well)	MSH receptor activity Specific binding (cpm/10 6 cells)	% Activity
Control	0.36	213 }5	100 }2
UVB @ 5mJ/ E	0.32	497 }96	233 }45
@ @ 10mJ/ E	0.32	431 }128	202 }83
20mJ/ E	0.30	496 }137	232 }64
ET-1	0.30	316 }146	148 }68
IL-1f¿	0.34	426 }26	200 }12
IL-1fÀ	0.31	442 }129	207 }60
TNF-f¿	0.40	202 }5	95 }2
ADF 1fÊg/ml	0.33	172 }6	80 }3
5fÊg/ml	0.30	606 }63	284 }29
NAC	0.31	477 }164	223 }76



Molecules involved in melanosome maturation and transfer to keratinocytes

- Coated vesicles
  - Clathrin, adaptor protein, small G proteins (Rab)

  Transport of melanosomes
  - actin/myosin-5, Rab 27a

  Transfer to keratinocytes
  - protease-activated receptor-2 (PAR-2)

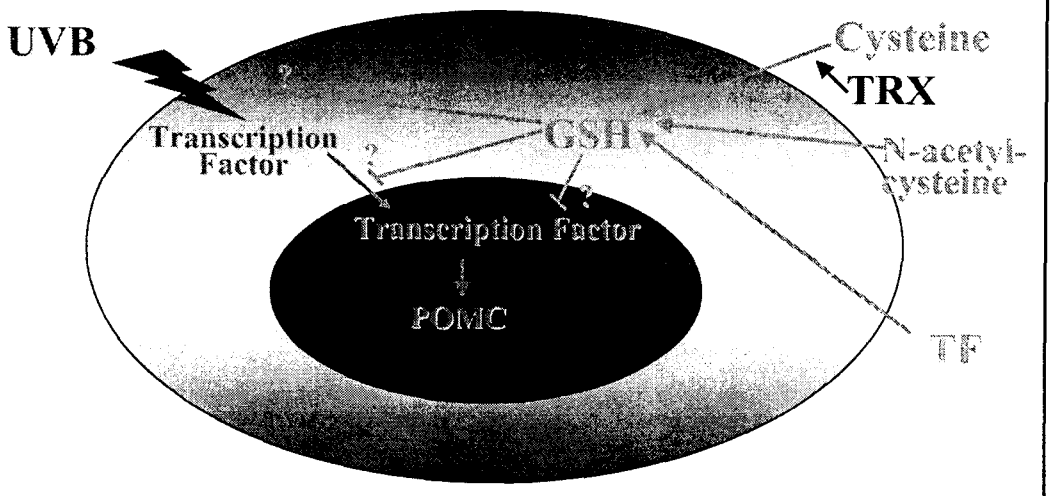
Evaluation methods of skin lightening agents-in vivo/clinical test-

1. Animal models (slightly pigmented hairless mice, guinea pigs, Yucatan miniature swine)
   - ä *lightening effect of UV-induced pigmentation
   - ä *protective effect of UV-induced pigmentation
2. Human
   - ä *healthy skin
   - ä *patients (melasma, solar lentigines, ephelides, etc)

Background- α-tocopherylferulate-

- α-tocopherylferulate inhibits melanin formation in human melanocytes
  - tyrosine hydroxylase activity
  - DHICA polymerase activity
  - TRP-2 activity
  - melanogenic protein amount
  - glycosylation of tyrosinase
  - direct inhibition of tyrosinase (-)

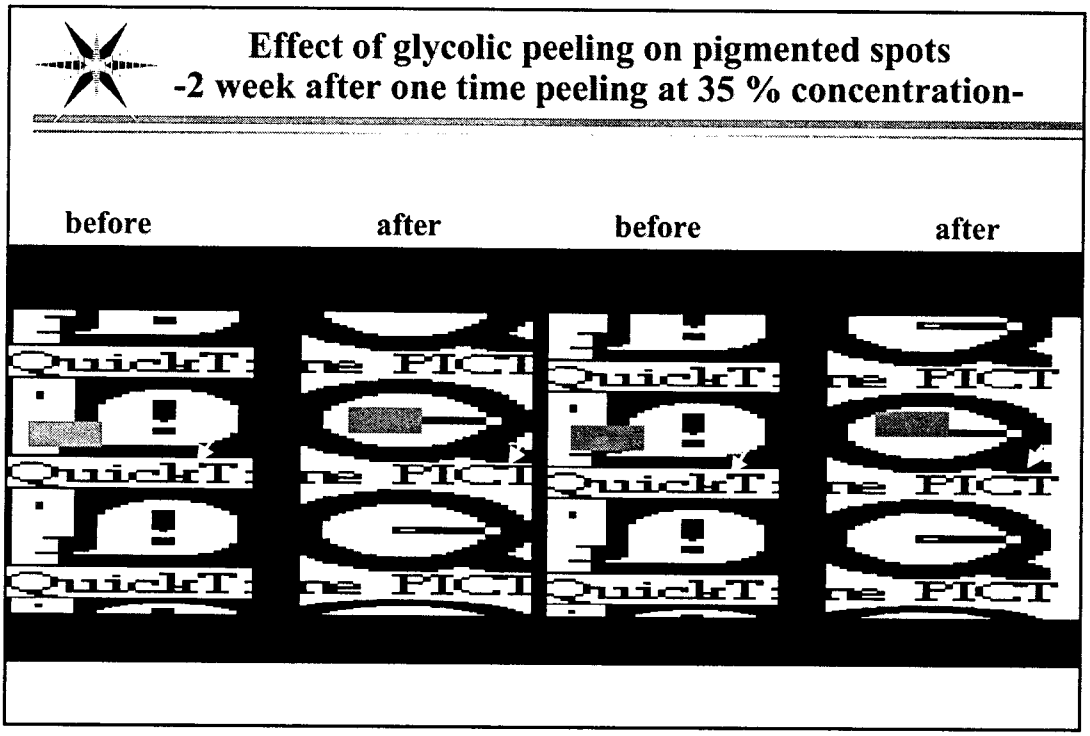
The Action of TRX/Cysteine/TF on POMC Expression



UV-induced DNA damage

- 1.Direct damage
  - cyclobutane pyrimidine dimers
  - (6-4)photoproducts
  - Dewer isomers
- 2.Indirect damage
  - 8-OHdG (8-hydroxydeoxyguanosine)
  - 8-OHdA
  - thimine glycol

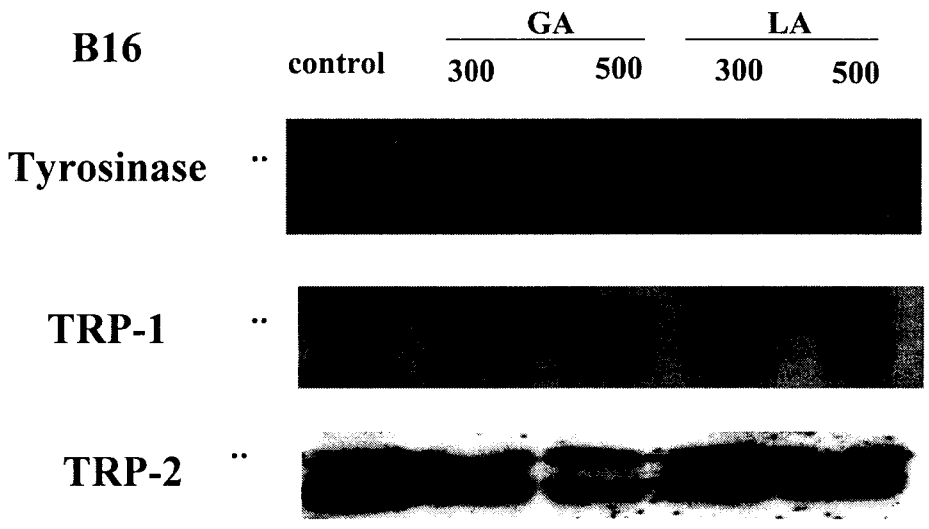
Effect of glycolic peeling on pigmented spots
-2 week after one time peeling at 35 % concentration-

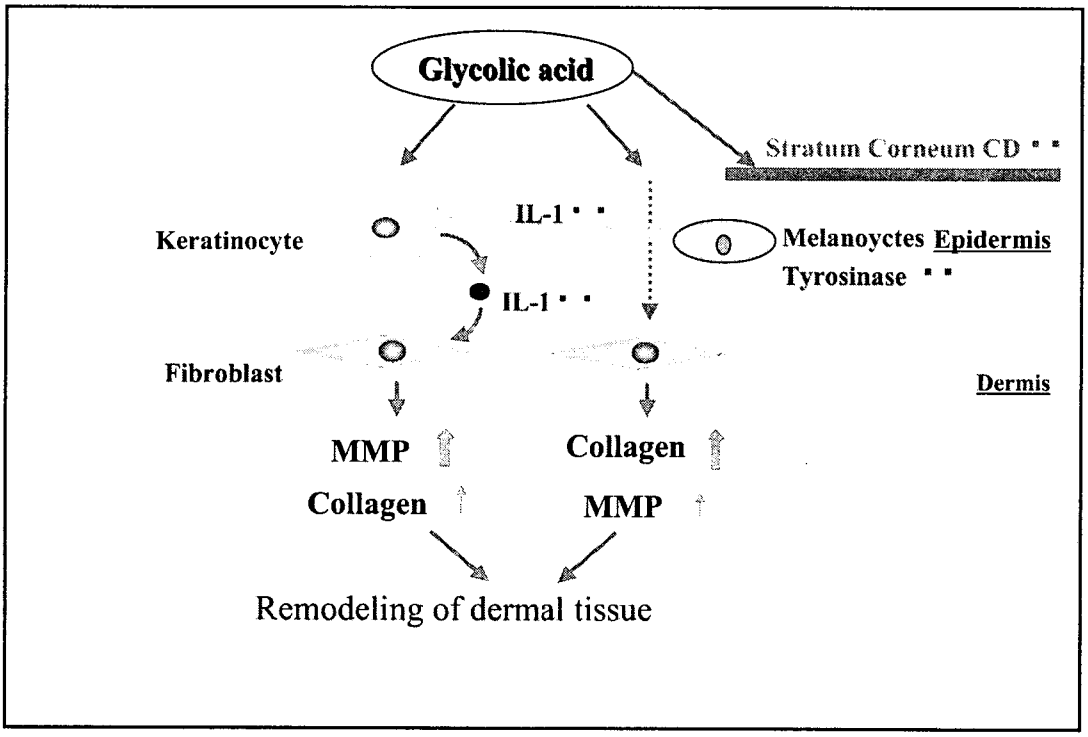


The improvement of pigmentary lesions by GA +/- HQ

	HQ(-) visit1-5	HQ(+) visit 6-9
Ephelides	@ ++ @@ ++++	
Melasma	@ ++ @@@@@ +++	
Nevus spilus	- @@@ ±	
Postinflammatory pigmentation	+	+++
Soloar lentigine		
under 30's	++	+++
40's	++	+++
50's	+	++
over 60's	-	+

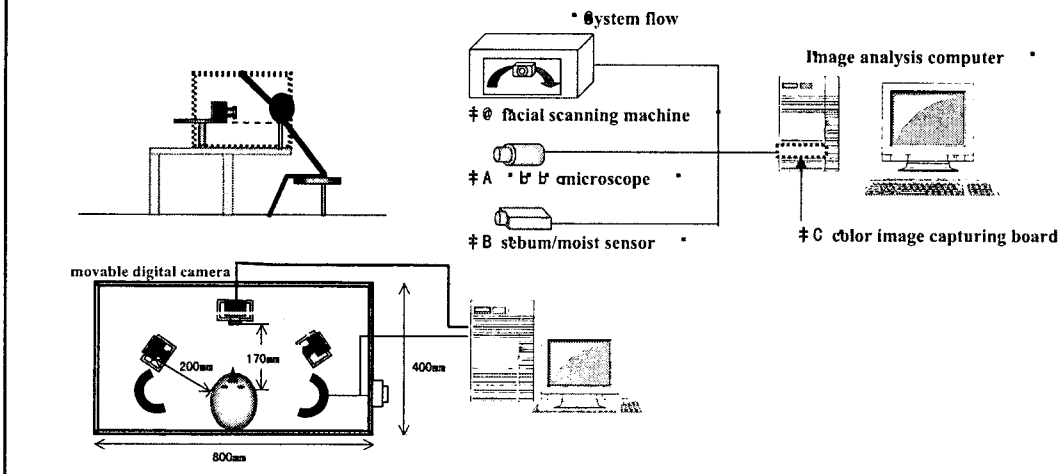
Western Blotting





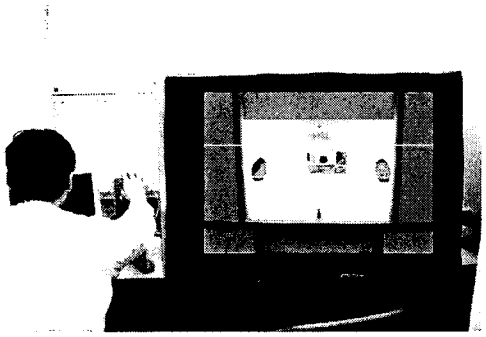
Facial Scanning System

Facial Scanning System is a digital photographic imaging system that takes digital images of the face and analyzes in a various skin surface conditions.
The system measures colors, wrinkles, textures, spots, acnes and pigmentation, then generates quantitative values through image analysis algorithm.

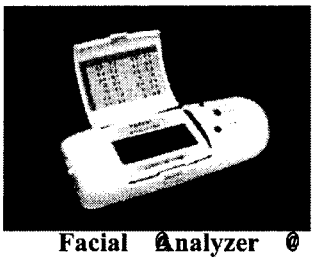


Outlook

Facial Scanner

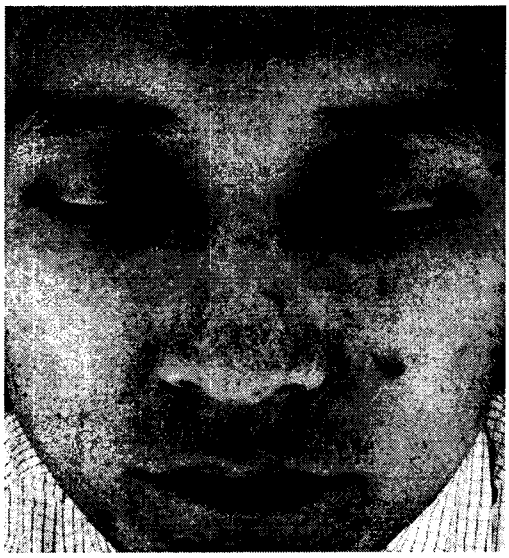


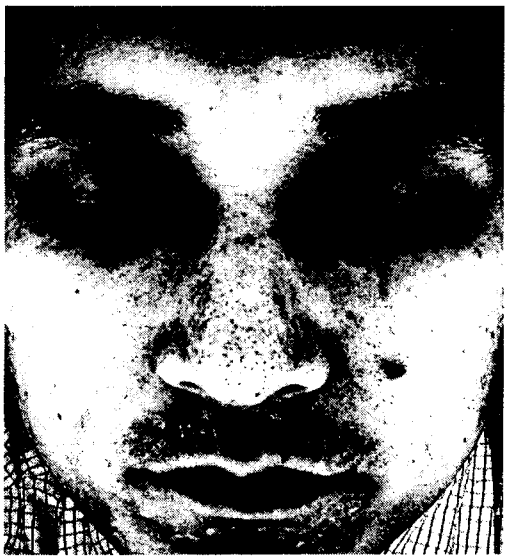
CCD Microscopic and Image analysis software



Facial Analyzer

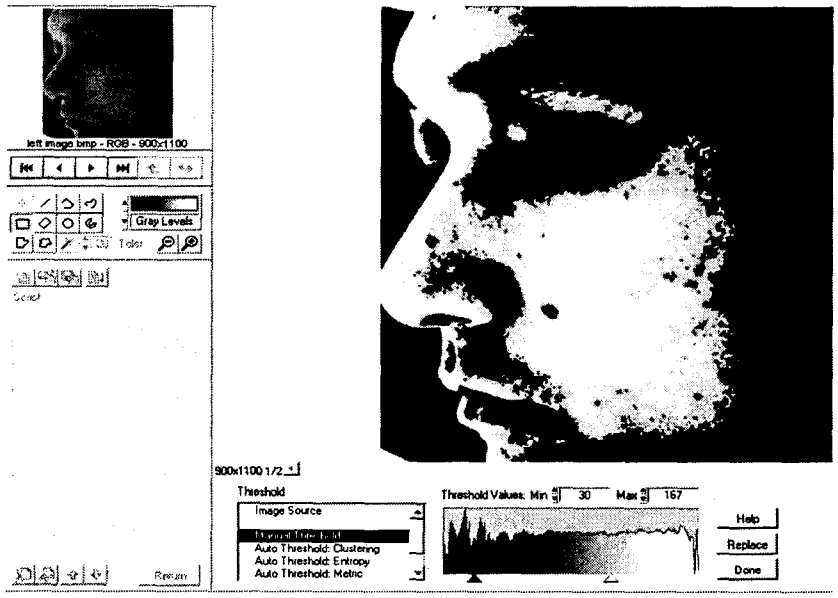
Clinical Image 1. (front view)



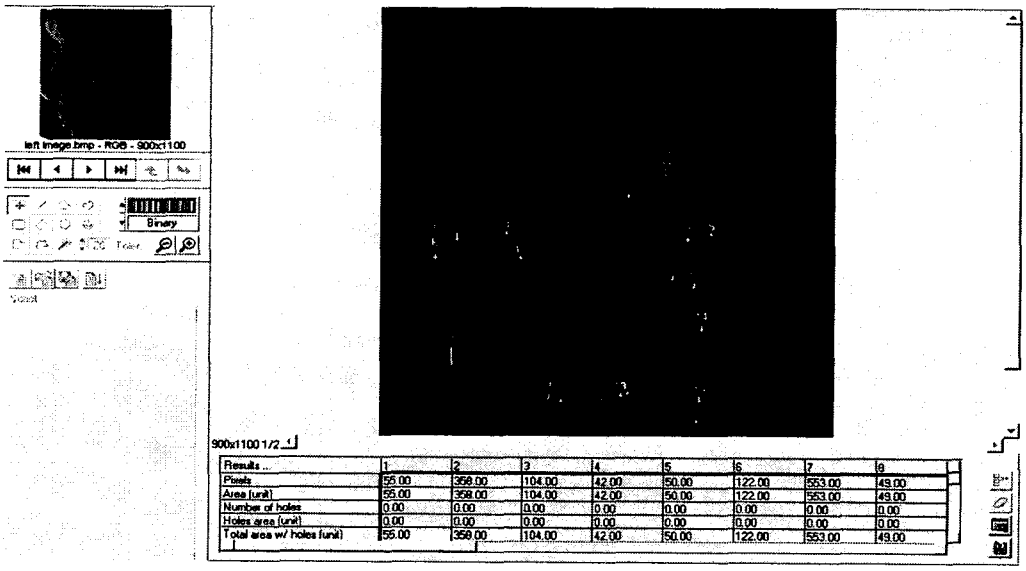


Clinical image 1. (side view)

Clinical image 1. (side view reddish area extract)



Clinical image 1. (side view reddish area quantification)



Whitening agents

- Topical
- arbutin
- kojic acid
- vitC derivatives
- oil-soluble licorice extr acts
- retinoid
- camomilla
- linoleic acid
- elagic acid
- 4-n-butylresorcinol
- AHA

Oral

- **vitC**
- **vitE**
- **tranexamic acid**
- **cysteine**
- **glutathione**