Atomic force microscopy as a tool for the evaluation of UV and botanical agents exposure on skin cells

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INTRODUCTION

Continuous exposition of the human skin to the UV irradiation accelerates skin ageing and inflammatory disorders. Therefore, the use of botanical agents to decrease the deleterious effect of UV radiation by photochemoprevention has increasingly gained attention. In view of this, to accurately valuate the dysfunction, the crucial is determination of fast and exact methodology.

AIM OF STUDY

In this work three botanical agents were used to check their effect on the fibroblast cells. The skin cell response was estimated through the measurements of cell mechanical properties (atomic force microscopy, AFM) and by the visualization of cytoskeleton structures (immunofluorescence microscopy).

MATERIALS AND METHODS

Cell culture

Tests were performed on fibroblast cell line (ATCC), cultured in DMEM medium supplemented with 4,5 g/L glucose, 10 % FCS, 10 mM HEPES, 2 mM L-glutamine and antibiotics (100 U/ml penicillin; 0,25g/ml streptomycin sulfate). All cells were grown in culture flasks then trypsinized using trypsin-EDTA solution, diluted 10 times in growth medium and spread on glass coverslips.

Cell treatment

Prepared cells were exposed to UVA (8 J/cm^2) and UVB (250 mJ/cm^2) radiation as well as treated with selected botanical agents (extracts: [A] which protects skin cells against environmental stress, [B] – antioxidant, showing anti-inflammatory activity, and flavonoid, antioxidant [C]. Subsequently, cells were kept at 37˚C in atmosphere of 95% air/ 5 % CO2 and taken for AFM measurements and fluorescent microscopy 48 h later.

Atomic Force Microscopy

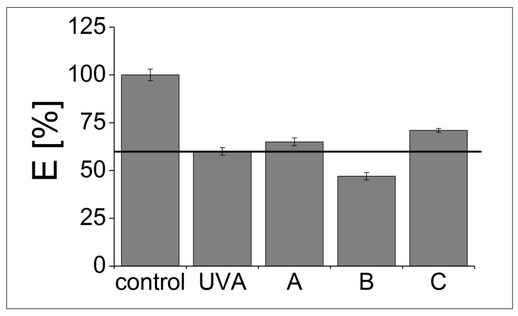
The elastic properties of studied fibroblasts were determined using the atomic force microscopy (Park Systems XE120 AFM). The silicon nitride cantilever MLCT (Brüker) were used as probes. Fibroblasts were measured in DMEM at RT. The relation was obtained by subtracting the approach part of the two curves recorded on a hard, non-deformable surface and on a soft cell. The resulting relation, *force-versus-indentation curve*, between the applied force and the produced indentation depth was fit with the function which corresponds to the Hertz model with the assumed conical shape of the probing tip. All determined elastic modulus values were normalized to the value obtained for the control measurements. The results are presented as a mean ± standard error obtained for a given number of cells measured for a particular sample.

Actin filaments visualization

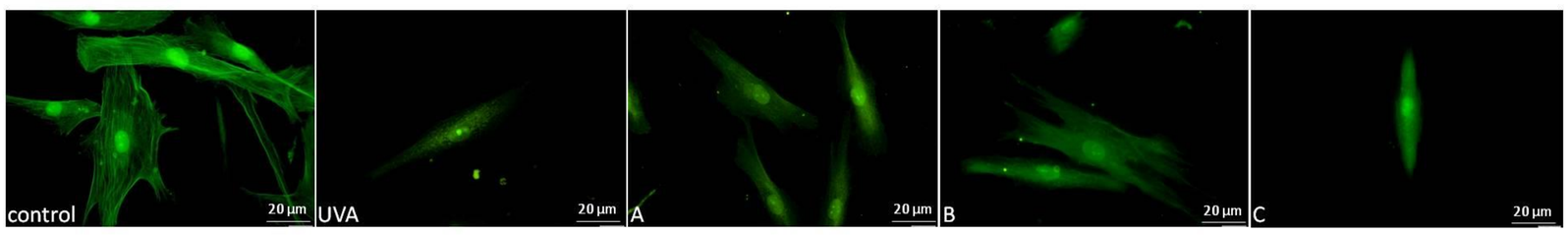
To visualize the organization of the actin filaments, fibroblasts were stained with phalloidin labeled with Alexa Fluor 488 (1:200) and imaged using the fluorescent microscope Olympus IX71 (Nikon, USA) equipped with the mercury lamp.

RESULTS

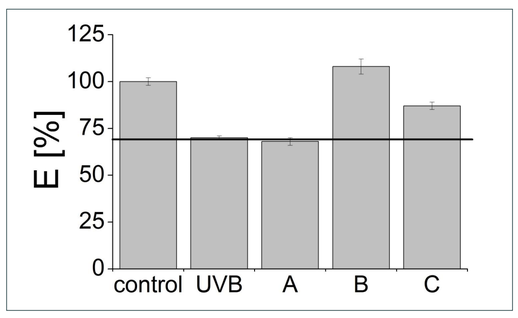
The AFM study shows a decrease of the cell stiffness after UVA or UVB radiation. The change in the elasticity in UV-irradiated cells correlates with the rearrangement of actin filaments observed by fluorescent microscopy. In cells exposed to UVA and post-treated with botanical agents no significant changes in actin filament organization were detected. In cells exposed to UVB and post-treated with botanical agents positive correlation between actin filament organization and AFM measurements was noticed only for the botanical agent - extract B.



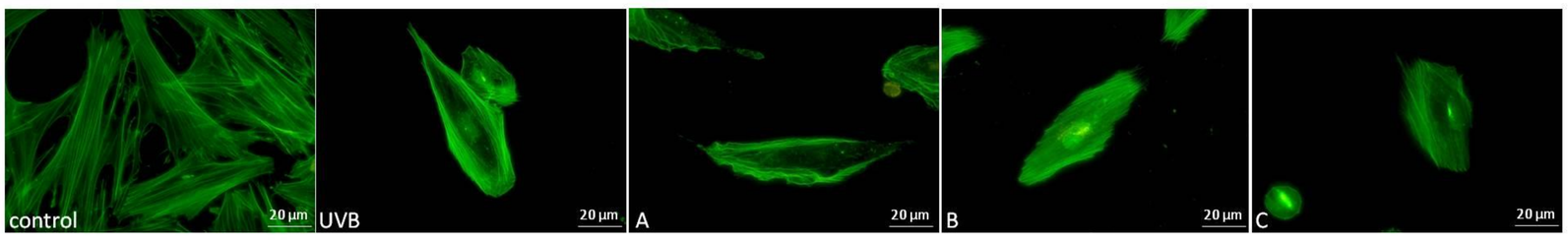
The mechanical properties of fibroblasts cells treated with the UVA radiation (8 J/cm^2) and three botanical agents: extracts A and B, and an active substance [C]



The visualization of actin filaments in fibroblasts treated with UVA radiation (8 J/cm^2) and three botanical agents: extracts A and B, and an active substance [C]



The mechanical properties of fibroblasts cells treated with the UVB radiation (250 mJ/cm^2) and three botanical agents: extracts A and B, and an active substance [C]



The visualization of actin filaments fibroblasts treated with the UVB radiation (250 mJ/cm^2) and three botanical agents: extracts A and B, and an active substance [C]

CONCLUSION

The cell stiffness measured by the atomic force microscope can be used to evaluate the effect of botanical agents against the UV radiation.

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