30TH IFSCC CONGRESS
18 – 21 SEPTEMBER 2018

P-S7-394

HAIR STRENGTHENING BY ANISOTROPIC OSMOLITE SOLUTIONS (INOSITOL + ARGININE): CROSS-TALK BETWEEN DERMAL PAPILLA FIBROBLAST AND KERATINOCYTES OF THE OUTER ROOT SHEATH BY A “PROTO-FOLLICLE” 3D MODEL

Gianni Baratto[1], Elisa Caviola[2], Marisa Meloni[2], Nicola Lionetti[5], Adriana Bonfigli[3], Maurizio Sironi[4], Stefano Pieraccini[4], Marc Oliver[6], Luisa Coderch[6], Luigi Rigano[5]

[1]Unifarco S.r.l., S. Giustina, Italy; [2]Vitroscreen S.r.l., Milano, Italy ; [3]ISPE S.r.l., Milano, Italy; [4]Dept. Chemistry University of Milano, Milano, Italy; [5]Rigano Laboratories S.r.l., Milano, Italy; [6]IQAC-CSIC, Barcelona, Spain

Aim of the study

The hanging drop technology produced a scaffold- free micro-tissue model applied to a DPF-ORSK co-culture in order to create a “proto-follicle” 3- dimensional model. This system was capable to evaluate the efficacy of anisotropic osmolite solutions on the increase of the follicle turnover and ‘health’. This process takes place by the strengthening of the hair follicle structure, morphology and metabolism, consequently improving also the hair characteristics. A multi-parametric approach (Morphological, IF/IHC, Gene expression analysis, Viability) has been adopted, in order to characterise the “proto-hair model” and have a comprehensive view of its evolution during the culture. Moreover, an in-silico model was used in order to screen the most promising combination of osmolite molecules and design a proper formulation strategy. In-vivo objective evaluations were finally carried out on volunteers with hair disorders.

Introduction

The hair follicle (HF) is a dynamic “mini-organ” which undergoes bi-continuous cycles of growth, destruction and rest. This is controlled by a group of specialized mesenchymal cells, located in the structure of the dermal papilla. The precursors of papilla cells are mesenchymal-cell aggregations, which form in embryonic skin dermis at the beginning of the follicle morphogenesis. The epithelial–mesenchymal interactions, and in particular the cross- talk between dermal papilla fibroblast (DPF) and the keratinocytes of the outer root sheath (ORSK) play a pivotal role. Aim of this study is the evaluation of the biological activity of anisotropic osmolite solutions (myo-inositol + arginine) on the HF cycle. The complexity of these interactions makes it unlikely the complete in vitro reproduction of such mechanism, as dermal papilla cells deeply modify their gene expression profile when cultured as monolayer. Their transcriptional pattern can be partially restored when they are cultured as 3D spheroids. The distribution of cells in the growth medium is fundamental in order to produce a verisimilar model.

Results and discussion

In-silico: myo-inositol induce the greatest effect on the microscopic structure of water, as shown by the increased magnitude of the first peak of the water-oxygen radial distribution function (fig 1), revealing a more ordered first solvation shell around water molecules. On the other hand, arginine has a stronger reducing effect on the coefficient of self-diffusion of water (fig 2), thus affecting water dynamics and its ability to act as the primary denaturant on proteins.

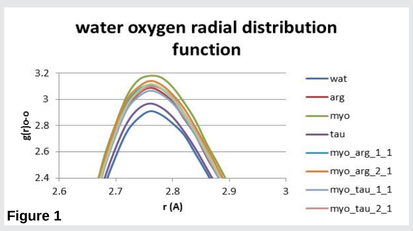

Figure 1

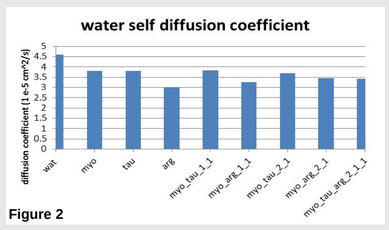

Figure 2

We investigated the interaction of a keratin model segment with solutions of the two most promising osmoprotectors. The 1:1 mixture of the two osmoprotectors retains their basic behaviour, with myo-inositol crowding around keratin, constituting a first osmotic protection layer on the protein, while arginine remains on average more distant, building up a second, more diffuse layer (Fig 3) so reducing the aggregation of myo-inositol, that otherwise would be more pronounced.

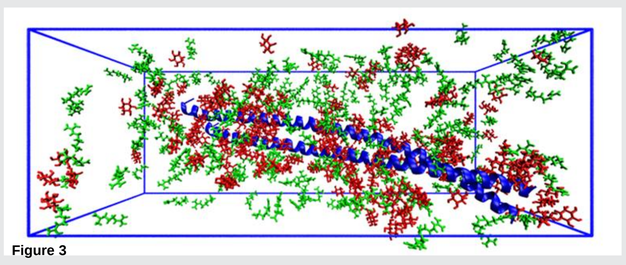
Figure 3

In-Vitro - Histo-morphological analysis: Mix myo-inositol+arginine 0.25%+0.25% - at T6 the morphology (fig 4) indicates an increased cells differentiation resulting in a partial detachment of epithelial layers from the dermal core. Keratinocyte layers present a better organization in lamellar structures and higher thickness compared to the negative control.

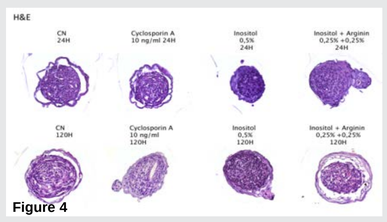

Figure 4

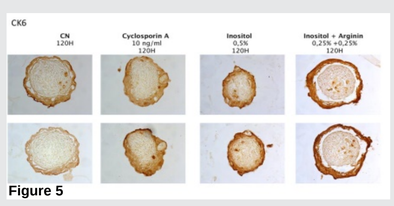

Figure 5

Ex-Vivo - Protein degradation analysis: When the hair is irradiated without any treatment, the protein degradation increases (+25.41%). In contrast, in all other groups, with HSP treatment the protein degradation is reduced, even if at different intensities. This confirms that the irradiation increases the protein degradation, and the treatment with HSP 1 h before the exposition not only protects the hair but also decreases the level of protein degradation in respect to NN (non-irradiated & non-treated) (Fig 7).

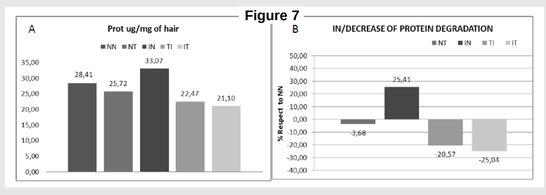

Figure 7

In-Vitro - Immunostaining: Mix myo-inositol+arginine 0.25%+0.25% - in the treated samples, the KC layer results more differentiated in comparison with Cyclosporine A and myo-inositol alone (fig 5). The most relevant result is the strong CK6 signal, corresponding to an overexpression of CK6 in comparison with all other samples. The mix forms well organized layers and produces a high quantity of structural keratin CK6. This could lead to an increase of hair shaft thickness and strength in-vivo. The expression of the protein IV (fig 6) is significantly increased. The application of the mix induces an increase of COLIV protein expression, indicating a role in the production of structural protein of the basal lamina and, as a consequence, of the firmness and strength of the hair follicle.

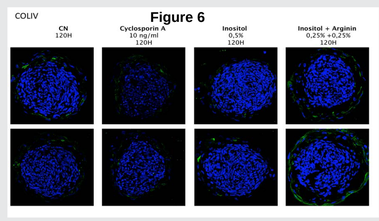

Figure 6

In-Vivo - After 6 weeks of use of the hair-care treatment, the pull test demonstrated a significant reinforcing effect ($p<0.05$) with 67.1 % increase in hair strength against the pulling action (Fig 8). A tendential increase in the hair diameter, of 5.1% was also evident (Fig 9).

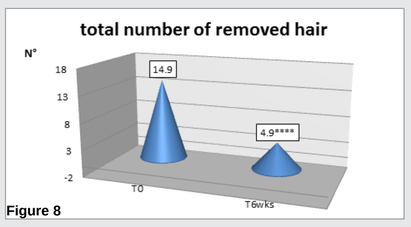

Figure 8

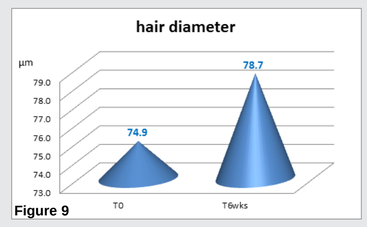

Figure 9

Materials and methods

In-silico: A water-osmolyte system has been built by placing the osmolytes in random positions into a 50 Å simulation box. Subsequently, the boxes have been filled with water. The 3D structure of a helical keratin segment has been obtained from Protein Data Bank. The protein has been inserted in a virtual box and amounts of osmolyte (1 M) molecules (arginine, myo-inositol, taurine or their mixture) have been added. Electrostatic interactions have been described with the Particle Mesh Ewald algorithm, while a 14 Å cut off has been applied to van der Waals interactions. The simulations have been run with the Gromacs 4.5.3 version. All the systems have been submitted to a geometry optimization of 10000 steps employing the steepest descent method. Afterward, they have been equilibrated for 100 ps at constant temperature (T) and volume and for further 100 ps at constant pressure (P) and T. The production phase of the simulations consisted in 100 ns of molecular dynamics at constant T (310 K) and P (1 bar). The LINCS algorithm has been used to constraint all the bonds to their reference length, in order to use a 2 fs time step. The protein is described with the AMBER99SB-ildn force field, while the osmoprotectors with the Generalised Amber Force Field.

In-vitro: Human Hair Follicle Dermal Papilla Cells (HDPC) and Human Hair Follicle Keratinocytes (HHFK) have been used to reconstruct proto-hair micro-tissue using the hanging drop method into plates from InSphero. The Proto-hairs have been further transferred in Gravitytrap for cultivation and treatment with Cyclosporin A (reference for hair growth), Myo-inositol or a mix of myo-inositol:arginine 1:1. In order to evaluate the effect of anisotropic osmolytes solution on the structure and morphology of reconstructed models, the proto-hairs were harvested at different time points after 24 and 120h of treatment and characterized on the basis of the following parameters: **Histo-morphological analysis**: Hematoxilin-Eosin (Histoline) staining of 7µm formalin fixed paraffin embedded (FFPE) sections; **Immunostaining**: performed on FFPE sections using anti-human CK6 (Sigma) primary antibody and DAB chromogenic detection method (Life-technologies) or anti-human COLIV (Santa Cruz) and fluorescent secondary antibody to monitor the presence and the maintenance of type-specific features. Images have been captured using Leica Instruments (DM2500 microscope or SP2 confocal microscope) and LASX software.

Ex-vivo: All studies have been performed with 1g tufts of same lot of Caucasian hair (CAU). To evaluate the protective effect of the product (hair spray product HSP), different samples were evaluated: Non Irradiated & Non Treated (NN): CAU sample without treatment or irradiation; Irradiated & Non Treated (IN); Non Irradiated & Treated Sample (NT); Irradiated sample after Treatment (TI); Irradiated sample before treatment (IT)
Irradiation: Irradiation was carried out for 5 h using a SUNTEST CPS+ before protein degradation analysis. **Treatment administration**: Treated samples (NT, TI & IT) were impregnated with 650 mg of HSP. After treatment, or Irradiation in TI sample, all shafts were stored overnight (at 25ºC 50% HR). **Protein extraction + Bradford analysis**: Hair extracts were diluted to 0.01% SDS concentration and the Bradford colorimetric assay was used to quantify the proteins and peptides solubilised. A complex between the dye, Brilliant Blue G and proteins is formed. Bovine Serum Albumin is the standard to calculate the amount of protein.

In-vivo: A hair care treatment, made by shampoo, spray, serum and hair conditioner formulae was set. The objective of the study was to evaluate the strengthening effect on hair of the twice-weekly treatment, through pull test and electronic microscope. At the end of the test, volunteers were asked to express their opinion on the efficacy of the treatment by a five-point scale questionnaire. The study was conducted on 30 female volunteers (35-65 yrs, long, aged, breakable, damaged and weak hair). Volunteers washed their hair 48 hours before each visit. The evaluation of benefits was made by clinical and instrumental technique performed at the baseline and after 6 weeks of treatment; **Pull test**: Clinical evaluation of the resistance of hair to traction was performed by applying a constant traction on hair tuft in 3 different areas of the scalp. Growing hairs remain rooted in place while hair at the end of telogen phase (exogen) are extracted easily. The hair resistance to traction was evaluated by the total number of removed hair in the three areas. **Diameter** : Instrumental evaluation of the hair diameter was performed by Scanning Electron Microscope.

Conclusions

A multi-parametric approach (Morphological, IF/IHC, Gene expression analysis, Viability) has been adopted to characterise the “proto-hair model” described and have a comprehensive view of its evolution during the culture. Moreover, an in-silico model was used to screen the most promising combination of osmolite molecules and design a proper formulation strategy. Ex-vivo and in-vivo evaluations were finally carried out in order to confirm the performances of the mixture of myo-inositol+arginine 0.25%+0.25%. The use of spheroidal co-culture of DPF and ORSK represents an optimal efficacy evaluation method for checking the hair growth effects.