

Raman spectroscopic analysis of skin penetration and moisturizing effects of Bionics vernix caseosa cream compared with Vaseline

Hong Meng^a, Yating Yin^a, Wenhai Wu^b, Yuhong Liu^c, Li Li^a, Yinmao Dong^a, Yi Fan^a, Yue Li^d and Yifan He^{a,*}

^a*Institute of Regulatory Science, Beijing Technology and Business University, Beijing 100048, China*

^b*Beijing Academy of TCM Beauty Supplements, Beijing 100048, China*

^c*Nutri-Woods Bio-Tech (Beijing) Co., Ltd., Beijing 100048, China*

^d*Beijing International Studies University, Beijing 100000, China*

Abstract.

BACKGROUND: The stratum corneum (SC) is the outermost layer of human skin and deemed as barrier against chemical exposure and water loss. Moisturizers have beneficial effects in treating dry skin, especially the SC. Confocal Raman spectroscopy (CRS) was used to evaluate the efficacy of moisturizers on skin hydration and penetration, with such agents posing inherent characteristics of being noninvasive, nondestructive, timesaving, and cost effective. Bionics vernix caseosa (BVC) cream mimics the composition of vernix caseosa (VC), which could protect the newborn skin.

METHODS: This research applied CRS to evaluate the penetration depth and water content variation during the intervention with two moisturizers, BVC cream and Vaseline. Volunteers received the 2 h application of BVC cream and Vaseline on the forearms. The evaluations on 0 h, 2 h, 4 h and 6 h were performed clinical assessment. Experimental data was processed by least square method and analysis of variance (ANOVA).

RESULTS: The penetration depth of Vaseline was deeper than that of Bionics vernix caseosa cream. Specifically, BVC cream penetrated 18 μm into human skin, while Vaseline penetrated at least 20 μm . Compared with Vaseline, only BVC cream increased skin hydration, with a moisturizing effect lasting for 4 h. At 6 h, the Vaseline moisturizing effect decreased significantly.

Keywords: Bionics vernix caseosa cream, Vaseline, confocal Raman spectroscopy, hydration, penetration

1. Introduction

The skin is the largest organ of human being and accomplishes multiple defensive functions, such as protection against exogenous chemical and physical factors. The stratum corneum (SC) is the top layer of the epidermis, with the function of a barrier against the penetration of exogenous substances and the loss of water [1–5]. Skin barrier in proper condition is necessary to regulate evaporative water loss while

*Corresponding author: Yifan He, Institute of Regulatory Science, Beijing Technology and Business University, Beijing 100048, China. Tel.: +86 18310737514; Fax: +86 10 68984917; E-mail: heyifan@btbu.edu.cn.

maintaining the level of hydration essential for the enzyme reactions that facilitate stratum corneum desquamation and maturation [6]. Skin moisturization is important for flexibility and other aspects of a healthy appearance [7]. If the SC's water content (10–20%) decreases, the protective layer of the skin starts to become dry. In this case, cosmetic products are usually applied to healthy promotion, moisturized skin and overcome signs of dryness [8].

Moisturizers prevent evaporative water loss to the environment by placing an oily substance on the skin surface where water cannot penetrate, thus replenishing the SC moisture by water moving from the lower viable epidermal and dermal layers [9]. Vaseline, an oil-soluble ingredient in cosmetic formulas, has been proposed as the best treatment for dryness, among its various occlusive properties. However, Vaseline is a thick and waxy material that makes it difficult to handle and inconvenient for general use, especially over large areas of the body [10].

Vernix caseosa (VC) is a white and creamy substance, which naturally occurring biofilm covering the skin of the fetus during the last trimester of pregnancy. VC coating on the new born baby skin, facilitates extrauterine adaptation of the skin in the first postnatal week if not washed away after birth and protects the neonatal skin [11]. VC consists of water (81%), lipids (9%), and proteins (10%) [12], which can be deemed as a baby's natural skin cream with multiple functions such as skin miniaturization, protection against bacterial infection, pH adjustment and epidermal barrier repair [13–15]. As a result of bionic technology, Bionics vernix caseosa (BVC) cream could be used for a better improvement of the moisturizing process.

Confocal Raman spectroscopy (CRS) is used as noninvasive method to determine depth profiles of water concentration of skin seems to be a highly sensitive technique to monitor changes of molecular [16–20]. Currently, commonly used instruments are capacitance or conductance principles designed to evaluate the barrier function of stratum corneum, including trans epidermal water loss (TEWL) and hydration. Because highly hydrated skin is high conductivity and low capacitance, use oil products will hinder the flow of ions in the skin [21]. Therefore, it is more accurate to evaluate the influence of oily substances on skin moisture content with CRS. Caspers et al. [22] proposed a method to calculate the concentration profile of water mass, based on the ration of integrated region from 3350 cm^{-1} to 3550 cm^{-1} and that of integrated from 2910 cm^{-1} to 2965 cm^{-1} , represent the intensity of OH vibration and that of keratin. This method has been commonly used in water-related researches, e.g. stratum corneum thickness the determination. Vyumvuhore et al. [23] also illustrated the different water molecules types, namely primary bound water, partially bound water and unbound water, which can be calculated with the Gaussian function-based deconvolution of the Raman OH band ($3100\text{--}3700\text{ cm}^{-1}$). In this study, we investigated the skin penetration and moisturizing effect of BVC cream and Vaseline by CRS.

2. Materials and methods

Six healthy volunteers, 3 females and 3 males, aged between 23 to 25 years were recruited. The volunteers were informed not to shower or bathe at least 4 h and not to use any skin care products on the forearms for at least 72 h prior to the experiments. After 20 mins acclimation time, 2 skin areas (each of $2 \times 2\text{ cm}^2$ size) were marked on the volar forearms under constant temperature and humidity room ($23 \pm 2^\circ\text{C}$, $45 \pm 2\%$ relative humidity). First of all, the product was topically applied on one targeted area of the forearm and rubbed homogenously. Secondly, the intact skin area was measured at two measuring points of each person by confocal Raman microscopy as a control. After the product was applied for 2 h, the remaining product was cleaned by soft filter paper and each targeted area was measured using confocal Raman microscopy (CRM). Application times of 2, 4 and 6 h before measurements were chosen.

2.1. Moisturizer lotion

Vaseline (TeFuWang, China).

BVC cream lotions include refined jojoba oil 10 ~ 30 wt%, jojoba ester 10 ~ 30 wt%, hydrogenated lecithin 1 ~ 25 wt%, plant sterol 5 ~ 30 wt%, cetaceanol 5 ~ 30 wt%, ceramide 30.1 ~ 5 wt%.

2.2. Confocal Raman spectroscopy

Samples were placed in a constant temperature and humidity chamber under the microscope interfaced to a CRS (Horiba Jobin Yvon, Villeneuve d'Ascq, France). The sample video image of was used for accurate positioning of the laser spot on the sample. A 660 nm pumped Nd:YLF laser was utilized. The collected light was filtered through a notch filter and dispersed with an 8 cm^{-1} spectral resolution and a holographic grating of 600 gr/mm. Spectral acquisition was utilizing LabSpec 6.0 software (Horiba Scientific, Lille, France). Each volunteer was measured three times for each skin area. For each depth, a 2 seconds exposure time was used. The collection depth ranged from 0 to 20 μm . All spectra were designed to the same automatic preprocessing protocol and were smoothed using a Savitzky-Golay filter of nine points [24]. Fluorescence backgrounds were suppressed by a second derivative polynomial function [25].

2.3. Data analysis

2.3.1. BVC cream and Vaseline penetration measurements

Penetration was determined by using the classical least squares method, first obtaining the spectra of the samples and skin as well as the spectra after using the product, according to the following formula:

$$C(\text{after}) = \alpha * C(\text{skin}) + \beta * C(\text{sample})$$

where the coefficients for the skin and sample are α and β , respectively, and β/α is the transdermal absorption of samples. When the depth of transdermal absorption > 0 , it shows that the samples penetrate to that depth [23,26].

2.3.2. BVC cream and Vaseline influence skin moisture content measurements

According to Caspers et al. [22], the skin water content as a function of depth was detected by the ratio of the protein keratin integrated peak areas (between 2910 and 2965 cm^{-1}) and water molecules (between 3100 and 3620 cm^{-1}), resulting from CH and OH stretching vibrations. The following equation was used to evaluate the water content:

$$(3100 \sim 3620)\text{cm}^{-1}/(2910 \sim 2965)\text{cm}^{-1}$$

2.4. Statistical analysis

The ANOVA test was used for measurement of time changes among the subjects at 0 h, 2 h, 4 h, 6 h. The P value < 0.05 was considered as statistical significance.

For each time, the average results if the measurements on all volunteers' stratum corneum were presented as mean \pm standard deviation and an ANOVA test was performed.

3. Results

3.1. BVC cream and Vaseline penetration measurements

The results of skin retention of volunteers using BVC cream and Vaseline are shown in Fig. 1. The

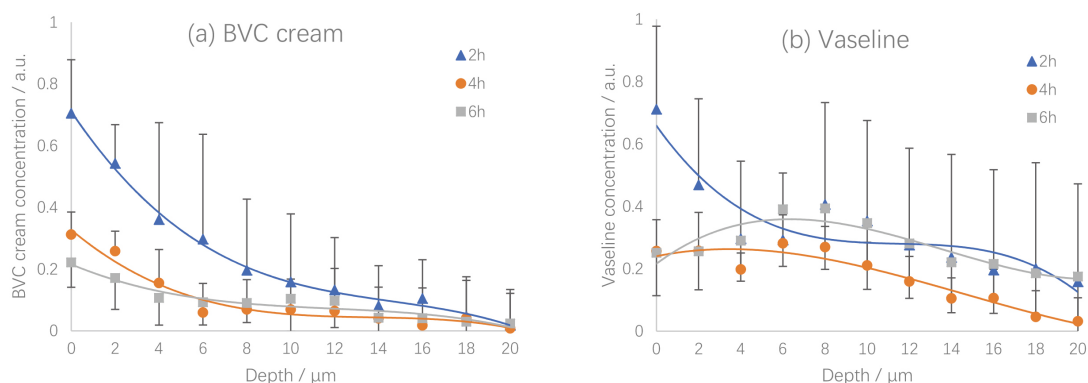


Fig. 1. (a) BVC Cream and (b) Vaseline penetration measurements.

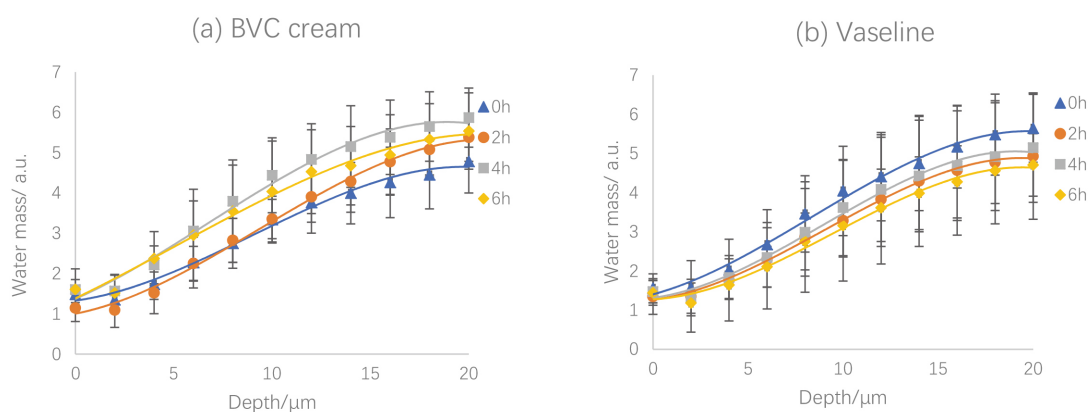


Fig. 2. (a) BVC cream and (b) Vaseline influence skin moisture content measurements.

x-coordinate is the skin depth, and the y-coordinate is the retention of the samples. When the percutaneous absorption at a certain depth is greater than zero, it indicates that the sample penetrates to that depth.

As shown in Fig. 1a, the penetration depth of the BVC cream is approximately $18 \mu\text{m}$. In the range of $0\text{--}10 \mu\text{m}$, BVC cream retention significantly decreases ($P < 0.05$) in 4 h and 6 h compared with 2 h. In Fig. 1b, Vaseline penetrates at least $20 \mu\text{m}$ because the curve tends to increase at $20 \mu\text{m}$. In the range of $0\text{--}2 \mu\text{m}$, Vaseline retention significantly decreases ($P < 0.05$) in 4 h compared with 2 h.

3.2. BVC cream and Vaseline influence skin moisture content measurements

The influence of the samples on the skin moisture content is shown in Fig. 2. The x-coordinate is the depth of the skin, and the y-coordinate is the ratio of the water peak ($3100\text{--}3620 \text{ cm}^{-1}$) to the keratin peak ($2910\text{--}2965 \text{ cm}^{-1}$). There was no significant difference in the baseline data of the 6 volunteers' water content ($P > 0.05$). Therefore, we used one-way ANOVAs to compare the water content of skin with different time periods under the same depth.

In Fig. 2a, the skin water content showed an upward trend after applying BVC cream for 2 or 4 h, but there was no significant difference ($P > 0.05$). In 6 h, the skin water content basically returned to the initial state.

In Fig. 2b, the water content of the skin decreased after applying Vaseline at 2 and 4 h, but there was

no significant difference at any depth ($P > 0.05$), while the water content decreased significantly in 6 h ($P < 0.05$).

4. Discussion

4.1. Comparison of the skin penetration depth

The maximum depth of collection was 20 μm . One reason is that a long integration time affects the stability and accuracy of the test. In addition, increasing the excitation wavelength can lead to the risk of skin burns. Under our test conditions, it was shown that Vaseline penetrates deeper than BVC cream.

Based on the above results, BVC cream and Vaseline mostly remain in the SC. The stratum corneum (SC) plays an important role in the skin barrier protection. However, when we use external intervention for skin care, we need to consider the target position of the active ingredients. Lipids of stratum corneum are with an important role in the regulation of the various compounds absorption from the surface of the skin [27].

BVC cream is a semipermeable film that allows the passage of water vapor [28]. Vaseline is an impermeable film that allows low water vapor transport, indicating that BVC cream is almost fully occlusive compared with BVC cream [28–30]. Georgios et al. [31] investigated Vaseline and found a significantly higher penetration depth. s Additionally, as for the high occlusion ability, Vaseline gives rise to an increase of water in the SC and causes a swelling effect. The stratum corneum swells to several times its normal thickness and exhibits increased cell membrane permeability when exposed to liquid water or high humidity. Occluded environments or Prolonged high humidity also contribute to poor skin conditions, e.g. diaper dermatitis [32]. Damaged stratum corneum treated with semipermeable films recovers more quickly than damaged stratum corneum treated under complete occlusion or no occlusion, as reported after tape stripping and in premature infants [33–36].

4.2. The moisturizing properties of Vaseline

The water content of Vaseline showed a significant difference by comparison of the results at 6 h with those at 2 and 4 h. Vaseline is a ready-made occlusive emollient [37]. It forms a film on the skin surface, fills the gap between a large number of exfoliating keratinocytes under dry skin conditions, smooths the rough surface of the cuticle, and increases the skin's moisturizing ability [37]. After applying moisturizers such as glycerin, Vaseline is applied to the skin, which can significantly reduce TEWL [38,39]. Based on the experimental design and equipment requirements, Vaseline was wiped for 2 h. As described in Section 4.1, Vaseline has the function of SC swelling and skin barrier disruption, leading to a significant decrease in water content after 6 h. Additionally, TEWL as a cell signal can induce the repair of the SC barrier and the synthesis of intracellular lipids. If TEWL is completely blocked, the repair of the SC barrier will be impeded. When the Vaseline is removed, the water content will return to the pretreatment level. However, other research also shows that Vaseline can penetrate SC to help repair the skin barrier, starting with the production of intercellular lipids, such as sphingolipids, free sterols, and free fatty acids [40].

4.3. The moisturizing properties of BVC cream

BVC cream is composed of 9 types of lipids, and some of its components form an oil film like that of Vaseline. Although each of the lipid species is important for stratum corneum homeostasis, ceramides are

of particular importance because of their large weight contribution and structural characteristics. Ali et al. [41] used Raman spectroscopy to study the organization of three synthetic ceramides (CER) and detect the evolution of the conformation differing from each other by their polar heads in nature (sphingosine, phytosphingosine and a hydroxyl sphingosine), namely CER 2, III and 5. CER III and 5 illustrated an ordered organization and more compact and stronger polar interactions at intermediate relative humidity values, while CER 2 illustrated opposite tendencies and results to those observed with CER III and 5 [41]. BVC cream adds Ceramide III, and its polar head may bind hydroxyl of water molecule. This may be responsible for increasing the skin water content. If the environmental humidity changes, BVC cream supplies the skin with ceramides. It has been illustrated that when free fatty acids, cholesterol, or ceramides are applied alone, they aggravate the barrier rather than improve. In contrast, a mixture of the three key lipids in appropriate molar ratios leads to normal barrier repair [42]. Thus, the present conclusion supports the scientific mixture of lipids in BVC cream lotions.

Compare the moisturizing properties of BVC cream and Vaseline, we find the two different kinds of mechanism. BVC cream reconstituted skin lipids, which include CER and unsaturated fatty acid. These materials can combine with hydroxyl group of water to increase water content and repair skin protection. While, Vaseline only contains saturated fatty acid that covers the skin to prevent water loss.

Our subject remaining to be explored is how to use CRS to compare skin penetration and the moisturizing effect of different moisture lotions. We used only healthy subjects to minimize confounding variables. Future research is suggested include testing subjects with damaged skin. The results of the present experiments indicate that BVC cream can be used in cosmetics for infant skin and sensitive skin, as well as an external reagent for fragile skin with thin cuticles.

Above all, Vaseline can penetrate deeper into the skin, meaning that it can be used as a substrate for medical purposes or for deeper targeted sites. In infant skin and sensitive skin, the SC is thin. Therefore, in the process of creating moisturizing products, skin physiological characteristics should be considered in the design principles.

5. Conclusion

The penetration depth of BVC cream is approximately 18 μm , and that of Vaseline is at least 20 μm . After applying Vaseline for 2 h and wiping it off, the effect on skin moisture content was negative. In contrast, BVC cream promoted the trend of increasing the skin moisture content.

Conflict of interest

The authors have no potential conflicts of interest to disclose.

References

- [1] Friberg SE, et al. Water permeation of reagggregated stratum corneum with model lipids. *J. Invest. Dermatol.* 1990; 94(3): 377–380.
- [2] Elias PM. Epidermal barrier function: intercellular lamellar lipid structures, origin, composition and metabolism. *J. Controlled Release.* 1991; 15(3): 199–208.
- [3] Schreiner V, et al. Barrier characteristics of different human skin types investigated with X-ray diffraction, lipid analysis, and electron microscopy imaging. *J. Invest. Dermatol.* 2000; 114(4): 654–660.

- [4] Golden GM, et al. Stratum corneum lipid phase transitions and water barrier properties. *Biochemistry*. 1987; 26(8): 2382–2388.
- [5] Elias PM, Friend DS. The permeability barrier in mammalian epidermis. *J. Cell Biol.* 1975; 65(1): 180–191.
- [6] Rawlings AV, Matts PJ. Stratum corneum moisturization at the molecular level: an update in relation to the dry skin cycle. *J Invest Dermatol.* 2005; 124: 1099–1110.
- [7] Jiang ZX, DeLaCruz J. Appearance benefits of skin moisturization. *Skin Res Technol.* 2011; 17(1): 51–55.
- [8] Tippavajhala VK, Magrini TD, Matsuo DC, et al. *In vivo* determination of moisturizers efficacy on human skin hydration by confocal raman spectroscopy. *AAPS PharmSciTech.* 2018; 19(7): 3177–86.
- [9] Wilhelm KP, Cua AB, Maibach HI. Skin aging: effect on transepidermal water loss, stratum corneum hydration, skin surface pH, and casual sebum content. *Arch Dermatol.* 1991; 127: 1806–1809.
- [10] Buraczewska I, Berne B, Lindberg M, Törmä H, Lodén M. Changes in skin barrier function following longterm treatment with moisturizers, a randomized controlled trial. *Br J Dermatol.* 2007; 156: 492–498.
- [11] Tollin M, Bergsson G, Kai-Larsen Y, Lengqvist J, Sjövall J, Griffiths W, Skúladóttir GV, Haraldsson Á, Jörnvall H, Gudmundsson GH, Agerberth B. Vernix caseosa as a multi-component defence system based on polypeptides, lipids, and their interactions. *Cellular and Molecular Life Sciences.* 2005; 62: 2390–2399.
- [12] Hoeger PH, Schreiner V, Klaassen IA, Enzmann CC, Friedrichs K, Bleck O. Epidermal Barrier lipids in human vernix caseosa: corresponding ceramide pattern in vernix and fetal skin. *Br J Dermatol.* 2002; 146: 194–201.
- [13] Visscher MO, Narendran V, Pickens WL, et al. Vernix caseosa in neonatal adaptation. *Journal of Perinatol.* 2005; 25(7): 440–446.
- [14] Moraille R, Pickens L, Visscher O, et al. A novel role for vernix caseosa as a skin cleanser. *Neonatology.* 2005; 87(1): 8–14.
- [15] Rissmann R, Oudshoorn MH, Kocks E, et al. Lanolin-derived lipid mixtures mimic closely the lipid composition and organization of vernix caseosa lipids. *Biochimica Et Biophysica Acta.* 2008; 1778(10): 2350–60.
- [16] Cabrera-Alonso R, Guevara E, Ramirez-Elias MG, et al. Detection of hydroquinone by Raman spectroscopy in patients with melasma before and after treatment. *Skin Research and Technology: Official Journal of International Society for Bioengineering and the Skin.* 2019; 25(1): 20–4.
- [17] Crowther JM, Sieg A, Blenkiron P, et al. Measuring the effects of topical moisturizers on changes in stratum corneum thickness, water gradients and hydration *in vivo*. *The British Journal of Dermatology.* 2008; 159(3): 567–77.
- [18] Egawa M, Sato Y. *In vivo* evaluation of two forms of urea in the skin by Raman spectroscopy after application of urea-containing cream. *Skin Research and Technology : Official Journal of International Society for Bioengineering and the Skin.* 2015; 21(3): 259–64.
- [19] Forster M, Bolzinger MA, Ach D, et al. Ingredients tracking of cosmetic formulations in the skin: a confocal Raman microscopy investigation. *Pharm Res.* 2011; 28(4): 858–72.
- [20] Mao G, Flach CR, Mendelsohn R, et al. Imaging the distribution of sodium dodecyl sulfate in skin by confocal Raman and infrared microspectroscopy. *Pharm Res.* 2012; 29(8): 2189–201.
- [21] Wang H, Zhang Q, Mao G, et al. Novel confocal Raman microscopy method to investigate hydration mechanisms in human skin. *Skin Research and Technology*, 2019.
- [22] Caspers PJ, Lucassen GW, Carter EA, Bruining HA, Puppels GJ. *In vivo* confocal Raman microspectroscopy of the skin: noninvasive determination of molecular concentration profiles. *J Invest Dermatol.* 2001; 116: 434–42.
- [23] Vyumvuhore R, Tfayli A, Manfait M, et al. Vibrational spectroscopy coupled to classical least square analysis, a new approach for determination of skin moisturizing agents mechanisms. *Skin Research and Technology.* 2014; 20(3): 282–292.
- [24] Steinier J, Termonia Y, Deltour J. Smoothing and differentiation of data by simplified least square procedure. *Analytical Chemistry.* 1972; 44(11): 1906–9.
- [25] Zhao J, Lui H, Mclean DI, et al. Automated autofluorescence background subtraction algorithm for biomedical raman spectroscopy. *Applied Spectroscopy.* 2007; 61(11): 1225–32.
- [26] Essendoubi M, Gobinet C, Reynaud R, et al. Human skin penetration of hyaluronic acid of different molecular weights as probed by Raman spectroscopy. *Skin Research and Technology.* 2016; 22(1): 55–62.
- [27] Pailler-Mattei C, Nicoli S, Pirot F, et al. A new approach to describe the skin surface physical properties *in vivo*. *Colloid Surf B Biointerfaces.* 2009; 68: 200–6.
- [28] Visscher M, Narendran V. Vernix caseosa: formation and functions. *Newborn and Infant Nursing Reviews.* 2014; 14(4): 142–6.
- [29] Gunt H. Water handling properties of vernix caseosa. Cincinnati, OH: University of Cincinnati. 2002.
- [30] Tansirikongkol A. Development of a synthetic vernix equivalent, and its water handling and barrier protective properties in comparison with vernix caseosa. Cincinnati, OH: University of Cincinnati. 2006.
- [31] Stamatias GN, De Sterke J, Hauser M, et al. Lipid uptake and skin occlusion following topical application of oils on adult and infant skin. *Journal of Dermatological Science.* 2008; 50(2): 135–42.
- [32] Dynamics of water transport and swelling in human stratum corneum. *Chemical Engineering Science.* 138: 164–72.

- [33] Schunck M, Neumann C, Proksch E. Artificial barrier repair in wounds by semioclusive foils reduced wound contraction and enhanced cell migration and reepithelization in mouse skin. *J Invest Dermatol.* 2005; 125: 1063–71.
- [34] Visscher M, Hoath SB, Conroy E, Wickett RR. Effect of semipermeable membranes on skin barrier repair following tape stripping. *Arch Dermatol Res.* 2001; 293: 491–9.
- [35] Bhandari V, Brodsky N, Porat R. Improved outcome of extremely low birth weight infants with Tegaderm application to skin. *J Perinatol.* 2005; 25: 276–81.
- [36] Mancini AJ, Sookdeo-Drost S, Madison KC, Smoller BR, Lane AT. Semipermeable dressings improve epidermal barrier function in premature infants. *Pediatr Res.* 1994; 36: 306–14.
- [37] Levi K, Weber RJ, Do JQ, et al. Drying stress and damage processes in human stratum corneum. *International Journal of Cosmetic Science.* 2010; 32(4): 276–93.
- [38] Brooks J, Cowdell F, Ersser SJ, et al. Skin cleansing and emolliating for older people: a quasi-experimental pilot study. *International Journal of Older People Nursing.* 2017; 12(3).
- [39] Friberg SE, Ma Z. Stratum corneum lipids, petrolatum and white oils. *Cosmet Toilet.* 1993; 107: 55–59.
- [40] Grubauer G, Feingold KR, Elias PM. Relationship of epidermal lipogenesis to cutaneous barrier function. *J Lipid Res.* 1987; 28: 746–752.
- [41] Tfayli A, Jamal D, Vyumvuhore R, et al. Hydration effects on the barrier function of stratum corneum lipids: Raman analysis of ceramides 2, III and 5. *Analyst.* 2013; 138(21): 6582–8.
- [42] Mao-Qiang M, Feingold KR, Thornfeldt CR, Elias PM. Optimization of physiological lipid mixtures for barrier repair. *J Invest Dermatol.* 1996; 106: 1096–101.