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Abstracts

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The Penetration Enhancement and the Lipolytic Effects of TAT-GKH, both in In-Vitro, Ex-Vivo, and In-Vivo

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It was demonstrated that the trans-activating transcriptional activator (TAT) protein from HIV-1 could enter cells when added to the surrounding media. TAT peptide chemically attached to various proteins was able to deliver these proteins to various cells and even at high levels in heart and spleen tissues in mice. In this study, the tri-peptide GKH (Glycine-Lysine-Histidine) derived from the parathyroid hormone, which is known as a lipolytic peptide, was attached to 9-poly Lysine (TAT) to be used as a cosmetic ingredient for eye-bag care product. When glycerol is released, expressed as the extracellular glycerol concentration (the so-called lipolysis index), TAT-GKH at 10^{-5} M induces a maximal lipolytic effect of approximately 41.5% in epididymal adipocytes isolated from rats, compared with basal lipolysis. In a microdialysis study, TAT-GKH was perfused into epididymal adipose tissues of anaesthetized rats in increasing concentrations in a Ringer solution. The glycerol concentration in each dialysate was measured using an ultra-sensitive radiometric method. The perfusion of TAT-GKH induced a lipolytic effect. A penetration study showed that TAT-GKH resulted in a 7-fold higher penetration into excised hairless mice skin than GKH. An in-vivo study showed that a TAT-GKH containing emulsion had a better effect upon the relative volume reduction of eye bag after 28 days of application on twenty-two healthy female volunteers than the placebo.
It was therefore concluded that TAT-GKH increased skin penetration, which resulted in enhanced lipolytic effects in in-vitro, ex-vivo and in volume reduction of eye-bags in in-vivo studies.

Role of Melanin and Artificial Hair Color in Preventing Photo-Oxidative Damage to Hair

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This research is a multifaceted study which investigates not only the role of melanin in providing photostability to natural hair color and hair proteins, but also the claim that the presence of specific artificial colors in hair slows down the rate of photodegradation of hair proteins. In earlier studies, the extractability of protein from photodegraded hair was investigated and showed that many of the cleaved proteins could not be extracted because of photo-oxidative cross-linking [1]. The current study investigates the effect of the amount of melanin in hair of different ethnicity and the presence of artificial hair colors on the extractability of the main classes of hair proteins. Furthermore, the data are used in the interpretation of the effect of these components in being able to prevent photo-oxidative damage to hair proteins. When exposed to sunlight, hair undergoes changes in chemical, mechanical and morphological properties. The UVB and UVA regions of the solar spectrum are the most damaging to human hair. Of these two, the UVA region is predominant in the solar spectrum at low altitudes. Hair of different ethnicity responds differently to the damaging radiation of the solar spectrum, because of different amounts of melanin present in hair. Melanin absorbs the impinging radiation (especially at the lower wavelengths (254-350nm), and converts it by some complex internal mechanism into heat [2]. Because of this, melanin provides a photochemical protection to natural hair color and hair proteins and prevents their photodegradation. However, the melanin pigments act sacrificially and become themselves degraded in the process of protecting the proteins from light. As a result, this »protective« effect of the melanin pigments does not last during long-term intense exposure, when, regardless of the amount of melanin in hair, most matrix, intermediate filament and high molecular weight hair proteins undergo

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photo-oxidative cross-linking into higher molecular weight species, and their extractability from hair decreases significantly. The goal of this study is to demonstrate how UV-radiation affects natural and artificial hair color during long-term exposures. Bright-field and UV-microspectrophotometry and an electrophoretic separation technique (SDS-PAGE) were chosen as investigative techniques for these studies, because they are well-suited to accurately and reproducibly investigate the initial properties of a specific hair sample and the changes in these properties as a result of long-term light-exposure. The goal of this paper is not to relate this to the content and type of melanin in hair. Electrophoresis, while not measuring the exact quantitative amount of protein extracted, is a semi-quantitative method, where increases in brightness of the bands represent increased amounts of proteins that were extracted of that specific protein from hair. This electrophoretic study attempts to determine whether the presence of natural or artificial color in hair influences the protein extractability in unaltered hair and the photo-oxidative cross-linking during light-exposure. The bright-field microspectrophotometric study showed that high concentrations of melanin provide protection to the melanin itself and that they prevent loss of natural hair color during light-exposure. However, neither large amounts of melanin in hair of different ethnicity, nor artificial hair colors (even a dye with an absorption in the UV region) provide protection to the hair proteins against photodegradation under the conditions used in this study. UV-microspectrophotometry has suggested the formation of high levels of photo-oxidized proteins as a result of light-exposure. Electrophoresis revealed photo-oxidative cross-linking of most matrix, intermediate filament and high molecular weight hair proteins into their higher molecular weight analogues, rendering them less extractable due to their lowered diffusivity. Only very low levels of low molecular weight matrix proteins could be extracted.

Improving Cellular Function Through Modulation of Energy Metabolism

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The ambivalent consequences of mitochondrial stimulation on cellular activity have been well established. Mitochondria supply the cell with energy through a process of oxidative phosphorylation but thereby generate free radicals, resulting in the accumulation of hydrogen peroxide in the cytoplasm. We have investigated the impact of cellular senescence as well as UV irradiation, on the balance between these two activities. The adenosine triphosphate (ATP) level, DNA and protein synthesis in fibroblasts obtained from donors between 30 and 90 years of age appeared to be significantly influenced by the aging process. Both DNA and protein synthesis could be stimulated by increasing intracellular ATP levels. *In-vitro* senescent fibroblasts showed a reduction in the level of ATP as well as a shift in mitochondrial membrane potential. At the same time, there was an increase in intracellular hydrogen peroxide with increasing population doubling, indicating a clear dysfunction of the metabolic machinery in the mitochondria of senescent cells. To counteract this degradation of the energy pool, we treated cells with creatine, which is known to restore the pool of phosphocreatine in the mitochondria. Creatine treatment significantly increased cell survival after UV exposure, stimulated the repair of UVB-induced DNA damage in keratinocytes and caused a significant reduction in the number of sunburn cells in a UVB-exposed reconstituted skin model. These results clearly indicate that restoration of the energy pool in mitochondria increased cellular self-defense mechanism. These data show the important role played by the mitochondrial energy metabolism on the aging process, and indicate a possible therapy that can be used to counteract this negative effect. Treatment with creatine seems to provide the necessary boost to the cellular metabolism, which leads to an induction of a significant amount of protection and repair to human skin cells.

Comparison of the Biomechanical and Biosynthetic Behavior of Normal Human Fibroblasts and Fibroblasts from a Forehead Wrinkle

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Wrinkles are the most obvious expression of skin aging and are manifested by numerous changes in the organization and structure of the dermis. To better understand if this tissue modification could be linked to a modification of cell function, contractile and synthesis capacities of normal aged human fibroblasts and those obtained from a biopsy of a forehead wrinkle were studied and compared. The capacity of fibroblasts to adhere to the collagen network and to maintain a three-dimensional structure of the dermis was studied using a three-dimensional model of a collagen gel. The metabolic activity of both cell types was determined immunochemically by quantifying collagen I synthesis. Human fibroblasts from the wrinkle contracted the collagen gel less than normal aged human fibroblasts and synthesized less collagen I. The results show that the metabolic activity of aging fibroblasts decelerates and that aging fibroblasts lose their capacity to adhere to collagen fibers, thus limiting the possibility of organizing dermal tissue. The potential of an active ingredient to compensate for the reduction of metabolic activity and to restore the contractile capacity of fibroblasts from the wrinkle was investigated. This effect was compared with a reference molecule, vitamin C.

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Investigating the Relationship Between the Hair Fiber Proteome and Hair Quality

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Many hair characteristics (e.g., color and curl) are genetically determined. Here, we investigated whether hair quality is also genetically controlled and related to differences in hair composition. Female, Caucasian subjects (n=292) were recruited into this study and segmented by self-perceived hair quality and by permanent colorant usage. Hair fibers were collected and characterized by amino acid analysis, dry tensile elastic modulus testing and 2-dimensional electrophoresis of hair protein extracts. Protein analysis revealed a string of 66kDa proteins that correlated with hair of high quality. Loss of low molecular weight (14-29kDa) proteins increased with colorant usage, particularly in hair of low quality. Amino acid analysis showed that the levels of serine and threonine across all the subjects followed bimodal frequency distributions suggestive of a genetic influence. Overall, data suggested that perception of quality was linked to high serine and threonine levels. In addition, where hair was colored, quality was associated with lower cysteic acid levels. Lower cysteic acid levels were not linked to lower colorant usage and our data suggest that high quality hair may be more resistant to colorant damage. Elastic modulus was significantly higher in hair of higher quality (4.65GPa) compared to all medium and low quality hair combined (4.3GPa), $p<0.02$. This suggests a link between altered hair composition and mechanical properties. In conclusion, the composition and mechanical properties of hair have been linked to hair quality. We hypothesize that hair quality is thus likely to be genetically determined. Understanding of the links between hair composition and its properties could be utilized in the future for designing products targeted to each individual's hair make-up, and for producing diagnostic tools for determining hair quality.

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