

Repair of photoaged dermal matrix by topical application of a cosmetic 'antiageing' product

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Summary

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cosmetic, fibrillin, matrix metalloproteinase, photoageing, procollagen I

Conflicts of interest

S.P.L. and S.P.B. are employed by The Boots Company, the manufacturer of the three commercially available preparations tested in this study.

Background Photoaged skin is characterized by coarse and fine wrinkles. The mechanism of wrinkle formation appears to involve changes to components of the dermal extracellular matrix. Topical treatment with all-trans retinoic acid (RA) can repair photoaged dermal matrix; this is regarded as the 'gold standard' against which repair agents are judged. To date, little is known regarding the ability of over-the-counter 'antiageing' products to repair photoaged skin.

Objectives We used a modified occluded patch test to ascertain whether topical applications of cosmetic 'antiageing' products are able to repair photoaged human skin.

Methods Commercially available test products [basic moisturizer, 'antiageing' cream containing different active complex levels (6% active: lipopentapeptide, white lupin peptides, antioxidants, retinyl palmitate; 2% active: lipopentapeptide, white lupin peptides, antioxidants)] were applied under occlusion for 12 days prior to biopsy and histological assessment in photoaged volunteers ($n = 9$). RA was used as a positive control.

Results In agreement with previous studies, the patch-test study revealed that RA produced significant fibrillin-1 deposition in the papillary dermis ($P < 0.01$) but had little effect on procollagen I or matrix metalloproteinase-1 expression. The 6% total active complex formulation, however, increased the deposition of fibrillin-1 and procollagen I ($P < 0.01$, $P < 0.05$, respectively).

Conclusions This study indicates that in an *in vivo* 12-day patch test an over-the-counter cosmetic product can induce changes in photoaged dermal extracellular matrix, which are indicative of repair.

Ageing of the skin can be thought of as two simultaneous processes: intrinsic and extrinsic. Intrinsic changes occur as a result of the passage of time and produce characteristic fine wrinkles.¹ Superimposed upon these are changes resulting from exposure to extrinsic or environmental factors. One such factor is chronic sun exposure, resulting in photoageing, which by comparison with intrinsic ageing produces coarse, roughened, and deeply wrinkled skin.^{2,3} Histologically, skin that is intrinsically aged has an atrophied extracellular matrix (ECM) and contains reduced levels of collagen and elastin.⁴⁻⁶ By comparison, photoaged skin exhibits other, numerous, alterations to the dermal ECM. These include deposition of dystrophic elastic fibres in the dermis,⁷ decreased levels of fibrillar collagens types I and III,^{8,9} reduced numbers of anchoring fibrils (collagen VII)¹⁰ and loss of the fibrillin-rich microfibrils in the papillary dermis.¹¹

Levels of matrix metalloproteinases (MMPs) are increased in both intrinsically and extrinsically aged skin and are major contributors to the remodelling of the dermal ECM in both conditions.¹²⁻¹⁴

Currently, the medical treatment of choice for photoaged skin is topical application of retinoids.¹⁵ Previous studies have shown that application of topical all-trans retinoic acid (RA) to photoaged skin partially restores levels of collagens I and VII (anchoring fibrils), reduces MMP-1 expression and ameliorates some of the clinical features such as wrinkles.^{8,16-20} In addition, treatment with RA partially restores the fibrillin-rich microfibrillar network of the papillary dermis.²¹ Using a short-term occluded patch-test protocol in human skin, we have shown that fibrillin is a useful biomarker of dermal repair in photoaged skin and may be predictive of successful repair in long-term clinical studies.²¹

Cosmetic 'antiageing' products for the most part target the undesirable clinical features of photoageing. Either one, or both, of two strategies is adopted: protection of skin against photoageing; or correction of the visible signs of photoageing. Protection, by the use of broad-spectrum ultraviolet (UV) radiation absorbing or reflecting filters, is well documented as a means of reducing total lifetime UV dose, thereby reducing the effects of extrinsic environmental factors on both the appearance and the biomechanical properties of the skin.²² Recent reviews have also highlighted research into other topical protective strategies, particularly antioxidants.²³ Cosmetic products target the stratum corneum and epidermis; there are few published *in vivo* data on the effects of such 'antiageing' products on the dermis.

In the current study, we have extended our previously described 4-day *in vivo* protocol for assessment of topical photoageing repair agents²¹ to simulate the effects of longer-term 'real life' use. We investigated whether three cosmetic products marketed by The Boots Company (Nottingham, U.K.) were able to repair the dermal ECM changes associated with photoageing. To assess repair, we examined the expression of three key biomarkers: fibrillin-1, procollagen I (pCI) and MMP-1.

Materials and methods

Test products

Three commercially available preparations were used in these studies: a basic moisturizer (oil in water emulsion containing paraffin, glycerin, caprylic/capric triglyceride, dimethicone, glyceryl stearate, polyethylene glycol-100 stearate, petrolatum, cetyl alcohol, phenoxyethanol, propylene glycol, methylparaben, xanthan gum, propylparaben, *Camellia oleifera* extract, carbomer, tetrasodium ethylenediamine tetraacetic acid, potassium hydroxide, *Aloe barbadensis* extract, butylparaben, isobutylparaben and ethylparaben); and two 'antiageing' products from the Boots range, namely an oil-in-water emulsion-based moisturizing formulation containing 2% total active complex (lipopentapeptide, white lupin peptides and antioxidants) and a water-in-silicone emulsion-based serum formulation containing 6% total active complex [lipopentapeptide, white lupin peptides, antioxidants and retinyl palmitate (< 0.2%)].

In vivo patch-test study

Nine healthy, photoaged volunteers were recruited (two men and seven women; age range 42–79 years). Test substances (20 µL) were applied separately to the extensor photoaged aspect of the forearm under standard 6-mm diameter Finn chambers on Scanpor tape (Epitest, Tuusula, Finland). The test substances were basic moisturizer, 2% total active content formulation and 6% total active content formulation. Test formulations were applied to clean skin on days 1, 4 and 8 of the assay. RA [0.025%; Retin-A®

cream (Janssen-Cilag Ltd, Beerse, Belgium); 20 µL] was applied to an untreated site on day 8 to avoid potential complications of irritancy caused by extended occlusion. On day 12, the Finn chambers were removed and 3-mm punch biopsies obtained under 1% lignocaine local anaesthesia from each test site. Biopsies were embedded in optimal cutting temperature compound (Tissue-Tek®; Miles Laboratories, Elkhart, IN, U.S.A.), snap frozen in liquid nitrogen and stored at -70 °C prior to immunohistochemical analyses. The Salford and Trafford Local Research Ethics Committee approved the study and all subjects gave written, informed consent.

Immunohistochemistry

Frozen sections (10 µm) were fixed in 4% paraformaldehyde and hydrated in Tris-buffered saline (TBS; 100 mmol L⁻¹ Tris, 150 mmol L⁻¹ NaCl; pH 7.4). Sections were pretreated with 0.5% Triton X-100 and endogenous peroxidase activity abolished by incubation with 0.6% hydrogen peroxide in methanol. Nonspecific binding was blocked by incubation with 3% bovine serum albumin plus 3% normal serum. Primary antibodies were applied overnight at 4 °C. These were: mouse antihuman fibrillin-1 (clone 11C1.3; NeoMarkers, Union City, CA, U.S.A.) diluted 1 : 100; rat antihuman pCI (clone M-58; Chemicon International, Temecula, CA, U.S.A.) diluted 1 : 1000; or mouse antihuman MMP-1 (Oncogene Research Products, Boston, MA, U.S.A.) diluted 1 : 100. Negative controls were by incubation of isotype sera at the appropriate concentration or omission of primary antibody. Sections were washed in TBS, prior to incubation with the appropriate biotinylated secondary antibody for 30 min. Antibody staining was visualized using a well-characterized immunoperoxidase reaction (VectaStain® Elite ABC system; Vector Laboratories, Burlingame, CA, U.S.A.) utilizing Vector SG® as chromogen. Following light counterstaining with nuclear fast red, serial dehydration and mounting, sections were randomized, blinded and examined on a Nikon Optiphot microscope (Tokyo, Japan). The degree of immunostaining for fibrillin-1 and pCI was assessed as previously described.²¹ In brief, a five-point semiquantitative scale was used where 0 = no staining and 4 = maximal staining within the experiment. The numbers of epidermal keratinocytes positive for MMP-1 were quantified per high-power field (×400). Four sections (including control) were examined per subject, per site, per treatment and the mean score calculated.

Statistical analyses

Differences in amount of immunostaining produced by the test formulations were assessed for significance using the repeated-measures analysis of variance. Results were considered significant if $P < 0.05$ (95% confidence level) and were calculated using SPSS+ v11.5 software (SPSS Inc., Chicago, IL, U.S.A.).

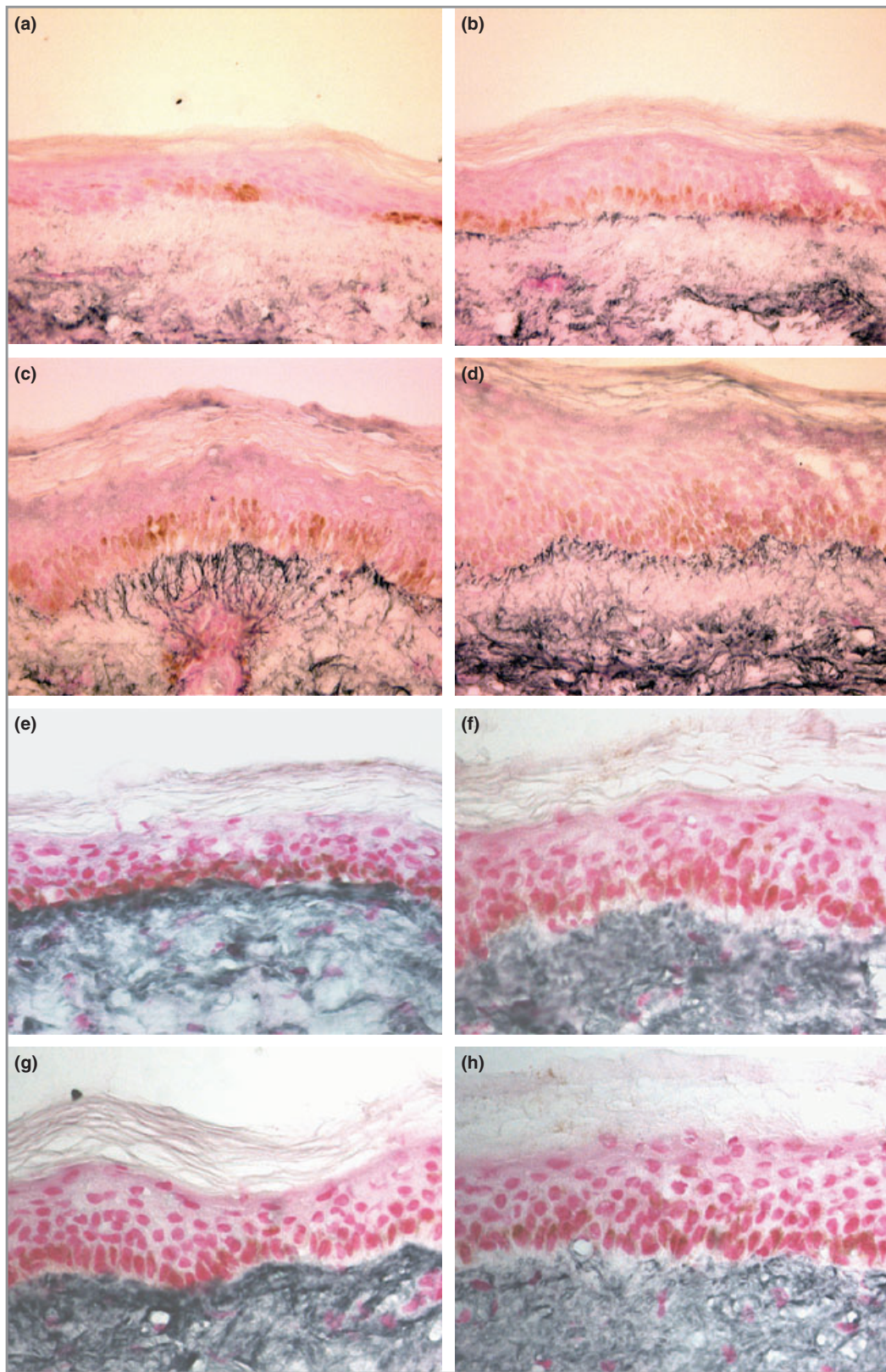


Fig 1. Expression of fibrillin-1 and procollagen I (pCI) in photoaged skin is increased following treatment with 6% total active complex formulation. Representative photomicrographs detail the occurrence of fibrillin (a–d) and pCI (e–h) in photoaged extensor forearm skin following either the extended 12-day patch-test protocol [(a, e) basic moisturizer; (b, f) 2% total active complex formulation; (c, g) 6% total active complex formulation] or the 4-day standard patch-test protocol [(d, h) 0.025% all-trans retinoic acid]. Original magnification $\times 400$.

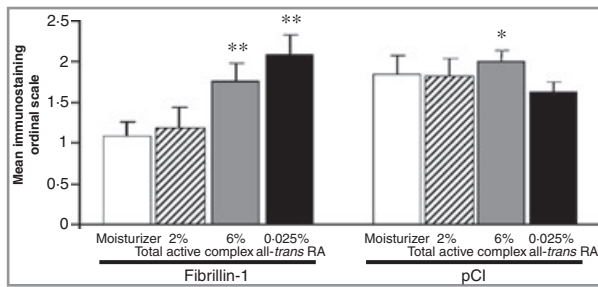


Fig 2. The 6% total active complex formulation has beneficial skin effects for photoaged dermis. Dermal extracellular matrix biomarkers were assessed by standard immunohistochemistry for putative changes following 12-day treatment with basic moisturizer (open bars), 2% total active complex formulation (hatched bars) or 6% total active complex formulation (grey bars), or 4-day treatment with the pharmaceutical 'gold standard' all-trans retinoic acid (RA) (filled bars). We identified significantly increased fibrillin-1 deposition with both 6% total active complex formulation and RA (** $P < 0.01$) plus significantly increased procollagen I (pCI) deposition following treatment with 6% total active complex formulation only (* $P < 0.05$). Results are shown as mean \pm SD.

Results

Immunohistochemistry

Application of RA produced significant ($P < 0.01$) deposition of fibrillin-1 in the papillary dermis, as compared with basic moisturizer, in eight of nine volunteers assayed, a finding consistent with previous studies.²¹ Topical treatment with 2% active complex formulation resulted in a small but insignificant increase in fibrillin-1 deposition. However, application of 6% active complex formulation resulted in a significant ($P < 0.01$) increase in fibrillin expression similar to that observed with RA treatment (Figs 1, 2).

Application of RA had little effect on the deposition of pCI in the papillary dermis following 4-day occluded application, in agreement with previous data,²¹ as compared with the basic moisturizer and the 2% total active complex formulation. However, 12-day application of 6% total active complex formulation resulted in a small, but significant ($P < 0.05$), increase in pCI deposition in the papillary dermis (Figs 1, 2).

MMP-1 staining was observed in both epidermis and dermis and was the most variable of all the parameters measured. Overall, topical application of RA for 4 days under occlusion had little effect on epidermal MMP-1 expression as compared with the basic moisturiser and both the 2% and 6% total active complex formulations.

Discussion

To ascertain whether a cosmetic product was capable of repairing the dermal ECM of photoaged skin we examined the effects of three cosmetic products in an extended *in vivo* patch-test assay, which simulates long-term topical use.²¹ We have

shown previously that topical application of RA under occlusion for 4 days results in a significant increase of fibrillin-1 mRNA and protein. Such an increase is similar to the partial restoration of fibrillin-1 that occurs when RA is used long term for up to 4 years.²¹ Fibrillin-1 is a sensitive biomarker for gauging dermal responses to topical photoageing repair agents. Using RA as our positive control, we applied products under occlusion for 12 days, and assayed samples for fibrillin-1, pCI and MMP-1.

Both of the active complex-containing products increased fibrillin-1 in the papillary dermis over and above that achieved with moisturizer alone, but this was significant only with total active complex at a concentration of 6%. The 6% product not only contains higher levels of lipopentapeptide and white lupin extracts, but also the retinyl ester, retinyl palmitate. Somewhat surprisingly, topical application of this formulation also resulted in increased pCI deposition in the papillary dermis whereas this did not occur with RA in this *in vivo* system. Identification of increased aminopropeptide for collagen I within the papillary dermis implies increased pCI synthesis. A lack of increase in pCI following application of RA in this assay may be due to the short application time: occlusion for longer than 4 days often results in significant side-effects, namely irritation and superficial erosions.^{24,25}

In the current study, RA was applied only for the final 4 days of the patch-test protocol to minimize the potential for skin irritation, whereas the cosmetic 'antiageing' formulations were applied for 12 days. Previous (unreported) studies using a 4-day patch-test protocol with cosmetic agents demonstrated that certain formulations were capable of increasing fibrillin-1 deposition, but that this response showed considerable inter-subject variation. The extended application protocol reported in the current study elicited more reproducible changes in components of the ECM after application of cosmetic products.

Among the active ingredients for the formulations, white lupin peptides are known to inhibit the activity of MMP *in vitro* (P. Oger, unpublished data). Therefore we examined the expression and distribution of MMP-1 – the major MMP acting on fibrillar collagens in the papillary dermis – to identify remodelling of ECM. RA when used either *in vivo* or *in vitro* has been shown to downregulate the synthesis of MMP-1^{7–19} but had little effect over 4 days in our *in vivo* system. Similarly, the commercially available cosmetic formulations did not significantly reduce keratinocyte expression of MMP-1.

It is common for cosmetic products to contain a number of complementary ingredients which purport to address photoageing. While the current study does not differentiate between the roles of specific ingredients, especially those of peptides and retinyl palmitate, it has shown that combinations of cosmetic ingredients in marketed products can induce changes in the dermal ECM similar to those brought about clinically by RA. Retinyl esters, such as retinyl palmitate and retinyl propionate, are the least active of the available topical retinoids, and must undergo significant oxidation to produce active RA.^{26–29} Experiments using animal models and human explant cultures

have shown that a relatively small proportion of retinyl ester is absorbed by human skin (approximately 18% in an acetone vehicle) and that approximately half of this goes on to be metabolized to retinol.^{30–32} Using these data, it is estimated that the relative amount of retinol available from the more active product tested here (6% total active complex formulation) would be approximately 0.01%. Such esters have been suggested as alternatives to RA for the treatment of photoaged skin,^{33,34} as they produce far fewer side-effects (irritation, erythema, xerosis), as does retinol (while resulting in cellular and molecular changes associated with RA treatment³³). However, when topical retinyl esters (at a concentration of 0.15%) were assessed in a rigorous, double-blind clinical trial they did result in clinical improvement of photoaged skin.³⁴ We cannot exclude and also cannot conclude that retinyl palmitate – present in this formulation at < 0.2% – may contribute to the changes in ECM seen in this occluded *in vivo* patch-test assay.

The 2% cosmetic formulation tested, which contained peptides – but not retinyl palmitate – produced results that were intermediate between those observed using the 6% active formulation and the basic moisturizer, suggesting a role in the partial repair of dermal matrix for this peptide combination independent of the retinyl palmitate action. There has been growing interest in the role of various peptides as cosmetic treatments for the signs of skin ageing.^{34,35} Robinson *et al.*³⁶ showed that use of a lipopeptide in a skincare base may improve skin appearance and periorbital wrinkles. To date, there have been few histological assessments of the effects of cosmetic skincare products; hence, the findings of this study add to our understanding of the benefits that such products can offer. It should be emphasized that our study only assessed the ability of a cosmetic product, when applied under occlusion, to repair photoaged skin at the histological level. What the study did not do was to assess whether the cosmetic products tested were capable of producing significant improvement in the clinical features of photoaged skin. A 6-month double-blind clinical trial is in process to ascertain whether these cosmetic 'antiageing' products are capable of producing significant clinical benefit.

The potential for corrective action by cosmetic products upon superficial manifestations of photoageing in the stratum corneum has been described for materials such as glycols, α -hydroxy acids and ceramides (see Leyden and Rawlings³⁷ for review). Such superficial changes are accepted to be within the terms of a cosmetic effect. The findings reported here, where a product has beneficial effects below the epidermis, provide evidence that cosmetic products may be capable of skin repair. There is debate in some quarters about whether such changes are within the definition of a cosmetic product. The effects reported, which control observations suggest are localized, address a cosmetic problem which is not medical. As such, the products can be described justifiably as cosmetics. Recent regulatory opinion recognizes the physiological effects of cosmetics (http://ec.europa.eu/enterprise/cosmetics/html/cosm_borderline_docs.htm (last accessed 21st November 2007))³⁸ and further re-establishes that it is pack or advertised claims that should be

used to judge these issues. The studies reported here show that claims for 'antiageing' products can be supported by assessment of changes in markers of photoageing using the modified patch-test protocol described.

In conclusion, the products tested from this range of over-the-counter cosmetic 'antiageing' products affect the expression of fibrillin-1 and pCI in photoaged skin at the histological level. These changes could equate to partial repair of photoaged human skin, thus implying clinical benefit.

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