

**Managing Skin Ageing as a Modifiable Disorder – The Clinical Application of
Nourella® Dual Approach Comprising a Nano-encapsulated Retinoid, Retilex-A®
and a Skin Proteoglycan Replacement Therapy, Vercilex®**

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Abstract

Skin ageing is a progressive but modifiable, multi-factorial disorder that involves all skin tissues. Pertaining to its wide range of physiological and psychosocial complications, skin ageing requires rigorous clinical attention. Topical retinoids and per-oral proteoglycans are promising, non-invasive, therapeutic modalities. To overcome the low bioavailability of conventional free retinoids, Nourella[®] cream with Retilex-A[®] (Pharma Medico, Aarhus, Denmark) was developed using a proprietary nano-encapsulation technology. The nano-encapsulation is a sophisticated ‘permeation/penetration enhancer’ that optimises topical drug delivery by increasing surface availability and net absorption ratio. Treatment adherence is also improved by minimising skin irritation. Interventional evidence supports the higher efficacy of Retilex-A[®] in improving skin thickness and elasticity compared with conventional free forms. It is also reported that the rejuvenating efficacy of Retilex-A[®] and tretinoin are comparable. Another skin anti-ageing approach is proteoglycan replacement therapy (PRT) with Vercilex[®]. Vercilex[®] in Nourella[®] tablet has the potential to ameliorate proteoglycan dysmetabolism in the aged skin by activating skin cells and improving collagen/elastin turnover. Replicated clinical trials evidenced that PRT can significantly enhance the density, elasticity and thickness of both intrinsically aged and photoaged skin. Evidently, Vercilex[®] and Retilex-A[®] share a range of bioactivities, which underlies their synergistic activity observed in a clinical trial. Dual therapy with Nourella[®] tablets and cream produced higher effect sizes on skin characteristics than monotherapy with each of the two treatments. In conclusion, Nourella[®] cream and tablets are safe and effective treatments for skin ageing; however, combining the two in a ‘dual skin rejuvenation system’ significantly improves treatment outcomes.

Keywords: Skin Aging, Skin delivery/penetration, Skin structure, Retinoid formulation, RetileX-A, Vercilex

1. Chronological (intrinsic) and accelerated (extrinsic) skin ageing

Ageing is a complex, multi-factorial and progressive process driven by both genetic programming and cumulative environmental damage. Detectable cellular ageing starts in cutaneous tissues somewhat earlier than most other tissues probably due to the fact that skin is exposed to environmental stressors and hazards. Visible signs of skin ageing gradually appear during the 3rd decade of life onwards at a rate that accelerates with age [1]. Two interacting and overlapping processes of skin ageing are recognised: intrinsic (chronological) and extrinsic (accelerated or premature) skin ageing. These processes run in parallel in most areas of the skin. Premature skin ageing is estimated to affect as much as 83% of adults under the age of 55 [2]. Intrinsic aetiologies that lead to chronological skin ageing include cellular ageing (Hayflick-Limit) and shortening of telomeres, mutations of mitochondrial DNA, oxidative stress, genetic mutations and the dropped level of several hormones. The rate and intensity of chronological processes can be accelerated by superimposed factors such as ultraviolet (UV)-light exposure, tobacco smoking, infrared radiation, environmental pollution, psychological stress and malnutrition. The major molecular mechanisms through which these factors accelerate the disintegration rate of skin components is presented in the Figure. In clinic, a chronologically aged skin is manifested by deepened frown and wrinkle lines of the forehead, glabella and lateral periorbital area and increased intensity of creasing at the chin, upper lip, nasolabial folds, nasal flare and platysma neck bands. The skin becomes conspicuously thinner, transparent and fragile and age spots begin to spread and magnify over decades. Expressions of accelerated skin ageing (photoageing) largely depend on the Fitzpatrick skin type and the cumulative exposure to stressors [3].

Distinctive clinical symptoms of skin ageing are external presentations of the underlying structural and functional deteriorations that occur to all histological layers of the skin. The outermost layer, epidermis, declines in thickness at about 6.4% per decade, and its turnover rate plummets to half when one hits his eighth decade of life. Rete pegs at epidermal–dermal

junction gradually flatten, which contributes to wrinkle formation and leaves the skin less resistant to shearing forces. In dermal parts, vascularity, cellularity and the volume of extracellular matrix (ECM) decreases over time. Secondary to the lowered number/activity of fibroblasts, the turnover of collagen and elastin, and thus skin flexibility and elasticity also decrease in the aged skin. Vascular network deterioration leads to considerable decline in cutaneous skin blood perfusion [1]. However, being overly exposed to UV light and other risk factors can extensively influence the course and path of skin histological alterations during ageing. For example, in sun-exposed areas, the epidermis is thicker with higher degrees of solar elastosis, perivascular inflammation and perifollicular fibrosis; whereas severe photodamage can elicit epidermal atrophy [4].

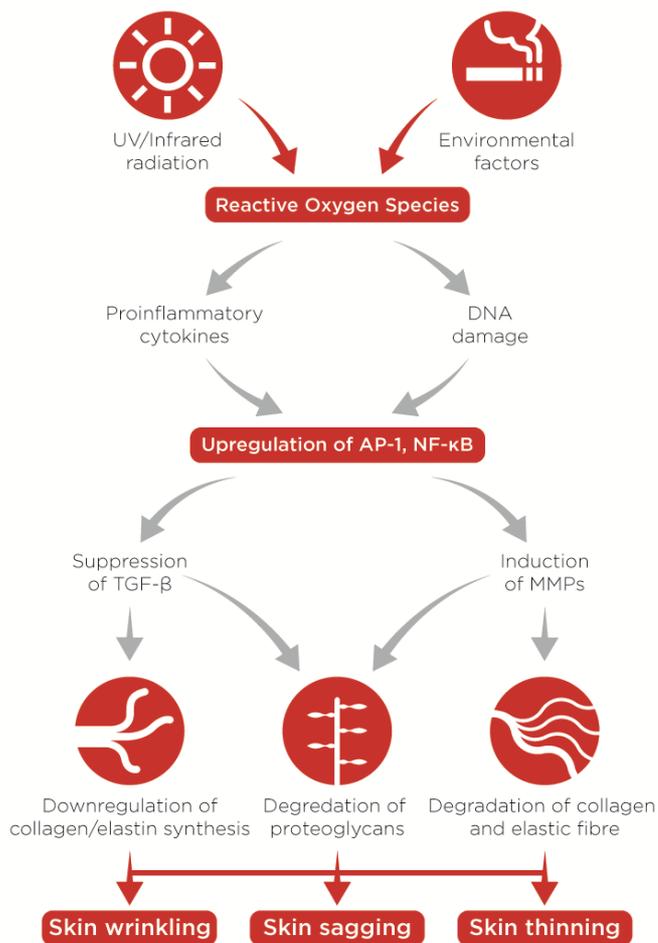


Figure. A simplified illustration of molecular mediators and underlying mechanisms of accelerated skin ageing [5]; Abbreviations: AP-1: activator protein 1; NF- κ B: nuclear factor- κ B; TGF- β : transforming growth factor beta; MMPs: matrix metalloproteinases

2. Approaching skin ageing as a modifiable disorder

Whether or not a specific condition is recognised as a ‘disease/disorder’ is greatly influenced by several cultural and historical factors as well as the existing level of scientific understanding of its pathophysiology and complications. Several conditions, such as osteoporosis and senile Alzheimer’s, that are incontrovertibly known as disorders at present were in the past labelled as normal results of ageing [6]. Major geriatric conditions such as congestive heart failure, chronic obstructive pulmonary disease and chronic kidney disease are in fact the end results of a chronological or accelerated decline in the function of a specific organ due to ageing.

Surprisingly, degenerative changes of the skin are still considered by some as components of the ‘normal ageing process’; whereas skin ageing fully meets the accepted criteria for a ‘disorder’, which is a disruption of the normal arrangement or functioning of the body. In terms of the skin, as long as there is no reference range for each skin function at a given age range, no loss of function or disrupted structure at any age can be marked as normal. Skin ageing involves several parallel pathological mechanisms that produce considerable loss of function and clinically significant complications (see below). This is particularly true in the case of premature skin ageing (dermatoheliosis) with recognised underlying risk factors and high risk of comorbidities [7]. During the past decade, an emerging trend has appeared to refer to the chronic skin fragility syndrome of ageing as ‘dermatoporosis’ in order to encourage timely treatment and to provide a nosological tool for the classification of this under-recognised condition [8].

Skin ageing involves a significant and progressive decline in almost all physiological functions of the skin ranging from biochemical to neurosensory alterations. Aged skin is drier with

decreased lipid content and higher cutaneous PH, Cell renewal, synthetic capacity and vascularisation of the aged skin are also waned leading to an impaired wound healing capability. Adding this to the gradual deterioration of superficial neurosensory perception makes the aged skin exceedingly vulnerable to injury which brings significant indirect risk of morbidity [9]. In addition to physical complications, skin ageing disorder can inflict a significant psychosocial burden on affected individuals. Research shows that the discoloured, blemished and slack appearance of the aged skin can negatively affect one's body-image and self-esteem setting the stage for anxiety and depression. Skin ageing can also repel physical contact by others, which may end in social withdrawal and disturbance of interpersonal relationships [10] with profound effects on one's overall quality of life. It can therefore be concluded that skin ageing qualifies to be recognised as a 'disorder' that requires targeted medical attention. In the same manner as other senile disorders, the process of skin ageing does not follow a predetermined, invariable time course; instead, its underlying pathologies and presentations may be delayed and/or reversed by avoiding modifiable risk factors (e.g., unprotected sun exposure and smoking) [11] and using proper pharmaceutical intervention [12].

A rich variety of medicinal and cosmetic anti-ageing products have been introduced to the market with variable efficacy profiles and action mechanisms. The main conventional, non-invasive treatments are sunscreens, topical and systemic antioxidants, topical retinoids, topical and systemic glycosaminoglycans and proteoglycans. Experimental evidence indicates that despite their claims, few anti-ageing ingredients have the capacity to penetrate far enough into the dermis to ameliorate deep wrinkles [13]. Minimally invasive resurfacing techniques, such as chemical peeling, laser/radiofrequency tightening and filler injections, and invasive surgical procedures are also used in selected cases [14]. However, effective prevention of skin ageing disorder is a long-term endeavour that requires interventions that are easy to apply, skin friendly, well tolerated, affordable and devoid of side effects. Accordingly, topical retinoids [8] and oral proteoglycans appear to be among the most appropriate candidates for long-term anti-

ageing therapy of the skin. In this paper, we strive to sketch an overview of these two successful approaches, which, in combination, form a targeted dual treatment known as ‘Nourella® Skin Rejuvenation System’ (developed by Pharma Medico, Aarhus, Denmark).

3. Natural retinoids as the ‘Gold Standard’ treatment of skin ageing

Retinoids are a group of natural and synthetic vitamers that share either molecular or functional similarities with all-trans retinol (vitamin A). Topical formulations of retinoids have been in cosmetic and clinical use since the 1940s, however, their prominent anti-ageing effects were not scientifically reported until the early 1980s [15]. Since then a growing pile of clinical research have clearly demonstrated their ability to improve fine wrinkling, skin elasticity and lighten uneven skin pigmentation [16]; thus, natural retinoids have become the ‘gold standard’ for both prevention and treatment of skin ageing.

Retinol and its storage form, retinyl ester (e.g., retinyl palmitate and acetate), are the most abundant, naturally occurring retinoids present in the body. Retinol is a transport form and a precursor that is converted via a two-step oxidation process to its bioactive metabolites, retinoic acids. Through interaction with several cytoplasmic and nuclear receptors, retinoids regulate a range of vital molecular pathways and cellular activities from embryogenesis to skin regeneration and maintenance. Skin is a major retinoid-responsive tissue in which retinoic acid directly and indirectly regulates the expression of multiple key genes [17]. Such a multifarious, gene-level bioactivity is being employed in clinical prevention and management of the skin ageing disorder. According to available evidence [18-22], long-term topical treatment with natural retinoids promotes pervasive histopathological improvements in all layers of the skin as listed below:

- A significant rise in epidermal thickness of the aged skin via amplifying the proliferation of epidermal keratinocytes by several folds

- A marked upturn in proliferation rate of dermal stromal cells particularly in papillary dermis compared to the placebo treated skin
- Improvement of dermoepidermal ECM microenvironment by two mechanisms. First, retinoids stimulate the activity of fibroblasts and boost the production of type-I and -III collagen, fibronectin and tropoelastin in both chronologically aged and photoaged skin; and second, these compounds can inhibit the expression of matrix metalloproteinases and thereby diminish the degradation of ECM components.
- A considerable increase in the proliferation of dermal endothelial cells and the formation of blood vessels, thus improving the skin's microcirculation and blood supply
- Efficient anti-inflammatory effects through suppressing the release of proinflammatory cytokines and the activity of leukocytes

In conclusion, after a sufficient course of retinoid therapy, the skin becomes thicker with improved fibroelastic properties, enhanced blood circulation and subsided inflammatory reactions. These cellular-level enhancements by natural retinoids give rise to the clinical improvements observed in short- and long-term, controlled clinical trials as detailed by Mukherjee et al. [18]. Based on published reports, 3 to 4 months of retinoid therapy can compact stratum corneum and fade both fine and coarse wrinkles making the skin to appear rejuvenated. In addition, the concentration of glycosaminoglycans will increase [23, 24]. As evidenced by long-term trials, extended courses of retinoid application, i.e. for six months or more, results in higher degrees of improvement in skin wrinkling, which plateaus after around 10 months and is maintained afterwards [25]. However, it appears that for appreciable dermal-level improvement and formation of new collagen, more than six months of retinoid treatment is required. It is of practical interest to note that in clinical studies, the skin condition of participants continued to improve even after cessation of the active treatment [18].

At present, there are two commonly used natural retinoids approved for the management of skin ageing: all-trans retinol (and its derivatives) and tretinoin. Despite the fact that retinol is

theoretically less potent than tretinoin, comparative clinical studies suggest that its efficiency in producing ‘retinoid-mediated histological changes’ is comparable with tretinoin. Of note, retinol and its derivatives induce much less skin irritation than tretinoin and is preferable in long-term treatment of skin ageing [26, 27].

3.1. Using nano-encapsulation to enhance the bioavailability and safety of retinoids

Despite their proven molecular efficacy, the ‘dose efficiency’ and ‘long-term patient compliance’ of topical free retinoids (the conventional form) is compromised by two major impediments. The first challenge is to penetrate deep enough into the skin to reach the primary action site of retinoids, i.e., the epidermal basal layer and upper papillary dermis with high density of cells and collagen fibres. Experiments by Roos et al. revealed that topically applied retinoids poorly penetrate into or through the skin. This is specifically true with gel-based formulations, which tend to trap the active drug molecules [28]. Poor penetration leads to around 80% of the applied active retinoid to remain on skin surface [29]. Long term entrapment of unprotected retinoid molecules on skin surface exposes them to environmental factors such as heat and UV radiation. Since retinoids are chemically and thermally unstable and sensitive to photoisomerization and degradation [30], a large share of the applied dosage will be lost to degradation. This reduces the overall ‘dose efficiency’ and ‘bioavailability’ of the treatment. In order to overcome such a critical shortcoming and improve topical absorption of retinoids, several techniques have been attempted over decades, such as developing retinol derivatives [31] and chemical penetration enhancers. However, the rate of success with penetration enhancers has been limited owing to their common skin irritancy and little enhancement effects [32].

The second impediment that hampers the effective use of topical free retinoids is their common irritation side effects. Retinoid-induced irritation is clinically similar to a mild irritant dermatitis and exhibits with excessive skin dryness, burning sensation, erythema, scaling and pruritus.

Retinoid reaction is time and dose dependent and frequently occurs when using an effective dosage of either free retinol or tretinoin for extended periods of time. Induced release of pro-inflammatory cytokines, e.g., TNF- α and IL-8, is reported to be a triggering factor for retinoid reaction [33]. Likelihood of skin reactions is higher with conventional products containing a free form of retinoid since these products release their active ingredients in a relatively high concentration within a short period of time. Such an unmodified release profile leads to a cycle of short-term overmedication followed by long-term undermedication, which maximises side effects and undermines clinical efficacy [34]. It is conceivable that by transforming the release and absorption profile of retinoids into a more gradual and steady form, the overload of biological membranes can be attenuated, thus, minimising the release of cytokines and the likelihood of skin irritation.

An efficient, nanomolecular skin delivery system that works as a 'permeation and penetration enhancer' of transdermal delivery of pharmaceuticals is nano-encapsulation with biomaterials. Examples of materials frequently used in biomedical industries are non-conjugated peptides, nanostructured lipid carriers, dendrimers and cyclodextrins. Based upon this practical knowledge and years of research and development, Retilex-A[®] has been developed through a proprietary technique of nano-encapsulation and optimised emulsification of retinyl ester by scientists at Pharma Medico (Aarhus, Denmark). This novel complex represents a next-generation topical retinoid with modified pharmacokinetic characteristics used as the main active ingredient of Nourella[®] anti-ageing cream (Pharma Medico, Aarhus, Denmark). Such a smart drug delivery system aims to overcome both of the aforementioned impediments of topical retinoid therapy of skin ageing. Improved efficacy and safety of Retilex-A[®] in Nourella[®] cream have been corroborated by controlled clinical trials (described in the next section).

There is enough ground to establish that the specific form of nano-encapsulation used in the formulation of Retilex-A[®] enhances topical drug bioavailability and treatment efficacy by:

- I. **Increasing drug availability at skin surface:** It is known that in aqueous solutions, lipophilic molecules, e.g., retinyl esters, compete for a space in carrier cavity, which forms a dynamic equilibrium between encapsulated and free drug molecules. At the surface, retinoid molecules that partition from carrier cavity and penetrate into the lipophilic skin barrier are replaced by newly partitioned active molecules (a buffering effect); such that a fresh pool of free, intact retinoid molecules is continuously available for absorption while the rest of the pool is protected from environmental factors inside the nano-capsules (a protective effect). The significant action of nano-encapsulation on producing a controlled, constant drug release profile has previously been documented [35].
- II. **Augmenting retinoid net absorption ratio:** Whether a result of improved availability or an independent effect, nano-encapsulated retinyl ester exhibits augmented absorbability as demonstrated by a comparative study on a model barrier system (Franz Diffusion Cell). Measurements demonstrated that retinoid molecules from Retilex-A[®] have a markedly higher penetration ratio into a skin model compared to a conventional, commercial formula in both water and isopropyl alcohol media [36].
- III. **Reducing skin irritation side effects:** As explained earlier in this section, it is well founded that specific forms of nano-encapsulation can alleviate the skin irritancy of chemicals. Clinical studies with various skin irritants have verified this integral benefit of nano-encapsulation [32].

In conclusion, nano-encapsulated Retilex-A[®] has improved skin bioavailability and tolerance due to a diminished molecular disintegration rate, constant release profile, increased absorption ratio and less skin irritancy compared to the conventional free forms of retinoids.

3.2. Supporting evidence for the anti-ageing and skin rejuvenating efficacy of Retilex-A[®]

Since its development around the turn of the 21st century, Retilex-A[®] has been subjected to several clinical studies on subjects with chronological and premature skin ageing with promising outcomes [37, 38, 27, 39]. The role of nano-encapsulation in the anti-ageing efficacy characteristics of Retilex-A[®] was clinically studied in a double-blind, clinical trial. Both encapsulated (Retilex-A[®]) and conventional test preparations contained 0.2% W/W of retinyl palmitate in an identical vehicle and were applied b.i.d. on a random side of the volar aspect of each participant's forearms (age range = 40-60 years). Skin thickness and elasticity were objectively measured by ultrasonic dermal scanning. After just 12 weeks of treatment, RetileX-A[®] significantly improved both skin thickness and elasticity (+31% and +18%, respectively, $P < 0.01$), while the conventional free form induced negligible effects (+2% and +1%, respectively). Participants' self-evaluation of treatment results on a visual analogue scale (VAS) also confirmed the objective assessments and showed a significant improvement only after using the nano-encapsulated RetileX-A[®] [37]. A follow-up evaluation has revealed that as much as 25% of therapeutic effects of RetileX-A[®] on skin thickness and 23% on skin elasticity were still present and measurable after one year of treatment cessation. This led the individuals treated with RetileX-A[®] to have significantly thicker ($P < 0.05$) and more flexible ($P < 0.05$) skin 12-months after the study [38]. Overall, this trial signifies that the specific nano-encapsulation technique in RetileX-A[®] can intensify and accelerate the anti-ageing effects of retinyl ester. A succeeding trial re-evaluated the clinical efficacy and safety of Nourella[®] with Retilex-A[®] and compared it with an equivalent dosage of tretinoin. 20 females participated in this clinical study with an average age of 51.4 years, each used both Nourella[®] and tretinoin on the volar aspect of their right or left forearms (chosen at random) b.i.d. for 3 months. Ultrasonic measurement of skin thickness and elasticity detected comparable significant increases in skin thickness and elasticity index at endpoint in both groups. Treated individuals have also judged both formulations to be equally effective, whereas the rate of side effects was much lower with RetileX-A[®] [27]. These findings invariably support, that in clinical treatment of skin ageing,

nano-encapsulated retinyl ester (RetileX-A[®]) is as effective as the prescription medication tretinoin.

A yet unpublished clinical research project performed by the author, Erling Thom, and an academic scholar, Allan Bertil Lassus, demonstrated the efficacy of RetileX-A[®] in treating premature skin ageing (photoageing). Study subjects were middle-aged volunteers with moderate to severe degrees of facial solar elastosis who applied Nourella[®] cream twice a day on affected parts of their skin for 12 weeks. Monthly clinical assessments pointed to a continuous improvement in skin hydration, wrinkling and mottling in the affected areas. Objective measurements consistently reported significant enhancements of 6%, 11% and 16% in lesional skin elasticity after 1, 2 and 3 months of Nourella[®] therapy, respectively. No clinically important side effect was reported during the course of this trial. The observations provide evidence for using Nourella[®] with Retilex-A[®] for the rejuvenation of immaturely aged skin.

4. Proteoglycan dysmetabolism in the ageing skin

Proteoglycans are bioactive macromolecules with a multiplicity of integral mechanical and biological functions. Skin proteoglycans are highly bioactive and contribute to tissue hydration, resistance and resilience by forming super-molecular structures with matrix proteins and regulating fibrillogenesis. They also control cell behaviour and interactions and form a major biological reservoir for various cytokines and growth factors [40, 41]. In excess of 40 different proteoglycans are discovered several of which are expressed in cutaneous tissues. Among the important skin proteoglycans, versican, decorin and biglycan are the most abundant [42, 43]. Versican is a large chondroitin sulphate (CS) proteoglycan, which binds to and modifies several ECM components via its active domains. Versican co-localises with elastic fibres in the dermis and influences cell proliferation and migration. Decorin and Biglycan are small leucine-rich proteoglycans with vital roles in modifying the organisation of dermal matrix. These proteoglycans bind to several types of collagens and elastic fibre components and regulate their

fibrillogenesis and interfibrillar distance [44, 45]. Therefore, any disruption in their turnover or structure may significantly interrupt the homeostasis and structure of dermal tissues. This fact is best demonstrated by decorin/biglycan knockout mice models, which exhibit thin and fragile skin with reduced tensile strength caused by irregular collagen fibrillogenesis [46, 47].

Degradative transformation of proteoglycans is an inherent part of the pathogenesis of both intrinsic and extrinsic skin ageing disorders. Naturally, the synthesis of extracellular matrix proteins by senescent fibroblasts tends to reduce. Research findings show a considerable decline in total amount of sulphated glycosaminoglycans in dermis (in both sexes) and epidermis (only in women) of intrinsically aged skin [45]. Tzellos et al. reported lower levels of versican, decorin and biglycan mRNA in sun-protected aged skin compared to juvenile skin [48]. The relative composition of proteoglycans also undergoes radical changes as the skin ages.

Observations evidence that concomitant to a decline in the proportion of large CS proteoglycans (e.g. versican), the proportion of small dermatan sulphate proteoglycans (e.g. decorin) increases during ageing [49]. Parallel with these quantitative deviations, the molecular characteristics of certain proteoglycans, i.e., the number and size of their side chains and cleavage products, are affected as a function of age [44, 49]. This phenomenon is allegedly a by-product of an age-associated functional loss in glycosaminoglycan synthesising enzymes. The maturity decline in the secretion of sex hormones may as well play a role in the dysmetabolism of skin proteoglycans [45, 44].

Proteoglycan dysmetabolism is presented differently in the photoaged skin that occurs together with the deposition of abnormal elastic tissue. The total amounts of dermal sulphated glycosaminoglycans and hyaluronic acid are elevated as a compensational recovery response to the cumulative damage of overexposure to UV light. However, the reaction of different proteoglycans to photoageing is not identical [45]. For example, versican, which co-distributes with elastic fibres, accumulates in the regions of solar elastosis, nonetheless, with an abnormal distribution pattern. While glycans are normally distributed diffusely in the dermis, these are

predominantly deposited on the abnormal elastotic material in affected areas. This abnormal localisation renders the glycans incapable of enhancing cell activities or serve as a source of hydration. Furthermore, similar to chronologically aged skin, the size and structure of glycosaminoglycans in sun-damaged skin is deviated affecting their water binding properties and ability to interact with other ECM components [50]. Contrarily, the expression of decorin is greatly decreased in the areas of solar elastosis [51]. These abnormalities in the expression, structure and distribution of skin proteoglycans are suggested to be a primary factor that contributes to the development of accelerated skin ageing.

4.1. Proteoglycan Replacement Therapy (PRT) of the aged skin using Vercilex®

Oral administration of natural proteoglycans and glycosaminoglycans is an emerging therapeutic method that has been tried for the treatment of a variety of human disorders including, but not limited to, hair loss disorders [52], osteoarthritis [53] and skin psoriasis [54]. Pertaining to the integral role of ‘proteoglycan dysmetabolism’ in the pathogenesis of both chronological and accelerated skin ageing, oral administration of specific bioactive proteoglycans is deemed to be a relevant anti-ageing approach. Certain proteoglycans and their moieties have proven oral bioavailability and ability to reach peripheral tissues after oral ingestion. In-vivo pharmacokinetic studies on other animals and humans have shown gastric acid survivability and gastrointestinal absorption of glycosaminoglycans such as CS [55] and hyaluronic acid [56]. Evidence suggests that a portion of orally administered proteoglycans is absorbed intact via endocytosis mostly in distal parts of the small intestine [57].

The concept of treating skin ageing with oral administration of proteoglycans has stemmed from the works of Wadstein and colleagues in the 80s and early 90s, which has later been developed into PRT. Throughout the past few decades, several independent research groups have presented supporting evidence for the efficacy of treating the skin with natural proteoglycans of marine origin. Both in-vitro and in-vivo research confirmed the enhancement of cellular function and

improvement of skin integrity with this treatment approach. However, available proteoglycan-based treatments on the market are of diverse composition and origin, and conceivably, different efficacy levels. Some, such as salmon nasal cartilage extract, have a simple composition mainly composed of aggrecan, while Nourella[®] tablet with Vercilex[®] (Pharma Medico, Aarhus, Denmark) has a more complex, multi-molecular nature, rich in several skin-specific proteoglycans. Sano et al. reported that a crude salmon nasal cartilage extract (mainly aggrecan) amplified the proliferation of human dermal fibroblasts through the activation of Erk1/2. This growth stimulating effect of aggrecan is presumably related to its epidermal growth factor-like module [58]. Another study confirmed that this complex has the ability to cause prominent upregulation in both the proliferation and migration activity of skin fibroblasts [59]. Other lines of research propose that certain proteoglycans and their moieties provide cell protection against oxidative stress. For example, CS retains an ability to reduce the generation of free radicals; and aggregating CS proteoglycans with negatively charged side chains can contribute to reducing local oxidative stress by scavenging and binding redox-active iron [60]. The importance of this feature can well be recognised in the light of the fact that oxidative stress has determining roles in both intrinsic [61] and extrinsic skin ageing [62]. In-vivo therapeutic potential of oral proteoglycan therapy was studied in a model of accelerated skin ageing. Treatment with either a proteoglycan extract (composed mainly of aggrecan) or CS was started 3 weeks prior to skin UV irradiation and continued for 11 weeks thereafter. Proteoglycan treatment appeared to mitigate various photoaging effects of UV-B irradiation including skin erythema, increased water loss and decreased skin hydration level. Compared with controls, a dose-dependent, suppressing effect was also observed on epidermal and dermal hypertrophy, while CS alone had no effect [63]. These significant findings were subsequently verified in humans through a randomized, controlled study. A 2-week treatment course could improve skin elasticity, periocular wrinkling, conspicuous pores and blotches and induce positive corneum structural changes in both men and women [64]. Since Vercilex[®] is a multi-molecular extract rich in several bioactive proteoglycans, e.g., versican, decorin and biglycan, its level and spectrum of

clinical efficacy is higher and wider compared to the more basic aggrecan-based cartilage extracts (see below).

Nourella® tablet is a per-oral anti-ageing treatment for both chronological and accelerated skin ageing. The primary active ingredient of Nourella® tablet is Vercilex®, a unique proteoglycan complex, refined and optimised over decades to prevent and ameliorate the degenerative changes of ageing in human skin. One of its crude, primal versions (at the time branded as Imedeen) was used by Lassus et al. to treat premature skin ageing in an open-label pilot followed by a controlled, blinded trial. Subjects with photoaged skin were treated with 500 mg/day for 3 months. The majority of participants experienced improvements in wrinkling, mottles and skin dryness starting from the second month of treatment onwards. PRT with Vercilex® increased both skin thickness and elasticity indices of patients with an average age of ≈50 years such that, after treatment, these parameters were comparable to individuals in their 20s-30s; Whereas, before treatment, skin characteristics of the patients were at the level of 60+ year old individuals [65]. In a succeeding controlled trial, the clinical efficacy of a first-generation form of Vercilex® (with the working title Nourelle) was studied on volunteers of 35 to 65 years old with UV-induced premature facial skin ageing. 3 months of full-dose PRT with Vercilex® (500 mg/day) significantly reduced skin thickening due to elastosis by 55% (compared to no change with placebo) and mitigated skin laxity by 76% (compared to 4% with placebo) and dryness by 78% (compared to 23% with placebo). All objectively measured skin parameters, i.e. skin density, elasticity, corneometer and erythema indices, were enhanced after 3 months of Vercilex® therapy demonstrating an all-around structural improvement the participants reported positive changes in their skin consistency after 1-2 months of treatment with full dose and after 3 months with low dose of Vercilex® [66]. Per-oral treatment with Vercilex® was also shown to be effective in treating chronological skin ageing. A group of subjects, of whom 55% had moderate to severe symptoms of skin ageing, received a modified version of the Nourella® tablet with Vercilex® for 6 months. Subjective and objective

assessments performed every second month demonstrated a prominent incremental improvement in skin structure throughout the intervention period. At endpoint, skin thickness and elasticity were enhanced by 34% and 32%, respectively, in contrast with a decline in both parameters in the placebo group. In line with this, self-evaluation of treatment outcomes using VAS revealed positive changes that reached statistical significance after 4 months compared to the placebo [67]. These pieces of high-quality evidence support the conclusion that PRT with Vercilex® can effectively be utilised for the treatment of intrinsic and extrinsic skin ageing and to prevent their multiple serious complications. This unique method is also proven safe with no related side effects reported in published clinical trials.

In explaining the mechanism of action of PRT with Vercilex®, both direct and indirect pharmacodynamic mechanisms have been proposed. As mentioned before, intact proteoglycans and their degradation products absorbed through the intestine are likely to implement direct modifications in bioactivity of skin cells and skin fibrillogenesis. There is, however, evidence that natural proteoglycans may as well influence cutaneous cells indirectly by changing the composition of gut microbiota and their active metabolites [68].

4.2. Synergistic anti-aging effects of Vercilex® with Retilex-A®

In an earlier section on the bioactivities of natural retinoids, we mentioned that topical application of Retilex-A® can stimulate the proliferation of epidermal keratinocytes as well as the activity of fibroblasts and thereby boosts the production of ECM components. According to the evidence summarised previously, PRT shares this bioactivity with Retilex-A® and exhibits significant abilities to activate skin cells and improve the synthesis and arrangement of ECM microfibrils. Beyond that, as Wolf et al. have demonstrated [22], topical retinoids possess a range of anti-inflammatory properties. This key pharmacological effect has also been reported with specific marine proteoglycans [69, 70]. Therefore, combining per-oral Vercilex® with topical Retilex-A® produce substantial synergistic efficacy in activating skin cells and

improving the quality of dermal fibrillogenesis, two primary anti-ageing properties. This original concept has been put on trial in a long-term, double-blind, randomised, controlled, parallel group study. Subjects in the intervention group received Nourella[®] cream with Retilex-A[®] together with Nourella[®] tablets with Vercilex[®] twice daily for 16 weeks. The rejuvenating effects of Nourella[®] dual therapy could first be observed after 8 weeks. At endpoint, treated individuals displayed significant improvements in both objectively measured skin thickness (42% increase) and skin elasticity (35% improvement), while these parameters worsened in the placebo group. Before treatment, volunteers in both groups (with an average age of around 48 years) had facial skin characteristics typical of individuals in their 60s indicating the presence of premature skin ageing. Through comparison with an age-stratified sample of persons with similar ethnicity as the participants, the authors concluded that dual therapy with Nourella[®] cream and tablets had induced a prominent skin age-reversing effect of over 20 years. The efficacy of this novel approach was also reflected in the results of subjective evaluations. When asked to express their global clinical evaluation of the treatment outcomes on a VAS, the participants gave a score of 8.1/10 (± 3.1) to the active treatment and 0.2/10 (± 0.8) to the placebo after 4 months ($P = 0.0001$) [39]. Since the effect sizes of the treatment in this trial are higher than the ones achieved from monotherapy with Vercilex[®] [67], it demonstrates Retilex-A[®] and Vercilex[®] indeed have complimentary and compound synergistic effects.

5. Conclusions

Skin ageing is a multi-factorial condition that leads to considerable loss of skin functionality and a broad range of somatic and psychosocial complications and thus, deserves to be recognised as a clinical disorder. Evidence demonstrates that the progression rate and quality of the skin ageing disorder can be modified by effective topical and/or systemic treatments. Topical application of natural retinoids as the gold standard and per-oral administration of proteoglycans as a safe approach are among the treatments of choice for this condition. Retilex-

A[®] is a unique proprietary nano-encapsulated retinoid with enhanced bioavailability and skin penetration profiles and improved skin compatibility indicated for the prevention and treatment of intrinsic and extrinsic skin ageing. Comparative research demonstrated Nourella[®] cream with Retilex-A[®] to be more effective than a conventional formulation of retinoid in counteracting the pathological changes of skin ageing and causing a more lasting therapeutic effect. The clinical effects of RetileX-A[®] is on par with tretinoin, but it causes less side effects. Overall, the aggregation of clinical evidence signifies that the specific nano-encapsulation technique used in RetileX-A[®] significantly intensify and accelerate the anti-ageing efficacy of retinol ester. According to the recently emerged evidence, the turnover of skin proteoglycans undergoes conspicuous alterations as a function of age leading to proteoglycan dysmetabolism in the aged skin. Clinical studies revealed that PRT with Vercilex[®] ameliorates the symptoms of skin ageing, i.e., skin dryness, elastosis and laxity. This treatment is hypothesized to work directly via upregulating the activity of skin cells, normalising the rate of fibrillogenesis and anti-inflammatory actions, and indirectly through changing the composition of gut microbiota. This range of pharmacological properties shares features with the bioactivity of RetileX-A[®] in Nourella[®] cream in a synergistic fashion. Combining topical and per-oral anti-ageing treatments in 'Nourella[®] Skin Rejuvenation System' is a novel approach that covers several aspects of skin ageing, which efficacy is supported by controlled clinical research.

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Please contact the corresponding author.

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