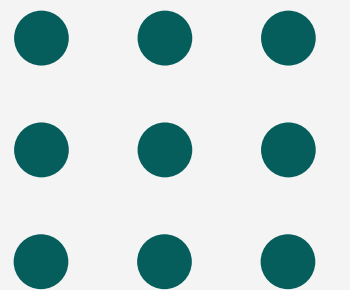# Well Shine® Biopolymer
Chitin/Chitosan

Well Shine Biotechnology Development Co., Ltd

What is Chitin / Chitosan

Chitosan, also known as chitin, poliglusam, or deacetylchitin, is produced through the deacetylation of chitin. It is a polymerized linear polymer compound composed of glucosamine and N-acetylglucosamine, connected by β-1,4 glycosidic bonds. Generally, materials that achieve a certain degree of deacetylation and are soluble in dilute acid solutions are referred to as chitosan.

1
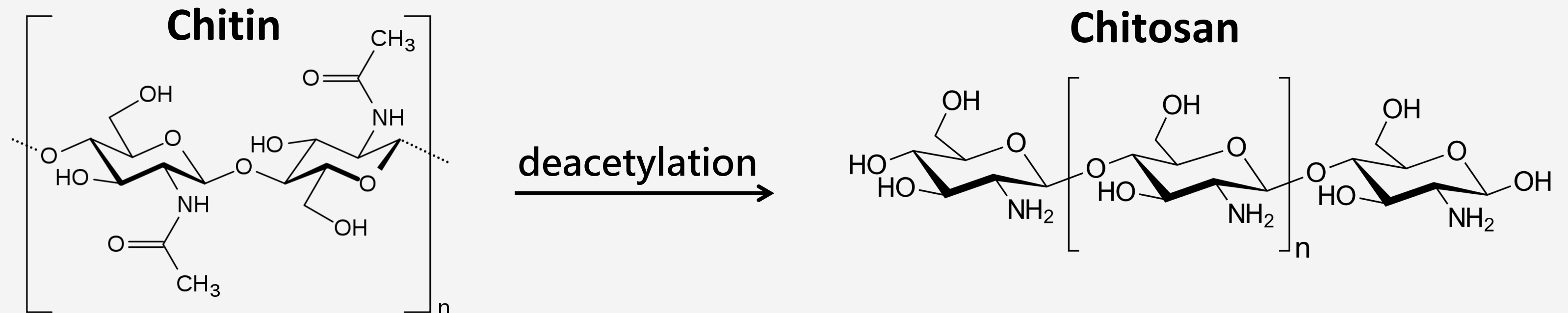
Applications of Chitin / Chitosan

Cosmetics

Chitosan has a moisturizing effect and finds widespread usage in facial masks, lotions, hair products, hair dyes, and coagulants.

Medical

1. Excellent hemostatic function
2. Promote wound healing
3. Utilized in drug transportation
4. As medical materials, such as surgical sutures

Supplements

1. Food Processing

Characteristics such as being non-toxic, odorless, bioadhesive, and biodegradable make it suitable for use as functional ingredients, thickeners, and stabilizers. Additionally, its antibacterial properties make it an ideal material for food packaging.

2. Health Supplements

1) Improve digestion
2) Reduce high blood pressure
3) Lower fat and cholesterol levels
4) Reduce the accumulation of heavy metals in the body

Industry

Chitosan possesses characteristics such as strong hygroscopicity, heavy metal absorption, and biodegradability, which make it a valuable material for wastewater treatment. Additionally, it can be applied in the textile industry to enhance its antibacterial effects.

Agriculture

1. The inducer of antibacterial substances produced by plants
2. Seed dressing or sanitizing
3. Increase the quality of the product after harvest
4. Soil amendments

2

Chitin / Chitosan

one of the most abundant organic substances in nature, with a storage capacity second only to cellulose.

Chitin is widely found in shrimp and crab shells, mollusk exoskeletons, cell walls of fungi, and arthropod exoskeletons. Its structure is similar to cellulose, making it the second most abundant polysaccharide in nature.

Mollusk exoskeletons

Shrimp & crab shells

Cell walls of fungi

Insect exoskeletons
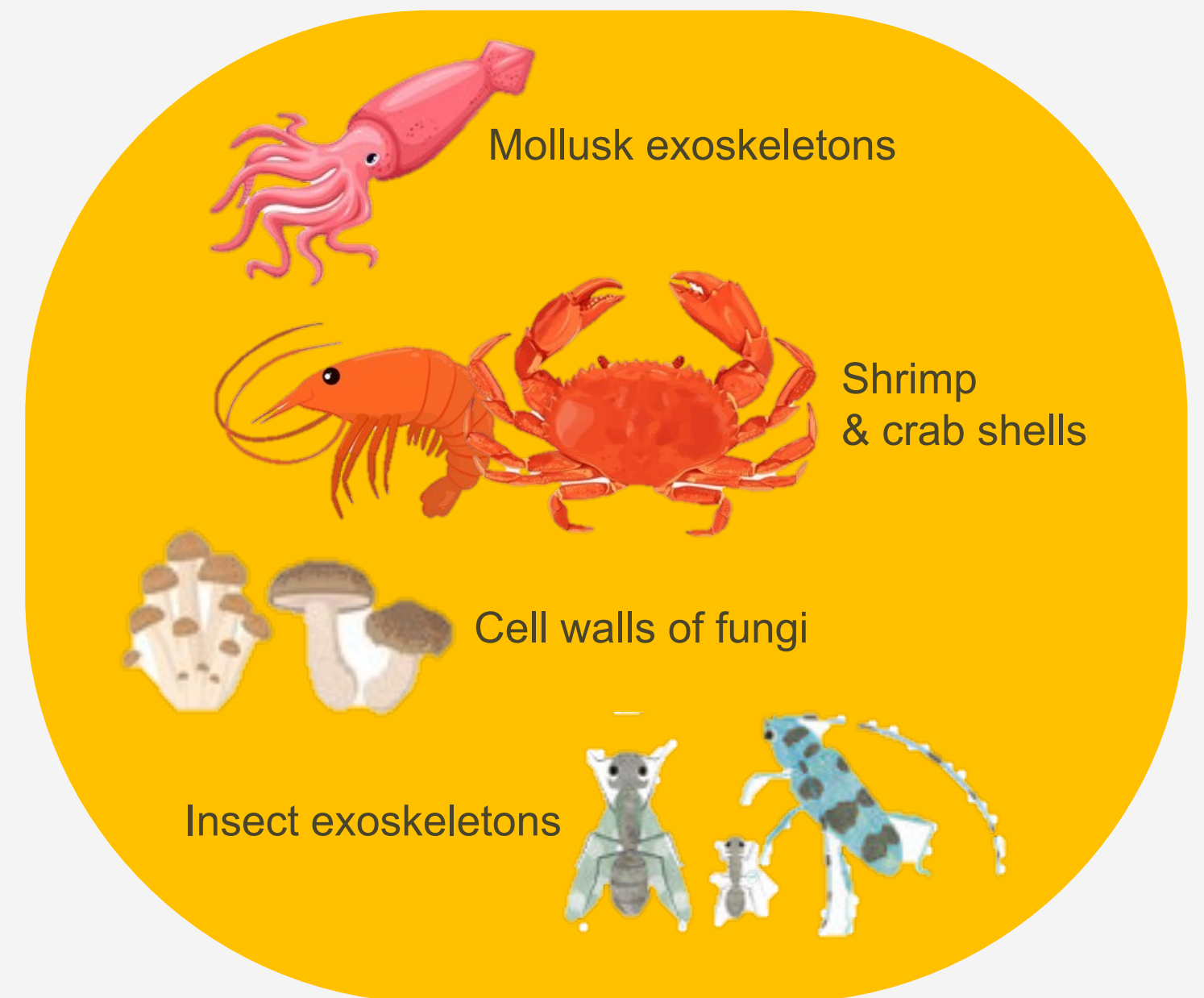
Animal Origin Chitin / Chitosan

Most of the chitin sold on the market is produced from shrimp and crab shells through the following steps: Collecting and cleaning the shells. → Using acid to remove calcium carbonate. → Using alkali to eliminate protein and fat.

Doubts：

Protein residue

Remnants of marine Pollution

Lack of traceability and quality control

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Mushroom origin Chitin / Chitosan

Botanical Origin Derived Chitosan Well Shine® Biopolymer

1. No risk of shrimp/crab shell protein allergies.

2. Derived from environmentally controlled mushrooms.

3. Free from pesticide and heavy metal pollution, and avoids the residue of environmental pollutants in the ocean.

4. Can reduce the need for chemicals in extraction and purification processes.

5. Product batches are consistent, and the quality is stable.

Well Shine Ganoderma Lucidum & Antrodia Camphorata

Antrodia Camphorata

Ganoderma Lucidum

◉ 100% made in Taiwan from pure fruiting bodies.

◉ The environmentally controlled cultivation farm employs strict cultivation techniques to prevent mosquito & germ pollution.

◉ Naturally cultivated without the use of pesticides.

Well shine® Biopolymer material offers consistent quality, is free from contamination, and is highly valuable.

Well shine® Biopolymer is extracted from the precious fruiting bodies of mushrooms and not only contains Chitin/Chitosan but also abundant mushroom β-glucan (β-1,3-1,6-glucan).
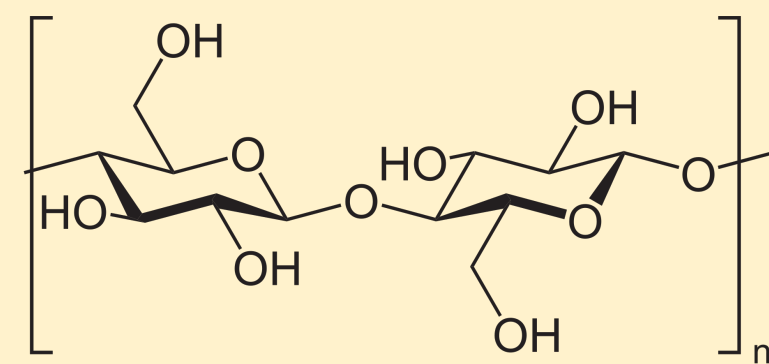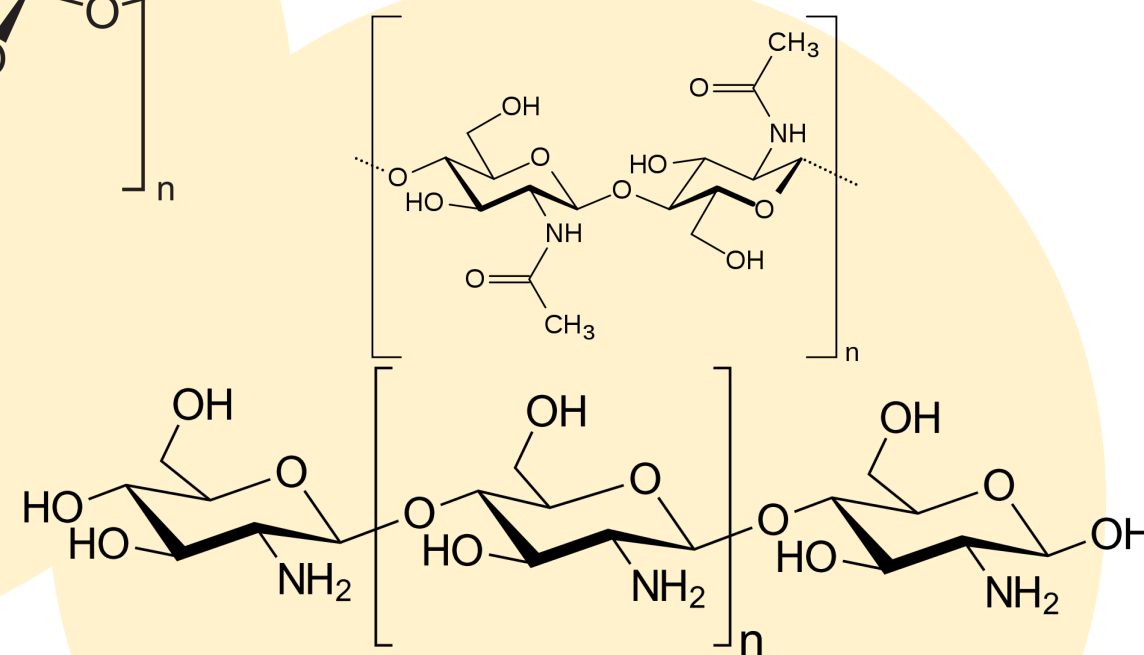
β-glucan

Chitin/Chitosan

Mushroom β-glucan Benefits:

Activates immune cells

β-glucan also reduces oxidative stress and activates immune substances called macrophages, which destroy potential invaders such as viruses.

Lowers cholesterol and helps alleviate the rise in blood sugar after meals:

According to research cited in 《Today's Dietitian Magazine》, a daily intake of 3 grams of β-glucan from either oats or barley can reduce blood cholesterol levels by 5 to 8%, and patients with high cholesterol levels are likely to see the greatest benefit.

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How to produce Botanical Origin Derived Chitosan ?

Health Supplements

Two-stage patent extraction

Triterpenoids

Polysaccharides

Ganoderma Lucidum & Antrodia Camphorata fruiting bodies

Alcohol extraction

Hot water extraction

The substrate after extractions

Well Shine® Biopolymer

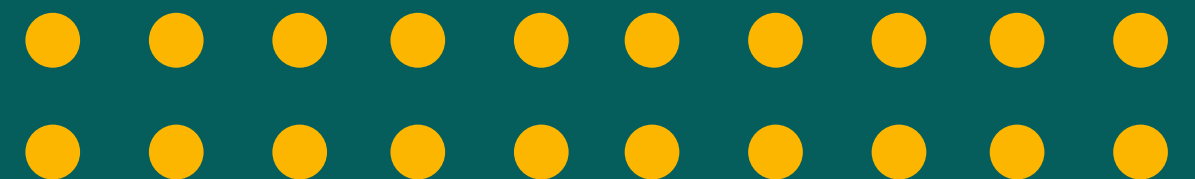**Washing** ④

Modified ③

Blenching ②
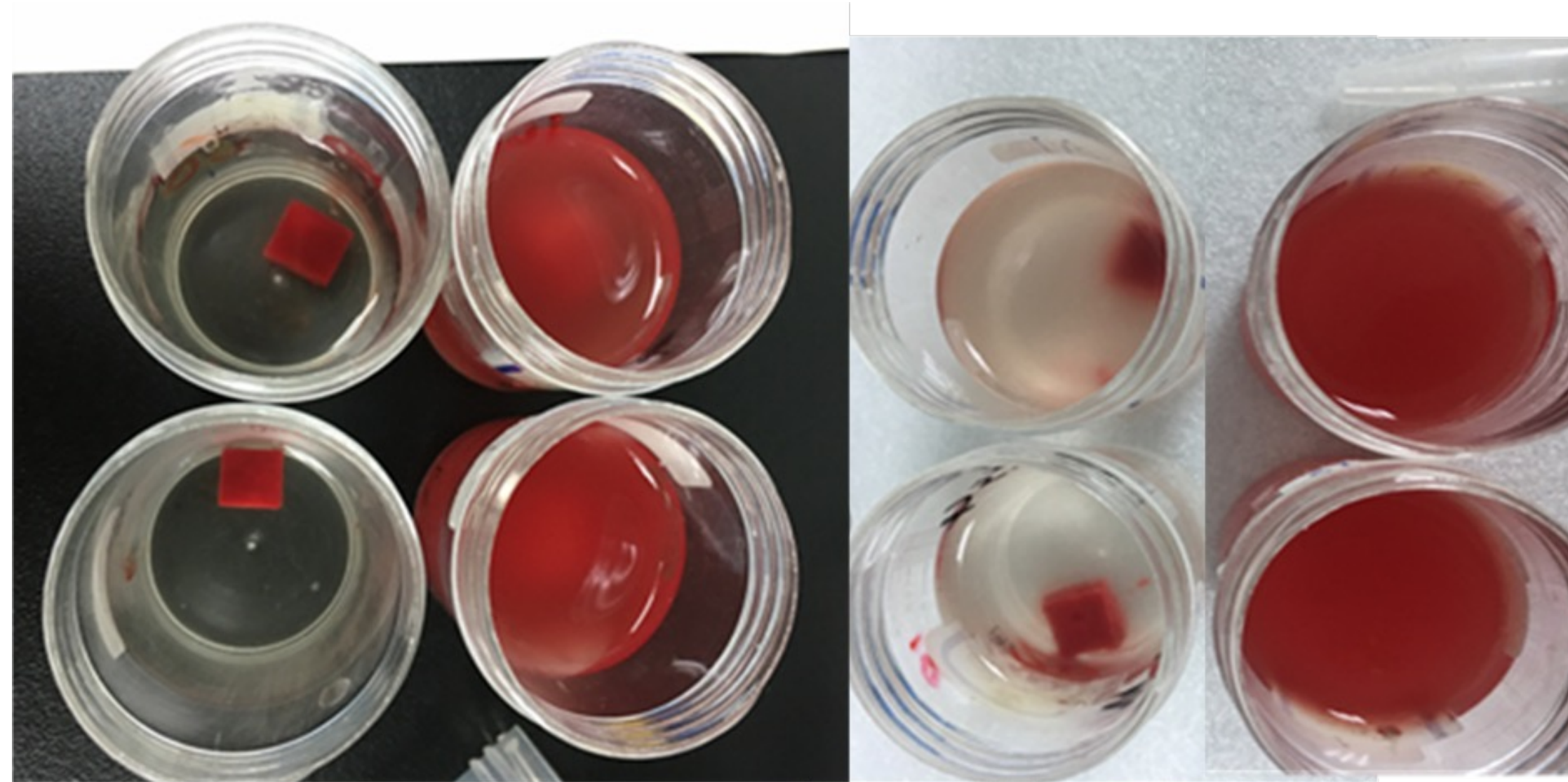
Protein removal ①

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Well Shine® Biopolymer Botanical Origin Derived Chitosan

- ✓ **Blood Clotting Index (BCI)**

- ✓ **Antibacterial Test**

Dressing Blood Clotting Index Test

Well Shine®
Biopolymer-DC

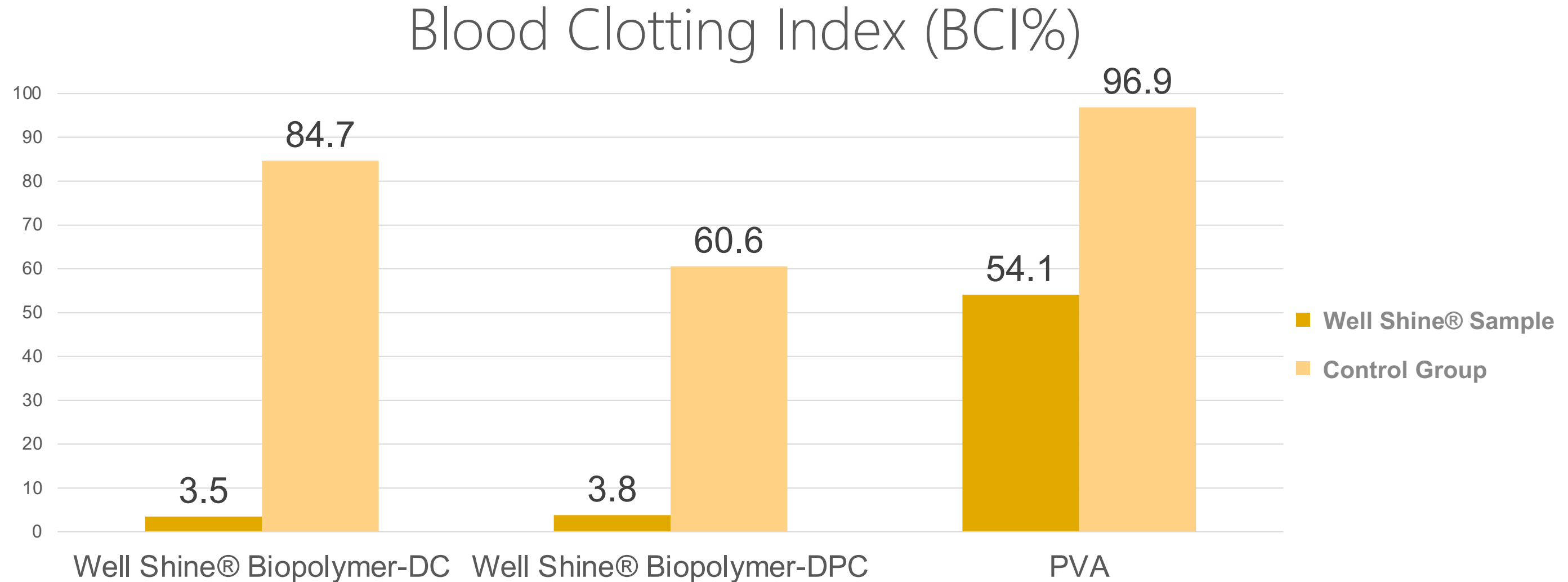Control Group

Well Shine®
Biopolymer-DPC

Control Group

After using Well Shine® Biopolymer to create dressings, cut them into a specific size and add blood. Let them stand for 5 minutes, then add a PBS solution in an amount 50 times that of the test blood sample. Shake the mixture at 100 rpm for 2 minutes and observe whether the blood redissolves into the PBS solution.

Dressing Blood Clotting Index Test

Blood Clotting Index (BCI)

$$= \frac{\text{Optical Density (OD) Sample}}{\text{Optical Density (OD) Control}} \times 100$$

Blood Clotting Index (BCI%)

Blood Clotting Index (BCI%) of Well Shine® Biopolymer and PVA foam products were measured. Both the control group and the test sample used gauze with the same weight.

With Well Shine® Biopolymer, the BCI is below 20, indicating a good coagulation effect. 11

Antibacterial Test

Referring to the method outlined in JIS Z2801:2010, we have made modifications. Antimicrobial activity (%) = (number of bacteria in the control group - number of bacteria in the experimental group) / number of bacteria in the control group × 100.

Antimicrobial activity(%)	
Strain category/Sample	0.1% DPC
Pseudomonas aeruginosa	99.99 %
Bacillus subtilis	92.33%
Candida albicans	90.19%

The antimicrobial activity against Pseudomonas aeruginosa, Bacillus subtilis, and Candida albicans are all over 90%.

Antibacterial Test

Refer to JIS L1902:2015 for the quantitative test using the bacterial liquid absorption method.

Test Item	Test Result	
	Antibacterial activity (A)	
	DPC	DC
Staphylococcus aureus (ATCC)6538	5.6	5.6

An antibacterial activity >2.0 indicates that the raw material has a good antibacterial effect on Staphylococcus aureus.

Botanical Origin Derived Chitosan Well Shine® Biopolymer

✓ **ISO 10993-5 Cytotoxicity Test**

✓ **in vitro Skin Irritation Test**

✓ **in vitro Eye Irritation Test**

✓ **The Skin Sensitisation Test**
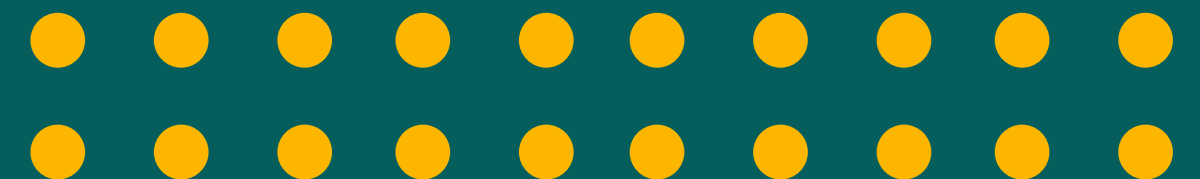
The Cytotoxicity Test : Qualitative Experiment (Observation of Cell Appearance)

Refer to ISO10993-5:2009 regulation, the cytotoxicity test was conducted on human fibroblast Hs68 cells using the sterilized and centrifuged DPC sample at 4000 rpm for 20 minutes, with the supernatant taken as 100%. The cells were treated with different concentrations for 24 hours.

A. Negative Control Group

B. 10% (v/v) DMSO

C. 20%

D. 40%

E. 60%

F. 80%

G. 100%
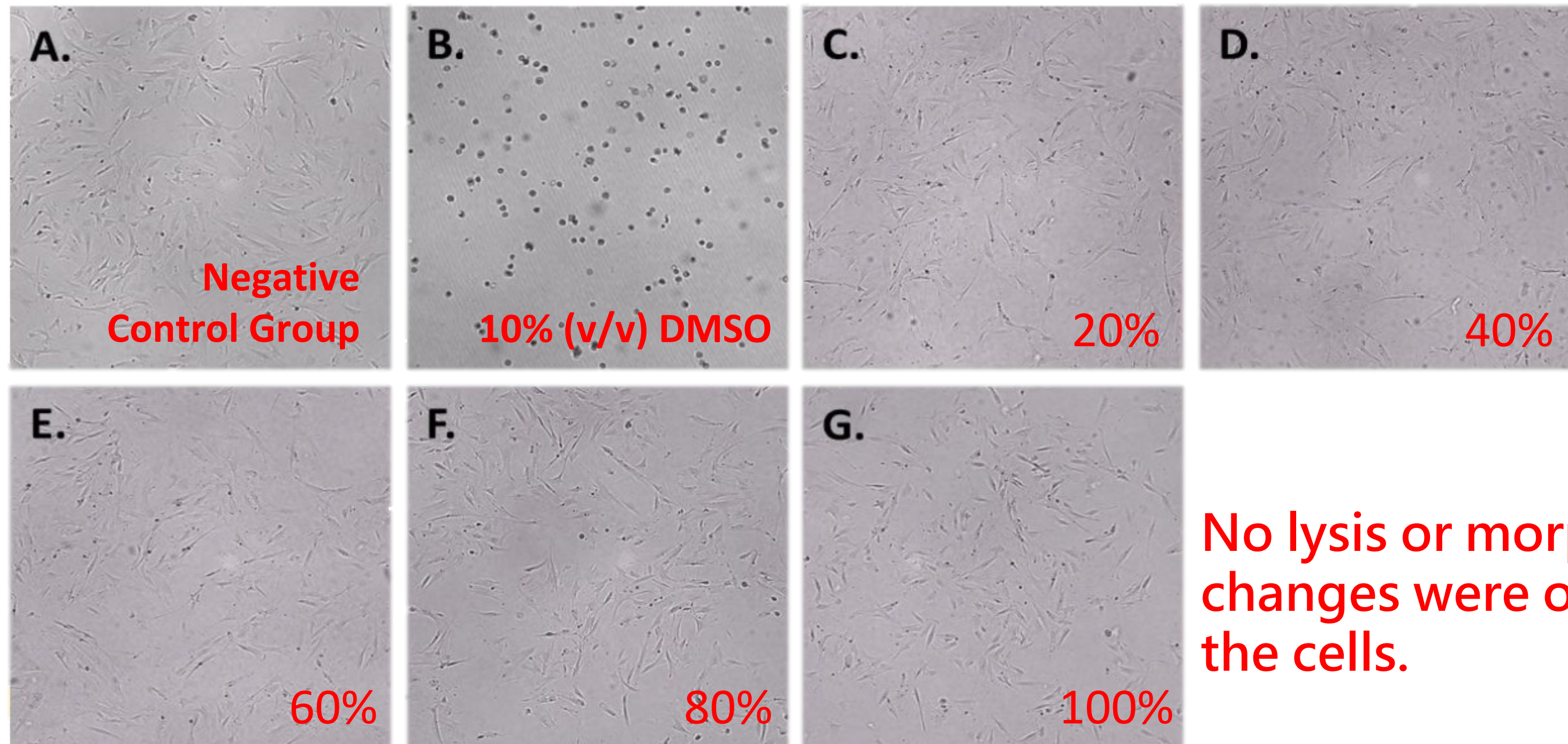
No lysis or morphological changes were observed in the cells.

The Cytotoxicity Test : Quantitative Test (**MTT assay**)

MTT Quantitative Test
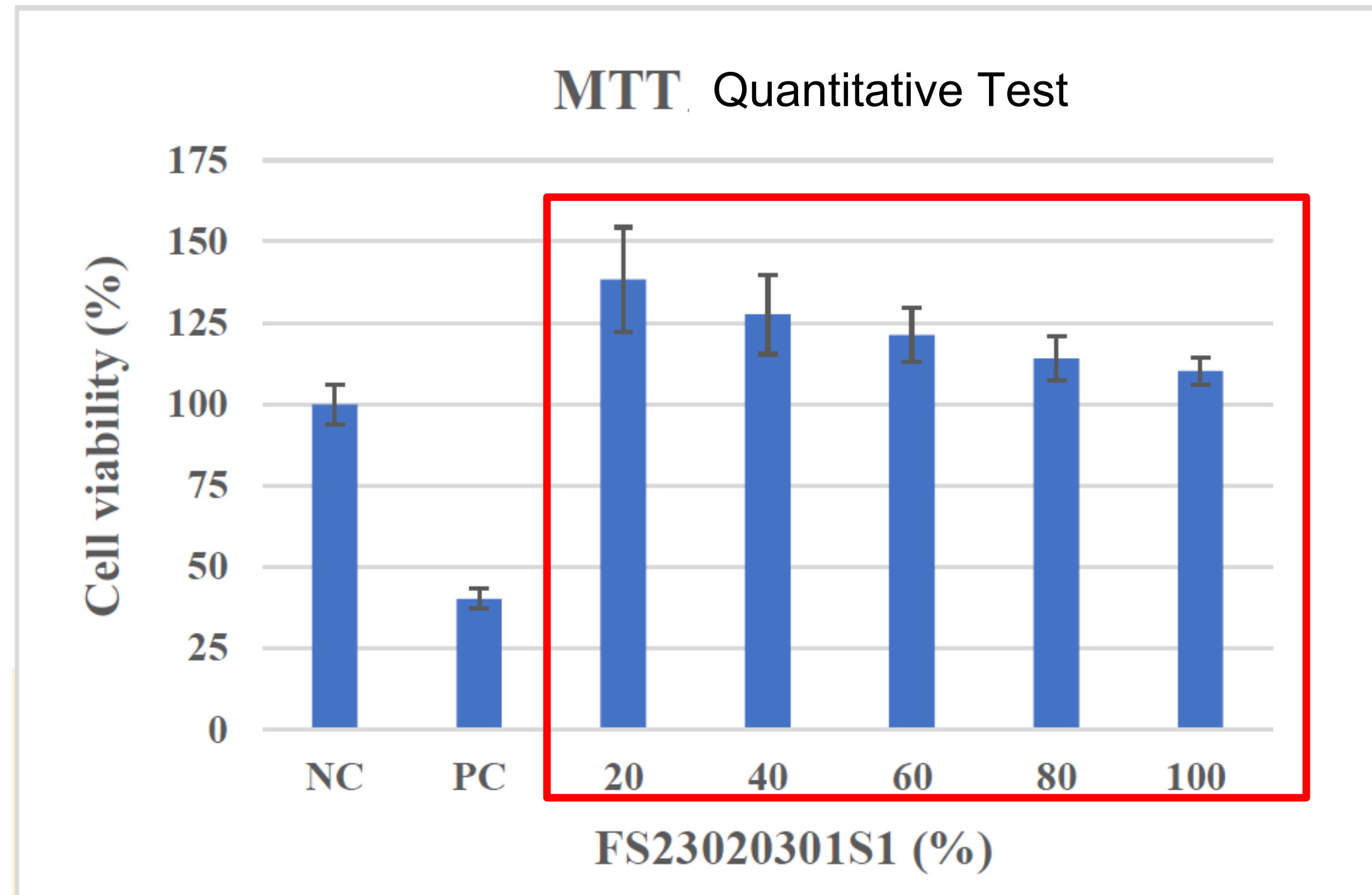
$$\text{Cell activity (\%)} = \frac{\text{Absorbance}_{\text{Test Group}}}{\text{Absorbance}_{\text{Control Group}}} \times 100$$

The cell viability is greater than 100% at different dilutions.

ISO 10993-5 Cytotoxicity Test

Sample	Dilutions (%)	Cell Morphology	Cell Viability	Cytotoxicity grade
NC	-	No lysis or morphological change	100 ± 6.1	0
PC	10% DMSO	Severe dissolution & deformation occur	40 ± 3.1	4
DPC	20	No lysis or morphological change	138 ± 16.1	0
	40		128 ± 12.2	0
	60		121 ± 8.3	0
	80		114 ± 6.9	0
	100		110 ± 4.3	0

The results of this experiment showed no obvious cytotoxicity.

in vitro Skin Irritation Test

OECD TG 439

in vitro Skin Irritation: Reconstructed Human Epidermis (RhE) Test Method

Sample	Concentration	Cell Viability	Model Prediction	Result
Negative Control Group	DPBS	100% ± 1.1	(NI, Non-irritant)	Non-irritant
Positive Control Group	5% SDS	1.8% ± 0.1	Irritant	Irritant
Test Group	5% DPC	87.8% ± 0.8	(NI, Non-irritant)	Non-irritant

OECD TG 439 Skin Irritation Test Result Benchmark Table

in vitro result	*in vivo* prediction
mean tissue viability ≤ 50%	GHS Category 2: Irritant
mean tissue viability > 50%	No Category: Non-irritant (NI)

The results of this experiment showed that the skin irritation was non-irritating.

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in vitro Eye Irritation Test

OECD Test No. 492
in vitro Eye Irritation: Reconstructed Human Cornea Epithelium (RhCE) Model
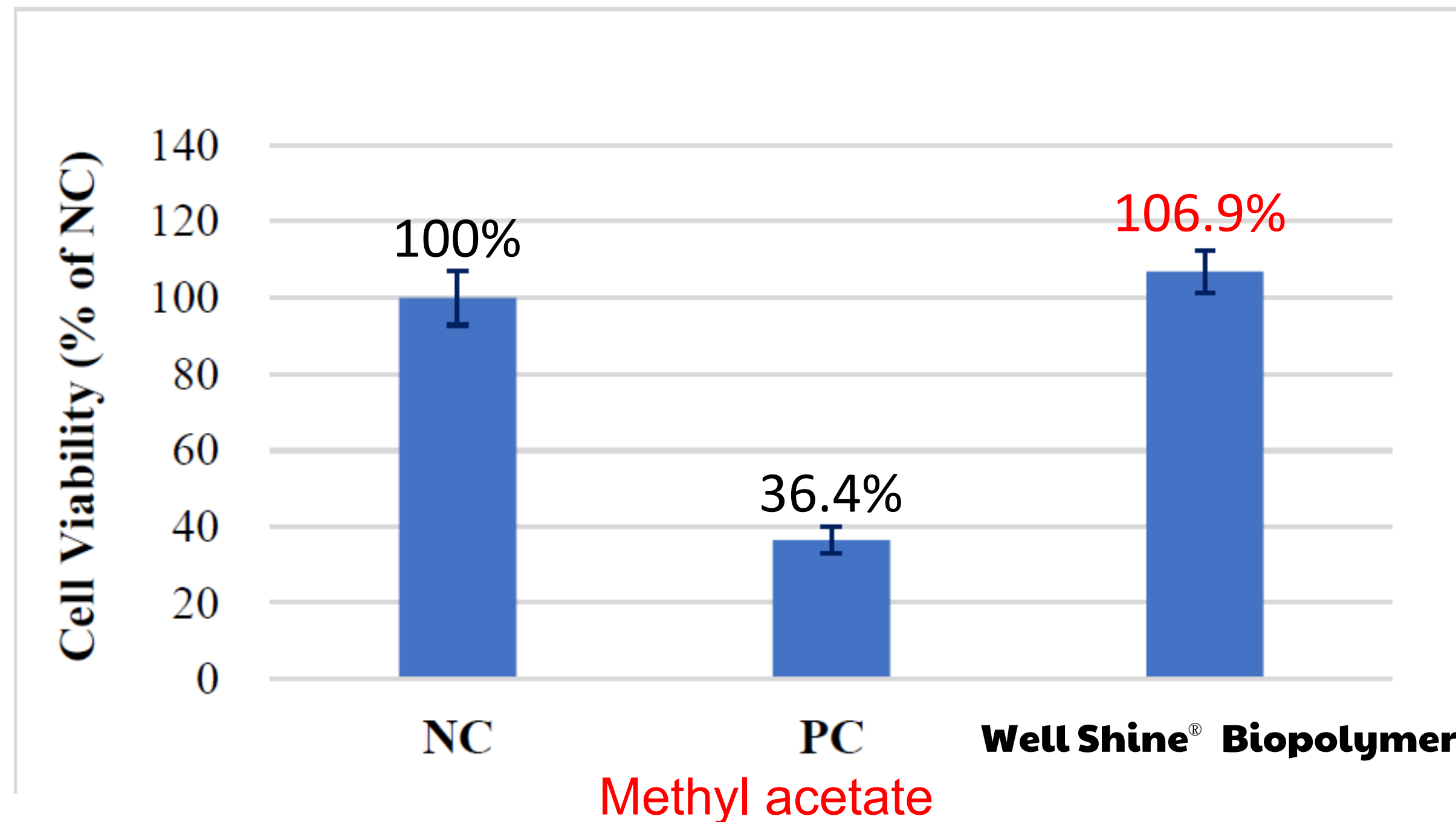
The results of this experiment showed no eye irritation.

The Skin Sensitisation Test

OECD 442C Skin Sensitisation Test Guideline

Sample	Concentration	Lysine peptide depletion (%)	Cysteine peptide depletion (%)	Peptide Average depletion (%)	Sensitisation Level
Cinnamicald ehyde	100 mM	40.99	71.24	56.11	Sensitiser (High Reactivity)
Lactic acid	100 mM	0.01	0.00	0.00	Non-sensitiser (Minimal Reactivity)
Well Shine® Biopolymer	**5 %**	**0.57**	**4.60**	**2.59**	**Non-sensitiser (Minimal Reactivity)**

OECD TG 442C Skin Sensitisation test model judged the result in the non-sensitisers category.

in vitro Anti-Inflammatory Test - TNF-a

Determination of *In Vitro* TNF-*a* Inflammatory Factor Content:
Raw264.7 Test Method for Lipopolysaccharide-Induced Macrophages

Sample	Concentration	IL-6 Expression (pg/mL)	% of Inhibition
Control Group	-	1.00±0.08 pg/mL	-
LPS Group	0.1 µg/mL	1.75±0.06 pg/mL	-
Dex Group*	1 µM	0.63±0.02 pg/mL	64%
	20%	1.85±0.07 pg/mL	no inhibition
Well Shine® Biopolymer* (FS23032401S1)	40%	1.05±0.03 pg/mL	40%
	60%	0.56±0.03 pg/mL	68%
	80%	0.28±0.02 pg/mL	84%
	100%	0.28±0.02 pg/mL	84%

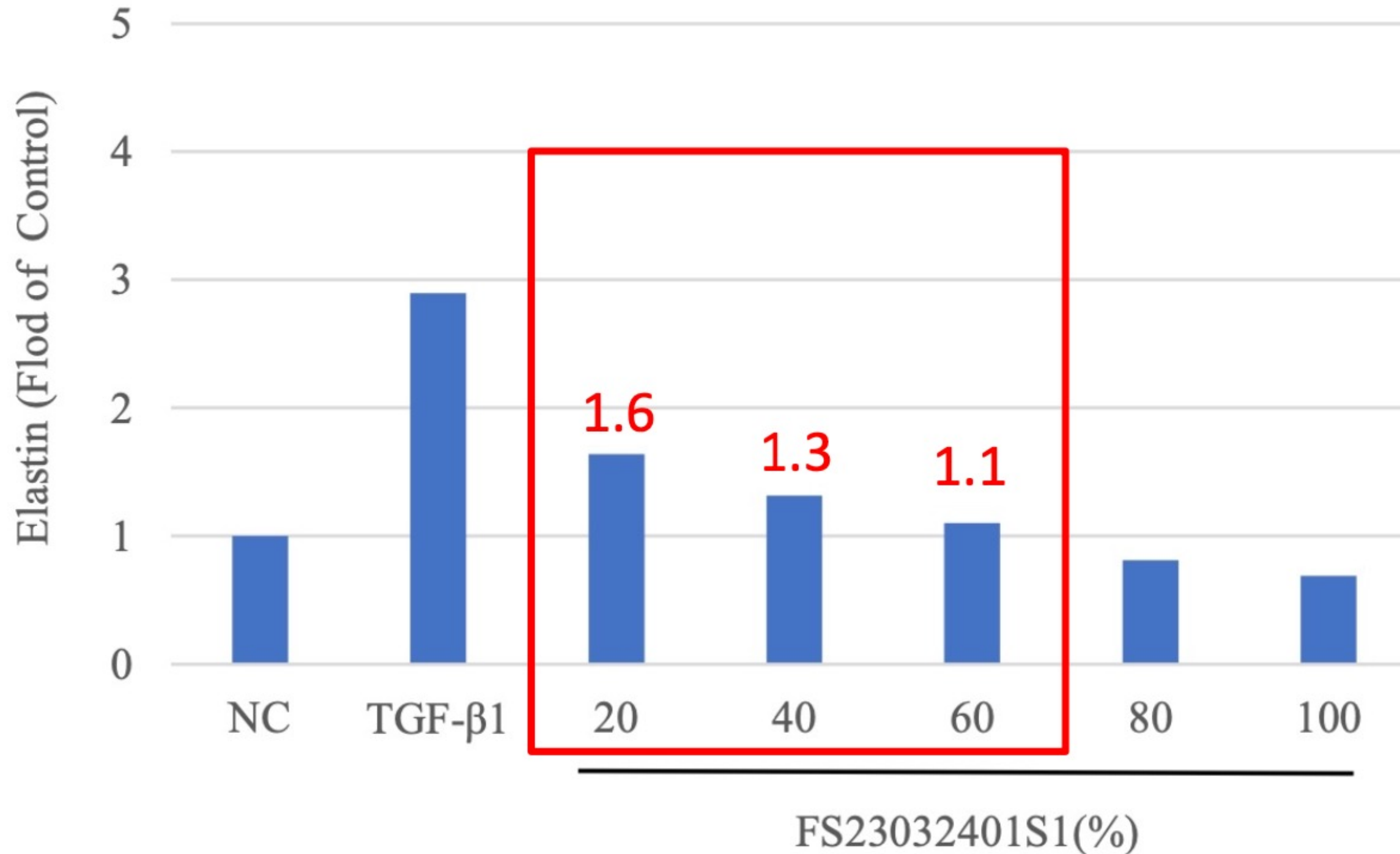The results showed that the concentration above 80% has a soothing effect.

in vitro **Anti-Inflammatory Test - IL -6**

Determination of *In Vitro* IL-6 Inflammatory Factor Content:
Raw264.7 Test Method for Lipopolysaccharide-Induced Macrophages

| Sample	Concentration	IL-6 Expression (pg/mL)	% of Inhibition
Control Group	-	1.00 ± 0.08 pg/mL	-
LPS Group	0.1 µg/mL	1.75 ± 0.06 pg/mL	-
Dex Group*	1 µM	0.63 ± 0.02 pg/mL	64%
	20%	1.85 ± 0.07 pg/mL	no inhibition
Well Shine®	40%	1.05 ± 0.03 pg/mL	40%
Biopolymer*	60%	0.56 ± 0.03 pg/mL	68%
(FS23032401S1)	80%	0.28 ± 0.02 pg/mL	84%
	100%	0.28 ± 0.02 pg/mL	84%

The results showed that at higher concentrations, there was higher IL-6 inhibition and reaching 40-84%.

in vitro Anti-Aging - Elastin

The results showed that the amount expression of Elastin produced in the low concentration group (20-60%) was significantly improved.

in vitro Anti-Aging Test - Type-1 Procollagen
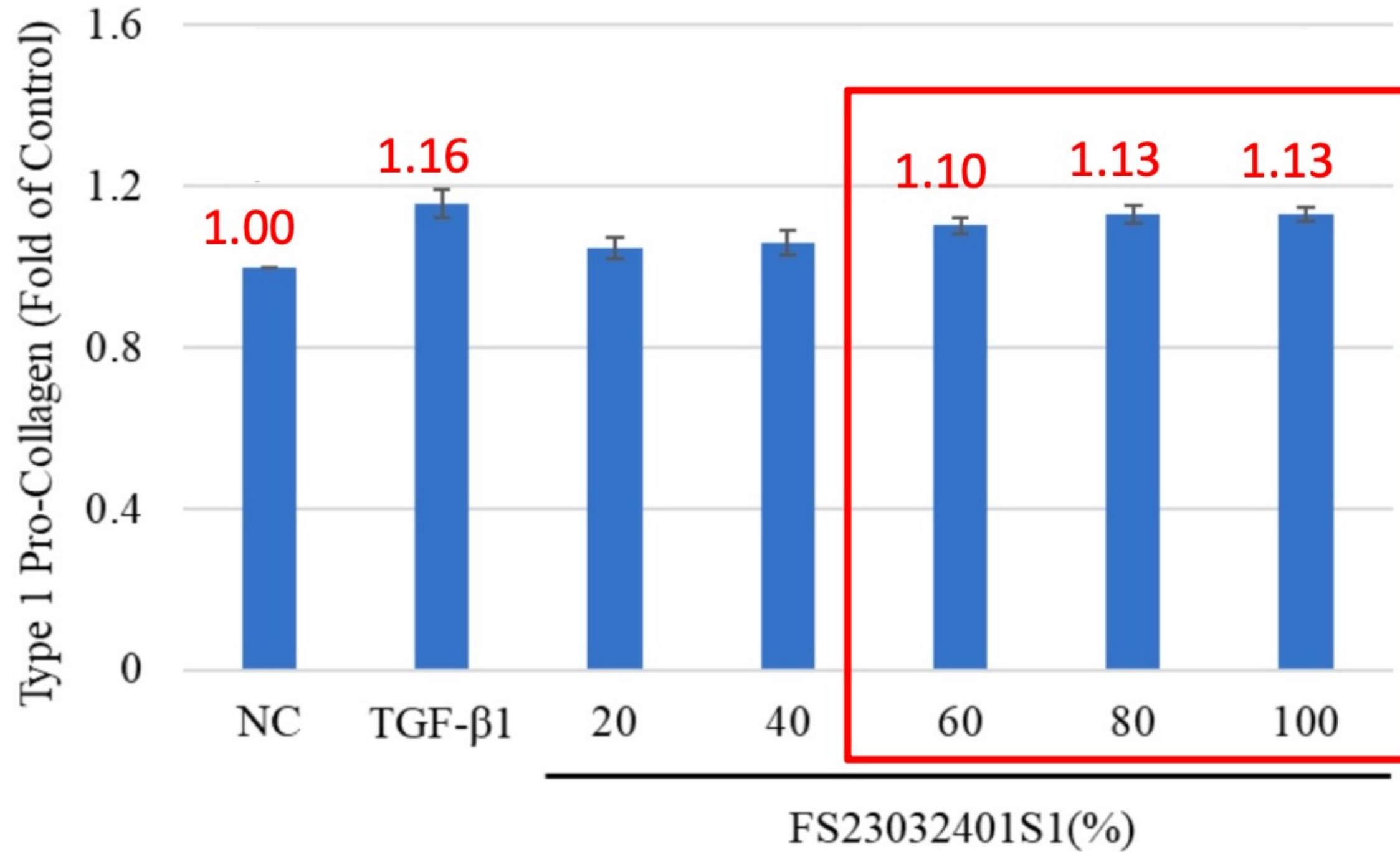
The results showed that the expression levels in each concentration group are significantly improved. Especially in higher concentrations, shown more than 1.1 times than the negative control group.

Thanks