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The Advantages of the Scanning Electron Microscope in the Forensic Studies of Hair in Domesticated and Wild Animals

Forensic Science

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ABSTRACT

Within the forensic identification the study of animal hair has remain limited to cross-species comparisons from the same family but there is also considerable changes in those animals like scale layer differentiation growth pattern, tensile strength, granulation and elemental profile of the animal hair. Here a rapid method is described for assessing a variety of morphological characteristics for identification of species that could be easily used by forensic investigators to identify the hairs from different species by just a fraction of single hair sample using Scanning Electron Microscope (S.E.M). Use of an optical light microscope gives very poor topographic resolution of hair features. While the scanning electron microscope gives better resolution, there is still the matter of replication of the surface of the sample to be investigated, which gives another possibility for error in investigation. The advantages of using the Scanning Electron Microscope (S.E.M.) for domesticated and wild animal's hair studies far surpass the technique of light or Transmission Electron Microscopy (T.E.M.) microscopic studies. Determinations of surface characteristics such as scale count, hair diameter, surface debris, hair shape, scale structure and surface damage, whether physical or chemical may be significant in in¬vestigative. The aim of this study is to investigate the hair in domesticated and wild animals, thereby making a contribution in filling the gap of knowledge in this field. As per knowledge, in many vetero-legal cases one fails to identify the hairs of animal and confuse them with those of some other small ruminants and wild animals. This investigation will be helpful to the field veterinarians as well as zoo veterinarians. Elemental analysis for animals can be done by using Energy Dispersive Spectrum coupled with SEM for analysis of Sodium, Potassium, Calcium and Sulfur. Significant results were obtained are can be further used to establish a concordance with the species identification.

INTRODUCTION

India is referred to have among richest biodiversity nations of the world having mixture of Palaearctic, Oriental and Afro-tropical fauna. India is home for 400 mammal species out of which 129 species are protected under Wildlife (Protection) Act 1972 (India) and a arbitrator to Convention on International Trade in Endangered Species. Illegal trade of the wildlife products are key risk to conservation and responsible for regional obliteration to these species. In forensic identification the study of animal hair has remain limited to cross-species comparisons from the same family but there is also considerable changes in those animals like scale layer differentiation growth pattern, tensile strength, granulation and elemental profile of the animal hair. The early pioneering work on species characterizations from hair includes studies by McCurtie (1886), Hausman (1920, 1944), and Hardy and Plitt (1940). Descriptive guides on microscopic hair characteristics for some important mammalian species of particular regions have been contributed by Brunner and Coman (1974), Moore *et al.* (1974), Appleyard (1978), and Teerink (1991).

MATERIALS AND METHODS

The hairs were taken using forceps with gloves to avoid contamination and were packed in zip-lock bags followed by paper envelops. These hair samples may be collected from near to Poaching area or Near to surroundings of dead animals. Hair samples were washed with absolute alcohol at room temperature for about 24 hours followed by packing in fresh ziplock bags and stored at 4° C till further analysis. For Scanning electron microscopy hair samples can be dissected in pieces of 5 mm size leaving 3 mm from root side and placed on sample holder. Finally, they were coated with gold by using JEOL JFC-1600 auto fine coater and observed under a JEOL JSM-6610LV scanning electron microscope.

The samples will be tested for measurements for all the species and difference in the measurements were further cross checked at various magnifications i.e. 500X, 1000X, 2000X simultaneously and raised up to 7500X for some samples to maintain the accuracy using annotations. The systematic examination using various instruments resulted in detection of the elements with their corresponding quantities. These have shown the constituent concentrations of the elements that are present in hair samples.

STRUCTURE OF HAIR

A typical mammal hair is comprised of three layers: the innermost core region or the medulla, an intermediate layer of cortex surrounding the medulla, and the outermost layer of overlapping and transparent scales known as the cuticle. (Fig 1 and Fig 2)

Perhaps the best way to explain the structure of hair is to compare it with a lead or graphite pencil, where the innermost lead or graphite layer represents the medulla, the wooden portion is the cortex, and the outermost paint layer is the thin layer of cuticle. Choudhary, O.P. *et al.* (2014)

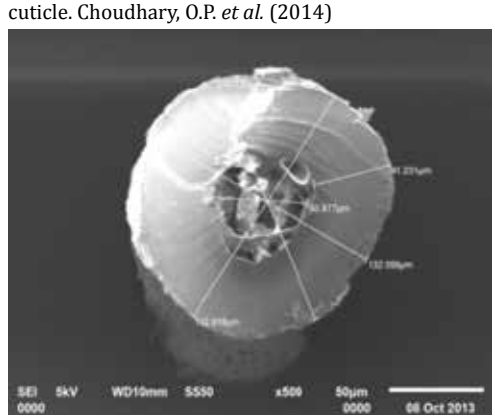

Fig.1 Scanning electron micrograph showing inner medulla and outer cortex part of hair

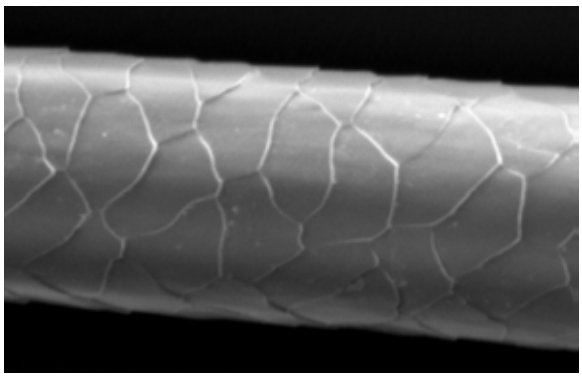

Fig.2 Scanning electron micrograph showing Outermost layer of overlapping and transparent scales of hair

ADVANTAGES IN THE FORENSIC STUDIES

The information on hair development, growth, and chemical components and research projects on hair of Domesticated and Wild animals fills volumes. The short article to follow will certainly not be able to cover all the information on hair, but will, perhaps, give a little insight into the characteristics of hair and the role the S.E.M. can play in bringing these characteristics into focus. Histological studies have shown certain structural relationships of hairs to be usually predictable, though not invariable. The normally existing correlations are cuticular scales, medullae, cortical cells, and pigment patterns.

Since growth is managed by cells arising from a mass of rapidly proliferating tissue near the base of the hair follicles, hair growth patterns show up on the surface scale patterns. These cells grow by differentiating into a core of keratinized material composed of two interlocked parts—the hair proper and its internal root sheaths. During growth, the core passes upward through the wall of the follicle, the external root sheath. Root sheaths and dermal papilla are important in determining hair shape, size, and movement. Several glands and skin cells are involved (which will not be delved into, since the above description of hair growth is not the prime concern, but shows some of the things involved in influencing hair structure besides hormones, vitamins, gland functions, environment and genetic factors). Nutritive and traumatic factors can modify normal rhythm of hair growth. Thyroid hormones have a physiologically stimulating action on hair growth. The lack of adrenocorticotrophic hormones (ACTH), disease, injury or environmental stress causes a depression on growth of hair. Of the eighteen orders of Domesticated and Wild animals, all grow differently structured hair, (Figures 1 and 2) which reveals either the outer structure, or the size, or the shape of the hair differently in each case. Some animals grow hair in wave patterns; others grow hair continuously, as in sheep and humans.

In addition to growth mechanisms previously described, there are other individual and diagnostic pathological, chemical and abnormal conditions which affect surface characteristics of hair, and may be used as criteria for identifying hair of animal's individuals.

The variability of animal's hair in each race is greater than the variability of hairs on a single individual's head. The hair of the dog is most nearly circular in cross-sections; coarser and straighter hair lends itself to a more circular shape. Goat hair is the roost flattened being quite curly in nature, as tends to be the case with flattened ovaloid cross-sectioned hair. The elephant hair sections are intermediate in cross-section shape.

Stereo photographs can be taken on the S.E.M. simply by taking one picture of the sample at one angle, then, tilting the sample an additional 2° to 10° to another angle, and taking another picture, litis is the angle at ‹which your eyes would normally see the sample, since all depth is seen by the brain interpreting two images from two angles in relation to the two eyes. The stereo image can be seen by focusing at a distance, holding the picture about 10 inches from the eyes, and viewing the stereo pair. Stereo glasses era also be used to view the image. Stereo photographs show the great depth of field, not achieved with light optical instruments since the depth of field would not be the same and resolution would be very poor with the optical instruments in general use. With the stereo means, scaley structure and surface features not readily noticed or determined before literally pop into view. This, ''too, is a great advantage in hair identification, image can be seen by focusing at a distance, holding the picture about 10 inches from the eyes, and viewing the stereo pair. Stereo glasses era also be used to view the image. Stereo photo-•graphs show the great depth of field, not achieved with light optical instruments since the depth of field would not be the same and resolution would be very poor with the optical instruments in general use. With the stereo means, scaley structure and surface features not readily noticed or determined before literally pop into view. This, ''too, is a great advantage in hair identification. Elemental analysis using SEM-EDS have revealed about the percentage of Sodium, Sulfur, Calcium and Potassium on the basis of weight % and Atomic % respectively.

SUMMARY

Hair identification is a complex and important .problem in view of forensic investigation. It entails many tests and uncertainties still exist in drawing fetal conclusions to determine whether a single hair is identifiable from a certain individual. The Scanning Electron Microscope alone may not be able to cause final conclusions to be drawn, as however, with farther tests and comparative studies, this type of study certainly will eventually lead to a more positive identification of the hairs, since it definitely shows improvement over the optical means of identification regarding structural, surface morphology, coupled with other scientific data. The use of scanning electron microscopy in wildlife forensic cases has been described for species identification. The surface cuticular pattern, cross section and medullary index provides the information regarding the species. Researchers have revealed the scale architecture of regular mosaic with smooth margins of shahtoosh wool. But when the hair evidence from different species from the same family were found to be blended together than the investigation becomes quite tough and typical so the present study which incorporates the scale layer difference between closely related species can provide the information regarding its species to the forensic expert. Elemental analysis through EDS provides significant information of the hair sample of specific sample which can further be used as a geographical region and species identification tool. Dahiya, M.S. *et al.* (2013). The elements present inside the hair were tested for both intra and inter animal differences using element Percentage and atomic percentage analysis like Sulfur (S), Iron (Fe), Potassium (K) and Calcium (Ca).

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