TECHNICAL NOTE

Miwa Tanaka,[1] B.D.S.; Takashi Yoshimoto,[1] B.S.; Hideki Nozawa,[2] M.D.; Hiroyuki Ohtaki,[1] B.S.; Yoshiki Kato,[3] B.D.S.; Keizo Sato,[3] M.D.; Toshimichi Yamamoto,[1] Ph.D.; Keiji Tamaki,[4] M.D.; and Yoshinao Katsumata,[1] M.D.

Usefulness of a Toothbrush as a Source of Evidential DNA for Typing

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ABSTRACT: We investigated the usefulness of a toothbrush as a source of DNA for an unidentified cadaver. Ten toothbrushes were obtained from ten individuals along with their peripheral blood. We recovered from 10 to 430 ng of DNA from all but one of the toothbrushes. All ten toothbrushes, including the one containing no detectable DNA by fluorometry, were typed correctly at all of the loci tested, including nine STRs. Three toothbrushes obtained in two actual deaths also identified two victims and one suspect. Therefore, toothbrushes seem to be useful as a source of evidential DNA for personal identification.

KEYWORDS: forensic science, DNA typing, toothbrush, polymerase chain reaction, LDLR, GYPA, HBGG, D7S8, GC, HLA-DQA1, short tandem repeats

Examination of severely decomposed cadavers is sometimes required in forensic practice. The effectiveness of DNA analysis for personal identification is well established (1), and can be applied even to skeletal remains or hairs (2–4). However, the reference DNA samples for the cadavers themselves, such as bloodstains, or biopsy materials remaining with their medical records, are rarely available. The DNA profiles of close biological relatives, such as parents or siblings, can be used to help identify the cadavers. In instances where there are few or no living biological relatives, one way of obtaining the reference samples would be to find personal effects containing the DNA of the particular individual. A toothbrush has been reported as one such possible personal effect (5).

[1] Postgraduate Student, Research Students, Associate Professor, and Professor, respectively, Department of Legal Medicine and Bioethics, Postgraduate School of Medicine, Nagoya University, 65 Tsuruama-cho Showa-ku, Nagoya 466-8550, Japan.

[2] Associate Professor, Department of Legal Medicine, Hamamatsu University School of Medicine, 3600 Handa-cho, Hamamatsu 431-3124, Japan.

[3] Research Student and Professor, respectively, Department of Legal Medicine, Showa University School of Medicine, 1-5-8 Hatanodai, Shinagawa-ku, Tokyo 142-8555, Japan.

[4] Lecturer, Department of Genetics, University of Leicester, Adrian Building, University Road, Leicester LE1 7RH, UK.

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In the present paper, the usefulness of toothbrushes for personal identification of unidentified cadavers was investigated.

Materials and Methods

Sample Preparation—Ten toothbrushes were obtained from each of ten unrelated individuals who had used them from three months to one year. One of those toothbrushes had been left unused for six months before this experiment. Blood samples were also taken for reference DNA. Three toothbrushes were obtained also in actual deaths; one from the house of a possible victim of drowning, and two (a son and a father) from the house in a murder case in which a father strangled his son. Blood samples from the heart of both the drowning and homicide victims were obtained at autopsy, and a buccal swab (6,7) of the culprit (father of the victim) was obtained after his arrest.

DNA Extraction—The head of each toothbrush was removed by heated scalpel, immersed in 30 mL of TE-buffer, and incubated with vigorous shaking in cold water for three hours. Cell debris were pelleted by centrifugation at 3000 rpm for 10 min. The cell pellet was digested in 168 μL lysis buffer, containing 100 mM Tris-HCl (pH 8.0), 5 mM EDTA (pH 8.0), 0.1 M sodium chloride, 39 mM dithiothreitol, 0.5% sodium dodecylsulfate, and 200 μg/mL of proteinase K, and incubated overnight at 55°C. DNA was prepared by phenol-chloroform extraction and ethanol precipitation (8). DNA was also prepared from blood (8) or buccal swab (9) as described elsewhere.

Quantitative and Qualitative Analysis of Extracted DNA—The amount of extracted DNA was determined by fluorometry using bisbenzimidazole (Hoechst 33258: Polysciences Inc., Warrington, PA) on the TKO 100 Dedicated Mini Fluorometer (Hoefer Scientific Instruments, San Francisco, CA), and by spectrophotometry using absorption at 260 nm. Human DNA was also quantified using the QuantiBlot™ kit (Perkin Elmer, Branchburg, NJ) (10). The quality of the extracted DNA from toothbrushes was analyzed by gel electrophoresis. Approximately 100 ng of the DNA was electrophoresed on 0.7%(w/v) agarose gels and then stained with ethidium bromide.

DNA Typing of Various Genomic Markers—Six loci (DQA1, LDLR, GYPA, HBGG, D7S8, and GC) were typed using the Am-

pliType® PM + DQA1 kit (Perkin Elmer, Branchburg, NJ). Nine STR loci (D3S1358, vWA, FGA, TH01, TPOX, CSF1PO, D5S818, D13S317, and D7S820) as well as the amelogenin gene were amplified using the AmpFISTR Profiler PCR Amplification kit (PE Applied Biosystems, Foster City, CA). The PCR product was analyzed by capillary electrophoresis with the Genetic Analyzer 310 (PE Applied Biosystems, Foster City, CA).

Results and Discussion

DNA amounts recovered from nine toothbrushes tested ranged between 10 to 430 ng determined by fluorometry. The tenth toothbrush tested showed a concentration of less than the lower limit of the fluorometric method (0.5 ng/μL). DNA amounts recovered were further determined in some specimens by spectrophotometry and by the QuantiBlot™ method (Table 1). The DNA amounts recovered from toothbrushes showed smaller values by fluorometry than by spectrophotometry, with the smallest values being shown by the QuantiBlot™ method. The quality of DNA recovered from the toothbrushes was analyzed by gel electrophoresis, and two samples from actual deaths are shown in Fig. 1. DNA recovered from toothbrushes always showed the smear pattern without high-molecular DNA. The principle of spectrophotometry is based on the absorption at 260 nm of ultraviolet by the four bases, while that of fluorometry is based on the specific binding of the dye to the double-strand structure of DNA. Thus, fluorometry is theoretically more sensitive to degradation of DNA which could explain the smaller values obtained than by spectrophotometry. Using buccal cells obtained by either scrubbing or just rinsing, we clearly showed this phenomenon recently (9). In that paper, we showed that the ratio of DNA amounts determined by spectrophotometry and fluorometry (S/F) was much lower with the DNA obtained by scrubbing (1.45) than that with the DNA obtained by rinsing (3.95), and the former showed a band of high molecular weight DNA with smears when analyzed by agarose gel electrophoresis although the latter showed usually only smears. Also, note that neither spectrophotometry nor fluorometry is species specific. The QuantiBlot™ is primate specific so no bacterial DNA will be detected, which could explain the very small values obtained by QuantiBlot™ when compared with the spectrophotometry and fluorometry (Table 1).

All of the experimental toothbrushes, including the one containing undetectable DNA as well as the three toothbrushes in the two cases described above, could be typed at all loci tested. Each typing result showed a complete match with the referred blood or buccal swab, and the combined frequencies in the Japanese population were 8.99×10^{-12} to 1.45×10^{-16} using the allele frequencies of these loci reported in the Japanese population (11,12). Although DNA specimens recovered from toothbrushes were inevitably degraded, and some of the toothbrushes yielded very low DNA recovery, all of the specimens tested in this study could be typed at all loci. Therefore, a toothbrush could be a useful source of individual DNA for typing using PCR. We showed in the previous paper that the typing of minisatellite loci, MS32, was impossible with DNA specimens of up to 800 ng obtained by rinsing (9). Therefore, it will be difficult to type very large minisatellite loci with the DNA prepared from toothbrushes.

TABLE 1—*DNA recovery from toothbrushes.*

Sample	DNA Amounts (ng) Spectrophotometry	Fluorometry	QuantiBlot
Toothbrush 1	280	60	30
Toothbrush 2	496	40	10
Case 1 toothbrush	11620	600	60
blood	365	325	160
Case 2 victim's toothbrush	2900	540	26
victim's blood	23400	37350	27000

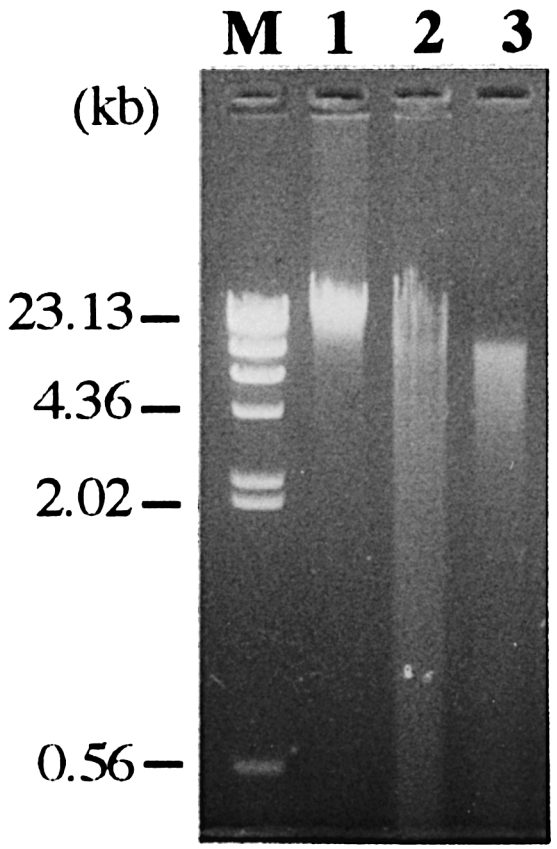

FIG. 1—*Agarose gel electrophoresis of DNA extracted from toothbrushes and blood. M: Size marker (λ DNA digested by Hind III). 1: blood. 2: toothbrush of case 1. 3: toothbrush of case 2.*

Since people usually have their own toothbrushes and seldom use others, one can usually expect to obtain DNA of the person in question from his/her toothbrush without any contamination by other people's DNA. This was true with the ten toothbrushes tested and with the three in the two actual cases discussed previously. Thus, we were able to identify these two individuals in the Japanese population (1.5×10^8) at frequencies between one in one trillion and one in ten quadrillion. The present results suggest that a toothbrush could be used as an excellent reference sample for personal identification, and should be sought early in the investigation of such cases.

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Additional information and reprint requests:
Miwa Tanaka
Department of Legal Medicine
Postgraduate School of Medicine
Nagoya University
65 Tsuruma-cho, Showa-ku,
Nagoya 466-8550
Japan