Age-Reversing Drugs and Devices in Dermatology

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The quest for youth and beauty is an ongoing one. No organ conveys youth and beauty to the extent that skin does. Advances in research over the past several decades have yielded a tremendous amount of information on the molecular pathways involved in both intrinsic aging (natural) and extrinsic aging (photoaging). In this article, we aim to describe the molecular pathways that lead to an aged appearance and to describe the latest and most commonly employed drugs and procedures to reverse skin aging and stimulate the production of new collagen. With enhanced understanding of these molecular pathways, drugs and devices used to treat aging skin can be more precisely tuned.

It is well known that the skin is the largest organ in the human body. It provides a protective barrier against the environment, has a role in immunoprotection, and is an indicator of overall well being and health. One's skin is the outward appearance that one displays to the world. For this reason, it can be considered to be an organ associated with physical attractiveness and beauty. Albeit controversial, in most cultures in the world, beauty is associated with youth, and it is desirable to retain youth in professional and personal realms. Youth is conveyed through actions and attitude and, most importantly, through physical appearance.

Koblenzer has written that the quest for youthful beauty is considered a universal fact of life and aging is not considered desirable in today's world.[1] Others have posed the question of whether antiaging is a scientific concern or a social trend.[2] In the book *Face It*, two former models, now psychotherapists, argue that, for a woman, dealing with age-associated changes in appearance can be as daunting as dealing with financial loss or divorce. It is not necessarily about vanity, say the authors, but rather about the perceived loss of potential and self-doubts about one's future place in the world.[3] Whether or not one buys into such a value system, the area of enhancement and preservation of beauty will continue to grow exponentially in the foreseeable future.

Beauty is a subjective notion, yet there are standards that seem to remain consistent across cultures and societies. In general, beauty is associated with a youthful appearance. Young skin is characterized by homogeneous color, smooth texture, tautness, dewiness, a lack of irregular pigmented lesions (lentigines), and the absence of wrinkles. It is supple to the touch and lacks dryness. Aged skin is rough and wrinkled and has irregular pigmentation and areas of erythema (telangiectases). Such skin may be atrophic or hypertrophic and is dry on palpation (**Figure 1**).

The upkeep and maintenance of a youthful appearance have become more accessible through the invention and development of drugs and devices to reverse the changes seen in aging skin. This quest for youth has spawned an enormous industry in cosmetic agents and procedures as evidenced by the accelerated financial growth in this industry.

Why a particular person looks old or young for his or her age is poorly understood. It is likely that a combination of genetically inherited factors such as variants in the *MC1R* gene, hair graying, and lip height are coupled with extrinsic factors to produce a perceived clinical age.[4,5] Light-skinned individuals or those with Fitzpatrick skin types I and II, defined as skin that burns easily/never or rarely tans, tend to show premature aging much more noticeably than those with Fitzpatrick types IV–VI. Those who have had extensive exposure to ultraviolet (UV) light have prematurely aged skin. Darker skin types age more slowly because of the protective effects of melanin.

INTRINSIC VS. EXTRINSIC AGING

Intrinsic aging, also known as chronological aging or natural aging, is clinically manifested as fine wrinkles and a crepe-like texture to the skin on sun-protected sites such as the upper inner aspect of the arm or on the hip (**Figure 2**). The skin has an atrophic and dry appearance, and the color is generally homogeneous without dyspigmentation.

The hallmarks of extrinsic aging or photoaging are wrinkles and a rough texture on sun-exposed sites such as the

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Received 15 July 2010; accepted 19 September 2010; advance online publication 24 November 2010. doi:10.1038/clpt.2010.259

Young	
Clinical	**Histologic**
Homogeneous color	Organized, intact collagen
Smooth texture	"Stretched" fibroblasts
Few to no wrinkles	
Taut	
Hydrated	
Old	
Clinical	**Histologic**
Dyspigmentation	Decreased collagen
Wrinkles	Fragmented collagen
Dynamic	"Collapsed" fibroblasts
Fine	
Coarse	
Rough texture	
Dry	
Patchy erythema	

Figure 1 Clinical and histologic features of young skin and old skin.

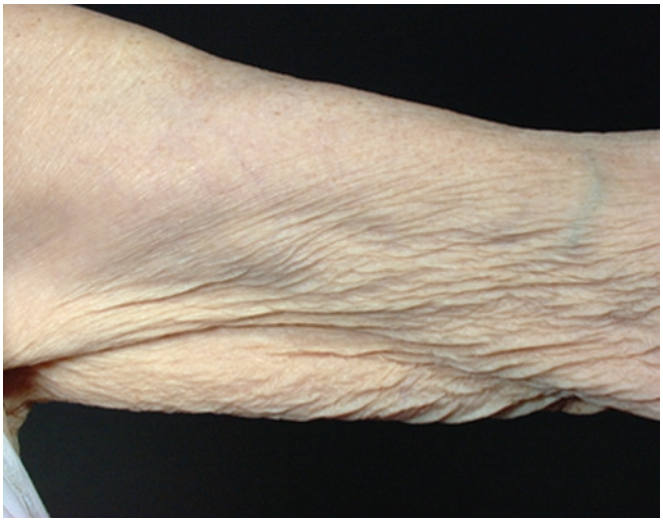

Figure 2 Intrinsic aging. Intrinsic aging, also known as natural aging, is clearly demonstrated on the upper inner aspect of the arm. The salient features are homogeneous color, atrophy, and fine wrinkling.

face and forearms. UV light is the major driver of extrinsic aging, but other factors such as tobacco use may be contributory. Two clinical phenotypes of extrinsic photoaging have been observed. In the more common type, namely, atrophic photoaging, the individuals have numerous fine wrinkles on the face, epidermal atrophy, telangiectases, and focal depigmentation. They also tend to have skin cancers and precancers and typically belong to Fitzpatrick skin type I or II. In the less common type, hypertrophic photoaging, individuals have deeper, coarser facial wrinkles and lentigines but few skin cancers. They tend to have Fitzpatrick type III or IV (**Figure 3**).[6,7]

Intrinsic and extrinsic aging are superimposed on each other. Intrinsic aging is characterized by subtle changes occurring over a lifetime, whereas extrinsic aging tends to occur in the setting of intrinsic aging and generally accelerates and exaggerates the process of intrinsic aging. Extrinsic aging is seen in skin of color but to a much lesser extent. In individuals with

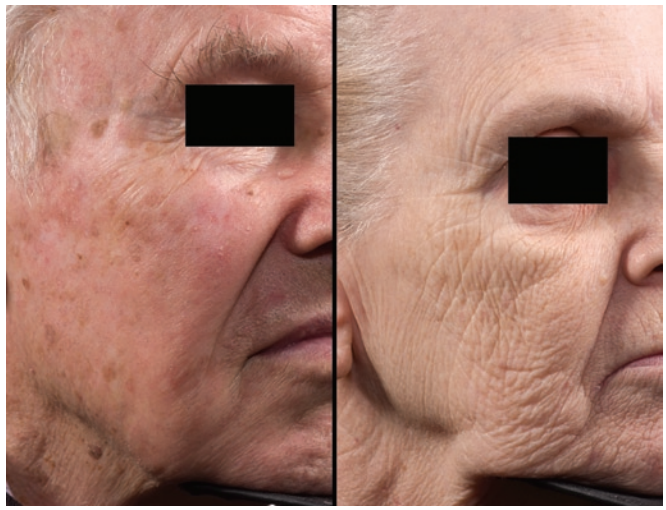

Figure 3 Extrinsic aging. Extrinsic aging is also known as photoaging and is superimposed on intrinsic aging. Ultraviolet light accounts mainly for the changes that are characteristic of this type of aging, although cigarette smoking is also a contributory factor. On the left, the more common form of photoaging is shown, namely, atrophic photoaging, which is characterized by lentigines, patchy erythema, telangiectases, fine wrinkling, and actinic keratoses. The hypertrophic variant is seen on the right, with deep, coarse wrinkles, and little dyspigmentation or erythema. Fewer actinic keratoses and skin cancers are seen in this variant.

darker skin, extrinsic aging is visible to a much lesser extent, with pigmentary changes predominating over textural changes (**Figure 4**).

MOLECULAR MECHANISMS IN AGING SKIN

In recent decades, much work has been done to elucidate the molecular mechanisms of aging in skin. A major factor that determines the appearance of the skin is the condition of the dermal collagen. Although there are differences between young and aged skin with respect to the condition of the epidermis, dermal collagen is the most important factor in whether the skin appears to be young or old. The dermal extracellular matrix is composed of type I collagen, proteoglycans, and glycosaminoglycans produced and secreted by fibroblasts. Type I collagen is the most abundant protein in the dermal extracellular matrix. It is responsible for the support and structure of the skin, and its loss and degradation are thought to result in the clinical phenotype of aged skin, specifically with regard to fine and coarse wrinkles.[8–10] Elastic fibers are another key protein in skin and reside in the papillary and reticular dermis. They provide elasticity and recoil capacity to skin. Certain diseases of elastic fibers, such as Marfan's syndrome (fibrillin-1 mutation) and Williams syndrome (elastin mutations), are characterized by loss of elasticity, much like that seen in aged skin. Clearly, loss and damage of elastic fibers are contributory to the clinical and molecular changes seen in aged skin, but, for practical reasons, the majority of the research work being carried out is focused on collagen.

Collagen fibers provide "scaffolding" for fibroblasts; this scaffolding is critical for the maintenance of the collagen network because it allows the fibroblasts to exist in a "stretched"

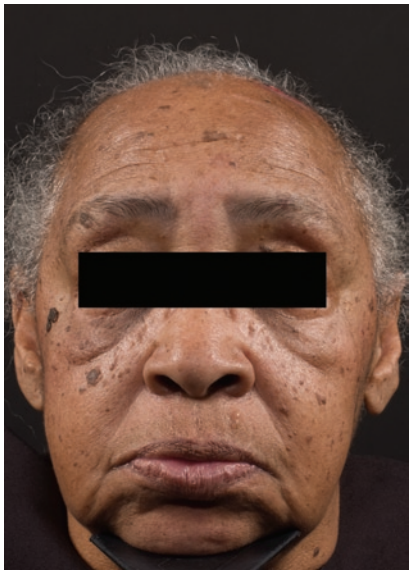

Figure 4 Skin aging in skin of color. In darker skin types, aging skin may be characterized more by pigmentary changes than by wrinkles.

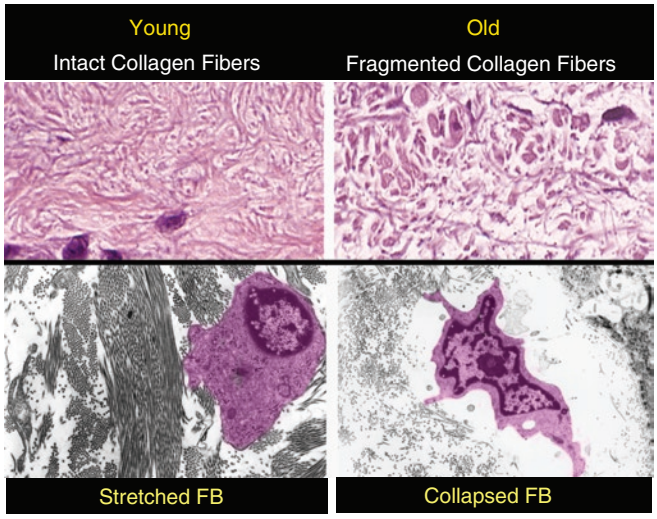

Figure 5 Electron micrographs (EMs) of young skin and old skin. Young skin is characterized histologically by intact, organized collagen fibers in the dermis. On scanning the EMs, it is seen that the fibroblast is in a "stretched" configuration and is in close proximity to collagen fibers. Abundant endoplasmic reticulum can be visualized in the cytoplasm, indicating active protein synthesis. In old skin, collagen is fragmented. The fibroblast structure has collapsed, and very little cytoplasm is apparent. The fibroblast has lost its connections to the surrounding collagen fibrils.

configuration, as is typical of young, healthy, non-sun-damaged skin.[11] The fibroblasts appear elongated, with abundant endoplasmic reticulum indicative of the active collagen protein–producing machinery. Collagen fibrils are intimately associated with these elongated fibroblasts and are seen in close proximity to them. In contrast, the fibroblasts in old and sun-damaged skin exist in a "collapsed" configuration, and the cells appear much smaller than the stretched fibroblasts. There is minimal endoplasmic reticulum in these fibroblasts as seen by electron micrography, indicating that the collagen-producing machinery is minimally productive. In old, sun-damaged skin, collagen fibrils are not seen in close proximity external to the fibroblasts and are fragmented. The relationship between the fibroblasts and the collagen matrix is an interdependent one, with the fibroblasts serving as the structure or anchor for the collagen fibers and the collagen fibers allowing the fibroblasts to exist in their "stretched" configuration (**Figure 5**).[10]

Extrinsic aging is associated with episodic photodamage, which culminates in permanent photoaging. Minimal erythema dose refers to the amount of UV radiation that causes barely perceptible skin reddening. A mere 0.01 minimal erythema dose leads to statistically significant injury that will induce the molecular sequence of events resulting in collagen deficiency, collagenase excess, and micro- and macro-scarring leading to solar scars. These additive solar scars are associated with the clinical presentation of wrinkles. Both UVB and UVA radiation lead to an increase in reactive oxygen species (ROS). Specifically, exposure to two minimal erythema doses leads to the production of H_2O_2, which gives rise to other oxygen free radicals within a matter of minutes. ROS play an important role in direct cellular damage (cell walls, lipid membranes, mitochondria, and DNA) as well as in molecular signaling. Through ROS signaling, transforming growth factor-β, a cytokine that promotes collagen production, is blocked, and the formation of new collagen is reduced. Also mediated through ROS signaling is the epidermal growth factor receptor pathway, which signals activator protein-1, a transcription factor responsible for upregulating collagenase, a matrix metalloproteinase that is important in collagen breakdown. The upregulation of collagenase leads to an increase in collagen fragmentation that in turn leads to a decrease in the mechanical tension of the fibroblast. Loss of mechanical tension leads to perpetuation of the cycle in which collagenase concentration is increased, leading to further collagen fragmentation and, ultimately, permanent collagen loss clinically manifested as permanent photoaging. Each UV insult results in solar scars, which manifest as a wrinkle[8,12,13] (**Figure 6**).

The sequence of events in intrinsic aging is similar but with a few notable exceptions. The damage in natural aging is continuous rather than episodic and is caused by the passage of time rather than by UV damage. The increase in ROS signaling leads to a decrease in collagen formation mediated by transforming growth factor-β, as in extrinsic aging. The increase in collagenase level, however, is mediated through the JNK pathway rather than through the epidermal growth factor receptor pathway.

The above discussion is focused on collagen in aging skin because most of the ongoing research is focused on this vital skin protein. There have, however, been findings to suggest that there is a difference between photoprotected and photodamaged skin with regard to extracellular protein matrix. The extracellular matrix is important because of its water-binding capacity. Four glycosaminoglycans were identified in skin samples: hyaluronic acid (HA), heparan sulfate, dermatan sulfate,[14] and chondroitin sulfate. In a study of photodamaged facial skin as compared with photoprotected postauricular skin used as a control, photoexposed skin was observed to have a significant increase in

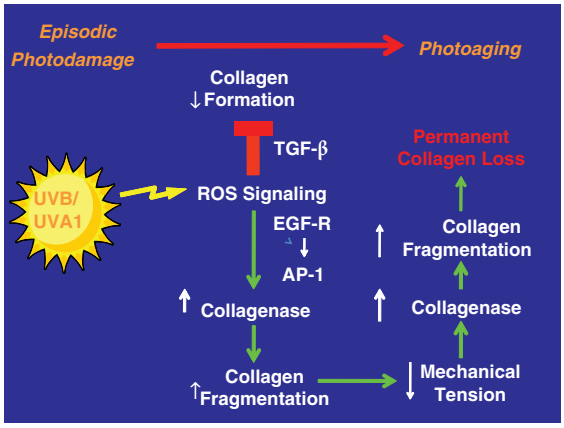

Figure 6 Sequence of events culminating in photoaging. Episodic ultraviolet (UV) B and ultraviolet A1 exposure leads to the generation of ROS including H_2O_2. ROS signaling leads to blockade of the TGF-β pathway, and new collagen formation is blocked. ROS signaling also induces EGF-R to signal AP-1, which upregulates the matrix metalloproteinase collagenase, the major enzyme responsible for collagen breakdown. The induction of collagenase results in an increase in collagen fragmentation, leading to a breakdown in the collagen scaffolding that supports the fibroblast. This loss in mechanical tension promotes further increases in collagenase level, resulting in collagen fragmentation. Repeated episodes culminate in permanent loss of collagen, clinically manifested as wrinkles and laxity of the skin. AP-1, activator protein-1; EGF-R, epidermal growth factor receptor; ROS, reactive oxygen species; TGF-β; transforming growth factor-β.

the content of HA and DS as compared with photoprotected skin. The increase in HA was associated with an increase in the amount of fragmented HA, which is abnormal and does not interact in the usual manner with water.[15]

THE SOLUTION

Medical and procedural interventions are available to improve aging skin. Medical interventions typically refer to topical agents that will prevent sun damage or stimulate the production of new collagen. These treatments are long-term solutions, and the results, although measurable, can be subtle and occur over fairly long periods of time. Medical treatments focus mainly on the issue of abnormal collagen; such treatments include the use of topical retinoids, select antioxidants, and, more recently, drugs that are used primarily to resolve precancerous skin lesions known as actinic keratoses. Medical treatments are usually in the form of prescription medications, but some are available as over-the-counter preparations.

Procedural treatments, on the other hand, tend to lead to more dramatic changes, and the effects are noted sooner than with medical therapy alone. These procedures are often employed in conjunction with medical therapy to combat the effects of aging skin. Procedures such as laser surgery can address dyspigmentation, redness, and textural changes. Soft-tissue fillers address the volume loss associated with aging. Botulinum toxin injections are used to treat dynamic wrinkles associated with repeated muscle movement and that ultimately lead to "fixed" wrinkles at rest. These procedures are administered by trained professionals and can be associated with significant side effects. Appropriate identification of individuals qualified to carry out these procedures is of the utmost importance (**Figure 7**).

Therapy	Main action
Medical	
Sunscreens	Prevent UV damage
Topical retinoids	Repair dermal collagen
Antioxidants	
N-acetylcysteine (NAC)	
Genistein	
Vitamin C	
Idebenone	
Peptides	Collagen?
5-Fluorouracil	Stimulate wound healing
Imiquimod	Restore epidermal thickness
Lasers/light based	
Ablative	Stimulate wound healing
Conventional CO_2	
Erbium:yag	
Nonablative	
Vascular	Resolve telangiectases
Q switched	Resolve dyspigmentation
Intense-pulsed light	Resolve telangiectases and dyspigmentation
Radiofrequency	Heat stimulates collagen production
Fractionated	
CO_2	Stimulate wound healing
Fraxel	
Injectables	
Botulinum toxin type A	Dynamic rhytides
Onabotulinum toxin	
Abobotulinum toxin	
Soft tissue fillers	Restore volume
Temporary	
Bovine collagen	
Porcine collagen	
Human collagen	
CL-HA	Restore volume
	H_2O retention
	Increase "stretched" fibroblast state
Semipermanent	
Poly-L-lactic acid	
Calcium hydroxylapatite	
Permanent	
Liquid silicone	
Polymethylmethacrylate suspended in collagen	
Miscellaneous	
Microdermabrasion	Stimulate wound healing

Figure 7 Age-reversing therapies and their main sites of action. CL-HA, cross-linked hyaluronic acid.

PHOTOPROTECTION

The only reliable way to prevent photoaging is through adequate protection against UV radiation from sunlight. Individuals should be educated to avoid the strong midday sun and to use sun-protective devices such as UV-protective clothing, broad-brimmed hats, umbrellas, and sunglasses. It is also strongly recommended that the use of artificial sources of UV light (i.e., sunlamps and tanning beds) be avoided because they emit UVA, which is known to be associated with premature skin aging and skin cancer.[16]

Sunscreens are broadly defined as agents that protect against UV damage, sunburn, wrinkles, and pigmentary change and should be considered to be adjuvant to the above

recommendations. They are rated in terms of their sun protection factor (SPF), which is an indicator of how long a person can remain in the sun before starting to develop erythema as compared with not using a sunscreen. SPF pertains only to UVB protection, not UVA protection. The SPF of a sunscreen increases the inherent time to sunburn or suntan. SPFs of sunscreens currently range from 8 to ~60–70. A higher SPF theoretically means that a sunscreen requires less frequent application. Sunscreens need to be applied frequently and abundantly because, in real-life situations, they tend to rub and wipe off with moisture from sweat and activities involving water.

Sunscreens may consist of physical or chemical blockers. Physical blockers work by blocking or reflecting UVA and UVB. Examples of physical blockers include titanium dioxide and zinc oxide. These agents are associated with a chalky opaque white appearance when applied, but newer formulations have improved the cosmesis of these agents. Specifically, nanoparticle technology has revolutionized drug delivery systems for these sunscreens by imparting a light, nongreasy feel to titanium dioxide and zinc oxide sunscreens. It also decreases the white, chalky appearance and allows these blockers to be spread more evenly over the skin's surface. There has been some concern about long-term risks relating to nanoparticle absorption and consequent systemic toxicity because nanoparticles have been associated with oxidative stress leading to protein, DNA, and mitochondrial damage and inflammation. To date, however, there is no evidence of dermal penetration of titanium dioxide and zinc oxide formulated in nanoparticles, and no particles have been found in the interfollicular epidermal tissue below the stratum corneum.[17,18]

Chemical sunscreens initially conferred protection only against UVB, but recent-generation chemical sunscreens protect against both UVB and UVA. Some examples of UVA blockers include oxybenzone and avobenzone. Recently, ecamsule, marketed as Mexoryl, received US Food and Drug Administration approval, and a daily cream containing ecamsule and avobenzone was found to protect against photoaging.[19] Properly applied sunscreens that protect against UVB and UVA can prevent UV-induced upregulation of matrix metalloproteinases and subsequent collagen breakdown (JJ. Voorhees and G. Fisher, unpublished data).

Polypodium leucotomos is a chemical extracted from a tropical fern that is found in abundance in Central and South America. In a study of the oral formulation of this chemical, a significant decrease in UV-induced erythema, sunburn cells, and cyclobutane pyrimidine dimers was observed, leading to the conclusion that it is an effective systemic chemoprotective agent for skin against UV radiation.[20]

RETINOIDS

Retinoids are vitamin A derivatives that have been used in dermatology over many decades to treat acne, psoriasis, and aging skin. The finding that patients being treated for severe acne with isotretinoin, an oral retinoid associated with significant birth defects, prompted great interest in topical forms of vitamin A for cosmetic improvement of aged skin. Despite the abundance of topical agents claimed to improve aging skin, there is substantial evidence of effectiveness only for topical retinoids. Kligman was one of the first to note that tretinoin ameliorates the signs of aging skin.[21] These drugs are typically applied every night and are associated with some irritation and desquamation during the early stages of therapy. These side effects tend to lessen with time, but, because the treatment involves continued use of the drugs, some patients find the side effects unacceptable. Topical retinoids have been considered to be phototoxins, but this classification is probably less clear than was initially thought. Several studies have suggested that they are neither phototoxic nor photoallergic *in vivo*.[22] In controlled studies, when skin is pretreated with tretinoin and irradiated with UV light, there is no effect on the minimal erythema dose, indicating that tretinoin has no phototoxic activity and lacks sunscreen properties as well.[8]

Tretinoin (all-*trans*-retinoic acid) is a topical vitamin A derivative that binds to and activates the nuclear retinoic acid receptor RAR; it is derived from sequential oxidation of all-*trans* retinol and all-*trans* retinaldehyde.[23] It is available in several strengths and formulations in the United States by prescription only. It is dispensed in several forms: cream, gel, and in microsphere technology as well as in an emollient base. Numerous studies with tretinoin have confirmed its antiaging properties. It has been noted to impact several features of photoaged skin; surface roughness, dyspigmentation, and fine wrinkles are consistently and significantly improved with tretinoin therapy.[24] The wrinkle improvement feature of this drug has generated much interest, including among physicians. Within the first week of tretinoin therapy, tactile smoothing of the skin is noted, corresponding with the histologic changes of compactness of the stratum corneum and spongiosis in the epidermis.[25,26] Wrinkle improvement is not seen until after 2–4 months of therapy. It has been noted that despite continued treatment with tretinoin the epidermis reverts to its pretreatment state. Therefore, the wrinkle improvement is attributed to dermal changes rather than epidermal ones.[27,28] The dermal changes resulting from tretinoin therapy are significant and well reported. As discussed earlier, type I collagen is the major structural protein of the dermis, and it is reduced and fragmented in aged skin. In an open-label study of treatment with tretinoin 0.05% cream to facial skin for a minimum of 6 months, the drug was demonstrated to thicken the collagen band in the papillary dermis.[29] In a 16-week, double-blind, vehicle-controlled study of 30 subjects with photoaged forearms and facial skin, there was significant improvement in photodamaged skin as measured in relation to clinical and histological end points.[25] A landmark study of topical tretinoin applied to sun-damaged forearm skin and sun-protected buttock skin demonstrated significantly less collagen type I formation at sun-damaged sites as compared with sun-protected sites. Treatment of the sun-damaged sites for 10–12 months with topical tretinoin led to an 80% increase in extracellular collagen I staining as compared with vehicle-treated skin, which showed a 14% reduction in collagen I staining (**Figure 8**).[30]

Retinol, retinol derivatives, tazarotene, and adapalene for aging skin are discussed in detail in the **Supplementary Data** online.

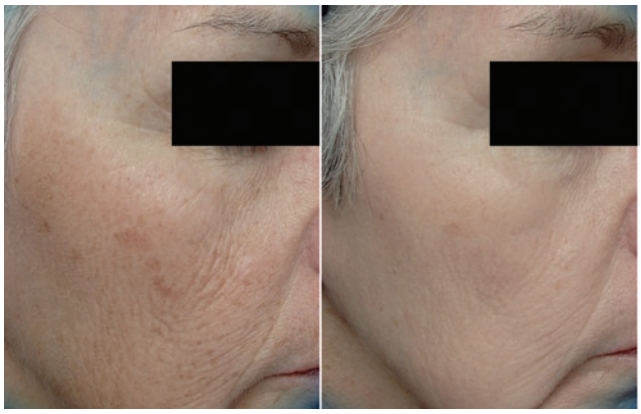

Figure 8 Tretinoin therapy for photoaging. After a course of tretinoin 0.05%, signs of photoaging are reduced. The subject has clinically significant reduction in lentigines and wrinkling, and the skin has a smoother and younger appearance after the treatment.

ESTROGEN

The effects of estrogen on skin and changes seen in menopause are discussed in the **Supplementary Data** online.

A recent study evaluated the effectiveness of topical estradiol in stimulating production of collagen I and III in naturally aged and photoaged skin in postmenopausal women and age-matched men. The subjects underwent a 2-week topical application of various strengths of estradiol or vehicle to photoaged or naturally aged skin, and biopsies were performed after the treatment. In this study, collagen production in women was seen to be stimulated in sun-protected skin only. This effect was also observed in sun-protected skin of the male controls but to a lesser degree. Photoaged skin of the forearms in both women and men showed no increase in collagen production with this regimen.[31]

ANTIOXIDANTS

Antioxidants are natural or synthetic agents that protect cells from damage and scavenge free radicals. As discussed earlier, ROS play critical roles in skin-aging pathways, and targeting them is an attractive therapeutic consideration. Much interest has been generated in these compounds for the treatment of aging skin.[32] In general, oral supplementation with antioxidants has not been shown to be effective in improving photoaged skin. There are many pitfalls in carrying out this type of research activity. The designing of antioxidant trials involves identifying appropriate candidate agents, determining dosing schedules and duration of therapy, and allowing for potential side effects such as toxicity and drug interactions.

A few antioxidant compounds have drawn more attention than others; these include *N*-acetylcysteine, genistein, vitamin C, and the synthetic coenzyme Q10, known as idebenone. A discussion about the important studies with these antioxidants can be found in the **Supplementary Data** online.

PEPTIDES

Peptides are amino acid chains that are fragments of large proteins such as collagen. There is evidence in wound-healing models that certain peptides penetrate into the dermis and stimulate the production of collagen. Because of this property, there is interest in using peptides in topical antiaging therapies. One such peptide, Pal-KTTS, marketed as Matrixyl (Sederna, France), is an ingredient in a number of cosmeceuticals. It promotes a youthful appearance of the skin and has been shown to stimulate collagen production in wounds.[33] Another peptide used in cosmeceuticals is a tripeptide–copper complex (GHK–copper peptide) that has been shown to increase collagen levels in wounds.[34] However, none of the peptide-containing agents has been rigorously evaluated by means of double-blinded, vehicle-controlled studies for their effects on aging skin.

5-FLUOROURACIL

Topical 5-fluorouracil (5-FU) has been used since the 1960s to treat actinic keratoses and certain superficial nonmelanoma skin cancers. Early findings were that patients treated with topical 5-FU had a more youthful appearance to their skin and that wrinkles were reduced after treatment,[35] leading to an excellent cosmetic and therapeutic result. In recent work on the use of topical 5-FU in the treatment of actinic keratoses and photoaging, these early observations were confirmed by means of molecular measurements. In a study of 21 subjects with moderate to severe photoaging and actinic keratoses, topical 5-FU was applied twice daily for 2 weeks in accordance with the manufacturer's recommendations. Skin biopsies were performed at baseline, immediately after the last application of 5-FU, and at several points over a 6-month period. Gene expression of the effectors of epidermal injury, inflammation, and extracellular matrix degradation was significantly increased. Levels of type I procollagen mRNA and protein were significantly increased at weeks 4 and 24, respectively. After a course of topical 5-FU, the predicted series of events associated with wound healing occurred. This suggests that even minor epidermal perturbation can lead to statistically significant clinical and molecular improvement in aged skin. The severity of wrinkles, dyspigmentation, and overall global photoaging, as well as of actinic keratoses, was reduced by week 24.[36] For patients unwilling to undergo costly laser resurfacing procedures and for those with actinic keratoses, topical 5-FU can be considered part of the antiaging armamentarium.

IMIQUIMOD

Imiquimod 5% cream is a topical immune modulator approved by the US Food and Drug Administration for the treatment of genital warts, superficial basal cell carcinoma, and actinic keratoses. A few small studies have examined the antiaging properties of imiquimod. In one study, 10 women with moderate facial photodamage but without actinic keratoses applied imiquimod cream 5 times a week for 4 weeks. Global clinical assessments by a dermatologist and by the subjects themselves revealed that imiquimod led to wrinkle reduction and improvement in dyspigmentation. The epidermal changes characteristic of aging skin were diminished after therapy, notably atrophy and atypia; however, dermal changes were not observed in this short study.[37] In another study, pre- and post-treatment biopsy specimens were examined for the histologic changes associated

with daily imiquimod therapy for 3 months for lentigo maligna. Significant improvements were noted in papillary dermal fibroplasia and melanization. Epidermal thickness was restored to normal following therapy.[38]

ABLATIVE LASER RESURFACING

For many years, laser procedures have been used to reverse sun damage and enhance the condition and appearance of aged skin. The use of lasers is based on the principles of selective photothermolysis. This concept was put forth by Anderson and Parrish in 1983 and proposes that thermal tissue injury is selective to laser light generated with pulsed lasers.[39] Ablative laser resurfacing refers to procedures that remove skin in a controlled manner, leading to wound healing and a subsequent improved appearance of the skin. Descriptions of the molecular changes seen in the skin after CO_2 laser ablation and of the use of the Er:YAG laser are available in the **Supplementary Data** online**.**

Ablative laser resurfacing is especially useful for improving fine facial rhytides of the perioral and periorbital regions. Coarser wrinkles are reduced but, typically, not eradicated. The tightening effect does not seem to significantly improve dynamic rhytides. Ablative resurfacing is associated with a recovery time on the order of 2 weeks for skin re-epithelialization, with discomfort requiring sedation, and with the potential for scarring. Also, the method cannot be used to resurface medium and darker skin types because of the risk of hypopigmentation or even hyperpigmentation. Post-procedure erythema can persist for weeks to months after resurfacing. Vigilant post-procedure wound care is needed to ensure proper re-epithelialization. Typically, antivirals and antibiotics are prophylactically prescribed given the increased susceptibility to herpes simplex virus and bacterial infections during the period immediately after laser treatment. Isotretinoin therapy within the previous 12 months is a contraindication to laser resurfacing of any type.

NONABLATIVE LASER REJUVENATION

Nonablative techniques to improve the skin's appearance include light sources to (i) target dermal remodeling, (ii) alleviate erythema and telangiectases associated with aged skin, (iii) reduce dyspigmentation, and (iv) reduce textural irregularities associated with rhytides. Because of the side effects and the considerable recovery time associated with ablative laser resurfacing, there has been tremendous interest in recent years in developing lasers that use nonablative techniques to stimulate dermal collagen to induce remodeling. These lasers are typically in the mid- to longer infrared wavelengths. The 1,320-nm Nd:YAG is the prototype of a nonablative laser system and is considered a mid-infrared laser along with the 1,450-nm diode and 1,540-nm Er:glass lasers. In several studies with the 1,320-nm Nd:YAG laser, neocollagenesis was noted at 6 months after the treatment, along with minimal to mild improvement with respect to rhytides and scars.[40,41] Using a single treatment to photoaged forearm skin with either the 585-nm pulsed dye laser (PDL) or the 1,320-nm Nd:YAG, Orringer *et al*. were able to demonstrate statistically significant increases in type I procollagen mRNA expression of 47% ($P < 0.05$) and 84% ($P < 0.05$) above baseline for the two laser treatments, respectively. Although forearm skin was treated rather than facial skin, and it was a single treatment rather than serial treatments as would be performed in real life, quantifiable changes were seen.[42] It is not generally believed, however, that the changes resulting from nonablative techniques are similar to those seen with ablative laser resurfacing.

Light sources that target erythema and vessels include the 532-nm potassium titanyl phosphate laser and the 585- or 595-nm PDLs. The 532-nm potassium titanyl phosphate laser is especially useful for the treatment of facial telangiectases that are clinically visible. PDLs demonstrate the most significant reduction in facial erythema and superficial vascular lesions such as spider angiomas. PDLs can be adjusted to purpuric or nonpurpuric settings. The purpuric settings typically yield slightly better results in treating facial erythema, but this treatment is associated with a recovery period of ~2 weeks. In addition to the increase in type I procollagen mRNA expression shown in the study discussed earlier, PDL treatment was also reported to result in a reduction in rhytides, with histologic evidence of collagen remodeling and increased cellularity.[43]

Q-switched lasers, intense pulsed light, and radiofrequency for the treatment of aging skin are discussed in the **Supplementary Data** online.

FRACTIONAL RESURFACING

Fractional resurfacing, or fractional photothermolysis, describes the technology in which only a portion of skin is thermally altered, leaving normal, untreated skin surrounding the treated zone.[44] In fractional resurfacing, microthermal treatment zones are produced, representing columns of thermally injured skin. This technique was designed with the idea of ensuring greater safety throughout the laser resurfacing procedure. Both ablative and nonablative fractional resurfacing systems are available. Ablative fractional systems target water in the epidermis and the dermis and create wounds that heal over a period of ~ 1 week. Ablative fractional systems include CO_2 (10,600 nm), yttrium scandium gallium garnet (YSGG) (2,790 nm), and erbium-doped yttrium aluminum garnet (2,940 nm). The indications for ablative fractional resurfacing are the same as for traditional ablative resurfacing: rhytides, photodamage, and acne scarring. As compared with traditional ablative resurfacing, ablative fractional resurfacing is associated with a shorter recovery period with less discomfort for the patient because the surface area that is thermally damaged is smaller than the area involved in conventional resurfacing. Patients undergoing fractional resurfacing experience less oozing, crusting, edema, and erythema, although scarring has been reported in more aggressive settings.[45] The reduction in rhytides and photodamage is less impressive than with traditional ablative resurfacing but is nevertheless considered quite good, given the briefer and more comfortable recovery period.

Nonablative fractional systems also target tissue water but without ablation. Examples of these systems include 850–1,350-nm infrared, 915-nm radiofrequency, 1,440-nm neodymium-doped yttrium aluminum garnet lasers, and 1,550-nm erbium lasers. Nonablative fractional lasers are used to treat periorbital rhytides, textural problems associated with

photodamage, acne scarring, and melasma. With nonablative fractional systems, serial treatments are required at 2- to 4-week intervals. The risks of nonablative fractional rejuvenation of the skin are low with respect to permanent scarring. Patients should be cautioned to expect redness, swelling, and a "gritty" feeling to the skin that lasts for up to 1 week. Darker-skinned individuals with Fitzpatrick skin types III–V may experience some degree of hypopigmentation and should be cautioned about this side effect.

MICRODERMABRASION

Microdermabrasion is a technique performed by physicians, nurses, and estheticians that is used for very superficial skin resurfacing. It has been touted to be useful in reducing acne, scars, and wrinkles and is considered a minimally invasive procedure with very little associated risk. Microdermabrasion systems differ based on the source of the abrasive component. Inert crystals such as aluminum oxide or sodium chloride are propelled at the skin through a handpiece; this is the most popular type of system. These are closed-loop systems consisting of a receptacle of unused crystals attached to a source of compressed air that propels crystals through a tubing and then through a disposable or sterilizable handpiece. Such a system is designed to prevent cross-contamination between patients. Another system employs a handpiece that is embedded with an abrasive stimulus, such as diamond fragments, that contacts the skin directly and produces mechanical dermabrasion.

There have been only a handful of studies examining the efficacy of microdermabrasion for wrinkle reduction. There is some evidence that microdermabrasion works to improve wrinkles and skin texture. In one study, subjects who underwent weekly facial microdermabrasion treatments every 8 weeks reported improvement in skin texture. Review of pre- and post-treatment facial photographs did not demonstrate improvement when evaluated by medical professionals, but lay observers noted reduction in fine wrinkles. Biopsies performed after 8 weeks demonstrated a thickening of the epidermis, with an increase in collagen content as evidenced by Masson's trichrome staining test.[46] In another study, subjects noted reduction in textural irregularities, roughness, and mottled pigmentation but did not notice any change with regard to wrinkles.[47] More rigorous studies of the technique were performed by Karimipour *et al.*, who studied the effects of a single aluminum oxide microdermabrasion treatment on sun-protected skin on the buttock and found that there was a statistically significant elevation in the levels of molecules that regulate matrix metalloproteinases that degrade collagen. Importantly, and interestingly, there was minimal disruption of the stratum corneum, suggesting that significant dermal changes can be seen in the absence of epidermal interruption. Specifically, concentrations of the proinflammatory transcription factor activator protein-1 and cytokines interleukin-1β and tumor necrosis factor-α were elevated. The elevation in levels of matrix metalloproteinases allows the removal of damaged collagen and the placement of new collagen. In this study, 20% of the subjects had an increase in type I collagen production as evidenced by immunohistochemical staining.[48] In a study involving photodamaged forearms, Karimipour *et al.* used a diamond-studded handpiece with varying degrees of abrasiveness and demonstrated that the coarse-grit, rather than medium-grit, handpiece was associated with rapid induction of cytokeratin 16 and activation of activator protein-1. Dermal remodeling was noted to follow this wound-healing response, highlighted by induction of types I and III procollagen.[49]

SOFT-TISSUE INJECTABLES

Dermal fillers improve the appearance of the skin with respect to lines, wrinkles, and certain types of scars by "filling in" areas that have experienced loss of collagen and structure. This is the second most commonly performed cosmetic procedure, botulinum toxin injection being the most common. Typically, dermal fillers are injected into the lower two-thirds of the face; the most common sites are the nasolabial creases. Other commonly injected sites that show improvement with this treatment include the "marionette lines," or lines between the angles of the mouth, the chin, and the tear troughs. Deficiency in lip volume can be corrected with these fillers. The prejowl sulcus, between the chin and the mandible, is often a site of volume loss, which can be satisfactorily corrected with soft-tissue fillers.

The procedure for administering soft-tissue injections typically involves a topical anesthetic or regional nerve blocks. Injections of the agents are performed using either a serial puncture technique or a linear fanning technique. Temporary swelling and bruising may result, especially when patients are taking blood thinners such as aspirin, nonsteroidal anti-inflammatory drugs, warfarin, and vitamin E. The many agents that are available for use as dermal fillers can be categorized as temporary, semipermanent, and permanent fillers. Temporary fillers include bovine collagen (Zyderm and Zyplast), porcine collagen (Evolence), and human collagen (Cosmoderm and Cosmoplast) as well as crosslinked HA fillers (Perlane, Restylane, Juvederm). Semipermanent fillers include poly-L-lactic acid (Sculptra) and calcium hydroxylapatite (Radiesse). Permanent fillers include liquid silicone and polymethylmethacrylate suspended in collagen (Artecoll).

HA fillers are the most popular; they are widely available in several variations, each distinguished by its crosslinking properties. HA is an endogenous polysaccharide that binds water and imparts turgor to the skin. When injected into the skin in its native form, it hydrolyzes rapidly. With crosslinking, however, it is stabilized and degrades at a much slower rate in the dermis. HA fillers are derived from bacteria and rooster combs, and generally last for 4–5 months. "NASHA" is a term used to describe the nonanimal stabilized form of HA.

Anecdotally, physicians have observed that injecting collagen and HA fillers repeatedly leads to patients requiring less of the product over time. Perhaps the effect of such agents goes beyond a mere "filling" effect. Recent work has suggested that the mechanism of action of HA fillers extends well beyond their space-filling properties.

In a study of 11 subjects with photodamaged forearm skin, HA filler was injected into one forearm and saline vehicle into the other forearm. Biopsies were taken at 4 and 13 weeks, and it

was found that the increase in the level of type I procollagen protein at the HA-injected site was >90-fold higher than that at the saline-injected site. Electron microscopy of the HA-injected sites demonstrated the fibroblast to be in a "stretched" configuration and the endoplasmic reticulum to be in an activated state, implying that the collagen-producing machinery of the fibroblast was active. The saline-injected site showed the fibroblast remaining in its collapsed state, with minimal evidence of the endoplasmic reticulum being activated, as in baseline sun damage.[50]

The mechanism of action of HA fillers is multifactorial and includes mechanical filling of the space, water binding (due to inherent properties of the polysaccharide), preservation of mature collagen (by decreasing the production of collagenase), and an increase in production of new collagen (by means of mechanical tension on the fibroblast).

BOTULINUM TOXIN

Dynamic wrinkles result from overactive movements of underlying muscles leading to skin creases; these are fairly easily corrected with botulinum toxin type A. This has become the most common cosmetic procedure in the United States, and extensive clinical experience confirms that it is both safe and effective.[51] Dynamic wrinkles are typically seen in the top third of the face, particularly in the glabellar complex, the forehead, and the lateral periorbital sites. The skin at these sites becomes creased because of repeated contraction of the muscle. Another site that is prone to dynamic wrinkles is the perioral region.

The US Food and Drug Administration has approved *Clostridium botulinum* toxin type A injections for cosmetic treatment of glabellar rhytides. Two versions of botulinum toxin type A have been approved by the agency: onabotulinum toxin (Botox) and abobotulinum toxin (Dysport). The dosing schedules of the two products are different. The neurotoxin—a purified protein derived from botulinum—works by inhibiting the neurotransmitter acetylcholine at the neuromuscular junction. The heavy chain of the protein binds the toxin to the presynaptic cholinergic nerve terminal. The light chain cleaves SNAP25, which prevents vesicles from fusing with the membrane and prevents the release of acetylcholine into the neuromuscular junction. Collateral sprouting of new nerve terminals over time leads to restoration of function. When injected into the glabellar complex, which consists of the procerus and corrugator muscles, the treatment paralyzes these dynamic wrinkles or "frown" lines. Repeated injections lead to softening and smoothing of the lines at rest. The injections are also used for dynamic rhytides of the forehead (frontalis) muscles, as well as for those in the lateral periorbital regions (orbicularis oculi), commonly known as "crow's feet." Other common uses of the neurotoxin include treatment of the orbicularis oris in very small doses to reduce perioral rhytides and injection into the mental crease and platysmal bands.

The injections are associated with temporary discomfort and potential bruising, if some of the small facial blood vessels are inadvertently injected. This is especially true in patients taking aspirin, nonsteroidal anti-inflammatories such as ibuprofen, and certain over-the-counter vitamins such as vitamin E, which can lead to an increase in bleeding time. Inadvertent injection of botulinum toxin into the region of the mid-pupillary line can lead to eyelid ptosis; typically, this resolves within a few weeks. The duration of the beneficial effect of botulinum toxin injections is ~4 months.

SUMMARY

Our understanding of skin aging, both natural aging and photoaging, has advanced significantly over the past several decades. The desire to maintain a youthful appearance is pervasive in our society, and the demand for medical and procedural treatments to achieve a youthful appearance continues to increase. With better understanding of the molecular events culminating in the breakdown and repair of collagen, it is hoped that newer and more sophisticated therapies will be developed for its repair and restoration. There are undesirable side effects and economic costs associated with the treatments, both medical and procedural, that limit their widespread use.

SUPPLEMENTARY MATERIAL is linked to the online version of the paper at http://www.nature.com/cpt

CONFLICT OF INTEREST

The authors declared no conflict of interest.

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