TECHNICAL NOTE

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Epidermal Cells on Stubs Used for Detection of GSR with SEM-EDX: Analysis of DNA Polymorphisms

REFERENCE: Torre, C. and Gino, S., **"Epidermal Cells on Stubs Used for Detection of GSR with SEM-EDX: Analysis of DNA Polymorphisms,"** *Journal of Forensic Sciences,* JFSCA, Vol. 41, No. 4, July 1996, pp. 658–659.

ABSTRACT: DNA from epidermal cells attached to the adhesive tape of stubs employed to collect and identify gunshot residue (GSR) with scanning electron microscope (SEM) was extracted, amplified with PCR and typed.

The method allowed identification of specimens when attribution to a definite person was uncertain. These results also suggest that adhesive tape could be used as a non invasive method for obtaining biological material suitable for DNA analysis from the skin surface.

KEYWORDS: forensic science, DNA, polymerase chain reaction, HLA-DQA1, epidermal cells, tape lift, gunshot residue collection, scanning electron microscope

Adhesive tape has been widely used to collect various samples for forensic purposes (1). It is still the most suitable and convenient method for detecting gunshot residue: double-sided adhesive tape is affixed to the SEM stub, which is then used to collect samples from different surfaces (in particular, from the skin of hands and face of the suspected shooter).

The carbon-coated sample can then be readily examined with SEM. When samples collected from the skin of living individuals and cadavers are examined with SEM, cells from the outer layers of the epidermis are usually observed. Occasionally, these cells are so numerous that they conceal most of the GSR, making it difficult to detect the residue (2).

A few years ago in casework, the problem of attribution of a stub for detection of GSR to a definite person arose in that the defense alleged that different samples had been confused by mistake. This experience led to the investigation of whether the cellular material, which constantly sticks to the adhesive tape on stubs when collecting GSR, could be used for DNA analysis.

Materials and Methods

Six different samples were obtained from three subjects. The adhesive tape was pressed and rubbed on the skin of the back of their hands in the method employed to collect gunshot residues. One of the samples was carbon coated with the usual technique for observation with SEM, as a morphologic control; another sample was attached to a slide and stained with May Grunwald Giemsa in order to detect nucleated cells. Observation with SEM was performed with Cambridge 110.

Blood and hair samples were obtained from the same individuals for a comparative study of DNA. DNA was extracted from blood and hair using the procedure suggested by Walsh et al. (3), employing 5% Chelex 100.

The extraction of DNA from samples on the adhesive tape was performed as follows: adhesive tape was cut in small pieces and placed in 1.5 microcentrifuge tube with 200 μL of 5% Chelex 100. The specimens were incubated at 56° for 2–3 days. These samples were vortexed at high speed for 15 s. Then they were put in a boiling water bath and vortexed again. They were microcentrifuged for 3 min at 13,500 × g.

The HLA DQα sequences were amplified in samples using the

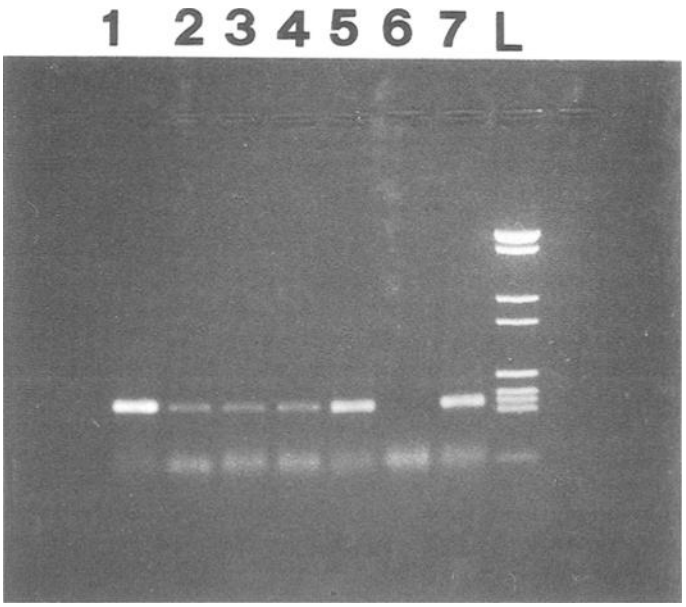

FIG. 1—*Ethidium bromide-stained agarose gel showing DNA amplified from blood (1; 2), epidermal cells on adhesive tape (3; 4), positive control (5; 7), negative control (6), and Bio Rad DNA size standards-low range (L).*

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Received for publication 29 June 1995; revised manuscript received 11 Oct. and 28 Dec. 1995; accepted for publication 2 Jan. 1996.

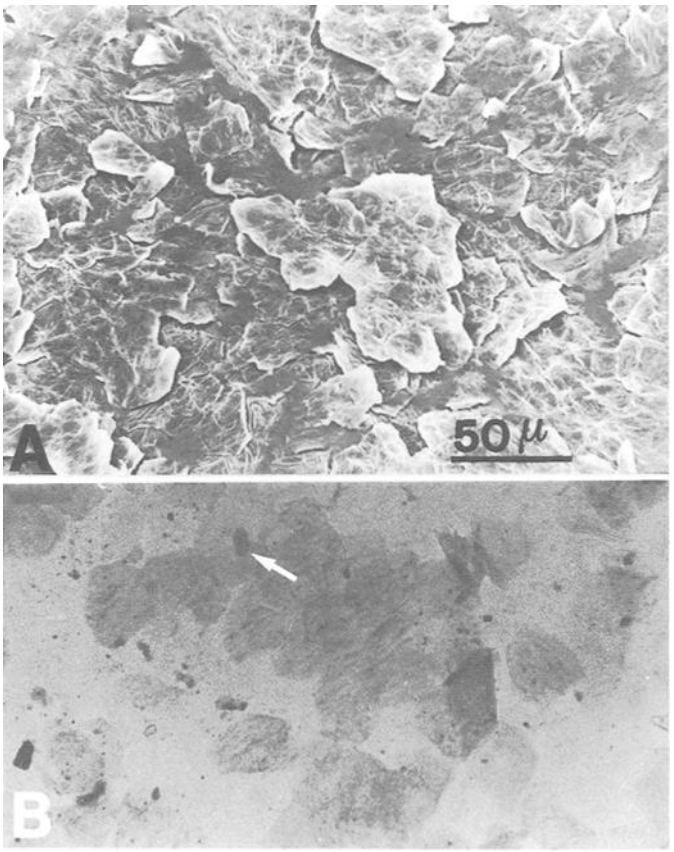

FIG. 2—*Epidermal cells on adhesive tape observed with SEM (A) and with light microscope (B-May Grunwald Giemsa's staining). The arrow shows nucleus.*

AmpliType HLA DQα kit (Cetus). The final volume of the reaction was 120 μL: 50 μL PCR reaction mix, 50 μL $MgCl_2$, 20 μL DNA.

PCR amplification was accomplished using 40 cycles consisting of 1 min denaturation (94°C), 1 min annealing (60°C) and 1 min extension (72°C) steps. The final cycle included a 7 min. extension step. Amplifications were carried out in a TECHNE PHC-3 thermal cycler.

PCR products were analyzed by gel electrophoresis on 2% agarose gel at 150 V for 50 min. The gels were stained with ethidium bromide and the fragments were visualized by fluorescence under ultraviolet light. All samples were typed with the reverse dot blot method.

Results

The amplification of DNA at the DQA1 locus (Fig. 1) was observed in all samples examined (including the carbon coated sample). Typing results on the amplified DNA were consistent with typing data on blood and hair from the same individuals; a clear C dot was obtained for all samples.

The morphological control with SEM (Fig. 2-A) showed the usual appearance of specimens collected for identification of gunshot residues with a large quantity of cells from the stratum corneum. Examination of the morphological control of the samples with light microscope demonstrated that, besides many cells without a stained nucleus, there were a few nucleated epithelial cells (Fig. 2-B; the image is obviously poor, as the samples include the adhesive tape and dirt which became attached to the tape during collection).

Discussion and Conclusions

The attempt to extract, amplify and type DNA from epidermal cells found on the adhesive tape employed to collect GSR from the skin was suggested by a previous case, in which a questionable sample was attributed to a defendant.

In our experimental study, all the examined samples were correctly attributed. This result was obtained using a very small piece of adhesive tape (about a quarter of the amount attached to a one inch stub normally employed to collect GSR). It is worth noting that the sample is not completely destroyed by the investigation and can be examined again for the detection of GSR.

Observation performed with a light microscope confirms that the few nucleated cells which were clearly observed contained enough extractable DNA which was suitable for amplification with PCR and consequent typing.

This method could be successfully employed in casework, when a sample for GSR detection is uncertainly attributed. It could also be used in general as an alternative procedure to obtain samples for DNA analysis, which would avoid the invasive technique of taking blood samples.

Acknowledgment

We wish to thank Professor M. De Marchi, M.D., for his helpful discussions and criticisms.

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