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***In vivo* optical investigation of short term skin water contact and moisturizer application using NIR spectroscopy**

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Abstract— Nowadays, a number of noninvasive methods and instruments are available to inspect the biophysical properties and effects of various applicants on human skin, providing quantitative measurements and more details regarding the interactions between skin and various products. Such methods include Near Infrared Spectroscopy (NIRS), a technique which over the years, has gained quite a reputation in being able to accurately determine moisture levels and water contents due to its sensitivity to hydrogen bonding. This paper reports preliminary results of an *in vivo* study carried out on the skin of a small number of human participants, investigating the optical response of human skin after direct short-term contact with water followed by application of a moisturizer, using a highly advanced spectrophotometer in the region of 900-2100nm, and equipped with a reflectance fibre optic probe. Results obtained here certainly raise some questions regarding the optical characteristics of different skin types and the influence of frequent moisturizer use, as well as the varying response between different water bands in the NIR region. Future work will focus on gaining more knowledge about these, in order to further improve optical skin measurements, and hopefully support the design and development of a portable and/or miniaturized optical device that could provide reliable, accurate and fast skin hydration readings in real time.

I. INTRODUCTION

The outermost layer of the skin, known as the Stratum Corneum (SC) is composed of several layers of dead keratin-filled squamous cells called corneocytes, embedded in an intercellular lipid matrix, whose role is vital in controlling the level of transcutaneous water loss and acts as an essential permeable barrier [1, 2]. Moreover, the hydration state of this layer or its water content is an important factor in maintaining a series of intrinsic and extrinsic factors that regulate the epidermal barrier function [1]. SC hydration levels are highly influenced by the structure and arrangement of the intercellular lipid matrix, and by the Natural Moisturization Factors (NMFs) which are comprised of water-soluble substances, and by the permeation path length through the SC. In turn, barrier function is influenced by the water content of the skin which regulates hydrolytic enzyme activities involved in SC maturation and desquamation of corneocytes.

Even though water plays a critical role in the homeostasis of skin, it is potentially a strong irritant, can disrupt barrier function and even cause skin damage [3, 4]. Prolonged skin contact with extrinsic water is not innocuous and can strip the skin off its natural oil and moisturizers, and lead to a number of profound effects such as SC swelling, increasing SC suppleness, weakening SC corneocyte cohesion, and increasing the permeability of all substances that penetrate the skin [5, 6]. For this reason, moisturizers are often applied on dry skin to lock water inside the SC and create a barrier that prevents oils from escaping and harmful elements from entering.

However, moisturizers are also commonly used for cosmetic purposes, and so far, some studies have shown that long-term use of certain moisturizers can weaken skin barrier function, enhance susceptibility to irritants and influence skin barrier recovery [7], although more knowledge about the effect of moisturizing agents on skin and its barrier function is required.

This paper reports initial findings of an *in vivo* experiment conducted on a small number of volunteers, where the optical properties of skin were assessed after direct short-term contact with extrinsic water, followed by application of a moisturizer and using a highly advanced spectrophotometer equipped with a fibre optic probe to obtain Near Infrared (NIR) reflectance spectra of skin.

This particular technique was employed here due to its ability to directly and accurately detect water inside the skin using the intensities of overtone and combination bands of OH and HOH water bonds occurring in the NIR region, which are good indicators of the level of skin hydration and water content [8-11]. NIR spectra can also provide additional insight regarding other skin constituents including lipids and proteins, it can differentiate between different types of water in the skin [9, 10], and its instrumentation can easily be equipped with fibre optic probes for *in vivo* measurements.

Unfortunately, a reliable instrument based on NIR spectroscopy designed specifically for skin hydration measurements is not yet available, although a few recent studies have looked at ways to improve or miniaturize this technique [12-14]. Results from this experiment will be used in conjunction with our previous work [15], to aid the future design and development of a portable NIR spectroscopic device that would allow accurate, fast and non invasive *in vivo* measurements of skin hydration and water content.

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II. MATERIALS AND METHODS

City University London Senate Research Ethics approval was sought prior to performing any measurements on individuals or collecting any personal details.

A. Experiment Design

Twenty healthy volunteers were recruited and asked to complete a skin health questionnaire prior to performing any tests. Then, measurements were taken by placing the probe slightly above the ankle on the lower left leg of each volunteer, taking 3 spectrophotometric scans for each measurement for averaging purposes. Overall, 3 measurements were recorded. The first was a control measurement where the skin of the individual was at its natural state (no application of tape-stripping, moisturizer or wet patch). Next, a wet patch previously immersed in water was placed on the test site for 10 minutes, removed, and then the same site was scanned again, then finally, a moisturizing agent was applied on the same area, and scanned once more 30mins after. From those participants, 3 groups, each consisting of 5 volunteers each, were identified and categorized as follows: 5 who moisturize on a daily basis and have normal skin (Group 1), another 5 with normal skin but do not at all use moisturizers (Group 2), 5 who moisturize everyday or most days but have dry skin type (Group 3). Further analysis was later carried out on the group spectra from each category to examine and compare their response.

B. Instrumentation

NIR skin spectra were collected using the Lambda 1050 dual beam UV/Vis/NIR spectrophotometer (Perkin Elmer Corp, Massachusetts, USA) at increments of 2 nm in the spectral region of 900-2100 nm, with an InGaAs detector operating throughout the entire region. Light was provided by a tungsten lamp and gain settings were also added to measure the energy in the single mode beam and give better quality spectra. The scanning period for each interval was set at 0.2 secs, as this value seemed to give a reasonable balance between sensitivity and scan time.

Attenuator settings were kept constant at 1% for the reference beam to improve noise levels at high absorbance, and at 100% attenuation for the sample beam. Slit size controls for the InGaAs detector which allow one to adjust the amount of light entering was set on "servo mode" so that the system could oversee the reference beam energy and select the slit size accordingly.

Initial baseline corrections were performed to eliminate irrelevant bands and background noise especially evident in highly absorbing media such as skin. These corrections were carried out at 100 % T/OA baseline to correct maximum sample and reference beams, and at 0 % T/blocked beam to regulate the beams at 0 % for the sample and 100 % for the reference.

In order to acquire reflectance measurements, it was necessary to replace the sample holder compartment with a universal reflectance accessory that permitted the attachment of a fibre optic probe (Ocean optics, Duiven, The

Netherlands). This probe consists of a bundle of 7 optical fibres in a stainless steel ferrule, with 6 of them as illumination fibre, all around 1 light detecting fibre. However, depending on the connection of the probe to the spectrophotometer, it was possible to use the single fibre as the source of radiation and the other 6 as detectors. The fibre core diameter was 600 μm , allowing a fairly reasonable area of skin to be sampled.

It was essential to introduce a small gap between the probe and the test site to prevent blockage of light leaving and entering the probe, and also since occlusion can lead to build up of water and increase hydration, thereby falsely raising skin water content measurements. Therefore, the reflectance probe was slightly modified by enclosing its tip with a Perspex tube layer that was longer than the end of the probe by 1.5 mm. This coating ensured that the desired separation distance was maintained throughout all tests. In addition, this particular coating was overlaid with another white coating to eliminate interference from ambient light whilst scanning.

Pre-treatment processes such as averaging and smoothing were performed on all the spectra obtained using the UVWinlab Data Processor and Viewer software (Perkin Elmer Corp, Massachusetts, USA), and were later transferred to Matlab (Mathworks Inc., Novi, MI) to carry out Standard Normal Variate (SNV) scatter corrections.

III. RESULTS AND DISCUSSION

All skin reflectance spectra were converted into apparent absorption after smoothing and averaging using the equation $\text{Log } 1/R$, and then transferred to Matlab to perform scatter corrections using the SNV method. For the 3 set of spectral measurements taken from each participant (control, after application of a wet patch and then after treatment with a moisturizer for 30 mins), the average was calculated again



Figure 1. Mean of the mean apparent absorption graph of all volunteers before, and after application of a wet patch then followed by a moisturizer.

for each set to give the mean of the mean of all data. This graph is shown in Fig 1. As can be seen, the overtone band of the OH stretching fundamental around 1450 nm and the weak water combination band near 1780 nm are greater for the first measurement (control), recorded before application of the wet patch or moisturizer, which indicates higher water contents. In contrast, other known water bands in this spectrum do not adhere to the same behavior, as seen at the weak combination band of water around the 1200 nm region where the control spectrum is in fact slightly lower than after skin contact with water and moisturizer use, and whereas at the combination band of OH and HOH bending in the region of 1900-1920 nm all 3 spectra are nearly equal, but with the average spectrum obtained after applying the wet patch being slightly dominant in this particular region.

Next, peak values occurring around 1200 nm, 1450 nm and 1900 nm from the averaged spectra of each volunteer were noted and tabulated separately for each of the 3 tests performed. Then, the mean was calculated for the 3 groups classified earlier in the methods section according to the type of skin of 15 participants and their daily use of moisturizers so that: Group 1, represented 5 volunteers who moisturize daily and have normal skin type, Group2, 5 participants who do not at all moisturize their legs but also have normal skin type and lastly, Group 3, another 5 volunteers who moisturize daily or regularly but have dry skin type. Mean values from each group were then averaged and transformed into a chart including their standard deviation.

The first of these is shown in Fig 2 representing the arbitrary peak values for the categorically different groups at the weak combination band of water near 1200 nm. Looking at this chart, all three groups gave similar results at the initial control measurement. However, after applying the wet patch for 10 mins on the test site, both groups who moisturize frequently showed a considerable increase,

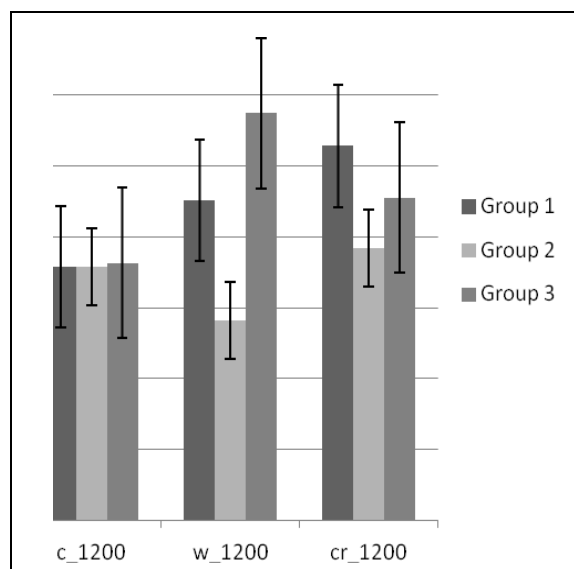


Figure 2. Mean peak value variability between groups 1, 2 and 3 at the weak water combination band near 1200 nm region.

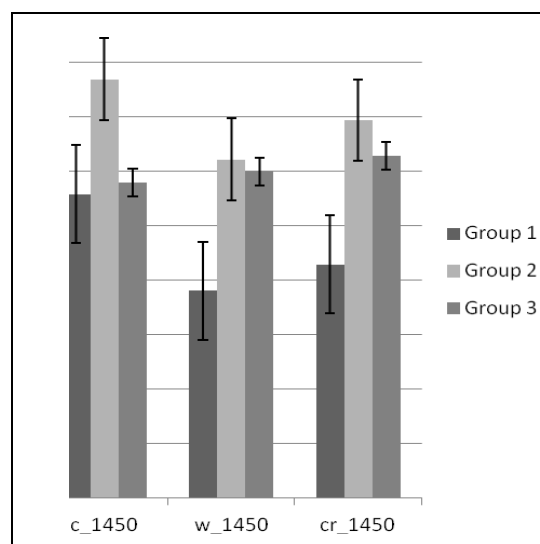


Figure 3. Mean peak value variability between group 1, 2 and 3 at the OH overtone band around 1450 nm region

especially in those with dry skin whereas the opposite is shown for the second group who do not moisturize at all. This peak rise is also seen after treatment with the moisturizing agent, this time affecting all groups, but with the second group showing the least increment. The smallest deviation was also expressed by group 2 throughout all tests in this region.

As for the overtone band near 1450 nm, the reaction here was quite different in comparison to the former wavelength and is shown in Fig 3. This time, group 1 which comprised of those with normal skin and who moisturize on a daily basis constantly showed lower values compared to the remainder of groups and a higher standard deviation. Nevertheless, their peak value was highest at the control reading, which decreased after water contact then slightly increased again after using the moisturizer, although it did not return to its original value, which was probably due to the slight damage introduced to the skin barrier function by

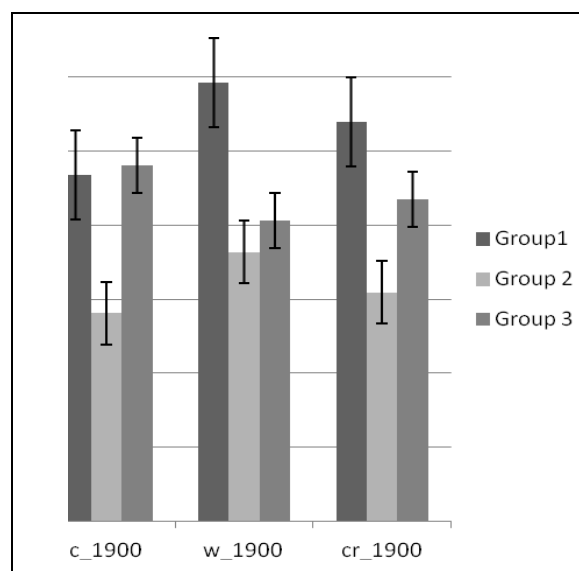


Figure 4. Mean peak value variability between groups 1, 2 and 3 at the water combination band between the 1900-1920 nm region.

direct contact with water for a short period of time.

The second group exhibited the highest peak values in all tests at this region whilst the third group demonstrated the least amount of variability between the different tests, and peak value changes observed in the water combination band between 1900-1920 nm. Analogous to the variation pattern seen in the 1200 nm region chart, peak values for group 1 are seen to ascent after applying the wet patch as well as the moisturizer though the wet patch apparently caused a larger increase in this specific region, as oppose to the third group whose control value decreased, mostly after water contact with skin but again expressed the least amount of standard deviation overall. Results from the second group show the same pattern as group one in this band, but opposite to that seen in the 1200 and 1450 nm region charts, and their values at this band also reacts contrarily to those from the 1450 nm region, in this case having the lowest values throughout all tests.

IV. CONCLUSIONS AND FUTURE WORK

A state of the art spectrophotometer equipped with a fibre optic reflectance probe was employed to perform an *in vivo* experiment investigating the effects of short term direct water contact with skin followed by application of a common moisturizer on a small group of participants divided into categories depending on their type of skin and frequency of typical moisturizer use. Looking at the mean spectra of all participants for the 3 tests, it was evident that the resulting spectrum from recordings taken prior to contact with water or treatment with a moisturizing agent showed higher peak values around the water overtone and weak combination bands near 1450 nm and 1780 nm, which is probably expected since the majority of volunteers were frequent users of moisturizers. However, this was not the same for other known water bands although peak values from all tests were quite similar in those regions.

In terms of group analysis, a similar pattern in response to water contact and moisturizer application was seen between the first group which comprised of individuals with normal skin type and who moisturize daily, and the second group which included those who do not moisturize at all but also have normal skin type. Nevertheless, actual peak values differed quite largely, which may possibly relate to the effects of long-term moisturizer use on skin barrier function and recovery. Results from the group of individuals with dry skin were the most conflicting in comparison to the first two groups, perhaps indicating the nonconformities in skin barrier function characteristics and sensitivity in individuals who suffer from dry skin.

Overall, these results raise questions about the optical properties of different skin types and the influence of frequent moisturizer use, as well as the varying response between different water bands in the NIR region. However, since this experiment was based on a small number of participants, future work will focus on extending this work to include data from a larger population to allow a fairer

statistical meaning, and on gaining more knowledge about the response of different water bands inside the skin, in order to further improve optical skin measurements, and hopefully support the design and development of a portable and/or miniaturized optical device that could provide reliable, accurate and fast skin hydration readings *in vivo* to eliminate the use of current bench-top instruments yet perform to the same standard.

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