

Diatoms: A Review on its Forensic Significance

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Abstract

Diatoms also called as the 'jewels of sea' are microorganisms which are extensively found in the aquatic system. These unicellular organisms make up nearly half of the biological material in the water body. It is also one of the most significant biological evidence that is obtained in case of drowning. The diatoms that infiltrate inside the body of the deceased may serve as a corroborative or even conclusive evidence to support the diagnosis of death. These diatoms also help in ascertaining whether the drowning is ante-mortem or post-mortem. The review discusses the current extraction procedures and microscopic examination techniques used in forensic science for diagnosis of death by drowning.

Keywords: Ante-mortem, Biological Evidence, Diatoms, Drowning, Forensic Science

Introduction

Diatoms are unicellular, non-motile micro-algae. They are the most common type of phytoplankton which belong to the kingdom of Protista and are classified under Bacillariophyceae. Diatoms are the most successful organisms which can thrive in large numbers in almost every aquatic environment. They are also responsible for the carbon fixation in the environment. Typical diatoms come in the range of 10 μm – 20 μm ¹. They have cell wall made of silica, which makes the outer lining of the cell harder. The siliceous covering of the cell is inert and indestructible². Diatoms are broadly classified into two categories based on their structure: Centrales and Pennales. Centric diatoms are radially symmetrical, found drifting near the surfaces of the oceans and are wheel shaped whereas pinnate diatoms are laterally symmetrical and live in fresh water streams, swamps, or bottoms of shallow water³.

There are more than 10,000 species of diatoms that have been discovered. Therefore, they come in various shapes and sizes. Due to these variants, these microorganisms are advantageous for the diagnosis of death by drowning⁴. Perpetrators could often be seen disposing the corpse into water to mimic the cause of death and raise a suspicion of suicide during the investigation. During drowning

there is a struggle for the expiratory process due to which the water enters the respiratory tract. Only a living body with an active circulation could transport the diatoms from the lungs and rupture the alveolar wall and pass through the lymph nodes, pulmonary veins, heart (left side) and systemic circulation that include the bone marrow, kidney and brain⁵. It is a challenging situation for a forensic pathologist to examine these specimens and identify the cause of the death. But due to the presence of diatoms, it can be identified if the death has occurred before drowning or after drowning⁶.

Organs for the Examination

The success of the conclusive tissue analysis helps in the qualitative and quantitative diagnosis for the presence of diatoms in suspected cases of drowning. The distribution of diatoms in each organ varies; therefore it is essential for the forensic scientist to identify the appropriate specimen for the process of examination. For many decades the conventional practice was to perform the analysis by using lungs, bone marrow or long bones, heart, liver and blood. In a control experimental study on laboratory rats to determine the number of diatom cells in each organ, it was reported that the highest number of diatom cells were

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identified in the stomach and lungs due to the direct entry of the water through the mouth and the nostrils⁷. During the postmortem examination, the samples are collected and preserved using suitable preservatives. Formalin used to fix lung tissue in the autopsy was found to be effective. The samples preserved using formalin can be utilized for long term examination purposes as these microorganisms resist putrefaction⁸. However, formalin is not suggested for the preservation as it destroys the fine structure of the cells. Control samples collected for the examination

process should be preserved using Lugol's iodine solution or ethanol⁹.

Extraction Methods

A great number of techniques are proposed by various studies to compare the efficiency of different extraction methods for forensic practice. The primary goal of each method is to extract diatoms from the postmortem

Table 1. Procedure for different methods of extraction for diatoms

Extraction Method	Procedure
Acid digestion	It is one of the oldest techniques accepted for the extraction of diatoms. In this method the tissue is digested in nitric acid. The residue that is obtained is then centrifuged to make a pellet. These pellets consist of nitric acid resistant material which is then smeared on a microscopic slide for further examination. To overcome the practical complication an instrument called 'can' was developed. The procedure involves liquidating the tissue with a strong acid and then subjecting to a high temperature. The special feature of this instrument is that it simple to operate, less time consuming and it is practically more efficient ¹¹⁻¹³ .
Enzymatic digestion	This method involves the application of proteinase K. The tissue sample is minced and rinsed in proteinase K and Tris HCl buffer solution. The solvent is incubated overnight and the sample is then centrifuged. The solid residue is then removed and observed under the microscope which is mounted using Naphrax ¹⁴ .
Soluene-350	Soluene-350 method of extraction is effective for fresh water diatoms. The tissue sample is washed thrice with distilled water and centrifuged. The residue obtained is suspended in Soluene-350 solution and incubated at room temperature overnight. This solution is then centrifuged and the resultant pellet is smeared on the microscopic slide and observed under the light microscope ¹⁵ .
Microwave digestion	It is a highly sensitive novel extraction method for diatoms. The sample is digested using microwave digestion apparatus containing a mixture of tissue sample and acid solution. The technique is highly efficient and there is less contamination. The digested solution is then subjected to SEM analysis ^{16,17} .

Table 2. Description for different microscopic methods for observation of diatoms

Microscopic Techniques	Description
Light microscopy	Light microscopic examination is not used widely because of its limitations in the resolution or magnification of the microorganism which is smaller in size. The efficiency of distinguishing smaller species is difficult with this microscopy. Therefore it is not recommended for forensic purposes ¹⁸ .
Scanning Electron microscopy	SEM gives a 3D image of the diatom with high resolution. In recent years Zhao et al., 2017 developed a new novel method called microwave digestion- vacuum filtration -automated SEM analysis which is highly sensitive and specific. One of the disadvantages of this technique is that it is time consuming. Therefore to overcome this, deep learning based algorithm was developed which could automatically detect the diatoms from the SEM images ¹⁹ .
Transmission electron microscopy	TEM is a technique of choice for the analysis and the evaluation of micro structures which generates a 2D image of the sample ²⁰ .
Atomic Force microscope	Atomic force microscope also called as scanning probe microscope is a newer technique developed to observe objects or materials which are in nano scale. The image processed from the AFM has high resolution and also the image can be scanned in both vertical and horizontal axis ^{21,22} .

tissue. This includes acid digestion, enzymatic method, Soluene-350 and microwave digestion¹⁰.

Microscopy Techniques

Diatoms are made up of tiny cells which could be observed using a microscope for detection and identification. Microscopic examination is the oldest and conventional method of diatom test. The taxonomical features can be observed to identify the species which is necessary for medico legal purposes. Presence of diatoms helps to differentiate a death by submersion from an immersion of a body. During the extraction process, the foreign materials are removed from the tissue sample to avoid any interference during the microscopic examination. The most commonly used microscopes are Light microscope, Scanning electron microscope, Transmission electron microscope and Atomic force microscope.

DNA Barcoding of Diatoms

In case of drowning, many small living microorganisms present in the water source gets deposited into the victim's internal organs because of the continuous flow of water in and out of the body. DNA barcoding is a method of characterizing a particular target region in a DNA strand. The advancement in DNA barcoding has many applications in forensics and other bio assessments. The amplification of specific sequence is generated to identify an organism to their definitive taxa. It provides additional information on the diatom species meticulously than the conventional microscopic techniques or the culturing techniques for analysis²³. Examination of diatom using a microscope is a time-consuming process and requires taxonomic expertise. At present there are many molecular based technologies to efficiently detect the diatom. A novel microarray based on specific 18sRNA using Sanger sequencing was developed for single cell rDNA²⁴.

Conclusion

On a broader aspect, diatoms can be studied efficiently using the various techniques that are available. Specifically, in forensic science diatoms can be used in the identification of death due to drowning, their extraction and examination can be done with the various techniques as discussed above. Comparisons with the

present databases could possibly also help in the region-wise identification of diatoms. Diatoms are a boon to the identification of death due to drowning.

Conflict of Interest

The authors have no conflict of interest.

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