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Advances and Prospects in the Application of Modern Biological Techniques in Forensic Science

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Abstract: With the rapid advancement of biological technologies, their application in forensic science has become increasingly widespread, serving as a crucial pillar for case investigation and judicial identification. This paper systematically reviews the progress in the application of DNA technology, proteomics, and microbiological techniques in forensic science. It compares the advantages and limitations of various technologies and explores the prospects for their integrated application. The study indicates that DNA extraction and analysis techniques, protein mass spectrometry imaging, and microbiome analysis hold significant value in individual identification, time-of-death estimation, bodily fluid identification, and drowning diagnosis. Research indicates that methods such as DNA extraction and analysis, protein mass spectrometry imaging, and microbiome analysis hold significant value in individual identification, time-of-death estimation, bodily fluid identification, and drowning diagnosis. Looking ahead, the integration of automation, artificial intelligence, and multi-omics technologies will propel forensic biology towards greater efficiency, precision, and intelligence, providing stronger scientific foundations for judicial practice.

Keywords: Forensic Biology; Time of Death Determination; DNA Extraction; Microbiome.

1. Introduction

Given current technological advancements, forensic science increasingly emphasises the development and application of biological techniques. This research was undertaken to better apply diverse biological technologies across different case scenarios.In everyday life, biological technologies utilise organisms (including microorganisms, animal cells and plant cells) or their components (organelles and enzymes) to produce useful substances or provide services for human benefit. Within forensic science, however, biological techniques assist investigators in reconstructing case sequences, establishing victim profiles, and analysing interpersonal relationships. Examples include bloodstain analysis, paternity testing, DNA comparison, and even determining the time of death.This study employs comparative analysis to highlight the distinct advantages of various biological technologies across different domains. DNA extraction and analysis, alongside proteomics, may prove crucial for individual identification and determining time of death. Microbiome analysis holds significant value in drowning investigations and injury assessment. With the advent of personalisation, automation, and digitalisation, biological technologies can furnish more precise evidence for case analysis and forensic evaluation.

2. DNA Technology

Common forensic DNA techniques broadly fall into two categories: DNA extraction and DNA analysis.

2.1. DNA Extraction Techniques

Certain environments are unsuitable for DNA extraction. As time passes, corpse decomposition worsens, reducing the available DNA for forensic collection. Consequently, DNA is typically extracted from hard tissues—bones and teeth.

2.1.1. Phenol/Chloroform/Isopentanol Method

This organic chemical DNA extraction method is relatively antiquated. Its principle involves using organic solvents to remove impurities such as proteins and polysaccharides, exploiting differences in solubility between DNA and impurities in various solvents to achieve separation. Ultimately, a series of steps including centrifugation and washing yields purified DNA.Christian Haarkötter et al., through comparative studies with other extraction methods, concluded that the phenol/chloroform/isopropanol method yields the highest quantities and efficiency for both small-molecule and large-molecule human DNA extraction. However, it requires substantial sample volumes, complex procedures, and the use of toxic compounds[1] .

2.1.2. Magnetic Bead Method

This method employs the principle of reversible solid-phase immobilisation (SPRI) technology using magnetic beads as a solid-phase carrier. It leverages the specific adsorption between carboxyl groups on the bead surface and DNA molecules, alongside the effects of PEG and salt ions on DNA conformation, to achieve DNA separation and purification. Chandra Finaughty et al. demonstrated through comparative analysis that while magnetic bead methods yield comparable DNA quantities to organic chemical methods, they produce more complete DNA typing profiles.In terms of performance, magnetic bead methods demonstrate superior STR typing success rates compared to nearly all commercially available DNA extraction techniques. This excellence stems from their unique technical mechanisms. The automated operation and significant proportion of demultinification steps in magnetic bead methods are both positively correlated with DNA typing success rates [2] .

2.2. DNA Analysis Techniques

2.2.1. Single-Cell Gel Electrophoresis (SCGE)

This technique enables the estimation of post-mortem interval (PMI) through qualitative and quantitative analysis of DNA fragmentation. Its core principle relies on the fact that following biological death, intracellular nucleases progressively fragment DNA, with the extent of

fragmentation increasing with elapsed time[3]. Zheng et al. extended the PMI range to 72 hours. By analysing DNA fragmentation in mouse and pulp cells, they demonstrated that SCGE comet parameters exhibit high correlation with PMIs within 72 hours, proving the technique's applicability even during prolonged post-mortem intervals [4]. Summarising the aforementioned research, SCGE's advantages lie in its enhanced sensitivity, greater reliability, and superior convenience [5].

2.2.2. DNA Profiling Analysis Technology

This technology involves analysing specific genetic markers within DNA to construct an individual's genetic profile, serving as a core technique for personal identification and kinship testing. Its essence lies in utilising DNA polymorphism, visualising these variations through specific processes to create a unique "identity card" for an individual. DNA profiling proves particularly crucial where traditional forensic methods prove inadequate. It becomes indispensable when dealing with severely damaged or decomposed remains where soft tissue samples cannot be obtained [6].

3. Proteomics Technology

Historically, for most forensic scientists, proteins within samples were considered optional components. They preferred to focus on the separation, extraction, and purification of nucleotides, often viewing proteins as elements requiring removal[7-8]. However, proteins within samples exhibit high complexity and harbour substantial information [9], capable of providing additional connections and robust leads for cases. Consequently, proteins can be utilised to identify tissue origins[10, 11].

3.1. Luminol Reaction

This reaction is applied in forensic bloodstain detection. Utilising its characteristic emission of blue light during oxidation, forensic scientists devised a method to react it with haemoglobin in blood. Luminol (5-amino-2,3-dihydrophthalazine-1,4-dione) is a highly sensitive blood detection chemical capable of identifying traces diluted to ratios as high as 1:10,000,000 ([12,13]). Researchers have developed a novel artificial blood substitute (referred to as YFBS). This alternative not only reacts with luminol but also highly mimics the rheological properties of human blood, a characteristic that can enhance the reliability of forensic laboratory and educational applications [14].

3.2. Proteomics Mass Spectrometry

It identifies molecules by measuring the mass-to-charge ratio of ions, calculates electronic structures and properties, and simulates molecular structures and protein sequencing.

3.2.1. Liquid Chromatography-Mass Spectrometry Coupling Technology

This technique enables multi-reaction detection and quantitative analysis of ions, offering high sensitivity. Lorenzo Toma et al. developed a high-throughput shotgun proteomics strategy based on this technique for analysing post-mortem skin ecchymoses. The sole protein specifically identified across all ecchymosis samples was glycoprotein A (GYPA), which maintains stability over time post-mortem. This renders it an effective marker for corpses exhibiting late-stage putrefaction[15] .

3.2.2. Mass Spectrometry Imaging (MSI)

It scans samples using lasers or high-energy ion beams, then employs a mass analyser ( ) to obtain the mass-to-charge ratio ( ) and ( ) of the sample surface ( ). Utilising mass spectrometry imaging software, it generates a two-dimensional distribution map of corresponding molecules or ions across the sample surface. Subsequently, the aforementioned software processes the data further to determine the three-dimensional spatial distribution of the target substance within the sample.When illicit actors conceal abused drugs within herbal mixtures to evade detection, MSI enables rapid visual localisation of the drugs without inefficient random sampling or microscopic morphological examination. MSI also holds significant value in analysing exogenous drugs within fingerprints and drugs/metabolites within the fingerprints of drug users.Hair possesses unique advantages for illicit drug detection in forensic toxicology, with segmented hair analysis providing insights into drug usage patterns and information. Currently, MSI technology is employed to monitor illicit drug distribution throughout hair samples. Finally, MSI finds applications in whole-animal analysis, brain tissue analysis, and renal and hepatic tissue analysis[16]. Thus, MSI holds significant importance for forensic science.

4. Microbiological Techniques

By extracting microbial samples from environments or corpses, compelling evidence relevant to a case can be obtained, such as the primary crime scene and the time of death of the victim. In specific environments—such as those characterised by high temperatures, humidity, or extreme cold—unique microorganisms present in these conditions can yield distinctive evidence.

4.1. Drowning Deaths

Historically, forensic scientists utilised diatoms as an adjunct to drowning diagnosis. However, this approach is now contested due to diatoms dispersing throughout various organs during aspiration [17, 18].Through further research and development, forensic scientists are increasingly adopting planktonic bacteria as a potential criterion for confirming drowning deaths. Analysis employs molecular PCR detection methods using fluorescently labelled TaqMan oligonucleotide probes[19] .

4.2. Child Abuse

Distinguishing traumatic injuries from certain naturally occurring infectious diseases in children presents particular challenges. For instance, differentiating skin haemorrhages caused by post-streptococcal toxic shock syndrome from those resulting from physical abuse requires careful differential diagnosis. Toxic shock syndrome is a systemic infectious disease manifesting as erythematous lesions and skin necrosis, caused by Staphylococcus aureus strains producing exotoxins or Streptococcus pyogenes strains producing exanthemines[20]. However, from a forensic pathology perspective, there may be grounds to suspect the presence of child abuse. Thus, microbiological examination plays a crucial role here, identifying all microorganisms consistent with the observed lesions[21]. This necessitates bacterial and viral microbiological analysis of multiple matrices (blood, urine, cerebrospinal fluid, brain, throat, lungs, heart, spleen, and kidneys).For each isolated microbial spectrum, a comprehensive assessment should be conducted, integrating signs of ante-mortem reactions observed in histological examinations, C-reactive protein levels, and ante-

mortem clinical information [22]. Through this integrated analysis, the forensic pathologist can determine the presence or absence of traumatic injury.

5. Conclusion and Outlook

Over the years, DNA technology, proteomics, and microbiology have each played distinct yet vital roles within forensic science. DNA techniques prove particularly suited to individual identification and post-mortem interval estimation; proteomics excels in determining time of death, tissue provenance, and detecting substance distribution within the body; while microbiome analysis is most effective for drowning diagnosis and injury assessment. In certain cases, the combined use of different techniques can expedite the provision of valuable evidence. For instance, the integration of DNA and proteomics can enable faster and more accurate identification of the deceased and estimation of the time of death. It is anticipated that in future criminal cases, these forensic techniques will be automated, enabling swifter determination of the primary crime scene and more precise identification of suspects or murder weapons. Furthermore, the incorporation of AI-powered intelligent modelling could facilitate automated reconstruction of the crime scene.

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