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"Smart Skincare Formula for a Cheerful Day: Detect and Repair Damaged Skin Areas"

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1. Introduction

The condition of our skin can significantly influence our mood throughout the day, but unintentional skin issues often arise. Signs of skin damage are often minute and vague, making them difficult to notice and address, potentially worsening the damage. Conversely, when skin damage becomes noticeable, nearby unaffected areas may receive excessive care, potentially inducing new skin problems. Is there a way to address these skincare frustrations? A formulation that detects and repairs damaged areas, regardless of size, could offer a more targeted solution. This could be achieved with ingredients that preferentially adhere to damaged areas and promote recovery.

To create such a smart formulation, it is essential to first consider the physicochemical properties of the skin surface, which vary with skin condition. The zeta potential of the skin surface has been reported to be associated with skin barrier [1][2][3]. However, these results are inconsistent, with measured values differing in sign and magnitude across studies. These inconsistencies may arise from different measurement devices, including custom-made instruments, a lack of discussion on skin environment factors such as pH, and circadian variation. To clarify this property, understanding the pH dependence of the zeta potential of the stratum corneum (SC) may be a key factor. This is because the SC is the outermost layer of the skin and is composed of elements with charges that vary depending on pH.

Next, effective components for skin recovery related to zeta potential should be explored. Polymers with negative charges can promote skin barrier recovery [4]. This is presumed to be due to their effects on ions that regulate the skin barrier and cellular activity. However, the effects of pH and ionic strength during application have not been thoroughly discussed. Additionally, the shape and size of polymers should also influence their presence on the skin. Microgels, which have a defined size range and shape, may be advantageous for selective adhesion.

In this study, we first aimed to understand the surface potential and its pH dependence of isolated SC sheets and cells, providing a basis for evaluating preferential adhesion to the skin surface. We then investigated the effects of ionic species and sizes of microgels on the zeta potential of SC and evaluated the preferential adhesion of candidate microgels to damaged skin areas. Furthermore, we clarified the effects of microgels applied under damage response conditions on skin recovery.

2. Materials and Methods

The study was approved by the internal ethics committee (approval numbers: B10037, B10534, C10577, C10611, and C10613). Written informed consent was obtained from all participants. Statistical significance was assessed using Student's t-test.

2.1 Skin, SC sheets, and corneocytes (SC cells)

Human ex vivo skin was obtained by thawing frozen dermatomed skin (Biopredic International). SC sheets were isolated from ex vivo skin using previously reported methods [5]. Adhesive sheets (D-Squame D-100, Clinical & Derm) were used for flat collection of corneocytes from living human skin. A dispersion of corneocytes was obtained from the heel using a heel file.

2.2 Surface potential measurement

The zeta potentials of SC sheets and corneocytes collected on tape were measured using an electrokinetic analyzer (SurPASS 3, Anton Paar), while dispersion was measured with a particle analyzer (Zetasizer Nano ZS, Malvern Panalytical). The pH was adjusted using potassium hydroxide and hydrogen chloride.

2.3 Induction of artificial skin damage

Tape-stripping was performed using an adhesive tape (Cellulose Tape, Nichiban). An aqueous solution of sodium dodecyl sulfate (SDS) at the desired concentration was applied with a patch for a specified time and then rinsed with tap water. The mixture of acetone and diethyl ether (AE) was prepared in a 1:1 weight ratio for treatment.

2.4 Components used for efficacy evaluation

The polymers used are shown in Table 1. The polyion complex of DAA and AHC (DH-PIC) and the polyion complex of ATB and MPC (ATB-M) were prepared to have a negative charge. Fluorescently labeled ATB (ATB-DiI) was obtained by labeling ATB with 1'-dioctadecyl-3,3,3',3'-tetramethylindocarbocyanine perchlorate (DiIC18(3), λex/λem = 550/565 nm, FUJIFILM Wako). Fluorescently labeled DH-PIC (DH-PIC-FITC) was prepared by labeling AHC with fluorescein isothiocyanate (FITC, λex/λem = 495/520 nm, Sigma–Aldrich) as referenced in previous reports [6], followed by complexation with DAA. The formulation was designed to maintain the desired pH for over one minute after application to the SC or skin.

Table 1. Polymers used in this study.

INCI Name (chemical name)	Charge	Functional group	Abbreviation
dimethylacrylamide/sodium acryloyldimethyltaurate crosspolymer	negative	SO_3^-	DAA
ammonium acryloyldimethyltaurate/beheneth-25 methacrylate crosspolymer	negative	SO_3^-	ATB
ammonium acryloyldimethyltaurate/VP copolymer	negative	SO_3^-	ATV
polyquaternium-51 (2-methacryloyloxyethyl phosphorylcholine/butyl methacrylate copolymer)	negative positive	PO_4^- $N^+(CH_3)_3$	MPC
polyquaternium-10 (o-[2-hydroxy-3-(trimethylammonio)propyl]hydroxyethyl cellulose chloride)	positive	$N^+(CH_3)_3$	AHC
carbomer (carboxyl vinyl polymer)	negative	COO^-	CVP
cellulose gum (sodium carboxymethyl cellulose)	negative	COO^-	CMC

2.5 Observation of skin, SC sheets, and corneocytes

An optical microscope (BX-53, Olympus) and a video microscope (RH-2000, Hirox) were used to obtain optical images. Fluorescence images were acquired using appropriate excitation filters. The distribution of OH groups on the skin was observed using a NIR camera system [7], detecting the OH band of water at approximately 1920 nm and visualizing it in intensity levels.

2.6 Practical use test

Irritation of the forearm of ten healthy male participants was induced using SDS, and samples were applied immediately afterward, in the evening, and the next morning. The SC moisture content was measured using a corneometer (Courage+Khazaka) to calculate the recovery rate, the ratio of the recovery amount of moisture to the decrease caused by irritation. The damage-responsive formulation was applied twice daily to the cheeks of nine healthy male participants for four weeks, and the levels of squamous cell carcinoma antigen 1 (SCCA1) were compared. SCCA1 levels were measured with modifications to previously reported methods [8], which involved evenly collecting corneocytes on a specific area of tape and detecting their quantity with the ELISA method.

3. Results

3.1 Surface potential of the corneocytes and SC sheets

The pH dependence of the zeta potential of corneocytes collected from the same individual using tape is shown in Figure 1. The zeta potential of corneocytes changed with pH. The cells were negatively charged at pH values higher than the isoelectric point (IP), where the zeta potential is zero, while at pH values lower than the IP, they were positively charged.

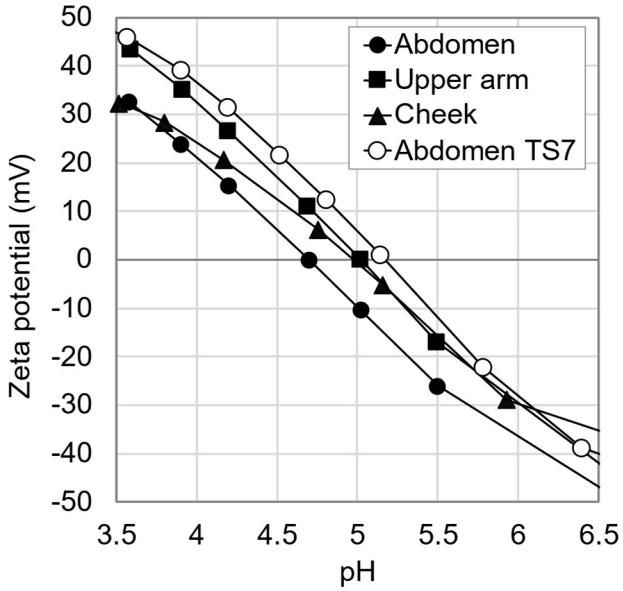


Figure 1. pH dependence of the zeta potential of corneocytes from the same individual. TS7 refers to the 7th layer obtained from tape-stripping.

The average IPs of corneocytes derived from specific sites of five individuals are presented in Table 2. The IPs of corneocytes from the first layer of the abdomen, inner arm, and cheek were pH 4.55, pH 4.70, and pH 4.76, respectively. Additionally, the IP of the corneocytes from the 7th layer of the abdomen after tape-stripping was 4.86. Each value for the cheek and the 7th layer of the abdomen was significantly higher than that for the first layer of the abdomen.

Table 2. Isoelectric points (IPs) of corneocytes. Values are the average IP of corneocytes collected from the same five individuals at each site. TS7 refers to the 7th layer obtained from tape-stripping. Different letters indicate statistically significant difference ($p<0.05$).

	IP (pH)	SD
Abdomen	4.55 [a]	0.09
Upper arm	4.70 [ab]	0.21
Cheek	4.76 [b]	0.17
Abdomen TS7	4.87 [b]	0.19

The zeta potential of SC sheets isolated from human ex vivo skin is shown in Figure 2 . An increase in IP was observed after tape-stripping, similar to that seen with corneocytes collected from the living skin. Additionally, the values increased with the number of tape-stripping events. For example, the back side of the same sheet, which was the deepest layer, showed the highest value. SC sheets treated with SDS or AE to induce skin irritation also exhibited an increase in IP, similar to that observed with tape-stripping, with a concurrent decrease in potential. However, some SC sheets showed little change in IP (data not shown).

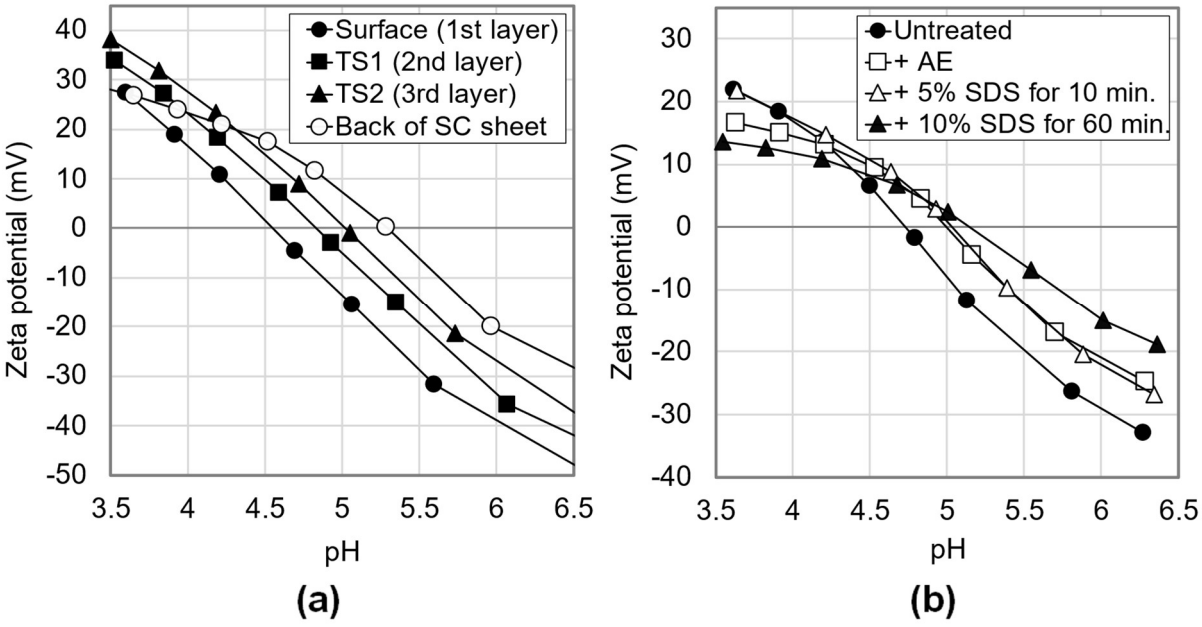


Figure 2. pH dependence of the zeta potential of SC sheets. (a) Effects of barrier disruption and SC depth on the pH and zeta potential of SC sheets. TS2 and TS3 refer to the 2nd and 3rd layers obtained after tape-stripping, respectively. (b) Effect of treatment on the pH and zeta potential of SC sheets. The "+" in the legend indicates that the treatment was applied.

These results revealed that the IP of the SC surface, which is normally keratinized and unaffected by external environments or barrier damage, is approximately pH 4.6. When the skin is disturbed or insufficiently keratinized, the IP tends to rise. Additionally, the pH–zeta potential curve indicated that at pH 4.6, normal SC shows zero charge, while damaged SC surfaces exhibit a positive charge. Therefore, the pH range centered at pH 4.6 can be considered a responsive condition that allows negatively charged components to preferentially adhere to damaged areas.

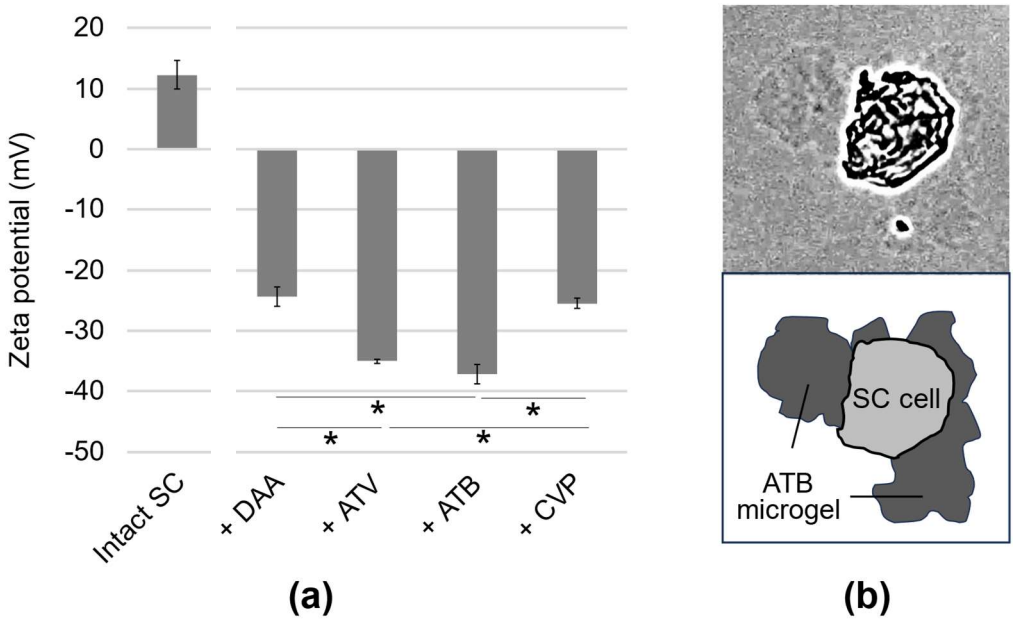


Figure 3. (a) Changes in the zeta potential of corneocytes after applying each microgel at pH4.0. Symbol indicate a significant difference ($p<0.05$). (b) State of ATB adhering to corneocyte.

3.2 Selection of Effective Components

Negatively charged microgels were considered candidate components to create adhesion differences and impart a negative charge to the skin surface. Figure 3 shows the changes in charge when each microgel was applied to corneocytes at pH 4.0, the pH at which cells exhibit a positive charge. Applying negatively charged microgels reversed the charge of corneocytes from positive to negative at the same pH. Observations indicated that this reversal arose from the adhesion of the microgel to the surface of the corneocytes. Among the microgels, ATB and ATV induced the most significant change in charge. In contrast, DAA, which also contains sulfonic acid groups, as ATV and ATB, but has a smaller particle size, induced a significantly smaller change in charge. CVP, which has negatively charged carboxylic acid groups, also induced a markedly smaller change in charge. The quantity of functional groups due to particle size and the strength of the charge of these groups likely influenced the extent of charge reversal, although the number of charges per microgel volume was not identical.

On the basis of these results, ATB was selected as one candidate in the following experiments. Additionally, aggregated DAA microgel, which addressed the disadvantage of DAA's small size, was used as another candidate. The aggregated DAA microgel is a polyion complex formed by combining DAA and AHC at a specific ratio to exhibit a negative charge (DH-PIC), and it was prepared to have a particle size similar to that of ATB (Figure 4) .

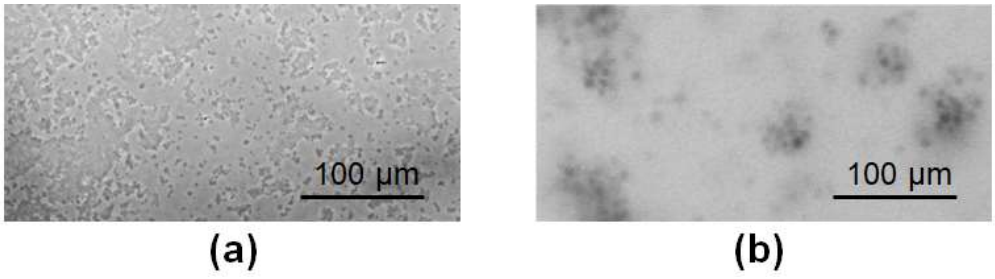
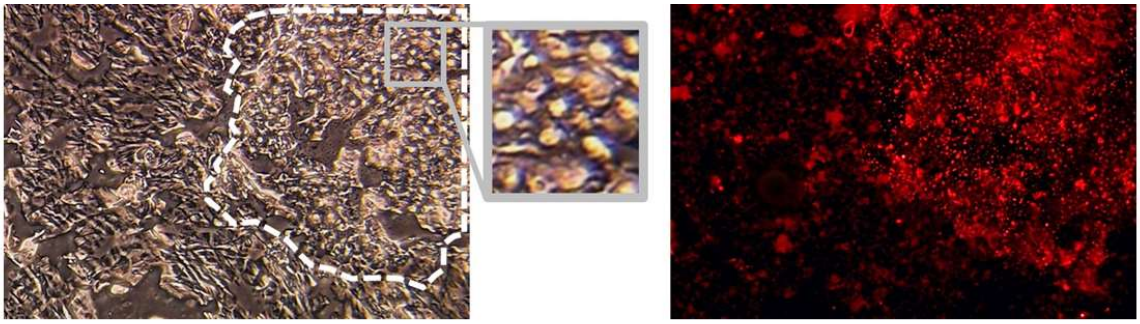


Figure 4. Optical microscopy images of (a) ATB and (b) DH-PIC.

3.3 Preferential Adhesion to Damaged Areas of the SC

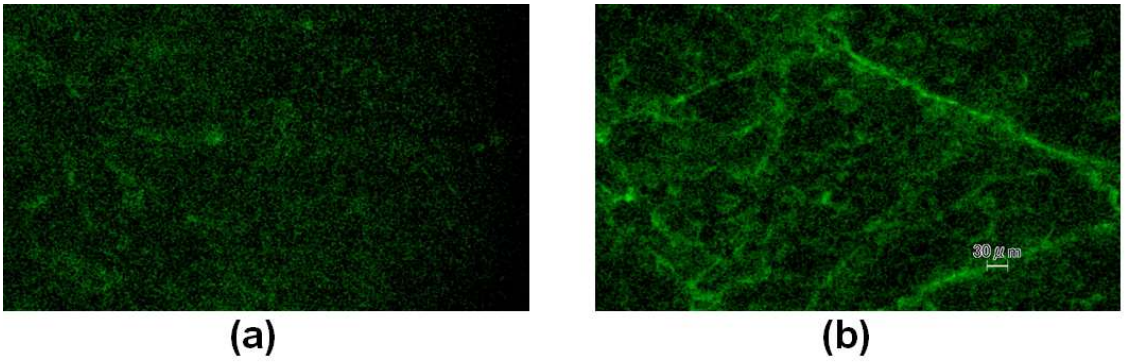
To determine the adhesion state of active ingredients applied to the SC, fluorescently labeled ATB (ATB-DiI) and DH-PIC (DH-PIC-FITC) were applied to corneocytes of skin surface and to ex vivo skin.The images obtained after washing with water are shown in Figures 5 and 6.



(a) (b)

Figure 5. Microscopy images showing the adhesion state of ATB-DiI applied to corneocytes of the skin surface at pH 4.6. (a) Optical microscopy image and (b) fluorescence microscopy image of the same area. The area enclosed by the dotted line in (a) is an incompletely keratinized region containing numerous nucleated cells.

Fluorescence microscopy images of ATB-DiI applied to the corneocytes collected on the tape from the skin surface revealed that the fluorescence intensity was significant in the incompletely keratinized area, which contained numerous nucleated cells. Conversely, the fluorescence intensity was low in normal keratinized areas on the same tape.



(a) (b)

(c)

Figure 6. Fluorescence microscopy images showing the adhesion state of DH-PIC-FITC applied to the surface of ex vivo skin. (a) Area without barrier disruption, treated with microgel at pH 4.6. (b) Area with barrier disruption by tape-stripping, treated with microgel at pH 4.6 (c) Area with barrier disruption treated with microgel at pH 6.5, outside the responsive range.

Fluorescence microscopy images of DH-PIC-FITC applied to untreated ex vivo skin within the damage-responsive pH range revealed that the fluorescence intensity was high in few areas.

In contrast, areas where the barrier was disrupted by tape-stripping showed a significantly higher fluorescence intensity when the microgel was applied. No adhesion was expected at pH 6.5, and fluorescence was indeed undetectable, even if the barrier was disrupted.

These results suggest that applying ATB and DH-PIC at pH 4.6 promotes the preferential adhesion of these microgels to the skin surface in a damaged state, such as incomplete keratinization or barrier disruption. Thus, formulations containing these microgels at around pH 4.6 were defined as "damage-responsive formulations.

3.4 Recovery Effects on Skin Condition

Applying a damage-responsive formulation promoted the recovery of artificially irritated skin. The recovery rates of the SC moisture content and skin texture are shown in Figure 7.

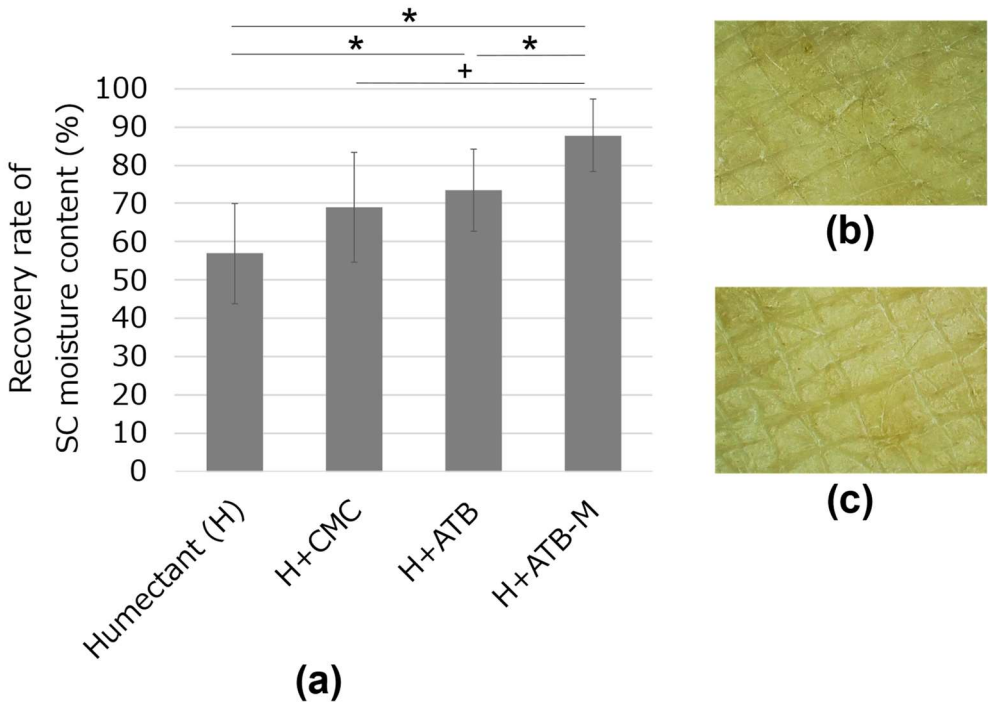


Figure 7. Recovery of skin with induced irritation after sample application. (a) Recovery rate of SC moisture. Symbols indicate significant difference (*p<0.05, +p<0.1). (b, c) Skin condition 24 h after applying humectant solution (b) and damage-responsive formulation (c).

The recovery rate of the SC moisture content 24 h after applying the formulation containing ATB at pH 4.6 was 72.0%. This was higher than the recovery rate of 53.6% observed after applying the humectant solution alone ($p<0.05$). Furthermore, the recovery rate was 87.4% after applying ATB-M, a composite of ATB and MPC in a fixed ratio, which was significantly higher than that after applying both the humectant solution and the formulation with ATB alone ($p<0.01$ and $p<0.05$, respectively). Additionally, there was no significant difference between the recovery effects of CMC and the humectant solution ($p>0.1$). ATB-M tended to promote a higher recovery rate than CMC ($p<0.1$). Skin treated with the humectant solution only continued to exhibit desquamation, and the skin texture appeared indistinct after 24 h, although applying ATB significantly improved both aspects.

The relationship between the adhesion state of the damage-responsive components and moisture content was examined by analyzing the distribution of the peak of OH groups observed under the NIR camera (Figure 8).

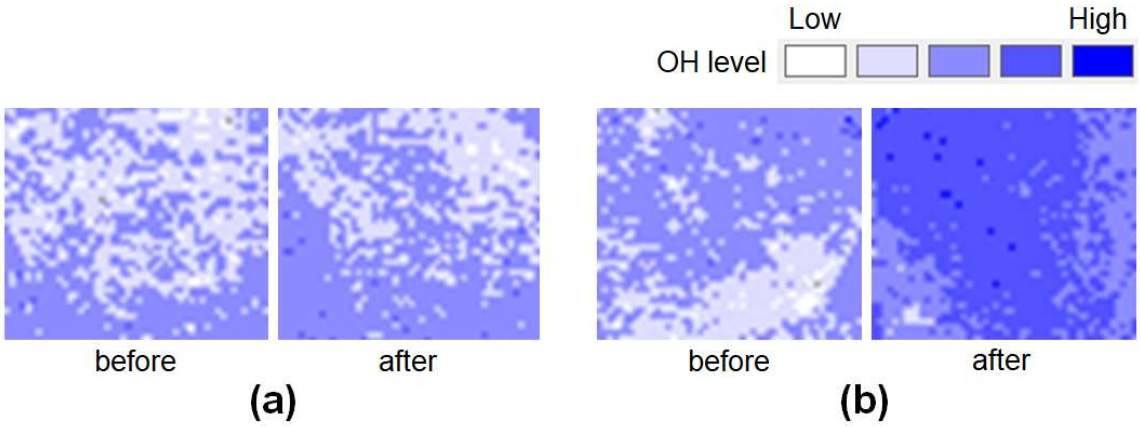


Figure 8. Distribution of OH groups on the surface of skin with induced damage after sample application. (a) Application of a moisturizer solution. (b) Application of a damage-responsive formulation containing DH-PIC. The left image was acquired before sample application, while the right image was acquired 30 min after sample application and water rinsing.

The irritated area showed desquamation and a decreased SC moisture content, along with a reduction in the amount of OH groups and an increase in brightness deviation. When the humectant solution was applied and then rinsed off, the moisturizer was removed and dryness returned within 30 min. In contrast, when the damage-responsive formulation containing DH-PIC was applied, the amount of OH groups increased and the distribution of OH groups was homogeneous, even 30 min after rinsing. These findings suggest that the damage-responsive formulation can adhere to and repair damaged areas of the SC, leading to a more uniform and improved skin condition that is resistant to external forces such as washing.

The effects of continuously using the damage-responsive formulation on skin condition were examined (Figure 9). The formulation was applied twice daily for four weeks, and SCCA1, an indicator of impaired keratinization, was monitored. After two and four weeks, the SCCA1 level decreased from the initial level. This suggests that the damage-responsive formulation immediately repairs daily irritation factors to enhance skin condition, thereby preventing the accumulation of damage.

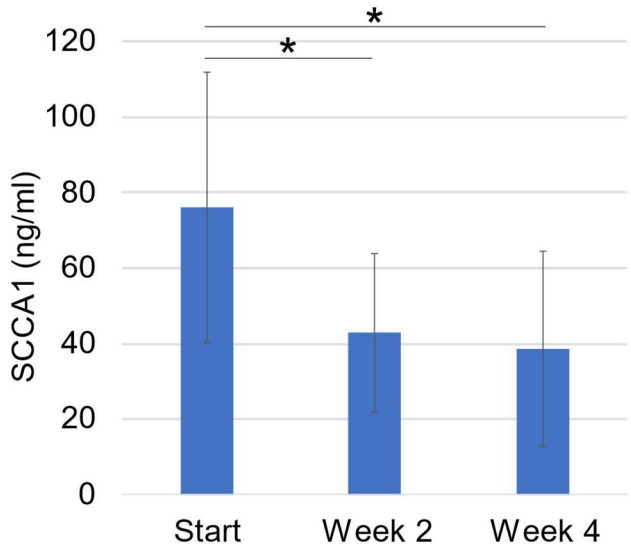


Figure 9. SCCA1 level of the SC with repeated use of the damage-responsive formulation. Symbol indicates significant difference ($p<0.05$).

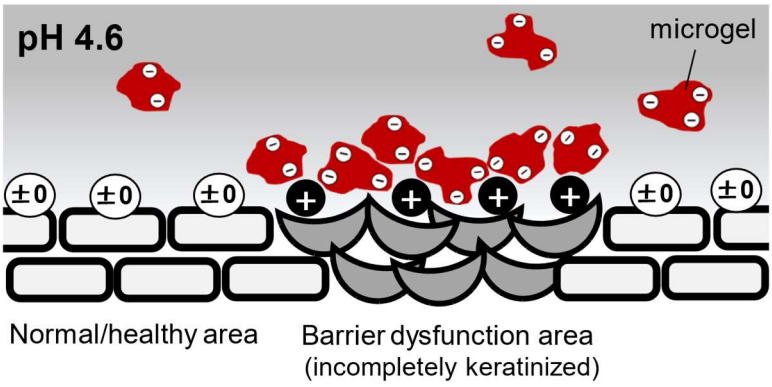


Figure 10. Conceptual diagram of preferential adhesion of barrier-responsive formulation.

4. Discussion

In this study, we aimed to develop a formulation that preferentially targets areas of damaged skin. First, we attempted to gain a detailed understanding of the surface potential of the SC, the outermost layer of the skin, where differences arising from damage were anticipated to occur. The method used in this study to examine the pH dependence of surface potential was an effective approach when targeting the SC, which contains both positive and negative charge elements. We found that the IP of normally keratinized SC is approximately pH 4.6, and that barrier disruption and incomplete keratinization increase the IP and surface potential. Theoretically, at pH 4.6, damaged areas have a surface potential of zero, while normal areas have a positive surface potential, indicating that negatively charged components would preferentially attach to damaged areas (Figure 10). In reality, various positive and negative charges coexist on corneocytes, and the surface potential reflects these collective charges. Therefore, even with slight fluctuations from pH 4.6, negatively charged components would still preferentially attach to the damaged SC. Thus, the pH range centered at 4.6 can be considered a "responsive condition" that allows for the preferential attachment of negatively charged components to damaged skin areas.

Ion fluctuations occurring in epidermal living keratinocytes, which are located beneath the SC, have been suggested to influence the differences in surface potential of the skin caused by damage [11]. In this study, it was found that differences in surface potential can arise even in corneocytes and in SC derived from frozen ex vivo skin, which do not contain living cells such as lower keratinocytes. This suggests that factors other than the ionic fluctuations of living organisms may also influence the surface potential of the SC. Possible factors include the depletion of fatty acids in intercellular lipids in damaged skin [9], the incompleteness of lipid membranes in immature SC, and the immaturity of the cornified envelope [10]. These factors warrant further investigation. Furthermore, a decrease in the quantity of potential was also observed in the cheek and SDS-treated areas, suggesting that the removal of amino acids and fatty acids by surfactant washing may also be a factor.

Negative charges promote the recovery of damaged skin, while positive charges delay recovery [4,12]. In this regard, the responsiveness of damaged skin to negative charges observed in this study was considered ideal. Polyelectrolytes that can impart a strong negative charge were considered effective components. Additionally, when aiming for preferential attachment to areas with micro-level damage, components with shapes maintained within a specific range were also advantageous. On the basis of these considerations, it was considered that microgels with stronger negatively charged functional groups and sizes close to corneocytes were the most suitable for achieving the aims of this study.

Fluorescence microscopy images revealed that the candidate components applied under responsive conditions preferentially attached to areas of incomplete keratinization and skin damage. Furthermore, their application to the skin promoted the recovery of the moisture content and skin texture 24 h after damage. This recovery effect was higher than that of CMC, which similarly promotes the recovery of skin conditions [4], further highlighting the importance of the strength of ionic functional groups. The recovery from damage due to negative charges is suggested to occur through the acceleration of lamellar body secretion [12]. Ion strength was likely to influence this effect as well. Additionally, the observed recovery effects of the complexes of ATB with MPC and DAA with AHC (data not shown) suggest the possibility of synergistic effects beyond the size effect of the polyion complex, which we aim to clarify through further investigations. Although these effects were short-term, two weeks of continuous use resulted in a decrease in the level of SCCA1, which has been reported to be associated with long-term impairment of various SC barriers [8]. This suggests that the damage-responsive formulation not only facilitates immediate repair of damaged skin but also has the potential to suppress damage accumulation and enhance skin quality in the future.

5. Conclusion

1. Normally keratinized SC has an IP of approximately pH 4.6, and damage or incomplete keratinization induces an increase in both IP and charge. The pH range centered at 4.6 can be considered a "damage-responsive condition" that allows for the preferential attachment of negatively charged substances to damaged areas.

2. Microgels with a high degree of negative charge and a consistent size efficiently adhered to damaged areas under these conditions. Formulations containing these microgels at the specified pH were termed "damage-responsive formulations."

3. The damage-responsive formulation exhibited excellent short-term recovery effects and the potential for preventing long-term damage accumulation.

This “smart” formulation can detect and repair signs of damage, whether they are noticed or unnoticed, achieving balanced skin care. The peace of mind from having one's skin monitored, along with the comfort of maintaining healthy skin, will contribute to a cheerful day.

6. References

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