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Convenience biosensing approach of lactic acid in stratum corneum for skin care assessment

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Abstract

Background: Lactic acid in the stratum corneum contributes to skin flexibility, making it a useful indicator of skin conditions. It is this useful to devise a simple technique to measure the lactic acid in the stratum corneum. To this aim, a printed chip biosensor was developed to analyze lactic acid in tape stripped (TS) stratum corneum samples.

Materials and Methods: Lactic acid was extracted from TS stratum corneum samples. A normal lactic acid sensor was prepared by applying lactate oxidase (LOD) to a printed chip. Another lactic acid sensor was prepared using LOD and a mediator osmium polymer immobilized on a printed chip. The amount of lactic acid in the extracted solutions was quantified using either the prepared biosensors or an existing analysis method.

Results: The results measured using the normal lactic acid sensor show low correlation with the results measured using an existing analytical method as a comparison, but those of the mediator osmium lactic acid sensor show high correlation.

Conclusions: The amount of lactic acid in samples extracted from the stratum corneum using a simple TS technique can be simply analyzed with high accuracy using a printed chip biosensor.

KEYWORDS

biosensor, lactic acid, stratum corneum, tape strip

1 | INTRODUCTION

The stratum corneum is a layer located on the outermost surface of the epidermis, the physical condition of which, such as roughness, can be directly assessed via touch. When the outer skin is rough, desquamation and fine cracks occur in the stratum corneum^{1,2} and it loses its softness, and subsequently its flexibility,^{3,4} so to prevent this from occurring skin must be kept soft and smooth. Water and natural moisturizing factor (NMF), a low-molecular weight water-soluble substance that exists in outer layer of the skin, are known to contribute toward the flexibility of the stratum corneum, as it is known that this layer becomes soft as the water content increases.⁵⁻⁷ NMF mainly comprises amino acids, pyrrolidone carboxylic acid (PCA)

derived from filaggrin and sweat-derived lactic acid, and urea.⁸⁻¹⁰ In NMF, amino acids and PCA contribute to flexibility through increasing the water content.^{9,11,12} However, the amount of lactic acid in the stratum corneum is not dependent on the water content, suggesting that lactic acid itself improves the flexibility of this layer of skin.¹³ In a literature study, it was found that the flexibility of isolated stratum corneum increases when immersed in an aqueous solution of lactic acid aqueous solution, even though there is no increase in its water content. This shows that the flexibility if the stratum corneum is related to the amount of lactic acid adsorbed, thought to proceed via a mechanism in which the interactions between polar sites are weakened by adsorbing lactic acid to keratin molecules present in the skin. With this information in mind, it is important to be able to

appropriately gauge the amount of lactic acid that contributes toward the flexibility of the stratum corneum as described above and to use the knowledge for better skincare, and it would therefore be beneficial to have the ability to measure the amount of lactic acid in the stratum corneum at a skincare counseling sites or at home.

Raman spectroscopy is a method that is used to measure the amount of lactic acid in the stratum corneum,¹⁴ but is an expensive and complex technique that requires operation by specialized personnel. An alternative method is to extract the lactic acid from the stratum corneum by immersing it in water using the tape strip (TS) method and then quantify it using high-performance liquid chromatography (HPLC). However, HPLC is also a fairly complex method, its apparatus is relatively expensive, and measurement and preparations such as column processing are complicated, meaning that a certain amount of knowledge is required for its operation. There is also a measurement method that involves enzyme reaction and color development,¹⁵ but this is also a fairly involved method as it requires a plate reader to quantify the color development reaction, and also operations such as pipetting need to be carried out.

Biosensing is a simple method for detecting a component in solution,¹⁶ where a biosensor is a probe that is used to detect the electrical signal intensity of a component as the result of the release of electrons from the reaction between an enzyme that specifically reacts with the substrate being analyzed. If the biosensor is in the form of an inexpensive disposable chip, the amount of components can be detected using a relatively simple electrical device. For this reason, various biosensors are currently being put into practical use, with a good example being blood glucose sensors.¹⁷ Thus, it is beneficial to develop a lactic acid sensor for easy use in a skincare counseling sites or at home.

Hence, a detection method was devised using a printed chip biosensor that can be used to detect the amount of lactic acid in a solution containing stratum corneum extracted using the TS method.

Two studies were carried out, with the second study based on the observations of the first. In the first study, a standard lactic acid enzyme-based printed sensor chip was investigated. Using the stratum corneum extract and standard lactic acid, the accuracy of the sensor was verified using other existing measurement methods such as HPLC and an F-kit as a reference. However, it was found that some there were some issues encountered in the extraction that needed to be improved. Therefore, in the second study, to solve the problems observed in the first study, in addition to the lactic acid enzyme, a sensor that can measure with greater accuracy was devised using an osmium polymer (Os polymer) as a mediator.

2 | MATERIALS AND METHODS

2.1 | Tools used for stratum corneum tape stripping

Oriented polypropylene (OPP) tape (DIA HALLO TAPE, Mitsubishi Plastics Inc) was used as an adhesive tape. An OPP tape tool was

prepared by fixing the tape with its adhesive surface facing the holes of a plastic plate (Figure 1A).

2.2 | Obtaining stratum corneum TS samples from subjects

Before carrying out any work, the study was approved by the Ethics Committee of POLA Chemical Industries and written consent was obtained from all subjects.

2.3 | Acquisition of facial stratum corneum TS samples

Eleven healthy Japanese women were used as subjects, with an average age of 43 years. The faces of the subjects were washed prior to extracting the skin samples using specialized makeup remover and face wash in a prescribed manner. Fifteen minutes after washing, TS was performed on the facial cheeks using the OPP tape tool by the same operator to ensure that the same pressure was used each time (Figure 1B).

2.4 | Acquisition of inner upper arm stratum corneum TS samples

Fifteen healthy Japanese women were used as subjects, with an average age of 39 years. The inner upper arms of the subjects were washed using the specified face wash mentioned in the previous section in the prescribed manner. Fifteen minutes after washing, TS was performed on the inner upper arms using the OPP tape tool by the same operator to ensure that the same pressure was used each time.

2.5 | Preparation of the stratum corneum TS sample extracts

Ion exchange water (250 μ L) was dropped onto a disposable petri dish. Then, an OPP tape sample, cut to a diameter of 2 cm after TS, was placed with the stratum corneum adhering side facing the water. The petri dish was then sealed and left to stand at room temperature for 24 hours (Figure 1C). Thereafter, all of the liquid was collected using a pipette.

2.6 | Measurement of the lactic acid content in TS stratum corneum extraction samples using HPLC and an F-kit

Quantification of the lactic acid in stratum corneum TS sample extracts was performed in two ways: with a commercially available

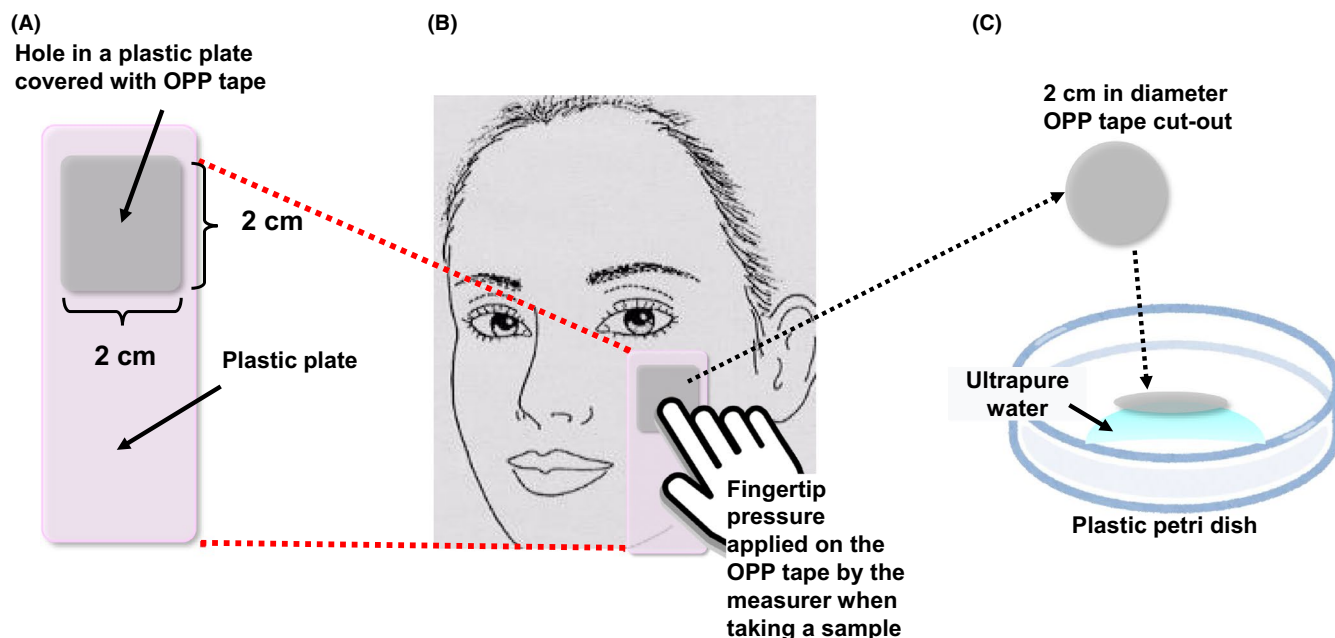
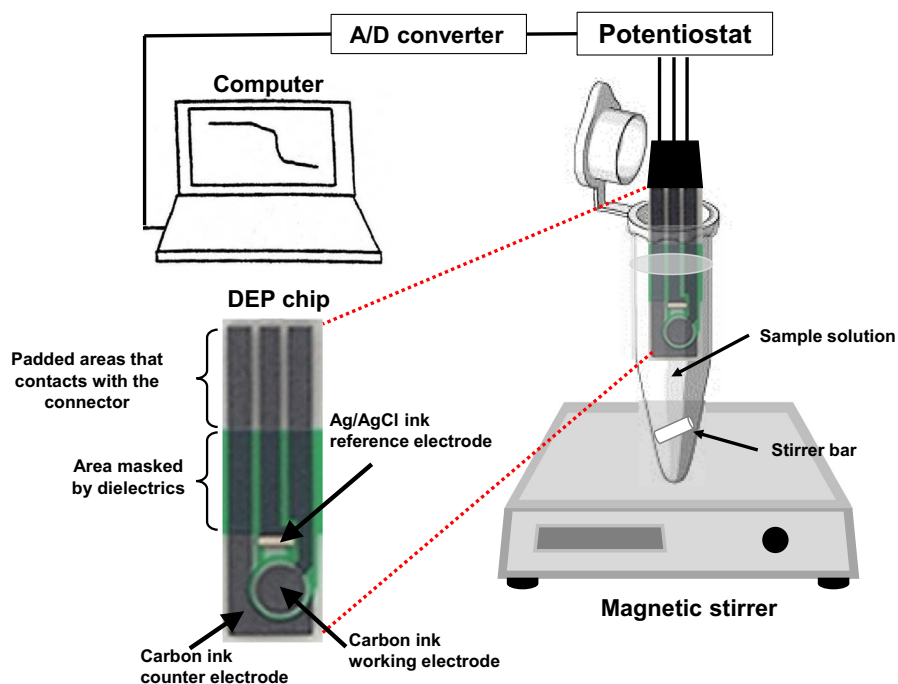


FIGURE 1 Image showing the extraction of lactic acid from skin, where (A) is the tool used in the stratum corneum tape stripping. The plastic plate shown has a 2 cm square hole covered with OPP tape. B, The OPP tape shown in (A) is applied to the left cheek of the subjects' face and firmly pressed by the measurer to take a stratum corneum sample. C, Circles of 2 cm in diameter were cut from the OPP tape samples collected in (B). Lactic acid was then extracted by applying the adhesive surface of the tape onto pure water dropped into a plastic petri dish

FIGURE 2 Disposable print sensor chip (DEP chip) used in the experiments and an image of the measuring system used



quantitative reagent kit using an enzymatic method (F-kit, Boehringer Mannheim) and HPLC (Agilent 1100 HPLC, Agilent Technologies, Inc). HPLC was used to measure the extracts from the face and the F-kit was on the extracts from the inner upper arm. For HPLC analysis, the analytical column used was an Imtakt Unison UK-C18 (3 μ m, 3 $\times$ 25.0 mm; Imtakt Corporation) and the mobile phase was

20 mmol/L sodium phosphate buffer solution containing 5 mmol/L tetrabutylammonium phosphate adjusted to pH 6.9 with NaOH and acetonitrile (98:2). The flow rate used was 0.4 mL/min, the measurement wavelength was 210 nm, the column temperature was 40°C, and the injection volume was 20 μ L. L-(+)-lactic acid (L1750-50G, Sigma-Aldrich) was used as the standard lactic acid reagent.

2.7 | Printed sensor chip and potentiostat used for the study, and measurement method

An image of the measurement system used in this study is shown in Figure 2. The disposable electrode-printed chip (DEP chip), consisting of a miniaturized three-electrode system including a carbon or gold working electrode, a carbon counter electrode, and an Ag/AgCl reference electrode, used in the measurements was purchased from Bio Device Technology Co. Ltd. The total length of the DEP chip was 12.5 mm, and the area of the working electrode was 2.64 mm². The method used to produce each lactic acid sensor using the DEP chip is described in the following section. The prepared DEP chip sensor was inserted into a 1.5-mL conical microtube. The solution to be measured was placed in the tube, and measurements were performed while stirring with a magnetic stirrer bar. Current values were measured using a potentiostat (MODEL 1112, Fuso Seisakusho) connected to an analog-to-digital (A/D) converter and a personal computer. All measurements were performed at room temperature (about 25°C).

2.8 | Development of the lactic acid sensor

2.8.1 | Producing a normal lactate sensor

A sensor was produced with an active amount of enzyme of 10 units per cm² applied to the working electrode of a DEP chip. Lactate oxidase (LCO-301, Toyobo), hereinafter abbreviated as LOD, was used as an enzyme for detecting lactic acid with an enzyme activity of 101 units per mg of solid. The LOD was weighed and dissolved in pH 7.0 phosphate buffer (PB), and then an equal amount of enzyme immobilization resin (Biosurfine SPH, TOYO GOSHI Co., Ltd) was added and mixed in. Then, 0.5 µL of the prepared solution was dropped onto the working electrode of the DEP chip and spread across the entire surface. At 4°C, the DEP Chip was irradiated with a fluorescent lamp for 1 hour to cure the resin and the resulting sensor was stored in a refrigerator until use.

An overview of the reaction of lactic acid with the LOD generated on the lactic acid sensor is shown in Figure 3A. The hydrogen peroxide generated from the reaction between lactic acid and the LOD was oxidized on the carbon electrode via the applying of potential and the current flow was measured.

2.8.2 | Production of a lactic acid sensor using an osmium polymer as a mediator (Os polymer lactic acid sensor)

On the DEP chip working electrode, 0.5 µL of a polymer (Bioanalytical Systems) composed of Os polymer and surfactant was applied uniformly and it was then kept overnight at a temperature of 4°C. 0.1 mg of LOD was dissolved in 10 µL of PB and further mixed with 10 µL of Biosurfin SPH. 0.5 µL of the resulting solution was then dropped onto the sensor chip working electrode

and uniformly spread across its surface (10 units per cm²), and this resin was cured via irradiation with a fluorescent lamp for 1 hour at 4°C. Figure 3B shows the reaction on the lactic acid sensor based on the Os polymer.

2.8.3 | Performance evaluation and calibration characteristics of both types of lactic acid sensor

The performance evaluation and calibration characteristics of the produced lactic acid sensors were measured as follows. L-(+)-lactic acid (L1750-50G; Sigma) was used as a standard lactic acid reagent and dissolved in PB to prepare 10, 50, 100, 500, and 1000 µmol/L solutions. 100 µL of each of the concentrations of lactic acid were respectively injected into microtubes. The prepared lactic acid sensor was then inserted into each microtube, and in the case of the normal lactic acid sensor, a potential of +550 mV was applied and for the Os polymer lactic acid sensor 0 V, while the solutions were stirred with a magnetic stirrer bar. The current response over 180 seconds was measured three times, and the average current response values of each sensor were calculated between 120-180 seconds and used as the measured values. This measured data were then used to plot a calibration curve of the current response for each concentration of lactic acid.

2.9 | Measurement of various samples using the different lactic acid sensors

2.9.1 | Examinations common to studies 1 and 2

Evaluation of the lactic acid concentration in the stratum corneum TS sample extracts

The TS extracted stratum corneum sample solution of 100 µL was added to a 1.5-mL microtube, and the prepared lactic acid sensor was introduced into the microtube while the solution was stirred with a magnetic stirrer bar. Under an applied voltage +550 mV in the first study and 0 mV in the second study, three measurements were performed and the average value of the current response between 120 and 180 seconds, where the measured value was stable after the start of the measurement, was calculated and used as the measured lactic acid value for each of the samples.

2.10 | Measurement of the changes in the lactic acid sensor current response for various concentrations of lactic acid added to TS stratum corneum extract samples and only OPP tape samples extracted in water

In each of the two cases, TS stratum corneum extract samples or only OPP tape samples extracted in water, 100 µL aliquots of the two types of sample solutions were added to two separate groups of three 1.5-mL microtubes, to which 5.3, 5.8, and 6.5 µL of a 1 mmol/L aqueous

FIGURE 3 Reaction formula of the lactic acid sensor (A) Reaction of the normal lactic acid sensor. B, Reaction of the lactic acid sensor based on the osmium polymer

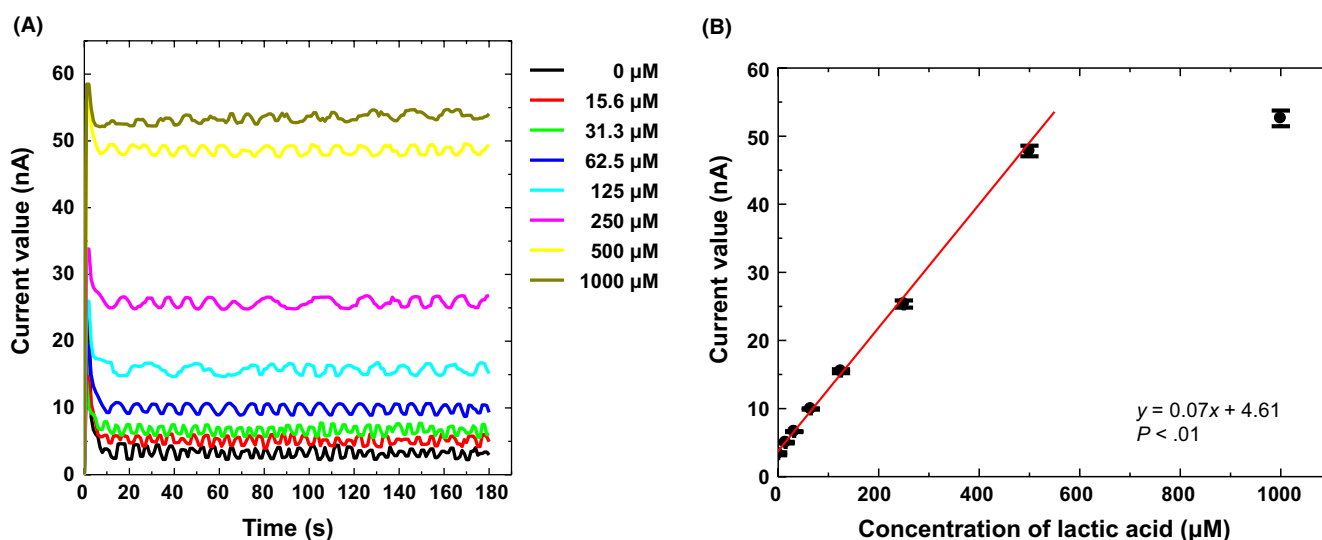
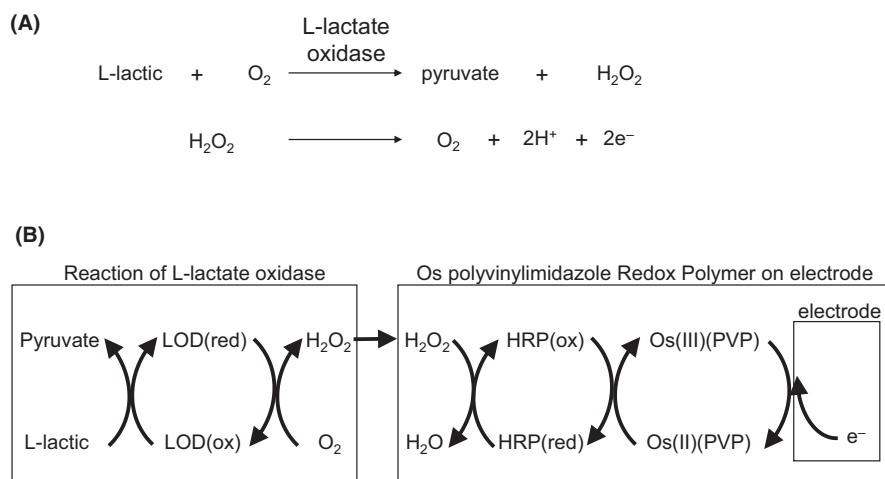


FIGURE 4 A, Typical response currents for each concentration of lactic acid (0, 15.6, 31.3, 62.5, 125, 250, 500, and 1000 $\mu\text{mol/L}$) using a normal lactic acid sensor and (B) calibration curve plotted using the data shown in (A). Linear regression lines were calculated using 500 $\mu\text{mol/L}$ or less data

solution of lactic acid were added, respectively. As a result, the total amount of lactic acid in the microtubes was increased by 50, 100, and 150 $\mu\text{mol/L}$, respectively (the total solution volume was 117.6 μL at 150 $\mu\text{mol/L}$). Thereafter, 117.6 μL of PB was added, halving the concentration of lactic acid in the microtubes. For all of the solutions prepared using the conditions described above, the current responses were measured using the prepared normal lactic acid and Os polymer lactic acid sensors under the same conditions as in the evaluation of the lactic acid concentration of the aforementioned TS stratum corneum extract samples and only OPP tape samples extracted in water.

2.11 | Statistical analysis

Correlation coefficients were calculated using JMP Ver9 software (SAS institute).

3 | RESULTS

3.1 | Study 1

3.1.1 | Examination of the normal lactic acid sensor

Evaluation of the sensor performance and calibration characteristics

Results of the measurement of 0, 10, 50, 100, 500, and 1000 $\mu\text{mol/L}$ lactic acid using L-(+)-lactic acid dissolved in PB as a standard lactic acid reagent using detected with the normal lactic acid sensor at an applied voltage of +550 mV are shown in Figure 4A. Figure 4B shows the plotted average current values at each lactic acid concentration, showing that the current increases linearly upon an increase in the lactic acid concentration up to 500 $\mu\text{mol/L}$.

3.1.2 | Relationship between the measurement results of the normal lactate sensor and F-kit method for a TS stratum corneum sample extracts

Figure 5 shows the relationship between the measurement results of the normal lactic acid sensor and F-kit for the TS stratum corneum sample extracts from the inner upper arm. Although linearity can be observed between the two variables, the correlation was low, with a correlation coefficient of 0.38.

3.1.3 | Measurement of the changes in the current response of the normal lactic acid sensor for various concentrations of lactic acid solutions added to the TS stratum corneum sample extracts

A change in the current response was examined when different amount of lactic acid was added to the TS stratum corneum sample extracts inner upper arm, the results of which are shown in Figure 6. In simple PB as a control, it was shown that the current response value increased linearly upon the addition of lactic acid and that the current response was almost halved when the solution was twofold diluted after the addition of the lactic acid. However, in all of the TS stratum corneum sample extracts, there were no clear changes observed in the current response characteristics due to the change in the lactic acid concentration. Also, the twofold diluted solution after the addition of lactic acid showed a lower current response for all of the samples than for the TS stratum corneum sample extracts alone.

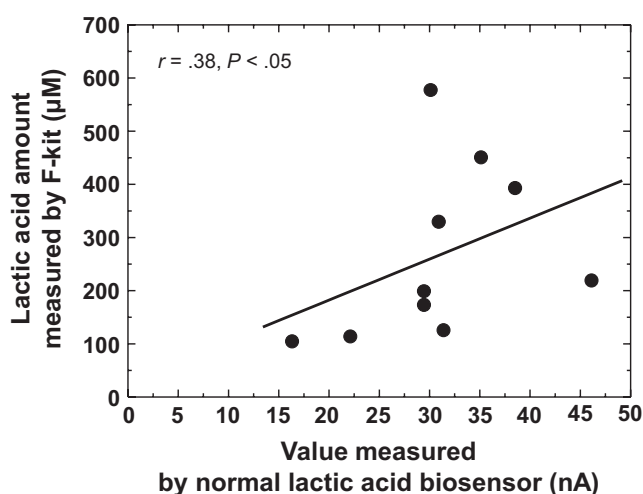


FIGURE 5 Relationship between the measurement results of a normal lactic acid sensor and F-kit of TS stratum corneum sample extract solutions

3.1.4 | Measurement of the current response of a solution in which only OPP tape was extracted with water using the normal lactic acid sensor

The change in the current response was examined when different amounts of lactic acid were added to solutions by carrying out extraction of only the OPP tape, the results of which are shown in Figure 7. In the measurement with only the extracted solution, the response current value was about 11.5 nA, which was higher than the current response measured using PB as a control. When lactic acid was added, the current response tended to increase in proportion to the lactic acid concentration. Also, since the lactic acid concentration in the solution was 75 μmol/L after twofold dilution, it was assumed that the current response after the addition would be between the values observed for the 50 and 100 μmol/L concentrations. However, the current response of all three specimens examined showed a tendency to be lower than in the absence of the reagent.

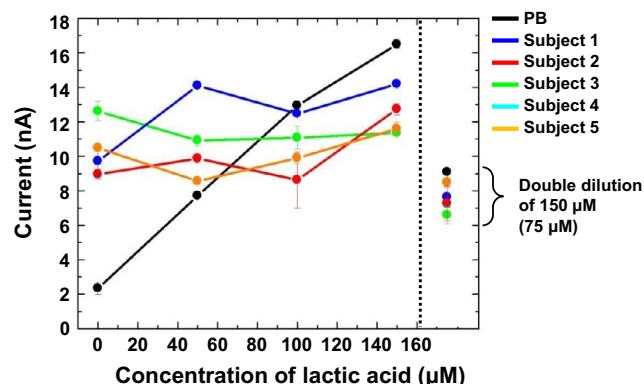


FIGURE 6 Changes in the current response of the normal lactic acid sensor when lactic acid was added to the TS extracted stratum corneum samples or PB with concentrations of 0, 50, 100, or 150 μmol/L, and when a 150 μmol/L solution was twofold diluted (75 μmol/L)

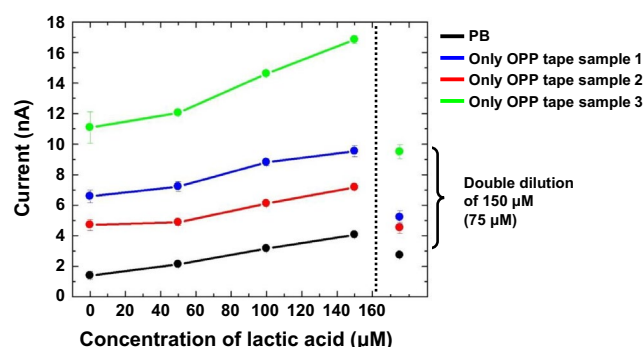


FIGURE 7 Change in the current response of a normal lactic acid sensor when lactic acid was added to 0, 50, 100, and 150 μmol/L solutions and PB and the sample extraction of only the OPP tape, and when a 150 μmol/L solution was twofold diluted (75 μmol/L)

3.2 | Study 2

3.2.1 | Examination of the Os polymer lactic acid sensor

Evaluation of the sensor performance and calibration characteristics

Results of the measurement of 0, 10, 50, 100, 500, and 1000 $\mu\text{mol/L}$ lactic acid solutions containing L-(+)-lactic acid dissolved in PB as a standard lactic acid reagent using the Os polymer lactic acid sensor are shown in Figure 8A. Figure 8B shows the results of the average current values plotted at each lactic acid concentration and that the absolute current value increases linearly upon an increase in the lactic acid concentration up to around 500 $\mu\text{mol/L}$.

3.2.2 | Relationship between the measurement results of the normal lactate sensor and HPLC method for the TS stratum corneum sample extracts

Figure 9 shows the relationship between the results measured using the Os polymer lactic acid sensor and the HPLC for the facial TS stratum corneum sample extracts, and it can be seen that linearity is observed between the two methods, and the correlation is high, with a coefficient of 0.93 ($P < .01$).

3.2.3 | Measurement of the changes in the Os polymer lactic acid sensor current response when different lactic acid concentrations were added to the TS stratum corneum sample extracts

The change in the current response was examined when different amounts of lactic acid were added to the cheek TS stratum corneum sample extracts, the results of which are shown in Figure 10.

It can be observed that the current response values increase linearly upon an increase in the lactic acid concentration for all five specimens examined, similarly to the results observed in the presence of the control PB.

However, a twofold dilution test of the solution after the addition of lactic acid showed a lower current response for all of the samples than for the TS stratum corneum sample extracts alone.

In the control PB, there was no decreasing trend observed compared to the case of no addition.

3.2.4 | Measurement of the current response of a solution in which only OPP tape was extracted with water using the Os polymer lactic acid sensor

The change in the response current was investigated when different amounts of lactic acid were added to the extracted solution obtained from OPP tape alone, the results of which are shown in Figure 11. Upon

the addition of lactic acid, for all samples the increase in the current response was observed to be dependent on the lactic acid concentration using PB as a control. Also, after twofold dilution to give a lactic acid concentration of 75 $\mu\text{mol/L}$, it was assumed that the current response after the addition would be between the responses observed for the 50 and 100 $\mu\text{mol/L}$ concentrations. However, the current response was lower than expected for all three of the specimens examined.

4 | DISCUSSION

Lactic acid contributes to the flexibility of the stratum corneum; therefore, the development of a method that can easily analyze the amount of lactic acid is useful for skincare counseling. Hence, a detection method was devised using a biosensor made up of a disposable print sensor chip that can be used to easily detect lactic acid during the TS stratum corneum extraction method.

In the first study, a normal lactic acid sensor was investigated using a disposable print sensor chip (DEP chip) and a basic reaction system of a lactic acid enzyme (Figure 3A). It was confirmed that the current response of the prepared normal lactic acid sensor showed good concentration dependency on lactic acid at an applied voltage of +550 mV (Figure 4). The relationship between the measured results obtained by the normal lactic acid sensor and the F-kit was examined for the TS stratum corneum sample extracts. As a result, although linearity was observed between the two sets of results, the correlation was shown to be low, with a coefficient of only 0.38 (Figure 5). The normal lactic acid sensor may detect non-specific current originating from non-lactic acid components in the TS stratum corneum extract. Therefore, the change in current was measured when different amounts of lactic acid were added to the TS stratum corneum sample extracts. As a result, no clear change in current response due to the addition of lactic acid was observed, as shown in Figure 6. Furthermore, the twofold dilution resulted in a lower current response compared to that of the TS stratum corneum sample extract itself. From these results, the non-specific response current was attenuated when the extracted solution was diluted. In the extraction solution itself, the presence of some substances that inhibit the enzyme reaction on the electrode was considered. Furthermore, to investigate the characteristics of non-specific current response, the current response was measured upon the addition of various concentrations of lactic acid to solutions obtained from extracting only OPP tape (Figure 7), which shows that the current response is around 11–5 nA, higher than that of PB. When lactic acid was added to the extract, there tended to be an increase in the current response in line with an increase in lactic acid concentration. Furthermore, when the extract was twofold diluted, the current tended to be lower than when no additional lactic acid was added. From the above observations, it is considered that the non-specific current response of the solution extracted from the TS stratum corneum samples may be derived from the tape. However, the intensity of the current response showed a proportional increase upon the addition of lactic acid, and therefore, the cause of the non-specific current response was considered to be due to the influence of other

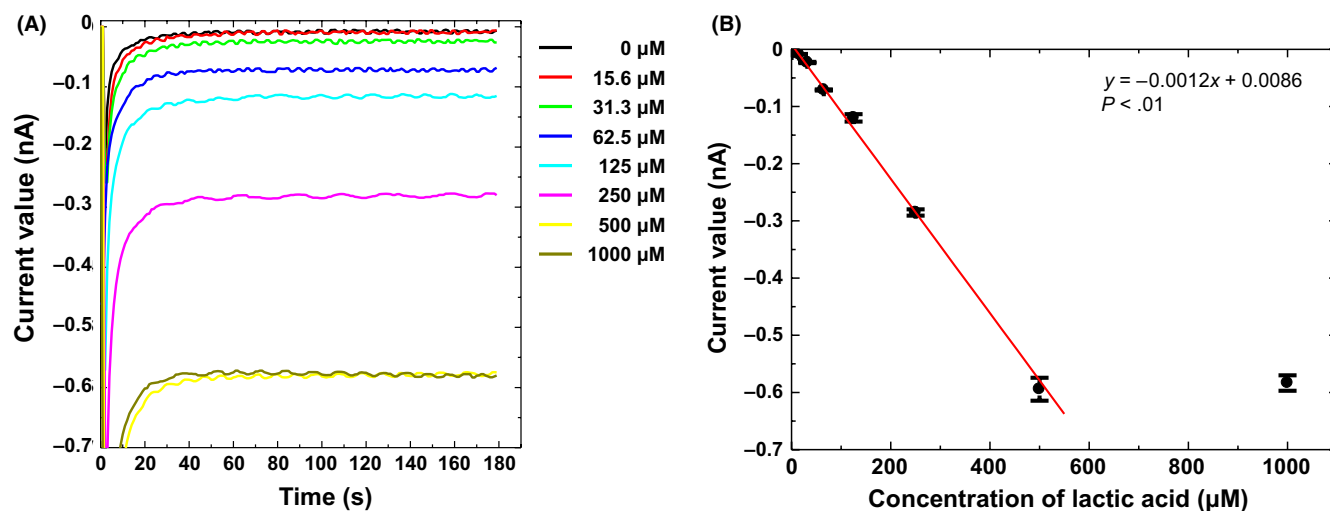


FIGURE 8 A, Typical response current for each of the concentrations of lactic acid (0, 15.6, 31.3, 62.5, 125, 250, 500, and 1000 $\mu\text{mol/L}$) of the Os polymer lactic acid sensor and (B) calibration curve plotted using the data shown in (A). Linear regression lines were calculated using 500 $\mu\text{mol/L}$ or less data

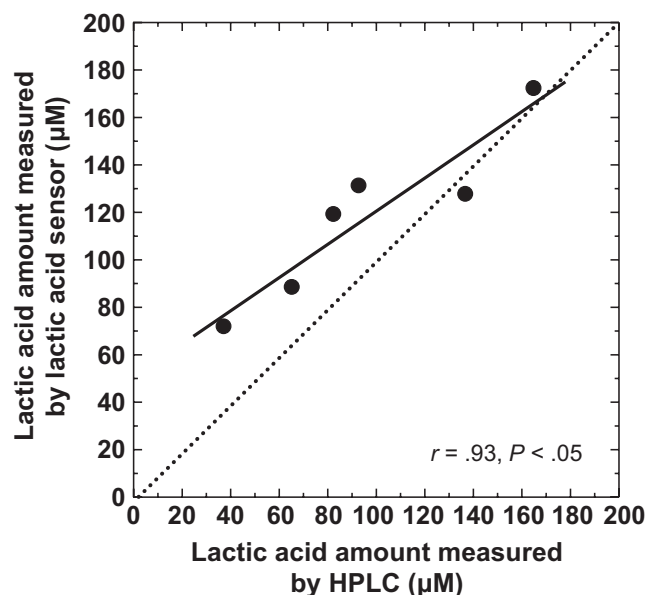


FIGURE 9 Relationship between the measurement results of the Os polymer lactate sensor and HPLC for TS stratum corneum sample extract solutions

components of the stratum corneum. The methods that have been tried so far require the application of a voltage (potential difference) to electrolyze H_2O_2 produced by the enzymatic reaction and charge extraction. The application of this voltage is considered to be one of the main reasons behind the generation of a non-specific current response other than that of the target being measured.

Therefore, a method of measuring the current response without applying a voltage was investigated using osmium as a mediator. The method utilizes a redox reaction involving the immobilization of peroxidase (HRP) and osmium on the working electrode, therefore making it possible to generate charge without applying a voltage (Figure 3B). It was hypothesized that using this method the

non-enzyme reaction-specific current response that arises as a result of applying a voltage can be reduced.

Hence, in the second study, an Os polymer lactic acid sensor was used in the measurements. Experiments using the Os polymer lactic acid sensor to detect various concentrations of lactic acid showed that there was a linear increase in the current response upon an increase in lactic acid concentration (Figure 8). To evaluate the performance of the fabricated sensor, the relationship between the HPLC and Os polymer lactic acid sensor measurement results for solutions extracted from TS stratum corneum samples was evaluated. As shown in Figure 9, the correlation between the two methods was shown to be high, with a coefficient of 0.93 ($P < .01$). Compared to the results measured by the normal lactic acid sensor without Os polymer (Figure 5), the correlation to the amount of lactic acid measured by the standard method was greatly improved. To confirm the presence of a non-specific current response, various different concentrations of lactic acid were added to the from TS stratum corneum sample extracts and the change in the current response was measured. For all of the samples, there was an observed lactic acid concentration-dependent increase in the current response, as shown in Figure 10.

However, the current response was observed to be lower when the extracted solution was twofold diluted than when the extraction solution was not added. Also, in the above-mentioned comparison with HPLC (Figure 9), the measured values obtained using the Os polymer lactic acid sensor are higher than the results from HPLC, although the correlation between the Os polymer lactic acid sensor and HPLC results is very good. From the results above, it is considered that some non-specific current response is generated in the stratum corneum extract even when measuring using the Os polymer lactic acid sensor. Furthermore, it was thought that there was a reduction in the current response because the concentration of the substance at fault was decreased in the process of double dilution. The change in current response was when different amounts of lactic acid were added to a solution extract of only OPP tape was

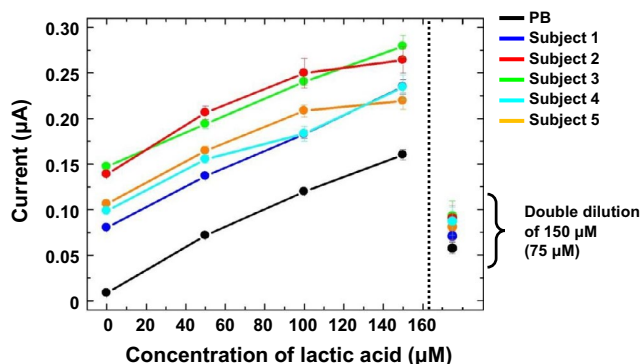


FIGURE 10 Changes in the current response of the Os polymer lactic acid sensor, when lactic acid was added to the TS extracted stratum corneum samples and PB with concentrations of 0, 50, 100, 150 $\mu\text{mol/L}$, and when 150 $\mu\text{mol/L}$ of solution was twofold diluted (75 $\mu\text{mol/L}$)

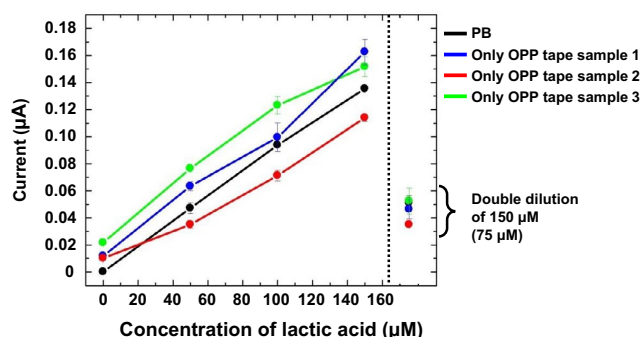


FIGURE 11 Changes in the current response of the Os polymer lactic acid sensor, when lactic acid was added to 0, 50, 100, and 150 $\mu\text{mol/L}$ in PB and the sample extraction of only the OPP tape, and when 150 $\mu\text{mol/L}$ solution was twofold diluted (75 $\mu\text{mol/L}$)

investigated (Figure 11). Upon the addition of lactic acid, the current response was observed to be dependent on the lactic acid concentration for all samples containing PB as a control. Since the lactic acid concentration in the solution twofold diluted was 75 $\mu\text{mol/L}$, the current response after dilution was thought to be between the values observed for the 50 and 100 $\mu\text{mol/L}$ concentrations; however, the current response was lower than expected for all three of the samples examined. At present, looking at the current data, the reason behind this observation is not clear. It is thought that an unknown component that dissolves into the water from the tape itself may have some influence on the current value, even when no voltage is applied.

5 | CONCLUSIONS

The results of this study show that the amount of lactic acid in a solution obtained by simple extraction of a TS stratum corneum sample with water can be analyzed easily and accurately using a Os polymer lactic acid sensor. However, there are still some factors that have an

effect on the current response of the stratum corneum extract or the tape itself, which could not be overcome using the Os polymer method, but analysis using a biosensor was shown to be a promising and simple method. Water-soluble components in the stratum corneum such as lactic acid can be easily extracted with water; therefore, extraction from stratum corneum samples can be carried out in water-containing microtubes. This study shows that specific components can be quantified easily using the disposable sensor and that this technology may be useful for skin health care, especially implementation at skincare counseling sites.

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