Facial Rejuvenation in the Triangle of ROS

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Received June 19, 2009; Revised Manuscript Received July 24, 2009

ABSTRACT: Recently, we introduced into the conventional catalogue of biological aging a new determinant: ordered interfacial water layers. The discovery of their tunability with skin-tolerated levels of 670 nm light inspired a model, which suggested that the light, by interaction with ordered interfacial water layers in the extracellular matrix, would reverse elastin degeneration. We validated the model in a 10 month self-experiment and arrived at an effective facial rejuvenation program. Importantly, during the experimental phase we avoided extreme oxidative stressors, in particular exposure to extensive ultraviolet and infrared radiation as well as air pollution. Here we report on the adaptation of our model to the extreme oxidative stress levels prevalent in numerous polluted megacities. The results of the extension comprise a new understanding of the protective function of the skin acid mantle, new predictive insight into effects of reactive oxygen species (ROS) on interfacial water layers, and their implication in processes of biological aging, including depletion of follicular stem cell reservoirs and telomere shortening, and led to the design of an accelerated skin rejuvenation method.

Earlier we demonstrated that extended periodic irradiation with intense 670 nm light, generated by light-emitting diodes (LED), significantly reduces facial wrinkle levels.1 Our previous study was inspired by the results of laboratory experiments performed on model surfaces suggesting that the irradiation of elastin fibers will be instrumental in restoring skin elasticity. Elastin is the protein that provides elasticity to our skin, heart, and arteries. Natively, elastin is hydrophobic but becomes progressively hydrophilic with the physiological changes in the extracellular matrix associated with biological aging. Prerequisite for the functional performance of the elastin fibers is the preservation of a contrast in polarity between their surface and their direct environment. Diminishing contrast promotes dysfunction. The conversion from hydrophobic to hydrophilic is mediated by deposition of an interlayer consisting principally of amino acids, fatty acids, and calcium salts.2 Native elastin is necessarily coated with a predominantly crystalline interfacial water layer, as predictable from theory2 and follows from laboratory experiments performed on hydrophobic model surfaces, including polystyrene3,4 and hydrogenated nanocrystalline and natural diamond.5–8 The bonding stability of interfacial water molecules on solids depends on their affinity to the solid surface. The affinity is associated with a curvature-dependent asymmetry in charge distribution (for water molecules, the surface charge is H bond, this result offers an explanation to the origin of the extremely low friction coefficients reported on hydrogenated diamond-like carbon surfaces.9 The relevance of interfacial water layers is not limited to nanoscale processes in the extracellular microenvironment, where we exploited the finding that 670 nm light increased the fluidity of interfacial water layers masking hydrophilic surfaces.3 The process of liberation, re-establishment of the native surface polarity, and eventually restoration of the functionality of the elastin fibers received support from the simultaneous activation of cellular metabolic processes in the dermis. The activation of cellular metabolic processes by light (laser or LED) is routinely exploited in clinical practice to facilitate the uptake of properly administered chemical substances by the skin, for instance, to accelerate the healing of complicated wounds, complementary to the actual light effect. The cooperative interplay between the physicochemical and biological effect of light is based on ample evidence obtained individually for each part, by us and other groups, in laboratory experiments and clinical studies, respectively.

Clearly, the biological relevance of the order of interfacial water layers is not limited to nanoscale processes in the extracellular matrix. The order is believed to play a key role in modulating a variety of bidirectional flow processes in the cell, for instance, in nuclear pores, where water and water-soluble molecules are selectively transported in and out of the nucleus across the nuclear envelope. Correlations between metabolic flow processes across the cell membrane and traffic of cargo across the nuclear membrane, with water layers lining the channels’ (pores’) entrance and/or wall, represent an unexplored field, specifically with regard to the order of the interfacial water involved, and importantly, the impact of the environment on the order of the interfacial water, for instance, oxidative stress and chemical alterations related to climate change and air pollution — a highly attractive multidisciplinary arena. Therefore, the focus on the facial skin — a major target of environment-provoked oxidative stress — is not a coincidence.

Triangle of ROS. Natural ultraviolet and infrared radiation and air pollution, including but not limited to hydrophobilized carbon particles,16 with sizes ranging from a few nanometers to several micrometers, cause independently significant structural damage to the exposed skin — stratum corneum, viable cells, and extracellular matrix — partly by contributing to an increase of ROS levels. The total damage caused by the interplay of these components appears, however, to exceed that of their sequential

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DOI: 10.1021/cg900688g
Radiative energy transfer. In a worst case scenario (megacity with intense air pollution) the order of interfacial water layers, both on the epidermis and deep in the dermis, will be coated by a nanoscopic, negatively charged ROS, which, as shown for a variety of hydrophobic surfaces, will instantly be depleted when exposed to visible light of the intensity of the solar radiation.23,24 In contrast, on hydrophilic surfaces the same light intensities only increase the fluidity of the nanoscopic water layers, however, without depleting them. This observation is crucial for understanding the role of the acidic pH of our skin. Considering that the largest fraction of the airborne pollution particles is hydrophilic, it becomes clear that by accentuating the glue-like nature of the interfacial water, a neutral or alkaline pH of the skin (converting the top skin layer practically to hydrophilic) would reinforce the adhesivity of the particles eventually landing on the skin. Because of the specific size range, such particles have the capacity to effectively block the pores of the skin (both hair follicles and sweat pores). At higher concentrations, they may completely set off the thermoregulatory function of the skin. By casting shadows on the skin, the particles are instrumental in preserving the glue-like character of the interfacial water layers established between them and the skin. This mechanism is not only valid for pollution particles originating from anthropogenic sources — it holds also for volcanic soot, sand, organic debris, pollen, bacteria, viruses, and in general all kinds of pathogens. Probably, the immobilizing potential of the shadow increases with particle size. Obviously, by keeping its pH acidic, our skin is perfectly protected both against overheating by potentially pore-blocking particles and against infections caused by the attachment of microorganisms. Presumably, we are facing here an extraordinarily efficient evolutional adaptation. In summary, the biological relevance of ordered interfacial water layers is not restricted to the functionality of the elastin — in humans they also play a role in skin defense. By comparison, the skin of cats and dogs, whose skin is more or less protected by fur, presents pH values close to neutral.

**Accelerated Facial Rejuvenation.** From the aforementioned scenario, it becomes evident that prolonged bombardment with ROS, as for instance, stimulated by a persisting interplay of the oxidative stressors represented in Figure 1, will convert hydrophobic native elastin to hydrophilic, and/or accentuate the hydrophilic character of matured elastin, thereby transiently restricting the elastic function of the elastin fibers, encouraging their immobilization. The transition is mediated by changes in the order of the interfacial water layers enveloping the elastin fibers. Surprisingly, the generation of ROS in the dermis is not only triggered by the left (UV) and right (IR) side of the triangle of

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**Figure 1.** Hydrophilized airborne pollution particles — nanoparticles (NP) and microparticles (MP) — can block the pores of the skin, thereby inhibiting skin respiration. The sun, emitting ultraviolet (UV), visible, and infrared (IR) radiation, is the principal factor in skin aging. As all the assumptions entering the triangle of ROS is useful for modeling the interplay between different oxidative stressors and their synergistic impact on biological aging. As all the assumptions entering the triangle of ROS (Figure 1) and derived from the interplay of its components with elements of the biosystem are of a qualitative character (do not depend on the assumption of special sets of numerical data) we are justified in expecting, if the whole way of considering is coherent, accurate model predictions.

**Skin Acid Mantle.** The outermost layer of healthy skin is hydrophobic with a slightly acidic pH between 4 and 5.20 present with a slighty acidic pH between 4 and 5.21 which changes to negative at neutral pH.21 Whereas the acid mantle of the skin has been described in the literature for more than 100 years, its provenance and intrinsic functions are intensively discussed. New research ascribes to it, however, two vital protective functions: epidermal permeability barrier and antimicrobial barrier. Interestingly, these functions are consistent with those of ordered interfacial water layers on hydrophobic surfaces in general, and because of the anticipated excess protons at the acid mantle, on healthy skin in particular.2 Whereas on hydrogenated diamond the bond and polarization of the first layer of interfacial water molecules is determined by the C-H bond (in agreement with experiments), it is reasonable to assume on healthy skin a similar ordering, induced by the interfacial layer of excess protons. Figure 2 illustrates the principle and compares the organization of water molecules on hydrogenated diamond with that on healthy skin. Lacking the stabilizing C-H bond, the ordering on skin will be less pronounced (more statistical) than on hydrogenated diamond. However, as long as the skin maintains its acidic pH, its outermost layer will be coated by a nanoscopic, predominantly ordered water layer, which, as shown for a variety of hydrophobic surfaces, will instantly be depleted when exposed to visible light of the intensity of the solar radiation.23,24 In contrast, on hydrophilic surfaces the same light intensities only increase the fluidity of the nanoscopic water layers, however, without depleting them. This observation is crucial for understanding the role of the acidic pH of our skin. Considering that the largest fraction of the airborne pollution particles is hydrophilic, it becomes clear that by accentuating the glue-like nature of the interfacial water, a neutral or alkaline pH of the skin (converting the top skin layer practically to hydrophilic) would reinforce the adhesivity of the particles eventually landing on the skin. Because of the specific size range, such particles have the capacity to effectively block the pores of the skin (both hair follicles and sweat pores). At higher concentrations, they may completely set off the thermoregulatory function of the skin. By casting shadows on the skin, the particles are instrumental in preserving the glue-like character of the interfacial water layers established between them and the skin. This mechanism is not only valid for pollution particles originating from anthropogenic sources — it holds also for volcanic soot, sand, organic debris, pollen, bacteria, viruses, and in general all kinds of pathogens. Probably, the immobilizing potential of the shadow increases with particle size. Obviously, by keeping its pH acidic, our skin is perfectly protected both against overheating by potentially pore-blocking particles and against infections caused by the attachment of microorganisms. Presumably, we are facing here an extraordinarily efficient evolutional adaptation. In summary, the biological relevance of ordered interfacial water layers is not restricted to the functionality of the elastin — in humans they also play a role in skin defense. By comparison, the skin of cats and dogs, whose skin is more or less protected by fur, presents pH values close to neutral.
ROS (Figure 1), but apparently also by the visible part of the spectrum. In vitro studies indicated a dependence of the ROS effect on three light parameters: wavelength, intensity, and dose. As a general tendency, smaller doses generated ROS levels that were beneficial for cellular processes, whereas higher doses generated levels with potentials reaching from the inhibition of cellular functions to bactericidal effects. To compensate for a possibly extensive ROS generation by the intense LED light and subsequent inhibition of cellular processes, we included into our facial rejuvenation program a powerful ROS scavenger; epigallocatechin gallate (EGCG) extracted from green tea. Orally administered EGCG is known to compensate for environment-induced oxidative stress. Topical effects are ROS compensation and extension of the survival rate of the cells involved in the incorporation and transport of extracellular metabolic waste from the basal membrane across the epidermis to the top skin layer.

It is instructive to recapitulate: Between November 2007 and September 2008 one of us irradiated the skin around the corner of the eyes with intense LED light (WARP 10, Quantum Devices, Inc. WI): Central wavelength 670 nm, integral intensity 728 W m$^{-2}$, and dermal dose $4 \times 10^4$ J m$^{-2}$. The resulting change in wrinkle levels for 10 consecutive months of daily irradiation was communicated earlier. The second phase of the facial rejuvenation program started December 2008. First, we continued the previously established protocol. The difference to the previous phase was residence in megacities: in China (one month) and Africa, including Cairo (one month), directly prior to phase two of the experiment. This exposed the skin to massive environmental stresses, including high levels of ultraviolet, and infrared radiation causal for heat stress reinforced by its interplay with extreme particulate matter concentrations in the air. Figure 3 (left) shows the condition of the facial skin in Africa, one day before the end of the journey. Evidently, two months of extreme sun and air pollution were sufficient to neutralize the success of 10 months of light treatment. Phase two of our facial rejuvenation program started upon return from Africa and persisted for a further two months without a visible change in wrinkle levels. Here we modified the routine and introduced topical application of green tea (3 g of dry leaf mass per 250 mL of water, brewing temperature 100 °C, cooling time 30 min), applied onto the skin around the corner of the eyes 20 min before irradiating the wrinkled zones according to the protocol. The temporal coordination between use of ROS scavenger and light, and the coupled functional complementarity between the biological and physicochemical processes in the skin offers an explanation to the accelerated rejuvenation of the facial skin displayed in Figure 3 (right). In our study, we exploited the protective effect of EGCG. However, it is clear that the combination polyphenolic component of green tea and red light is not the only possible one. An arsenal of powerful ROS scavengers can be found in the literature.

Depletion of Follicular Stem Cell Reservoirs and Telomere Shortening. The new understanding emerging from a systematic analysis of the implication of ordered interfacial water layers in conserving or converting the polarity of biological surfaces is not limited to the function of elastin fibers. It applies likewise to the design of practical strategies allowing us to prevent and reverse topical skin deteriorations related to anomalous ROS levels provoked by the environment. This opens the door to a multitude of novel biomedical and cosmetic applications related to biological aging, for instance, skin rejuvenation formulas compatible with pulsed light, promising to improve their transdermal penetration. As a general recommendation, our model suggests to use for long-lasting topical cosmetic applications exclusively hydrophobic formulas. Hydrophilic formulas, for instance, nutritive creams, are beneficial when applied for shorter periods, but are preferably to be avoided for long-lasting protective-topical applications. This is, however, not the end of the list of biological implications, which are potentially controlled by the order of interfacial water layers. In vitro work showed that the graying of human hair is caused by $\text{H}_2\text{O}_2$-mediated oxidative stress, the process of oxidation involving the entire hair follicle, proximal to the sebaceous gland producing and containing the hydrophobic sebum. One may speculate that prolonged ROS bombardment in the narrow follicular space might not only turn the hair gray but also, by transiently changing the order and pH of the interfacial water layers, dramatically affect the lifespan and differentiation of the keratinocyte, thereby successively depleting the reservoirs.

Figure 3. Representative photographs show wrinkles subsequent to 2 months of extreme oxidative stress (left) and after 3 months of daily LED treatment (right): initially 2 months of LED only, followed by 1 month of green tea assisted LED. The change resulting from 1 month of combinational treatment (less pronounced wrinkle levels, shorter wrinkle valleys, and juvenile complexion) was previously realized in 10 consecutive months.
The effect of ROS on the order and pH of interfacial water layers might also affect processes in the nucleus. Whereas the mechanism and control of cargo migration through nuclear pore complexes is still poorly understood, it is clear that prolonged ROS bombardments could transiently accentuate hydrophilic aspects of the nuclear pore complex, thereby disturbing the selective transport of vital cargo in and out of the nucleus. Likewise, macromolecules representing the cargo could transiently change their surface polarity. The result is likely a temporary nutritive deprivation in the nucleus. Possibly, interfacial water layers and interfacial pH play a central role in processes of stress-mediated telomere shortening — one key factor in cellular aging. Telomere shortening can be induced by various causes, including both external and internal stressors, and has already been linked to an overproduction of ROS by mitochondria. Chromosomes are known to attach to the nuclear matrix via telomeres. Polarity contrasts between nuclear matrix and telomere segments seem to serve the telomeres as anchors, which protect chromosomes from unfavorable interactions. It is now clear that minimal changes in polarity can maximally affect space-mediated build-up of a glue-like interfacial water layer, thereby to retard and reverse biological aging. Finally, we wish to put forward a strategy to possibly minimize the impact of ROS. Further exploration of ROS-related changes in hydrophobic surfaces and extremely high viscosities of hydrophilic water layers on hydrophilic surfaces — equivalent to reducing their glue-like character.

References


(28) Cf. table of contents image and cover image related to ref 1.


