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Review article

Current and emerging tools for the recovery of genetic information from post mortem samples: New directions for disaster victim identification

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ABSTRACT

DNA profiling has emerged as the gold standard for the identification of victims in mass disaster events providing an ability to identify victims, reassociate remains and provide investigative leads at a relatively low cost, and with a high degree of discrimination. For the majority of samples, DNA-based identification can be achieved in a fast, streamlined and high-throughput manner. However, a large number of remains will be extremely compromised, characteristic of mass disasters. Advances in technology and in the field of forensic biology have increased the options for the collection, sampling, preservation and processing of samples for DNA profiling. Furthermore, recent developments now allow a vast array of new genetic markers and genotyping techniques to extract as much genetic information from a sample as possible, ensuring that identification is not only accurate but also possible where material is degraded, or limited. Where historically DNA profiling has involved comparison with ante mortem samples or relatives, now DNA profiling can direct investigators towards putative victims or relatives, for comparison through the determination of externally visible characteristics, or biogeographical ancestry. This paper reviews the current and emerging tools available for maximising the recovery of genetic information from post mortem samples in a disaster victim identification context.

1. Introduction

The primary and most reliable means of identification for disaster victim identification (DVI) are fingerprint analysis, dental comparison and DNA analysis [1]. DNA profiling has become the gold standard for the identification of victims in both mass casualty incidents and forensic cases where human remains are highly fragmented and/or degraded [2]. This is due to the relatively low cost and high degree of discrimination DNA-based identification can provide. In addition to identifying victims, DNA profiling also offers the ability to reassociate body parts and can aid in the identification of offenders where human activity has led to a mass casualty event [2]. Challenges associated with the sampling of remains can include the number of victims, mechanisms of body destruction, extent of body fragmentation and body accessibility [3]. Disaster locations considered hostile environments can also pose additional challenges for the recovery effort.

2. International standards in disaster victim identification

The 2004 tsunami in South East Asia and subsequent DVI effort highlight the necessity of standards in the DVI process; it was during this mass disaster that forensic scientists and police organisations started to develop standards for the identification process based on their practical experience [4]. The Tsunami Evaluation Report [5] documents the workflow, responsibilities and other significant issues influencing the decision making process, as well as the aspects of the operation that would influence the efficiency of the identification process. The INTERPOL Standing Committee on DVI [6] would go on to develop guidelines for all aspects of the DVI process, with the inclusion of three working groups: forensic pathology, forensic odontology and police. International standards have continued to develop [4,7–9] with strong evidence following disaster events that local structures should adopt international standards and recommendations, as well as be provided with more detailed guidance regarding appropriate DVI responders [10].

Goodwin [9] highlights two International Organisation for

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Standardisation (ISO) standards having high relevance to the identification of human remains. These include ISO/IEC 17020:2012 'Conformity assessment - Requirements for the operation of various types of bodies performing inspection'; ISO/IEC 17025:2018 'General requirements for the competence of testing and calibration laboratories' and; the Forensic Science ISO/IEC 17025 Application Document and ISO 18385:2017 'Minimising the risk of human DNA contamination in products used to collect, store and analyse biological material for forensic purposes – Requirements'. These standards have applicability usually in the recovery of evidence at a crime scene and as a technical standard for forensic genetics. The supplementary publication ILAC G19:08/2014 Modules in a Forensic Science Process, by the International Laboratory Accreditation Cooperation Organisation (ILAC) helps to bring a specific forensic application to the broad scope of the ISO standards. Additionally, both practical and simulated quality exercises are becoming more common in an attempt to standardise DVI procedures internationally [11,12].

3. Sources of DNA

DNA can be recovered from a range of biological sources. Depending on the circumstances of a mass casualty incident, some sources may be more ideal for the purposes of DNA-based identification than others. Factors such as resistance of the source to degradation or damage due to its natural structure can also affect DNA recovery.

3.1. Current approaches

3.1.1. Blood and saliva

Current INTERPOL Guidelines [1] recommend the collection of blood or saliva on Flinders Technology Australia (FTA®) paper or a swab in complete, non-decomposed bodies. It can be difficult to collect blood and saliva from deceased individuals once the blood ceases to circulate, and saliva ceases to be produced. The collection of blood from a body cavity becomes labour intensive and may require surgical recovery [2]. Blood is the recommended sample if the body is a complete or mutilated non-decomposed body [1], with DNA from blood usually being less degraded than saliva [13]. Blood vessels have been found to yield better short tandem repeat (STR) typing results than muscle samples in decomposed, dismemberment cases [14]. Buccal smears on FTA® cards are recommended only if the body condition is complete and non-decomposed [15].

3.1.2. Skin and muscle

Deep-seated red muscle tissue is currently recommended if the remains are mutilated and incomplete [1]. During decomposition the soft tissues of the body will begin to decompose much earlier than hard tissues. Consequently, DNA in soft tissues tends to degrade faster than in hard tissues [2,7,15,16]. When sampling soft tissue, skeletal muscle is recommended [3,17–19]. In severely burnt corpses, smears from the bladder have been shown to be a particularly effective alternate source of DNA [20].

3.1.3. Bone and teeth

DNA is well preserved in bone cells and teeth [15], making them reliable sources of DNA, particularly in adverse environmental conditions and for long-term sampling [7,21]. Current recommendations suggest the collection of bone is most appropriate for compromised remains due to a higher success rate of DNA recovery from femur shafts and teeth as compared to blood, buccal and tissue samples [2,7,15,16,22,23]. Specifically, where bodies are complete and decomposed or mutilated, INTERPOL Guidelines [1] recommend the collection of a sample from long, compact bones (4–6 cm window section without shaft separation), healthy teeth (preferably molars) or any other available bones (~10 g if possible; preferably cortical bones with dense tissue). This is limited, however, by the ability to isolate sufficient quantities of DNA from the skeletal samples [24]. Due to the poor quality and/or quantity of nuclear DNA (nDNA) in samples such as bone and teeth, the analysis of nDNA markers may fail to yield a reportable profile [25–27]. In these instances, mitochondrial DNA (mtDNA) is an alternative target due to its higher resistance to degradation and high copy number per cell [2].

Structurally the major proportion of bone is matrix, consisting of both an inorganic (principally hydroxyapatite) and an organic fraction, which is composed chiefly of type I collagen and extracellular matrix proteins, such as glycosaminoglycans and osteocalcin [28,29]. The collagen provides a soft framework and the minerals add strength and harden the framework. Approximately 70% of bone consists of the inorganic mineral hydroxyapatite which includes calcium phosphate, calcium carbonate, calcium fluoride, calcium hydroxide and citrate [30].

Different skeletal elements have been found to vary in the way they preserve DNA and consequently, yield different amounts of DNA [15,31]. Historically, sampling advice suggested that although spongy and cancellous bone can be rich in DNA, preservation is not reliable and the dense cortical bone (preferably weight bearing long leg bones) should be collected preferentially [7]. This is due to the DNA being protected by the physical and chemical structure of compact bone within the calcium (Ca^{2+}) matrix, which is not present in spongy bones [23]. During the identification efforts of the World Trade Center disaster (9/11) in 2001, it was determined that skeletal samples from femur and metatarsal bones offered more DNA, while skull bones were less suitable [32]. The International Commission on Missing Persons [31] has also identified weight bearing bones such as femur, tibia, pelvis, metatarsal and talus as some of the most suitable skeletal elements for sample collection.

The unique composition of teeth and their location in the jawbone provide additional protection from environmental and physical conditions that accelerate post mortem (PM) decomposition and DNA decay [33,34]. The total DNA content of teeth varies considerably between individuals and also within the same individual [35–37]. Pulp and cementum are the most valuable sources of nDNA in the tooth, as well as being good sources of mtDNA [38]. The pulp provides the richest source of DNA in teeth due to the relatively high cellularity [39]; however, pulp may be limited or even absent in aged and/or diseased teeth [38]. Teeth with the largest pulp volume provide the best source of DNA [40,41] and Higgins and Austin [38] suggest teeth with the largest root surface area (i.e. molars) should be targeted. Factors such as tooth type, tooth health and chronological age of the donor will have an effect on the relative proportions of DNA present in the tooth [38].

3.1.4. Hair

Hair is associated with use as an ante mortem (AM) sample rather than as a good source of DNA for the purposes of identification of a PM sample [1,7]. Because of its nature, hair has limited value for a conclusive identification, especially if separated from the body. This is further complicated in decomposed remains, particularly following hair loss during the bloat stage [42].

The difficulty with hair samples is being able to recover enough nDNA for a useable DNA profile. This is due to the structure of hair itself which is made up mostly of keratinised proteins, with little or no undegraded source of DNA [43]. In nDNA testing, the success of DNA profiling using hair samples is attributed to the presence of the root, and adhering epithelial cells [44,45]. As for bone, mtDNA can provide an alternative target.

3.1.5. Nails

Nails can be a valuable source of DNA because they have been shown to be resistant to decay and preserve their DNA content well [46–48]. DNA in fingernails is assumed to adhere to the underside of the nails but DNA is also preserved within the keratin structure of the nail [49]. STRs were successfully recovered from nails after one month