Safe-B3™ is extremely well tolerated

- Niacinamide always contains traces of unwanted nicotinic acid which can causes skin flushing with a dose-response relationship [1)]

RESULTS

Acute testing: In forearm testing, topical niacinamide did not induce a skin flushing response. In contrast, a wide range of other nicotinates did induce an often intense flushing response, with onset times as early as 2 minutes post application and duration as long as 12 hours (see table below).

CONCLUSIONS

- For niacinamide formulations it is important to use only high purity material (3)

DETAILED DESCRIPTION

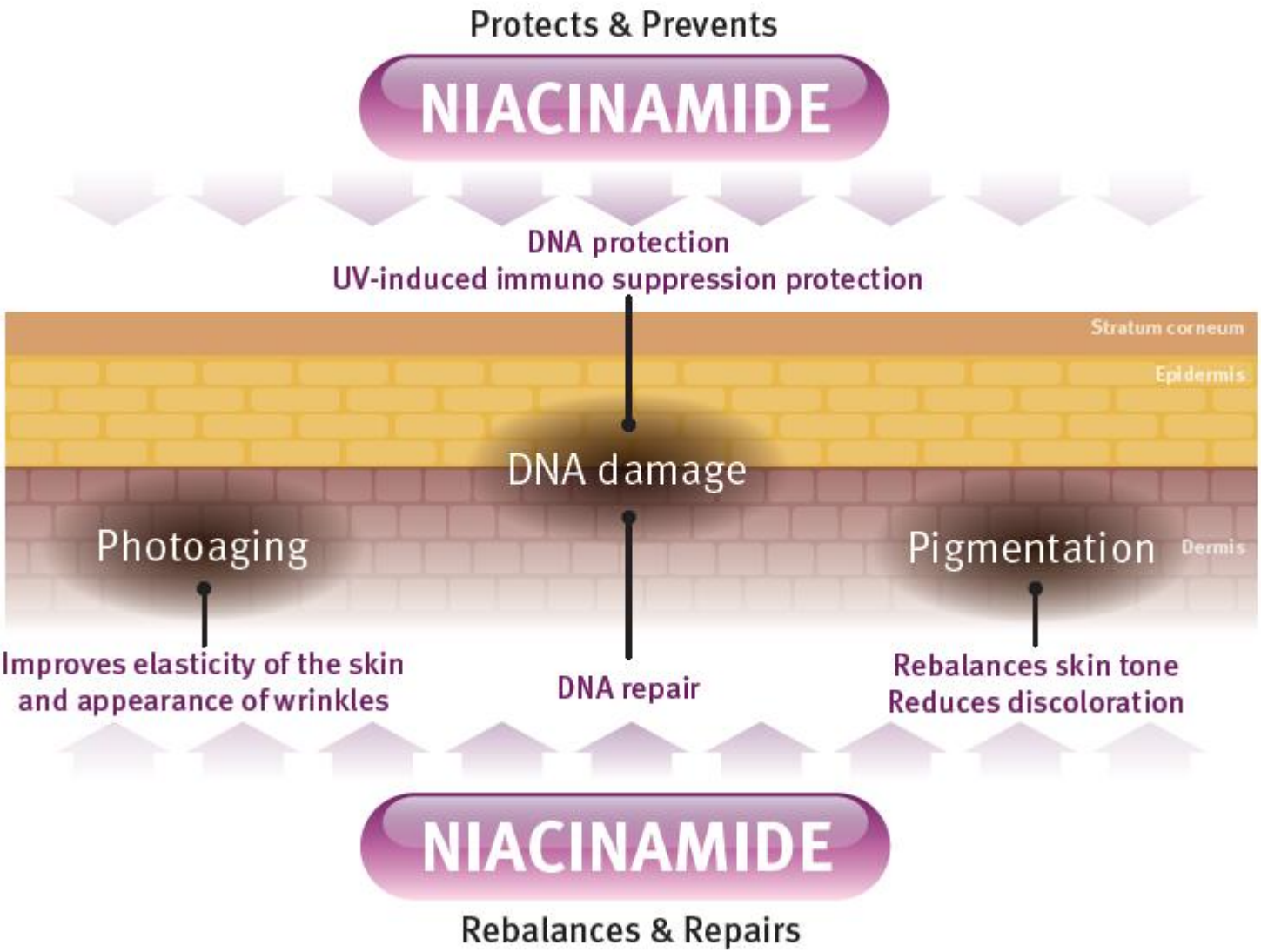
Applicant has found that skin flushing and reddening adds to the effect of reducing enlarged pores, minimizing fine lines, penetration of moisturizer ingredients, shrinking of pimples, removal of blackheads and other unwanted dirt or oxidents under the skin, tightening of the skin due to the flushing and topical circulation of the reddening. Applicant has found through experience and testing that even 0.06% of niacin (nicotinic acid) or its esters can cause reddening or flushing of the skin for people with sensitive skin and people who use glycolic acids or other peeling chemicals. For over-the-counter and mass marketing, it would be very difficult to analyze everyone's skin type to provide a cream or lotion which would produce a desirable degree of redness, blush or flushing.

(2)

Safe-B3™- the guaranty of minimal nicotinic acid.

(1) Muller B et a, 2003 (2) Benyo et al, (3) Bissett et al,

UV-stressed Skin Care with **Safe-B3™**



UV-stressed Skin Care
Skin Tone efficacy of **Safe-B3™**

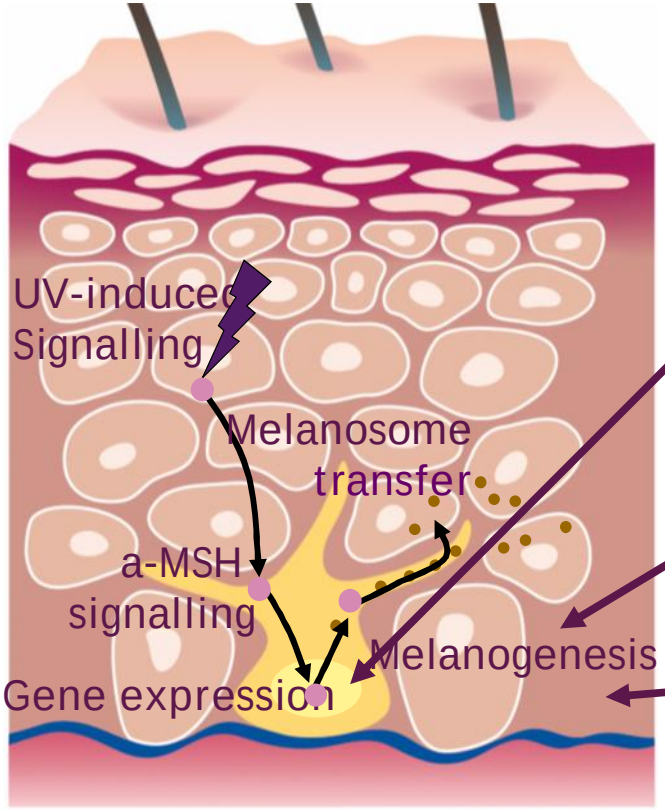
Consumer level

- Rebalances uneven skin tone
- Reduces discoloration

Functional level

- Safe-B3™ prevents the pigments from surfacing on the skin
  - Inhibition of melanosome transfer
- Safe-B3™ reduces cutaneous pigmentation in 3D skin model
- In clinical trials: Safe-B3™ shows significant skin lightening effects, as effective as hydroquinone with a safe mode of action
- Safe-B3™ is safe and effective in helping even out skin tone

Safe-B3™ prevents the pigments from surfacing on the skin



Effective skin brightening requires to attenuate visible pigmentation at multiple points.

Melanocytes

- part of the basal cell layer of the epidermis
- form long, finger-like dendrites which reach to neighboring keratinocytes
- create enzymes (e.g. tyrosinase) and the amino acid tyrosine

Melanosome

- is an organelle (specialized subunit within a cell) containing melanin
- converts tyrosine into Eumelanin (brown – black) and Pheomelanin (yellow – red)
- migrates upward into the dendrites and are taken up by keratinocytes

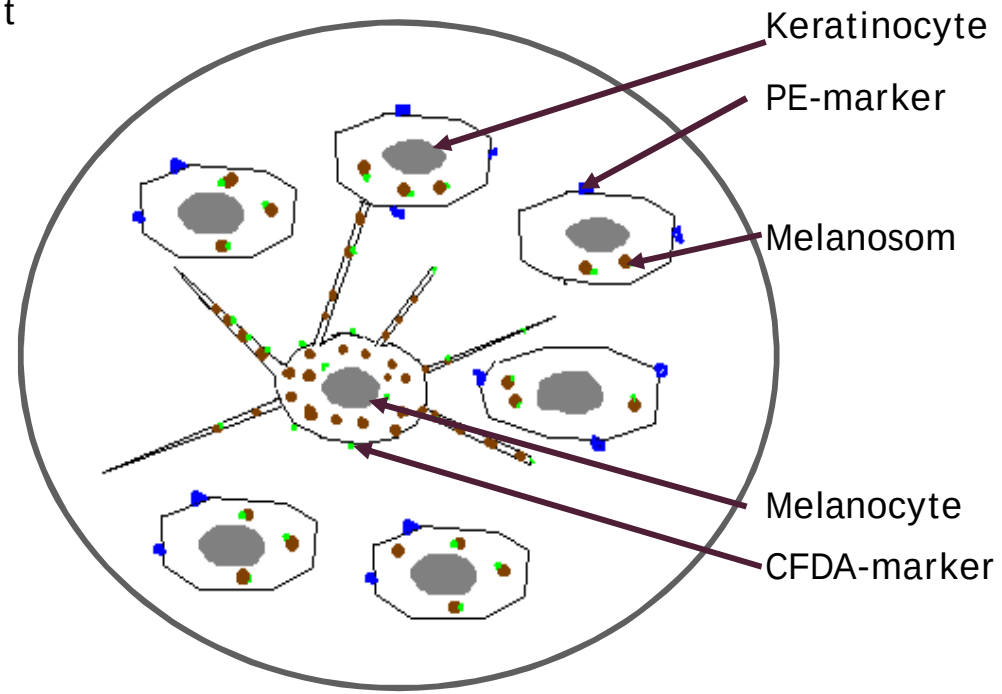
Safe-B3™ interferes in the transfer of melanosomes to keratinocytes.

Safe-B3™ inhibits melanosome transfer

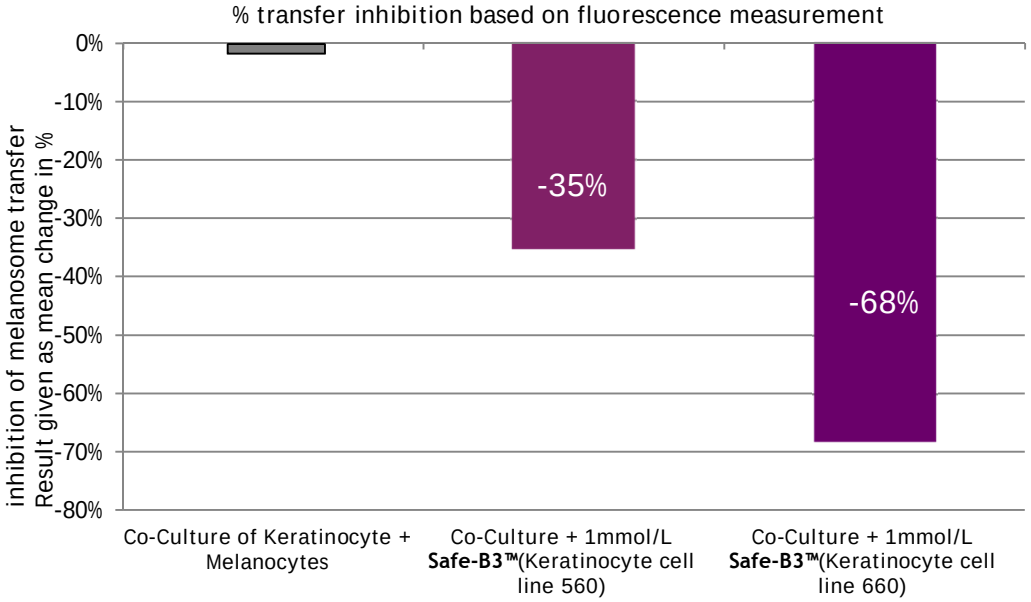
Test system: co-cultures of melanocytes and keratinocytes
Differentiation between melanocytes and keratinocytes is essential and done by staining the cells

- Melanocytes and thus melanosomes are stained with a fluorescent marker (CFDA)
- Keratinocytes are labeled with a different fluorescent marker (PE)
- Both cell-lines are cultivated together
- If melanosomes are taken up by keratinocytes the melnosome- specific fluorescent marker will be detected inside the cell
- Cells were cultivated together with or without **Safe-B3™**
- After 7 days measurement of fluorescent signals

Coculture of melanocytes and keratinocytes (scheme)

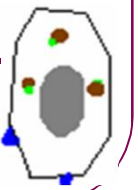


Skin Tone: **Safe-B3™** prevents pigments from surfacing on the skin



- Co-cultures of fluorescence-labeled melanocytes/ melanosomes and keratinocytes were supplemented with Safe-B3™ (1mmol/l).
- After seven days fluorescence measurement of keratinocytes carrying.

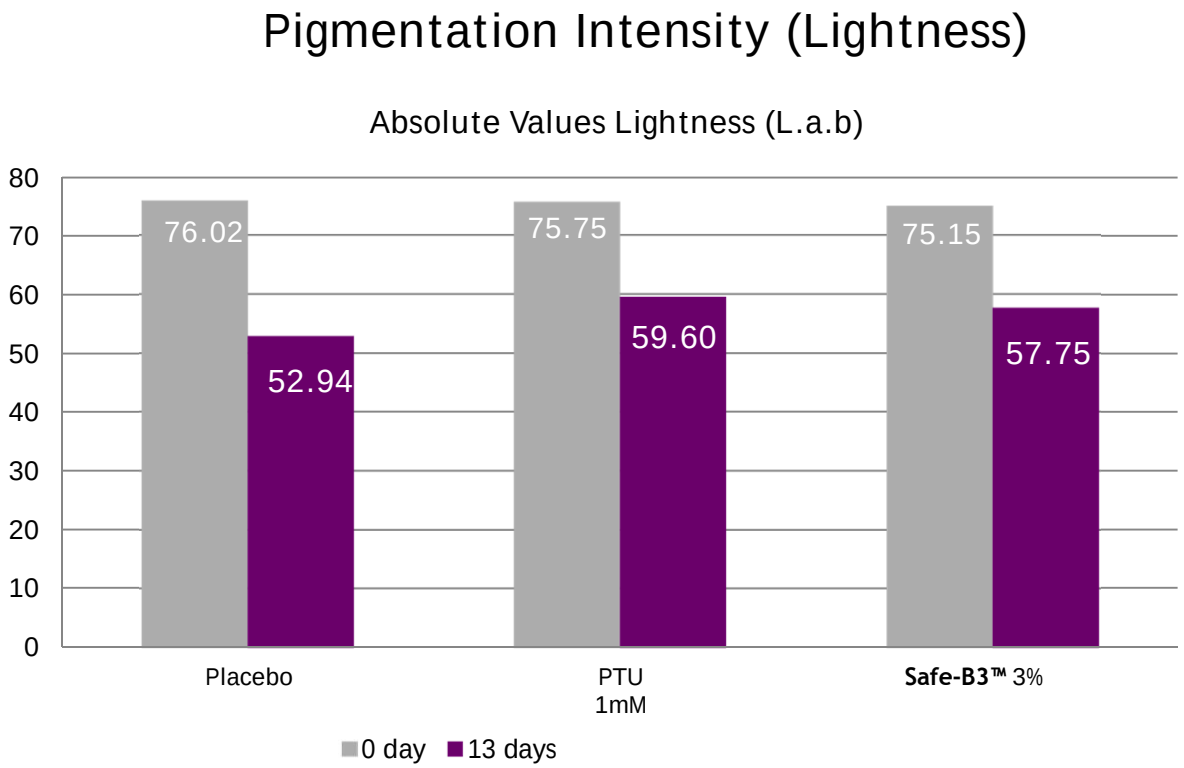
melanosome specific label
+ keratinocyte specific label



Safe-B3™ inhibits the melanosomes tranfer by up to 68% (1).

(1) Hakozaki et al 2002

Safe-B3™ reduces cutaneous pigmentation in 3D skin model



- Positive control PTU and the test substance Safe-B3™ are applied topically on a daily base during 13 days.
- Change of pigmentation monitored with Minolta chromameter CR 300. Measured in luminosity units [L*(D65)] compared to a white reference (L = 100).

Safe-B3™ PC performs excellently after 13 days in 3D-human reconstituted epidermis.

Safe-B3™ is safe and effective in helping even out skin tone

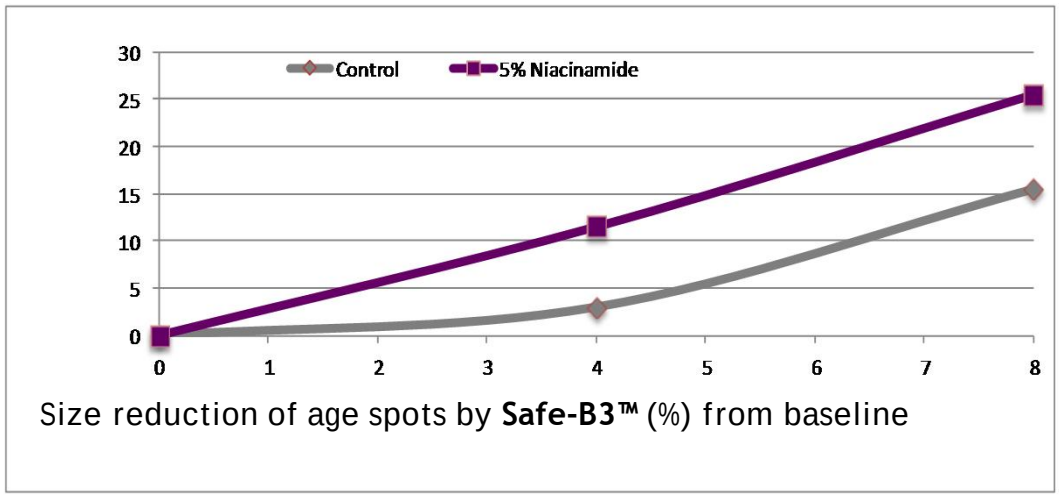
- Safe-B3™ is an effective skin lightening compound showing in several high quality clinical trials
  - effects at 2 and 5%, significant at 5%(1,2)
  - 4% Safe-B3™ being as effective as 4% hydroquinone(3)
- Safe-B3™ shows no side effect

5% Safe-B3™, Japanese female (2)

Click

4% Safe-B3™, melasma patients at week 0 (left) and week 8 (right) (3)

Click



Size reduction of age spots by **Safe-B3™** (%) from baseline

(1) Hakozaki et al 2002, (2) Hakozaki et al 2005, (3) Navarrete-Solis, J et al, 2011

UV-Stressed Skin Care
Protection and Repair

Consumer level

- Well-protected from UV damage for beautiful and healthy skin

Functional level

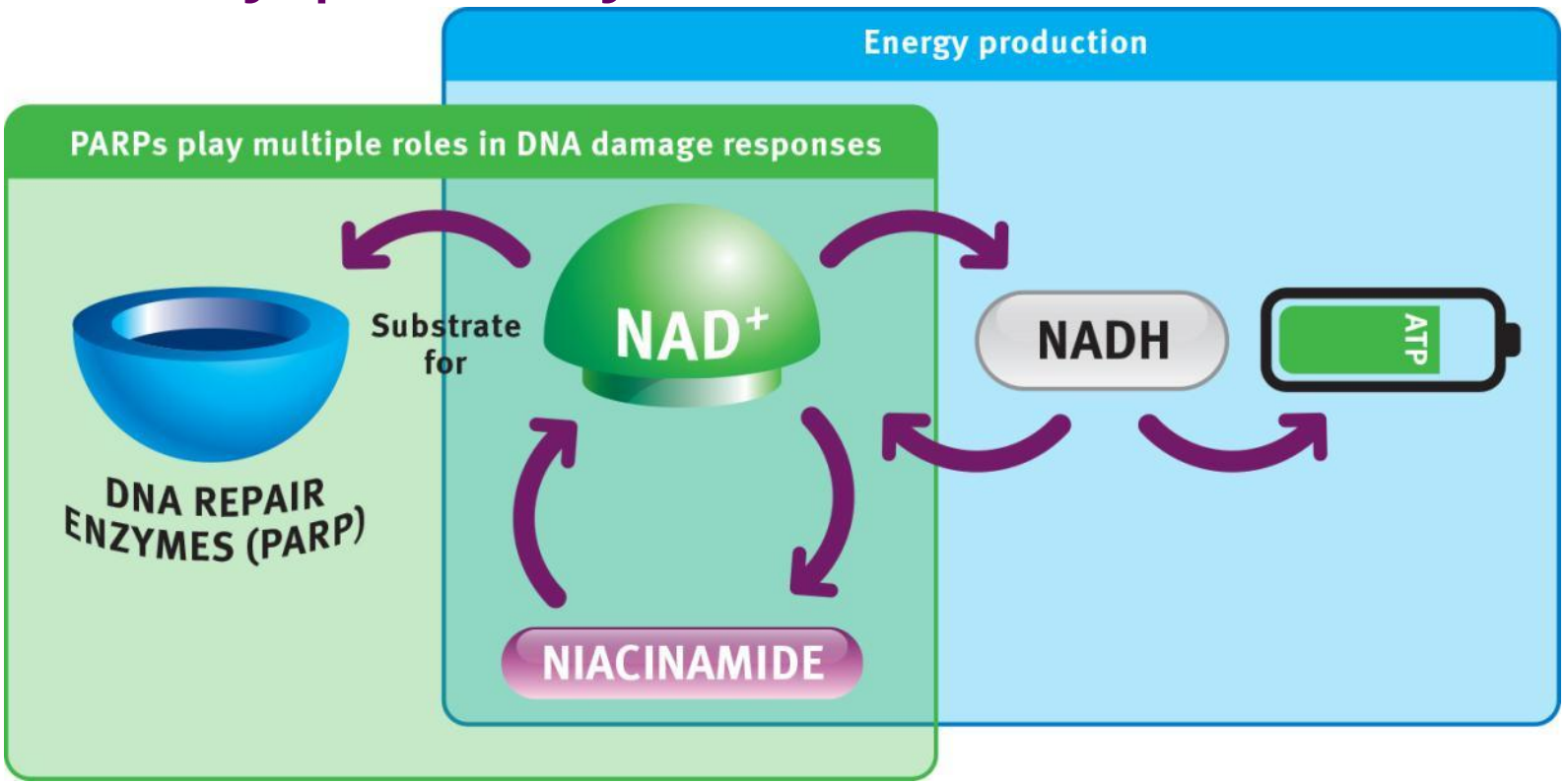
Repair

- Safe-B3™ increases physiological DNA repair

Protection

- Safe-B3™ prevents UV-induced ATP decline →cell energy crisis prevention
- Energy refueling allows → significantly increased DNA repair → significantly reduced induction of immunosuppression

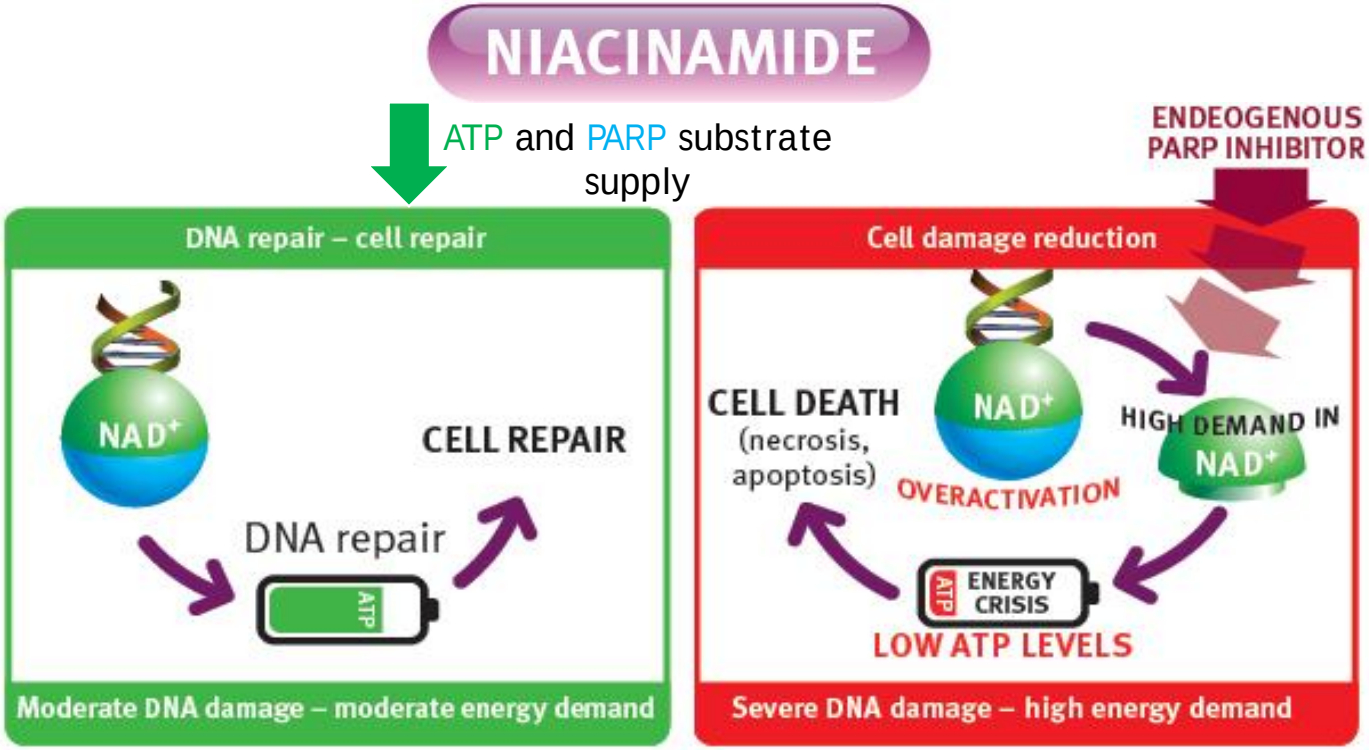
Safe-B3™ is the essential NAD+ precursor in two key pathways



- Simplified pathway of cellular energy production (ATP production) with Safe-B3™ and NAD +
- NAD+ is a substrate for poly(ADP-ribose)polymerases (PARP). PARP play multiple role in DNA damage responses including DNA repair and maintenance of genomic stability

Roles of **Safe-B3™** in DNA repair and protection

Safe-B3™ plays a dual role in DNA repair and maintenance of (genomic) stability



- In moderate DNA damage, PARP activation enhances DNA repair
- In severe DNA damage Safe-B3™ plays the role of PARRP inhibitor for cellular protection →avoid over-activation of PARP and energy crisis

Safe-B3™ enhances repair of UV-induced DNA damage

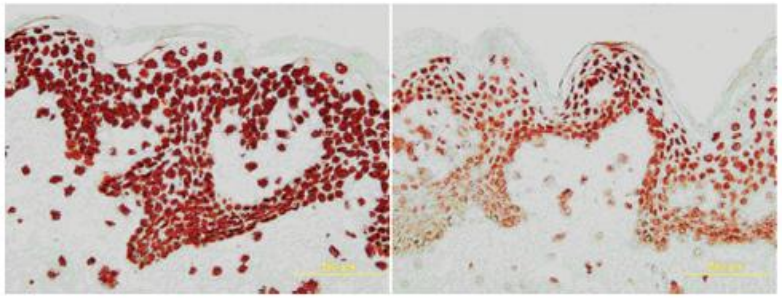
Ex vivo skin irradiated with ssUV at low sub-erythemal dose (4J/cm^2)

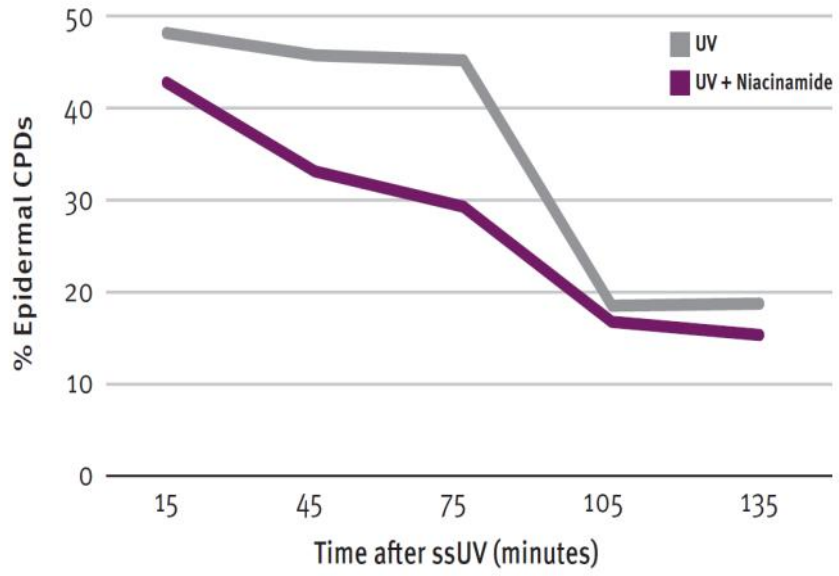
- Cell viability was not affected
- DNA damage was increased by 3 fold!

- Read out parameter : CPD staining as an indicator for level of DNA damage

Stained CPDs after irradiation

Control **Safe-B3™**





Safe-B3™ significantly enhances the repair of CPD photolesions in human skin.

CPD=Cyclobutane Pyrimidine Dimer. ssUV= solar simulated UV

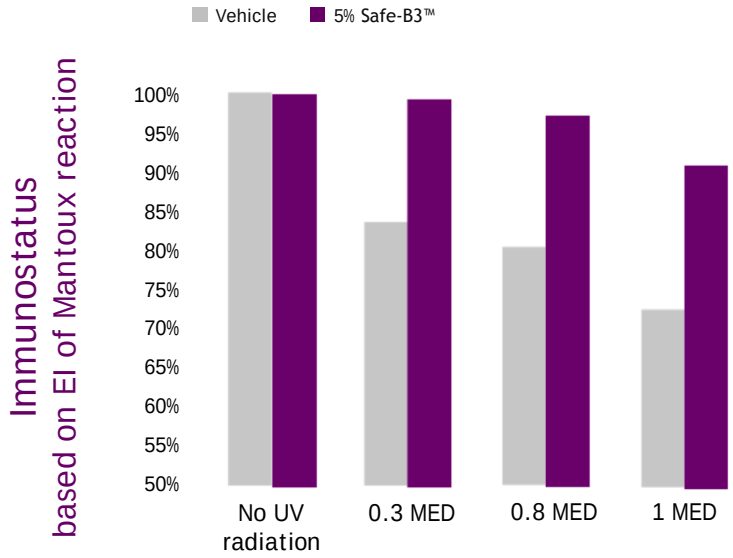
Surjana et al, 2013

Safe-B3™ interferes with photo-immunosuppression

Mantoux testing, a skin immunity model tc assess UV-induced local immunosuppressic

- 20 healthy, Mantoux-positive volunteers, skin type I-III: 5:9:6
- During 3 consecutive days: application of 5 % Safe-B3™ on lower back followed 15 minutes later by UV exposure (SSR)
- Mantoux tests at irradiated and unirradiated test sites immediately after final irradiation
- Read out of erythema 72 h later to evaluate immunosuppression

Influence of **Safe-B3™** on UV-induced immunosuppression



Safe-B3™ significantly protects against erythemal and sub-erythemal induced immunosuppression, a major risk factor of melanoma and non melanoma skin cancer.

SSR=Solar Simulated Radiation
Damian et al, 2007

UV-Stressed Skin Care
Anti-Ageing

Consumer level

- Reduces the appearance of lines and wrinkles
- Improves the elasticity of the skin

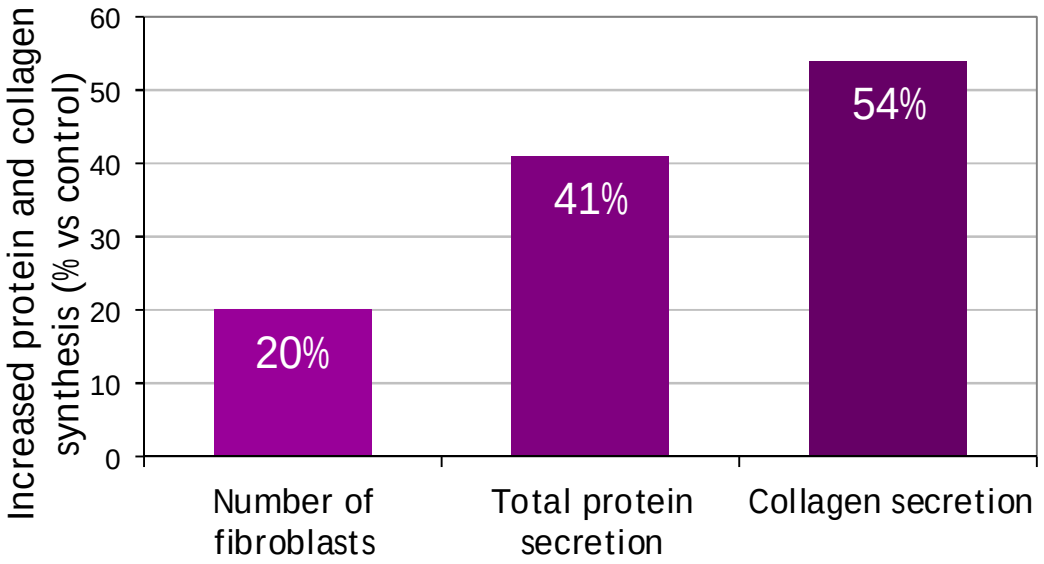
Functional level

Exposure to sun light prematurely ages our skin. Safe-B3™ is the ideal candidate for day care and sun care products as it:

- Stimulates collagen synthesis
- Improves the appearance of lines and wrinkles

Safe-B3™ stimulates collagen synthesis

Safe-B3™ stimulates collagen synthesis selectively compared to other proteins: significant 35% increase in total collagen/ total protein ratio vs. control.



- Cultured human dermal fibroblasts from aged donors were supplemented with 500µM Safe-B3™ for 48h.
- 14C-hydroxyproline as marker for collagen, 14C-prolin as marker for total protein.

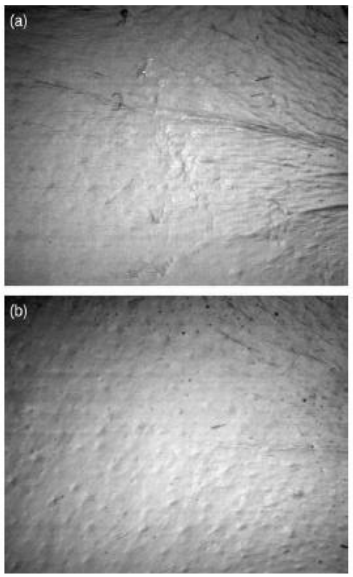
Safe-B3™ may help retain skin elasticity.

Bissett et al, 2003

Safe-B3™ improves the appearance of lines and wrinkles

Significant wrinkles decrease in eye area

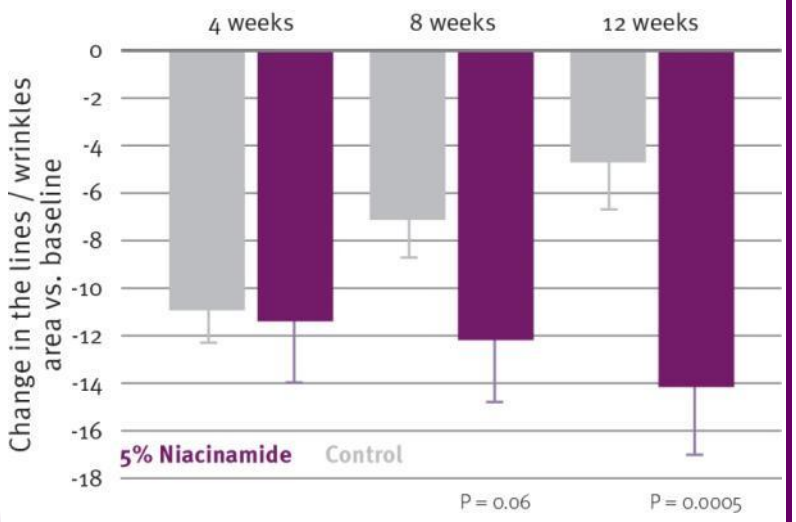
Japanese female volunteer, Kawada, 2008



Skin replica in a 44-year-old woman (a) before and (b) 8 Weeks after application of 4% Safe-B3™ preparation.

Significant reduction of fine lines

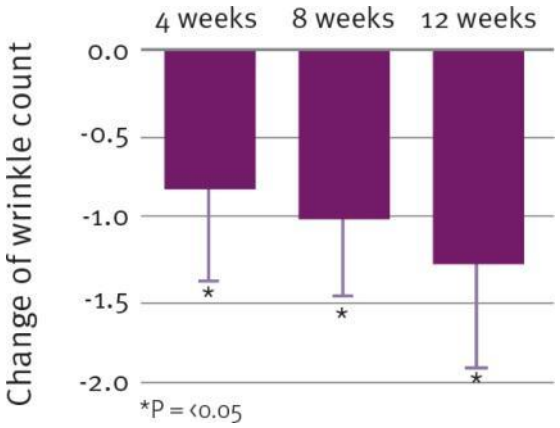
Caucasian female volunteer, Bissett, 2004



Treatment with a Safe-B3™-containing moisturizer shows significant improvement in fine lines and wrinkles following both 8 and 12 weeks of treatment.

Significant wrinkle reducing effects

Taiwanese female volunteer Chiu, 2007



Significant decrease (-51.6%) of wrinkle count with 4% Safe-B3™ after 12 weeks. vs baseline.

Blemish Care with **Safe-B3™**

Consumer level

- Blemish-free, shine free refined skin appearance

Functional level

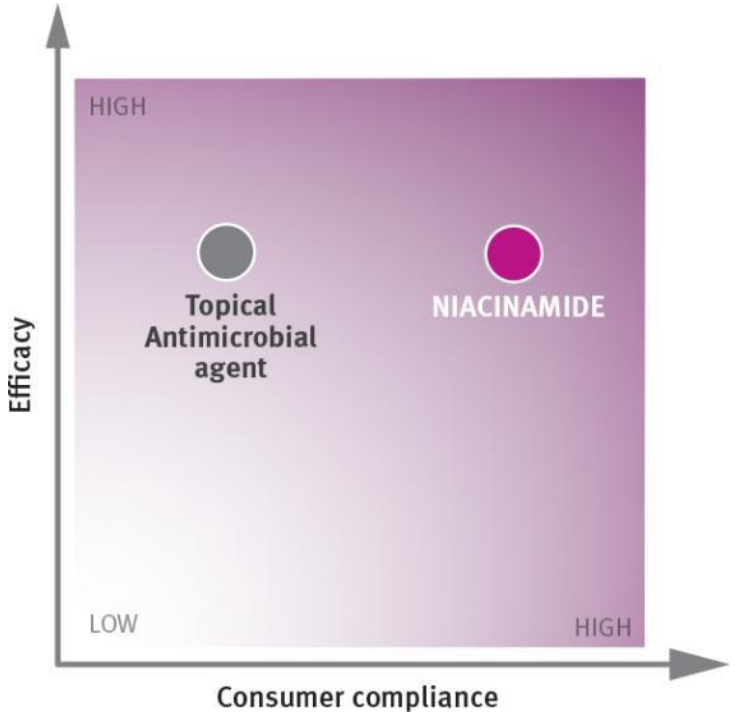
- Safe-B3™ reduces acne condition, including reduced severity (grade) and reduced lesions (number of papules, pustules)
- Safe-B3™ shows trend of reduced sebum formation and excretion

Safe-B3™ in Blemish Care
Spot the opportunity!

Common problems

Sebum on the face
→ Shiny, oily looking skin enlarged pores
→ Blackheads and inflammatory acne lesions

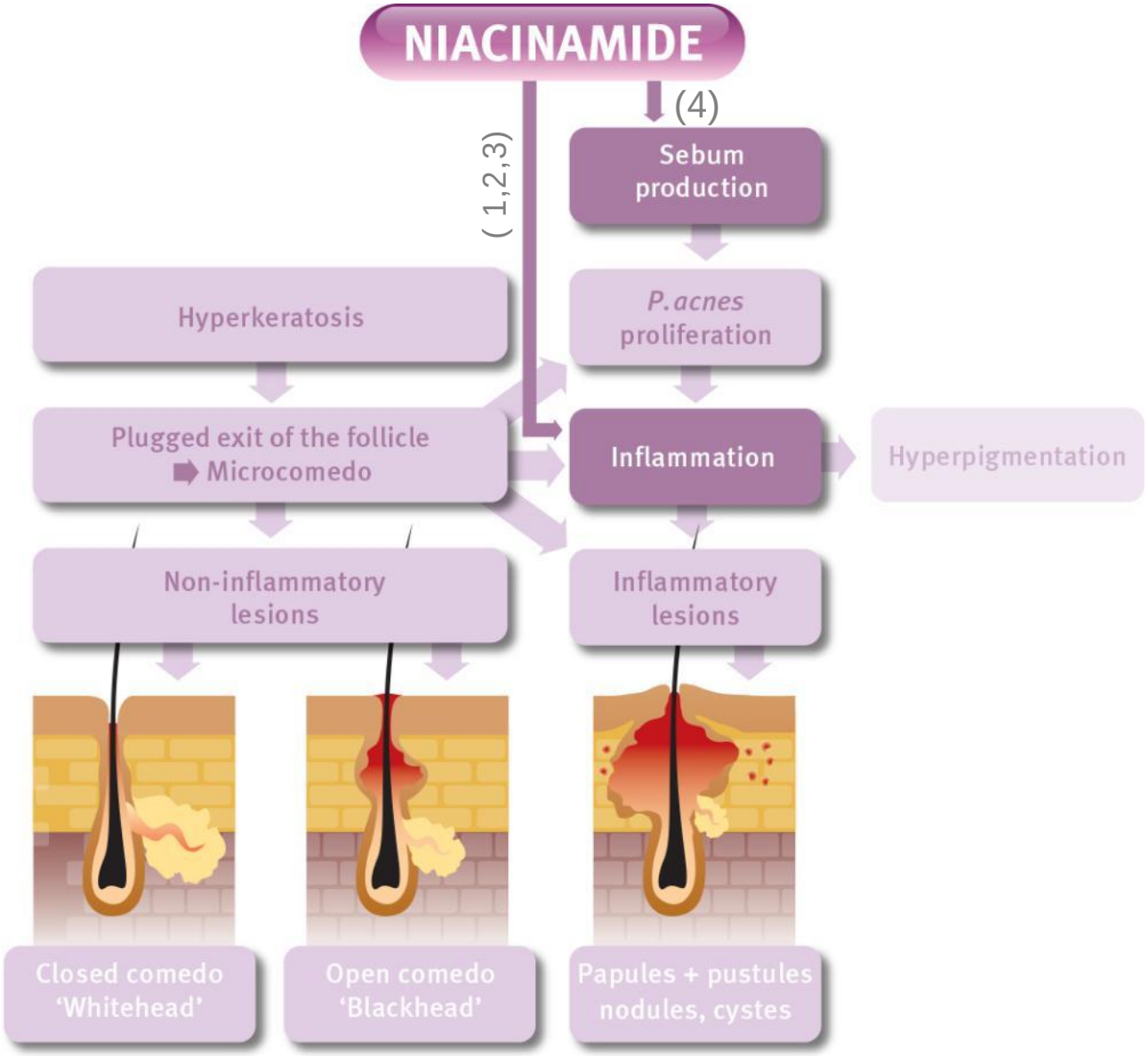
→ Enlarged pores due to accumulation over time



Use of topical antimicrobial agent
→ Skin irritation, especially for adult acne with dry skin
→ Risk of inducing bacterial resistance

→Problematic consumer compliance

Safe-B3™ and Blemish Care



(1) Shalita et al, 1995 (2) Fouladi et al, 2013 (3) Kaymak et al, 2008 (4) Draelos et al, 006

Results of several clinical trials with 4% **Safe-B3™** vs 1% clindamycin

	Safe-B3™ (4%)	Clindamycin (1%)
Overall improved acne skin (1)	82% of volunteers after 8 weeks	68% of volunteers after 8 weeks
Reduced acne severity (2)	-32% (4 weeks), -64% (8 weeks)	
Reduced acne lesions (1,2,3)	Inflammatory acne reduced by 60%	Inflammatory acne reduced by 43%
	Significant reduction of papules and pustules	

(1) Shalita et al, 1995 (2) Fouladi et al, 2013 (3) Kaymak et al, 2008

Safe-B3™ just as effective as clindamycin, but without side effect.