Study of the migration of small molecules through the hair fiber to the scalp

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Abstract

Human hair is a mainly keratin structure that packages itself in a highly complex way. This high complexity leaves free spaces (vacancies) that allow substances to permeate the hair fiber. The permeation is influenced by different factors including pH of the environment, hair porosity, and quality of the hair. The study of the permeation of molecules through the hair fiber to the scalp is important for the development of cosmetic products with actives that could potentially have efficacy no just on the fiber but on the scalp. On this study scalp explants from rhytidectomy with hair shafts of 6 cm were cut in pieces with 6mm diameter. The hair tips were submersed in an aqueous solution of fluorescent dye eosin at 3 different times. After that, the skin explants were included on TOC compound and cut with cryotome. The cuts were analyzed by fluorescence microscopy to understand if the dye was able to migrates to the scalp. The results show migration of the fluorescent dye in all the times tested. The presence of fluorescent molecules on the skin cuts means that the actives absorbed on hair fiber are capable of achieving the hair bulb and in contact time higher than 30 min they can even reach the inner structures of the scalp.

Keywords: Hair Permeation, pH, Hair Porosity, Hair Evaluation, Scalp.

Introduction.

The human hair is a bio-composite polymer with a complex structure primarily composed of keratin chains that organize it self into a highly complex structure with three main components; cuticle, cortex and medulla that are cemented by the cell membrane complex (CMC) [1][2][3][4]; the organization of the protein chains results ins a natural formation of low-density regions (microvoids), mainly on the amorphous phase domain [5]. Those regions are used as paths for in/out of small molecules to hair; Potsch et al (1996), showed that at room temperature a Rhodamine B solution start to spread on the hair fiber by the cuticle, but after 30min the dye enter into the inner part of the fiber predominantly by the CMC regions and after 60min the dye was already diffused into the cortex and trough the all fiber [1].

The permeation of substances into hair fibers is influenced by various factors, including the pH of the environment, hair porosity, and the overall quality of the hair [4][6]. Understanding the intricacies of hair structure and porosity is fundamental to effective hair care development, as many active ingredients use these pathways to enter the hair. In recent years, research into hair structure and porosity has gained significant traction within the cosmetic industry. The growing demand for treatments that target both the hair fiber and the scalp has sparked interest in developing such products. This burgeoning field holds immense promise for

revolutionizing hair care product development and formulation. By delving deeper into the molecular composition of hair and understanding how porosity affects its behavior, cosmetic scientists can tailor formulations to address specific needs and concerns, ultimately leading to more effective and targeted solutions for consumers. Therefore, it is crucial to study the migration of active ingredients within the hair fiber and their movement to the hair bulb.

To assess porosity and the diffusion of molecules through hair fibers, various methods have been explored. Chandrashekara and Ranganathaiah (2009) studied the diffusion of liquid dye molecules using positron lifetime spectroscopy [5]. Han et al. (1985) investigated the diffusion of dyes by microspectrophotometry [6], Al-Kindy et al. (2003) studied the detection of surfactant TW-80 on by accoupling it with fluorescent dye Eosin B [7], Gruber et al. (2001) & Faucher et al. (1978) studied the deposition and sorption of surfactants on human hair marking the surfactant molecule by adding chemically a fluorescent marker [8][9] while Worthmann et al. (2004) examined the diffusion of surfactants in human hair using EDX mapping [10]. However, few studies have focused on the diffusion of molecules to the scalp. However, few studies have focused on the diffusion of molecules to the scalp. It is well known that molecules can migrate from the scalp to the hair fiber (this principle is used on drug abuse detection) [1]; given that migration can occur in both directions, it is reasonable to hypothesize that active ingredients in hair care formulations can migrate from the hair fiber to the scalp.

In this context, this article aims to explore the migration of small molecules from the hair fiber to the scalp by analyzing transversal cuts of explant scalp using fluorescence microscopy. The fluorescent dye eosin was used as an example of a small particle due to its ease detection and its high solubility in water.

Materials and Methods.

Skin explants from elective rhytidectomy surgery, from a single donor, were cleaned and cut into circular sections measuring 6cm in diameter. Three sections were kept in culture medium and the hairs from each of the skin segments were cut to 6 cm in length. The ends of the wires (1cm) were submerged in a solution of the fluorescent dye yellow eosin (C.I 45380) with water at pH 6 (selected from previous experiments performed in hair fibers) by 20min, 30min and 1h respectively. The experimental scheme is showed on Figure 1.

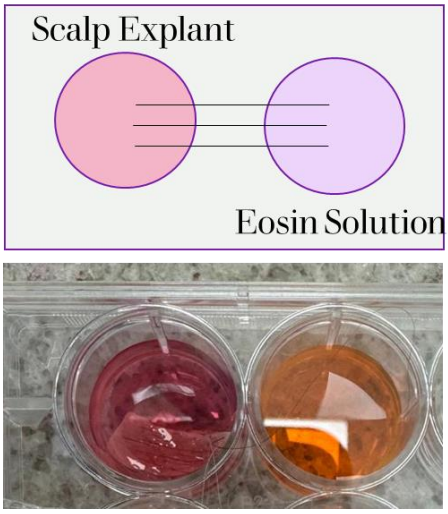


Figure 1. Experimental scheme.

After the exposure time, the hair fibers were removed from the fluorescent dye and the skin was included in O.C.T compound Tissue-Tek and histological sections were made on a Leica CM18670 cryostat at a temperature of -30°C, with a thickness of 8 µm and using a steel knife.

After cutting, the cell nucleus was marked with DAPI dye to facilitate visualization. The sections were then taken to an Olympus BX53 fluorescence microscope coupled to a DP74 digital camera and observations were made with a 10X objective. The images obtained were analyzed with the ImageJ 1.47v program to quantify the fluorescence intensity of the eosin dye.

To verify if there were differences between the measurements, statistical comparations were performed on the software GraphPad Prism, version 8.3.4, using ANOVA one-way test followed by Tukey´s post-test. Statistically significant differences between samples were checked with a p-value ≤0.05.

Results.

Some of the images obtained from cross-sections of the scalp are shown in Figures 2. Here it is possible to observe the hair follicle structure marked in blue and some red points referent to the fluorescent dye eosin that migrates from the tip of the hair to the hair follicle.

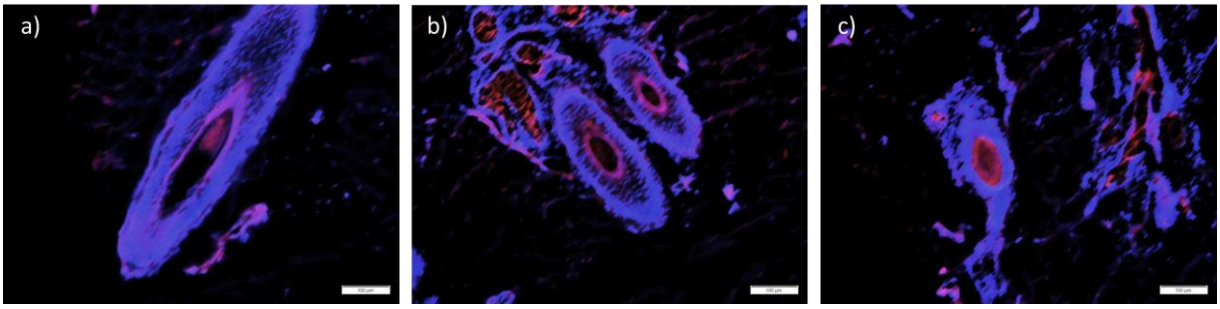


Figure 2. Scalp transversal cuts. Images of follicles (blue) after the ends of the hairs were immersed in eosin (red) dye by **a)** 20min **b)** 30min **c)** 60min.

Within 20min, it is possible to observe the eosin dye more located in the hair shaft, with little migration to other structures. On the other hand, the images of the follicles whose hair were exposed to eosin during 30 min showed the fluorescent dye not only on the hair shaft but, on the follicle structure. In creasing the exposure time to 60min, it is possible to visualize the eosin not only in the hair shaft and follicle, but in other structures of the skin.

The images were then analyzed with the software ImageJ, in order to quantify the fluorescence intensity between the groups; the values of maximum intensity and medium intensity were plotted. Figure 3, surmise the results. Same colors mean no statistically significant differences between the groups.

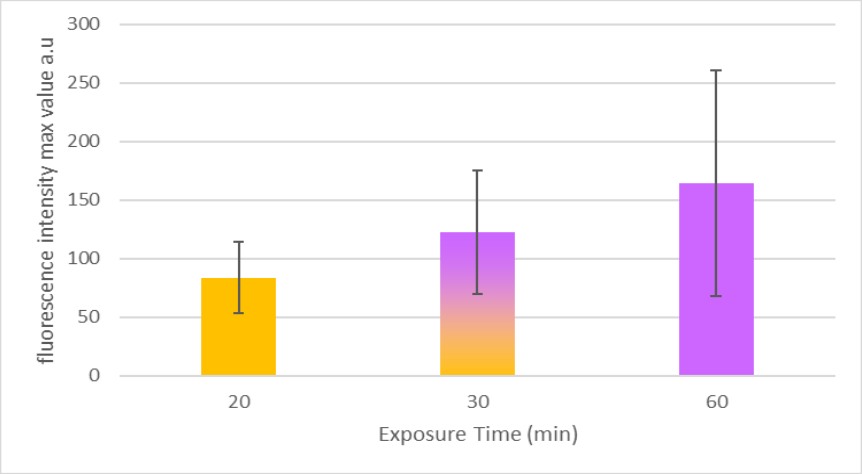


Figure 3. Maximum eosin fluorescence intensity. 10 images analyzed per group. Different colors mean statistically significant differences.

Discussion.

The results showed the presence of eosin dye in the scalp after exposure of the ends of the hair strands at all times studied; that is, after 20 minutes the dye had already managed to migrate to the hair follicle, confirming the hypothesis that small particles can be transported through the hair strand to the scalp.

Figure 5 shows the average of the maximum intensity of the eosin fluorescence trough the images. Here is possible to observe that the fluorescence intensity increases with the exposure time, this is, the amount of eosin is increasing on the scalp with time. Also is possible to observe that the standard deviation increases with exposure time; this shows that the dye is more dispersed on the scalp surface with some points with high intensity (more concentrated).

Eosin is a fluorescent anionic dye, soluble in water [11] and its adsorption has been studied in the past in several substrates as: Chitosan [12], activated carbon [13], nacre [11] between others; those studies shows that the main factors that influence the sorption/desorption of the dye are the pH of the solution, temperature and the pore size of the subtract. On this study those parameters were maintained stable, changing just the time of sorption; the pH selection of 6 was made taking in account the isoionic point of hair (pH 5.6-6.2), and results obtained in previous experiments performed on the hair fiber by our research group that showed a lower diffusion of the dye on the hair fiber at basic pH. Those results are in accordance with the experiments performed by Han (1985), where the pH 6 showed the highest uptake rates for the dye Red 3 [6].

Conclusion.

The results of the experiments shows that the hair fiber is capable of absorb eosin dye from aqueous solution from the tip to the scalp; here 6cm fiber was tested, but the results let to think that the dye could travel even highest distances. The diffusion of eosin on hair is pH dependent, then probably the amount of dye that is transferred to the scalp could change with the pH of the hair or the solution. At this experiment the pH of the eosin solution was maintained at pH6 and no product was applied on the fiber; at this pH the hair fiber is in their isoinoic point (pH 5,6 – 6,2) [6], minimizing the ionic interactions between the eosin and the hair.

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This study was conducted entirely at Kosmoscience group, Rua Italia 274, Valinhos, SP, Brazil.

Conflict of Interest Statement.

NONE.

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