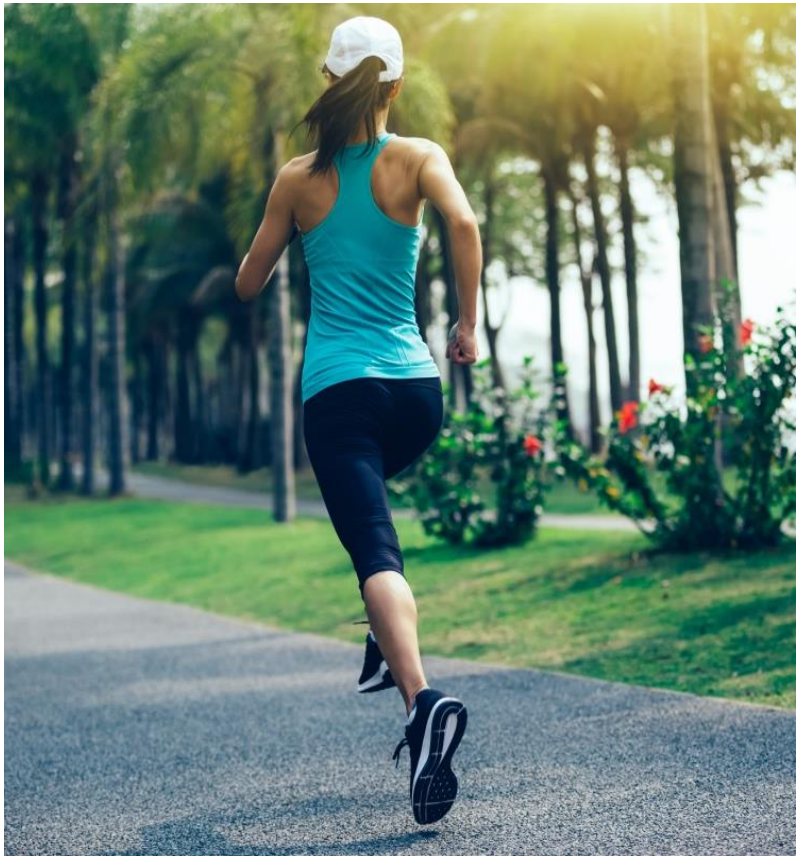
MARUZEN PHARMACEUTICALS CO.,LTD.

Topics

- ✓ Market and consumer survey on a relationship of exercise, beauty, and chlorine
- ✓ Basic research on mechanisms of chlorine damage to the skin and hair
- ✓ Our solution- Peach Leaf Extract and its efficacy proven by *in vitro* investigation and clinical trial with unique protocol

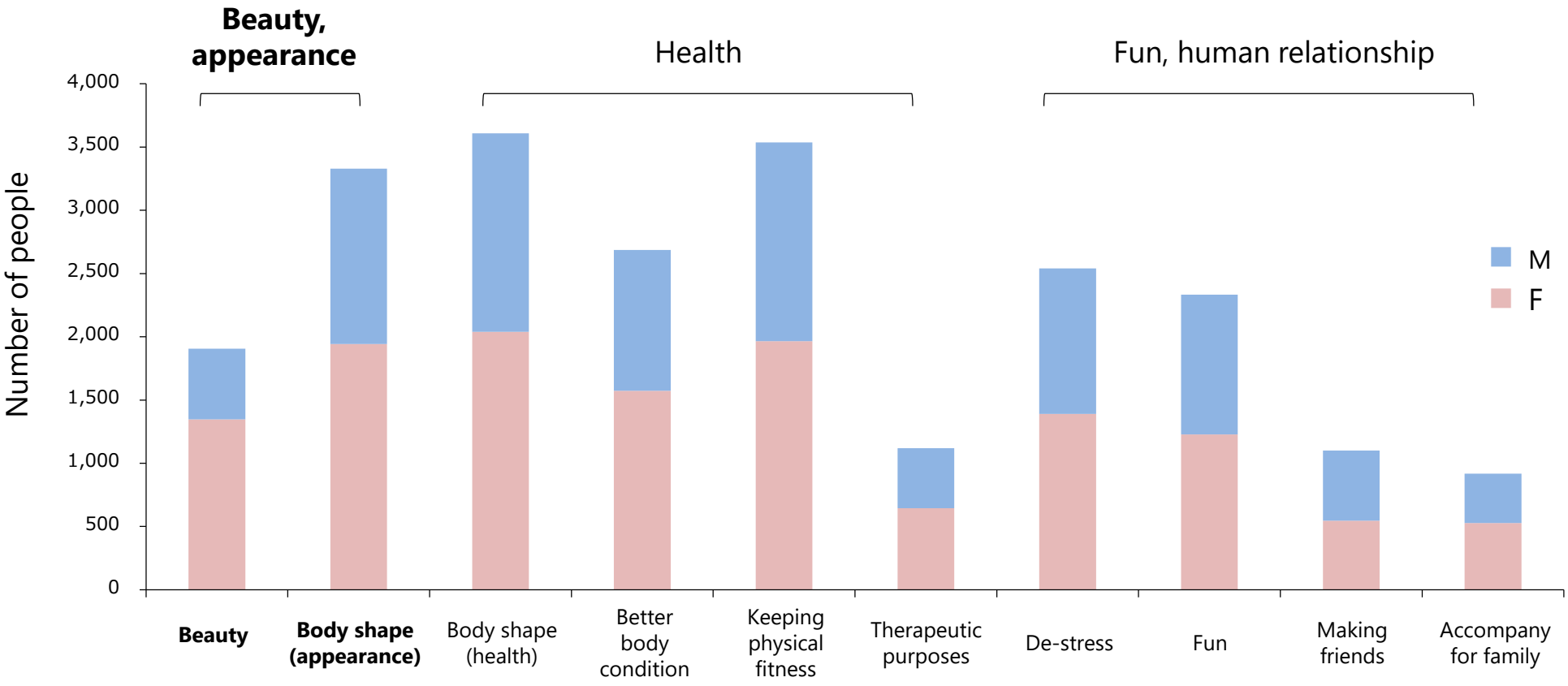
Active Beauty

A number of consumers are aware of the relationship between exercise, health, and **beauty**



Consumer survey revealed that **“Beauty”**,**“better appearance”** are among the biggest motivations to do sports and exercise

Q：What is the reason you do sports ??



The consumer survey was conducted by Maruzen Pharmaceuticals and out-sourcing research company in January, 2019, in Japan

n=5,021 (M：2,384、F：2,637)

We have proposed beauty care botanical extracts which are able to apply the concept of “Maximizing the benefit of exercise for beauty”

Outdoor sports

Rose Myrtle
“Biological UV screen”

- ✓ Cutting edge DNA damage care
- ✓ Newest data: Mitochondria damage care

Urban workout

Silver Vine
“Anti-pollution in daily care”

- ✓ Inhibiting car exhaust gas damage
- ✓ Enhancing skin translucency

Our new target

The reason why we focus on swimming is not only its popularity as an exercise but also….

chlorine

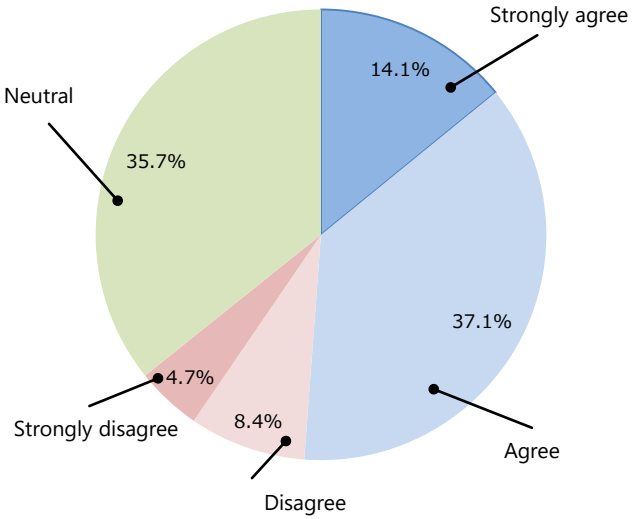
Our hypothesis- Chlorine may reduce the benefit of exercise for beauty

Consumers have a **negative image of chlorine** and are concerned about its effects on their skin and hair which are directly in contact with the chlorinated water

Q：What is your image of chlorine ??

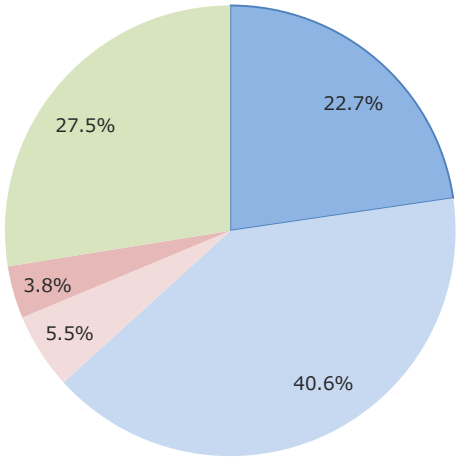
Bad for **health**

Agree：51.2%



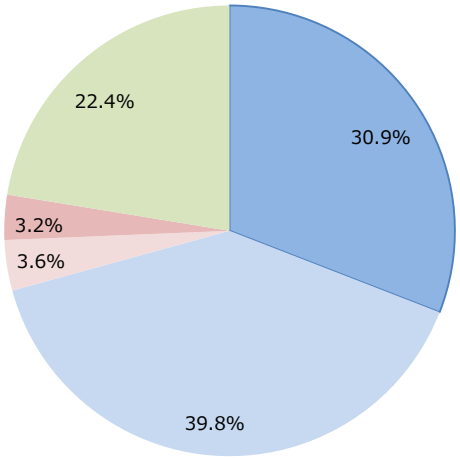
Bad for **skin**

Agree：**63.3%**



Causes damage to **hair**

Agree：**70.7%**



The consumer survey was conducted by Maruzen Pharmaceuticals and out-sourcing research company in January, 2019, in Japan

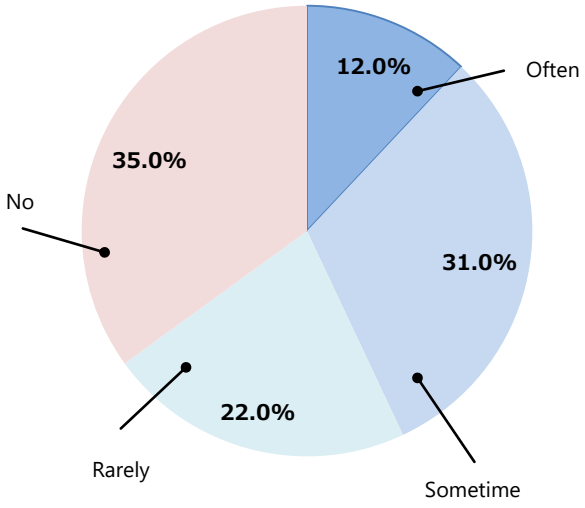
n=5,021 (M：2,384、F：2,637)

Consumers who regularly visit swimming pools **recognize** the damage from the pool water to their **skin** and hair

Q： Have you ever recognized any deterioration of your **skin condition** because of continuous pool exercise ?

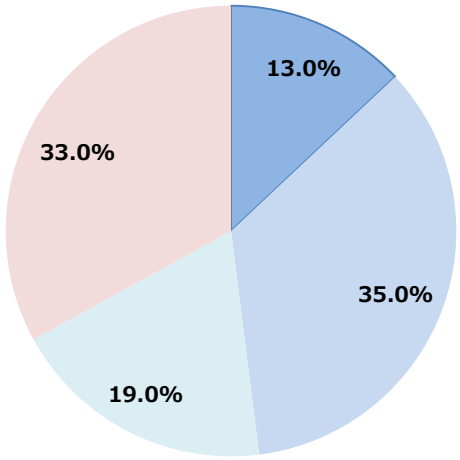
Hypersensitivity
(itching etc.)

YES：**65.0%**



Dryness

YES：**67.0%**



The consumer survey was conducted by Maruzen Pharmaceuticals and out-sourcing research company in January, 2019, in Japan

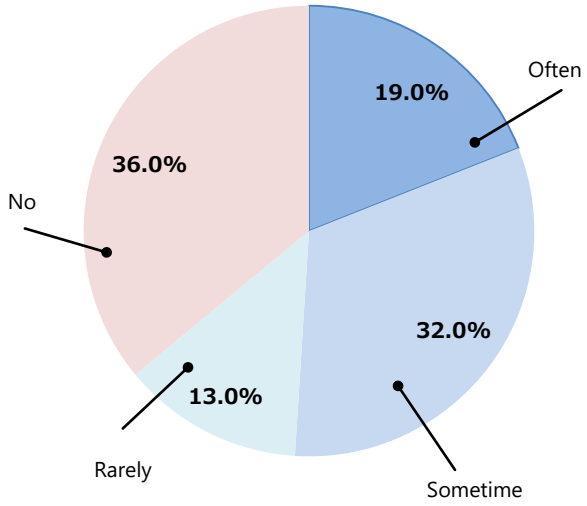
n=100 (M：55、F：45)

Consumers who regularly visit swimming pools **recognize** the damage from the pool water to their skin and **hair**

Q： Have you ever recognized any deterioration of your **hair condition** because of continuous pool exercise ?

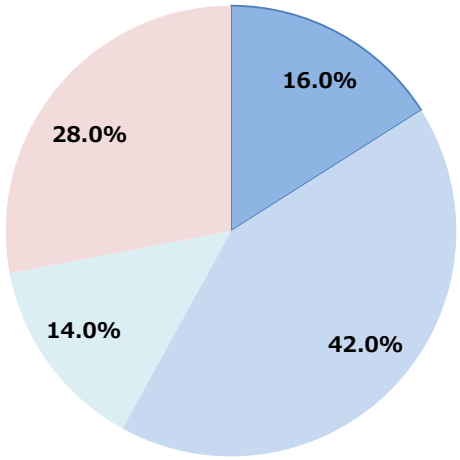
Decoloring

YES：**64.0%**



Hair damage

YES：**72.0%**



The consumer survey was conducted by Maruzen Pharmaceuticals and out-sourcing research company in January, 2019, in Japan

n=100 (M：55、F：45)

Our hypothesis- Chlorine may reduce the benefit of exercise for beauty

Verification

Low concentration chlorine which we may be exposed to in daily life could have negative effects on our skin and hair condition ??

Regulations on chlorine concentration

Country	Information source	Subject	Regulation / evaluation value	URL
US	Centers for Disease Control and Prevention	Pool Spa	Pool: more than 1 ppm. Spa (hot water): more than 3 ppm.	https://www.cdc.gov/healthywater/swimming/residential/disinfection-testing.html
Canada	National Collaborating Centre for Environmental Health	Pool Spa	0.8～5ppm	http://www.ncceh.ca/documents/practice-scenario/pool-chlorination-and-closure-guidelines
Japan	Ministry of Education, Culture, Sports, Science and Technology	Pool	More than 0.4 ppm. Less than 1ppm is preferable.	http://www.mext.go.jp/b_menu/hakusho/nc/t19920428001/t19920428001.html
Japan	Tokyo Metropolitan Government Bureau of Waterworks	Tap water	More than 0.1 ppm by water works law. Controlled 0.1 to 0.4ppm.	https://www.waterworks.metro.tokyo.jp/faq/qa-13.html#7
Thailand	Metropolitan Waterworks Authority (governmental company)	Tap water	Quality of water in Bangkok city was measured and chlorine content was 0～2ppm	https://www.e3s-conferences.org/articles/e3sconf/pdf/2018/05/e3sconf_iwa2018_01011.pdf

Chlorine concentrations we may be exposed in any pool are around 1 to 5 ppm

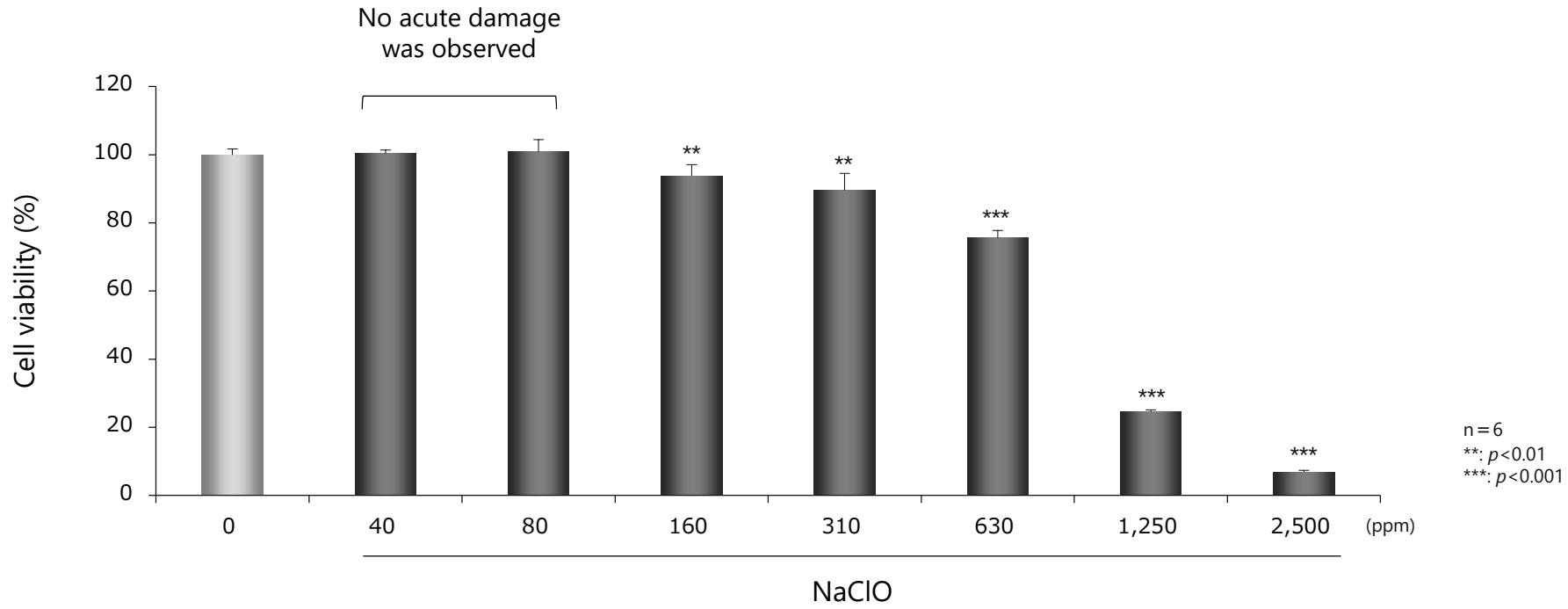
Investigation on effects of low concentration chlorine (1)

Test method

NHEKs → Apply multiple concentration of chlorine (as NaClO) → 24 hours → MTT assay

Results

Low concentration chorine did NOT show cytotoxicity on NHEKs. It suggested that the damage caused by low concentration chorine dose NOT induce visible skin disorders <u>acutely</u>, like strong UV ray exposure.



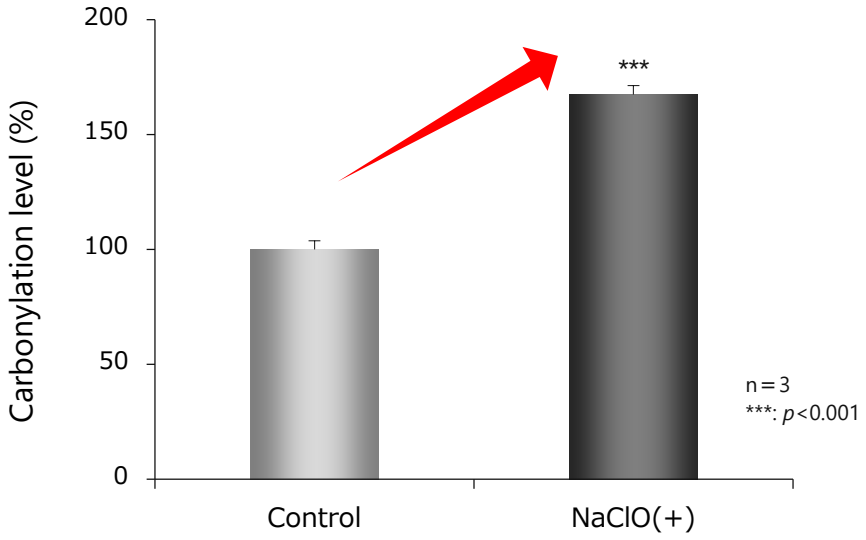
Investigation on effects of low concentration chlorine (2)

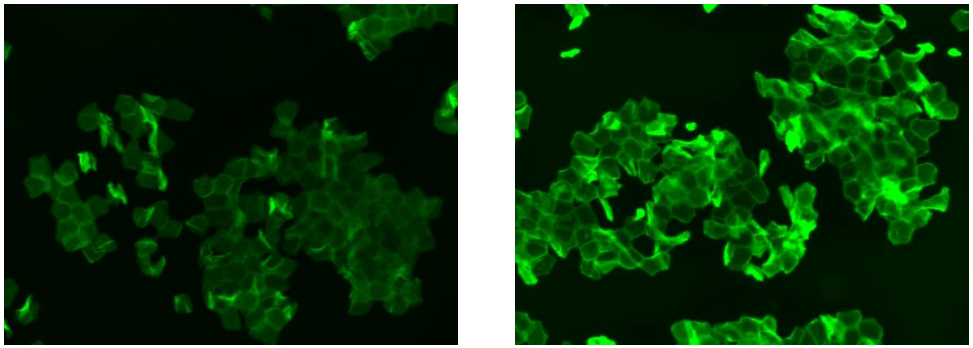
Test method

Tape-stripped SC → Apply **1ppm** chlorine (as NaClO) → 16 hours→ Stain Carbonylated proteins, a biomarker of oxidative stress

Results

Carbonylation level significantly increased by application of low concentration of chlorine





Control NaClO(+)

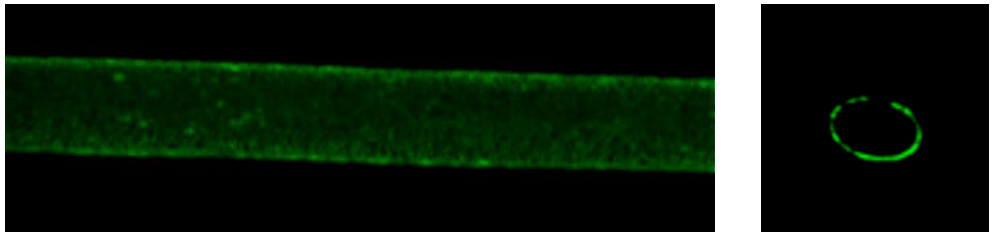
Low concentration chlorine causes oxidative stress, which triggers skin and hair trouble

Carbonylation is confirmed in the hair as well

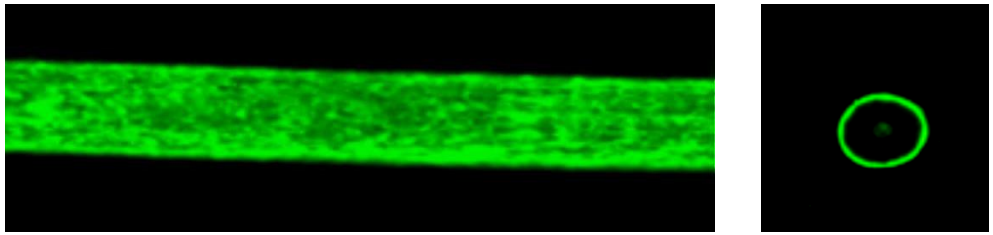
Test method

Human hair → Soak in chlorine solution → 2 hours→ Stain Carbonylated proteins

Chlorine (-)



Chlorine (+)



Oxidative stress is considered to cause deterioration of hair condition

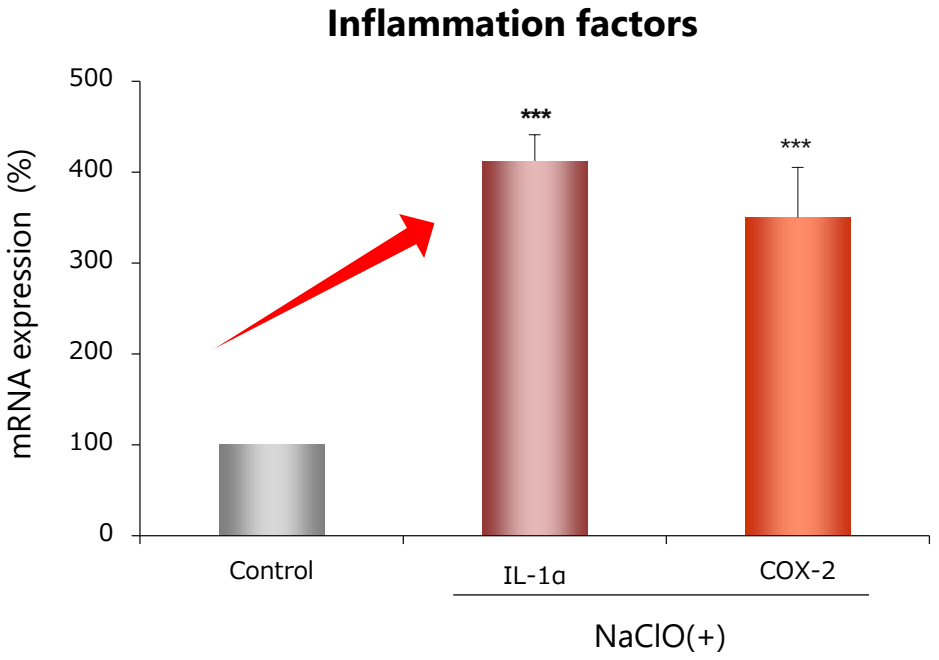
Investigation on effects of low concentration chlorine (3)

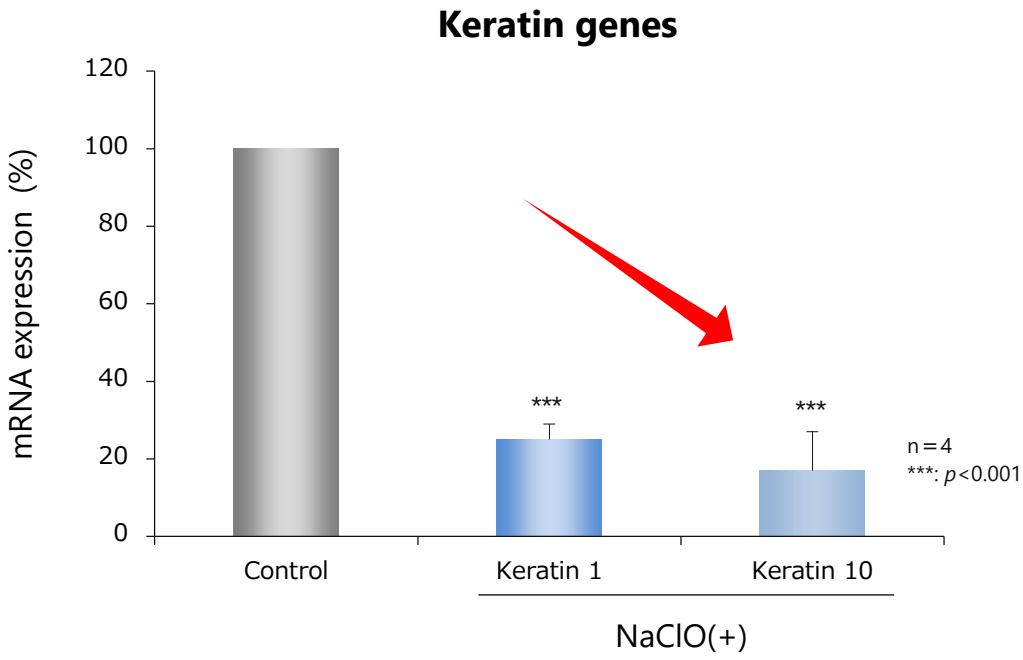
Test method

NHEKs → Apply chlorine (as NaClO) to be approx **0.2 ppm** in culture medium → Culture 24 hours → Real time RT-PCR

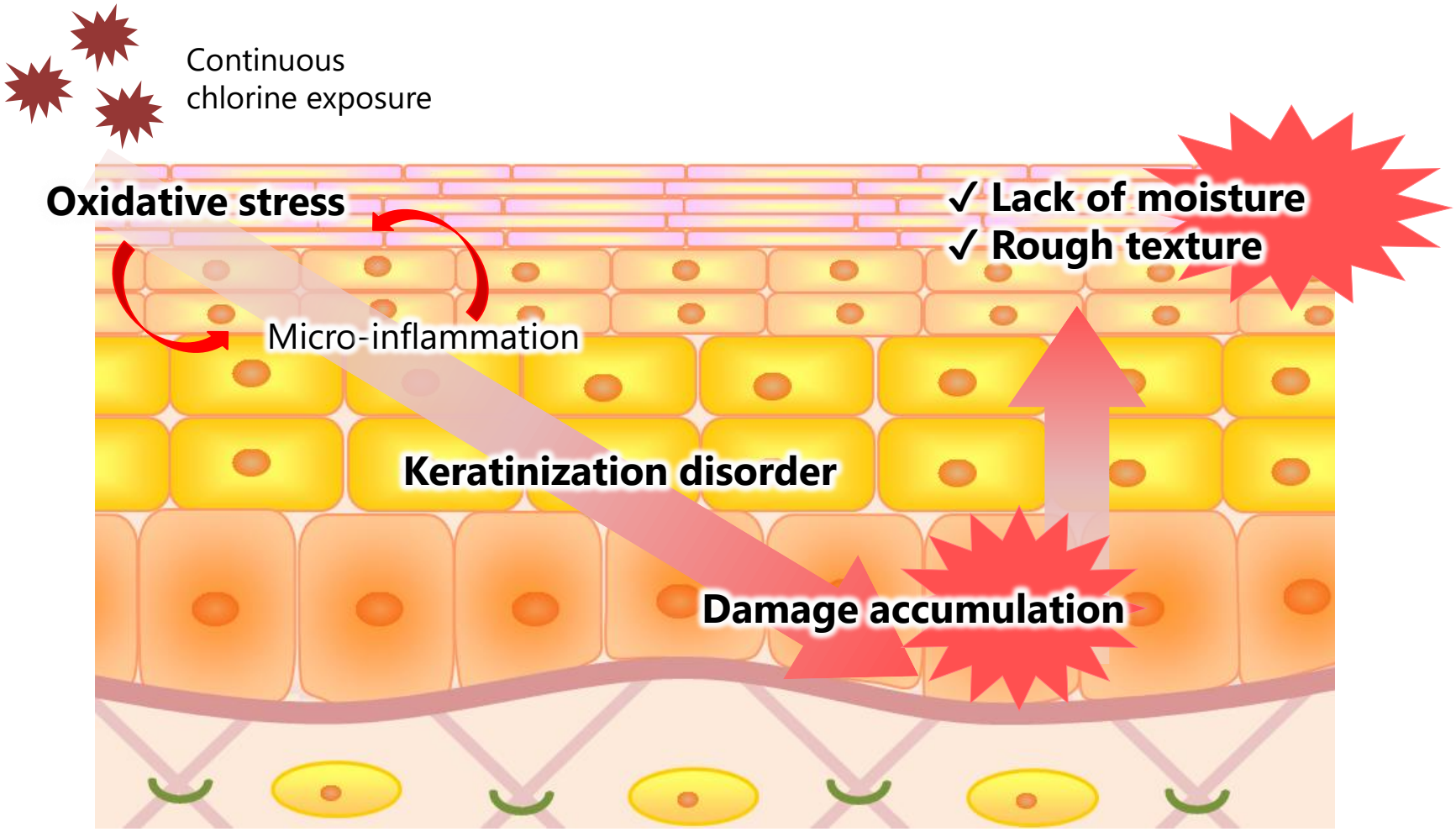
Results

Genes of inflammation factors increased and keratin related genes decreased by application of a low concentration of chlorine.





Suggested damage flow caused by low concentration chlorine exposure



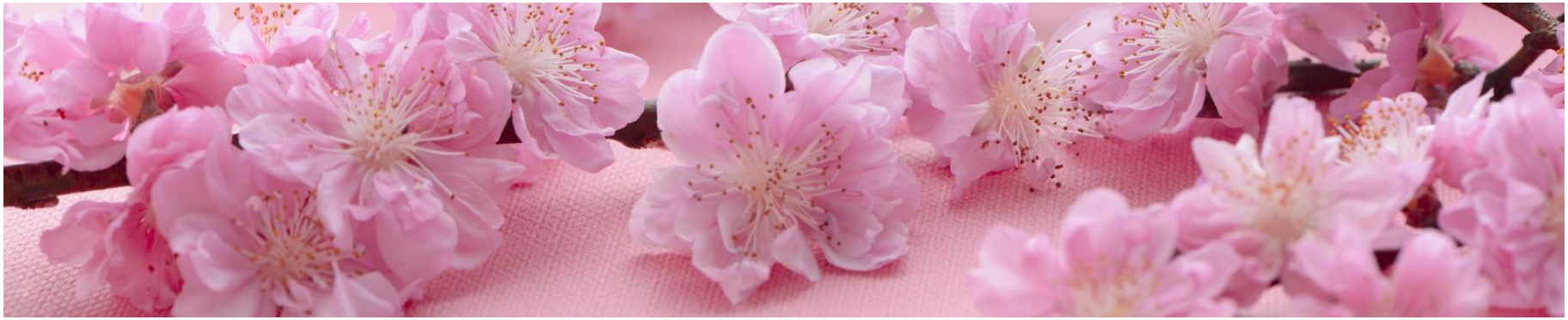
Accumulation of low concentration chlorine damage leads to perceivable skin deterioration

Summary

Market information and basic research

- ✓ Active Beauty concept has been a big trend in the beauty industry recently
- ✓ Consumers are encouraged to do exercise not only for health but also for better appearance
- ✓ Consumers who regularly visit a swimming pool recognize chlorine damage on their skin and hair
- ✓ Low concentration chlorine induces oxidative stress leading to abnormal keratinization

Solution



PEACH LEAF EXTRACT

Peach & Asian culture; Protective flower of Girls' Day

Hina-Matsuri (also known as Girls' Day or the Peach Festival) on March 3 is a traditional ceremony in Japan, which has its origins in the Heian period (8^{th}-12^{th} Centrury), cerebrates the health and growth of girls.

Even now, most families with girls display graceful dolls in ancient aristocratic costumes representing the Empress (called Hina), Emperor, and servants on platforms covered with a scarlet carpet.

As one of the ornaments on the platforms, peach flowers are traditionally used. The reason is not only their beautiful appearance, but also as it blooms from February to March as if heralding the arrival of spring.

Historically, peach has been regarded as a charm which protects from sickness, ill-fortune and drives away evil spirits, based on Chinese ancient traditions.

As a traditional remedy

Peach leaf has been utilized as a home remedy in Japan, especially, it is said to be effective for dermatitis.

In the Edo period (17th-19th Century), there was a custom to take **Peach leaf baths** during the midsummer to prevent heat rash in children.

Today, peach leaf extract is widely formulated in sensitive skin care as well as a wide range of skin care products.

Harvested at the slopes of Mt. Fuji

The raw material for this product comes from peach leaf harvested in **Yamanashi** Pref., which slopes **Mt. Fuji**. It is the NO.1 peach production site in Japan.

Product information

Product name : **PEACH LEAF EXTRACT BG30-06 (YAMANASHI)**
Scientific name : *Prunus persica* Batsch (*Rosaceae*)
Japanese name : 桃
Part used : Leaf
JSQI 2006 : Quasi-drug additive
IECIC (2015.12) : 桃(PRUNUS PERSICA)叶提取物

INCI NAME	Composition
Prunus Persica (Peach) Leaf Extract	0.20%
Butylene Glycol	29.94%
Water	69.86%

Safety tests: Eye irritation (HCE), Ames, h-RIPT

In vitro efficacies

for chlorine induced damage inhibition

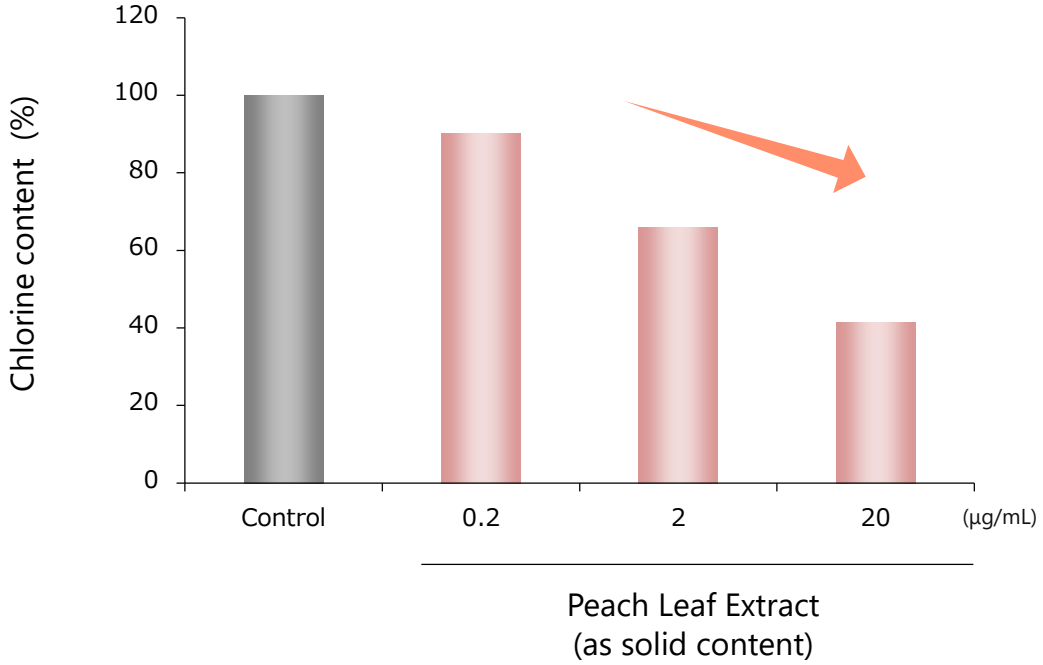
Chlorine-scavenging effect

Test method

Chlorine containing solution → Add test sample and DPD reagent → Absorbance (510nm)

Results

Peach Leaf Extract showed a chlorine-scavenging effect in a dose dependent manner, leading to a reduction in the generation of chlorine induced damage to skin and hair



Test sample: Peach Leaf Extract BG-J. a similar product of the product, Peach Leaf Extract BG30-06(Yamanashi). 20 μg/mL of the solid content is equivalent to 1% of the product

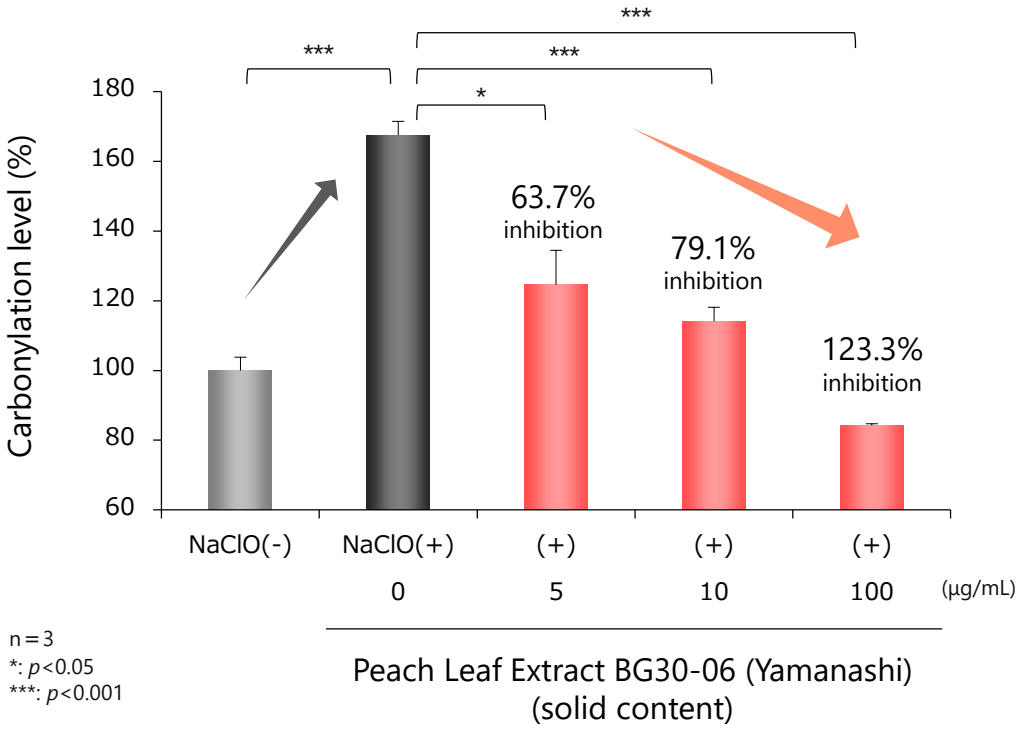
Inhibition of protein carbonylation induced by chlorine

Test method

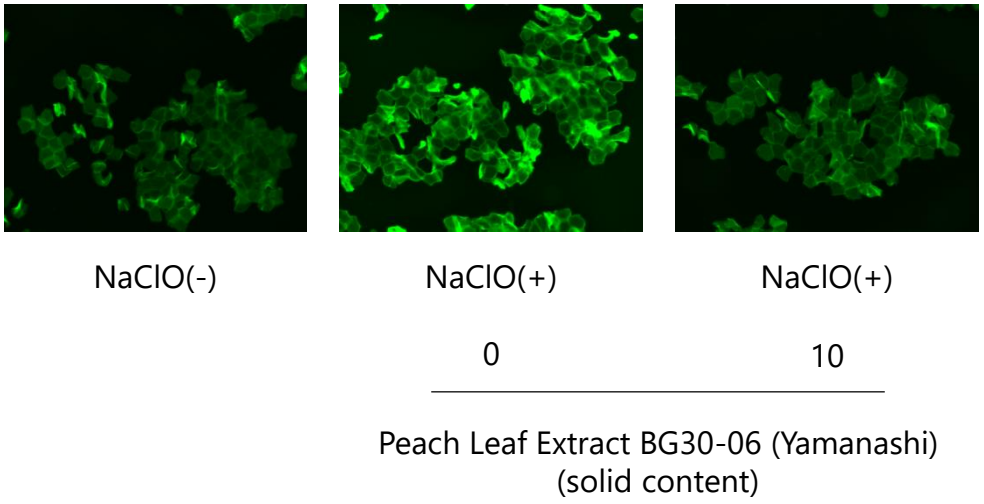
Tape-stripped SC → Add test sample → Apply **1ppm** chlorine (as NaClO) → 16 hours→ Stain Carbonylated proteins

Results

Peach Leaf Extract prohibited protein carbonylation, an indicator of oxidative stress in the skin and is expected to reduce biological damage induced by repeated chlorine exposure.



n = 3
*: $p<0.05$
***: $p<0.001$



10 μg/mL = 0.5% as product

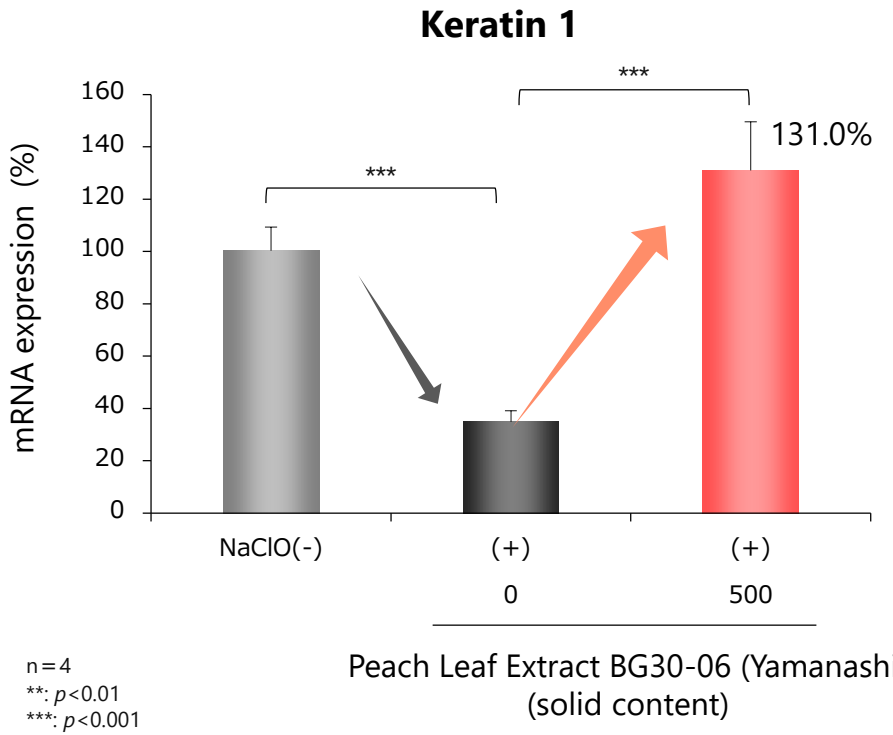
Recovery effects on the decrease of Keratin 1 and 10 mRNA expression

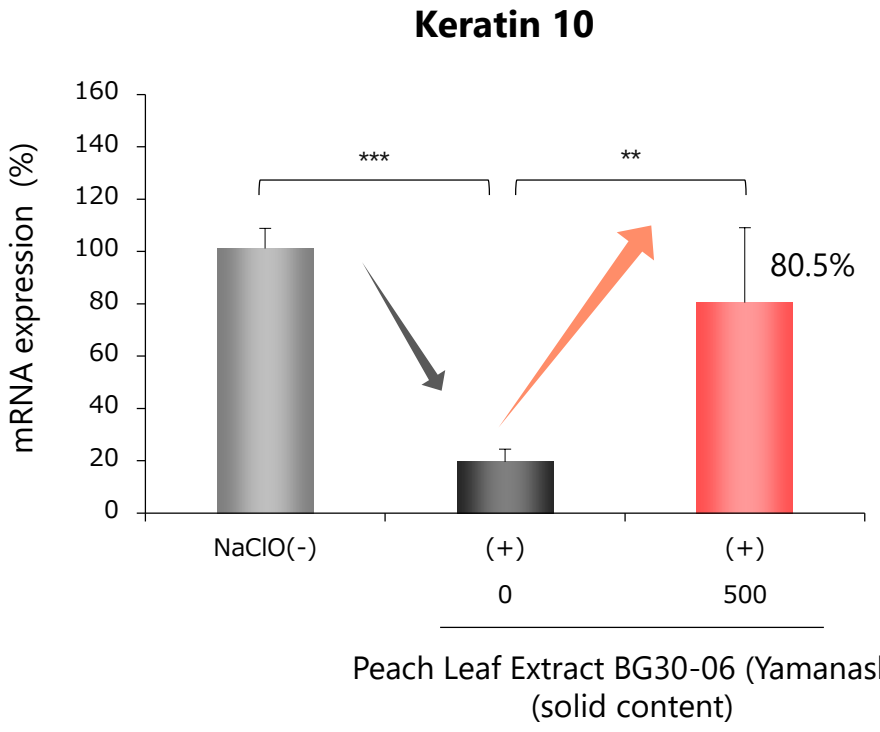
Test method

NHEKs → Apply chlorine (as NaClO) to be approx **0.2 ppm** in culture medium and add test sample→ Culture 24 hours → Real time RT-PCR

Results

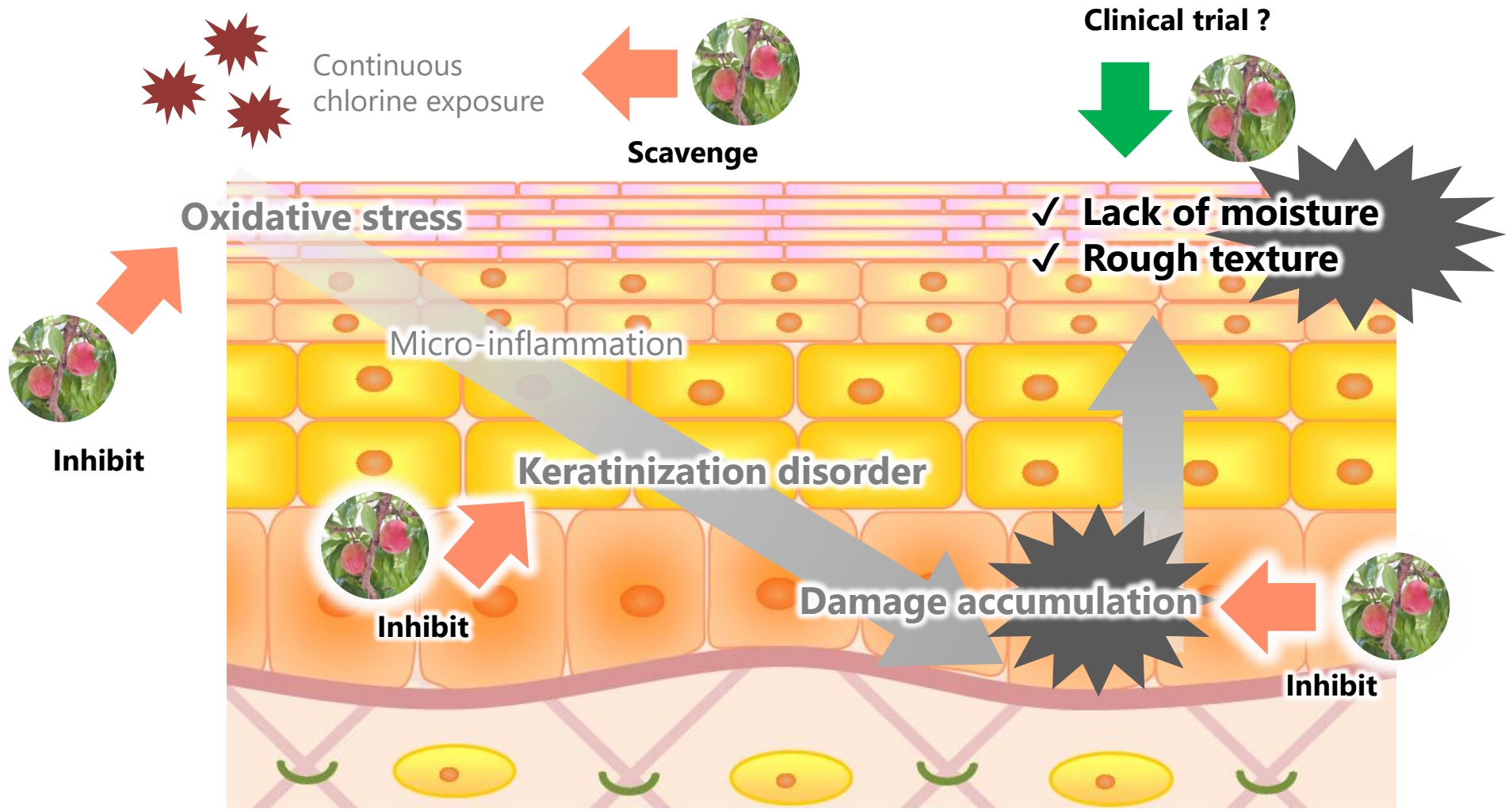
Expression of keratin related genes were recovered by the effects of Peach Leaf Extract from the damage induced by low concentration of chlorine .





n = 4
**: $p<0.01$
***: $p<0.001$

Efficacies of Peach Leaf Extract on the damage flow caused by chlorine



Clinical study;

with volunteers who visit a swimming pool a minimum of twice a week

Test protocols

Subjects : 29 female volunteers
- Aged from 32 to 57, mean age of 46
- Practice water sports twice a week as minimum
- Phototype (Fitzpatrick) I to III
- Straight hair at least 10 cm in length
- Skin and hair damage by assessment of dermatologist.

Test sample : Lotion containing 1% PEACH LEAF EXTRACT BG30-06 (YAMANASHI) and placebo lotion

Application : Twice daily for 2 months to hair and face (hair: by spray, skin: by hand)
- 15 volunteers: Test lotion
- 14 volunteers: Placebo lotion

Measurement : Skin
- Skin moisture (Corneometer)
- Skin texture (Primos, 3D skin analyzer)

: Hair
- Damaged area of hair (Scanning electron microscope)
- Friction force (Instron)
- Hair luminosity (L*) & color change (b*) (Chromameter)

Formulations

Ingredients	Test lotion	Placebo lotion
Butylene Glycol	3.00	3.00
10% Citric Acid	0.10	0.10
10% Sodium Citrate	0.35	0.35
Phenoxyethanol	0.40	0.40
Peach Leaf Extract BG30-06 (Yamanashi)	**1.00**	0.00
Water	95.15	96.15
Total	100.00	100.00

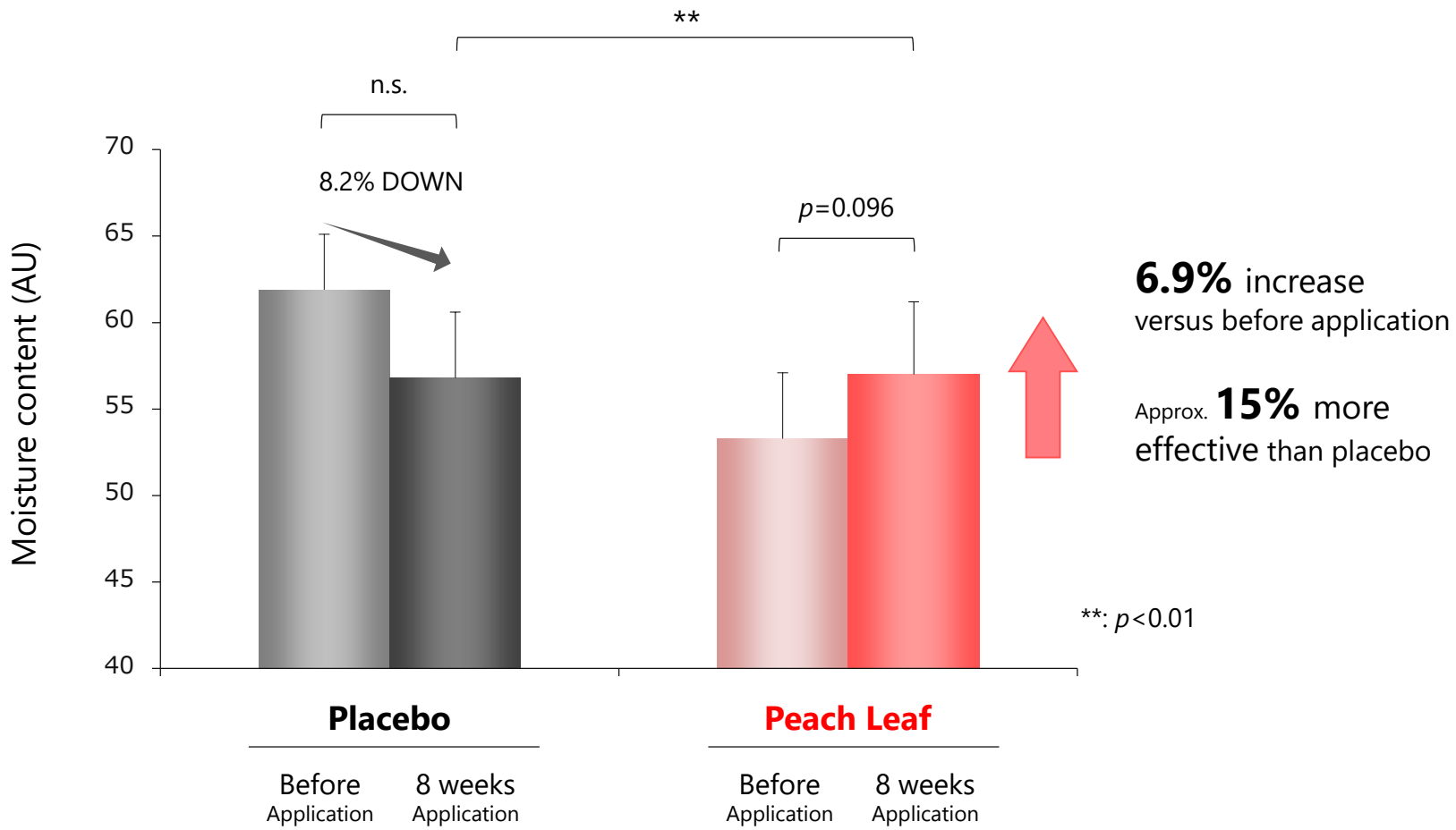
(%)

Results on skin

✓ Improvement of moisture content

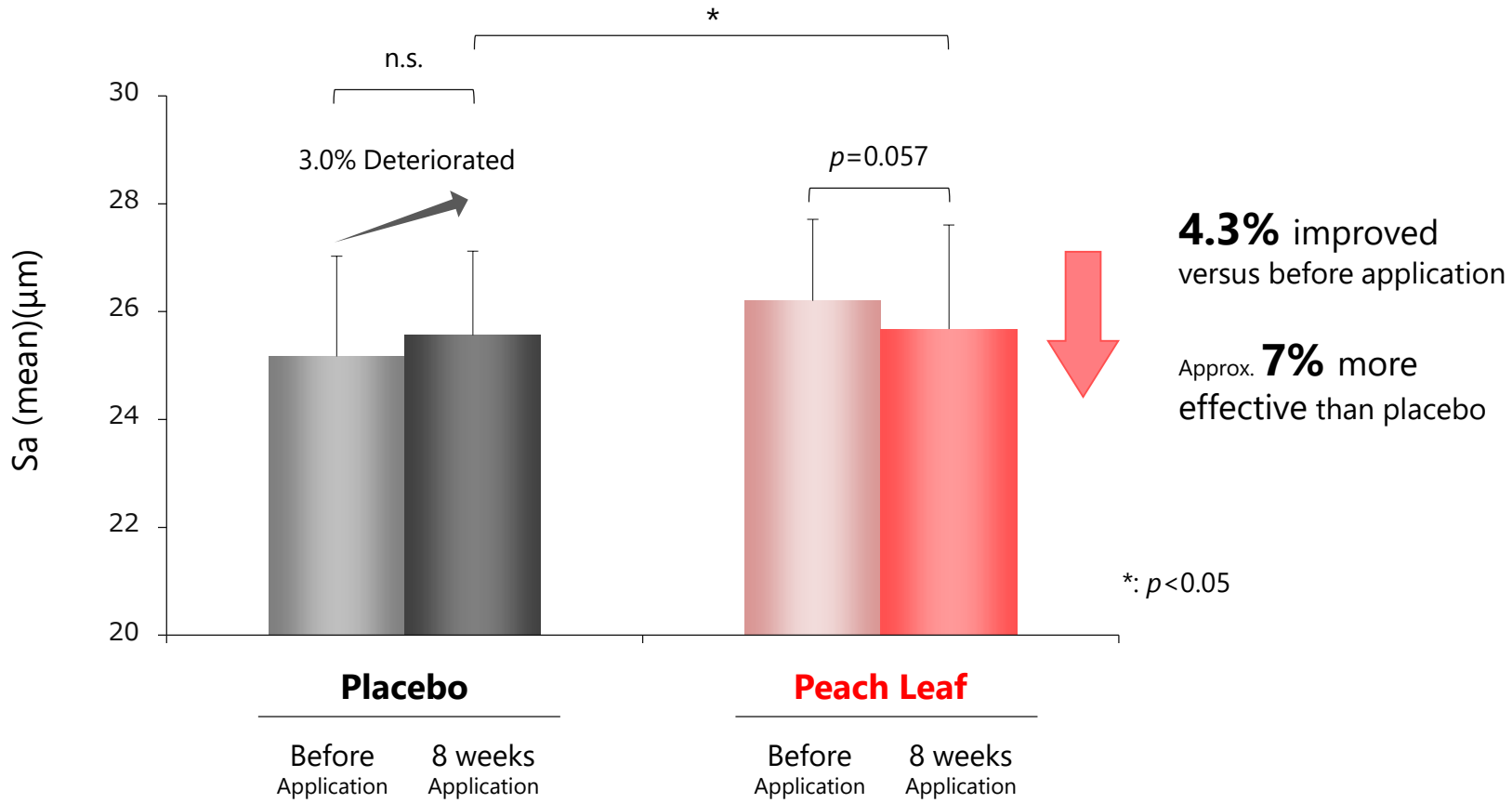
✓ Improvement of surface texture

1) Improvement of skin moisture content



Skin moisture content was increased by application of lotion containing Peach Leaf Extract BG30-06 (Yamanashi)

2) Improvement of skin surface texture



Sa value, an indicator of skin roughness, was decreased by application of lotion containing Peach Leaf Extract BG30-06 (Yamanashi).

Results on hair

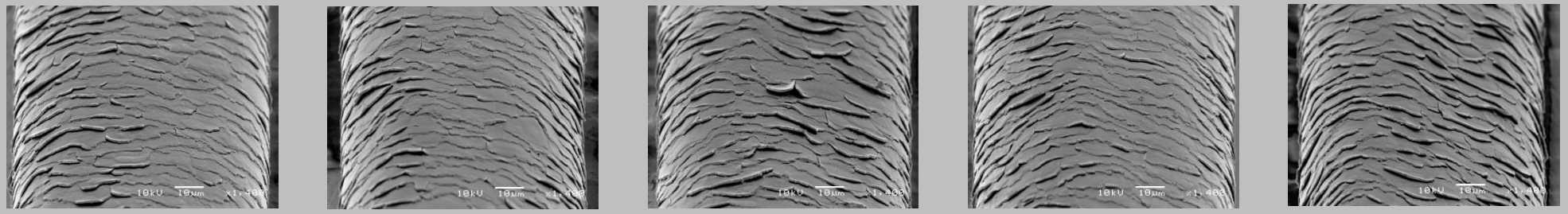
✓ Pictures of representative volunteers

✓ Improvement of texture
(reduction of damaged area and friction force)

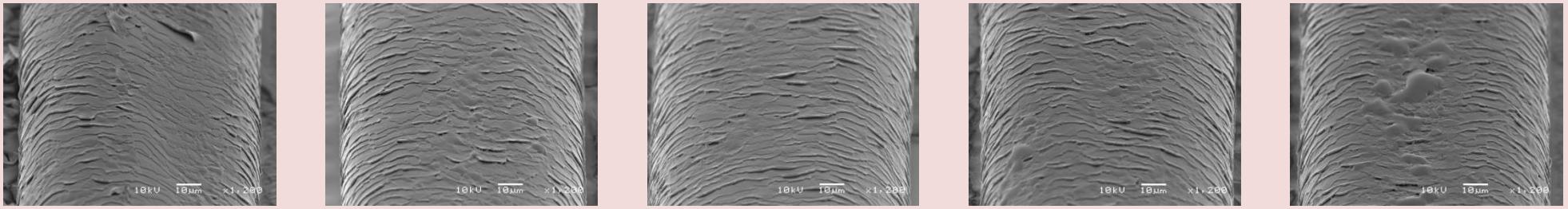
✓ Improvement of color (L* and b* value)

Representative pictures (SEM, volunteer A)

Before application



4 weeks after application of Peach Leaf Extract

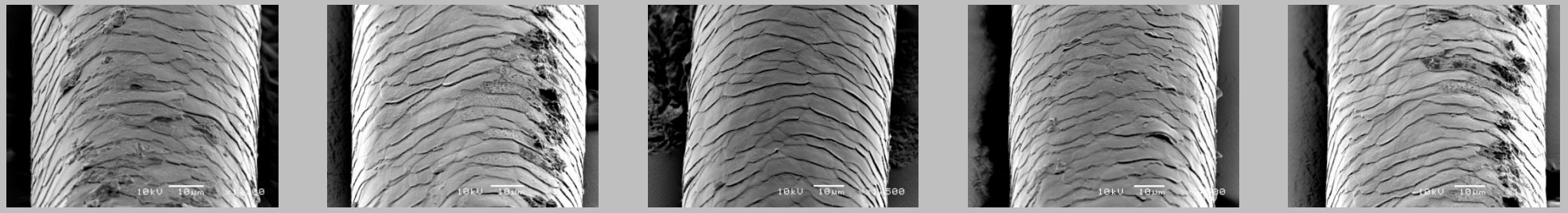


By application of lotion containing Peach Leaf Extract BG30-06 (Yamanashi), texture of cuticles and surface condition of hair were improved according to SEM picture observation

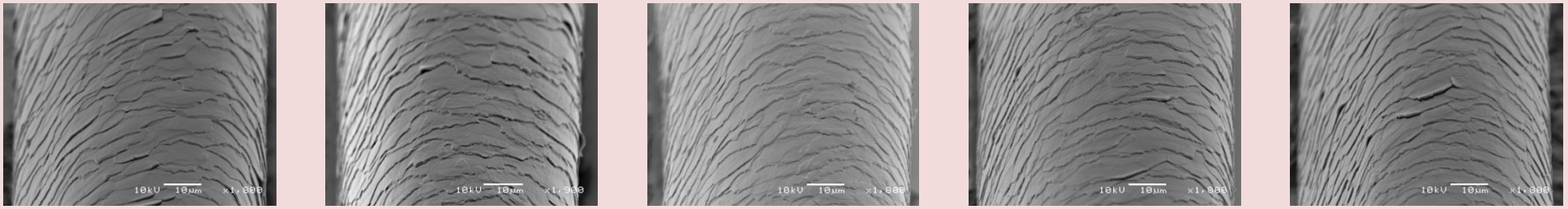
Randomly taken hair of from volunteers

Representative pictures (SEM, volunteer B)

Before application



4 weeks after application of Peach Leaf Extract

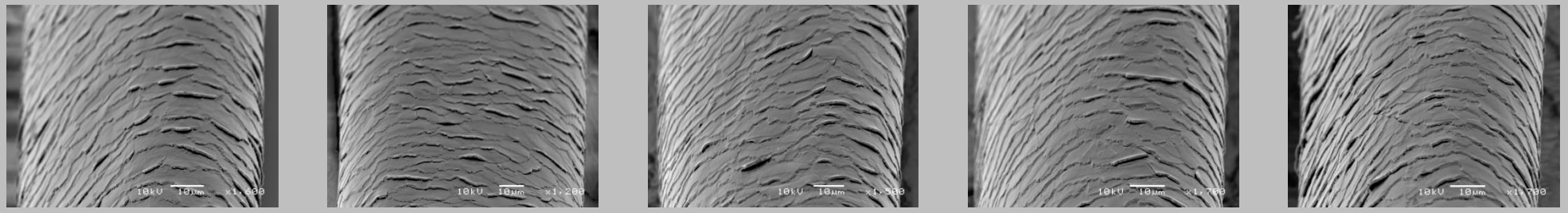


By application of lotion containing Peach Leaf Extract BG30-06 (Yamanashi), texture of cuticles and surface condition of hair were improved according to SEM picture observation

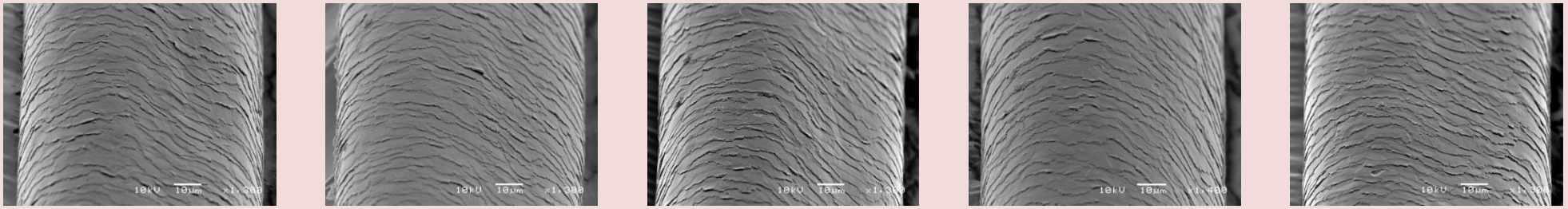
Randomly taken hair of from volunteers

Representative pictures (SEM, volunteer C)

Before application



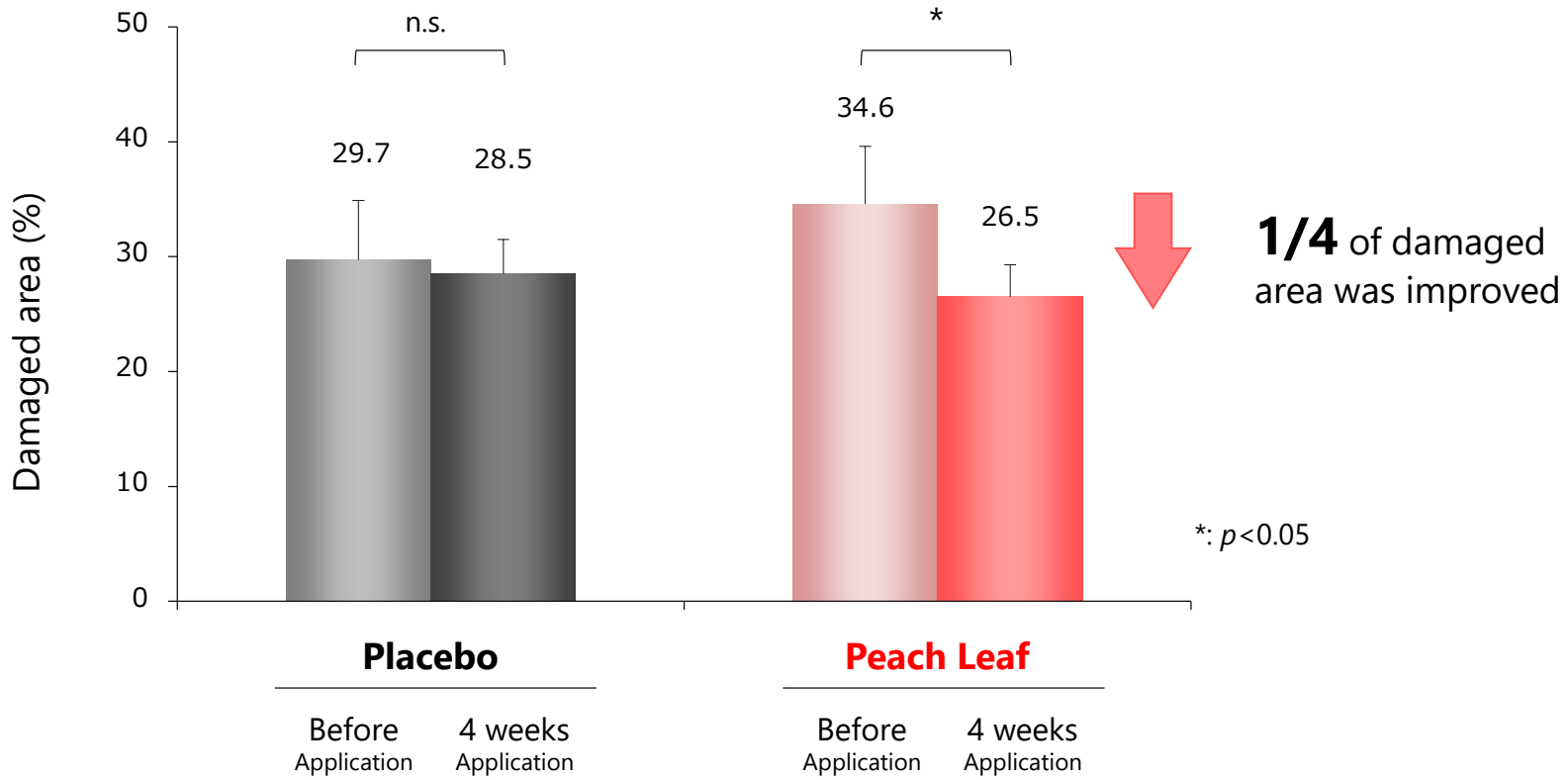
4 weeks after application of Peach Leaf Extract



By application of lotion containing Peach Leaf Extract BG30-06 (Yamanashi), texture of cuticles and surface condition of hair were improved according to SEM picture observation

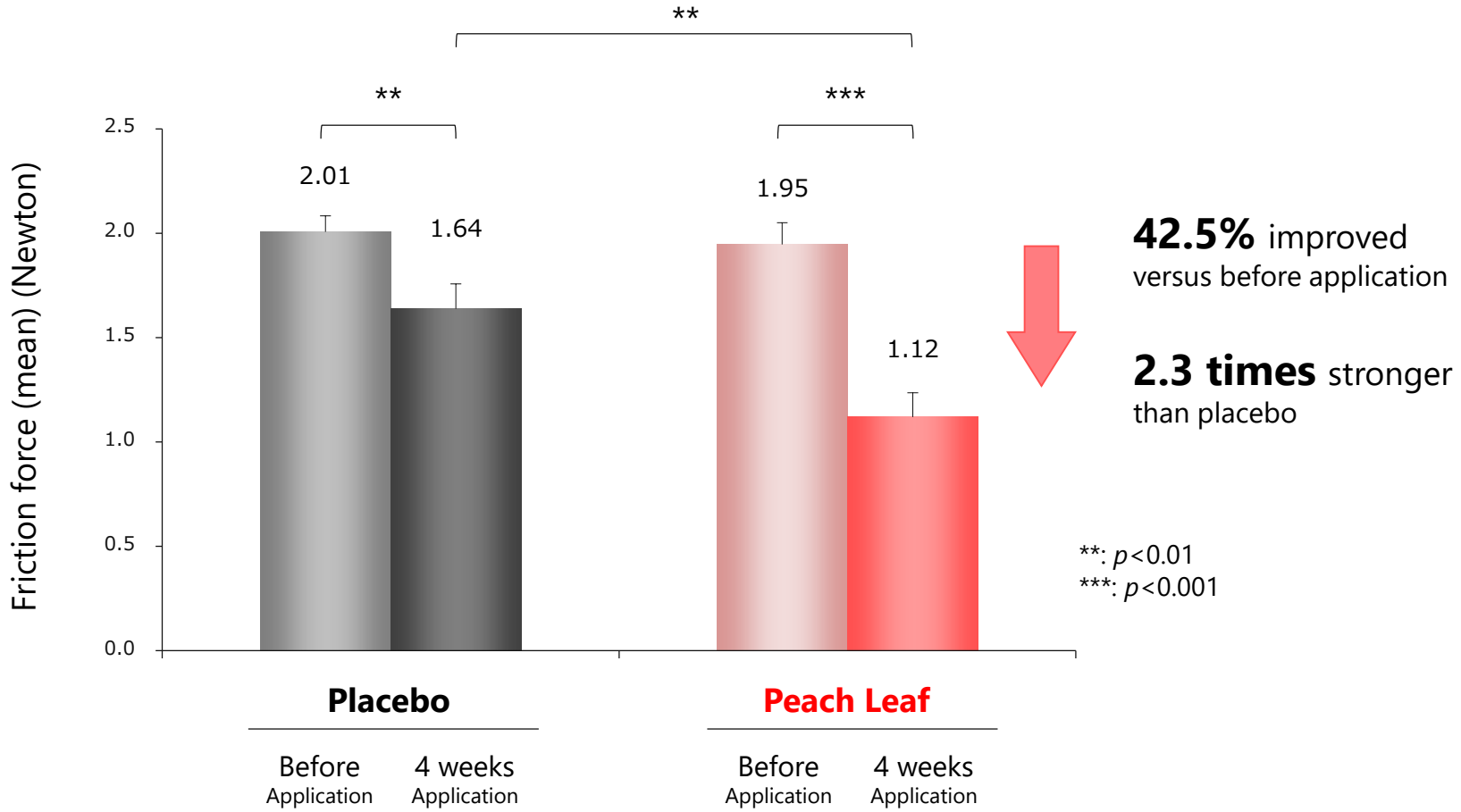
Randomly taken hair of from volunteers

1) Reduction of damaged area of hair



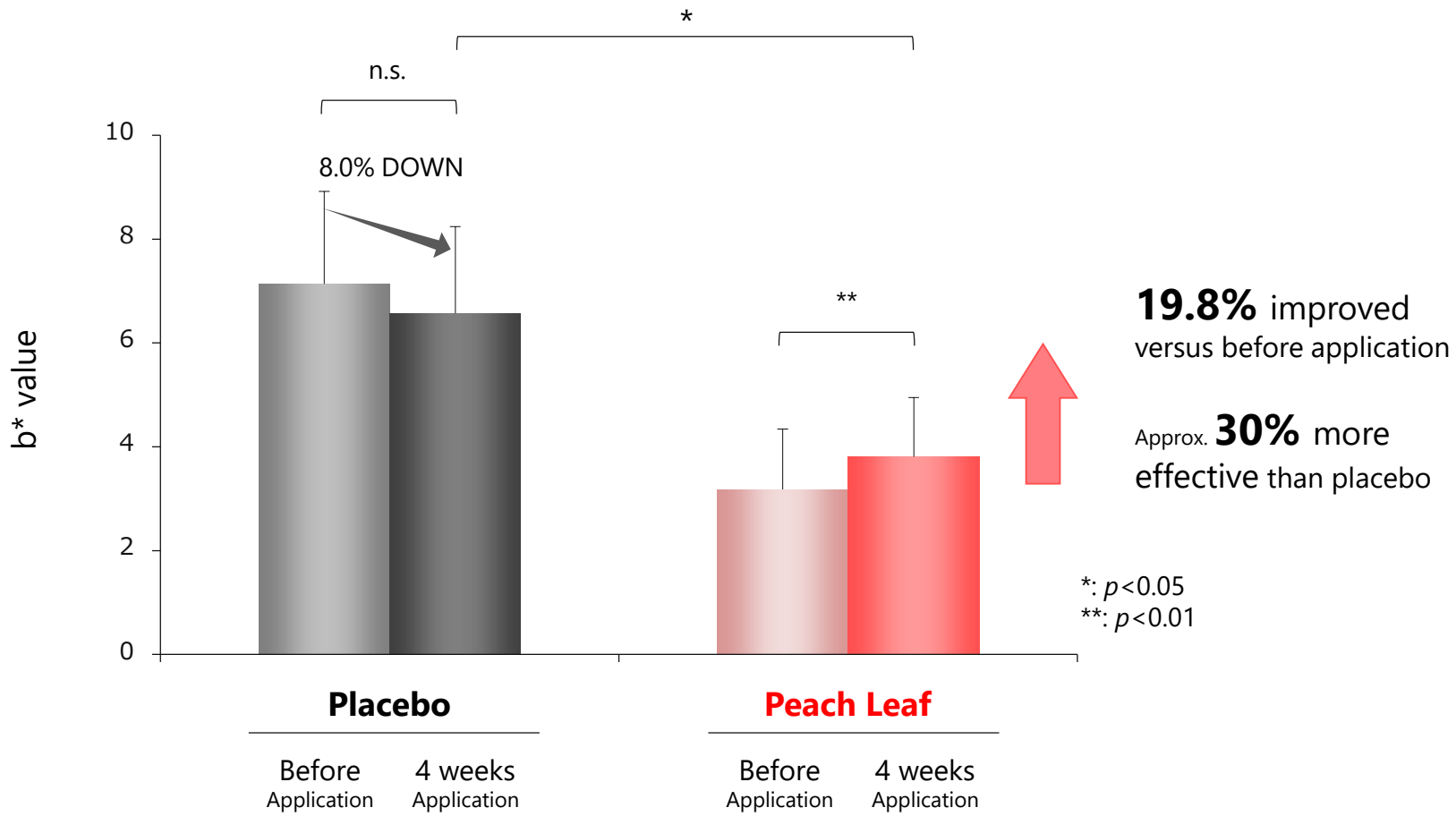
Damaged area of hair was significantly decreased by 4 weeks application of lotion containing Peach Leaf Extract BG30-06 (Yamanashi).

2) Reduction of friction force of hair



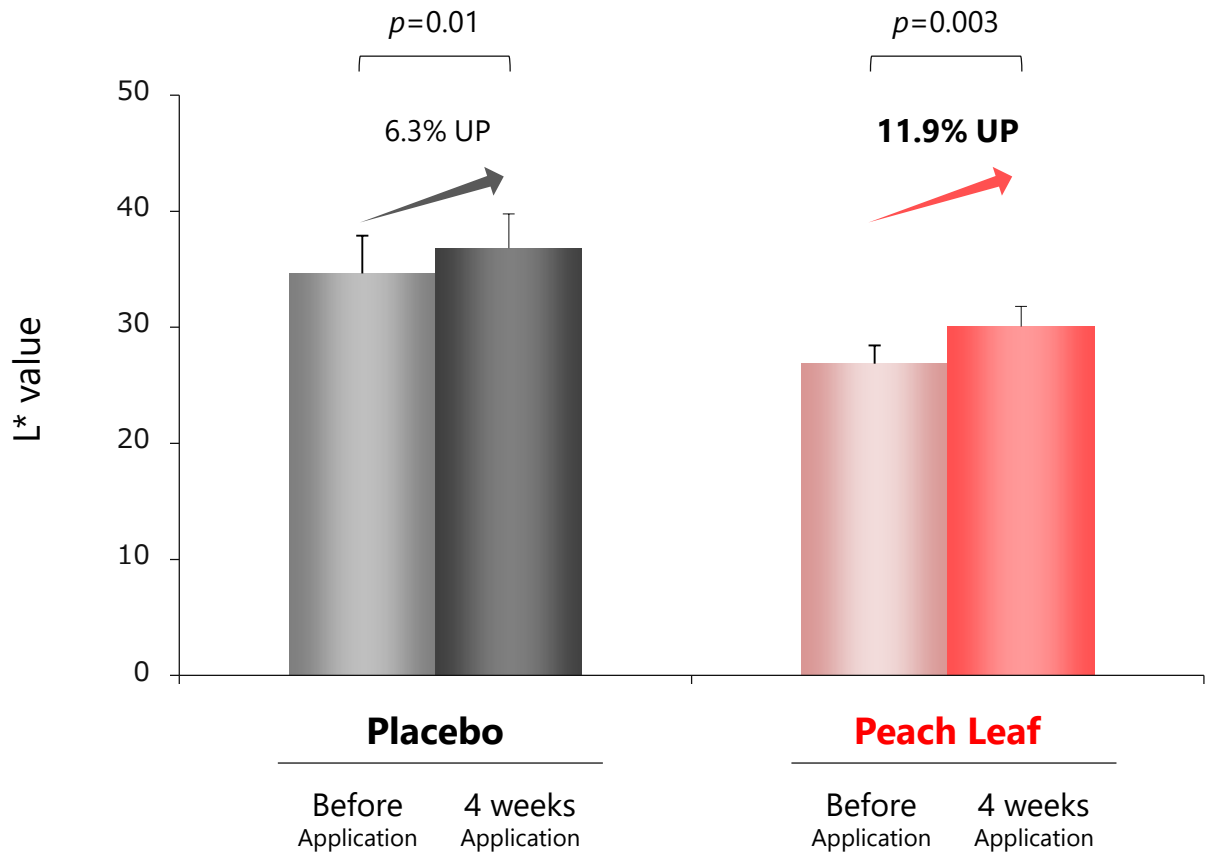
Friction force, an indicator of roughness and unsmoothness, of hair was significantly decreased by application of lotion containing Peach Leaf Extract BG30-06 (Yamanashi), in comparison with the placebo application.

3) Improvement of vividness of color of hair



Vividness of hair color was significantly improved by application of lotion containing Peach Leaf Extract BG30-06 (Yamanashi) and a deterioration was observed by placebo application.

4) Improvement of luminosity of hair



Application of lotion containing Peach Leaf Extract BG30-06 (Yamanashi) showed a stronger improvement effect of hair luminosity compared with placebo application

Representative pictures (back of head, volunteer A)

Before application

4 weeks after application of Peach Leaf Extract

Visible differences in hair condition, such as manageability, luster, and texture were observed

Summary

Background and efficacies of Peach Leaf Extract BG30-06(Yamanashi)

- ✓ Peach has unique cultural background in Asia and has been traditionally used as a remedy
- ✓ Maruzen adopted peach leaf harvested at the slopes of Mt Fuji for as the raw material for the extract
- ✓ Maruzen's Peach Leaf Extract prohibited chlorine-induced oxidative damage and reduction of Keratin related genes expression
- ✓ Clinical trail with volunteers who do pool workouts regularly revealed that Peach Leaf Extract BG30-06(Yamanashi) significantly improved the condition of their skin and hair, frequently exposed to chlorine

MARUZEN
PHARMACEUTICALS CO.,LTD.

Thank you for your attention

MARUZEN PHARMACEUTICALS CO.,LTD.

14703-10 MUKAIHIGASHI CHO ONOMICHI CITY HIROSHIMA 722-0062 JAPAN
TEL: 81-848-44-2200 FAX: 81-848-20-6114
http://www.maruzenpcy.co.jp/english/

Consumer surveys for chlorine and pool workout

Conducted in: January, 2019

Conducted by: Maruzen Pharmaceuticals / out-sourcing research company

Method: Internet survey

Subjects:

Primary survey: 5,021 (M: 2,384, F: 2,637)(age:20s-40s)
Secondary survey : 100 (M : 55, F: 45)

* Secondary survey was conducted to subjects who go to pool more than once per a month according to primary survey result.

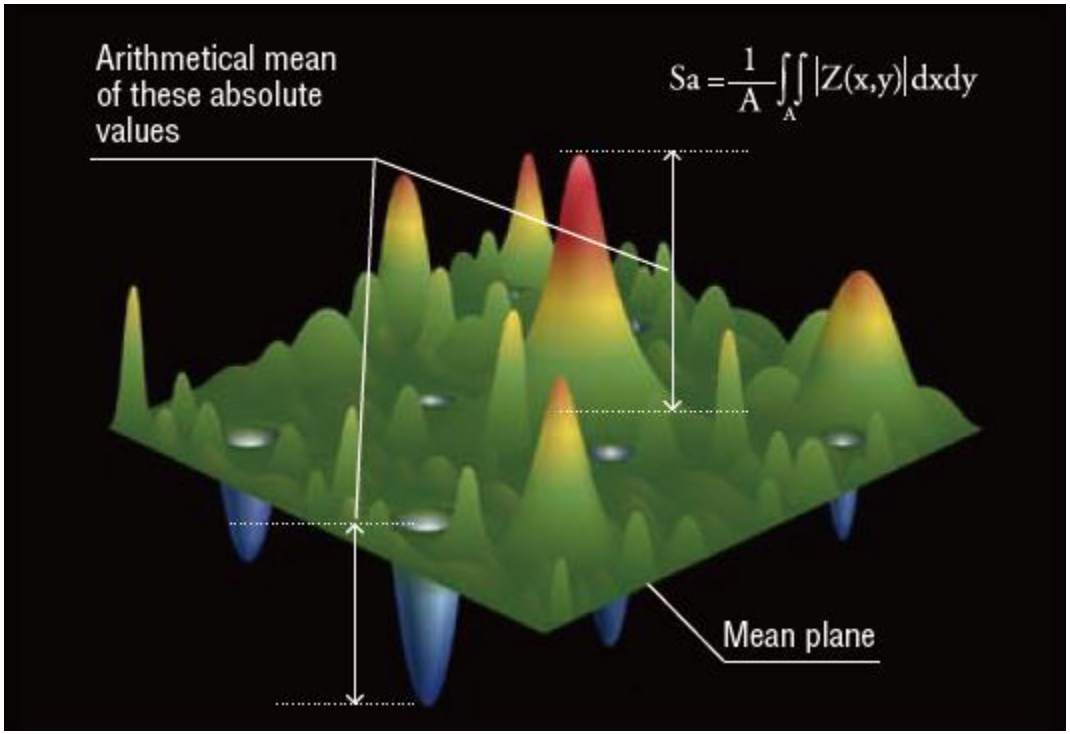
Supportive efficacies of Peach Leaf Extract BG30-06 (Yamanashi)

- ✓ Promotion of SPT mRNA expression (ceramide production)
- ✓ Promotion of Profilaggrin mRNA expression (NMF Production)
- ✓ Promotion of Transglutaminase-1 production (CE formation)

Sa (arithmetical mean height)

Sa is the extension of Ra (arithmetical mean height of a line) to a surface.
It expresses, as an absolute value, the difference in height of each point compared to the arithmetical mean of the surface.
This parameter is used generally to evaluate surface roughness

https://www.keyence.com/ss/products/microscope/roughness/surface/parameters.jsp



Life & Style / Health

Yes, you can put too much chlorine in a pool. Here's what to know.

Chlorine is a pesticide used in pools to destroy germs, but excessive exposure can cause health problems, experts warn. (Getty Images)

HealthDay

JUNE 6, 2018, 5:00 AM

記事のポイント

✓ Over the past 10 years, more than 500 people in California have been exposed and sickened by too much chlorine while swimming, according to the California Department of Pesticide Regulation (DPR).

カリフォルニアでは過去十年間で500人以上が遊泳中の過剰な塩素曝露による疾病に陥ったと報告されている。

✓ More than half of those affected were at public pools, and about 66 percent of the incidents weren't caused by faulty equipment.
"Whether you are a homeowner with a pool or you run a public water park or community pool, you must follow all of the manufacturer's instructions and not exceed the amount of chlorine specified," said Brian Leahy, director of the DPR.

事故の多くはパブリックプールで発生しており、66%は機器の故障によるものではない。
当局者コメント：「決められた塩素量を超えないよう、製造者の取扱説明書の記載に従ってほしい」

http://www.chicagotribune.com/lifestyles/health/sc-hlth-too-much-chlorine-dangers-0613-story.html

Representative result (back of head, volunteer D)

Before application

4 weeks after application of Peach Leaf Extract

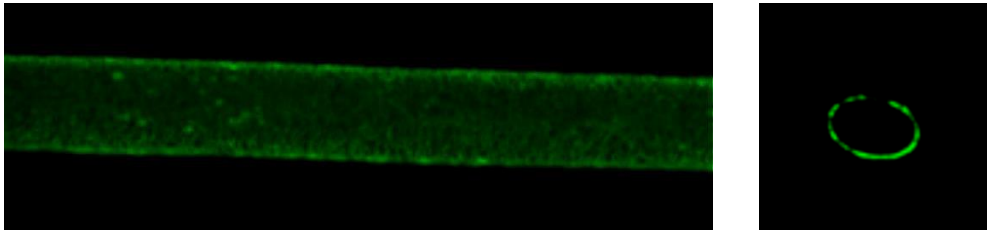
Visible differences in the hair condition, such as manageability, luster, and texture were observed

Inhibition of protein carbonylation on hair

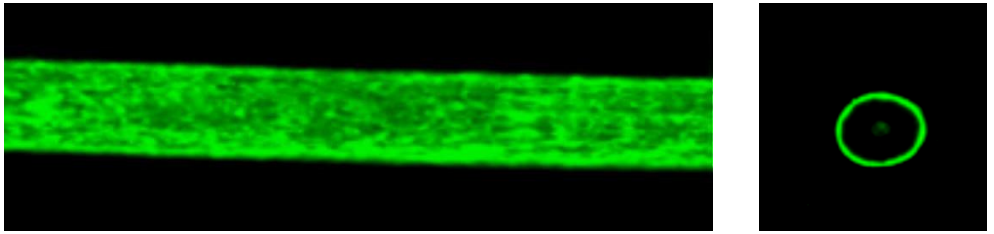
Test method

Human hair → Soak in solution containing chlorine and test sample → 2 hours→ Stain Carbonylated proteins

Chlorine (-)



Chlorine (+)



Chlorine (+)
+
1% Peach Leaf Extract
BG30-06(Yamanashi)