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CONTENTS

RESEARCH

CASE REPORTS

A NEW METHOD OF MARKING DENTURES USING MICROCHIPS

M. Rajan[1], R. Julian [2]

1. *Rajah Muthiah Dental College and Hospital, Chidambaram Tamilnadu, India*
2. *Tamilnadu Govt. Dental College & Hospital, Chennai-3 Tamilnadu, India*

ABSTRACT

Over the years various methods of denture marking have been reported in the literature. They include surface marking and inclusion techniques using metallic or non-metallic materials, microchips and microlabels. The microchips are preferred because of their small size and aesthetic acceptability. They are not however widely used due to the high cost of manufacture and data incorporation. This article details the procedures involved in inscribing a microchip using the photochemical etching process used in the electronics industry. The resulting microchip was cosmetically appealing, cost effective and was able to satisfy all the forensic requirements for a suitable denture marker.
(J Forensic Odontostomatol 2002:20;1-5)

Keywords: Denture marking, Microchips, Photochemical etching

INTRODUCTION

A person must be identified in the event of death or loss of memory. Dental identification plays a major role in this process as the teeth and restorations often outlast all other body tissues after death.[1,2] When the teeth are lost however this becomes impossible and it is important that any prosthesis such an edentulous individual may be wearing be marked.

Over the years various denture marking systems have been reported in the literature and have been broadly divided into surface marking and inclusion methods. The surface marking methods include engraving the casts, scribing the denture or writing on the denture surface[3,4,5] while the inclusion methods involve incorporation of metallic or non-metallic labels or microchips into dentures.[6,7,12-14] Microchips may be electronic memory chips or simply vehicles for imprinting miniaturized letters and digits. The advantages of using chips for denture marking are that they are small, they are cosmetically acceptable and more easily inserted. The 'Swiss identification system'*uses small metal, plastic or ceramic discs for denture marking.[8]

Gold microchips embossed with intelligence data were used by the U.S. Armed Forces[9] while the 'MIN-I-DENT'** identification strip is made of plastic.[10] A metal denture marking microchip has also been described by Cotter *et al.*[11]

In spite of its advanced technology, the microchip has not been popular because of its unavailability and high cost. An attempt was therefore made to develop a denture marking chip with a view to overcoming some of the previous difficulties, which was simple, inexpensive and effective.

MATERIALS AND METHODS

The chip† used was a modification of an already existing technique of computer chip manufacture. In this case instead of circuit diagrams used in making computer chips, patient data information were fed into the computer for printing on paper.

The chip consists of a base laminate on which the required data is incorporated using a photochemical etching process. The base laminate is composed of high quality woven E-glass sandwiched between

* *I.dent, Switzerland*

** *Super Tooth Products, Minnesota, USA*

† *Zeta Microsystems, Chennai, India*

epoxy resin with copper cladding which is designated in industry as FR-4 and is used in the manufacture of printed circuit boards in the electronics industry.

The data were incorporated on the base layer in a series of steps:

1. The required data were printed by high quality laser printer on paper.
2. A photographic reduction was then carried out to the required size (0.5mm) and the reduced pattern photographed.
3. The 0.6 mm copper cladded FR-4 sheet was coated with a photosensitive polymer film by lamination and the pattern on the photographic film transferred to the photopolymer by UV exposure. It was then developed and hardened.
4. The surface containing the pattern was now subjected to tin electroplating after which the polymer layer was removed.
5. The surface was then etched with ferric chloride solution and the areas not covered by tin etched off .
6. The surface was cleaned leaving the inscription clearly visible as copper patterns coated with tin. The chip was then ready for incorporation into a recess created in the acrylic of the dentures (Figs.1 and 2).

FORENSIC TESTS ON THE CHIP

Forty six heat processed acrylic resin blocks (3x2x1cm) were prepared. A chip was placed into a 5mm depression cut into each block and covered with a mixture of clear autopolymerizing acrylic resin.[††] The blocks were polymerized in a pressure pot for 15 minutes at 25psi and 40°C.

Each chip was clearly visible through the clear acrylic resin after polishing and 30 samples were used for testing, with one sample kept exclusively for reference. The reference/control sample was used for visual and radiographic comparison of extent of visibility of data on the samples before and after the forensic tests.

The specimens were prepared differently for tensile testing. Fifteen heat processed acrylic resin blocks (3x2x1cm) were prepared. A depression of 5mm was made in a side of a resin block allowing for a 4cm chip to be partly buried and partly to protrude after.

Autopolymerizing acrylic resin was flowed into the depression and the chip placed in the depression and covered with resin. The blocks were polymerized in a pressure pot for 15 minutes at 25psi and 40°C.

Fig.1: *Denture-marking microchip with inscription. Dimensions 5mm x 5mm x 0.6mm*

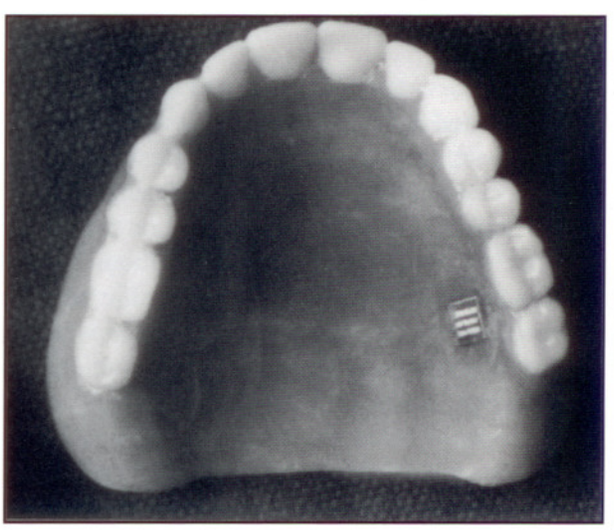

Fig.2: *Microchip incorporated in the posterior palatal region of maxillary denture*

[††] *Stellon, DPI, India*

1. Heat resistance

Fifteen specimens were placed in a porcelain crucible individually in a muffle furnace. The temperature was gradually raised to 1000°C and changes observed visually, noted and tabulated. The temperature at which the markings were unreadable was recorded as the maximum heat resistance (Fig. 3) .

2. Acid resistance

A further 15 specimens were immersed in 99.98% sulphuric acid kept in separate glass beakers and the markings observed every week for 10 weeks. The point at which the markings were visually unreadable due to the opacification of the clear acrylic resin surface was recorded as the maximum acid resistance (Fig.3).

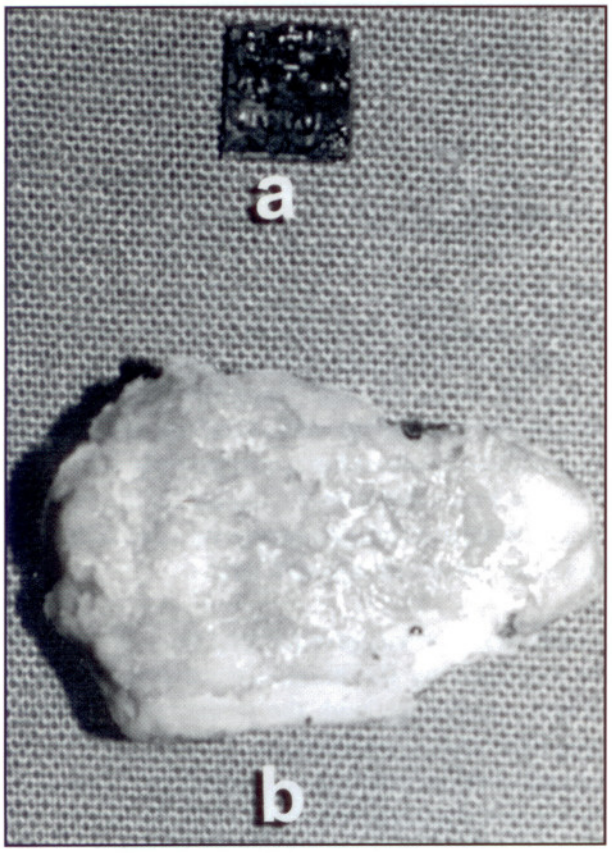

Fig. 3: *Microchip embedded in acrylic resin blocks (a) after heating at 600°C, (b) after immersion in sulphuric acid at eight weeks*

3. Radiography

Radiographs of 15 samples were taken on periapical dental film*** at 70Kv for 0.5 seconds with a conventional dental X-ray machine#. Radiographs were also taken of the visually unreadable 30 samples subjected to heat and acid tests. The ability to read clearly the data on the radiographs was noted (Fig. 4).

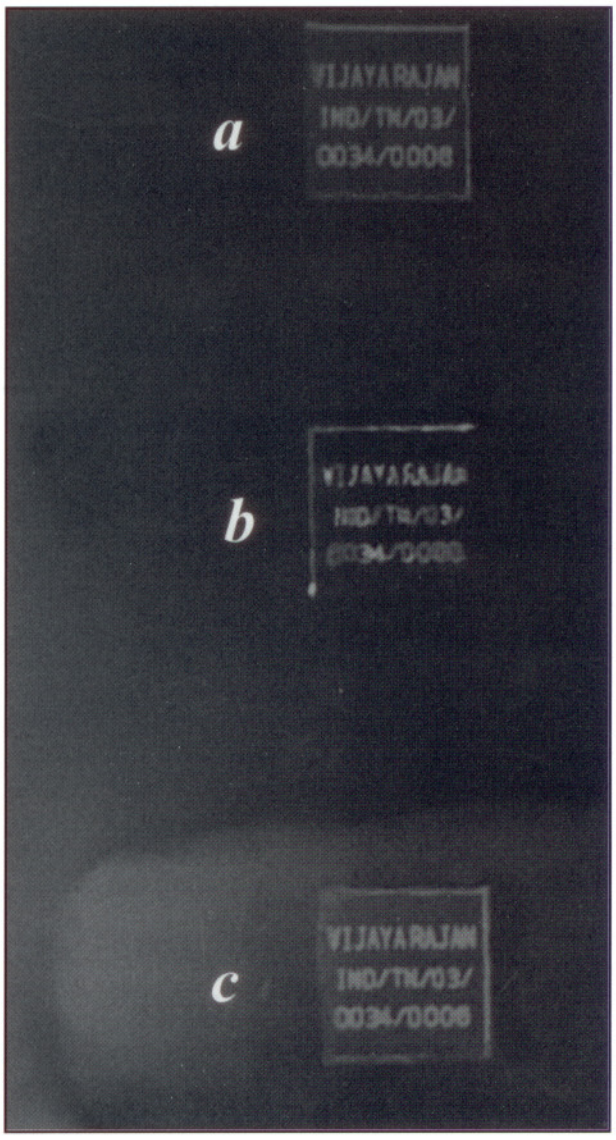

Fig. 4: *Microchips radiographed on dental film and by conventional dental X-ray machine (a) Microchip under normal conditions, (b) Microchip after heating at 600°C, (c) Microchip after immersion in sulphuric acid at eight weeks*

*** *Kodak EktaSpeed,Eastman Kodak Company ,USA*

Satelec, Italy

4. Tensile bond strength

The fifteen specimens made for tensile testing were mounted in the jaws of a Universal testing device[##] (Fig. 5) and pulled to breaking point. The values were noted and also the point of failure, whether at the chip/acrylic bond interface or within the chip itself.

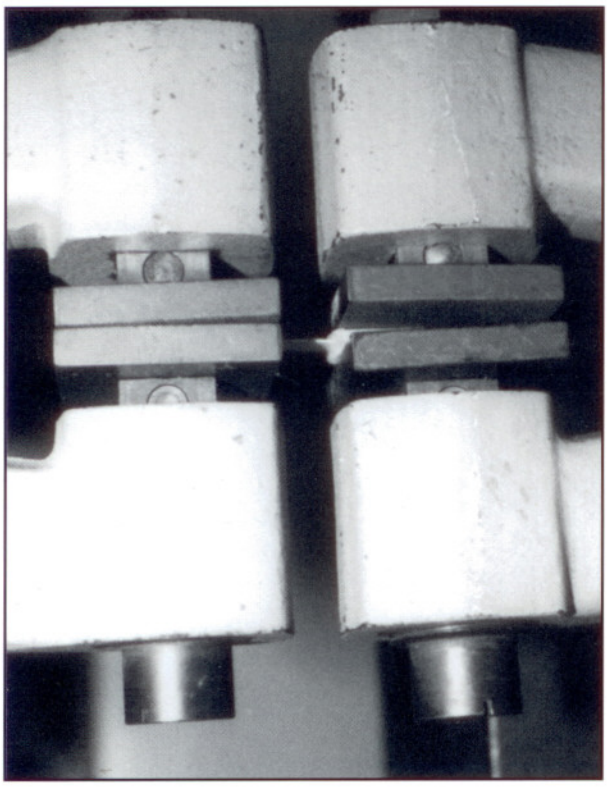

Fig. 5: *Tensile bond strength testing in a universal testing machine*

Tests	n	Mean values
Heat resistance (°C)	15	600±25.24
Acid resistance (weeks)	15	8
Tensile bond strength (kg/m_2)	15	15.07±0.45

Table 1: *Mean heat resistance, mean acid resistance and mean tensile bond strength of the chip*

RESULTS

1. **Maximum heat and acid resistance and tensile bond strength.**

   Table 1 shows the mean maximum heat resistance (^{0}C) and acid resistance (wks), and the mean maximum tensile bond strength. All the specimens failed at the chip/acrylic resin bond interface.

2. **Radiography**

   The data were clearly visible on all the samples because of the radiopacity of the markings. The data were also clearly visible on the radiographs taken on the visually unreadable samples subjected to heat and acid tests.

DISCUSSION

The study conducted to evaluate the resistance of the chip showed that it was able to withstand a temperature of up to 600^{0}C, had excellent acid resistance, was radiopaque and bonded with acrylic resin. Because of this specific bonding characteristic as both the chip and dentures are made of resin, there is no weakening of the denture as would be expected with the metallic markers. The chip could also store a lot of personal information in a small area and was cosmetically pleasing. The data inscribed could be read radiographically or directly with naked eye or with a magnifying glass and no special devices are necessary to read the chip. The data were also clearly visible on the radiographs taken on the visually unreadable samples subjected to heat and acid tests. This can be attributed to the copper-tin inscriptions on the epoxy resin base rendering the data radiopaque and resistant to heat and acid tests.

The main disadvantage of the chip is that it can only be inscribed by the manufacturer and not by a dentist. However, with the proliferation of chip manufacturers, availability ought not to be a problem and dentists simply need to specify their requirements to a company of their choice. Presently, the cost of manufacture of 100 chips is about Rs250 (US$5), making the marking procedure inexpensive. Although initial results appear encouraging further long term clinical studies are needed to demonstrate the success of the technique.

[##] *Instron, Massachusetts, USA*

REFERENCES

1. Gladfelter IA, Smith BE. An evaluation of microdisks for dental identification. J Prosthet Dent 1989; 62: 352-5.

2. Fischman SL. The use of medical and dental radiographs in identification. Int Dent J 1985; 35: 301-6.

3. Thomas CJ. The role of denture in identification: A review. J Forensic Odontostomatol 1984; 2: 13-6.

4. Harvey W. Identity by teeth and the marking of dentures. Br Dent J 1966; 121: 334-40.

5. Johanson G, Ekman B. Denture marking. J Am Dent Assoc 1984; 108:347-50.

6. Kruger Monsen A. The marking of dentures for identification purposes. Plenary session IIA. Third International Meeting on Forensic Sciences, London 1963.

7. MacEntee MI, Campbell T. Personal identification using dental prostheses. J Prosthet Dent 1979; 3: 377-380.

8. Muhlemann HR, Steiner E, Brandestini M. Identification of mass disaster victims: the Swiss identification system. J Forensic Sci 1979; 24:173-81.

9. Wilson DF, Kolbinson D. The heat resistance of a data-encoded ceramic microchip identification system. Am J Forensic Med Pathol 1983; 4: 209-15.

10. Toolson LB, Taylor TD. Method for denture identification. J Prosthet Dent 1989; 61: 114-5.

11. Cotter WA, Chaney SA. A conservative cosmetic identification concept for prosthetic appliances. J Can Dent Assoc 1988; 54: 601-4.

12. Bernitz H , Blignaut J. An inclusion technique for marking dentures. J Forensic Odontostomatol 1998; 16: 14-6.

13. Thomas CJ, Mori T, Miyakawa O, Chung HG. In search of a suitable denture marker. J Forensic Odontostomatol 1995; 13: 9-13.

14. Rotzscher K, Bedrich MR, Jurisch R, Peitsch P. Electronic denture marking. J Forensic Odontostomatol 1999; 17:27-9.

Address for correspondence:
Dr Manoj Rajan
33/1, Sri Padam
C.V. Raman Road, Alwarpet
Chennai - 600018
Tamilnadu, India
Tel: 044-4995629
E-mail: rajanmanoj@hotmail.com

POPULATION STUDIES OF THE Y-CHROMOSOME OF LOCI DYS390, DYS391 AND DYS393 IN BRAZILIAN SUBJECTS AND ITS USE IN HUMAN IDENTIFICATION

R.N. Oliveira,[1] F.D. Nunes,[1] E.K. Anzai,[1] E. Daruge[2]
R.A. Mesquita,[3] A.N. Ozaki,[4] R.D.C. Hirata,[4] M.H. Hirata[4]

1. School of Dentistry, University of São Paulo, Brazil
2. School of Dentistry, Campinas State University, Brazil
3. School of Dentistry, Minas Gerais Federal University, Brazil
4. School of Pharmaceutical Sciences, University of São Paulo, Brazil

ABSTRACT

Human identification can be accomplished by several technical procedures, especially by the comparative analysis of dental documents. Recent advances in molecular biology have now widened the scope for human identification and defined the protocols for the collection of biological material. Allele patterns of a population are particularly useful and they should be verified since they vary in different populations. We have studied the frequency of the alleles in three STR loci of the Y chromosome (DYS390, DYS391 and DYS393) in a group of Brazilian caucasian subjects. Results presented alleles 21, 22, 23, 24, 25 and 26 in locus DYS390, alleles 8, 9, 10, 11 12 and 13 in STR DYS391 and alleles in STR DYS393 were 11, 12, 13 14 and 15. The highest frequencies were 24 (0.46), 11 (0.37) and 13 (0.45). Each of the three STR systems had a PE (power of exclusion) of 0.6764 (DYS390), 0.5988 (DYS391) and 0.6136 (DYS393), The combination of the three STR systems revealed a PE of 0.9498, suggesting that this data can help in human identification.
(J Forensic Odontostomatol 2002:20;6-9)

Keywords: Short tandem repeats, Y-chromosome, population data, Brazilian

INTRODUCTION

Modern technology is being used extensively in forensic analysis to produce scientifically reliable data to serve as acceptable evidence. The search for the best evidence has been fundamental in supporting criminal investigation and the law, and thus contributing to the high quality of justice. Physical evidence such as the dental profile from dentists' records of patients has proved to be a very useful tool in identification but many are incomplete or absent, sometimes because the victim's dentist cannot be identified or located. In developing countries this is a common problem because changing dentist is frequent in these populations. Clark,[1] analyzing 10 mass disasters in Great Britain, where dental records were used, came to the same frustrating conclusion.

This limitation and others have stimulated the search for new and improved human identification techniques.[2] Blood groups defined by antigens on the surface of red cells is a popular technique but is not discriminatory enough because of the high frequency of recurrence of blood groups in the population. DNA typing on the other hand has replaced ABO, Rh and Lewis blood group tests, as well as a variety of enzymes such as phosphoglucomutase (PGM), esterase D (EsD), glyoxylase 1 (GLO 1), erythrocyte acid phosphatase (EAP), adenosine deaminase (ADA), adenylate kinase (AK), carbonic anidrase (CAII), peptidase A (PepA) and glucose-6-phosphate dehydrogenase (G6PD) that had been routinely used.[3] Restriction Fragment Length Polymorphisms (RFLP) was the first system used in human identification, known as DNA fingerprinting, but the most revolutionary advance was in DNA amplification by PCR of specific hyper-variable regions, called VNTR (variable number of tandem repeat) and STR (short tandem repeat). These regions have been explored with increased interest because they are very polymorphic and give good discrimination. DNA amplification can be applied to very small amounts of material, decomposed tissues, bone, tooth and

other organic materials found at disaster or crime scenes such as blood, saliva and hair.[4,5] In the past 5 years STR from Y chromosome has been used to identify a rapist or murderer in a sexual abuse case.[6,7] There are many advantages in using Y chromosome loci and most importantly, that female DNA does not interfere with the analysis.

An important aspect of population studies is to compile a database of allelic frequencies using DNA analysis, which can assist in the calculation of statistical probabilities[8-16] for forensic applications or studies of evolution. In Brazil there is no study describing Y chromosome STR loci with allelic frequency to use as reference in creating an index of probability.

The object of this study was to set up a statistical database for a local population. Three STR loci from Y chromosome DYS390, DYS391, DYS393, in the population of São Paulo, Brazil, were analyzed.

MATERIALS AND METHODS

DNA extraction

DNA was obtained from white blood cells of 100 Caucasian individuals living in São Paulo, SP, Brazil, using the salting out procedure of Salazar *et al.*[17] DNA from saliva was obtained from 5 women as a negative control group using the technique described by Hochmeister et al.[18]

DNA amplification using Polymerase Chain Reaction

Based on the sequence from the Y-STR haplotype reference database,[14] primers for DYS390, DYS 391, DYS 393 were designed. Of each pair of primers described below, one was labelled with Cy5 at 5' end*.

DYS390
Primer A: TAT ATT TTA CAC ATT TTT ggg CC
Primer B: TgA CAg TAA AAT gAA CAC ATT gC

DYS391
Primer A: CTA TTC ATT CAA TCATAC ACC CA
Primer B: gAT TCT TTg Tgg Tgg gTC Tg

DYS393
Primer A: gTg gTC TTC TAC TTg TgT CAA TAC
Primer B: AAC TCA AgT CCA AAA AAT gAg g

The reaction conditions were set up using 10mM of each nucleotide (dNTPs), 80nM of each primer, 2mM MgCl2, 50 mM KCl, 10 mM TRIS-HCl, and 2,5 U of Taq DNA polymerase to a final volume of 25 µL. A thermocycler model PTC-100** was programmed for 35 cycles of 94°C for 1 min, 56°C for 1 min and 72°C for 2 min with a final extension of 72 °C for 10 min. These PCR conditions were used for the three STRs.

Fragments identification by electrophoresis

The ALF Express System¥ was utilized for fragment analysis in a 20% polyacrylamide gel using denaturing conditions. The electrophoresis separation and analysis of fragments were evaluated using a program from the ALF express system fragment manager. A ladder from Pharmacia Biotech¥ (ALF Express Sizer™ 50-500, cat. # 27-4539-01) was used as a pattern to determine the allelic size.

The PCR products were diluted with 40% of a loading dye (100% formamide and 0.05% Dextran blue 2000, and 10 ml were applied to a 20% polyacrylamide gel (acrylamide/bis:19/1) in 1X TBE buffer and electrophoresed at 500 volts, 60mA at 55°C, for 2 hours. Peak patterns were analysed using the ALF running gel software (ALFwin Instrument Control Version 2.00)¥ and fragment manager software (Allele Locator).¥

Statistical analyses

The exclusion index for each locus and the combination of three STRs was then calculated by $P(Y) = 1 - \sum(Pi)^2$.and $A = 1 - (1\text{-}PE_1).\ (1\text{-}PE_2).\ (1\text{-}PE_n)$ respectively,[13] and for population differentiation we used the Raymond & Rousset[16] test.

RESULTS AND DISCUSSION

Six different alleles were found in STRs DYS390 and DYS391, and in DYS393 there were five alleles (Table 1). Alleles 21, 22, 23, 24, 25 and 26 were

* *Synthegen, Houston, Tx., USA*

** MJ Research, Watertown, MT, USA
¥ Pharmacia Biotech, Uppsala, Sweden

Table 1: *Allele distribution of DYS390, DYS391 and DYS393 (n=100)*

DYS390	Allele	21	22	23	24	25	26
	Length (bp)	203	207	211	215	219	223
	Frequency	0.02	0.15	0.29	0.46	0.07	0.01
DYS391	Allele	08	09	10	11	12	13
	Length (bp)	275	279	283	287	291	295
	Frequency	0.01	0.05	0.16	0.37	0.34	0.07
DYS393	Allele	11	12	13	14	15	
	Length (bp)	115	119	123	127	131	
	Frequency	0.05	0.42	0.45	0.07	0.01	

discovered in locus DYS390, the most frequent one was 24, the locus DYS391 has shown alleles 8, 9, 10, 11, 12, 13. Allele 11 was the most frequent. Allele distributions in locus DYS393 were 11, 12, 13, 14 and 15 and the most frequent was number 13. The observed frequencies ranging in the spectrum previously described [8,9,11,13,14] showed apparent differences in the allele distribution. Comparing the loci found in the Brazilian population with the ones described in New Guinea/Australia[8,9,14] there was a significant difference ($p < 0.001$),[16] suggesting that the data base for statistical analysis can modify the PE results in different populations. However, it is necessary to increase the number of studies to create a representative database in order to confirm these preliminary results.

In Brazil there are people from different ethnic origins including Europeans, Asians, Africans and Amerindians and we used the general classification of Caucasian described by the Brazilian Institute of Geography and Statistics (IBGE – Instituto Brasileiro de Geografia e Estatística).[19] In further studies we intend to study other ethnically diverse Brazilian populations in order to create a more representative database for application in forensic analysis.

Employing a positive and negative control in each reaction enabled us to validate the amplification parameters used for the Y chromosome in this study. However, the absence of amplification of the negative control in all experiments validates the primers used as a choice in forensic cases. Further, a positive control with a known allele in each PCR reaction and gel was run.

The calculated individual PE[13] for each locus was 0.6764, 0.5988 and 0.6136 for DYS390, DYS391 and DYS393 respectively. The combined PE result of 0.9498 shows that these three STRs provide important data that can be used in conjunction with other STRs for forensic analysis, as an initial distinction between suspects of crimes such as rape cases or in human bitemark, as most victims are females and the male specific Y chromosome STRs have been shown to be a powerful tool in solving these cases.

REFERENCES

1. Clark DH. An analysis of the value of forensic odontology in ten mass disasters. Int Dent J 1994; 44:241-50.

2. Potsch L, Meyer U, Rothschild S, Schneider PM, Rittner C. Application of DNA techniques for identification using human dental pulp as a source of DNA. Int J Legal Med 1992;105:139-43.

3. Froede RC, Froede SM, Birkby WH. Systems for human identification. Pathol Annu 1981; 16:337-65.

4. Corach D, Sala A, Penacino G, Iannucci N, Bernardiz P, Doretti M, Fondebrider L, Ginarte A, Inchaurregui A, Somigliana C, Turner S, Hagelberg E. Additional approaches to DNA typing of skeletal remains: The search for 'missing' persons killed during the last dictatorship in Argentina. Electrophoresis 1997;18:1608-12.

5. Nicolás PS. Canela PR. Restos biológicos de interés forense, In: Jarreta MBM. La prueba del ADN en medicina forense. Madri: Masson,1999:138-51.

6. Dekairelle AF, Hoste B. Application of a Y-STR-pentaplex PCR (DYS19, DYS389I and II, DYS390 and DYS393) to sexual assault cases. Forensic Sci Int 2001;118:122-5.

7. Betz A, Baszler G, Dietl G, Steil X, Weyermann G, Pflug W. DYS STR analysis with epithelial cells in a rape case. Forensic Sci Int 2001;118:126-30.

8. Kayser M, Cagliá A, Corach D, Fretwell N, Gehrig C, Graziosi G, Heidorn F, Herrmann S, Herzog B, Hidding M, Honda K, Jobling M, Krawczak M, Leim K, Meuser S, Meyer E, Oesterreich W, Pandya A, Parson W, Penacino G, Perez-Lezaun A, Piccinini A, Prinz M, Schmitt C, Roewer L. Evaluation of Y-chromosomal STRs: a multicenter study. Int J Legal Med 1997;110:125-33, 141-9.

9. Knijff P, Kayser M, Cagliá A, Corach D, Fretwell N, Gehrig C, Graziosi G, Heidorn F, Herrmann S, Herzog B, Hidding M, Honda K, Jobling M, Krawczak M, Leim K, Meuser S, Meyer E, Oesterreich W, Pandya A, Parson W, Penacino G, Perez-Lezaun A, Piccinini A, Prinz M, Roewer L. Chromosome Y microsatellites: population genetic and evolutionary aspects. Int J Legal Med 1997;110:134-49.

10. Moura-Neto RS, Budowle B. Fixed bin population data for the VNTR Loci D1S7, D2S44, D4S139, D5S110, D10S28 and D14S13 in a population sample from Rio de Janeiro, Brazil. J Forensic Sci 1997;42:926-8.

11. Junge A, Madea B. Population studies of the Y-chromosome specific polymorphisms DYS19, DY389 I+II, DYS390 and DYS393 in a Western German population (Bonn area). Forensic Sci Int 1999;101:195-201.

12. Pena SDJ. Single-tube single-colour multiplex PCR amplification of ten polymorphic microsatellites (ALF10): a new powerful tool for DNA profiling. Pure Appl Chem 1999;71:1683-90.

13. Graw M, Seitz T. Y chromosomal short tandem repeat (STR) loci in a representative group of males living in South Württemberg: a database for application in forensic medicine. Forensic Sci Int 2000;113:43-6.

14. Y-STR single locus information, http://ystr.charite.de/index_gr.html, (2002).

15. Goodwin W, Scoular C, Linacre A. 13 STR loci frequency data from a Scottish population. Forensic Sci Int 2001;116:187-8.

16. Reymond M, Rousset F. An exact test for population differentiation. Evolution 1995;49:1280-83.

17. Salazar LA, Hirata MH, Cavalli SA, Machado MO, Hirata RDC. Optimized produre for DNA isolation from fresh and cryopreserved clotted human blood useful in clinical molecular testing. Clin Chem 1998;44:1748-50.

18. Hochmeister MN, Rudin O, Ambach E. PCR analysis from cigarette butts, postage stamps, envelope sealing flaps, and other saliva-stained material. In: Lincoln PJ, Thomson J. Forensic DNA profiling protocols. Totowa:Humana Press,1998:27-32.

19. IBGE – Instituto Brasileiro de Geografia e Estatística, http://www1.ibge.gov.br/english/estatistica/populacao/condicaodevida/indicadoresminimos/conceitos.shtm (2002).

Address for correspondence:
Dr. R N Oliveira
Department of Social Dentistry
Faculty of Dentistry, University of São Paulo
Av. Prof. Lineu Prestes, 2227 - 05508-900
São Paulo, SP - Brazil.
E-mail: rogerion@usp.br

A CASE OF BITTEN BETTONGS

H. James,[1] A.B. Acharya,[1] J.A. Taylor,[1] M.J. Freak[2]

[1] *Forensic Odontology Unit, Adelaide University, Australia*
[2] *Department for Environment & Heritage, National Parks & Wildlife, SA.*

ABSTRACT

Australia has a unique collection of native fauna, which is often threatened by physical harm or the destruction of its habitat and conservation of endangered species is a primary concern. Investigation of the recent deaths of Bettongs in Lincoln National Park, South Australia was undertaken by the Forensic Odontology Unit, Adelaide University to determine the likely perpetrator. Feral domestic cats were deemed to be responsible, as indicated by bitemarks on the radiotransmitter collars. **(J Forensic Odontostomatol 2002:20;10-2)**

Key Words: Bettong, Bitemarks, Forensic Odontology, South Australia

INTRODUCTION

The Bush-tailed Bettong (*Bettongia penicillata*) is a small, fur-covered Australian marsupial belonging to the Family *Potoroide*[1] (Fig. 1). It is bipedal and omnivorous and its habitat is located across southern and western Australia, where dense ground vegetation required for protection and food can be found. Due to shrinking habitat and increasing prevalence of predators these Bettongs are now considered an endangered species.[1,2]

Fig.1: *Brush-tailed Bettong (Bettongia penicillata)*

In the past decade vigorous breeding programs have been established by both the South Australian and Western Australian government departments and by private conservationists, and stable populations of Bettongs now exist in a number of conservation parks. In September 1999 Bettongs were re-introduced into a large area of natural bushland in Lincoln National Park (Fig.2) after previously being classified as extinct from the area.[2] The large area and unusual shape of the park meant that, unlike releases in other areas of the state, a predator fence was not utilized.

The first Bettongs to be released were all fitted with radio collars so that their location could be tracked by radio telemetry. Initial success allowed other Bettongs to be translocated to the area but by March 2001 it was obvious that a significant level of predation was occurring (Fig. 3).[2]

MATERIALS

In April 2001, the Forensic Odontology Unit received five radio-transmitter collars retrieved from Bettongs killed in Lincoln National Park from a representative of the South Australian Department for Environment and Heritage. Eight additional collars from the same area were received in May 2001.

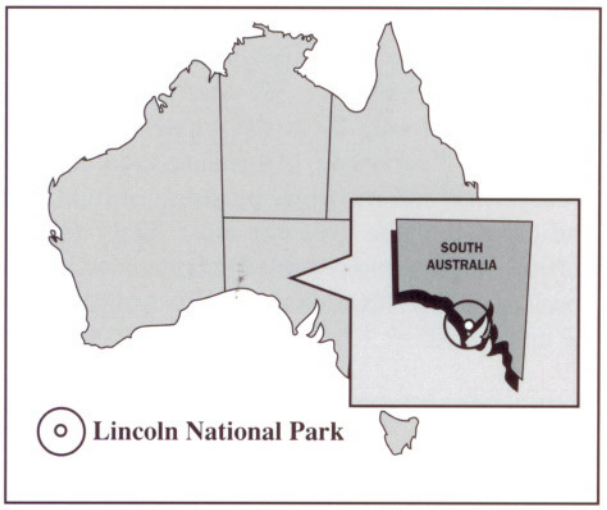

Fig.2: *Location of Lincoln National Park*

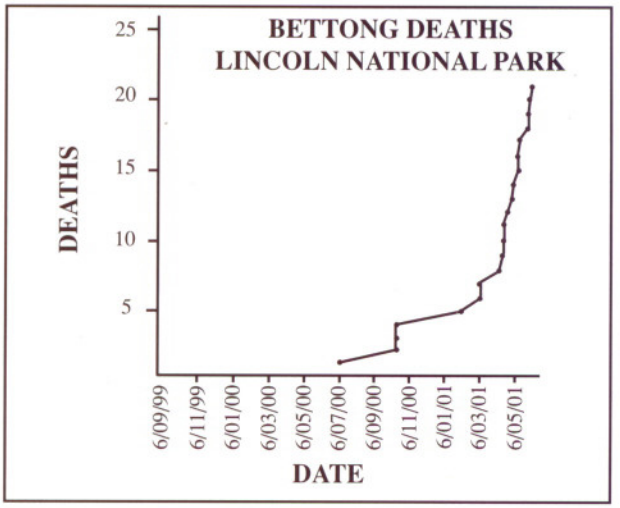

Fig.3: *Bettong mortalities in Lincoln National Park from release date in September 1999 to May 2001. Each point represents one death starting from 6/7/2000*

Examination of the collars revealed evidence of marks consistent with tooth morphology (Fig. 4) and the bitemarks on all the collars were similar in appearance, pointing to the possibility of one species of predator being responsible.

A comparison of these marks was made with various species thought to be the most likely predators of the region and which wildlife rangers considered would be either feral cats or foxes. Feral dogs and dingoes are also found in the area, along with several birds of prey. Skulls of each species were obtained from the South Australian Museum.

The cat family has a dentition that is specialized for grasping and killing prey by slicing the flesh. They display a short muzzle, with the incisor teeth making an approximately straight line across the front of the mouth and large canines at the corners.[3] Carnassial teeth (P^4 and M_1) form sharp cutting blades (Fig. 5) while dogs and foxes have larger jaws, with teeth designed for tearing and crushing.

RESULTS

The arch shape, inter-arch distance, and tooth shape were consistent with the species *Felis catus* (Fig. 6). *Vulpes vulpes* (fox) and *Canis familiaris* (dog/dingo) were excluded primarily on tooth size and arch width.

DISCUSSION

Studies in comparative anatomy of various species have shown that mammals are characterized by a

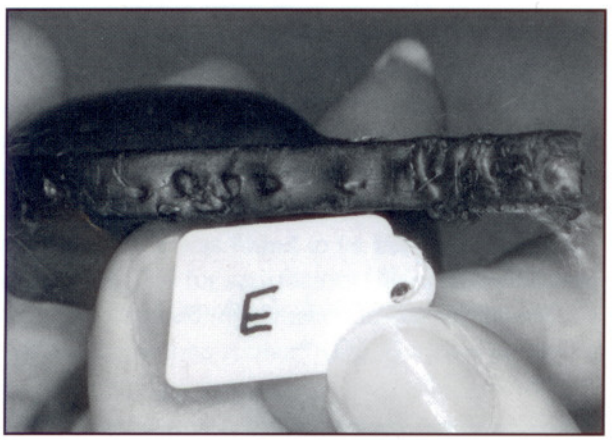

Fig.4: *Radio transmitter collar*

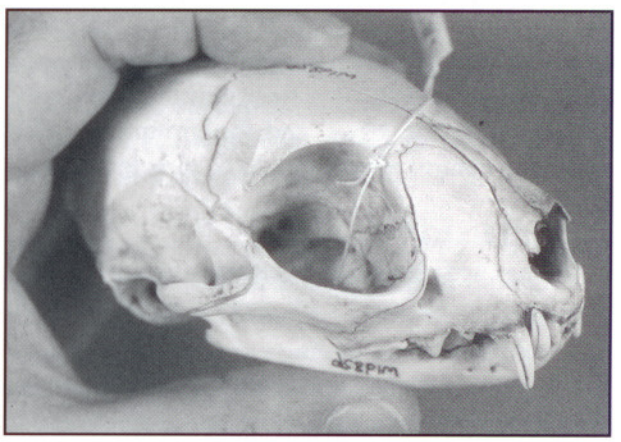

Fig.5: *Skull of domestic cat (Felis catus)*

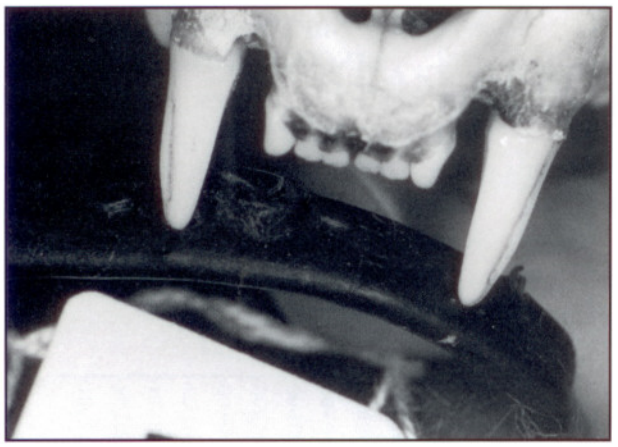

Fig.6: *Match of tooth shape and inter-arch distance between bitemark on collar and Felis catus*

heterodont dentition, with teeth varying in form in different locations within the mouth.[4] The typical mammalian dentition is generally considered to have the formula $I^3C^1P^4M^4$ and it is recognized that certain individual or groups of teeth are adapted to meet the requirements of specialized function, including mastication, fighting, defence, manipulation and holding of young.[4]

Introduced species from five mammalian groups, including carnivores such as dog, cat and fox live in a feral state in Australia[1] and were not introduced accidentally but were brought in as pets or to be hunted for sport. Neglect has allowed them to breed and have a huge impact on the local fauna and environment, in some circumstances rendering native fauna extinct in certain regions.

Reintroduction projects, coupled with targeted local predator control measures, are aimed at restoration of the population balance in favour of indigenous animals. Identification of specific species responsible for the death of native animals assists in determining the areas, and the groups, to which predator control efforts should be directed.

CONCLUSION

Bettongs had previously been classified as extinct in the Lincoln National Park area of South Australia. A reintroduction program, commenced in late 1999, was proving successful but predation rates were found to be extensive. By March 2002, it was estimated that only 20 of the initial 113 Bettongs released had survived.[5] An examination of radio-transmitter collars worn by these animals has indicated that the predator most likely to have inflicted the bitemarks was a feral cat and as a direct result of this investigation a control program will now be introduced.

ACKNOWLEDGEMENTS

South Australian Museum
Mr Murray Billett, Forensic Science Centre, Adelaide

REFERENCES

1. Strahan R, ed. The Australian Museum Complete Book of Australian Mammals. London: Angus and Robertson Publishers, 1983: 184, 482-9.

2. Freak MJ, Cotsell N, Tilley J. Re-introduction of Bush-tailed Bettongs (*Bettongia penicillata)* to Lincoln National Park. June 2001 Report, National Parks & Wildlife SA.

3. Osborn JW, ed. Dental Anatomy and Embryology. Vol 1, Bk 2. Oxford: Blackwell Scientific Publications, 1981: 406-8.

4. Scott JH, Symons NBB. Introduction to Dental Anatomy. 8th Ed. London: Churchill Livingstone, 1977: 430-4.

5. Cotsell N. Interim report, March 2002, National Parks & Wildlife SA.

Address for correspondence:
Helen James
Forensic Odontology Unit
The University of Adelaide, 5005
South Australia
Tel: +61 8 303 5431
Fax: +61 8 303 4385
E-mail: helen.james@adelaide.edu.au

COMPARISON MICROSCOPE IDENTIFICATION OF A CHEESE BITEMARK: A CASE REPORT

H. Bernitz[1], BA. Kloppers[2]

1. *Department of Oral Pathology and Oral Biology, Faculty of Dentistry, University of Pretoria, P.O. Box 1266, Pretoria 0001, Gauteng, South Africa*
2. *Forensic Science Laboratory, Silverton, Private Bag X 620, Pretoria 0001, Gauteng, South Africa*

ABSTRACT

Police investigating the murder of a farmer recovered a piece of cheese containing bite- marks. The local dental practitioner used white plaster to make casts of the bitemarks in the cheese and also of the teeth of three suspects. The cheese specimen was retained by the police and seven months later the case was referred to the Forensic Odontology Unit at the University of Pretoria where a silicone rubber cast of the bitemarks in the cheese was made. A lack of concordant features present in a conventional pattern-associated comparison was overcome with the aid of a Leica DMC comparison microscope. Individual features observed under 6.3x magnification aided in the positive identification of the suspect, who when confronted with the evidence, admitted guilt at his first court appearance. **(J Forensic Odontostomatol 2002:20;13-6)**

Keywords: Bitemarks, cheese, DMC comparison microscope

INTRODUCTION AND CASE REPORT

On 16 November 1999 a farmer was robbed and shot dead in the Richmond area of Kwa-Zulu Natal, South Africa. A piece of cheese containing bitemarks was found at the crime scene and the local dental practitioner who was consulted took an impression of the cheese bite and made casts in white plaster. Three suspects were arrested shortly afterwards and the same dental practitioner was then requested to make dental study models of the suspects. Using alginate impression material for the impressions, casts were once again poured in white plaster and the exhibits were transferred to the Silverton Forensic Science Laboratory in Pretoria.

Fig.1: A silicone cast of the cheese made 7 months after the crime. The defect on the 11 is clearly visible

On the 7 June 2000, three sets of study casts marked A, B and C, a piece of bitten cheese, and a plaster cast of the bitemarks was brought to the Faculty of Dentistry, University of Pretoria for forensic analysis. Upon receipt, the white plaster cast of the cheese surface was found to be porous and insufficiently detailed for satisfactory observation. It was therefore decided to re-make the cast of the cheese surface in silicone rubber* (Fig.1). This yielded a good quality replica considering the seven month age of the cheese specimen. Fortunately it had been stored in a sealed plastic container and kept in a refrigerator at approximately 4^0 C.

The cheese bite was classified as a type 3^1 in which the teeth bite right through or almost through the bitten material. However, a limited number of concordant features in a pattern-associated comparison of the silicone impression and the models of the suspects forced us to consider alternative methods of observation. Microscopic analysis[2] as well as scanning electron microscopy[3] were considered. The

* Light body President, Polyvinyl siloxane, Coltene Co, Switzerland

Ballistic Unit of the Silverton Forensic Science Laboratory offered their DMC (Das Microskop Comparison) comparison microscope* to assist in the analysis.

METHOD

A two-stage approach was used to examine and compare the bitemarks in the cheese with the study models of the three suspects. A pattern-associated comparison[4] in which the tooth features and their relationships were analyzed acted as an initial screening tool. Suspects A and C were excluded as the result of obvious mismatches and suspect B was considered to be a possible match.

In the second stage the models of the possible suspect were analyzed under the comparison microscope and a defect situated centrally on the incisal edge of tooth 11 was the area focussed on (Fig. 2). The two images visible on the microscope could be brought into juxtaposition and a direct comparison of the tooth (11) and the silicone cast of the cheese bite was made. An oblique light source was used to illuminate both casts and highlight the defect.

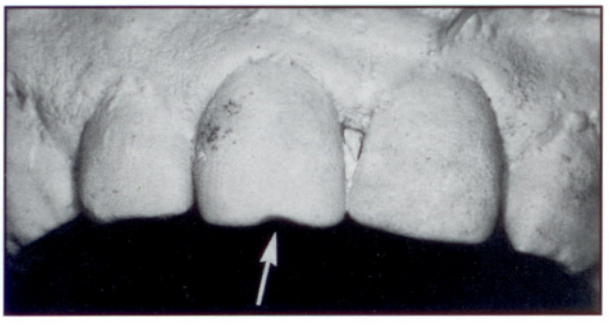

Fig.2: *A defect situated centrally on the incisal edge of the 11 is clearly visible*

RESULTS

The forensic team was satisfied that the bitemark was of human origin. During stage one it was furthermore clear that the models belonging to suspects A and D did not match the silicone cast of the cheese but that there was a possible match with suspect B.

* Leica, Mirosystems Wetzlar GmbH, Germany

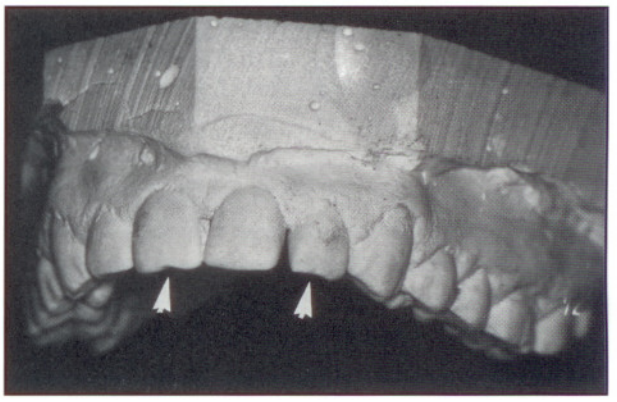

Fig.3: *The maxillary model of suspect B showing the defect on the incisal edge of the 11 and the concave incisal edge of the 22*

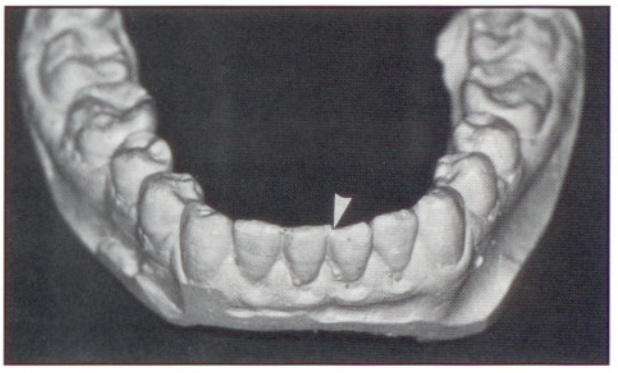

Fig.4: *The lower model of suspect B, showing the 31 labially placed in relation to the 41. This was also evident on the silicone cast.*

The following concordant features were observed in the pattern-associated comparison between the silicone cast and the model of suspect B (Figs. 3 and 4):

1. Centrally positioned defect on incisal edge of 11.
2. The defect on the 11 extended towards the lingual surface of the tooth.
3. The incisal edge of the 22 was concave.
4. The 31 was labially placed in relation to the 41.

Stage two was a microscopic comparison showing the following concordant features (Fig. 5):

1. The slope of the mesial edge of the defect formed an acute angle with the incisal edge.
2. The distal edge of the defect was shorter than the mesial edge, and formed an obtuse angle with the mesial edge.
3. The corner between the distal edge of the defect and the incisal edge of the tooth was rounded and bulbous.

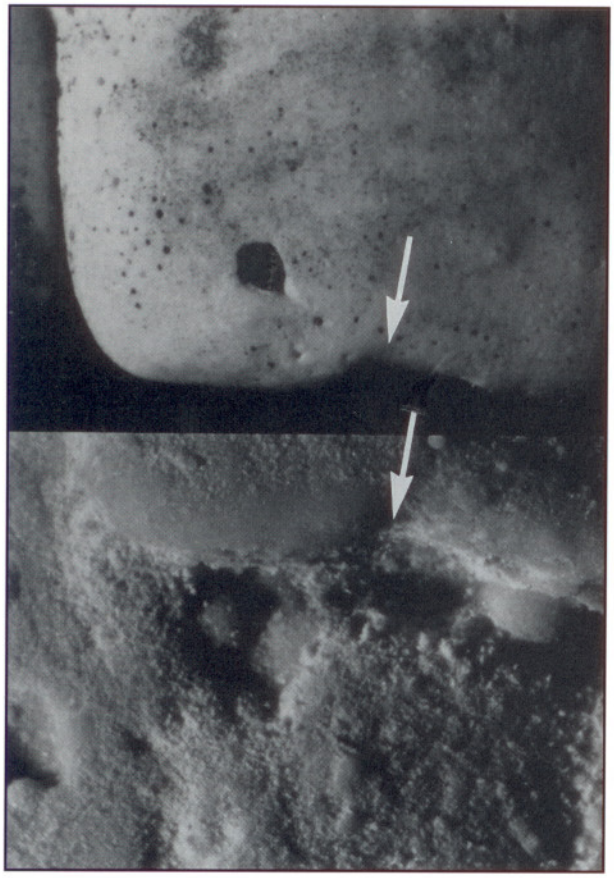

Fig.5: *The two incisal edges (tooth 11) as seen under the DMC comparison microscope. The two images are brought into juxtaposition and each point of concordance can be observed*

4. The incisal edge between the defect and the distal corner was slightly concave.
5. The defect was continuous with the lingual aspect of the tooth.
6. The mesial slope of the defect had characteristic mammilar protrusions (Fig 6).

It was concluded that in the presence of multiple concordant features suspect B was the probable perpetrator of the crime. The results of the above analysis were submitted to the Pietermaritzburg High Court and when confronted with the evidence at the first court hearing the suspect admitted guilt.

DISCUSSION

A bitemark can be defined as the registration of tooth cutting edges on a substance caused by jaw closure, and a tooth mark the print or impression registered on a substance by one or more teeth.[5] Each human dentition is unique, and will leave unique prints in

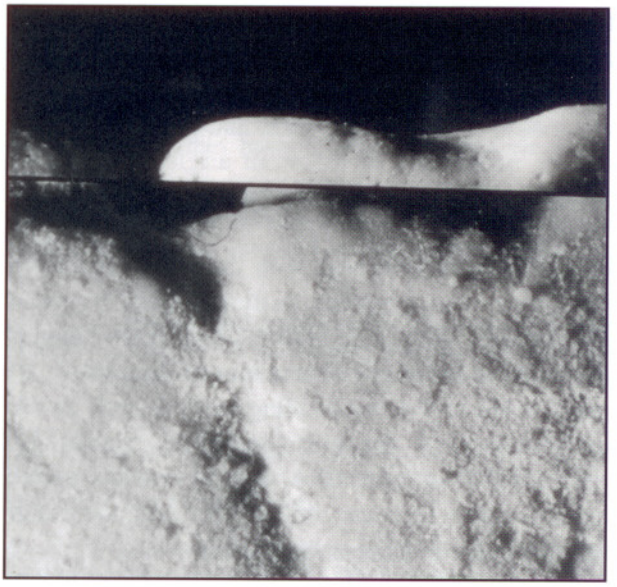

Fig.6: *The mesial slope of the defect shows the mammilar protrusions*

the objects bitten.[6] Bitemarks can be inflicted by humans or animals and can be found on skin or inanimate objects.[7] A variety of bitten foodstuffs have been associated with crime scenes, such as cheese,[8] cake,[9] chocolate,[2] a bread sandwich[10] and apples.[11]

In spite of the initial blunder of making the cast from the impression of the cheese in white plaster, the time it took before the cheese was examined, and a lack of sufficient dental features in the cheese bite, a satisfactory comparison was nevertheless possible. The use of a pattern-association technique allowed the authors to utilize a cheese specimen even though it had been kept by the police for seven months. Obvious shrinkage and a degree of distortion had taken place. Fungal growth, was also observed on the cheese. Any attempt at measurement would have failed to stand up to cross-examination in court. Cheese should always be kept in a sealed plastic container or sealed plastic bags and stored in a refrigerator at 4^0C[12]. Refrigerators should be periodically checked as current may fail or mechanical faults can cause temperature changes.

Unique imperfections only seen under magnification were observed, compared and analyzed with the aid of a comparison microscope at a magnification of 6.3 X, which is well suited to studying bitemarks. The images can be moved independently allowing

the examiner to position the specific features as required and the images can be photographed during all stages of the examination. Lens inserts containing metric scales can be used and the light source can be positioned at different angles to pick up details not seen under direct lighting.

Dental features observed with the naked eye were used to screen possible matches from obvious mismatches. Bite mark identification relies heavily on the three dimensional structure of each tooth, its relationship to the surrounding teeth as well as the relationship of the maxilla to the mandible. Individual characteristics, clearly visible under magnification increase the number of concordant features present in the comparison.

CONCLUSION

The DMC comparison microscope can be used to complement a pattern-associated analysis of bitemarks and by including individual characteristics only seen under magnification, the number of concordant features in the comparison can be increased. It should be borne in mind that the number of concordant features necessary to satisfy the judicial requirements will vary from country to country.[13]

REFERENCES

1. Webster G. A suggested classification of marks in foodstuffs in forensic dental analysis. Forensic Sci Int 1982; 20:45-52.

2. Mc Kenna CJ, Haron ML, Brown KA, Jones AJ. Bitemarks in chocolate: A case report. J Forensic Odontostomatol 2000; 18:10-4.

3. Solheim T, Torger IL. Scanning electron microscopy in the identification of bite marks in foodstuffs. Forensic Sci 1976; 6: 205-15.

4. Bernitz H, Piper SE, Solheim T, van Niekerk PJ, Swart TJP. Comparison of bitemarks left in foodstuffs with models of the suspect's dentitions as a means of identifying a perpetrator. J Forensic Odontostomatol 2000; 18:27-31.

5. Beckstead JW, Rawson RD, Giles WS. Review of bite mark evidence. J Am Dent Assoc 1979; 99: 69-74.

6. Rawson RD, Ommen RK, Kinard G, Johnson J, Yfantis A. Statistical evidence for the individuality of human dentition. J Forensic Sci 1984; 21: 245-53.

7. Ligthelm AJ, van Niekerk PJ. Comparitive review of bite mark cases from Pretoria, South Africa. J Forensic Odontostomatol 1994; 12: 23-9.

8. Layton JJ. Identification from bite mark in cheese. J Forensic Sci Soc 1996; 6: 76-80.

9. Aboshi H, Taylor JA, Takei T, Brown KA. Comparison of bitemarks in foodstuffs by computer imaging: a case report. J Forensic Odontostomatol 1994; 2: 41-4.

10. Simon A, Jordan H, Pforte K. Successful identification of a bite mark in a sandwich. Int J Forensic Dent 1974; 2:17-21.

11. Marshall W, Potter J, Harvey W. Bite marks in apples - Forensic aspects. The Criminologist 1974; 9: 21-34.

12. Bowers CM, Bell GL. Manual of Forensic Odontology. American Society of Forensic Odontology 1995:172.

13. Osterburg JW. An inquiry into the nature of proof. J Forensic Sci 1964; 9: 413-27.

Address for correspondence:
Dr. H. Bernitz
Department of Oral Pathology and Oral Biology,
Faculty of Dentistry,
University of Pretoria,
P.O. Box 1266,
Pretoria 0001, Gauteng,
South Africa.