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Review Article

Topically applied KTTKS: a review

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Synopsis

Skin ageing is an irreversible process that is caused by both intrinsic and extrinsic factors. The possibility of arresting or delaying skin ageing represents a large research area and has a big potential in the cosmetics sector. Recently, the polypeptide lysine–threonine–threonine–lysine–serine (KTTKS) has attracted a lot of attention and it features in numerous up-market cosmetic products where it has become erroneously associated with the term 'pentapeptide'. In this study, we review in detail KTTKS and its major derivatives, in terms of the limited information in the literature and an appraisal of its physicochemical and theoretical skin permeation properties. There appears to be a sound *in vitro* basis for its action on fibroblasts due to its stimulatory effect on extracellular matrix synthesis, where the stimulatory effect of KTTKS is specific to collagen types I and III and fibronectin expression. However, there is a surprising absence of *in vitro* skin penetration data in the literature, and there are relatively few clinical studies using these materials.

Résumé

Le vieillissement cutané est un processus irréversible causé par des facteurs tant intrinsèques qu'extrinsèques. La possibilité d'arrêter ou de retarder le vieillissement cutané représente un grand secteur de recherche et grand potentiel dans le secteur cosmétique. Récemment, le polypeptide lysine-thréonine-thréonine-lysine-serine (KTTKS) a attiré l'attention. Il est présent dans de nombreux produits cosmétiques haut de gamme où il est faussement associé au terme de 'penta peptide'. Dans cette revue, nous examinons en détail KTTKS et ses dérivés majeurs, avec dans la littérature des informations limitées quant à l'évaluation de ses propriétés physicochimiques et celles de perméation cutanée théoriques. Il semble y avoir un fondement à propos de son action *in vitro* sur des fibroblastes avec un effet de KTTS stimulant de la synthèse de la matrice extracellulaire, avec une activité spécifique aux collagènes de type I et III et à l'expression de la fibronectine. Cependant, il y a, dans la littérature, une absence surprenante de données concernant la pénétration cutanée *in vitro* et il y a relativement peu d'études cliniques utilisant cet ingrédient.

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Introduction

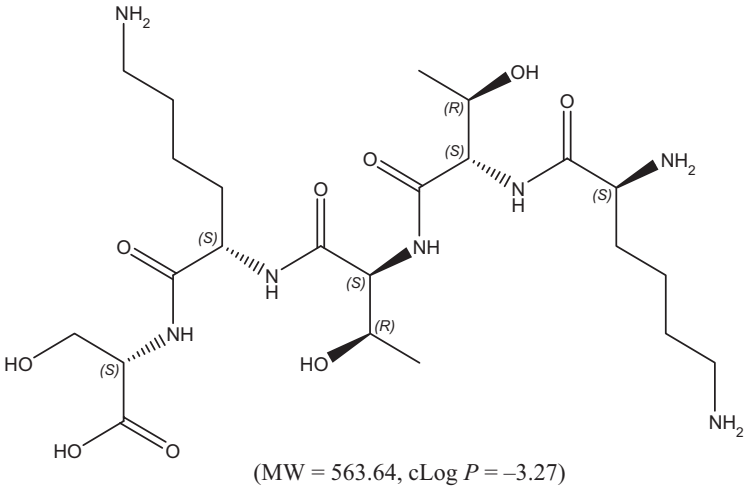
Skin appearance contributes a major social impact to human beings especially on self-esteem and quality of life, and the most apparent hallmarks of ageing are reflected by the skin. The overall functions of the skin decrease with age, and are a result of a combination of intrinsic and extrinsic factors [1–3]. Intrinsic (chronological) change occurs naturally over time [2, 4, 5] causing structural and functional changes in all layers of the skin [5]. Such effects are mostly functional, with less impact on skin appearance and can be described clinically as smooth, dull and fine wrinkles [1, 6]. Intrinsic factors may be compounded by exposure to extrinsic factors which are largely environment- and lifestyle-related such as pollution, smoking, and chronic sun exposure [2, 6, 7]. Ultraviolet (UV) irradiation from sun exposure is widely held to be the primary factor in premature skin ageing, or photoageing [3, 4, 7, 8]. This extent to which an individual is prone to photoageing depends mainly on the degree and duration of exposure to the sun and on skin pigmentation [4]. Coarse, rough and deep wrinkles are characteristics of photoaged skin [2, 3, 8]; clinical conditions such as dyspigmentation and telangiectasia are also associated with the problem [1, 8, 9].

In recent years, the cosmetic sector has delivered numerous products and chemical compounds in an effort to provide the user with an arrestive effect on skin ageing processes. In this study, we aim to review the scientific basis behind and published research on lysine–threonine–threonine–lysine–serine, KTTKS (sometimes erroneously referred to as 'pentapeptide') and its major derivatives.

Peptides intended for topical application

Short amino acid sequences, of less than 50 amino acids, can possess bioactivity and these include components or precursors of larger proteins such as collagens [10, 11]. The theoretical benefits of applying peptides to the skin were discovered during wound healing research as the compounds produced beneficial effects to skin components, particularly collagens and fibroblasts [10, 12–14]. The healing effect was theorized to be produced by up-regulation of cellular growth factors caused by angiogenesis, granulation tissue stimulation and new collagen synthesis [12]. The commercial potential of exploiting the enhanced biological activity of such compounds within the dermis was soon realized and numerous products launched with the aim of improving ageing skin condition. It appears that only the topical route of administration has been studied in depth.

Generally, peptides intended for topical application may be classified into four main categories based on their mechanism of actions:



(MW = 563.64, cLog P = –3.27)

Figure 1 The chemical structure of lysine-threonine-threonine-lysine-serine (KTTKS).

signal peptides, enzyme-inhibitor peptides, neurotransmitter-affecting peptides and carrier peptides [12, 15]. The general area was recently the subject of an excellent review by Gorouhi and Maibach, 2009 [15].

KTTKS

Biological properties

The most widely promoted peptide for topical application is KTTKS (Fig. 1) which is comprised of five amino acid residues (hence 'pentapeptide'), KTTKS is regarded as a 'signal peptide' – a short amino acid sequence that possesses the ability to enhance dermal remodelling by triggering cellular processes, such as inhibiting collagenases activities and increasing extracellular matrix (ECM) production [12]. KTTKS is a subfragment of type I collagen propeptide and was discovered in 1993 by a group of researchers at the University of Tennessee [16]. *In vitro* studies have suggested that amino ($-NH_2$) or carboxyl (-COOH) terminal propeptides of collagen, once removed from their parent procollagen molecules, may exhibit auto-regulatory feedback control of their own synthesis [13, 16–18]. KTTKS was described as being the minimum sequence able to retain 80% of the original activity of the much larger parent peptide necessary for stimulating ECM synthesis [13, 16, 17]. The stimulatory effect of KTTKS appeared to be specific to collagen types I and III and fibronectin expressions [16, 17, 19]. However, *in vitro* studies described the effect as cell density-dependent because it has been shown to be optimal in subconfluent fibroblasts cell culture and gives no response as the cells become fully confluent [16, 17]. Furthermore, the action occurred in both dose- and time-dependent modes; as was found to be neither cell specific nor species-restricted [17].

Mechanism of action

It has been suggested that the stimulatory effect of KTTKS on collagen types I and III and fibronectin relates primarily to the biosynthetic pathway, rather than the export or degradation pathways [16]. This can be explained as no changes were observed either in the ratio of secreted/cell-associated collagen or in the rate of intracellular degradation. Furthermore, neither effects on total protein synthesis are detected, nor is there any changes in the specific activity of the intracellular proline pool because of peptide stimulation [16, 17]. Proline content may directly reflect collagen synthesis primarily because of its co-translational conversion to hydroxyproline which constitutes a relatively high proportion of the total amino acid composition of collagens [20].

The steady-state levels of the messenger RNAs (mRNAs) encoding type I procollagen and fibronectin were not measurably modulated by treatment with KTTKS, which lead to the conclusion that KTTKS was active during the post-transcriptional phase [16, 17]. Recently, KTTKS has been documented as promoting the synthesis of type I collagen by maintaining the stability of mRNA in a process associated with the up-regulation of transforming growth factor-β (TGF-β) expression [18]. Despite such knowledge, the mechanism of action of KTTKS has yet to be fully established.

Topical delivery of KTTKS

Peptides generally possess several physicochemical properties that make their systemic delivery inappropriate, including susceptibility to extensive proteolytic degradation (by hepatic first-pass metabolism), sensitivity to extreme pH condition (stomach) and risk of inducing an immune response. Topical application offers a seemingly more direct route to the site of action provided sufficient compound can be delivered into the targeted area. Furthermore, greater bioavailability could be achieved as a result of much reduced metabolism.

However, there are several issues to consider when formulating peptides as topical ingredients, particularly their passive permeation and penetration potential across the skin. The major obstacle facing transcutaneous delivery is the skin anatomy itself. Between the area of application and the site of action in the dermis, there are numerous physicochemical barriers and issues. Firstly, KTTKS must be absorbed or partition into the outermost layer, the *stratum corneum*. Once the compound has diffused through the *stratum corneum* it must permeates through the viable epidermis, without significantly interacting with keratinocytes and proteolytic enzymes. Once it has reached and traversed the basement membrane, would KTTKS be able to elicit its effect in the growing cells of the dermis; provided it has evaded clearance by bypassing the microvasculature of the dermis.

Each of the above is capable of providing a formidable obstacle. However, the *stratum corneum* is considered to provide the major rate-limiting barrier for KTTKS skin permeation. This constitutes

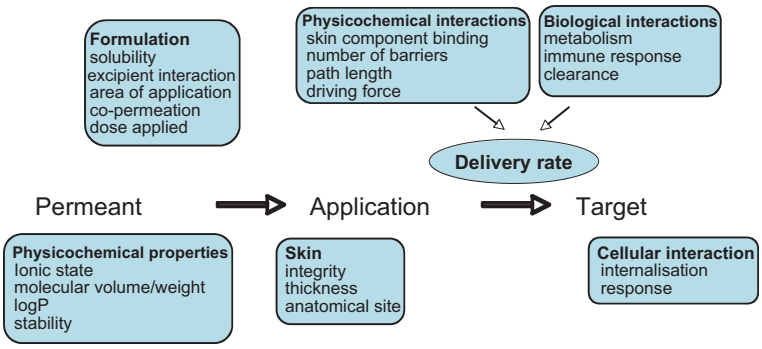


Figure 2 General factors influencing the permeation of a compound across skin.

only 10% of the entire skin but contributes a significant role (80%) in the cutaneous barrier function [21]. Comprising corneocytes in a lipoidal matrix, the barrier is essentially lipophilic in nature and provides excellent barrier for passive permeation of exogenous molecules, especially those that are polar and hydrophilic, as is KTTKS. Figure 2 shows the major aspects that may generally influence the permeability of a particular compound across the skin. A compound intended for transcutaneous delivery should have optimal physicochemical properties, including a log $P_{(\text{octanol/water})}$ in the range of ~1–3 [15, 22, 23], a molecular weight <500 [15, 22, 23], aqueous solubility >100 μg mL^{-1} [23], melting point <200°C and ≤4 polar centres [15]. As the compound becomes more lipophilic, its permeability across the skin increases as a result of better partitioning and as it size becomes larger, its diffusion in the skin is reduced. However, beyond the optimum lipophilicity, permeation decreases as a result of the increasingly aqueous environment of the viable epidermis.

Peptides generally have high-to very high molecular weights and they are typically charged at physiological pH which making them hydrophilic, thus contributing to their poor passive skin permeation [23, 24]. In addition, peptides contain multiple amide bonds (as hydrogen bond donor and acceptor groups) which have potential to affect their diffusion across skin [25]. Mathematical models can be used for estimating the permeation of a compound across human skin. One of the models most commonly utilized is the Potts and Guy equation [26]:

$$\log K_p = 0.71 \log P_{(\text{octanol/water})} - 0.0061\text{MW} - 2.74 \quad (1)$$

In Equation 1, K_p is the permeability coefficient (cm h^{-1}), $P_{(\text{octanol/water})}$ is the octanol/water partition coefficient of the permeant, and MW is the molecular weight of the permeant. The log $P_{(\text{octanol/water})}$ value can be predicted from numerous software and online algorithms. KTTKS is a peptide with a molecular weight of 563.64, comprised of five amino acids and highly hydrophilic with a predicted log P(clog P) of −3.27 [27]. The calculated value for K_p of 3.16×10^{-9} cm h^{-1} [28] is extremely low, and it is immediately apparent that this KTTKS is a poor candidate for topical delivery. Furthermore, unlike many other cosmetic products that act only on the *stratum corneum* and/or epidermis [2, 29], the intended target of KTTKS is the dermis which will necessitate the surmounting of further barriers. Overall, these properties would be expected to severely limit the potential of KTTKS reaching its site of action.

Penetration enhancers

One of the strategies available to improve topical delivery is to use a co-administered chemical penetration enhancer [30]. It has been suggested that, fatty acids (FAs) act as enhancers through several possible mechanisms: (i) disrupting the packed structure of lipids in the *stratum corneum* layer to enhance 'fluidity' or; (ii) increase skin/vehicle partitioning of a permeant or; (iii) increase solvent transport into or across the skin [31]. However, for the latter mechanism it has been suggested that eicosapentaenoic acid (and ethanol, 1,8-cineole) exerts an enhancing effect by a pull or co-permeation mechanism [32].

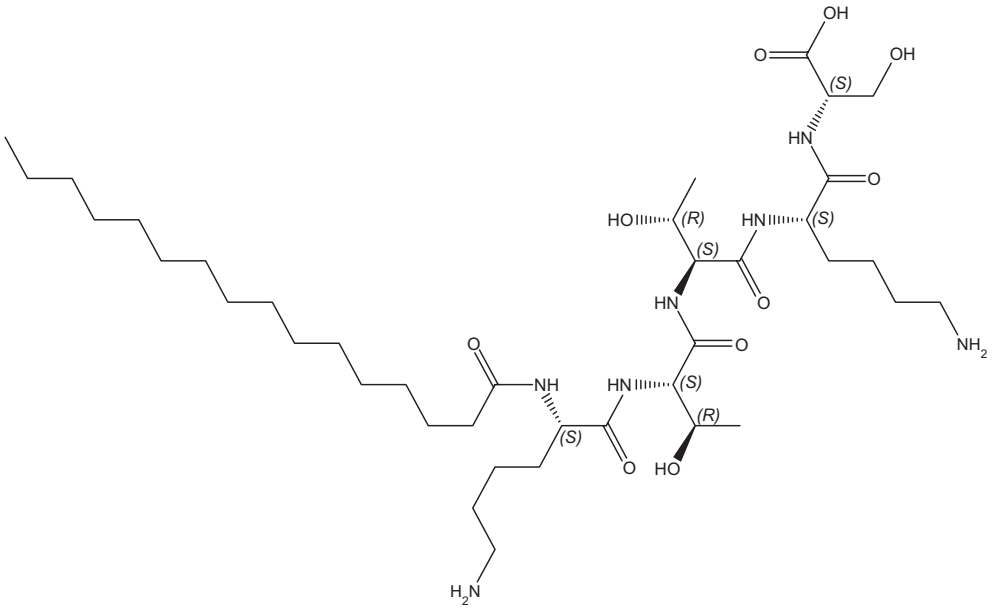
Human *stratum corneum* contains a complex mixture of intercellular lipids with major components including ceramides, cholesterol, free FAs [33] as well as derivatives of cholesterol and glucosylceramide [21, 23]. FAs, have been shown to enhance permeant flux [23], although this is typically as a distinct co-administered compound. Covalently bonded compounds have also been used. For instance, the cutaneous absorption of palmitoyl (ester) derivatives of interferon-alpha was found to be 5–6 times higher than the unmodified parent compound [33]. Lipophilic peptides were reported as having 'no significant' transcutaneous penetration [13], a property that is considered desirable for cosmetic purposes, as systemic activity is unwanted [34]. However, in the case of KTTKS, permeation beyond the viable epidermis is essential if it is to have any effects at its site of action.

The above rationale appears to have been applied to KTTKS as a means of improving its delivery across the epidermis, involving the attachment of a lipophilic group (i.e. palmitic acid) to the N-terminal group of lysine through the formation of an amide bond to form *N*-palmitoyl-KTTKS, available in a solution commercially known as Matrixyl™ [10, 13, 35]. The presence of the palmitic acid moiety, as one of the principle *stratum corneum* lipids, would be expected to enable KTTKS to penetrate the epidermal barrier to a greater extent and act as a 'permeant localizer'. Once incorporated into the skin, they could disrupt the lipid packing of the intercellular lipids within the skin layer and decrease diffusional resistance of the skin towards their conjugated drug. Additionally, a study conducted on the relative skin irritancy of free FAs of different chain lengths revealed that palmitic acid is among the FAs which are least irritant to the skin [36].

Currently, the literature describes two major KTTKS derivatives which are claimed to be able to enhance the delivery of KTTKS across the skin: Palmitoyl-KTTKS (Fig. 3) and ascorbyl conjugated KTTKS (Fig. 4).

Palmitoyl-KTTKS

Palmitoyl-KTTKS would be expected to demonstrate increased permeation across the skin compared to KTTKS (clog P = −3.27), because it has a major improvement in terms of its predicted log P value (clog P = 3.32) [27]. However, a study suggested that an



(MW = 802.05, cLog P = 3.32)

Figure 3 The chemical structure of palmitoyl-KTTKS.

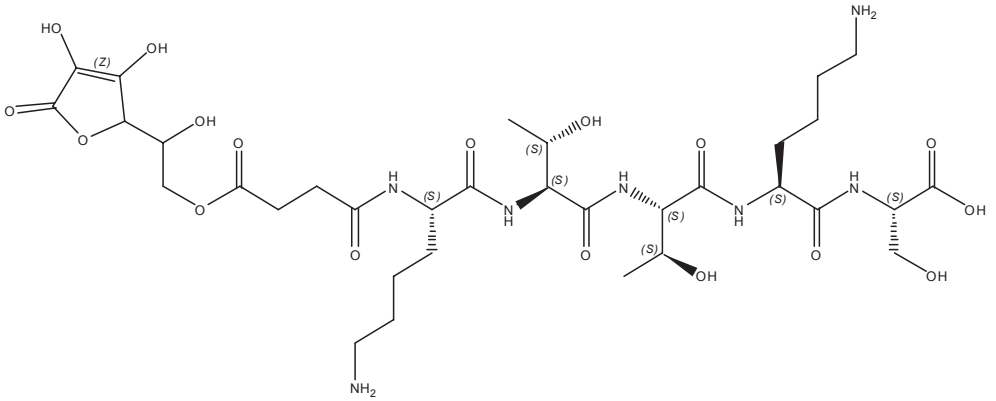


Figure 4 The structure of stabilized ascorbyl pentapeptide (SAP).

octanol/water partition coefficient is only able to predict semi-quantitatively a compound's permeability coefficient [37]. Molecular weight of the compound should be taken into consideration as well. Any modified compound will by definition possess increased molecular weight (Palmitoyl-KTTKS, 802.05) compared with its parent compound (KTTKS, 563.64). Even though experimental results have indicated that the effect of lipophilicity is more dominant than the molecular weight [23, 34, 37, 38], this principle is more applicable for compounds that lie within a narrow range of molecular weights (~100–500) [23]. For palmitoyl-KTTKS which is a much larger molecule, the molecular weight dependency on permeation flux is more important. The calculated K_p value for palmitoyl-KTTKS generated based on the Potts and Guy equation is 5.46×10^{-8} cm h^{-1}, and is some 17 times higher than the predicted value for the parent compound, KTTKS ($K_p = 3.16 \times 10^{-9}$ cm h^{-1}).

It is highly likely that palmitoyl-KTTKS would need to degrade and liberate KTTKS *in situ* in order to become biologically active. As such, palmitoyl-KTTKS may be considered a pro-drug, although it contains an amide bond (-CONH-), whereas ester linkages (-COO-) are the norm in such cases [22]. Amide bonds are considerably more stable to chemical hydrolysis and degrade at a much slower rate than ester bonds [39, 40]. This can be explained by carbonyl carbon of the amide bond being less electrophilic (the -CO-NH- bond has considerable double-bond character) than the ester bond, and an amine is a weak leaving group. Furthermore, ester bonds can be labile to endogenous esterases present in mammalian extrahepatic tissues including lung and skin [41].

L-ascorbic acid and peptide conjugate

Recently, a group of Korean researchers has developed a novel compound based on KTTKS and ascorbic acid which is known as stabilized ascorbyl pentapeptide (SAP) [42]. It was introduced as an active anti-wrinkle agent for cosmetics use and patented by a Korean company Peptron Inc. (Daejeon, South Korea) under the name Ascotide™ [43]. The new compound is a complex of ascorbic acid and KTTKS linked together by a succinoyl linker [42]. It was discovered that the conjugated form has a better ability to stimulate *in vitro* human fibroblast cells to produce more collagen than its parent compounds alone. When SAP was subjected to *in vitro* enzymatic hydrolysis using rat skin extracts, it was found to be stable under the experimental conditions used. Even though they could not rule out the exact mechanism behind those observations, the succinoyl linker was suggested to contribute to the enhanced activity and stability of the newly synthesized compound.

Potential cutaneous metabolism of KTTKS and palmitoyl-KTTKS

As peptides, the metabolism and proteolytic activity of the skin is potentially of significance to the transcutaneous delivery of both KTTKS and palmitoyl-KTTKS. The skin contains enzyme systems comparable with those found in other tissues for example the liver [44, 45]. Endogenous enzymes such as deaminases and esterases are present in the extracellular compartment of the *stratum corneum*, sebaceous glands and near hair follicles [21, 23], although different anatomical sites of the skin have different levels of enzymatic activities [46, 47]. Aminopeptidase activity is evenly distributed in the viable epidermis layer, and less pronounced activity is observed in the *stratum corneum* and dermis [48], although skin was reported as the weakest enzymatic barrier to the absorption of peptides [49]. Among the peptidases detected in the skin are endopeptidases and exopeptidases which are known metabolize polypeptides, such as collagens and elastin [46]. The physicochemical characteristics of palmitoyl-KTTKS would be expected to be of significance in relation to metabolism in the skin, as a low rate of penetration and high retention in the skin tissues [22, 50] would be expected to increase the rate of conversion [47, 51]. However, the presence of long chain FA moieties, such as hexadecyl (C16), can influence enzyme active site docking. Overall, the net sum of such processes would dictate the bioavailability of free KTTKS hence its pharmacological activity.

Reported *in vitro* and *in vivo* investigations of palmitoyl-KTTKS

At the time of writing, there is a paucity of literature reports into the topical delivery of KTTKS-based compounds. Indeed, KTTKS and palmitoyl-KTTKS are unusual in that no supporting *in vitro* data have ever been published supporting their successful transcutaneous delivery. This may lie in the fact that typical in-use doses are in the order of 3 parts-per-million (ppm). In fact, even using saturated solutions and penetration enhancers has yielded no detectable permeation across excised skin in the author's laboratories (unpublished data). Lintner (2002), however, claims to have confirmed penetration of the peptide into the skin but not through it, although no data were presented [13].

Current literature reports are primarily concerned with clinical effects. Several clinical published studies stated that topical formulations containing palmitoyl-KTTKS have a capacity to reduce fine lines, wrinkles and improve skin texture significantly which may help to delay the ageing process in the skin [35, 52, 53]. A double-blind, placebo-controlled, split face, left-right randomized trial involving 93 subjects was carried out to assess the clinical efficacy of palmitoyl-KTTKS, with fine lines or wrinkles improvement as the parameter of interest [35]. Following a 2-week washout period, subjects were supplied with two facial oil-in-water moisturisers: one containing placebo control and the other one, 3 ppm palmitoyl-KTTKS. Digital images of each side of the face of subjects were taken at baseline and at weeks 4, 8 and 12 using a Rapid Evaluation of Anti-ageing Leads (REAL 1.0) facial imaging system. Image analysis was performed quantitatively using non-commercial algorithms developed by Procter & Gamble (Cincinnati, Ohio, USA). The algorithms were used to determine the total linear depression area around the eye (crow's feet area) and the total linear depression area on the cheek (skin texture) of the subjects. The images were also evaluated in a treatment blind-coded study known as a Visual Perception Study; expert graders were asked to determine which side of the face looked better, based on specific skin attributes and graded them according to a given scale. Additionally, subjects were also required to complete a self-assessment questionnaire in terms of their skin appearance at weeks 4, 8 and 12. Measurement of transepidermal water loss (TEWL) was also determined to assess if the palmitoyl-KTTKS has any effect on the skin barrier. TEWL measurement is influenced by the concentration of water in the epidermis, cell integrity, relative humidity, diffusivity of water in the *stratum corneum* and thickness of the *stratum corneum* [21]. Impairment of the permeability barrier function as a result of mechanical or chemical assault to the *stratum corneum* can be directly monitored using this technique [54]. An increase in TEWL reflects impairment of the water barrier which may lead to the skin irritation. Topical application of palmitoyl-KTTKS, twice daily for 12 weeks was reported to reduce the total length of fine lines and wrinkles in comparison with the placebo-treated skin [35]. The magnitude of the effects was reported to be small, but reached a level of significance at weeks 8 and 12 to support the claims. For the self-assessment study, the subjects were reported to document some skin benefits including age spot reduction, improvement of dark circle under the eyes and skin firmness. There were no significant differences in TEWL between skin treated with palmitoyl-KTTKS formulation and vehicle (placebo-control), which the authors interpreted as suggesting palmitoyl-KTTKS is 'gentle' on the skin/skin barrier. palmitoyl-KTTKS was therefore claimed to be well tolerated and safe, with low incidence of undesired skin irritation such as redness, dryness, burning, stinging or itchiness commonly associated with formulations containing retinoids.

In 2005, a poster describing an *in vivo* study was presented in American Academy of Dermatology Annual Meeting [53]. The study aimed to evaluate the beneficial use of a facial moisturizer containing palmitoyl-KTTKS on the appearance of facial texture, fine lines and wrinkles of photoaged skin. The double-blinded, 8-week, split face, randomized round robin study was conducted on women aged 35–65 years with 60 panelists per product. Subjects were asked to apply a serum-type facial moisturizer product containing 3 ppm palmitoyl-KTTKS which also contained niacinamide, panthenol and vitamin E. Responses were compared with a moisturizer base vehicle. Analysis was performed qualitatively by expert and self-grading, as well as quantitatively using an image analysis system based on digital facial images captured at baseline and at 4 and 8 weeks. Palmitoyl-KTTKS treatment at a concentration of 3 ppm, twice daily was reported to improve the appearance of bumpy facial texture, fine lines and wrinkles in the under-eye and cheek areas of the subjects.

Another study was carried out to evaluate histological effects of palmitoyl-KTTKS onto the skin components [55]. This 4 months, double-blind study was sponsored by Sederma in which 49 women were directed to apply either palmitoyl-KTTKS (n = 22) or vehicle (n = 27) twice daily to the face. The end results showed that palmitoyl-KTTKS exhibited significant improvement in skin roughness, wrinkle volume and wrinkle depth compared with the vehicle. Histological evaluation showed that palmitoyl-KTTKS was associated with increment in elastin fibber density, thickness and improved regulation of collagen type IV at the dermal–epidermal junction.

A paper summarising several clinical studies conducted specifically for palmitoyl-KTTKS was published in 2002 [13]. The author briefly described the visible benefits of the palmitoyl-KTTKS based on *in vivo* studies conducted by his group: two studies comparing the palmitoyl-KTTKS to vehicle sample, one versus vitamin C and one versus retinol. However, only palmitoyl-KTTKS and retinol demonstrated a desirable effect – wrinkle reduction. The study conducted preferably comparing between palmitoyl-KTTKS and retinol in the same vehicle for efficacy evaluation. The topical use of retinoids has been studied and documented, both histologically and clinically, more than any other compound in dermatology [4]. Topical retinoids treatment particularly retinol can repair photoaged dermal matrix; this is regarded as the 'gold standard' against which repair agents are judged [2, 56]. Sixteen volunteers were subjected to either creams containing 3 ppm of palmitoyl-KTTKS or 700 ppm retinol for durations of 2–4 months [13]. Results were analysed based on digital image analysis, visual scoring by a dermatologist and echography. Both formulations were reported producing similar effects by reducing the volume of wrinkle depth and length according to the quantitative image analysis. The dermatologist graded analysis showed progressive improvement of the skin appearance. Furthermore, the echography results showed an increase in the skin thickness of about 9% after 4 months of both treatments. However, the effect exhibited by the palmitoyl-KTTKS only can be seen after 2 months of treatment. DNA array studies showed that both KTTKS and retinol-activated genes associated with wound healing mechanisms [55, 57]. The lipophilic peptide was reported able to stimulate about 16 genes in skin cells, which are only a fraction of the number of genes that retinol favourably stimulates in the skin, but it does not elicit the pro-inflammatory actions associated with retinol [57]. The author also considered toxicological aspects of the palmitoyl-KTTKS – studies including oral and ocular irritation, skin sensitization and mutagenicity were conducted on palmitoyl-KTTKS and its various solutions [13]. The results showed that this compound is safe for cosmetic purposes even at a concentration of about 30 times (100 ppm) higher than its recommended concentration. The recommended concentration of palmitoyl-KTTKS for cosmetic purposes is between 2 and 8 ppm (2–8 mg kg^{-1}) [7]. Based on a previous investigation, palmitoyl-KTTKS was claimed to be capable producing the observed benefits at such low concentrations, suggesting that palmitoyl-KTTKS is a very potent compound [35]. The rationale behind this suggestion is based on two factors: (i) several previous studies reported effective clinical benefits can be observed at concentrations as low as 3 ppm (3 mg kg^{-1}); and (ii) economic practicability as palmitoyl-KTTKS is quite costly. This factor has a significance impact for commercial market as the price of an active ingredient affects how much of it can be included into a final formulation.

A study was conducted to assess clinical efficacy of a cosmetic moisturizer regimen versus a prescription regimen containing 0.02% tretinoin in reducing facial fine lines and wrinkles [58]. Skin tolerability towards the product regimens was also investigated. The cosmetic regimen contained combinations of niacinamide, palmitoyl-lysine-threonine (abbreviated as Palmitoyl-KT), palmitoyl-KTTKS and retinyl ester. An 8-week, randomized, parallel-group facial appearance study was conducted on 196 women aged between 40 and 65 years. Subjects must exhibited Fitzpatrick skin types I to III and moderately severe periorbital wrinkles on both sides of their face to be eligible for this study. Subjects were randomly assigned to either cosmetic regimen or tretinoin regimen. Prior to the study start, 25 subjects were self-selected on each product regimen agreed to continue treatments for an additional 16 weeks (cohort, 24 weeks study). After 2 weeks of washout period, subjects on cosmetic regimen (n = 99) were instructed to apply a sun protection factor (SPF) 30 moisturizing cream containing 5% niacinamide, peptides and antioxidants, a moisturizing cream containing niacinamide and peptides and a targeted wrinkle product containing niacinamide, peptides and 0.3% retinyl propionate. For prescription regimen, subjects (n = 97) were asked to apply a moisturizing SPF 30 sunscreen and 0.02% tretinoin. Digital images of subject's faces were captured using the REAL 3.0 system taken at baseline and at weeks 8 and 24 (cohort study). Images of the infraorbital/crow's feet areas were also captured with VISIA CR imaging system. Improvement in the appearance of fine lines and wrinkles around the eyes were evaluated by both expert-grading and image analysis of the digital images. Product tolerance was determined via clinical grading of erythema and dryness, skin barrier integrity (TEWL) and changes in the level of protein analytes of *stratum corneum*. Additionally, subjects were also required to complete a self-assessment questionnaire by evaluating certain skin attributes such as fine lines and wrinkles around the eyes, age spots and skin firmness at designed time points. The study concluded that the cosmetic regimen significantly improved wrinkle appearance after 8 weeks of treatment relative to the tretinoin regimen, with comparable benefits after 24 weeks. Furthermore, the cosmetic regimen had better tolerability than tretinoin through 8 weeks of the product tolerance study.

In the first work published by the Katayama group regarding the benefits of KTTKS, subconfluent cells were treated with the peptide at 0.5, 5, 15 and 50 μM concentrations [16]. The group reported that the stimulatory effect of ECM production could be observed at a dose as low as 0.5 μM (0.3 ppm). However, the optimal and more pronounce effect could only be achieved at higher doses of >5 μM (2.8 ppm). Even though the concentration recommended by the manufacturer for palmitoyl-KTTKS is within the range of the earlier reported concentrations for KTTKS, the enhanced ECM production effect was obtained based on the *in vitro* work. The peptide was applied directly on to the monolayer cells with no other factors such as permeation or metabolism barriers were taken into consideration.

Most of the reported clinical benefits were obtained by using a formulation containing palmitoyl-KTTKS and other active ingredients (e.g. niacinamide and vitamin E), or at least in the presence of a basic moisturizer. For example, combination or addition of cosmetic ingredients in a moisturizing vehicle is claimed to magnify the enhance benefits produced by the main ingredients and profoundly improve skin appearance [14, 58]. Previous *in vivo* published studies did not differentiate the role of palmitoyl-KTTKS specifically with other ingredients presence in the formulation except for the study carried out by Robinson *et al.* [35]. Thus, the observed benefits cannot be claimed as solely produced by the conjugated peptide as the effect could be due to or partially contributed by other ingredients. Even though there are several published reports regarding the clinical benefits of palmitoyl-KTTKS from multiple independent *in vitro* or *in vivo* investigations, only two

studies were found to describe their study design and results evaluation in detailed – Robinson *et al.* and Fu *et al.* [35, 58].

Conclusions

Peptides are not new to the world of cosmetics, but their role as cellular messengers is a more recent concept. KTTKS is a small, highly specific biologically active peptide which has been reported to produce stimulation of elastin and collagens, specifically types I and III. However, it is very chemical nature places severe restrictions on its deliverability across skin. From a physicochemical standpoint it is clear that KTTKS and palmitoyl-KTTKS possess unfavourable properties for significant skin permeation. The reported beneficial clinical effects are not readily explained, but may be related to reservoir build up following many re-applications. One current question concerns how it may be possible to surmount the formidable barrier to the permeation of such compounds and thereby enhance the concentrations delivered to the target site in the dermis. Currently, there is a great deal of research taking place into physical enhancement techniques such as iontophoresis, electroporation and microneedle technology [24, 59, 60]. However, the damage potentially inflicted to skin by such techniques seems anathema to cosmetic application. Perhaps, the future lies in the new generation penetration enhancement methodologies involving drug-loaded carriers and nanotechnology. There is growing evidence to suggest that loaded nanoparticles are capable of high levels of skin penetration enhancement [61–63].

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References

1. Chung, J.H., Seo, J.Y., Choi, H.R., *et al.* Modulation of skin collagen metabolism in aged and photoaged human skin *in vivo*. *J. Invest. Dermatol.* **117**(5), 1218–1224 (2001).
2. Watson, R.E., Long, S.P., Bowden, J.J., Bastrilles, J.Y., Barton, S.P. and Griffiths, C.E. Repair of photoaged dermal matrix by topical application of a cosmetic 'antiageing' product. *Br. J. Dermatol.* **158**(3), 472–477 (2007).
3. Tzaphlidou, M. The role of collagen and elastin in aged skin: an image processing approach. *Micron* **35**(3), 173–177 (2004).
4. Fisher, G.J., Kang, S., Varani, J., *et al.* Mechanisms of photoaging and chronological skin aging. *Arch. Dermatol.* **138**(11), 1462–1470 (2002).
5. McCullough, J.L. and Kelly, K.M. Prevention and treatment of skin aging. *Ann. N. Y. Acad. Sci.* **1067**(1), 323–331 (2006).
6. Ramos-e-Silva, M. and da Silva Carneiro, S.C. Elderly skin and its rejuvenation: Products and procedures for the aging skin. *J. Cosmet. Dermatol.* **6**(1), 40–50 (2007).
7. Graf, J. Anti-aging skin care ingredient technologies. In: *Cosmetic Dermatology*. (Burgess, C.M., ed.), pp. 17–28. Springer-Verlag Berlin and Heidelberg GmbH & Co. KG, Berlin (2005).
8. El-Domyati, M., Attia, S., Saleh, F., *et al.* Intrinsic aging vs. photoaging: a comparative histopathological, immunohistochemical, and ultrastructural study of skin. *Exp. Dermatol.* **11**(5), 398–405 (2002).
9. Goldsmith, L.A. *Physiology, Biochemistry, and Molecular Biology of the Skin, Vol. II*, 2nd edn. Oxford University Press, New York (1991).
10. Choi, C.M. and Berson, D.S. Cosmeceuticals. *Semin. Cutan. Med. Surg.* **25**(3), 163–168 (2006).
11. Barrett, G.C. and Elmore, D.T. *Amino acids and peptides*. Cambridge University Press, Cambridge (1998).
12. Lupo, M.P. and Cole, A.L. Cosmeceutical peptides. *Dermatol. Ther.* **20**(5), 343–349 (2007).
13. Lintner, K. Promoting production in the extracellular matrix without compromising barrier. *Cutis*. **70** (6 Suppl.), 13–16; discussion 21–23 (2002).
14. Draelos, Z.D. The latest cosmeceutical approaches for anti-aging. *J. Cosmet. Dermatol.* **6**(s1), 2–6 (2007).
15. Gorouhi, F. and Maibach, H.I. Role of topical peptides in preventing or treating aged skin. *Int. J. Cosmet. Sci.* **31**(5), 327–345 (2009).
16. Katayama, K., Armendariz-Borunda, J., Raghow, R., Kang, A.H. and Seyer, J.M. A pentapeptide from type I procollagen promotes extracellular matrix production. *J. Biol. Chem.* **268**(14), 9941–9944 (1993).
17. Katayama, K., Seyer, J.M., Raghow, R. and Kang, A.H. Regulation of extracellular matrix production by chemically synthesized subfragments of type I collagen carboxy propeptide. *Biochemistry* **30**(29), 7097–7104 (1991).
18. Tsai, W.C., Hsu, C.C., Chung, C.Y., Lin, M.S., Li, S.L. and Pang, J.H. The pentapeptide KTTKS promoting the expressions of type I collagen and transforming growth factor-beta of tendon cells. *J. Orthop. Res.* **25**(12), 1629–1634 (2007).
19. Namjoshi, S., Caccetta, R. and Benson, H.A. Skin peptides: biological activity and therapeutic opportunities. *J. Pharm. Sci.* **97**(7), 2524–2542 (2008).
20. Opsahl, W.P. and Ehrhart, L.A. Compartmentalization of proline pools and apparent rates of collagen and non-collagen protein synthesis in arterial smooth muscle cells in culture. *Biochem. J.* **243**(1), 137–144 (1987).
21. Pouillot, A., Dayan, N., Polla, A.S., Polla, L.L. and Polla, B.S. The stratum corneum: A double paradox. *J. Cosmet. Dermatol.* **7**(2), 143–148 (2008).
22. Lau, W.M., White, A.W., Gallagher, S.J., Donaldson, M., McNaughton, G. and Heard, C.M. Scope and limitations of the co-drug approach to topical drug delivery. *Curr. Pharm. Des.* **14**(8), 794–802 (2008).
23. Williams, A. *Transdermal and Topical Drug Delivery: From Theory to Clinical Practice*. Pharmaceutical Press, London (2003).
24. Benson, H., Caccetta, R., Chen, Y., Kearns, P. and Toth, I. Transdermal delivery of a tetrapeptide: Evaluation of passive diffusion. *Int. J. Peptide Res. Therap.* **10**(5), 615–620 (2003).
25. Hadgraft, J. Skin, the final frontier. *Int. J. Pharmaceut.* **224**(1–2), 1–18 (2001).
26. Potts, R.O. and Guy, R.H. Predicting skin permeability. *Pharmaceut. Res.* **9**(5), 663–669 (1992).
27. ACDLabs. *ACD/ChemSketch*, 12.01 edn. Advanced Chemistry Development, Inc, Toronto (2009).
28. NIOSH. *Skin Permeation Calculator*. Centers for Disease Control and Prevention, Atlanta (2010).
29. Morganti, P., Ruocco, E., Wolf, R. and Ruocco, V. Percutaneous absorption and delivery systems. *Clin. Dermatol.* **19**(4), 489–501 (2001).
30. Kanikkannan, N., Kandimalla, K., Lamba, S.S. and Singh, M. Structure–activity relationship of chemical penetration enhancers in transdermal drug delivery. *Curr. Med. Chem.* **7**, 593–608 (2000).
31. Aungst, B.J., Blake, J.A. and Hussain, M.A. Contributions of drug solubilization, partitioning, barrier disruption, and solvent permeation to the enhancement of skin permeation of various compounds with fatty acids and amines. *Pharmaceut. Res.* **7**(7), 712–718 (1990).

32. Heard, C.M., Kung, D. and Thomas, C.P. Skin penetration enhancement of mefenamic acid by ethanol and 1,8-cineole can be explained by the ''pull'' effect. *Int. J. Pharmaceut.* **321**(1–2), 167–170 (2006).
33. Foldvari, M., Attah-Poku, S., Hu, J., *et al.* Palmitoyl derivatives of interferon alpha: potential for cutaneous delivery. *J. Pharm. Sci.* **87**(10), 1203–1208 (1998).
34. Lintner, K. and Peschard, O. Biologically active peptides: from a laboratory bench curiosity to a functional skin care product. *Int. J. Cosmet. Sci.* **22**(3), 207–218 (2000).
35. Robinson, L.R., Fitzgerald, N.C., Doughty, D.G., Dawes, N.C., Berge, C.A. and Bissett, D.L. Topical palmitoyl pentapeptide provides improvement in photoaged human facial skin. *Int. J. Cosmet. Sci.* **27**(3), 155–160 (2005).
36. Stillman, M.A., Maibach, H.I. and Shalita, A.R. Relative irritancy of free fatty acids of different chain length. *Contact Dermatitis* **1**(2), 65–69 (1975).
37. Sloan, K.B. *Prodrugs: Topical and Ocular Drug Delivery*. Marcel Dekker, New York, (1992).
38. Lin, R.-Y., Hsu, C.-W. and Chen, W.-Y. A method to predict the transdermal permeability of amino acids and dipeptides through porcine skin. *J. Control. Rel.* **38**(2–3), 229–234 (1996).
39. Testa, B. and Mayer, J. *Hydrolysis in Drug and Prodrug Metabolism: Chemistry, Biochemistry and Enzymology*. Helvitica Chimica Acta Verlag, Weinheim (2003).
40. Yoshioka, S. and Stella, V.J. *Stability of Drugs and Dosage Forms*. Plenum Publishing Co., New York (2000).
41. Satoh, T. and Hosokawa, M. THE MAMMALIAN CARBOXYLESTERASES: from molecules to functions. *Annu. Rev. Pharmacol. Toxicol.* **38**(1), 257–288 (1998).
42. Choi, H.-I., Kim, H.-J., Park, J.-I., Shin, E.-H., Kim, D.-W. and Kim, S.-S. Design and efficient synthesis of novel ascorbyl conjugated peptide with high collagen biosynthesis stimulating effects. *Bioorg. Med. Chem. Lett.* **19**(7), 2079–2082 (2009).
43. Park, J.-I., Kim, H.-J., Kim, D.-W., *et al.* Stabilized vitamin C derivatives having a peptide molecule, preparation method thereof, and composition containing the same. South Korea patent WO/2007/148847. World Intellectual Property Organization, (2007).
44. Hadgraft, J. and Guy, R.H. *Transdermal Drug Delivery: Developmental Issues and Research Initiatives*. Dekker, New York (1989).
45. Bronaugh, R.L. and Maibach, H.I. *Percutaneous Absorption: Drugs, Cosmetics, Mechanisms, Methodology*, 4th edn. Taylor & Francis, Boca Raton, FL, London (2005).
46. Steinsträsser, I. and Merkle, H.P. Dermal metabolism of topically applied drugs: pathways and models reconsidered. *Pharmaceut. Acta Helvetiae* **70**(1), 3–24 (1995).
47. Martin, R.J., Denyer, S.P. and Hadgraft, J. Skin metabolism of topically applied compounds. *Int. J. Pharmaceut.* **39**(1–2), 23–32 (1987).
48. Boderke, P., Merkle, H.P., Cullander, C., Ponec, M. and Bodde, H.E. Localization of aminopeptidase activity in freshly excised human skin: direct visualization by confocal laser scanning microscopy. *J. Investig. Dermatol.* **108**(1), 83–86 (1997).
49. Zhou, X.H. and Li Wan Po, A. Comparison of enzymic activities of tissues lining portals of drug absorption, using the rat as a model. *Int. J. Pharmaceut.* **62**(2–3), 259–267 (1990).
50. Storm, E.J., Collier, W.S., Stewart, F.R. and Bronaugh, L.R. Metabolism of xenobiotics during percutaneous penetration: Role of absorption rate and cutaneous enzyme activity. *Toxicol. Sci.* **15**(1), 132–141 (1990).
51. Williams, F.M. Potential for metabolism locally in the skin of dermally absorbed compounds. *Human Exp. Toxicol.* **27**(4), 277–280 (2008).
52. Lupo, M.P. Cosmeceutical peptides. *Dermatol. Surg.* **31**(s1), 832–836 (2005).
53. Osborne, R., Robinson, L., Mullins, L., Raleigh, P., Li, J. and Miyamoto, K. *Use of a Facial Moisturizer Containing Pal-KTTKS Peptide. 63rd American Academy of Dermatology Annual Meeting*, New Orleans, (2005).
54. Levin, J. and Maibach, H.I. The correlation between transepidermal water loss and percutaneous absorption: an overview. In: *Percutaneous Absorption: Drugs, Cosmetics, Mechanisms, Methodology* (Bronaugh, R.L. and Maibach, H.I., eds), 4th edn, pp. 583–593. Taylor & Francis, Boca Raton, FL; London (2005).
55. Guttman, C. Studies demonstrate value of procollagen fragment Pal-KTTKS. *Dermatol. Times.* **23**, 68 (2002).
56. Rona, C., Vailati, F. and Berardesca, E. The cosmetic treatment of wrinkles. *J. Cosmet. Dermatol.* **3**(1), 26–34 (2004).
57. Fernandes, D. Evolution of cosmeceuticals and their application to skin disorders, including aging and blemishes. In: *Dermatologic, Cosmeceutic, and Cosmetic Development: Therapeutic and Novel Approaches* (Walters, K.A. and Roberts, M.S., eds), pp. 45–60, Ilustrated edn. Informa Healthcare USA. Inc., New York (2008).
58. Fu, J.J.J., Hillebrand, G.G., Raleigh, P., *et al.* A randomized, controlled comparative study of the wrinkle reduction benefits of a cosmetic niacinamide/peptide/retinyl propionate product regimen vs. a prescription 0·02% tretinoin product regimen. *Br. J. Dermatol.* **162**(3), 647–654 (2010).
59. Prausnitz, M.R., Mitragotri, S. and Langer, R. Current status and future potential of transdermal drug delivery. *Nat. Rev. Drug Discov.* **3**(2), 115–124 (2004).
60. Naik, A., Kalia, Y.N. and Guy, R.H. Transdermal drug delivery: overcoming the skin's barrier function. *Pharm. Sci. Technol. Today* **3**(9), 318–326 (2000).
61. Alvarez-Román, R., Naik, A., Kalia, Y.N., Fessi, H. and Guy, R.H. Visualization of skin penetration using confocal laser scanning microscopy. *Eur. J. Pharmaceut. Biopharmaceut.* **58**(2), 301–316 (2004).
62. Singka, G.S.L., Samah, N.A., Zulfakar, M.H., Yurdasiper, A. and Heard, C.M. Enhanced topical delivery and anti-inflammatory activity of methotrexate from an activated nanogel. *Eur. J. Pharmaceut. Biopharmaceut.* **76**(2), 275–281 (2010).
63. Samah, N.A., Williams, N. and Heard, C.M. Nanogel particulates located within diffusion cell receptor phases following topical application demonstrates uptake into and migration across skin. *Int. J. Pharmaceut.* **401**(1–2), 72–78 (2010).