



Poster ID 228

Yuko Ito, Tsuyoshi Shoda, Hayato Yamaoka, Takahito Nakai, Hideki Nishiura Skin Research Center, NIHON KOLMAR CO., LTD., Japan

Introduction:

Although preservatives are essential ingredients to maintain the quality of cosmetic products, these are considered to be one of the causes of skin irritation. In the IFSCC 2020 Yokohama Congress, we reported that several preservatives, such as phenoxyethanol, increase the levels of inflammatory cytokines in our skin. On the other hand, many ingredients, which have antibacterial effects and are not listed as preservative on the positive list of cosmetic standards, are used in most cosmetics for sensitive skin. However, there are no reports investigating the expression of inflammatory cytokines induced by these ingredients. Thus, we examined the inflammatory cytokines induced by preservatives commonly used in cosmetics worldwide and their alternatives. Furthermore, we evaluated anti-inflammatory cytokine and how these ingredients affect the epidermal differentiation.

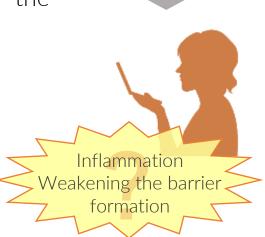
Table 1. 15 preservatives and their alternatives

1	Potassium Sorbate (POT)	9	Butylparaben (BUT)
2	Caprylyl Glycol (CAP)	10	Propylparaben (PRO)
3	Sodium Benzoate (SOD)	11	Methylparaben (MET)
4	Bisabolol (BIS)	12	Pentylene Glycol (PEN)
5	Butylene Glycol (BUG)	13	1,2-Hexanediol (HEX)
6	Benzyl Alcohol (BEN)	14	Ethylhexylglycerin (ETH)
7	Glyceryl Caprylate (GLY)	15	Phenoxyethanol (PHE)
8	Ethylparaben (ETP)		-

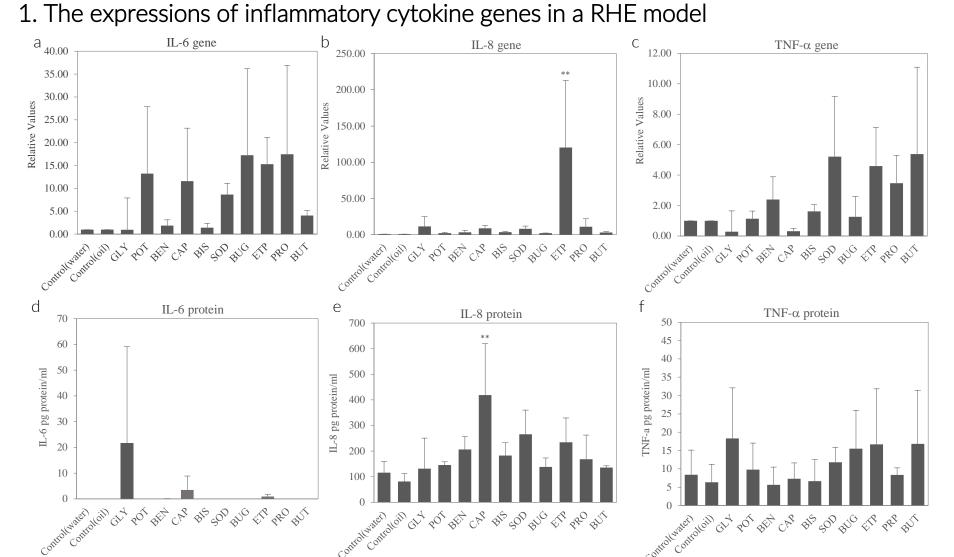


Preservatives and their alternatives

Inflammatory cytokines (IL-6, IL-8, TNF-α) & Anti-inflammatory Cytokine (IL-37)

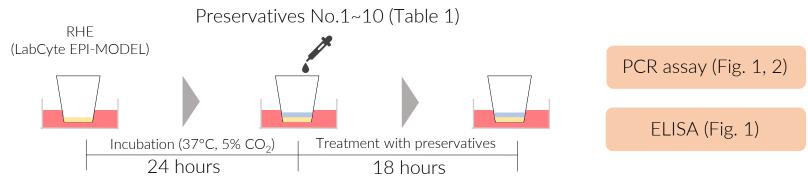


Results & Discussion:



Materials & Methods:

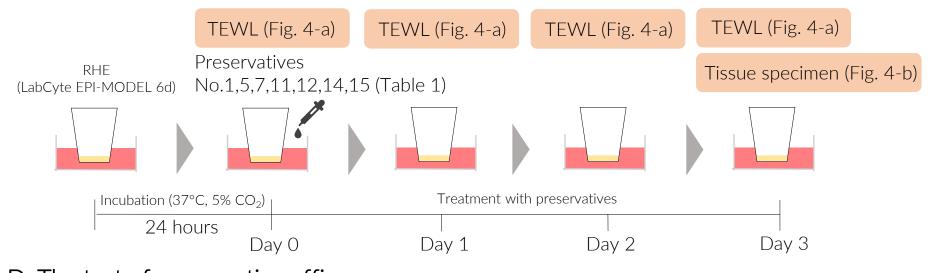




B. Gene and protein assay with normal human epidermal keratinocytes (NHEK)



C. Effects of preservatives in an immature RHE model



D. The test of preservative efficacy

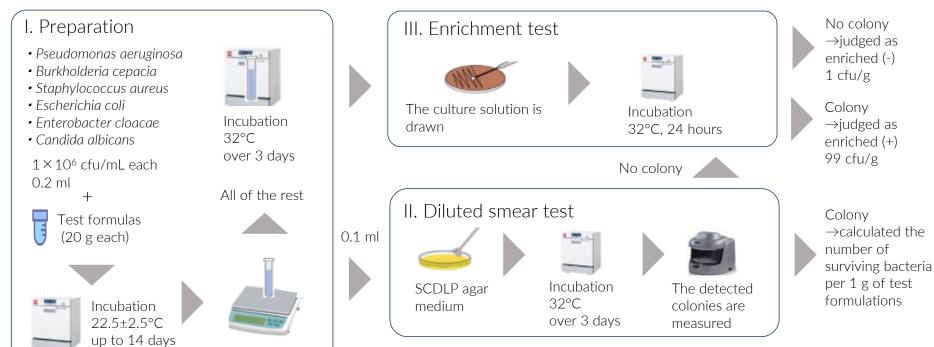
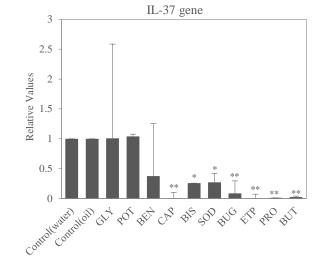


Fig.1 The relative mRNA expression level (a~c) and protein expression level (d~f) of inflammatory cytokines, IL-6, IL-8 and TNF- α , by 0.2% GLY, 0.3% POT, 0.3% BEN, 0.25% CAP, 0.5% BIS, 0.4% SOD, 20% BUG, 0.2% ETP, 0.1% PRO and 0.01% BUT for 18 hours treatment in a RHE model. GAPDH mRNA was used as an internal control. Dunnett tests were conducted with all preservatives except for GLY vs control (water), t-tests were conducted with GLY vs control (oil), N=3, **p < 0.01, Values are mean ± SD

► ETP significantly increased the gene expression of IL-8, and CAP significantly increased the protein expression of IL-8.

2. The expressions of anti-inflammatory cytokine gene in a RHE model



► CAP, SOD, ETP, BUT, PRO, BIS, BUG, and GLY significantly decreased the gene expression of IL-37.

Fig.2 The relative mRNA expression level of anti-inflammatory cytokine, IL-37, by 0.2% GLY, 0.3% POT, 0.3% BEN, 0.25% CAP, 0.5% BIS, 0.4% SOD, 20% BUG, 0.2% ETP, 0.1% PRO and 0.01% BUT for 18 hours treatment in a RHE model. GAPDH mRNA was used as an internal control. A Dunnett test was conducted with all preservatives except for GLY vs control (water), a t-test was conducted with GLY vs control (oil), N=3, *p < 0.05, **p < 0.01, Values are mean ± SD

3. The expressions of inflammatory cytokine proteins in NHEK

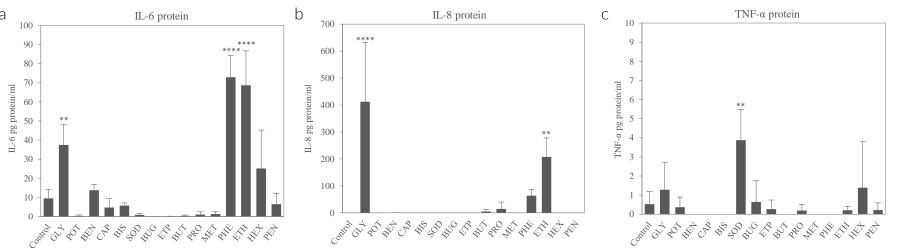


Fig.3 The protein expression level of inflammatory cytokines IL-6 (a), IL-8 (b) and TNF- α (c) by 0.012% GLY, 0.4% POT, 0.1% BEN, 0.05% CAP, 0.003% BIS, 0.75% SOD, 1.6% BUG, 0.03% ETP, 0.005% BUT, 0.0075% PRO, 0.1% MET, 0.2% PHE, 0.015% ETH, 0.4% HEX and 0.6% PEN for 18 hours treatment in NHEK. GAPDH mRNA was used as an internal control. N=3, **p < 0.01, ****p < 0.0001, vs control, Dunnett, Values are mean ± SD

- ▶ GLY, PHE and ETH significantly increased the protein expression of IL-6.
- ► GLY and ETH significantly increased the protein expression of IL-8.
- \triangleright SOD significantly increased the protein expression of TNF- α .

4. The antimicrobial efficacy and the summary of inflammatory cytokines expressions

Table 2. The results of the antimicrobial efficacy test and the summary of expressions of inflammatory cytokines which had significant increase by each preservative and its alternative for 18 hours treatment in NHEK and RHE models, *p < 0.05, **p < 0.01, ****p < 0.0001, vs control, Dunnett

	The antimicrobial efficacy					The summary of inflammatory cytokines expressions									
	Concentrati on (%)	Log reduction				IL-6			IL-8			TNF-α			
		Day0	Day7	Day14	Result	gene (RHE)	protein (RHE)	protein (NHEK)	gene (RHE)	protein (RHE)	protein (NHEK)	gene (RHE)	protein (RHE)	protein (NHEK)	
Standard	-	0	2.5	3.5	-										
Potassium Sorbate (POT)	0.3	0	0	0	Fail										
Caprylyl Glycol (CAP)	0.25	0	3.8	6.1	Pass					**					
Sodium Benzoate (SOD)	0.4	0	0	0	Fail									**	
Bisabolol (BIS)	0.5	0	0	0	Fail		1								
Butylene Glycol (BUG)	20	0	6.1	-	Pass		1								
Depart (Aleebal (DEN)	0.2	0	1 5	1 /	Fail	l		1		l			l		

To evaluate the storage efficacy of the test formulations, we calculated the logarithmic reduction value from the number of surviving bacteria at each test day from the following equation.

Log reduction = Log (the number of inoculums in 1 g of formulation) - Log (the number of surviving bacteria in 1 g of formulation on each test day)

As an evaluation standard, a test formulation in which the number of bacteria decreased by 2.5 Log or more on the 7th day or the number of bacteria decreased by 3.5 Log or more on the 14th day was judged to have enough antimicrobial effect as a cosmetic product.

Conclusions:

To evaluate the number of surviving bacteria in each solution, the following microbiological

challenge tests, diluted smear test and

enrichment test, were conducted.

► The expressions of inflammatory cytokines were different depending on preservatives. There were also differences between RHE and NHEK, which is thought to be affected by the difference in permeability.

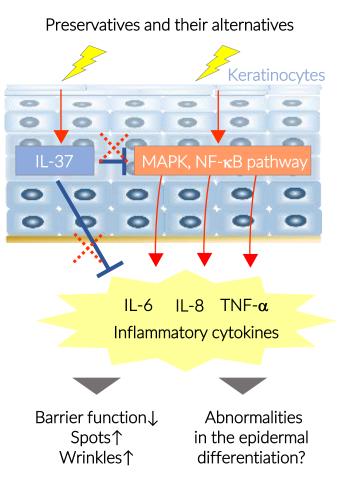
► CAP, SOD, ETP, BUT, PRO, BIS, BUG, and GLY significantly decreased the expression of IL-37, suggesting that these inflammatory response might be affected by anti-inflammatory response.

► BUG and PEN had enough antimicrobial efficacy as cosmetics without inducing inflammatory cytokines.

► The combination of POT, BIS and BEN might be a good option to develop cosmetics for sensitive skin.

▶ Preservatives and their alternatives caused abnormalities in the epidermal differentiation.

► To sum up, preservatives and their alternatives should be selected carefully, even though the alternatives are not defined as preservatives.



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Glyceryl Caprylate (GLY)	0.2	0	6.1	-	Pass		**			****		
Ethylparaben (ETP)	0.2	0	6.1	-	Pass			**				
Butylparaben (BUT)	0.1	0	6.1	-	Pass							
Propylparaben (PRO)	0.01	0	6.1		Pass							
Methylparaben (MET)	0.2	0	4.1	6.1	Pass				*			
Pentylene Glycol (PEN)	5	0	3.2	6.1	Pass							
1,2-Hexanediol (HEX)	1	0	1.9	2.4	Fail				*			
Ethylhexylglycerin (ETH)	0.05	0	0.8	0	Fail		****		*	**		
Phenoxyethanol (PHE)	0.5	0	2.4	5.2	Fail	*	****		*			

► BUG and PEN had enough antimicrobial efficacy as cosmetics without inducing inflammatory cytokines.

► The combination of POT, BIS and BEN might be a good option to develop cosmetics for sensitive skin.

▶ ETP, BUT and PRO contained 20% BUG and 4% Ethanol to dissolve in water and could not be conducted at the typical concentrations in cosmetics.

5. Effects of preservatives in an immature RHE model

(a) TEWL (Transepidermal water loss)

(b) Tissue specimen (hematoxylin-eosin staining)

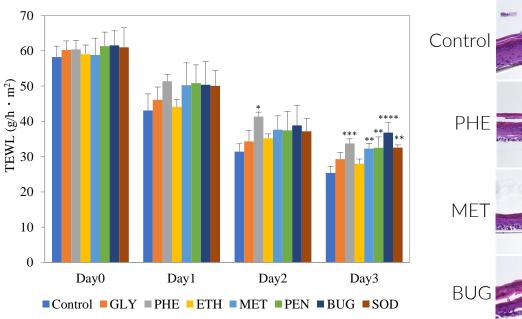
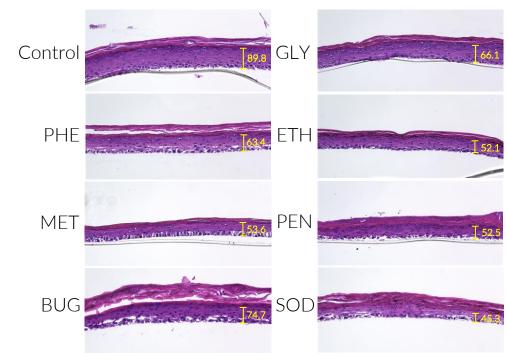


Fig.4 TEWL (a) and the epidermal thickness (b) by 0.012% GLY, 0.4% PEN, 0.075% PHE, 2.5% BUG, 0.008% ETH, 0.15% SOD, and 0.025% MET for 3 days treatment in an immature RHE model, N=3, *p < 0.05, **p < 0.01, ***p < 0.001, vs control, Dunnett, Values are mean ± SD

The formation of stratum corneum (SC) was disturbed by treating PHE, MET, PEN, BUG and SOD.



	Control	GLY	PHE	ETH	MET	PEN	BUG	SOD
Epidermal thickness excluding SC (μ m)	89.8	66.1	63.4	52.1	53.6	52.5	74.7	45.3

► The thickness of RHE model treated by preservatives, especially ETH, MET, PEN and SOD, was decreased.