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Review Article

Role of topical peptides in preventing or treating aged skin

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Synopsis

Ageing, a basic biological process seen in all living creatures, is not preventable. Surgical and topical modalities have been invented and substances were applied topically to alter the ageing process. Peptides and proteins, frequently used for this purpose, were categorized into four groups: signal peptides, enzyme-inhibitor peptides, neurotransmitter-inhibitor peptides and carrier peptides. We comprehensively review eligible studies -including controlled *ex vivo* or *in vivo* efficacy studies on any topical peptide or protein that has been administered to treat signs and symptoms of ageing.

Résumé

Le vieillissement, processus biologique concernant toutes les créatures vivantes est inévitable. Divers moyens chirurgicaux et topiques ont été inventés et plusieurs substances ont été appliquées pour modifier ce processus. Les peptides et les protéines fréquemment utilisés dans ce but sont regroupés en 4 catégories : les peptides signaux, les peptides inhibiteurs enzymatiques, les peptides inhibiteurs de neurotransmetteurs et les peptides transporteurs. Nous passons en revue les études recevables – y compris les études d'efficacité *ex vivo* ou *in vivo* – concernant les peptides ou les protéines mis en œuvre pour traiter les symptômes et les signes du vieillissement.

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Introduction

In the year 2000, individuals over the age of 65 represented 13% of the United States population; this is expected to increase to 20% by 2030. This preponderance of older individuals over younger ones will transform the shape of the age distribution graph into a rectangle rather than the current pyramid observed [1].

This demographic shift calls for increased efforts to prevent the ageing process and develop safe and effective drugs for the elderly. In cosmetic dermatology, experts are exploring better anti-solar, anti-ageing, anti-wrinkle and firming products. Pharmaceutical companies frequently use peptides as active ingredients in their creams. Peptides have different effects on the skin especially for cosmetics purposes, but one important hurdle to use them topically is their permeability to penetrate skin.

Generally, permeation ability depends on different factors: physicochemical properties of the substance (acid dissociation constant [pK_a], molecular size, stability, binding affinity, solubility and partition coefficient); the time-scale of permeation; integrity, thickness and components of the skin, cutaneous metabolism; site, area and duration of application; properties of the transdermal device and the creation of a local depot at the site of application [2, 3]. In summary, it is ideal to have a topical drug with parameters within the below-mentioned listed range:

1 Molecular weight less than 500 Da;
2 Moderate log of partition coefficient octanol/water between 1 and 3;
3 Melting point less than 200°C;
4 Reasonable aqueous solubility (>1 mg mL^{-1});
5 No or few polar centres [4, 5].

Diffusivity of the molecules in stratum corneum is related to the size and number of hydrogen bonding groups on a molecule, being maximal for small non-hydrogen bonding molecules and reaching a minimum with about four hydrogen bonding groups [6].

As peptides and proteins contain many amide bonds, (as hydrogen bond donor and acceptor groups), and because of their large molecular size, they have low diffusivity in skin. Furthermore, as they often charged at physiological pH, they are intrinsically hydrophilic. Hence, the lipophilic stratum corneum is a significant barrier to penetration [7].

Overall, topical peptides and proteins have been successfully and widely used. Patch test of purified protein derivatives tuberculin protein and more specific derivatives like MPB64 have been effectively addressed for active tuberculosis diagnosis [8, 9]. Tacrolimus and with lower permeability, pimecrolimus can penetrate through the skin to treat atopic dermatitis patients [10, 11].

Frech *et al.* [12] found a safe and protective vaccine patch containing heat-labile protein enterotoxin to prevent diarrhoea in travellers to Mexico and Guatemala. Even topical cyclosporine A with molecular weight of more than 1200 Da could be delivered into the skin with delivery enhancing methods [13].

The main barrier for topical drugs is stratum corneum, the outermost layer of epidermis. Several techniques overcome such a barricade. For example, chemical penetration enhancers might be useful for peptide dermal delivery [14].

One study [15] addressed usefulness of iontophoresis for topical insulin application. Other mechanisms like sonophoresis in liposomal peptides [16] and colloidal carrier systems [17] are considered helpful in this regard. Carrier peptides can increase and accelerate the permeation process. Chen *et al.* [18] reported that a short synthetic peptide (ACSSSPSKHCG) identified by *in vivo* phage display assay, facilitated efficient transdermal insulin delivery through intact skin. Although topical use of a peptide has potential to be effective, delivery across skin can be difficult because of the ionic nature of such materials. An approach to improving delivery is the use of fatty acid derivatives to increase the lipophilic property of the peptide. For example, the palmitoyl derivative of the polypeptide interferon α penetrates across human skin five- to six-fold greater than the simple peptide [19] and also, facial skin improvement has been reported after topical pal-KTTKS (palmitoyl pentapeptide-4) therapy [20].

Many peptides and proteins used for cosmetic indications; glycyl-histidyl-lysine (GHK)-Cu is one of the most widely utilized for wound healing and anti-ageing indications. Abdulghani *et al.* [21] revealed its enhanced but insignificant anti-ageing effects when compared with tretinoin, Vitamin C and melatonin.

Palmitoyl KTTKS, the other frequently used peptide, was shown to have more significant results than placebo and an active comparator [20, 22]. Acetyl hexapeptide-3 (Argireline®; Centerchem, Barcelona, Spain) had promising results. The depth of wrinkles was reduced more than 30% for Argireline® vs. 10% for placebo after 30 days [23].

All aforementioned peptides as examples can suggest that these agents may be effective. Note that in the only published systematic review [24] on interventions for ageing skin; no peptide therapy was included. Here, published peptides and proteins categorized in four categories, their characteristics, *in vitro* studies and *in vivo* efficacy data are presented.

Material and methods

Pubmed, EMBASE and Scopus were systematically searched from 1974 to June 15, 2008 (Appendix I). Different words have been used to locate any known peptides or proteins; find all possible topical therapies; locate all cosmeceutical-related papers and rule out irrelevant papers. The irrelevant studies were mainly on topical peptide application for skin cancers, inflammatory diseases, infectious diseases or blistering diseases. They also considered irrelevant if they used peptides as vaccines. We screened all references of relevant articles to find other eligible resources. In addition, some *in vitro* and *in vivo* data were collected from pharmaceutical companies' websites. The main purpose of this study was to evaluate available evidence on efficacy data of topical peptides and proteins for ageing skin and only controlled trials were included in this regard.

Results and discussion

Surprisingly, there were scarce data about permeation abilities of these topical peptides. Only permeation coefficients for three widely used topical cosmeceutical peptides (GHK and γ-L-glutamyl-L-cysteinyl-glycine (GSH); [25]) and Melanocyte stimulating hormone (MSH) ([26, 27]) and some amino acids [28, 29] as well as some of their Cu complexes were reported (Table I).

Braun *et al.* [30] reported relative superoxide dismutase content that has been absorbed in the epidermis and dermis after an 8-h penetration. The ratios of total concentration for epidermis and dermis were 0.009% and 0.010% after 4 h and 0.031% and 0.010% after 8 h respectively. The characteristics of included substances are shown in Table II. Eligible *in vivo* efficacy studies are shown in Table III.

We categorized topical peptides and proteins into four groups:

1 Signal peptides,
2 enzyme inhibitor peptides,
3 neurotransmitter-inhibitor peptides and
4 carrier peptides.

Signal peptides

Signal peptides stimulate matrix protein production in general and collagen synthesis in specific. They may be accomplished by stimulation and growth of different skin cells like human skin fibroblasts. Signal peptides can also increase elastin, proteoglycan, glycosaminoglycans and fibronectin proliferation. By increasing matrix cell activities and consequently collagen production, the skin looks firmer and younger.

Table I Permeation coefficients (K_p) of available relevant peptides

Peptide	Permeation coefficient (cm s^{-1})	Peptide	Permeation coefficient (cm s^{-1})
GHK*[1]	1.36×10^{-9}	Serine†[2]	1.00×10^{-8}, pH = 5.6
GHK-Cu*[1]	1.35×10^{-9}		8.33×10^{-9}, pH = 7.4
GSH*[1]	8.63×10^{-10}	Threonine†[2]	3.27×10^{-8}, pH = 6.2
GSH-Cu*[1]	1.5×10^{-9}		3.61×10^{-9}, pH = 7.4
Histidine†[2]	4.44×10^{-9}, pH = 7.6	Isoleucine†[2]	1.44×10^{-8}, pH = 6.0
	5.55×10^{-9}, pH = 7.4		3.61×10^{-9}, pH = 7.4
Histidine-Cu*[3]	$2.72 \times 10^{-6} \pm 0.05 \times 10^{-6}$	Leucine†[2]	4.44×10^{-9}, pH = 6.0
Alanine†[2]	1.03×10^{-8}, pH = 6.0		8.05×10^{-9}, pH = 7.4
	1.53×10^{-8}, pH = 7.4	Asparagine†[2]	1.14×10^{-8}, pH = 5.4
Alanine-Cu*[3]	$1.90 \times 10^{-6} \pm 0.16 \times 10^{-6}$		9.72×10^{-9}, pH = 7.4
Lysine†[2]	1.08×10^{-7}, pH = 9.8	Asparatic acid†[2]	2.38×10^{-8}, pH = 2.8
	5.83×10^{-9}, pH = 7.4		2.22×10^{-9}, pH = 7.4
Lysine-Cu*[3]	$1.66 \times 10^{-6} \pm 0.07 \times 10^{-6}$	Glutamine†[2]	8.88×10^{-9}, pH = 5.6
Glycine†[2]	3.30×10^{-8}, pH = 6.0		1.39×10^{-8}, pH = 7.4
	1.05×10^{-8}, pH = 7.4	Glutamic acid†[2]	1.36×10^{-8}, pH = 7.4
Glycine-Cu*[3]	$1.62 \times 10^{-6} \pm 0.06 \times 10^{-6}$		2.78×10^{-9}, pH = 7.4
Valine†[2]	1.25×10^{-8}, pH = 6.0	Phenylalanine†[2]	6.78×10^{-8}, pH = 5.4
	3.61×10^{-9}, pH = 7.4		8.33×10^{-9}, pH = 7.4
Valine-Cu*[3]	$1.59 \times 10^{-6} \pm 0.07 \times 10^{-6}$	Arginine†[2]	9.05×10^{-8}, pH = 10.8
α-MSH (hisetal)†[4]	1.55×10^{-8}		2.77×10^{-8}, pH = 7.4
α-MSH (hisetal)‡[5]	2.58×10^{-9}	Tyrosine†[2]	7.22×10^{-9}, pH = 5.6
Methionine†[2]	4.72×10^{-9}, pH = 5.6		4.44×10^{-9}, pH = 7.4
	8.61×10^{-9}, pH = 7.4	Tryptophan†[2]	5.28×10^{-9}, pH = 5.7
Proline†[2]	9.16×10^{-9}, pH = 6.3		4.17×10^{-9}, pH = 7.4
	7.50×10^{-9}, pH = 7.4	Cysteine†[2]	9.44×10^{-9}, pH = 5.2
			5.28×10^{-9}, pH = 7.4

GHK, glycyl-histidyl-lysine; GSH, γ-L-glutamyl-L-cysteinyl-glycine; MSH, Melanocyte stimulating hormone.
*Liposome model membranes were used.
†Hairless mouse skins were used.
‡Human skin was used.

Table II General characteristics of peptides and proteins

Peptides and proteins	Alternate/generic names	Source	Peptide type	Mechanism of action	Cosmeceutical applications
Copper tripeptide complex	Copper tripeptide-1 or GHK-Cu or lamin®	Synthetic	Signal and carrier peptide	Promotes 'extra large' collagen aggregates degradation, more regularly collagen synthesis, elastin, proteoglycans, glycosaminoglycans production and anti-inflammatory and antioxidant responses	Anti-ageing, anti-wrinkle, After-sun products, after skin resurfacing, skin moisturizer, hair growth stimulator
Biopeptide-CL	Pal-GHK	Synthetic	Signal peptide	Stimulates collagen and gylcosaminoglycans synthesis	Anti-ageing, anti-wrinkle, antisolar, firming, skin moisturizer
Syn®-Coll	Palmitoyl tripeptide-3/5	Synthetic	Signal peptide	Mimics thrombospondin I tripeptide sequence and promotes collagen formation	Improves stretch marks, anti-wrinkle, skin moisturizer, improves skin's firmness and tone
Peptamide-6	FVAPFP or phe-val-ala-pro-phe-pro	Biotechnological (*Saccharomyces* yeast fermentation)	Signal peptide	Increases collagen synthesis, upregulates growth factors, transmembrane, matrix and cell shock proteins	Firming peptide ideal for all face/body/eye creams, anti-ageing
Acetyl tetrapeptide-9	AcTP1	Synthetic	Signal peptide	Stimulates collagen I and lumican synthesis	Anti-ageing, Anti-wrinkle, firming peptide
Acetyl tetrapeptide-11	AcTP2	Synthetic	Signal peptide	Stimulates keratinocyte cell growth and syndecan-1 synthesis	Anti-ageing, Anti-wrinkle, firming peptide
Acetyl hexapeptide-3	Argireline® or acetyl hexapeptide-8	Synthetic	Neurotransmitter inhibitor peptide	Inhibits SNARE complex formation and catecholamine release	Anti-wrinkle especially periorbital, skin moisturizer, improves skin's firmness and tone
Pentapeptide-18	Leuphasyl®	Synthetic	Neurotransmitter inhibitor peptide	Mimics the natural mechanism of enkephalins and inhibits neuronal activity and catecholamine release	Anti-wrinkle (periorbital), skin moisturizer, improves skin's firmness and tone
Pentapeptide-3	Vialox®	Synthetic	Neurotransmitter inhibitor peptide	Competitive antagonist at the acetylcholine receptors	Alternative to Botox®, anti-wrinkle (against expression wrinkles), anti-ageing
Pal-KTTKS	Palmitoyl pentapeptide-4 or palmitoyl pentapeptide-3 or palmitoyl oligopeptide or Matrixyl®	Synthetic (Pro-collagen I fragment)	Signal peptide	Stimulates collagen I, III and VI, fibronectin, elastin and glucosamnoglycans production	Anti-ageing, anti-wrinkle
Tripeptide-10 Citrulline	Decorin-like tetrapeptide (Decorinyl™)	Synthetic	Signal peptide	Regulates collagen fibrillogenesis and influences diameter and placement of collagen fibres	Anti-ageing, firming agent
Human growth hormone	hGH	Biotechnological (recombinant)	Signal peptide	Increased IGF-1 production, fibroblast and keratinocyte activity, and sebum production	Anti-ageing, anti-wrinkle, after skin resurfacing

Table II Continued

Peptides and proteins	Alternate/generic names	Source	Peptide type	Mechanism of action	Cosmeceutical applications
Transforming growth factors	TGF-α and TGF-β	Biotechnological (recombinant)	Signal peptide	Reversibly inhibits keratinocytes and leucocytes growth, promotes keratinocyte migration, chemotactic for marcrophages and fibroblasts	Anti-photoageing, anti-wrinkle, post-laser uses
Interferon Alpha	IFN-α	Biotechnological (recombinant)	Signal peptide	Increases the concentration of dendritic cells and CD1a and HLA-DR-positive cells	Anti-ageing, anti-wrinkle
Heat shock protein (70)	Hsp70	Biotechnological (recombinant)	Signal peptide	Protects the cells against apoptosis, ageing and UV damage	Anti-ageing, anti-wrinkle
Syn®-Ake	Tripeptide-3 or dipeptide diaminobutyroyl benzylamide diacetate	Synthetic	Neurotransmitter inhibitor peptide	Mimics the effect of *Waglerin 1*, a peptide that is found in the venom of the Temple Viper, *Tropidolaemus wagleri*	Anti-ageing, intensive anti-wrinkles
Soybean protein/amino acids	Glycine Soja Protein or Preregen®	Natural (Soybean seed)	Enzyme inhibitor peptide	Inhibits the formation of proteinases, increases trichoblast and atrichoblast numbers, increases the number and length of the root hairs	Anti-ageing, skin moisturizer, used in cleansing detergents, sensitive skin care, anti-solar, regenerating effect. Hair-promoting agent
Keratin proteins/amino acids	Keramino 25®	Natural (human hair and sheep's wool)	Structural peptide	Improves hydration and elasticity of the skin and hair	Skin and hair moisturizer, firming agent, hair shiner
Decorinyl™	Tipeptide-10 Citrulline	Synthetic	Signal peptide	Mimics the sequences of decorinand regulates fibrillogenesis and control fibril growth and their uniformity	Anti-wrinkle, increases skin suppleness and tone
Silk protein	Sericin	Natural (moddle silk gland of the silkworm *Bombyx Mori*)	Antioxidant, enzyme inhibitor protein, copper chelator protein	Chelates with copper, inhibits lipid peroxidation and tyrosinase activity and keratinocyte apoptosis	Anti-ageing, anti-wrinkle, skin moisturizer
Aquaporin	AQP	Natural (extracted from *Ajuga turkestanica*)	Signal protein	Increases epidermal proliferation and differentiation. Makes stratum corneum thicker	Anti-ageing, anti-wrinkle, skin moisturizer

GHK, glycyl-histidyl-lysine; IGF, insulin growth factor; SNARE, soluble *N*-ethylmaleimide-sensitive factor attachment protein receptor.

Table III Efficacy data

Peptides	Study ID	Indication of topical use	Study design	Characteristics of subjects	Treatment arm(s)	Treatment protocol	Efficacy
Biotinyl-GHK	Lintner K [38]	Skin conditioning (anti-ageing)	Controlled *ex vivo* study	*Ex vivo* cell culture model containing dermal and epidermal cells	Biotinyl-GHK vs. control group	The solutions were applied to the cell culture	Biotinyl-GHK but not control solution stimulates collagen IV and laminin production and keratinocyte mitosis
GHK-Cu	Appa *et al.* [85]	Skin conditioning (anti-ageing)	Non-R, AC	Volunteer females	GHK-Cu containing liquid foundation and GHK-Cu containing cream concealer	Formulations were applied for 8 weeks	Sig. improvements in all evaluations were found for both products
	Leyden *et al.* [86]	Anti-ageing	DB, PC	71 Female volunteers with mild to advanced photodamage	GHK-Cu vs. placebo creams	The creams were applied on the faces b.i.d. for 12 weeks	Sig. improvements for GHK-Cu than placebo for all measurements by week 4
	Leyden *et al.* [88]	Anti-ageing (periorbital)	DB, PC	41 Female volunteers with mild to advanced photo-damage	GHK-Cu vs. Vitamin K creams	The creams were applied around the eyes b.i.d. for 12 weeks	Sig. improvements for GHK-Cu than placebo for all measurements by week 4
	Finkey *et al.* [87]	Anti-ageing	R, DB, PG, PC	67 Female volunteers aged 50–59 with mild to advanced photodamage	GHK-Cu vs. placebo creams	Creams were applied on the face b.i.d. for 12 weeks	GHK-Cu improved skin laxity, clarity and appearance, reduced wrinkles and increased density and thickness
	Finkey *et al.* [87]	Anti-ageing	Non-R, UC	5 Female volunteers aged 50–59 with mild-advanced photodamage	GHK-Cu vs. no treatment	The cream was applied on face b.i.d. for 12 weeks	GHK-Cu stimulated dermal keratinocyte proliferation
	Abdulghani *et al.* [21]	Anti-ageing	Non-R, AC, PG, WP	20 Healthy volunteers	Topical tretinoin, topical Vitamin C, Topical GHK-Cu, topical melatonin	20 Subjects received creams to the extensor surface of thighs for 1 month	In terms of increase of pro-collagen synthesis, 4/10, 5/10, 5/10 and 7/10 of patients showed response to tretinoin, Vitamin C, melatonin, and GHK-Cu respectively
Tripeptide-10	Puig *et al.* [40]	Anti-ageing	AB, PC, PG	43 Female volunteers aged 40–58	0.01% Liposomal tripeptide-10 citrulline cream vs. placebo cream	The creams were applied on the face (temple) daily for 28 days	Tripeptide-10 induced a sig. increase in skin suppleness. No sig. increase in placebo group
Leuphasyl® vs. Argireline®	Centerchem fact sheet	Anti-ageing	AC, PG	43 Healthy female volunteers aged 39–64	Cream containing 5% Leuphasyl® solution (0.05%) vs. cream containing 5% Argireline® solution (0.05%) vs. combination	Each cream was applied b.i.d. around the eyes of 14 volunteers for 28 days	Mean wrinkle reduction was 11.64% vs. 16.26% vs. 24.62% for Leuphasyl®, Argireline® and combination, respectively

Table III Continued

Peptides	Study ID	Indication of topical use	Study design	Characteristics of subjects	Treatment arm(s)	Treatment protocol	Efficacy
lipopentapeptide	Watson *et al.* [54]	Anti-ageing	R, AC	Nine healthy photoaged volunteers (2 men 7 women; aged 42–79)	6% vs. 2% total active complex cream (lipopentapeptide, white lupin peptide, antioxidants) vs. Retin-A	Substances were patch tested separately to the extensor aspect of forearm on days 1, 4 and 8. Patch tests were re moved on day 12.	6% Formula significantly increased fibrillin-1 and procollagen I deposition. Retin-A and 6% complex was the best triggers for fibrillin-1 and procollagen I deposition respectively
Peptamide®6	Arch personal care products technical sheet	Anti-ageing (periorbital)	PC, WP	25 healthy volunteers	2.80% Peptamide® 6 firming toner vs. control toner	Each cream was applied to half-face (periorbital and cheek) b.i.d. for 4 weeks	Initial skin elasticity and deformation response were improved at week 4
Pal-KTTKS	Robinson *et al.* [20]	Anti-ageing	R, DB, PC, WP	93 Caucasian female volunteers aged 35–55	Pal-KTTKS O/W moisturizer vs. placebo oil-in-water moisturizer	Each formulation was applied to the half-face skin b.i.d. for 12 weeks	Sig. better scores for expert grader assessment and subject self-assessment in age spots
	Osborne *et al.* [22]	Anti-ageing	R, DB, AC, PC, WP	180 female volunteers aged 35–65	Pal-KTTKS facial moisturizer vs. *Boswellia Serrata* extract vs. moisturizer base (vehicle)	Each formulation was applied to the randomly selected half-face skin b.i.d. for 8 weeks	Pal-KTTKS made sig. reduction in bumpy texture and fine lines/ wrinkles compared with other comparators and baseline
Acetyl tetrapeptide-9 (AcTP1)	Pauly *et al.* [42]	Anti-ageing	PC	17 healthy female volunteers aged 45–55 with loss of elasticity on the forearms	3% Cream containing AcTP1 vs. Cream containing placebo	The creams were applied b.i.d. for 112 days	Sig. increase in skin thickness and firmness for active cream. AcTP1 was more effective than placebo too
Acetyl tetrapeptide-11 (AcTP2)	Blanes-Mira *et al.* [23]	Anti-ageing	PC	19 healthy female volunteers aged 60–70 with loss of elasticity on the forearms	3% Cream containing AcTP2 vs. Cream containing placebo	The creams were applied b.i.d. for 112 days	Sig. increase in biomechanical parameters of the superficial layers of epidermis was observed for active cream. AcTP2 had 5–10% better effect than placebo
Acetyl hexapeptide-3 (Argireline®)	Blanes-Mira *et al.* [23]	Anti-ageing	PC, OL	10 healthy women volunteers	O/W emulsion containing 10% Argireline® solution	Solution was applied b.i.d. around the eyes during 30 days	Sig. more reduction in the depth of wrinkles for Argireline® group

Table III Continued

Peptides	Study ID	Indication of topical use	Study design	Characteristics of subjects	Treatment arm(s)	Treatment protocol	Efficacy
Syn®- Ake vs. Argireline®	Pentapharm fact sheet	Anti-ageing	PC	45 Healthy volunteers	4% Syn®-Ake vs. 10% acetyl hexapeptide-3 (Argireline®) vs. placebo	Each cream was applied to the skin of forehead b.i.d. for 28 days	Before-after measurements were sig. for Syn®-Ake only
Syn®-Coll	Pentapharm fact sheet	Anti-ageing	AC, PC	60 Healthy volunteers	2.5% Syn®-Coll vs. 10% Argireline® vs. placebo creams	Creams were applied to facial skin b.i.d. for 84 days	Syn®-Coll showed better sig. results for all parameters than the controls
Fibronectin-like peptide	dal Farra *et al.* [71]	Anti-ageing	R, DB, PC, PG	24 Healthy volunteers	1% Fibronectin-like peptide vs. placebo creams	Creams were applied to lips once and evaluations occurred 1 and 3 h after	Sig. improve in hydration, and smoothness in active group
	dal Farra *et al.* [71]	Anti-ageing	R, DB, PC, WP	12 Healthy volunteers	1% Fibronectin-like peptide vs. placebo creams	Each cream was applied to back of hands b.i.d. for 7 days	Active cream increased smoothness and lightening effects, and skin appearance
Soy extract	Südel *et al.* [72]	Anti-ageing	R, DB, PC, WP	21 Healthy females (55 ± 6 years) with skin types of II and III	2% Soy extract vs. placebo creams	Each cream was applied to volar forearm b.i.d. for 2 weeks	Papillae Index was more in creased by soy extract than placebo
	Andre-frei *et al.* [73]	Anti-ageing	Pseudo-R, PB, PC, WP	10 Healthy Caucasian females aged between 42 and 67	2% Soya biopeptide vs. placebo emulsions	Control emulsion was applied to left side of the face and soya emulsion to right side b.i.d. for 4 weeks	Collagen and glycosaminoglycan contents were significantly stimulated by soya extract
Silk protein vs. BSA	Zhaorigetu *et al.* [78]	Anti-ageing and anti-tumour	R, AC, PC	30 Four-week-old female Hos: HR-1 UVB-exposed hairless mice (three groups of five mice)	Single doses of 5 mg silk protein in 0.2 mL ethanol vs. 5 mg BSA in 0.2 mL ethanol vs. 0.2 mL ethanol	Each treatment group received its solution after single application of 180 mJ cm^{-2} UVB treatment	Silk protein significantly inhibited skin lesion formation and elevated expression of COX-2 protein more than BSA and vehicle
	Zhaorigetu *et al.* [78]	Anti-ageing and anti-tumour	R, AC, PC	30 Four-week-old female Hos: HR-1 UVB-exposed hairless mice (three groups of five mice)	5 mg Silk protein in 0.2 mL ethanol vs. 5 mg BSA in 0.2 mL ethanol vs. 0.2 mL ethanol	Each treatment group received its solution after 180 mJ cm^{-2} of UVB treatment daily for 7 days	Silk protein significantly inhibited skin lesion formation and elevated expression of COX-2 protein more than BSA and vehicle
Silk protein	Padamwar *et al.* [75]	Skin moisturizer	UC, WP	Six healthy human volunteers of both sexes (aged 22–25)	0.2 g sericin gel vs. no treatment	Sericin vs. control gel was applied on the skin of forearm	No sig. difference in all measurements

Table III Continued

Peptides	Study ID	Indication of topical use	Study design	Characteristics of subjects	Treatment arm(s)	Treatment protocol	Efficacy
	Daithankar *et al.* [76]	Skin moisturizer	AC, UC, WP	Six healthy volunteers	1% Fibrion vs. 3% fibroin vs. 5% fibroin vs. 5% silk-Pro-100 solutions	1 mL of each solution was applied to inner upper portion of the forearm for 15 min and lower portion was left untreated as control	For TEWL, 5% fibroin solution, Silk-pro-100 >1% and 3% fibroin solutions. Sig. drop in impedance observed for both 5% solutions
Keratin peptide	Barba *et al.* [70]	Skin moisturizer	R, AC, PC, WP	Six healthy Caucasian female volunteers phototype III–IV, aged 24–36	Keratin peptide aqueous solution vs. keratin peptide liposome solution vs. IWL liposomes vs. water vs. 0.9% NaCl solution	Each cream were applied onto marked areas of 9 cm^2 once a day for 4 days	Sig. differences of skin capacitance and elasticity parameters in keratin samples. IWL liposomal keratin showed a sig. beneficial effect
			R, PC, UC, WP	16 Healthy female volunteers aged 24–50 with skin types of III to V	3% Keratin peptide vs. 3% deionized water in base cream vs. untreatment	Each cream was applied to a 9 cm^2 area of hand once a day for 12 days.	Insignificant difference between topical therapies; elasticity results were significantly better for keratin
	Barba *et al.* [69]	Skin moisturizer	R, PC, UC, WP	9 Healthy females aged 24–50 with dry skin types of III to V	3% Keratin peptide vs. 3% deionized water in cream vs. untreatment	The treated areas were exposed to 2% sodium lauryl sulphate after 12-day daily application of creams on the hand	Significantly lower results for skin capacitance and TEWL in keratin group.
Aquaporins (protein)	Dumas *et al.* [41]	Skin moisturizer	UC, WP	15 Healthy female volunteers aged 22–56	*Ajuga turkestanica* extract formulated in a complex oil–water emulsion	The emulsion was applied to forearm skin b.i.d. for 21 days	Sig. TEWL decrease in treated site vs. untreated site (4.8 ± 0.4 vs. 5.4 ± 0.3)
CRS	Ehrlich *et al.* [52]	Anti-ageing	R, PC, WP, AB	12 Females with facial wrinkles (aged 42–74)	CRS cream vs. same cream without TGF-β1 component (Vitamin C base)	Each cream was applied b.i.d. for 3 months	Sig. improvement in wrinkle scores for CRS and non-sig. for Vitamin C
CRS vs. TNS	Ehrlich *et al.* [52]	Anti-ageing	R, AC, WP, AB	20 Healthy females with facial wrinkles (aged 29–74)	CRS cream vs. TNS cream	Each cream was applied b.i.d. for 3 months	Sig. improvement in wrinkle score for CRS and non-sig. for Vitamin C
PSP	Gold *et al.* [53]	Anti-ageing	R, DB, PC, WP	20 Females with demonstrable facial wrinkles (aged 35–65)	PSP cream vs. its physically identical placebo cream	Each cream was applied to the half-face skin b.i.d. for 2 months	Roughness parameters were significantly better in PSP group. No difference between the two groups

Table III Continued

Peptides	Study ID	Indication of topical use	Study design	Characteristics of subjects	Treatment arm(s)	Treatment protocol	Efficacy
Alpha -interferon	Ghersetich and Lotti [48]	Anti-ageing	BA with three inclusion protocols	15 volunteers who experienced a periauricular area surgery (5 aged 18–21, 5 aged 57–75, and 5 under went cycles of PUVA therapy over a year and aged 30–45)	Alpha-interferon cream (2 000 000 IU per day) in carboxymethylcellulose and glycerin	The cream was applied on periauricular area three times a day for 4 weeks	Expressed CD-1 and HLA-DR cell counts were sig. compared with baseline for aged and PUVA-exposed volunteers.
Hsp70	Cucumel *et al.* [57]	Anti-ageing	BA with two inclusion protocols	10 healthy volunteers aged 50–70, 5 healthy volunteers aged 31–40	Artemia extract	Artemia extract was applied to half of subjects before UV exposure	Sig. effect of Artemia extract on Hsp70 expression of aged skin

AB, assessor-blind; AC, active-controlled; b.i.d., twice daily; BA, before-after study; BSA, bovine serum albumin; COX-2, cyclooxygenase 2; CRS, cell rejuvenation serum; DB, double-blind; IWL, internal wool lipid; OL, open-label; O/W, oil-in-water; PB, patient-blind; PG, parallel-group; PC, placebo-controlled; PSP, processed skin cell proteins; PUVA, Psoralen + UVA; Sig., significant; R, randomized; TEWL, transepidermal water loss; TNS, tissue nutrient solution recovery complex; UC, untreated-controlled; UV, ultraviolet; WP, within-patient; HR, xxxxxx; TGF, transforming growth factor; GHK, glycyl-histidyl-lysine.

The tripeptide-1 (glycyl-L-histidyl-L-lysine or GHK) is primarily known as carrier peptides. It mainly helps to stabilize and deliver Cu. Carrier peptides are discussed later. However, GHK was originally isolated from human plasma in 1973 by Pickart and Thaler [31] and its wound repair properties were observed in 1985 by Maquart *et al.* [32] In 1999, Maquart *et al.* concluded that GHK or its Cu complex functioned as an activator of tissue remodelling [32]. It is also a signal peptide that promotes extra large collagen aggregates degradation in scars, regular collagen synthesis in normal skin, elastin, proteoglycans and glycosaminoglycans production, growth rate and migration of different cell types and anti-inflammatory and antioxidant responses [33–37]. In a controlled *ex vivo* study [38], biotinyl-GHK and vehicle were investigated. Biotinyl-GHK but not vehicle solution showed stimulation of collagen IV, laminin production and keratinocyte mitosis.

Tripeptide-1 can be also conjugated with palmitic acid and form pal-tripeptide-1 (Biopeptide-CL). *In vitro* and *in vivo* studies approved that Biopeptide-CL stimulates collagen and gylcosaminoglycans synthesis [39].

Palmitoyl tripeptide-3/5 (Syn®-Coll; Pentapharm, Basel, Switzerland) is a synthetic signal peptide. Thrombospondin I is a protein that binds to tissue growth factor beta (TGF-β) and makes it biologically inactive. Syn®-Coll mimics thrombospondin I tripeptide sequence to activate TGF-β. Therefore, it promotes collagen formation via TGF-β (Pentapharm). In a controlled trial, 60 healthy volunteers received 2.5% Syn®-Coll cream vs. 10% palmitoyl pentapeptide-3 cream vs. placebo cream twice daily for 84 days. Syn®-Coll significantly decreased average and maximum relief by −22 and −36 μm respectively; when compared with pal-pentapeptide-3, it showed better significant results for Ra, Rz and Rt parameters (Pentapharm).

Tripeptide-10 Citrulline (Decorinyl™; Centerchem) is a peptide with firming effects and mimics the sequences of decorin that binds to collagen fibrils. It also regulates fibrillogenesis and controls fibril growth and their uniformity (Centerchem). Puig *et al.* [40] presented a single-blind parallel group controlled trial comparing 0.01% liposomal Decorinyl™ and placebo creams. Tripeptide-10 induced a 54% increase in skin suppleness ($P < 0.001$). No significant changes were seen in placebo group.

Peptamide-6 (FVAPFP), a firming peptide that is biotechnologically derived from saccharomyces

yeast fermentation, increases collagen synthesis and upregulates growth factors, transmembrane, matrix and heat shock proteins. This peptide was applied onto half-face (periorbital and cheek) of 25 healthy subjects twice daily for 4 weeks. Initial skin elasticity and deformation response were improved at week 4.

Pal-KTTKS (palmitoyl pentapeptide-4), a synthetic signal peptide from pro-collagen I fragment, stimulates collagen I, III and VI and also fibronectin, elastin and glycosaminoglycan production [38] and has been frequently used as topical anti-ageing or anti-wrinkle agents. In a study [20] on 93 Caucasian female volunteers, Pal-KTTKS had significantly better scores than placebo for expert grader assessment and subject self-assessment of age hyperpigmented spots. Osborne *et al.* [22] showed a robust result for this peptide in reducing bumpy texture and fine wrinkles compared with other baseline and comparators.

Aquaporin is an epidermal water channel peptide that is upregulated by the extract of *Ajuga turkestanica*. One study [41] showed a significant transepidermal water loss (TEWL) decrease in aquaporin-treated forearms vs. untreated forearms (4.8 ± 0.4 g m^{-2} h^{-1} vs. 5.4 ± 0.3 g m^{-2} h^{-1}).

Pauly *et al.* [42] evaluated two new synthetic peptides: acetyl tetrapeptide-9 and -11 ((AcTP1, AcTP2, respectively). *In vitro* study revealed an increase in collagen I and lumican synthesis for AcTP1 and stimulation of keratinocyte cell growth and syndecan-1 synthesis for AcTP2. *In vivo* study showed significant increase in skin thickness (5.0%) and skin firmness (Ur/Uf = 7.5%) for AcTP1 cream. AcTP1 was also more effective than placebo. AcTP2 had significant effect on biomechanical parameters of the superficial layers of epidermis and 5–10% better effect than placebo [42].

Growth factors play an important role in reversing the ageing process on skin caused by extrinsic and intrinsic factors, although main use of growth factors is in wound healing. Recombinant human growth hormone has mitogenic effect on keratinocytes and fibroblasts [43] and increases insulin growth factor-1 and sebum production [44]. Cutaneous wound healing properties were confirmed by two trials on acute wounds [45, 46].

Interferon alpha increases the concentration of dendritic cells and CD1a and HLA-DR positive cells [47, 48]. Ghersetich and Lotti [48] conducted a before-after study with three different inclusion protocols: five individuals aged 18–21 years, five aged 57–75 years, and five underwent cycles of Psoralen + UVA (PUVA) therapy over a year and aged 30–45 years. Alpha-interferon cream (2 000 000 IU per day) in carboxymethylcellulose and glycerin was applied on periauricular area three times a day for 4 weeks. Counts for cells that expressed CD-1 and HLA-DR were significant compared with baseline only for aged and PUVA-exposed volunteers (5 ± 1.75 vs. 10 ± 4.47 and 6 ± 3.18 vs. 16 ± 2.15 for aged group and 4 ± 3.47 vs. 10 ± 3.53 and 3 ± 3.12 vs. 14 ± 1.75 for PUVA group respectively).

Transforming growth factor α and β are growth factors that reversibly inhibit keratinocytes and leucocytes growth, promote keratinocyte migration, chemotactic for marcrophages and fibroblasts [49–51]. Among major growth factors, transforming growth factor (TGF) α has the highest human keratinocyte pro-motility activity, reaching nearly 80% of the activity in serum [50].

Several growth factors and cytokines have been applied to treat ageing skin problems. Cell rejuvenation serum (CRS) contains liposome-encapsulated TGF-β1, ascorbic acid and *Cimicifuga racemosa extract* in a silicone base. In a trial [52], topical CRS was compared with placebo without TGF-β1 component to define the additive effect of TGF-β. TGF-β1 containing arm had 21.7% significant mean improvement in physician-rated wrinkle score and the other arm had 6.2% improvement ($P > 0.05$) compared with baseline. This trial [52] continued to compare topical CRS to another cream named tissue nutrient solution (TNS) in 20 patients. TNS contains growth factors and cytokines including vascular endothelial growth factor, platelet-derived growth factor alpha polypeptide (PDGF-A), granulocyte-colony stimulating factor, hepatyte growth factor (HGF), interleukin (IL)-6, IL-8 and TGF-β1 without Vitamin C. The results revealed that both creams produced significant improvement in wrinkle score.

Bio-restorative skin cream contains processed skin-cell proteins (PSP), a proprietary growth factor and cytokine mixture extracted from cultured first trimester foetal human dermal fibroblasts in a moisturizing cream. In a randomized placebo-controlled trial, Gold *et al.* [53] concluded that some skin roughness parameters were significantly improved in PSP group compared with the baseline but no statistical difference between the two groups was detected.

Lipopentapeptide, in combination with white lupin peptide and antioxidants had significant

effect on increasing fibrillin-1 and procollagen I deposition in its 6% concentration. It was also the best trigger for procollagen I deposition when compared with 2% concentration, Retin-A and untreated areas [54].

Heat shock proteins involve in one of the principal mechanisms of cell defence and protection from stress. Among its family, Hsp70 has protective effects against UV, apoptosis and ischaemia and recommended for wound healing and anti-ageing uses [55]. Hsp70 can effectively inhibit aggregation and assist in the refolding of denatured proteins. It can reduce cellular damage by retaining the damaged proteins in soluble form, as well as by binding to unfolded or misfolded proteins to assist in their proper refolding [55]. Hsp70 can be biotechnologically synthesized from yeasts [56]. Studies on both cultured human epidermal cells and ex vivo skin showed that induction or administration of Hsp70 prior to stress significantly diminished UV-related morphological changes and sunburn cell number [56–58]. It can also modulate inflammatory cytokine synthesis and reduce UV-induced inflammatory responses [59]. Hsp70 was proved to be able to block apoptosis by inhibiting signalling events upstream of stress-activated protein kinase (SAPK)/c-*Jun* N-terminal kinase. activation [60, 61]. Ageing alters the ability of cells to express Hsp70 in response to stress and any Hsp70 induction or application can reverse the process [61–67].

Generally, studies [62–68] showed that although aged skin exhibits a normal level of Hsp70 under non-stressful conditions, it fails to produce the typical protective Hsp70 increase, comparing with younger skin when exposed by UV. To sum up, Hsp70 can act against UV exposure especially in aged skin.

Keratin is a major protein in the structure of hair and skin that can be extracted from human hair or sheep's wool. Keratin's topical application can improve hydration and elasticity of the skin and hair. It is commonly used in skin and hair moisturizers, firming agents and hair shiners. Barba *et al.* [69] conducted a randomized trial comparing 3% keratin peptides with deionized water and untreated control in 16 healthy females. Keratin peptides were effective on disturbed but not undisturbed skin. In another recent trial [70], significant differences were achieved for skin capacitance (especially) and elasticity parameters with application of the keratin samples. Among all keratin containing creams, a combination of keratin peptide with the internal wool lipid liposomes had a significant beneficial effect compared with aqueous solution [70].

In two consecutive double-blind studies, dal Farra *et al.* [71] investigated the potential anti-ageing effects of a synthetic fibronectine-like peptide at 0.5% together with a booster molecule at 1%. Twelve volunteers applied the cream formula containing the active ingredient twice a day on the back of one hand and the placebo on the other hand. Evaluations were performed after 1 and 3 h, and after 7 days. Increased smoothness of skin surface and also lightening effect on the skin were noticed by volunteers at all time points. Volunteers estimated a 40% improvement on the peptide-treated side 1 h after application. A second study was performed on the lips and included two groups of 12 volunteers each. One group applied the cream formula with the active ingredient, and the other group applied the placebo. Evaluations were made at 1 and 3 h. Significant increase in smoothness, hydration and repulping effect were mentioned.

Enzyme inhibitor peptides

Enzyme inhibitor peptides directly or indirectly inhibit an enzyme. Soybean protein (Soja protein) or peptides, enzyme inhibitor peptides naturally extracted from soybean seeds, inhibit the formation of proteinases (Centerchem). Soy protein is frequently used as anti-ageing, skin moisturizer, anti-solar, cleansing detergent and hair-promoting agent. In a randomized, double-blind, placebo-controlled study [72], soy extract and placebo creams were applied to volar forearm of 21 healthy women. Papillae index was increased more by soy extract than placebo (3.76 vs. 4.56 in arbitrary units, $P < 0.05$). Another study with pseudo-randomized design in 10 Caucasian females [73], concluded the superiority of 2% soya biopeptide emulsion to placebo, in terms of collagen and stimulation of glycosaminoglycan contents.

Rice peptides/amino acids (Colhibin®) is another natural protein that inhibits matrix metalloproteinase activity and induces expression of hyaluronan synthase 2 gene in keratinocytes. Anti-ageing, film-forming and hair conditioning products may contain this protein [74].

Another enzyme inhibitor protein (silk protein, sericin), naturally extracted from Moddle silk gland of the silkworm *Bombyx Mori,* has antioxidant

properties with high affinity to chelate with Cu. In addition, it inhibits lipid peroxidation and tyrosinase activity and keratinocyte apoptosis. In a within-patient untreated-controlled study [75], 0.2 g sericin gel was compared with untreated site with hydroxyproline assay and TEWL measurement to evaluate its hydrating effect. For hydroxyproline assay, Sericin gel was applied on the dried skin of the forearm at the test site. For TEWL, the upper portion of forearm was used as the application site of sericin gel and lower portion of forearm for control. Although hydroxyproline content was slightly promising in all related parameters for sericin, no significant differences in hydroxyproline content, skin impedance and TEWL contents were seen when compared with no treatment. In another trial by Daithankar *et al.* [76], silk-protein 100 and different fibroin concentrations were tested. Five per cent firoin solution had similar TEWL content to 5% silk-protein-100 solution but more than 1% and 3% fibroin solutions. Significant drop in impedance was observed for both 5% sericin and 5% fibroin solutions within 1 h. Silk protein was compared with bovin serum albumin and vehicle and the results confirmed its superiority to both serum albumin and vehicle in reducing UV-B induced symptoms in short-term and long-term treatment courses [77].

Neurotransmitter inhibitor peptide

Neurotransmitter inhibitor peptides inhibit acetylcholine release at the neuromuscular junction and have curare-like effect. Seven types (A–G) of botulinum toxin target peripheral cholinergic neurons where they selectively proteolyse synaptosome-associated protein of 25 000 Da (SNAP-25), syntaxin 1 and synaptobrevin, the soluble N-ethylmaleimide-sensitive factor attachment protein receptor (SNARE) proteins responsible for transmitter release, to cause neuromuscular paralysis but of different durations. Type A toxin proteolytically degrades the SNAP-25 protein, a type of SNARE protein. The SNAP-25 protein is required for the release of neurotransmitters from the axon endings. Botulinum toxin specifically cleaves these SNAREs, and so prevents neurosecretory vesicles from docking and or fusing with the nerve synapse plasma membrane and releasing their neurotransmitters. botulinum toxin type A (BXT-A) paralysis lasts longer (4–6 months) among botulinum toxin subtypes, make it a good choice for anti-wrinkle uses.

Researchers have found less invasive topical equivalents of these toxins [78]. Acetyl hexapeptide-3 (Argireline®) is a synthetic peptide that is especially marketed as a component of eye care products and patterned from the N-terminal end of the protein SNAP-25 that inhibits SNARE complex formation and catecholamine release. Inhibition of noradrenaline and adrenaline release was also demonstrated. This small peptide exhibits the great advantage of its insignificant acute toxicity (2000 mg kg^{-1}) as compared with BTX-A (20 ng kg^{-1}) [23]. Argireline® (Centerchem) inhibits vesicle docking by preventing the ternary SNARE complex formation, which is involved in synaptic vesicle exocytosis [79, 80].

Another open label vehicle-controlled trial, 10% acetyl hexapeptide-3 and placebo creams were applied twice daily on 10 women and demonstrated a nearly 30% vs. 10% improvement in periorbital rhytids after 30 days as measured by silicone replica analysis respectively [23].

Pentapeptide-18 (Leuphasyl®) mimics the natural mechanism of enkephalins and as a result inhibits neuronal activity and catecholamine release. An active-controlled trial, compared cream containing 5% leuphasyl® solution (0.05%), cream containing 5% Argireline® solution (0.05%) and combination. Mean wrinkle reductions were 11.64% vs. 16.26% vs. 24.62% for Leuphasyl®, Argireline® and combination respectively. This study suggested a synergistic effect between Leuphasyl® and Argireline® (Centerchem).

Pentapeptide-3 (Vialox®), a synthetic peptide that is a competitive antagonist at the acetylcholine receptors, safely blocks the sodium ion release at the synaptic membrane on muscles so they cannot contract as frequently. *In vitro* tests showed muscle contractions reduced by 71% within 1 min after treatment and 58% 2 h later. Less frequent muscle contractions result in shallower lines. After 28 days of twice-daily use, wrinkle depth was reduced 49% (Centerchem).

Tripeptide-3 (Syn®-Ake) is used as an intensive anti-wrinkle agent and mimics the effect of Waglerin 1, a peptide that is found in the venom of the Temple Viper, *Tropidolaemus wagleri*. Syn®-Ake (at a concentration of 0.5 mmol L^{-1}) was able to reduce the frequency of innervated muscle cell contractions by 82% ($P < 0.05$) after 2 h of treatment.

In a study on 45 healthy subjects, Syn®-Ake, Argireline® and placebo were compared. Syn®-Ake

clearly showed a remarkable higher efficacy for all tested parameters. Before-after measurements were significant for Syn®-Ake only and not for Argireline®. Best results were seen on forehead skin by up to 52% (parameter Rt) (Lipotec, Barcelona, Spain). A high quality randomized controlled trial is needed to confirm these robust results for Syn®-Ake.

Carrier peptide

Carrier peptides belong to a general category that acts as a facilitator of an important substance' transportation, but their major application is to deliver important trace elements (like Cu and Mn) necessary for wound healing and enzymatic processes. Recently, several peptides and proteins have been developed to accelerate and facilitate the delivery of bioactive molecules into the skin. These peptides and proteins are known as penetrating peptides or membrane transduction peptides and have basic transduction domains in their structure [81]. A study [82], demonstrated that short arginine-rich intracellular delivery peptides facilitate the transport of various proteins into living cells. Hou *et al.* [82] also investigate whether arginine-rich peptide could serve as carriers for topical and/or transdermal drug delivery and concluded that protein penetration can be stimulated by such peptides even without fusion between carrier peptide and the protein. Another example of membrane transduction peptides is PEP-1 that can facilitate the skin penetration of an anti-ageing protein, ribosomal protein S3 (rpS3) [83, 84].

Abdulghani *et al.* [21] conducted a non-randomized four-arm active-controlled trial on 20 participants to compare GHK-Cu with topical tretinoin, Vitamin C and melatonin. Ten subjects received tretinoin and Vitamin C creams on the extensor surface of their right and left thighs respectively and the other ten subjects received GHK-Cu and melatonin creams to the extensor surface of their right and left thighs respectively, for 1 month. Tretinoin, Vitamin C, melatonin and GHK-Cu increased pro-collagen synthesis in 4/10, 5/10, 5/10 and 7/10 of patients respectively. Appa *et al.* [85] evaluated the efficacy of two cosmetic GHK-Cu containing formulations for skin conditioning. The skin treatment benefits of a GHK-Cu containing liquid foundation and cream concealer were evaluated over an 8-week period. Significant improvement in all visual evaluations of skin condition was found within first 2 weeks for both products. Skin viscoelastic properties significantly improved.

GHK-Cu was tested in a 12-week placebo-controlled study [86] on facial skin of 71 women with mild to advanced photodamage. By week 1, the active cream delivered significant improvement in skin laxity, clarity and overall appearance when compared with placebo. Significant improvement in fine lines was noted at week 2 and in wrinkles at week 4 over placebo. Significantly improved viscoelastic properties were consistent with ultrasound increase in overall skin density and thickness. Subjects indicated strong cream's performance acceptability. There were no adverse objective or subjective irritation findings.

In a randomized, double blind placebo-controlled study [87] that included 67 volunteers, GHK-Cu vs. placebo were applied twice daily for 12 weeks on facial skin. GHK-Cu Improved skin laxity, clarity and appearance, reduced fine lines, coarse wrinkles and mottled hyperpigmentation and increased skin density and thickness. Five included women also applied the cream to one forearm and leave the other forearm as untreated control. GHK-Cu Strongly induced dermal keratinocyte proliferation.

The efficacy and safety of GHK-Cu have been investigated in periorbital area of 41 female volunteers with mild to advanced photodamage. Within 4 weeks of this blind and controlled study with Vitamin K containing cream as the comparator group, there was significant improvement in all parameters, including fine lines, wrinkles and overall appearance of eyelids. The viscoelastic properties of periorbital skin – which was determined by ballistometer – exhibited statistically significant improvement by week 1. Increase in overall skin density and thickness was demonstrated with ultrasound and digital images captured noticeable improvement in appearance of periorbital skin [88].

Conclusions

Taken together, some peptides have notable effects on chronologically aged and/or photo-damaged skin. There is a large gap for permeability coefficient of major cosmeceutical peptides and proteins and researchers should focus on this ambiguity to find more efficient substances with better permeability. Although topical peptides are frequently

used in anti-ageing products, some do not have any well designed *in vivo* studies with adequate sample size. High quality randomized double-blind active-controlled large trials are needed to calculate exact effect sizes of main topical peptides or proteins to reach to these conclusions (i) whether they are effective enough to be approved, (ii) whether they can be utilized as an equivalent to current recommended treatments for ageing skin and (iii) at what doses they have maximum efficacy with acceptable safety profile.

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Appendix

Search strategy

Target of search	Search strategy
To locate all potentially used topical peptides for this indication	('peptide'/exp OR 'decapeptide' OR 'tripeptide'/exp OR 'octapeptide'/exp OR 'oligopeptide'/exp OR 'pentapeptide'/exp OR 'ghk' OR 'ghk cu' OR 'iamin' OR 'ghl' OR 'gsh'/exp OR 'gsh cu' OR 'neova' OR 'complex cu3' OR 'biopeptide' OR 'collagen pentapeptide' OR 'manganese tripeptide' OR 'egf' OR 'igf 1' OR 'fgf' OR 'growth factor'/exp OR 'thiorexidin' OR 'growth hormone'/exp OR 'hgh'/exp OR 'vegf'/exp OR 'kgf' OR 'tgf' OR 'ahk' OR 'argireline' OR 'hexapeptide'/exp OR 'pal kttks' OR 'matrixyl' OR 'lipospondin' OR 'elaidyl kfk' OR 'syn coll' OR 'syn ake' OR 'syn tacks' OR 'tetrapeptide'/exp OR 'vialox' OR 'fvapfp' OR 'vgvapg' OR 'leuphasyl' OR 'dipeptide'/exp OR 'serilesine' OR 'decorinyl' OR 'eyeseryl' OR 'saccharomyces lysate extract' OR 'oxy 229-bt' OR 'pepha timp' OR 'placentol' OR 'kinetin'/exp OR 'neuropeptide'/exp OR 'algae extract'/exp OR 'amaranth protein' OR 'fnk protein' OR 'gelatin protein' OR 'keratin protein' OR 'elastin protein' OR 'collagen protein' OR 'rh sod' OR 'superoxide dismutase'/exp OR 'bovine albumin'/exp OR 'pep-1 ribosomal protein' OR 'pl 14736' OR 'skin respiratory factor' OR 'becaplermin'/exp OR 'psp'/exp OR 'etaf' OR 'tns' OR 'glutathione'/exp OR 'secma' OR 'ctp complex' OR 'soy protein'/exp OR 'wheat protein' OR 'oat protein' OR 'rice protein' OR 'corn protein' OR 'vegetable protein'/exp OR 'milk protein'/exp OR 'silk protein' OR 'yeast extract'/exp OR 'honey protein' OR 'revitalin' OR 'immucell' OR 'sericin'/exp OR 'lipeptide' OR 'elhibin' OR 'colhibin' OR Hsp70 OR 'heat shock protein' OR melatonin OR MSH OR 'aquaporin' OR 'pyratine 6' OR 'AcTP' OR 'pal kt' OR 'snap 8')
To locate all topical therapies	AND ('topical'/exp OR 'skin'/exp OR cutaneous)
To locate all cosmeceutical indications	AND (cosmetic* OR cosmeceutical* OR hydrat* OR ('hair growth'/exp OR 'hair growth') OR ('hair loss'/exp OR 'hair loss') OR moistur* OR ('aged'/exp OR 'aged') OR ('aging'/exp OR 'aging') OR ('elderly'/exp OR 'elderly') OR senile OR photoaged OR photodamaged OR firm* OR lift* OR 'conditioner' OR 'hair conditioning' OR 'skin repair' OR ('rejuvenation'/exp OR 'rejuvenation') OR 'anti-wrinkle' OR 'hair remover' OR tightening OR 'hair care' OR 'scalp care' OR lightening)
Alternative search strategy obtained from embase emtree	OR (('skin'/exp OR 'skin') OR ('skin care'/exp OR 'skin care') OR ('cutaneous parameters'/exp OR 'cutaneous parameters') OR ('cosmetic'/exp OR 'cosmetic') AND (('proteomics'/exp OR 'proteomics') OR ('peptide'/exp OR 'peptide')))
To rule out all irrelevant articles	NOT (melanoma*:ti,ab OR cancer*:ti,ab OR carcino*:ti,ab OR malignan*:ti,ab OR onco*:ti,ab OR neoplas*:ti,ab OR tumor*:ti,ab OR tumour*:ti,ab OR sarcoma*:ti,ab OR lymphoma*:ti,ab OR 'c-reactive':ti,ab OR vaccine:ti,ab OR vaccines:ti,ab OR infectio*:ti,ab OR antimicrobial:ti,ab OR psoria*:ti,ab OR pemphig*:ti,ab OR *menopaus*:ti,ab OR replacement:ti,ab OR asthma*:ti,ab OR allerg*:ti OR sclerosis:ti,ab OR vasculitis:ti,ab OR arthritis:ti,ab OR obesity:ti,ab OR tuberculosis:ti,ab OR aortic:ti,ab OR lupus:ti,ab OR scleroderma:ti,ab OR alzheimer*:ti,ab OR 'sezary syndrome':ti,ab OR 'mycosis fungoides':ti,ab OR hypertens*:ti,ab OR bullous:ti OR onchocerc*:ti,ab OR polyneuropath*:ti,ab OR dialysis:ti,ab OR renal:ti OR kidney:ti OR apoptosis:ti,ab OR orthop*:ti,ab)

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