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Hair - A Drug Testing Substrate

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Abstract

Hair is a strong, stable tissue less affected by adulterants or short-term abstinence. It has the ability to capture long-term information about health and disease in an individual. Testing long hair offers a potential means of long-term monitoring of drug compliance, drug abuse, chronic alcohol abuse, and diagnostic biomarker discovery. The collection of samples is easier, risk of disease transmission is lower, and manipulation of samples is also not possible. Currently human hair testing is used for assessment of exposure to drugs of abuse, alcohol and environmental toxins. The possibility of using hair testing as a potential method to test drug compliance is being explored. Hair testing is also used in the detection of chronic stress and in the molecular assessment of cancer and neurodegenerative diseases. However there are difficulties in the analysis and interpretation of the results of hair testing. This review will give a brief overview on the physiology of hair and the routes of drug incorporation into hair strands, methods of hair analysis, the various applications of hair testing, the advantages and disadvantages of the same with a short note on the regulatory aspects of hair testing.

Keywords: Hair, Drug, catagen.

Introduction

The analysis of drugs in hair samples has become very popular in recent years, since it has potential applications in forensic and in clinical toxicology as well as in workplace drug testing procedures. Hair analysis has become both an alternative and a complementary approach to drug abuse and it is less embarrassing and intrusive for study subjects compared to urine testing. Hair analysis can yield a cumulative picture of long-term exposure and the window of detection ranges from a week to several months.[1] Hair testing is used for clinical and forensic investigations to evaluate drug exposure, to portray drug abuse history, for workplace drug testing, dope tests in sports and for assessment of prenatal exposure to drugs. It is also being tried in therapeutic

drug monitoring services.[2] The society of hair testing meets periodically to update guidelines on the analysis of hair samples. This review will attempt to cover the anatomy and physiology of hair and the drug incorporation routes, the steps in hair analysis, interpretation and applications of hair analysis, the disadvantages of hair testing and future perspectives.

Structure and growth of human hair[3]

Hair is a complex epidermal outgrowth synthesized in the hair follicle and is made up of keratinized cells. Human hair consists of an outer cuticle, inner cortex and central medulla. Human hair is composed of 65–95% proteins, 15–35% water and 1–9% lipids, 0.1–5% pigments (melanin), small amounts of trace elements, and polysaccharides. The growth rate of human hair is approximately 0.35 mm per day (1 cm per month) for both males and females.[3] The hair growth cycle is composed of four phases – the anagen or growing phase, the catagen or regression phase, the telogen or the resting phase and the exogen or shedding phase. The hair pigmentation occurs due to melanin formation in the hair follicle, a process called follicular melanogenesis. The hair bulb is the site of pigment formation for the entire hair shaft. The color varies depending on the type of melanin synthesized. Melanocytes and pigmentation play an important role in the incorporation of basic drugs into hair. Melanin has been found to have affinity for basic drugs. It has been found that pigmented hair contains higher concentrations of hair when compared to unpigmented hair. Each hair belongs to a sebaceous gland with the duct leading to the upper part of the root to ensure that the mature hair is bathed in sebum for two to three days prior to reaching the skin surface. The eccrine sweat glands wet the hair shaft and can contribute to the incorporation of hydrophilic drugs.

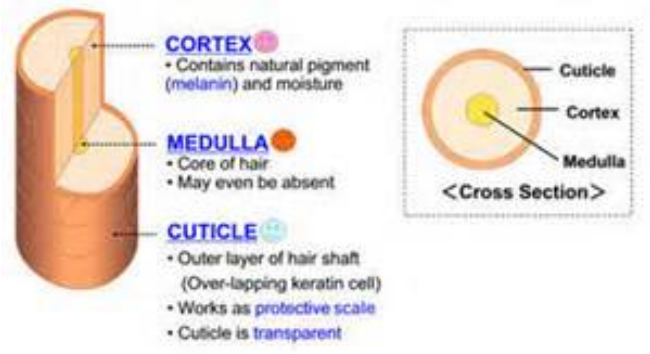

Fig 1: structure of human hair

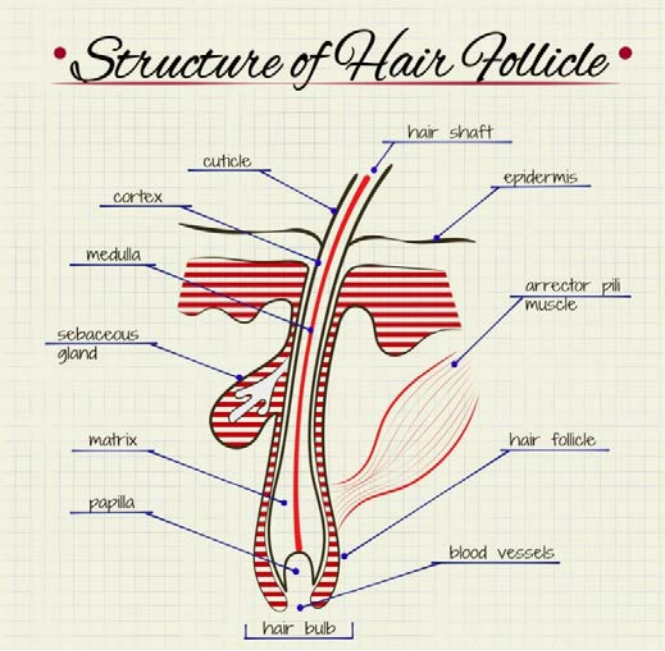

Fig 2: structure of human hair

Source: https://www.vectorstock.com/royalty-free-vector/structure-of-human-hair-vector-4718344

Mechanisms of drug incorporation into the hair[4]

There are three mechanisms by which drugs can enter the hair –

Incorporation from the bloodstream (Active or passive diffusion from the bloodstream feeding the dermal papilla)

Drugs circulating in the blood will be delivered to the hair follicle and diffuse across the cell membrane in order to enter the matrix cells. The rate of this transport is related to the lipid solubility of the drug and to the pH

gradient between the plasma and the cell. The pH of plasma is around 7.4 and the pH of matrix cells is 3-5. So basic drugs have a tendency accumulate in the matrix cells, as the diffusion into the cell is favored by the pH gradient. Once inside the cell, the molecule will be ionized and will not be able to diffuse back into the plasma. They cannot be removed or changed by the external environment also. Hence the physicochemical properties of the drugs seem to play a more important role in their incorporation than their plasma concentrations. Drugs in the bloodstream are entrapped by inaccessible regions of the hair during the hair growth process. After the hair emerges from the scalp, these drugs form bands in proportion to the concentrations present when the hair was formed. Because hair grows at a relatively constant rate hair analysis would provide a history of drug consumption in both time and amount.

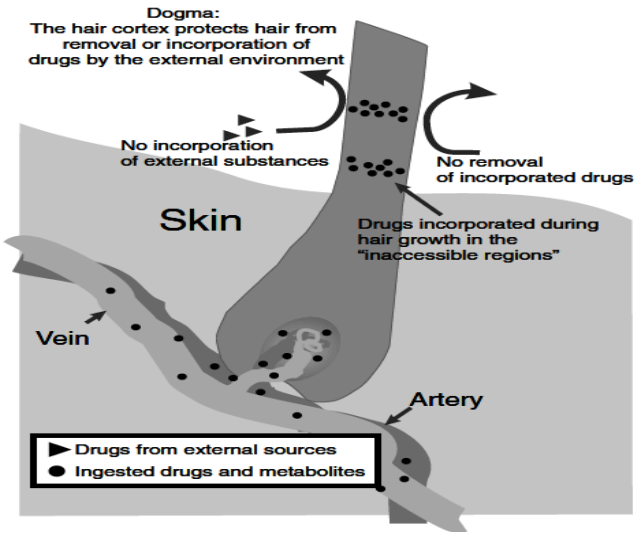

Fig 3: Drug incorporation through bloodstream

Source: Kintz et al. 2007

Incorporation from sweat and other secretions (Diffusion from sweat or other secretions bathing the growing or mature hair fiber)

Drugs and their metabolites are excreted in sweat and other secretions like sebum. Drugs persist in these secretions for a prolonged period and at higher concentrations than in plasma. The more lipophilic the drug, the better is its ability to cross cell membranes and the more it accumulates in hair.

Incorporation from external contamination

Drugs can be incorporated into hair from passive exposure of the hair to the drug, either from vapor phase (e.g., smoke) or solid-phase contact (e.g., drugs on furniture or clothing or skin-to-hair contact) followed by dissolution of the drug into drug-free sweat or other aqueous media. It will be extremely difficult to differentiate drug detection due to external contamination from those situations when the drug has actually been consumed.

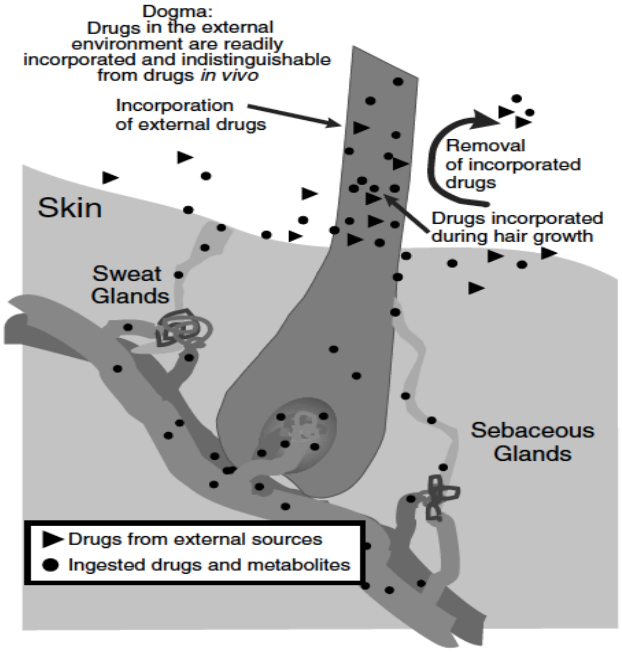

Fig 4: Drug incorporation through sweat and external contamination

Source: Kintz et al. 2007

Three factors influence drug incorporation into hair. These include the melanin content of hair and the lipophilicity and pH of the substance. Lipophilic (uncharged) organic molecules can penetrate membranes and diffuse according to the concentration gradient in

matrix cells. Basic or acidic drugs ionized to a high degree at physiological pH can reach matrix cells. The intracellular pH of keratinocytes and melanocytes has been found to be more acidic than plasma. Melanin also has an affinity for basic drugs. This leads to the accumulation of lipophilic and basic drugs in matrix cells. Acidic drugs are found only in very low concentrations in hair. Retention and stability of drugs in hair is considered good. Mean drug concentration slowly decreases with increasing distance from the hair root (i.e., increasing age of the hair).

Damage of the hair cuticle due to natural factors (e.g. sunlight, water, pollution) can be enhanced by hair cosmetic treatments, such as repeated perming, bleaching and dyeing of hair, leading to a loss of 50-80% respect to the original concentration.[5] The products used for cosmetic treatments are strong bases and can induce hair damage and affect drug content by loss or by affecting directly drug stability.

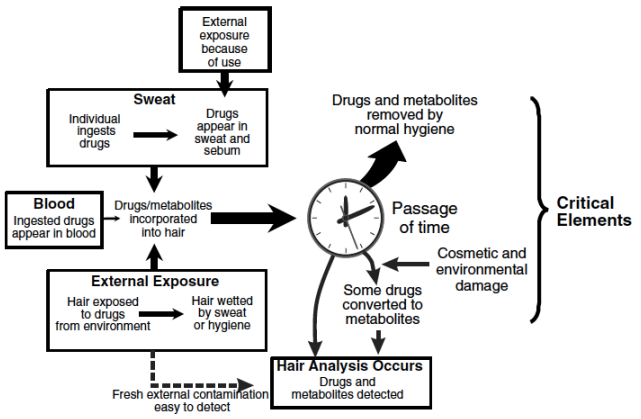

Figure 5: Factors affecting drug incorporation and detection

Source: Kintz et al. 2007

Steps in hair analysis[6,7]

Sample collection and storage

History collection and identification of the person are important in forensic cases. The date and site of collection (on the head), residual length on scalp, hair length and color must be noted down. A tuft of hair with a diameter of 3 to 4 mm is fixed by a string and cut at the skin surface. The preferred site on the scalp is vertex posterior because the proportion of telogen hair is lowest here and the growth rate is also relatively uniform. If scalp hair is not available, alternative sources including pubic, axillary and body hair should be collected. Hair samples should be stored under dry and dark conditions at room temperature.

Decontamination

The collected hair is divided into equal segments to try and analyze the time of drug intake. It is then decontaminated with the help of organic solvents like methanol. The purpose of decontamination is to remove residual hair care products, sebum, sweat and dust which can interfere with the analysis and to remove drugs adherent to hair due to external contamination.

Separation of drugs from hair matrix

The drugs must be extracted from the hair matrix by using methanol or aqueous acids or buffers or subjected to digestion by aqueous NaOH or enzymes like proteinase K. These extracts are then cleaned up using solid phase extraction procedures.

Detection or quantification of drugs

The drugs are detected using radioimmunoassay (RIA) or ELISA. Positive test results must be confirmed by gas chromatography – mass spectrometry (GC-MS). GC-MS is the most commonly used method in hair analysis. The prerequisite for this is that the substance should be volatile and stable at high temperature. The other methods used are LC-MS and HPLC.

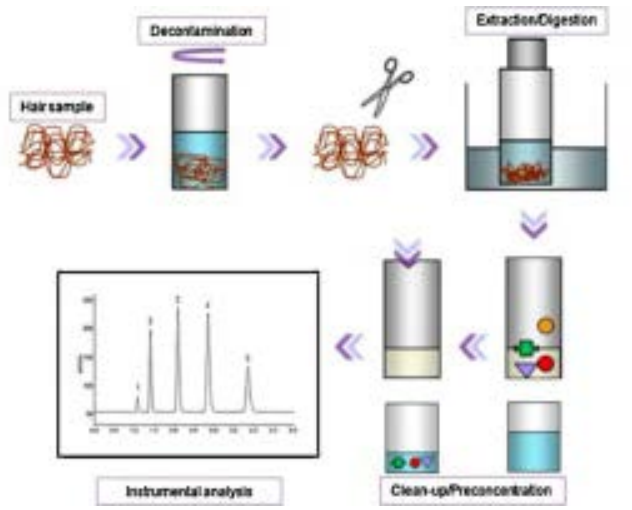

Figure 6: Steps in hair analysis

Source: Baciu et al, 2015

Interpretation of results

The intensity of drug use can be determined by cutoff values. Cutoff levels for drugs in hair have been assigned for the commonly tested drugs. There is no inter-individual correlation between frequencies of drug use or drug dose with hair concentration. Differences between consumption and external contamination can be made by comparing the concentration of drug in the hair with the concentration in the wash solution. If the concentration of the drug is higher in the latter, then the drug has been incorporated through external contamination. The position of drug molecules in a hair sample can be used to calculate the date of intake from growth rate, date of sampling and length of the residual stubble.

Figure 7: timing of drug intake

Applications of hair testing

Dope testing: Hair analysis has been tried to test the presence of, anabolic agents, peptide hormones/growth factors, β2-agonists, hormone and metabolic modulators, diuretics and masking agents, stimulants, narcotics, cannabinoids, glucocorticoids, and beta-blockers in sports personnel as part of the dope testing. In 1999, the Society of Hair Testing stated that hair drug analysis can supplement routine urine drug testing.[8]

Workplace drug testing: Employers make their staff undergo drug testing routinely to detect and prevent illicit drug use and excess alcohol consumption in an effort to create a drug free workplace. There are no accepted guidelines for using hair testing, but FDA has cleared certain firms to analyze hair for drugs of abuse. It was found that drug positive specimens were higher with hair samples than urine samples.[9,10]

Driving ability examination: In Germany and Italy, hair analysis is used as a supplementary test for re-granting a driver's license. The drugs analyzed include

opioids, cannabis and ecstasy. About 8 samples are collected over 40 days and analyzed by RIA. If the tests are positive, then urinalysis is done.[11]

Diagnosis of drug abuse and chronic intoxication: Hair samples of patients in withdrawal treatment were analyzed to determine the identity of abused drugs before treatment and to control change in consumption behavior after treatment. Segmental hair analysis can provide information about an individual's drug use or period of abstinence. Switching from one drug to another or mixing of drug combinations can also be detected.

Active smokers have higher concentrations of nicotine and cotinine in hair versus passive smokers. High concentrations are also found in the hair of children living in a smoker's house.

Alcohol, smoking and illegal drug use during pregnancy are hazardous to the fetus and may lead to miscarriage, premature birth, increased peri- and neonatal mortality rate, retarded physical and mental development, learning difficulty or hyperactivity. Meconium analysis is usually done to investigate in utero drug exposure. But analysis of hair obtained from the baby and mother can also be performed. Neonatal hair analysis has successfully detected nicotine, opioids and cocaine exposure in the mother in utero. [12,13]

Hair analysis for pesticides and adulterants are being tried and are in the testing stages.

Hair testing is also employed in postmortem toxicology and criminal assault (like sexual assault and child abuse).

Detection of excessive alcohol abuse can be made through analysis of its minor metabolites like fatty acid ethyl esters and ethyl glucuronide.

Therapeutic drug monitoring: Hair testing is unsuitable for adjustment of drug dosing. There is a very poor relationship between the drug dose, plasma concentration and hair concentration. There is also a huge intra and inter-individual variability due to which there could be no therapeutic hair concentration range. But hair testing can be done to analyze compliance of the patient. For this, multiple segments of a single strand can be analyzed, or repeated sampling of the proximal 1 cm hair can be analyzed. Hair testing has been employed for analysis of benzodiazepines, barbiturates like phenobarbital, antipsychotics like haloperidol, antiepileptics like phenytoin and carbamazepine. Other drugs tested include tricyclic antidepressants and antibiotic doxycycline.[14,15]

Advantages and disadvantages with hair testing:

The advantages are as follows:

1. Drugs and metabolites remain sequestered in the hair shaft with no observed time degradation, providing a window of detection that is much wider (weeks to several months) than that of serum or urine, in which drug levels decrease rapidly over a relatively short period of time (hours to days).
2. Hair collection is simple, noninvasive, and replicable for eventual confirmation of the original results.
3. There is low potential for evasion or manipulation of results from hair testing.
4. There is a low risk of disease transmission in the handling of samples.
5. Hair samples are stable indefinitely and very difficult to manipulate to alter their drug content.

The disadvantages of hair testing are as follows:

1. There is a high degree of external contamination and no sure way of differentiating true drug intake and contamination

2. There is no standard dose - response relationship and high intra and inter individual variability
3. The results obtained from hair testing procedures might not be reliable if any mistakes are made during decontamination and washing.
4. Results may also be affected by cosmetic treatments on the hair and environmental damage.
5. Bias with hair testing is very common because if inter-individual variability and living conditions of the persons.

Because of all these reasons hair testing remains a supplementary tool and is mostly used in forensic investigations and dope testing.

Conclusion

Hair as a testing substrate has received increased attention because of a wider detection time frame, less embarrassing circumstances of collection, and greater stability versus body fluids or other tissues. Improved analytical technology will further promote the use of hair analysis in clinical scenarios. Due to their high sensitivity and specificity, newer generations of GC–MS/MS and LC–MS/MS technologies will become standardized tools in toxicological laboratories. The Society of Hair Testing updates guidelines about specimen collection, decontamination and metabolites to be assayed. The main challenge however is overcoming the biologic variability including differences in hair growth and mechanisms of drug incorporation. Weak points in the present hair tests must be overcome by reliable reference standards, standardization of procedures for decontamination and extraction, establishment of performance standards for drug and metabolite identification and use of scientifically founded drug cut-off values.

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