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TECHNICAL NOTE

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PCR typing of human semen stains after SEM-EDX examination

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Abstract There is an increasing demand to use scanning electron microscopy in the forensic analysis of biological samples. Such analyses are routinely used for the investigation of blood stains, seminal stains, diatoms, residues on wounds and residues and trace elements in gunshot powder. The same samples are sometimes also required for identification via DNA analysis, e.g. blood stains, seminal stains or epidermal cells. The ionising radiation provokes damage to DNA and also to membrane and proteine structure. The question therefore arises whether the usual sequence of such an investigation, i.e. prior application of SEM and afterwards analysis of DNA, can affect the success rate of the DNA analysis. We have therefore experimentally exposed semen samples to a defined electron beam for different time intervals varying between 1 and 25 min and afterwards performed quantitative and qualitative DNA analysis. Our studies revealed that sample treatment with an electron beam does not interfere with subsequent DNA typing by various currently used forensic PCR systems.

Key words Semen · Scanning electron microscopy · Electron dispersive x-ray microanalysis · DNA typing · PCR

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Introduction

In sex crimes, the detection of semen is of prime importance as evidential material in the laboratory and ultimately in court. The most commonly used test for semen, the phosphatase test, can lead to a false negative result, especially in wet conditions, which can result in a complete deactivation of the enzyme after 48 h (Steinman 1995). Some plants (e.g. kiwi fruit, cauliflower, pea, or potato) can even give false positive results (Scheithauer and Luta 1988). The presence of semen can only be unequivocally demonstrated by microscopical examination. In the past few years, there has been significant progress in the morphological identification of semen traces using forensic

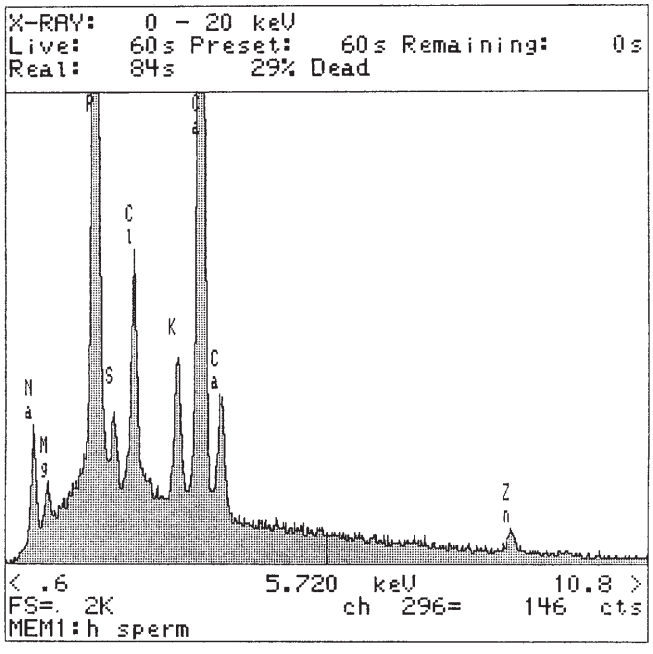

Fig. 1 Energy dispersion spectrum of a semen sample at a beam energy of 20 keV. Identification of the major constituents: Na, P, S, Cl, K, Ca, and Zn

techniques. One of the most popular is the scanning electron microscope visualisation of sperm heads, which is particularly helpful for old stains (Lachica and Garcia-Ferrer 1998). When an EDX (energy dispersive X-ray analysis) detector is coupled to the system, the characteristic zinc content of the sperm can be detected in addition to the morphology of the spermatozoa (Sótonyi and Darok 1995) (Fig. 1). It must be considered that electron radiation is very harmful to DNA, so the aim of the study was to determine whether semen stains subjected to scanning electron microscopy were still amenable to routine forensic DNA analysis or whether mistyping would result.

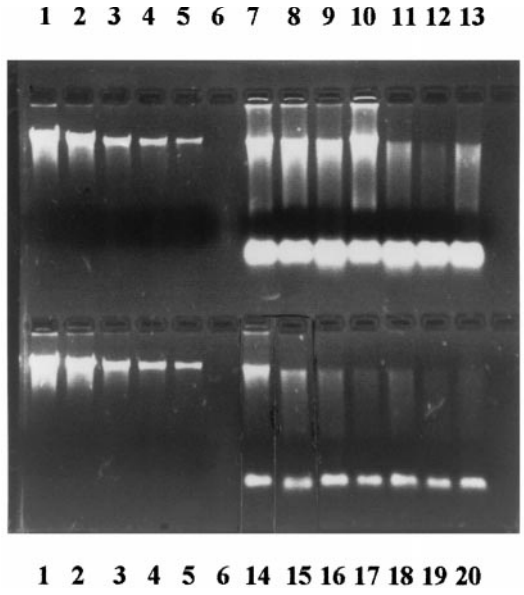

Fig. 2 Test gel of semen samples from two different subjects following an exposure to the electron beam for 1 min (Lanes 8 and 15), 5 min (Lanes 9 and 16), 10 min (Lanes 10 and 17), 15 min (Lanes 11 and 18), 20 min (Lanes 12 and 19) and 25 min (Lanes 13 and 20). Lanes 7 and 14 = untreated control semen stains; Lanes 1-5 = 200, 100, 50, 25 and 12.5 ng of high molecular weight DNA from calf thymus, respectively, lane 6 = blank

Materials and methods

Normospermic ejaculates were obtained from two healthy volunteers. Volumes of 100 µl each were pipetted on to sterile white cotton and dried at room temperature.

For further experimentation, a 1×1 cm piece was cut out from the middle of each spot and fixed to the SEM sample holder by carbon ribbon. The samples were covered with a ~ 50Å carbon layer by a 5 mm wide spectral graphite electrode heated by 45 A from a distance of 100 mm for 30 s in a JEOL JEE 4B vacuum evaporator at a pressure of 6×10^{-4} mm Hg. X-ray analysis was carried out in a DSM OPTON 940 digital scanning electron microscope (Zeiss, Germany) equipped with a Si(Li) detector and a Link AN 10/55 S (England) energy dispersive analyser system. The distance between detector and specimen was 24 mm, the take-off angle 35°. From each of the two series, seven samples were prepared and six were exposed to an electron beam of 20 keV in a vacuum of 10^{-5} mm Hg with 64 nA for 1, 5, 10, 15, 20 or 25 min. Unexposed samples were used as controls.

After exposure the cotton samples were cut into small pieces and placed into sterile microcentrifuge tubes and 500 µl of extraction buffer (0.01 M Tris base, 0.01 M EDTA, 0.1 M NaCl, 0.039 M DTT, 2% SDS, pH 8.0) and 12 µl of proteinase K (20 mg/ml) was added and incubated overnight at 56 °C. The samples were purified by a standard phenol/chloroform/isoamylalcohol (25:24:1) extraction method. After ethanol precipitation, the DNA was resuspended in 200 µl of sterile water. In order to control quality and quantity under UV-light, 1/10 of an aliquot was applied on a 0.8% agarose gel and stained with ethidium bromide (Fig. 2).

The DNA was amplified and investigated in one AmpFLP system (D1S80), four STR systems (CD4, TH01, VWA, F13B) and in one sequence polymorphic system (HLA DQα). Amplification and typing were performed using previously described methods (Rand et al. 1992; Szabo et al. 1998; Woller et al. 1996) and according to the manufacturers' instructions (Perkin Elmer USA and Serac Germany). Amplifications were carried out in a PTC 200 thermal cycler.

Results and discussion

In the quantitative and qualitative examination of the DNA on an agarose gel it was clearly visible that the se-

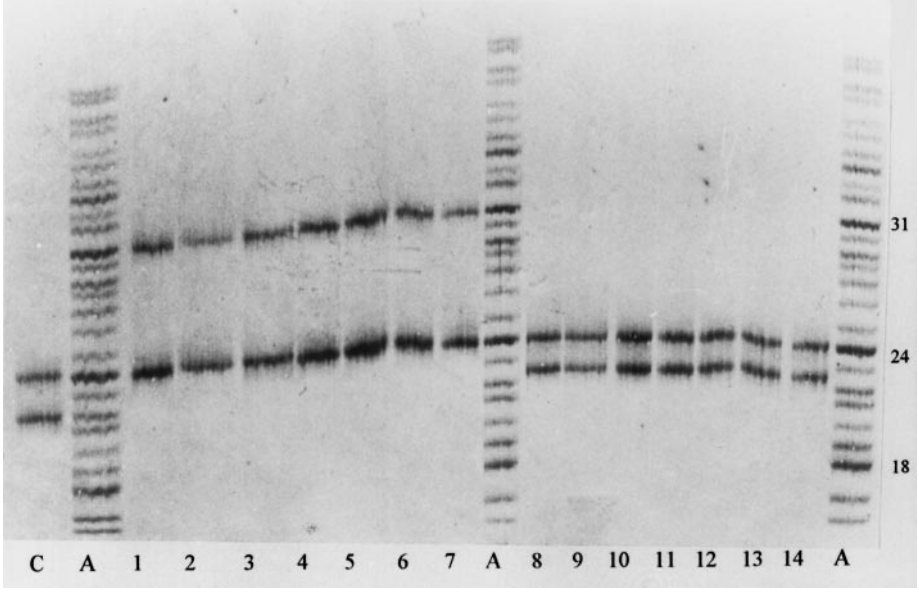

Fig. 3 Semen samples from two different subjects typed in the D1S80 AmpFLP system following an exposure to electron beam for 1 min (1 and 8), 5 min (2 and 9), 10 min (3 and 10), 15 min (4 and 11), 20 min (5 and 12), 25 min (6 and 13) and control samples without electron beam treatment (7 and 14). C: internal laboratory control, A: allelic ladder

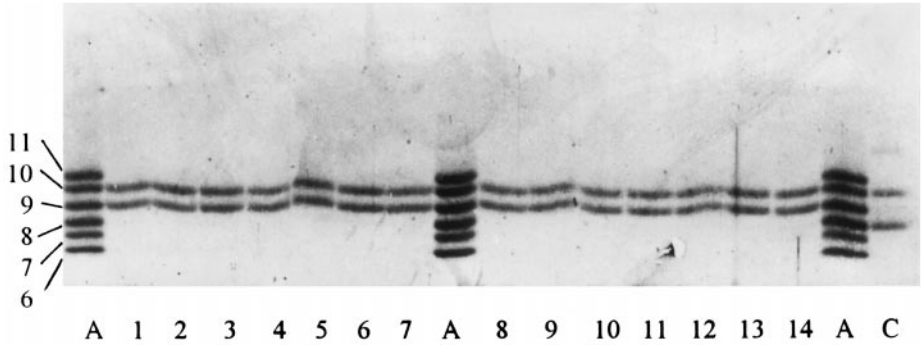

Fig. 4 Semen samples from two different subjects typed in a F13B STR system following an exposure to electron beam for 5 min (2 and 9), 10 min (3 and 10), 15 min (4 and 11), 20 min (5 and 12), 25 min (6 and 13) and control samples without electron beam treatment (7 and 14). C: internal laboratory control, A: allelic ladder

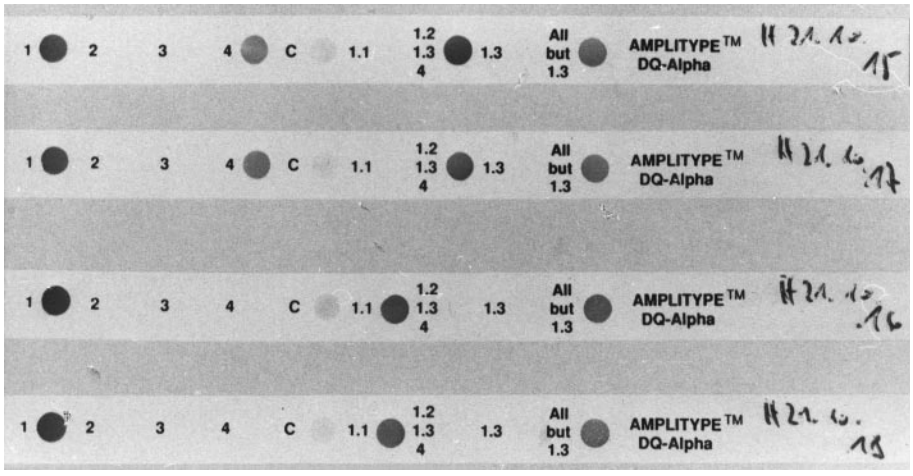

Fig. 5 Semen samples from two different subjects typed in a HLA DQα sequence polymorphic system. Samples 1 and 3 from the top following a 25 min exposure to an electron beam are shown as examples. Samples 2 and 4 from the top are the controls without electron beam treatment

men samples exposed to a high energy electron beam during our investigation did develop radiation-related degradation: samples exposed for 15, 20, and 25 min were highly degraded (Fig. 2). Approximately 10 ng DNA template of each sample, including the highly degraded ones, was successfully amplified in the AmpFLP, STR, and sequence polymorphism systems investigated (Figs. 3–5).

The energy dispersive x-ray element analyser, in connection with a scanning electron microscope, can detect elements from extremely small quantities of semen samples, without pre-treatment with chemical compounds (Hochmeister et al. 1991), within 100–120 s (Ingram et al. 1989; Goldstein et al. 1992). The secretion produced by the prostate has the highest concentration of zinc among all body fluids (140 μg/ml, in contrast to 1.2 μg/ml for blood serum). Whereas acid phosphatase degrades slowly in moist samples, zinc does not, making the latter a more useful standard in older specimens (Steinman 1995; Sótonyi and Darok 1995). Therefore, the morphology of spermatozoa and the characteristic zinc signal can be used to demonstrate sperm in a stain. The simplicity, rapidity of execution and high power of magnification make the SEM-EDX a useful tool in the preliminary study of semen stains.

Induction of DNA double-strand breaks in mammalian cells is dependent on the spatial distribution of energy deposition from the ionising radiation. It can therefore be expected that the distribution of double-strand breaks linearly along the DNA molecule, also varies with the type of radiation and the ionisation density (Lobrich et al. 1996). Radiation can also induce electron migration along the DNA molecule by which randomly produced stochastic energy deposition events can lead to non-random types of damage along DNA manifested distal to the sites of initial energy disposition. Fuciarelli and al. (1994) discovered that electron migration along oligonucleotides is significantly influenced by the base sequence and strandedness. Jeffreys (1997) emphasised that repetitive sequences – originally developed for forensic profiling – not only show high frequency spontaneous mutation in the germline, but also appear to be very sensitive to mutation induction by ionizing radiation. Even non-ionizing UV radiation can cause significant changes in the DNA molecule structure (e.g. oxidation of thymine in DNA to thymine glycol). In addition to DNA denaturation, the thermal energy released in samples exposed to electron beams may also induce single- and double-strand breaks, base and sugar modification and/or development of thymine-thymine and cytosine-cytosine dimers even in in vitro samples. A high degree of such modifications would make DNA typing impossible (Hüttermann et al. 1978; Fritz-Niggli 1988).

In the quantitative and qualitative examination of the DNA molecules on an agarose gel it was clearly visible that the semen samples exposed to a high energy electron beam during our investigation did develop radiation-related degradation. However, these changes had no detectable detrimental effects on the efficiency of PCR typing in some currently used forensic typing systems. Even

exposure to radiation for as long as 25 min failed to induce any significant deterioration of the quality of the samples for the purpose of typing. Thus, primary use of scanning electron microscopy in our series did not affect the efficacy of the subsequent DNA analysis and did not lead to any mistyping. Therefore the results presented in this study demonstrate that DNA analysis can be successfully performed on the original stains after SEM-EDX treatment.

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