Quantification of RNA degradation of blood-specific markers to indicate the age of bloodstains

Suaad Alshehhi (University of Strathclyde / Dubai Police) & Penelope R. Haddrill (University of Strathclyde)

Recently, there has been increasing interest in the use of Ribonucleic Acid (RNA) in the forensic science community, for various applications. Some RNA types such as messenger RNA (mRNA) and microRNA (miRNA) have shown the ability to reveal the activities of genes and their respective cells/tissues, which might help to give an indication of pathological states or any condition that leads to death[1]. These molecules have also proved to be potential tools to identify the origin of a body fluid and give some indication of the time it was deposited[2,3]. Since 1983, when RNA first appeared in forensic science, many studies have been carried out to try and understand how patterns of gene expression can be useful in forensic science. **Suaad was highly commended for her Presentation at the Postgraduate Conference held on 15th December 2017.**

Background

Identification of body fluids

Identification of the types of biological stains recovered from crime scenes can be very important to criminal investigations, i.e. whether stains originate from blood, saliva, semen, etc. This kind of information may help in reconstructing events that occurred at a crime scene. Current methods used in forensic laboratories for body fluid identification depend on catalytic or enzymatic tests, to identify proteins or compounds present in one specific body fluid, such as the Kastle-Meyer presumptive test for blood, which indicates the presence of haemoglobin. Some of these methods lack specificity and sensitivity, and may also destroy precious samples, precluding the ability to perform subsequent DNA profiling. As such, RNA is of increasing interest in forensic science as a novel means to identify body fluids, and the similar chemical structures of DNA and RNA mean they can be co-extracted from biological samples.

It has been shown that the identification of cell type-specific mRNAs can provide high specificity for body fluid identification, due to distinct gene expression patterns in different tissue types[3]. As a result, each body fluid has its own specific gene expression pattern that can be defined by the presence of mRNAs that encode for proteins with body fluid-specific functions.

In a small number of published gene expression association studies, it has been found that, similar to mRNA, a number of miRNAs exhibit expression restricted to one cell type, making them useful markers for body fluid identification. All miRNA assays exhibited different expression profiles across a range of tissues other than their specific body fluids, confirming the high degree of specificity of the selected potential markers.

Biological stain age determination

Knowing the time of body fluid deposition at a crime scene can be crucial for criminal investigations, as it can give information regarding when a crime occurred, or whether a stain is pertinent to a specific criminal investigation. Conversely, samples that do not correspond to the time when the crime is proposed to have occurred may be excluded. To date, a number of publications have revealed that the degradation rate of RNA can be useful for estimating the age of biological samples recovered from a crime scene[4,5]. Given that RNA is known to be unstable and gradually degrades in the environment, it has been proposed that quantifying the level of RNA degradation may be useful as a measure of stain age. This is an emerging area of research with, as yet, a small number of publications investigating the relationship between age and RNA decay in blood[4] and hair[5]. These studies have analysed the degradation level of RNA in an attempt to estimate the age of biological stains.

Project aims

The overall purpose of this project is to develop a method to estimate the deposition time of biological fluids commonly encountered in forensic casework – blood, saliva and semen – using the application of RNA analysis.

Initially, the degradation profiles of multiple RNA markers will be analysed, including reference genes, as well as mRNA and miRNA body fluid specific markers. The first aim is to look for a correlation between the age of the bloodstain and the degradation rate of these RNA markers in order to identify those most useful for body fluid stain ageing and to assess the possibility of using the degradation rate to determine time since deposition.

The expression level and degradation rate of multiple RNA molecules in blood samples were analysed; these were HBA, HBB, HMBS, miR16, miR451, ACTB, 18S and U6. The RNA markers have been selected from a thorough literature review, and have been shown to indicate the presence of bloodstains. After this, the stability of mRNA and miRNA was examined, by calculating the relative expression ratio (RER) of these two different RNA molecules. This was done in order to provide information about which markers are likely to be more accurate for use in

estimating the age of biological stains, both over the short- and long-term.

In order to use RNA markers for estimating the age of bloodstains, the degradation rate of these markers needs to be large enough that it is observable across ageing time periods. In this work, the degradation rate of blood specific RNA markers in aged samples was analysed. A total of 10 volunteers (six females and four males) were asked to give blood samples, which were deposited on cotton swabs and then stored in a dry dark place at room temperature to simulate natural ageing, until they reached a series of desired ages (0, 3, 6, 15, 30, 90, 180, 270 days). The degradation levels of eight RNA molecules were analysed and the RERs calculated in order to study the degradation behaviour and its relationship with age.

Key findings

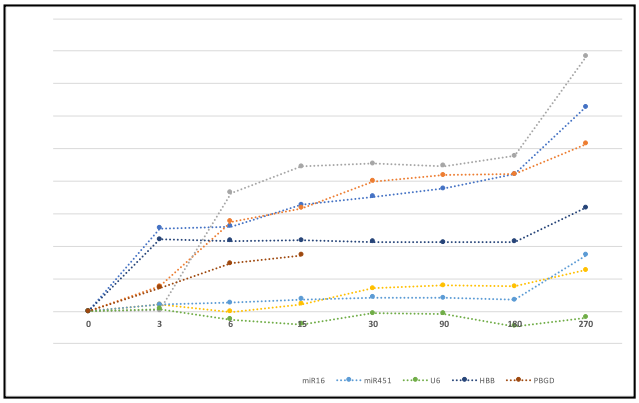

Figure 1. ΔC_q ($C_{q\ time\ x} - C_{q\ time\ 0}$) for HBA, HBB, PBGD, ACTB, 18S, miR16, miR451, and U6 in total RNA extracted from blood samples stored at room temperature for up to 270 days. Data represents the mean of n = 10. Error bars were omitted for clarity.

The outcomes of this research showed that different RNA molecules showed a unique pattern of degradation behaviour in bloodstains (Figure1), with miRNA markers exhibiting strong stability, likely due to their small size.

By applying correlation tests and regression analysis, the data indicate that the RERs of blood-specific markers have a positive correlation with ageing time points. The regression analysis of the relationship between the RERs of miR16/U6 and HBA/HBB with time produced the highest R^2 values (98.6% and 97.9% respectively) with narrow confidence intervals, using a non-linear model (Figure 2). These results indicate that these two ratios are the most reliable in bloodstain age estimation.

The RERs of blood-specific markers represent a potential method to estimate the age of bloodstains and therefore, the findings of this study emphasise that future methods using RNA have shown some success in accurate determination of the age of bloodstains.

References

[1] Maeda H, Fujita MQ, Zhu B-L, Ishida K, Quan L, Oritani S & Taniguchi M. (2003). Pulmonary Surfactant-Associated Protein a as a Marker of Respiratory Distress in Forensic Pathology: Assessment of the Immunohistochemical and Biochemical Findings. *Legal Medicine* 5, S318-S21.

[2] Bauer M. (2007). RNA in Forensic Science. *Forensic Science International Genetics* 1, 69- 74.

[3] Vennemann M & Koppelkamm A. (2010). mRNA Profiling in Forensic Genetics I: Possibilities and Limitations. *Forensic Science International* 203, 71-5.

[4] Anderson S, Howard B, Hobbs GR & Bishop CP. (2005). A Method for Determining the Age of a Bloodstain. *Forensic Science International* 148, 37-45.

[5] Hampson C, Louhelainen J & McColl S. (2011). An RNA Expression Method for Aging Forensic Hair Samples. *Journal Forensic Science* 56, 359-65.

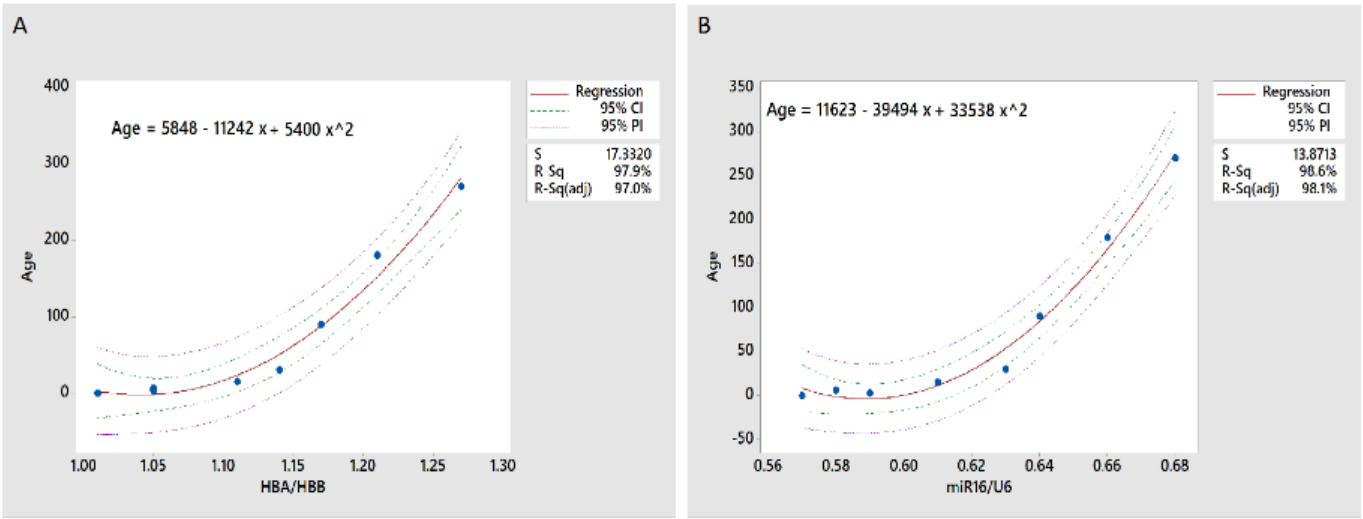

Figure 2. Regression analysis of the relationship between bloodstain age (up to 270 days) and RER. A second-order polynomial curve was applied to RERs: (A) HBA/HBB and (B) miR16/U6. Data represents mean of n = 10.