Review and Research on Cancer Treatment
Volume 5, Issue 1 (2019) ISSN 2544-2147

Application of the tape stripping method in the research on the skin condition and its diseases

Anna Sobiepanek*, **Tomasz Kobiela**

Laboratory of Biomolecular Interactions Studies, Chair of Drug and Cosmetics Biotechnology, Faculty of Chemistry, Warsaw University of Technology

Ryszard Galus,

Department of Histology and Embryology, Medical University of Warsaw

*Corresponding author: Anna Sobiepanek, Laboratory of Biomolecular Interactions Studies, Chair of Drug and Cosmetics Biotechnology, Faculty of Chemistry, Warsaw University of Technology, Noakowskiego 3, 00-664 Warsaw, Poland, asobiepanek@ch.pw.edu.pl

Abstract: Tape Stripping (TS) is a minimally invasive but yet a very useful procedure which enables the partial removal of the *stratum corneum* (SC) almost layer by layer. Being the outer layer of the epidermis, SC is responsible for the mechanical protection of the skin and deeper lying organs, as well as the protection against water loss from the body. In addition, the occurrence of the lipid coat on the surface of the SC regulates the process of absorption and penetration of various substances into the skin. The corneocyte samples obtained by the use of adhesive tapes may provide a lot of useful information concerning the skin condition and also its proper functioning as a barrier. This data could be helpful in the investigation and diagnosis of several skin diseases.
In this review, we present the history of the tape stripping method, some critical parameters of the procedure as well as the possible applications of the combined non-invasive tape stripping method with the other more advanced methods to analyze corneocytes taken from volunteers or patients.

1. Introduction

The minimally invasive procedure which allows the collection of the cells from the *stratum corneum* by a single or multiple use of the appropriate adhesive tape is the tape stripping method. It was invented in the 1940s and can be used in many studies carried out on humans as well as on animals such as pigs, rats, mice and guinea pigs (Arct, Koazyra et al. 2013). This method is based on the interaction of the adhesive forces of the tape with the cohesion forces occurring between the cells.

The general procedure (Fig. 1) of the tape stripping method is based on placing an adhesive tape on the surface of the skin (previously selected area – head, arm, leg), a gentle pressure on the tape ensuring a good contact of the tape with the cells and next by the removal of the tape with the cells through tearing the tape from the surface of the epidermis. The sample should be secured on a glass slide or in a tube. If more samples are needed or the study requires an analysis of certain part of the stratum corneum, the procedure should be repeated. Tests should be performed within 24-48 h for freshly collected cells or they should be frozen and stored at the temperature of -80°C.

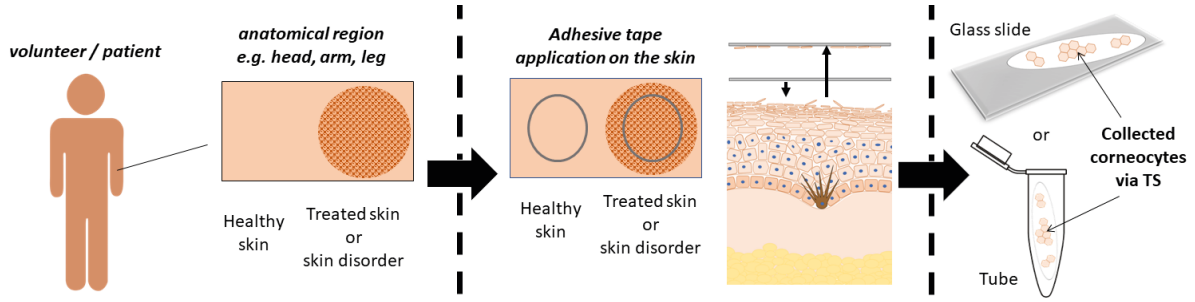

Figure 1. The basic procedure of the tape stripping method.

The procedure is painless and practically non-invasive, that is why it can be used routinely for testing skin conditions and in case of skin diseases in diagnostics. To receive high quality results, one must remember of some crucial parameters not only during the sample collection but also when measuring these kinds of samples.

2. Search strategy and selection criteria

Terms used for the preparation of this article are a combination of the words "tape stripping – skin/ corneocytes – selected methods – skin disorders – skin diseases".

As the selection criteria we have chosen articles in which the tape stripping method was used for some skin analysis mainly on human. For a better understanding of some issues some additional articles were also investigated.

3. State of the Art

The largest organ with a complex layered structure is the skin. Its surface in the adult human is around 2 m^2 and it weighs approximately 4 kg. The thickness of the skin depends on the place on the body that it covers e.g. on the eyelids it is the thinnest (0.5 mm), while on the palms and feet it is the thickest (4 mm). The skin consists of the epidermis, the dermis and the subcutaneous tissue. The surface of the skin is covered with exfoliated keratin and lipid coat (oil-water slurry), which protects the skin mainly against harmful chemical agents and microorganisms.

In addition, applying cream to the skin protects it to some degree against mechanical damage (Noszczyk, 2018).

The epidermis is the outermost layer of the skin. It is made of a constantly renewing multilayer epithelium (basic, spinous, granular and translucent) and an outer horny layer. The epidermis is basically built of keratinocytes (about 90%) and other cells, i.e. the melanocytes and the Langerhans cells (cells of the immune system) as well as the Merkel cells (cells of the nervous system). Depending on the type of cells and layer, epidermal cells differ in shape, size, structure and production of various chemical substances like the pigment melanin, hormones α-MSH (α-melanocyte-stimulating hormone) and ACTH (adrenocorticotrophic hormone), some cytokines from the interleukins families, the tumor necrosis factor (TNF) and the transforming growth factor (TGF) superfamily (Gasque, Jaffar-Bandejee, 2015; Hirobe, 2014). The process of keratinization, that is the passing of keratinocyte from the basal layer to the cornified layer and then subject to exfoliation, takes about 26-28 days. The daily amount of exfoliated epidermis is 6-14 g and it is caused by the continuous separation of corneous plaques from the cornified layer of the epidermis (Jabłońska, Majewski, 2010).

The most outer layer of the epidermis is the *stratum corneum*. It is composed of flattened corneocytes surrounded by the intercellular lipid cement. The dry mass of the corneocytes from the horny layer is about 50-80% lower than the cells from the granular layer. This is mainly due to two major changes that occur during the keratinization process while keratinocytes transform into corneocytes, i.e. they lose the cell nuclei and almost all of the cytoplasm (except for the intermediate filaments mostly keratin). The lipid matrix of the SC consists of ceramides, cholesterol and free fatty acids, that are responsible for the layer function as a barrier (Alikhan, Maibach, 2010). Moreover, the occurrence of the supramolecular structure, the glycosaminoglycan polymer hyaluronan (HA, hyaluronic acid) scaffold for the sulfated proteoglycans and the matrix proteins, enables water and ions trapping and prevents skin dehydration and turgor loss (Guzmán-Alonso, Cortazár, 2016).

As a consequence, corneocytes create a strong layer quite resistant to mechanical damage of the whole skin and to water loss from the body. In addition, the appearance of the lipid coat on the surface of the SC regulates the process of absorption and penetration of the substance into the skin (Jabłońska, Majewski, 2010). An important role in the SC formation, cohesion and desquamation is played by the expression of sugar moieties (glycans) on the surface of viable keratinocytes. Here, the formation of the permeability barrier in SC is possible due to the deglycosylation of glycosyl ceramides (Abdayem, Formanek et al. 2016).

Skin, equally like other organs, is affected by aging due to the intrinsic and extrinsic aging factors like thermal and radiation skin damage. This complex and multifactorial phenomenon progressively leads to a loss of structural integrity and physiological function of the skin (Farage, Miller et al. 2007). One can mention a few dermatological diseases like: the bacterial infection (e.g. impetigo); the fungal infection (e.g. nail fungus); the viral infection (e.g. herpes); the inflammatory and the allergic diseases (e.g. atopic dermatitis, psoriasis); the autoimmune diseases (e.g. systemic lupus erythematosus); moles, marks and benign tumors (e.g. fibroma); and last but not least the most dangerous skin malignancies like the basal cell carcinoma (BCC), the squamous cell carcinoma (SCC) and melanoma skin cancer (Burns, Breathnach et al. 2010). Some diseases may be easily diagnosed without sample collection just by observation. However, in some cases blood sample, skin swabs or scraps and even biopsy must be taken directly from the patient

for investigation. In these cases, the selection of a proper diagnostic biomarker covering the wide range of phenotypes and disease is obligate, but at the same time the analysis (e.g. mRNA) may be costly and quite laborious (Berekméri, Latzko et al. 2018). Other diagnostic possibilities randomly

used are allergy tests or skin imaging. Nowadays, a label-free imaging by the confocal Raman microscopy or the attenuated total reflectance Fourier transform infrared spectroscopy (ATR-FTIR) are often used for skin investigations performed both *ex vivo* (skin biopsy) as well as non-invasively *in vivo* (Ashtikar, Matthäus et al. 2013; Tosato, Orallo et al. 2015; Bindera, Kulovitsa, 2018).

In this way the profiles of human *SC* were obtained and different components of the tissue were detected. Nevertheless, one must be aware that developing non – or less – invasive (than biopsy) methods are more comfortable for the patient under the condition that they are still reliable. Especially, in case of the requirement of repetitive skin sampling before and after treatment or to prevent possible infections after biopsy (Clausen, Slotved et al. 2016). The introduction of the tape stripping method gave a significant expansion to experimental tools in the subject of skin research, both its condition and diseases. This is mainly due to the specificity of the method which enables one to obtain only the *stratum corneum* layers and in this way, it minimalizes the main side effects of other methods like biopsy – pain, itching or possible infections in case of improper wound dressing.

The first description of the method to remove cells from the epidermis with a cellophane tape was described by Wolf in 1939 (Wolf, 1939). With this method it was then possible to study the morphology of the cells. In 1951 with the use of the TS method Pinkus demonstrated that the removal of almost all of the horny layer stimulates the epidermal proliferation (Pinkus, 1951). Next, in 1953 Sper and Natzel used the TS to reduce the cornified layer before applying the patch test (a method to identify substances causing allergic reaction in patients). The reduction of the corneocyte layer increases the bioavailability of the substance to the deeper epidermal layers (Spier, Natzel, 1953; Dickel, Goulioumis et al. 2010). However, the basic tape stripping procedure could be further modified for wider examinations like: to study epithelial regeneration, keratinocytes' kinetics, skin microcirculation, cornified layer impermeability, the distribution of SC lipids (Berrutti, Singer et al. 2000), to confirm dermatological disorders, or to increase epidermal DNA/lipid synthesis and lamellar body production/secretion. Unfortunately, in some cases TS application may also suppress the mitotic activity of cells, increase the epidermal cytokine production and dermal inflammation (the occurrence of TNF and IL-1α in the skin) (Alikhan, Maibach, 2010).

4. RESULTS New insight

The application of the tape stripping method in the skin research should be followed by the selection of some crucial parameters like the place of sampling on the skin, the type of the adhesive tape and precise method of use. In some investigations the amount of the collected cells is a very important factor, that is why some methods enabling a direct cell counting or others based on the comparison of samples have been developed. Moreover, TS is used in the field of epidermal biology and dermatology.

5. The crucial parameters of the tape stripping procedure

In theory, the use of an adhesive tape should allow the removal of one full layer of corneocytes (about 0.5-1 μm). According to some sources, the complete removal of all the SC layers can occur after the removal of dozens of layers (about 60-100 strips) (Jacobi, Weigmann et al. 2005). However, some scientists claim that a complete removal of the horny layer with this method is impossible because still about one third of the layer remains (Schaefer, Schalla et al. 1982).

As research shows, in case of patients who had 30 layers removed the complete recovery of the SC was observed after two weeks (Berrutti, Singer et al. 2000). Nevertheless, the amount of the corneocytes removed by the TS in the same place on the skin decreases with each collected strip from different depths of the *stratum corneum* (Lademann, Jacobi et al. 2009). In the lower layers (*stratum compactum*), the corneocytes more closely adhere to each other using modified desmosomes, while in the upper layers (*stratum dysjunctum*) they are loosely laid on the surface, which allows them to be easily peeled off (Jabłońska, Majewski, 2010). If a long-term tape strip application is planned, the skin dressing becomes a very important issue. As in many cases, occasionally and in specific conditions at the site of the tape injury several pathological microorganisms may be colonized. For example, there is a described case of a 77-year-old patient, where tape stripping was applied and gave an inexpedient result when handling a postoperative wound. The patient sustained a TS type injury due to bandaging his periorbital after a cataract surgery and not having received any immunosuppressive medication. At the same time, he regularly used recreational hot tubs, where the injury was infected with achlorophyllic algae, one of the *Prototheca* species. Fortunately, the infection was completely cured after oral treatment with fluconazole, an antifungal medicine (Humphrey, Martinka et al. 2009).

The amount of collected corneocytes *via* TS method can be affected by numerous factors like skin moisturizing, cohesive forces occurring between the cells, the part of the body from which the corneocytes' samples were obtained, the individual features of the volunteer or patient (Arct, Koazyra et al. 2013). Before the main experiment the compatibility of the selected tape with the patients' skin should be checked.

Some important factors are also directly connected with the procedure of tape stripping: the way the tape is applied on the skin (creating folds), the pressure adjusted during tape application, the speed of the tape peeling off as well as the direct properties of the adhesive tape (Arct, Koazyra et al. 2013). The place for corneocytes sampling should be smooth and without scars. In case of furrows, their occurrence in the skin may cause difficulties, while performing the depth-penetration studies of SC (van der Molen, Spies et al. 1997). The skin should be previously prepared for the cells' collecting by hair removal with scissors and not with a razor that can also damage the skin layer. To create the optimal adhesive bond of the tape with the SC cells, a uniform pressure is crucial. It is also necessary to obtain comparable amounts of the removed SC layer. The high speed of the SC peeling may result in low amounts of corneocytes present on the tape, whereas low speed could cause an increase in the amount of corneocytes. What is more, to identically press the tape each time onto the skin surface as has been done up till now – spatulas, rollers as well as constant weights have been applied. Some tests might need a precise tape size. This may be provided by the usage of adhesive discs (e.g. the comparison of corneocytes dry mass), whereas for others it is possible to use tape from the spool. The tape transparency (e.g. for microscopic analysis) or its flexibility (e.g. for RNA or protein isolation) may be important features likewise (Lademann, Jacobi et al. 2009; Wong, Tran et al. 2006).

6. The determination of the amount of the collected corneocytes

For some tests the quantification of the collected corneocytes is essential for comparing samples, like those taken from the skin before and after the application of cosmetic/medical products as well as skin with or without dermatological changes. What is more, with this knowledge an interlaboratory comparison of the results would be possible. However, up till now a satisfactory method of determining the amount of collected cells has not been developed yet. The most common methods used to determine the amount of SC are:

a) the differential weighing – in this method, each tape must be weighed before and after stripping. This allows to determine the amount of tissue removed (m) on the basis of the difference in tape weights. For these experiments adhesive tapes with a defined area must be used (A) and the tissue density (ρ) can be known from the literature data. With these described factors, it is possible

to determine the depth or distance (x) of the interference in the tissue based

on the formula $x=m/(A*\rho)$ (Herkenne, Alberti et al. 2008). In general, the method is quite accurate and reproducible. However, it is time-consuming and, in some cases, due to the moisture content (e.g. topical application of the product) it might be deceiving. Only a few micrograms of the SC usually adhere to a single tape strip (Dreher, Modjtahedi et al. 2005).

b) the optical density measurements – in the optical spectroscopy, corneocytes exhibit pseudo--absorption during measurements using a UV-VIS spectrophotometer at the wavelength of 430 nm (Jacobi, Kaiser et al. 2005), but at the same time their aggregates reduce the transmission of radiation by the reflection, diffraction or dispersion of the light rays, which results in a linear increase in absorbance with a decrease in the wavelength (Arzt, Kozyra et al. 2013). The calculation includes the thickness of the completely removed SC (100%) by adding up values of the absorbance for every single tape strip taken. This allows one to obtain a non-linear correlation between the relative amounts of stratum corneum (y) removed with multiple tape stripping (n) as in the equation $y=a--be^{(-n/c)}$, where "a", "b" and "c" are variables (Jacobi, Weigmann et al. 2005). Also, for this kind of measurements the near-infrared (NIR) densitometry can be used. The beam of light at the 850 nm goes directly through the tape strips with cells and the decrease in light intensity is measured (Jacques-Jamin, Jeanjean-Miquel et al. 2017).

c) various microscopic techniques are used to determine the density of the cells on a specific area using a selected microscope. The cells can be viewed on a light microscope (like optical or fluorescent) after staining the cells with a selected dye. For the optical microscope, corneocytes may be stained with the solution of gentian violet or brilliant green (Lademann, Jacobi et al. 2009).

The intercorneocyte cohesion can be observed after the cells' incubation with the solution of toluidine blue and basic fuchsine in 30% ethanol (Gao, Wang et al. 2013). The fluorescent staining of corneocytes is possible with a fluorescein green dye (the general cell staining) or specific fluorescent antibodies (for example, corneodesmosomes staining) (Guz, Gaikwad et al. 2009; Igawa, Kishibe et al. 2013). In some cases, it is better to use the confocal laser scanning microscopy (CLSM) that

allows to obtain images of the cells/specimens' layer by layer. The sample must also be stained by the specific fluorophore, but the obtained images are sharper due to the removal of reflections not originating directly from the focal point (Lindemann, Wilken et al. 2003, Hanrahan, Harris et al. 2011). Results obtained with these methods (like images or calculations of corneocyte geometry) may also be influenced by artifacts occurring on the tape or skin exposed to stripping and the staining process. The manual data processing in such a situation might be time-consuming. That is why, a good automatic image analyzing software may be useful. Another problem is the occurrence of corneocyte clusters on the tape after the stripping procedure.

For the calculations of a single corneocyte area or geometry, clusters are a serious problem. To tackle this problem, it is possible to place a new adhesive tape on the first one (with the already collected cells) and next to peel it off creating another sample with a smaller number of cells (Li, Guz et al. 2011).

More advanced microscopic methods may be used like the scanning electron microscopy (SEM) and the atomic force microscopy (AFM) (Ezerskaia, Pereira et al. 2018; Li, Guz et al. 2011; Milani, Chlasta et al. 2018). These methods enable the visualization of the cells through imaging the cells' surface (topography). However, obtaining a proper number of images is time-consuming and requires proper operator training to achive this task.

d) the spectrophotometric determination of protein content in the collected corneocytes – it is possible to measure the maximum absorbance of keratin, which mainly remains in corneocytes, at the wavelength of 278 nm or 850 nm (Lademann, Jacobi et al. 2009; Mohammed, Hirata et al. 2014).

The absorbance can be meas red directly on a tape strip when taken with a proper transparent adhesive tape and by using a special UV-VIS spectrophotometer. Moreover, to increase the absorption of proteins and shift it to the visible range, corneocytes could be stained with dyes like Brilliant Blue R 250 (595 nm), crystal violet (540 nm) or trypan blue (652 nm). In practice, the more selective reagent applied to cells, the better (Lademann, Jacobi et al. 2009). Another accurate and reproducible method was developed for the soluble proteins' extraction from the SC by 1 M sodium hydroxide solution followed by the sonication process and next by the sample neutralization with 1 M hydrochloric acid. Finally, the amount of proteins is determined by a similar method to the Lowry assay one. The protein reacts with an alkaline copper tartrate solution as well as with the Folin phenol reagent. The appearing products are measured at 750 nm (Dreher, Arens et al. 1998). Furthermore, the method can be accustomed to 96-well plates, which shortens the time of analysis (Dreher, Modjtahedi et al. 2005). However, the modified Lowry assay may give a positive result in case of some adhesive tapes, especially those made of polyester and polyacrylate. That is why, a modified Bradford assay is also possible to apply with the absorbance read at 595 nm (Chao, Nylander-French, 2004).

On the contrary, the protein content in tape stripped samples may be investigated due to the activity of desq amatory proteases (for example, kallikrein 5 and kallikrein 7). The extraction with a Tris-HCl buffer enables the quantification of aminomethyl coumarin released from peptide substrates by the high-performance liquid chromatography (HPLC) (Mohammed, Hirata et al. 2014).

e) the transepidermal water loss (TEWL) measurements – though the SC is not homogenous, the corneocytes and the intercellular lipids should prevent the water loss from the skin and the loss of the skin barrier integrity. Water loss is carried out on the principles of Fick's laws of passive diffusion (Alikhan, Maibach, 2010). This parameter may be measured with an evaporimeter (tewameter®) directly on the tape stripped sample and with each tape strip number the TEWL value increases. Still, many factors may influence the result. TEWL measurements may be performed in open or closed chambers with similar results in values expressed in g/m^2h (Yosipovitch, Duque et al. 2007; Kikuchi, Asano et al. 2017).

7. Review and discussion

Due to the simplicity of the TS method and its possible usage with the more advanced methods, tape stripping can be used in various fields of epidermal biology. As the main advantage of TS, the ability to estimate the general condition of the *stratum corneum* and its re-analysis is made possible after the application of the skin care products (Escobar-Chávez, Merino-Sanjuán et al. 2008). The bioavailability, the bioequivalence and the penetration profiles are important assessments in the subject of the active substances. These substances being the potential ingredients for cosmetics or dermocosmetics are possible to detect with TS (Jacobi, Weigmann et al. 2005). Moreover, it is used in research of the disturbance of the skin barrier functioning usually together with the measurements of water loss (Kikuchi, Asano et al. 2017) or in the skin microbiome analysis (Ogai, Nagase et al. 2018). Valuable analysis can also be made on the tape stripped samples taken from patients

with various diseases like atopic dermatitis or psoriasis (Dyjack, Goleva et al. 2018; Berekméri, Latzko et al. 2018). TS may likewise be used prior to the direct *in vivo* measurements of the skin components, lipids and water with the short-wave infrared (SWIR) Raman spectroscopy (Ezerskaia, Pereira et al. 2018), ATR-FTIR spectroscopy (Bommannan, Potts et al. 1990) or the reflectance confocal microscopy (RCM) (Peppelman, van den Eijnde et al. 2015). On the other hand, skin after tape stripping may likewise be used as a model of the injury in the wound healing research (the study of epidermal growth kinetics) (Surber, Schwarb et al. 2001).

a) The investigation of the potential ingredients for cosmetic industry

The main ingredients in cosmetic formulations are water, emulsifiers, emollients, polymers, preservatives, fragrance compositions, natural and synthetic dyes, alcohols, protein hydrolysates, peptides, free amino acids, vitamins and plant extracts. Some commonly used moisturizing ingredients are glycerin and urea.

The *in vivo* 9-day treatment of the skin with glycerin resulted in the increase of the collected corneocytes geometrical characteristics like cell surface. Conversely, sensitivity to glycerin depends on the skin type (dry or oily skin) (Li, Guz et al. 2011). Moreover, low concentrations of the glycerin solution cause the mechanical deformation of corneocytes (the decrease in Young's modulus value measured by the AFM), whereas higher concentrations increase the adhesion forces between the cells (Yanagiya, Takahashi et al. 2015). On the other hand, the therapeutic effect of urea depends on its concentration which can be transferred from the formula to SC. The conventional radiocarbon (14C) or radioactive isotopes (RIs) labeling methods to determine urea concentration in the formula are complicated and rather carry a high risk for the analyst. That is why, TS method combined with colorimetry can easily be applied to measure the urea nitrogen, however, the accurate urea measurement requires the application of at least 1400 mg of a 20% urea solution on a 50 cm^2 skin area – the detection threshold (Goto, Morita et al. 2016).

Another widely used ingredient group are natural and synthetic colorants.

To obtain a golden color, curcumin alone or in the extract as well as fluorescein sodium (D&C Yellow NO. 8®) could be used (Modasiya, Patel, 2012; Winter, 2005). Their usage in microemulsions and hydrogels causes the need for the substance's penetration depth evaluation into the SC. The curcumin concentration measurement procedure is as follows: the application of the formula with curcumin on the skin, corneocytes collection via tape stripping, substance dissolvement in the pure ethanol during the sonication process and finally the UV/VIS spectroscopy analysis at 425 nm of the obtained curcumin solution. Similarly, in the fluorescein sodium quantification the salt from inside of the corneocytes is dissolved in a phosphate buffer during the substance release by sonication and the obtained solution is analyzed by the fluorescence spectroscopy at the excitation wavelength of 485 nm and the emission wavelength of 535 nm (Klang, Schwarz et al. 2012).

The ultraviolet (UV) radiation is a major risk factor of skin aging and many diseases including cancer, that is why protection of the skin barrier against the damage on the level of epidermis is very important. This may be provided by the usage of active compounds of dietary phytochemicals (Sobiepanek, Milner-Krawczyk et al. 2016) or the UV filters present in the day care creams and sun creams formula. The chemical filters are organic compounds with an aromatic structure that absorb radiation, whereas physical filters are inorganic compounds that reflect or scatter radiation (Tampucci, Burgalassi et al. 2018). The application of the potential UVA filters (like butyl methoxydibenzoylmethane) in the O/W emulsions has been determined in many investigations (Jacobi, Weigmann et al. 2005; Lindemann, Wilken et al. 2003).

The corneocytes may be collected with TS and the compound extraction can be made with ethanol during the sonication process. Next, the measurements are performed with the UV/VIS spectrophotometer or HPLC (Tampucci, Burgalassi et al. 2018).

The compound concentration in the collected tape strips can be determined due to the prepared calibration curve (Jacobi, Weigmann et al. 2005). Another possibility is to define the penetration profiles of the applied formulations with UV filters due to corneocytes image analysis performed with the laser scanning microscopy and light microscopy. The density of the stripped cells can be determined microscopically with the corneocytes pseudoabsorption (Lindemann, Wilken et al. 2003).

Significant cosmetic formula compounds are the organic solvents, which enable the dissolvement of various active compounds, as well as the anionic and non-ionic surfactants for their washing properties. The lower potential of irritancy has been assigned to nonionic surfactants, while anionic surfactants can damage the skin barrier (Hoppel, Holper et al. 2015). However, due to strong properties for both groups the penetration profile into the SC must be checked. For this purpose, the combination of the TS method with ATR-FTIR spectroscopy can be applied. The decrease in the relative surfactant or solvent concentration is visible with the following tape strip number (Bindera, Kulovitsa, 2018).

b) The investigation of the proper skin barrier function

The skin acts as a protective barrier for the whole body, but in case of the incorrect barrier function as the main result – the increase in the transepidermal water loss is mainly pointed out (Barba, Alonso et al. 2016). As it is known, cross-linked proteins ceramides, including transmembrane glycoproteins and proteoglycans, are covalently bound to the monolayer of the cell surface.

These structures participate in the maintenance of the SC barrier function (the hydration and the potential protective role against premature proteolysis) and in the regulation of SC desquamation. However, the presence and the distribution

of glycans on the corneocytes is still investigated. Samples collected via TS and labeled with antibodies or lectins may be later observed with SEM. As it was found, the peripheral distribution of α-D-mannosyl and N-acetyl-D-glucosamine-labelling patterns is higher than of α-linked fucose and α-(2,3) linked sialic acid and it is mainly concentrated in corneodesmosome (Abdayem, Formanek et al. 2016).

Gaining every knowledge about the SC barrier structure, the intercellular lipids properties and the level of hydration may help with the optimization of the damaged skin therapy and improve the design of percutaneous penetration enhancement strategies (Bommannan, Potts et al. 1990). For some cosmetics, to facilitate the pass through the barrier, skin penetration enhancers (SPEs) are used. Some examples are isopropyl myristate, propylene glycol or propylene glycol laurate. However, their exact effect on the skin is not yet fully understood. One of the possible investigation pathways in this research area is the SPE influence on the activity of the desquamatory proteases like kallikrein 5 and kallikrein 7 in healthy skin.

The amount of aminomethyl coumarin released during the extraction of TS samples with Tris HCl buffer was analyzed with HPLC. The result shows that propylene glycol may significantly elevate the kallikrein 7 activity to alter the skin barrier at the macroscopic level. At the same time TEWL was compared before and after SPE applications and the results suggest a significant extent of the TEWL value, which is consistent with the previous results (Mohammed, Hirata et al. 2014). Nowadays Nanoparticles (NPs) like magnetic nanoparticles (MNPs), magnetic beads (MB) and quantum dots (QDs) gain a lot of interest, especially in medicine.

This is due to their unique properties, for example, their significant affinity for cancer cells (Sobiepanek, Kobiela, 2018). Some investigations have been performed to study the penetration profile of the QDs from a proper formula applied topically.

Due to their fluorescent properties, the accumulation of QDs could be detected in the collected samples of corneocytes via TS method on the confocal microscope. The results show that the concentration of QDs progressively decreased with the investigated depth (15 tape stripped samples). Thus, QDs were able to penetrate through the SC, but not to the living epidermis which was revealed through the punch biopsy and the energy diverse X-ray (EDX) spectra to determine the elemental composition of particles assumed to be QDs (Jeong, Kim et al. 2010).

The barrier impairment associated with the SC lipid composition and structure changes, may also cause skin disorders. To confirm this statement, differences in intrinsic and extrinsic aging (UV light) were investigated on the corneocytes by comparing their shape and size with SEM, thickness with AFM and chemical changes with the time-of-flight secondary ion mass spectrometry (ToF-SIMS). Significant changes were observed in the sterol cholesterol sulfate, lignoceric acid and hexacosanoic acid (Starr, Johnson et al. 2016). On the other hand, for the inflammatory diseases glucocorticoids (GCs) like clobetasol proprionate or betamethasone diproprionate could be used, however, they might induce skin atrophy including excessive keratinocyte proliferation, synthesis of extracellular matrix proteins or the reduction of the skin barrier integrity. For a detailed lipidomic analysis the highly sensitive liquid chromatography–mass spectrometry (LC/MS) as well as the ultra-high performance liquid chromatography coupled to the time-of-flight mass spectrometry (UHPLC-ToF--MS) were used. Samples were collected via the TS method and lipids were extracted by homogenization in the chloroform/methanol mixture. The application of GC on the skin caused a significant reduction in lipids, whereas the most effected were ceramide classes consisting of the ester-linked fatty acids (Røpke, Alonso et al. 2017).

c) Diagnostics of skin diseases by means of the TS method

In inflammatory skin diseases, like psoriasis and atopic dermatitis (AD), several defects in the proper functioning of the skin barrier can be observed (Barba, Alonso et al. 2016). However, a proper diagnosis when the inflammation is minimal or located in certain anatomical regions may cause diagnostic difficulties. The current gold standard in their diagnosis is the histopathological examination of the tissue obtained through biopsy, but it is invasive and often unavailable in the primary care setting (Berekméri, Latzko et al. 2018).

As the main causes of AD filaggrin mutations, changes in the lipid composition and the altered antimicrobial response are pointed out. However,

due to the fact that the pathogenesis of AD is still unclear, research focused on SC barrier is required for a better understanding of these diseases (Clausen, Slotved et al. 2016).

The whole-transcriptome analysis of corneocytes collected via TS allowed the identification of the gene expression dysregulation in the AD molecular pathology by type 2 inflammation (Dyjack, Goleva et al. 2018). On the next posttranslational level, a significant decrease in the soluble and insoluble protein amount between AD lesional, AD non-lesional and healthy skin collected via TS was noted (Clausen, Slotved et al. 2016). The distribution patterns of corneodesmosomal components like desmoglein 1, corneodesm sin and desmocollin 1 can be followed by the immunofluorescent staining. In healthy skin they are detected at cell periphery. However, in case of AD skin peripheral, sparse diffuse, dense diffuse and partial diffuse pattern were observed (Igawa, Kishibe et al. 2013). Some reliable biomarkers for psoriatic and AD inflammation are the neutrophil-recruiting chemokines like (C-X-C motif) ligand 1 (CXCL1), (C-C motif) ligand 20 (CCL20) and interleukin IL-8, which can be detected on tape strip with the enzyme-linked immunosorbent assay (ELISA). What is more, with this approach it is possible to distinguish healthy skin from AD, and AD from psoriasis due to the increasing amounts of IL-36γ. In case of the first pair the difference was only 1.5 times higher, but in case of the second pair it was 10 times higher (Berekméri, Latzko et al. 2018). On the other hand, while comparing mRNA, profiled with the semiquantitative reverse transcriptase-PCR, the overexpression of TNFα, IFNγ, Krt-16, CD2, IL-23A, IL-12B, and VEGF in the psoriatic lesion can be traced. Additionally, samples collected via the tape stripping method revealed mRNA markers that were not detected in biopsy samples, that is why the application of both methods (non-invasive and invasive) to obtain more information is recommended (Benson, Papenfuss et al. 2006).

8. Short conclusion

The tape stripping method is a very simple and minimally invasive tool to obtain the maximum knowledge about the *stratum corneum* properties as well as to diagnose some skin disorders. In combination with other simple or more advanced methods it may help in the basic research as well as in the detailed one. It can be applied with a very low risk of side effects during a standard medical consultation. However, one must be aware that the selection of the crucial parameters of the tape stripping procedure (tape type and size, force and pressure during the skin stripping as well as the amount of the collected cells) are important factors for obtaining reliable results in any analytical method later used. That is why, it is recommended to optimize each parameter of the collecting procedure for one's own use. Collected samples may be analyzed directly via the microscope or they might be used for selected molecule isolation and analysis via spectrometry or chromatography.

Acknowledgements:

This work was supported by the Warsaw University of Technology and the Medical University of Warsaw.

References:

1. Abdayem R., Formanek F., Minondo A. M., Potter A., Haftek M. *Cell surface glycans in the human stratum corneum: distribution and depth-related changes.* Exp Dermatol. 2016; 25:865–71.
2. Akamatsu N., Nakajima H., Ono M., Miura Y. *Increase in acetyl CoA synthetase activity after phenobarbital treatment.* Biochem. Pharmacol. 1975; 24:1725–27.
3. Alikhan A., Maibach H. I. *Biology of Stratum Corneum: Tape Stripping and Protein Quantification.* In: Farage M.A., Miller K.W., and Maibach H.I. (Editors) *Textbook of Aging Skin.* 2010; Heidelberg: Springer Berlin Heidelberg (Berlin); ISBN: 978-3-540-89655-5 978-3-540-89656-2.
4. Arct J., Koazyra K., Mieloch M., Dzierzgowski S. *Metody strippingu w ocenie dostępności dermalnej surowców kosmetycznych.* Innowacyjność W Kosmetologii. 2013.
5. Ashtikar M., Matthäus C., Schmitt M., Krafft C., Fahr A., Popp J. *Non-invasive depth profile imaging of the stratum corneum using confocal Raman microscopy: First insights into the method.* Eur J Pharm Sci. 2013; 50:601–8.
6. Barba C., Alonso C., Martí M., Manich A., Coderch L. *Skin barrier modification with organic solvents.* Biochim Biophys Acta Biomembr. 2016; 1858:1935–43.
7. Benson N. R., Papenfuss J., Wong R., Motaal A., Tran V., Panko J., Krueger G. G. *An Analysis of Select Pathogenic Messages in Lesional and Non-Lesional Psoriatic Skin Using Non-Invasive Tape Harvesting.* J Invest Dermatol. 2006; 126:2234–41.
8. Berekméri A., Latzko A., Alase A., Macleod T., Ainscough J. S., Laws P., Goodfield M., Wright A., Helliwell P., Edward S. *Detection of IL-36γ through noninvasive tape stripping reliably discriminates psoriasis from atopic eczema.* J Allergy Clin Immunol. 2018; 142:988–91.

9. Berrutti L. E., Singer A. J., McClain S. A. *Histopathologic effects of cutaneous tape stripping in pigs.* Acad Emerg Med. 2000; 7:1349–53.
10. Binder L., Kulovits E. M., Petz R., Ruthofer J., Baurecht D., Klang V., Valenta C. *Penetration monitoring of drugs and additives by ATR-FTIR spectroscopy/tape stripping and confocal Raman spectroscopy – A comparative study.* Eur J Pharm Biopharm. 2018; 130:214–23.
11. Bommannan D., Potts R. O., Guy R. H. *Examination of stratum corneum barrier function in vivo by infrared spectroscopy.* J. Invest. Dermatol. 1990; 95:403–8.
12. Chao Y.-C. E., Nylander-French L. A. *Determination of Keratin Protein in a Tape-stripped Skin Sample from Jet Fuel Exposed Skin.* Ann Occup Hyg. 2004; 48:65–73.
13. Clausen M.-L., Slotved H.-C., Krogfelt K. A., Agner T. *Tape Stripping Technique for Stratum Corneum Protein Analysis.* Sci Rep. 2016; 6:19918.
14. Dickel H., Goulioumis A., Gambichler T., Fluhr J. W., Kamphowe J., Altmeyer P., Kuss O. *Standardized Tape Stripping: A Practical and Reproducible Protocol to Uniformly Reduce the Stratum Corneum.* Skin Pharmacol Physiol. 2010; 23:259–65.
15. Dreher F., Arens A., Hostýnek J. J., Mudumba S., Ademola J., Maibach H. I. *Colorimetric method for quantifying human Stratum corneum removed by adhesive-tape stripping.* Acta Derm. Venereol. 1998; 78:186–9.
16. Dreher F., Modjtahedi B. S., Modjtahedi S. P., Maibach H. I. *Quantification of stratum corneum removal by adhesive tape stripping by total protein assay in 96-well microplates.* Skin Res Technol. 2005; 11:97–101.
17. Dyjack N., Goleva E., Rios C., Kim B. E., Bin L., Taylor P., Bronchick C., Hall C. F., Richers B. N., Seibold M. A., *Minimally invasive skin tape strip RNA sequencing identifies novel characteristics of the type 2–high atopic dermatitis disease endotype.* J Allergy Clin Immunol. 2018; 141:1298–1309.
18. Escobar-Chávez J. J., Merino-Sanjuán V., López-Cervantes M., Urban-Morlan Z., Piñón-Segundo E., Quintanar-Guerrero D., Ganem-Quintanar A. *The tape-stripping technique as a method for drug quantification in skin.* J Pharm Pharm Sci. 2008; 11:104–30.
19. Ezerskaia A. A., Pereira S. F., Urbach H. P., Varghese B. *Depth resolved quantitative profiling of stratum corneum lipids and water content using short-wave infrared spectroscopy.* In: Choi B., Zeng H., (Editors) *Photonics in Dermatology and Plastic Surgery 2018*, 2018; SPIE (San Francisco, United States); ISBN: 978-1-5106-1419-2 978-1-5106-1420-8.
20. Farage M. A., Miller K. W., Elsner P., Maibach H. I. *Characteristics of the Aging Skin.* Adv Wound Care (New Rochelle). 2013; 2:5–10.
21. Gao Y., Wang X., Chen S., Li S., Liu X. *Acute skin barrier disruption with repeated tape stripping: an in vivo model for damage skin barrier.* Skin Res Technol. 2013; 19:162–8.
22. Gasque P., Jaffar-Bandjee M. C. *The immunology and inflammatory responses of human melanocytes in infectious diseases.* J Infect. 2015; 71:413–21.
23. Goto N., Morita Y., Terada K. *Deposits from Creams Containing 20% (w/w) Urea and Suppression of Crystallization (Part 2): Novel Analytical Methods of Urea Accumulated in the Stratum Corneum by Tape stripping and Colorimetry.* Chem Pharm Bull (Tokyo). 2016; 64:1092–8.
24. Guz N. V., Gaikwad R. M., Dokukin M. E., Sokolov I. *A novel in vitro stripping method to study geometry of corneocytes with fluorescent microscopy: example of aging skin.* Skin Res Technol. 2009; 15:379–83.
25. Guzmán-Alonso M., Cortazár T. M. *Water content at different skin depths and the influence of moisturizing formulations.* Dermatology. 2016; 11, 35–40.
26. Hanrahan O., Harris J., Egan C. *Advanced Microscopy: Laser Scanning Confocal Microscopy.* In: O'Driscoll L., (Editor) *Gene Expression Profiling.* 2011; NJ: Humana Press (Totowa); ISBN: 978-1-61779-288-5 978-1-61779-289-2.
27. Herkenne C., Alberti I., Naik A., Kalia Y. N., Mathy F.-X., Préat V., Guy R. H. ***In Vivo*** *Methods for the Assessment of Topical Drug Bioavailability.* Pharmaceutical Research. 2008; 25:87–103.
28. Hirobe T. *Keratinocytes regulate the function of melanocytes.* Dermatologica Sinica. 2014; 32:200–4.
29. Hoppel M., Holper E., Baurecht D., Valenta C. *Monitoring the Distribution of Surfactants in the Stratum Corneum by Combined ATR-FTIR and Tape-Stripping Experiments.* Skin Pharmacol Physiol. 2015; 28:167–75.
30. Humphrey S., Martinka M., Lui H. *Cutaneous Protothecosis following a Tape-Stripping Injury.* J Cutan Med Surg. 2009; 13:273–75.
31. Igawa S., Kishibe M., Honma M., Murakami M., Mizuno Y., Suga Y., Seishima M., Ohguchi Y., Akiyama M., Hirose K. *Aberrant distribution patterns of corneodesmosomal components of tape-stripped corneocytes in atopic dermatitis and related skin conditions (ichthyosis vulgaris, Netherton syndrome and peeling skin syndrome type B).* J Dermatol Sci. 2013; 72:54–60.
32. Jabłońska S., Majewski S. *Choroby skóry i choroby przenoszone drogą płciową.* 2010; Wydawnictwo Lekarskie PZWL (Warszawa). ISBN: 978-83-200-4154-5.
33. Jacobi U., Kaiser M., Richter H., Audring H., Sterry W., Lademann J. *The Number of Stratum corneum Cell Layers Correlates with the Pseudo-Absorption of the Corneocytes.* Skin Pharmacol Physiol. 2005; 18:175–79.
34. Jacobi U., Weigmann H.-J., Ulrich J., Sterry W., and Lademann J. *Estimation of the relative stratum corneum amount removed by tape stripping.* Skin Res Technol. 2005; 11:91–6.
35. Jacques-Jamin C., Jeanjean-Miquel C., Domergue A., Bessou-Touya S., Duplan H. *Standardization of an in vitro Model for Evaluating the Bioavailability of Topically Applied Compounds on Damaged Skin: Application to Sunscreen Analysis.* Skin Pharmacol Physiol. 2017; 30:55–65.

36. Jeong S. H., Kim J. H., Yi S. M., Lee J. P., Kim J. H., Sohn K. H., Park K. L., Kim M.-K., Son S. W. *Assessment of penetration of quantum dots through in vitro and in vivo human skin using the human skin equivalent model and the tape stripping method.* Biochem Biophys Res Commun. 2010; 394:612–15.
37. Kikuchi K., Asano M., Tagami H., Kato M., Aiba S. *Comparison of the measuring efficacy of transepidermal water loss of a reasonably priced, portable closed-chamber system device H4500 with that of rather expensive, conventional devices such as Tewameter ® and Vapometer ®.* Skin Res Technol. 2017; 23:597–601.
38. Klang, V., Schwarz, J. C., Lenobel, B., Nadj, M., Auböck, J., Wolzt, M., Valenta, C. ***In vitro*** *vs.* ***in vivo*** *tape stripping: Validation of the porcine ear model and penetration assessment of novel sucrose stearate emulsions.* Eur J Pharm Biopharm. 2012: 80:604–14.
39. Lademann J., Jacobi U., Surber C., Weigmann H.-J., Fluhr J. W. *The tape stripping procedure – evaluation of some critical parameters.* Eur J Pharm Biopharm. 2009; 72:317–23.
40. Lesiewicz J. L., Herson D. S. *A reinvestigation of the sites of transcription and translation of Euglena chloroplastic phenylalanyl-tRNA synthetase.* Arch. Microbiol. 1975; 105:117–21.
41. Li S., Guz N. V., Sokolov I. *A modified in vitro stripping method to automate the calculation of geometry of corneocytes imaged with fluorescent microscopy: example of moisturizer treatment: Stripping method for automated analysis of corneocytes.* Skin Res Technol. 2011; 17:213–19.
42. Lindemann U., Wilken K., Weigmann H.-J., Schaefer H., Sterry W., Lademann J. *Quantification of the horny layer using tape stripping and microscopic techniques.* J Biomed Opt. 2003; 8:601.
43. Lough A. K., Smith A. *Influence of the products of phospholipolysis of phosphatidylcholine on micellar solubilization of fatty acids in the presence of bile salts.* Br. J. Nutr. 1976; 35:89–96.
44. Milani P., Chlasta J., Abdayem R., Kezic S., Haftek M. *Changes in nano-mechanical properties of human epidermal cornified cells depending on their proximity to the skin surface.* J Mol Recognit. 2018; 31:e2722.
45. Modasiya M., Patel V. M. *Studies on solubility of curcumin.* International Journal of Pharmacy & Life Sciences. 2012; 3:1490–97.
46. Mohammed D., Hirata K., Hadgraft J., Lane M. E. *Influence of skin penetration enhancers on skin barrier function and skin protease activity.* Eur J Pharm Sci. 2014; 51, 118–22.
47. van der Molen R. G., Spies F., van 't Noordende J. M., Boelsma E., Mommaas A. M., Koerten H. K. *Tape stripping of human stratum corneum yields cell layers that originate from various depths because of furrows in the skin.* Arch. Dermatol. Res. 1997; 289:514–18.
48. Noszczyk M., *Kosmetologia pielęgnacyjna i lekarska*. 2013; Wydawnictwo Lekarskie PZWL (Warszawa). ISBN: 978-83-200-4561-1.
49. Ogai K., Nagase S., Mukai K., Iuchi T., Mori Y., Matsue M., Sugitani K., Sugama J., Okamoto S. *A Comparison of Techniques for Collecting Skin Microbiome Samples: Swabbing Versus Tape-Stripping.* Front Microbiol. 2018; 9(2362):1-10.
50. Peppelman M., van den Eijnde W. A. J., Jaspers E. J., Gerritsen M.-J. P., van Erp P. E. J. *Combining tape stripping and non-invasive reflectance confocal microscopy: an in vivo model to study skin damage.* Skin Res Technol. 2015; 21:474–84.
51. Pinkus H. *Examination of the epidermis by the strip method of removing horny layers. I. Observations on thickness of the horny layer, and on mitotic activity after stripping.* J. Invest. Dermatol. 1951; 16:383–86.
52. Røpke M. A., Alonso C., Jung S., Norsgaard H., Richter C., Darvin M. E., Litman T., Vogt A., Lademann J., Blume-Peytavi U. *Effects of glucocorticoids on stratum corneum lipids and function in human skin—A detailed lipidomic analysis.* J Dermatol Sci. 2017; 88:330–38.
53. Schaefer H., Schalla W., Zesch A., Stüttgen G. *Skin Permeability.* 1982; Heidelberg: Springer Berlin Heidelberg (Berlin). ISBN: 978-3-540-11797-1.
54. Smith R. P., Hoxsey R. J., Bayly M. A. *Monitoring of fetal heart rate and uterine activity in clinically uncomplicated pregnancy and labor.* IMJ Ill Med J. 1976; 149:58–62.
55. Sobiepanek A., Kobiela T. *Application of biosensors in cancer research.* Review and Research on Cancer Treatment. 2018; 4:4–12.
56. Sobiepanek A., Milner-Krawczyk M., Bobecka-Wesołowska K., Kobiela T. *The effect of delphinidin on the mechanical properties of keratinocytes exposed to UVB radiation.* J Photochem Photobiol B. 2016; 164:264–70.
57. Spier H. W., and Natzel R. *Industrial-dermatological and analytical contribution to chromate allergy and cement eczema.* Hautarzt. 1953; 4:63–65.
58. Starr N. J., Johnson D. J., Wibawa J., Marlow I., Bell M., Barrett D. A., Scurr D. J. *Age-Related Changes to Human Stratum Corneum Lipids Detected Using Time-of-Flight Secondary Ion Mass Spectrometry Following* ***in Vivo*** *Sampling.* Anal Chem. 2016; 88:4400–08.
59. Surber C., Schwarb F. P., Smith E. W. *Tape-stripping technique.* J Toxicol Cutaneous Ocul Toxicol. 2001; 20:461–74.
60. Tampucci S., Burgalassi S., Chetoni P., Monti D. *Cutaneous Permeation and Penetration of Sunscreens: Formulation Strategies and In Vitro Methods.* Cosmetics. 2017; 5:1.
61. Tosato M. G., Orallo D. E., Ali S. M., Churio M. S., Martin A. A., Dicelio L. *Confocal Raman spectroscopy: In vivo biochemical changes in the human skin by topical formulations under UV radiation.* J Photochem Photobiol B. 2015; 153:51–58.
62. Winter R. *A consumer's dictionary of cosmetic ingredients, 7th Edition: Complete information about the harmful and desirable ingredients found in cosmetics and cosmeceuticals.* 2009; Potter/Ten Speed/Harmony/Rodale (New York, USA). ISBN: 9780307459862, 0307459861.

63. Wolf J. *Die innere Struktur der Zellen des Stratum desquamans der Menschlichen Epidermis [The inner structure of the cells of the stratum desquamans of the human epidermis].* Z Mikr Anat Forsch. 1939; 46:170–202.
64. Wong R., Tran V., Talwalker S., Benson N. R. *Analysis of RNA recovery and gene expression in the epidermis using non-invasive tape stripping.* J Dermatol Sci. 2006; 44:81–92.
65. Yanagiya S., Takahashi A., Goto N. *Mechanical Properties of Stratum Corneum in Glycerin Solution by Atomic Force Microscopy.* E-Journal of Surface Science and Nanotechnology. 2015; 13:461–64.
66. Yosipovitch G., Duque M. I., Patel T. S., Ishiuji Y., Guzman-Sanchez D. A., Dawn A. G., Freedman B. I., Chan Y. H., Crumrine D., Elias P. M. *Skin barrier structure and function and their relationship to pruritus in end-stage renal disease.* Nephrol Dial Transplant. 2007; 22:3268–72.