Experiments in Raman Spectroscopy on Hair Follicles from Females with Androgenetic Alopecia

Medical Science

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ABSTRACT

Androgenetic alopecia in women is a frequent dermatological disease, characterized by shortening of ana-gen, hair thinning and hair loss.

Although the chemical structure of untreated hair from normal patients has been intensively studied, the hair structure in patients with alopecia or other hair pathology has not been well described in literature or studied in detail.

Our preliminary study investigated the potential of Raman micro-spectroscopy (RMS) in hair analysis, its capacity to point out differ-ences between spectral footprints to discriminate the composition of hair follicles from various regions of the scalp.

The spectral images were captured by Raman micro-spectroscopy, Bruker Sentera type The comparison of Raman spectra of hair follicles in the occipital and frontal areas showed a similar footprint.

The data obtained do not sustain the hypothesis that besides generating a physical change of the hair follicle's dimensions, the minia-turization process could also involve structural changes of the hair.

1. INTRODUCTION

Androgenetic alopecia in women is a frequent dermatological disease, characterized by shortening of the anagen stage of the hair cycle, telogen induction, miniaturization of the hair follicle, with hair thinning and hair loss, especially in the fronto-parietal area of the patient's scalp. [1,2]

Regarding the pathophysiology of the disease, studies have focused so far mostly on genetics, hormonal changes or al-teration of the hair cycle stages: anagen, catagen and telogen. [3,4] These cyclic changes involve rapid remodeling of both the epithelial and dermal components of the hair follicles [5,6]

The chemical structure of untreated hair from normal patients has been intensively studied through different methods. Still, the hair structure in patients with alopecia or other hair pathol-ogy has neither been well described in literature yet, nor stud-ied in detail. [7]

We started this study from the hypothesis that besides gen-erating a physical change of the hair follicle's dimensions, the miniaturization process in alopecia could also involve structural changes of the hair.

In this preliminary study, we have investigated the potential of Raman micro-spectroscopy (RMS) in hair analysis, and its capacity to point out differences between spectral footprints that would allow us to discriminate the composition of hair follicles from various regions of a scalp suffering from alope-cia.

OBJECTIVE:
Our study had a double purpose :
- to determine by using Raman micro-spectroscopy if there are significant differences in the structure of hair follicles taken from different female patients suffering of Androge-netic Alopecia.
- to compare the hair structure and properties from samples of the fronto-parietal area (scalp affected by alopecia) to hair samples of the occipital area (normal scalp) from the same patient and to measure the differences, in case they exist.

2. MATERIAL AND METHOD
2.1 Skin samples
Skin tissue sections were obtained from a Private Office of Der-matology Care in Cluj-Napoca. Prior to the study, approval was

obtained from the Research Ethics Committee of "Iuliu Hatie-ganu" University of Medicine and Pharmacy Cluj-Napoca. The samples were taken from 4 female patients with Androgenetic Alopecia, with diagnosis confirmed by personal history, clinical assessment and trichoscopy aspect. Tissue samples were taken from patients, by punch biopsy (4-mm, vertical sectioning), af-ter they had signed the informed consent.

Two skin biopsies were prelevated for each patient: one from the occipital area (used as control area, as this part of the scalp is not influenced by the disease). The other biopsy was taken from the fronto-parietal area, where the patient was clearly af-fected by alopecia. The fresh tissue sections were kept in the freezer, then cut in cryogenic system (20μm sections from the blocks removed during the punch biopsy procedure) for RMS investigations.

2.2 Instrumental set-up
The spectral images were captured by Raman micro-spectros-copy, Bruker Sentera type. Sample illumination was performed by a 785-nm continuous wave diode laser, a CCD receptor with thermo-electric cooler, coupled to an inverted Olympus micro-scope. The spectra images were taken under a 20xobjective lense (Olympus, LUCPlanFLN). The laser was set at 100mW.

2.3 Data acquisition
Data acquisition from the skin sections was done in collabora-tion with the Histology Department from UMF Cluj, the Histopa-tology Department of USAMV and the Chemistry and Physics Department of Babes University.

Areas of fresh tissue sections containing epidermis, dermis and hair follicles were raster scanned (5 μm step size). Each spec-trum was determined as a mean of two recordings. The acquisi-tion time for each position was 10 seconds.

2.4 Analysis
All spectra were analyzed using functions developed in MAT-LAB software (version 7.6.0.324, MathWorks). Cosmic rays were removed by semi-automatic customized MAT-LAB pro-grams. Spectral data reduction was performed by selecting the Raman spectral bands which provide maximum differences among the classes under study. Spectra were binned to ac-count for tissue heterogeneity, achieving a spatial resolution of 8 cm^{-1}.

3. RESULTS
3.1 Sample processing

Eight prelevated samples (from four patients) were sent for Raman examination. Only two were processed correctly on account of restrictions concerning the thickness of the tissue that can be laser-irradiated. Samples corresponding to patients number 1, 2 and 3 could not be investigated by Raman micro-spectroscopy as they were too thin (their size was the usual one for histological analysis) to avoid destruction by the laser beam. The two samples from patient number 4 were successfully used.

3.2 Classification model

Literature reports state that maximum spectral differences in tissue components were achieved by using different ratios of Raman bands [8].

Identified Raman bands and their correspondences are presented below :
- Raman bands at 850 and 950 cm-1 were associated to proline [9]
- Intensity of the 1003 cm-1 band corresponded to the ring breathing of phenylalanine
- Molecular Raman vibrations of 1093 cm-1 corresponded to PO4 vibrations in DNA [10]
- Amide III spectral regions from 1200 to 1350 cm-1 corresponded to molecular vibrations of collagen type I
- Raman band of 1447 cm-1 was associated to vibrations in proteins (CH2 deformation)
- Intensity of the 1662 cm-1 band corresponded to C=O vibrations from amide I structures

3.3 Graphical representation of results

Figure 1 presents comparatively the Raman bands of hair follicles located on the the two different areas of the scalp, for samples corresponding to patient number 4. Frontal area of scalp will be also marked as "forehead" and the occipital area of the scalp will be noted "neck".
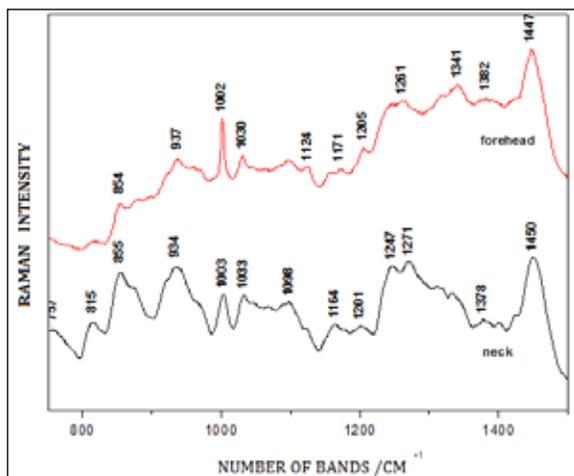

Figure 1. Raman Spectra of hair follicles from the forehead and the neck from samples number 4.

Both samples from the forehead and the occipital area (neck) had hair strong bands at 1447 cm-1, assigned to the CH2 deformation (protein vibration). A medium-strong band was found around 1662 cm-1, assigned to the in-plain-peptide carbonyl stretching vibration (C=O stretch). Another medium band was noted at 1003 cm-1, corresponding to the aromatic ring C-C stretching mode of phenylalanine. A medium broad peak was noticed at 1341 cm-1 (fig 1 and fig 2b) corresponding to molecular vibrations of collagen type I.

Figure 2 shows comparatively the Raman bands of FPF and FPC, pointing out different intensities and the general correspondence of signals in the field 800-1600 cm-1
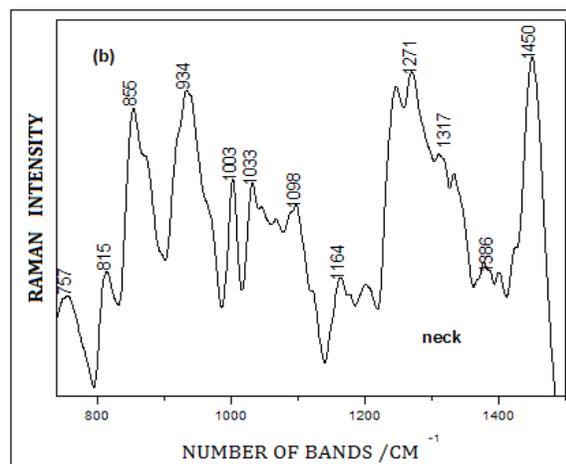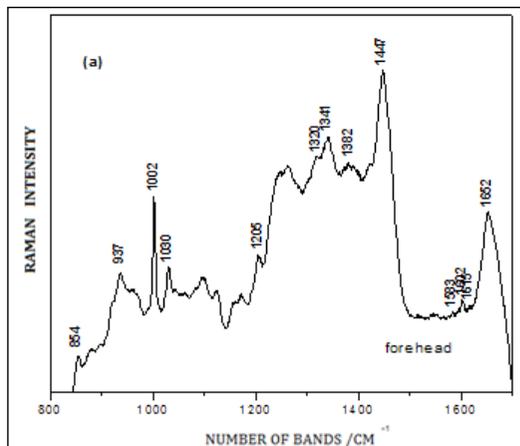

Figure 2. Comparative Raman bands, samples number 4 : a) forehead; b) scalp-neck

Smaller peaks were measured at 854 cm-1, 937 cm-1, 1030 cm-1, 1096 cm-1 and 1205 cm-1 and were interpreted as aliphatic and aromatic deformation of CCH, CH2 and CH3 rock or olefinic deformation of CCH, skeletal vibration of CC, COH deformation and CH2 deformation.

A shoulder was detected (in figure 2b) at 1317 cm-1 and this might be due to tryptophan.

There were no significant changes detected in the samples from the fronto-parietal and the occipital scalp area of patient number 4, with alopecia included in the study. The results suggested by the quantitative analysis of the Raman spectra were compared with other results found in similar hair studies already published in the literature.

4. DISCUSSION

Tissue images produced by spectroscopic techniques such as fluorescence, infrared (IR) or Raman provide chemical information. Fluorescence imaging is a qualitative assessment method with several disadvantages. Vibrational spectroscopy techniques such as infrared and Raman spectroscopy achieve high chemical specificity. Data obtained from a study performed in 2011 suggest that both IR and Raman imaging of molecular changes in a specific region of the hair are valuable tools for the understanding of the hair structure, its physiology and the effect of various stresses upon its integrity [11]. Infrared has also demerits, the major one being the sample thickness, which cannot be larger than 10 μm to avoid complete absorption of the incident radiation by the tissue [12,13]

Raman micro-spectroscopy (RMS) is better, because it allows both high chemical specificity and resolution on thick specimens.

Raman signal is produced by inelastic scattering of monochromatic light by the atoms and the molecules in the sample. Slight biochemical changes in cells, increased metabolic activity or alterations in lipid and protein levels are detected with RMS [14]

Until now many studies have acquired Raman images of human hair-cross sections or intact hair fibers from patients without hair pathology. Also, polarization-resolved Raman with near-infrared laser excitation has been applied to intact human hair to non-invasively investigate the conformation and orientation of the polypeptide chains. [15]

Over time, the study of hair follicles continued to gain interest, also regarding the dermal drug delivery. In 2013 a study on this topic made a comparison by Raman microscopy of normal human and porcine follicle composition and visualization of component distribution within follicle cross-sections. The results suggested that Raman microscopy is a noninvasive and chemically selective technique for the analysis of trans-follicular drug delivery [16].

RMS has revealed the spectral differences between hair follicles, hair, sweat ducts, capillaries, sebaceous glands, dermis and skin lesions [17, 18]. Literature reports from 2011 mention a model that used Raman bands corresponding to the largest spectral differences between the Raman spectra of BBC (basal cell carcinoma) and the normal hairy skin regions, associated mainly with nucleic acids and collagen type I [19].

No classification models capable to produce quantitative spectra images or automatic diagnosis have been developed so far. RMS studies mention that the histopathology diagnosis of different skin tissue excisions showed a good agreement between Raman spectral images and the gold-standard haematoxylin and eosin (HE).

Studies regarding the pathophysiology of the disease found that bald areas had the same number of stem cells as normal scalp in the same person and implied that there is a problem in the activation of stem cells converting to progenitor cells in bald scalp. Researchers (Cotsarelis et al, 2011) concluded that in alopecia, the hair follicles do not disappear, they decrease in size [20] These findings encouraged our study hypothesis, that besides generating a physical change of the hair follicle's dimensions, the miniaturization process from alopecia could also involve structural changes of the hair.

As far as our experimental set-up is concerned, the sample illumination was performed by a 785-nm continuous wave diode laser. We used it because literature reports from 2008 confirmed that the optimum excitation wavelength for spectral detail was 780nm and it resulted in little signal degradation over time. The same article suggested that near IR Raman spectroscopy offered the most suitable conditions to analyze the nature of secondary structural feature in hair fibers. [21]

Regarding our results, we did not detect significant changes in the fronto-parietal and the occipital biopsies taken from the same patient suffering from Androgenetic Alopecia. The Raman bands from the scalp biopsies (figure 1, figure 2a and 2b) were similar to the findings published earlier by other authors (M. Al-Arashi, M. Larraona Puy, W Akhar)[17,19, 22]. The Raman spectrum of the hair follicles were very similar to the measured spectrum of the epidermic layer [19]. This is explained by the fact that hair follicles are tubular invaginations of the epidermis, they extend deep into the dermis that surrounds the root of the hair. Also, the Raman spectra of hair and hair follicles is similar to the Raman bands of stratum corneum (outer-most epidermal layer), because it is composed mainly of keratin [23]. The mitotically active cells from the hair bulb, through differentiation and keratinisation generate the hair shaft. [24]

Regarding our second purpose, to determine if there are significant differences in the structure of hair follicles taken from different female patients suffering from Androgentic Alopecia, we could not draw a conclusion, because the skin biopsies were not processed correctly on account of restrictions concerning the thickness of the tissue that can be laser-irradiated.

5. CONCLUSION

The study data obtained until now do not sustain our hypothesis that besides generating a physical change of the hair follicle's dimensions, the miniaturization process could also involve structural changes of the hair.

The comparison of Raman spectra of hair follicles in the occipital (neck) and frontal (forehead) areas shows a similar footprint. For a better underlining of the differences between the Raman spectra of the hair follicles in the neck and forehead a significant number of samples is necessary in order to perform a multivariate statistical analysis, able to differentiate among the spectral profiles.

The results obtained confirm the potential of Raman microspectroscopy (RMS) in hair analysis in order to distinguish hair structure differences between samples taken from different regions of a scalp with alopecia.

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