ISSN 2219-6749

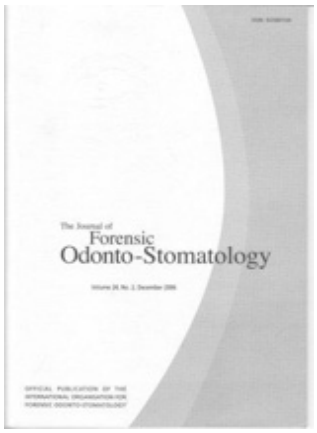
I·O·F·O·S

The Journal of Forensic Odonto-Stomatology

Volume 33, n. 1 - Jul 2015

OFFICIAL PUBLICATION OF THE
INTERNATIONAL ORGANIZATION FOR
FORENSIC ODONTO-STOMATOLOGY

JFOS
ISSN: 2219-6749



THE JOURNAL OF FORENSIC ODONTO-STOMATOLOGY

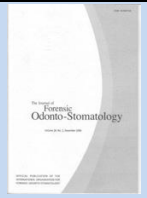
This Journal is a peer-reviewed on line publication of papers which should be broadly classifiable as original research, reviews, case reports or news (selected).

The Journal welcomes international material for publishing. We would also like to encourage submissions from new authors, recognising the importance of first publications. All papers should comply with the "Guidance to Authors" document printed on the IOFOS.eu website.

The JFOS is the official publication of the International Organisation of Forensic Odonto-stomatology (IOFOS)

Disclaimer

The views/opinions expressed in the publication are those of the authors and not the Editorial Board or Editor. The JFOS is not responsible for any consequences arising from the use of information contained in this issue.




JOURNAL of FORENSIC ODONTO-STOMATOLOGY

VOLUME 33 Number 1 July 2015

CONTENTS

Sections: Abuse Neglect (AN), Anthropology Archeology (AA), Age Estimation (AE), Bite Marks (BM), Case Report (CR), Dental Damage (DD), Disaster Management (DM), Editorial (ED), Facial Reconstruction (FR), Identification (ID), Jurisprudence/Litigation (JL), Tools and Techniques (TT), Review (RE), Virtopsy (VI).

JOURNAL of FORENSIC ODONTO-STOMATOLOGY
VOLUME 33 Number 1 July 2015

SECTION IDENTIFICATION

Personal Identification in Forensic Science Using Uniqueness of Radiographic Image of Frontal Sinus

Shital Sudhakar Nikam[1], Rajeev Madhusudan Gadgil[2], Ajay Ramesh Bhoosreddy[2], Karan Rajendra Shah[2], Vinayak Umesh Shirsekar[3]

[1]*Department of Oral medicine and Radiology, SMBT dental college Department, Sangamner, India.*
[2]*Department of Oral medicine and Radiology, M.G.V, K.B.H Dental College and Hospital, Nasik, India.*
[3]*Department of Oral Medicine and Radiology, TPCT's Terna Dental College, Mumbai, India.*

Corresponding author: drshital1987@gmail.com
The authors declare that they have no conflict of interest.

ABSTRACT

Frontal sinus pattern matching is a useful means of forensic identification. By the use of radiographs forensic scientists have recognized that there are diverse anatomical variations in the structure of the frontal sinus. Radiographs are a diagnostic tool, widely used in dental practices, hospitals and other health disciplines. Most health institutions possess the facility to store radiographs over long periods of time. Frontal sinus pattern matching technique can be applied in cases where ante mortem frontal sinus radiographs are available and dental matching cannot be carried out. Frontal sinus pattern matching technique may also be used to corroborate identifications based on other techniques such as fingerprints, teeth, or circumstantial evidence. The present study was carried out to assess the effectiveness of using the radiographic image of the frontal sinus for personal identification in studied population group. The results concluded that the appearance of the radiographic image of the frontal sinus is unique for each individual. On this evidence it is proposed that frontal sinus pattern matching can be used for personal identification when other methods have failed.

KEYWORDS**:** frontal sinus, postero-anterior skull radiograph (PA skull radiograph), personal identification forensic science.

INTRODUCTION

"The dead cannot cry out for justice, it is the duty of the living to do so for them." - Lois MeMaster Bujold".

Identity is the set of physical characteristics, functional or psychic, normal or pathological, that defines an individual. Since time immemorial, human identification has proven to be one of the most challenging tasks faced by mankind.[1] The application of radiology in forensic sciences was introduced in 1896, just one year following the discovery of X-rays by Roentgen where he used X-rays to demonstrate bullets lodged within the brain.[2] Radiographic analysis, in which ante mortem and post-mortem X-rays are compared, is frequently used for human identification purposes.[3] Radiographs obtained for diagnostic and clinical purposes always include skeletal features. The skeleton usually survives both natural and unnatural abuse or violation and is available for radiographic examination in most circumstances. It is often possible to obtain both ante mortem and post-mortem radiographs in cases where identification is required.[4] It is possible to use many different components of the skeleton for pattern matching in a similar way that finger prints are used for purposes of identification.[4,5]

Anatomically frontal sinus growth is completed before the age of 20 years and remains stable thereafter[6]. Additionally the anterior wall of the frontal sinus is thick and resistant to injury.[7]

Different radiographs can be used to study the pattern of the frontal sinus including both PA skull and Lateral skull views. Pattern matching is possible using both PA film projections (Caldwell orientation and Water's view). The Caldwell view allows better viewing of the frontal sinus, compared to Water's view which gives a slightly foreshortened image.[8] Hence this study was undertaken to determine the uniqueness of the radiological image of the frontal sinus for personal identification in a studied population group using the PA skull radiograph.

MATERIAL AND METHODS

109 individuals were randomly selected from OPD. The purpose of the study was explained to each of them and individual written and informed consent was obtained. Individuals less than 20 years of age, individuals with a history of trauma or surgery to the frontal sinus or individuals with pathology destroying or encroaching within the frontal sinus, were excluded from the study. A PA skull radiograph was taken of those individuals who met these initial criteria. Subsequently 9 of these individuals were excluded because of the absence of frontal sinuses or the presence of a unilateral frontal sinus demonstrated on the PA skull radiograph.

PA skull radiographs of the selected patients were taken after following all radiation protection measures using a Cephalometric machine (planmeca proline ec panoramic x-ray and cephalostat cm manufactured in Helsinki, Finland) with variable exposure parameters based on the manufacturer's guidelines. The patients were positioned facing the cassette with the head tipped forwards to ensure that the sagittal plane was perpendicular to the cassette and the floor, and that the cantho-meatal line was perpendicular to the cassette. The patient's head was stabilized in this position using the ear rod of the machine. Accepted protocols for exposure parameters were followed. The radiographs were taken and developed by a single operator using conventional techniques to minimize any margin of error. PA skull radiographs with the frontal sinus outlined clearly were selected for the study. The selected radiographs were traced manually using tracing paper and pre-defined metric variables were measured by both the researcher and an observer to minimize

inter-observer error and to check inter-observer variability.

A baseline was drawn across the superior margin of the orbits. A tangent was drawn to the baseline, segmenting the sinus area into quadrants. The tangent was located at the midpoint between two vertical orbital lines drawn at the most medial point of each orbit. The outline of the sinus above the baseline was traced by both the researcher and the observer. Variables of frontal sinus which were measured in this study were divided into (shown in fig. 1):

I) General sinus variables which include-
Number of complete sinus cavities,
Number of partial sinus lines,
Maximum overall height above baseline (Baseline-C),
Maximum overall width (A–B)

II) Variables on left side which include-
Number of complete sinus cavities left of septum,
Number of partial sinus lines in main cavity,
Number of scalloped arcades on main cavity,
Maximum height of quadrant above baseline (Baseline-C),
Maximum height of main cavity above baseline (Baseline-C),
Maximum width of main cavity from tangent line (G-A),
Maximum width of main cavity (F–A)

III) Variables on right side which include-
Number of partial sinus lines in main cavity,
Number of scalloped arcades on main cavity,
Maximum height of quadrant above baseline (Baseline-D),
Maximum height of main cavity above baseline (Baseline-D),
Maximum width of main cavity from tangent line (G-B),
Maximum width of main cavity (E-B).

A partial sinus line (shown by yellow arrow in figure 1) is defined as any line 1 mm or longer extending from the border of the cavity into the cavity area, but not completely dissecting the cavity into two cells. A scalloped arcade (shown by red arrow in figure 1) occurs any time the edge of a cavity arcs inward, then back out again; the change of direction (continuity) of the edge marks the end of one scallop and the beginning of another.[5]

To check for uniqueness of each sinus and to assess the role of the frontal sinus in forensic radiology, superimposition of both radiographs and tracings was carried out to ascertain whether the radiographic outline of any one sinus matched to any other included in the same study sample. Following measurement of all the metric variables including sinus, skull width and overall height in centimeters, data was tabulated, entered into Microsoft Excel and analyzed by SPSS Ver. 20.0.0 software. An independent sample t-test was used to check inter-observer variability.

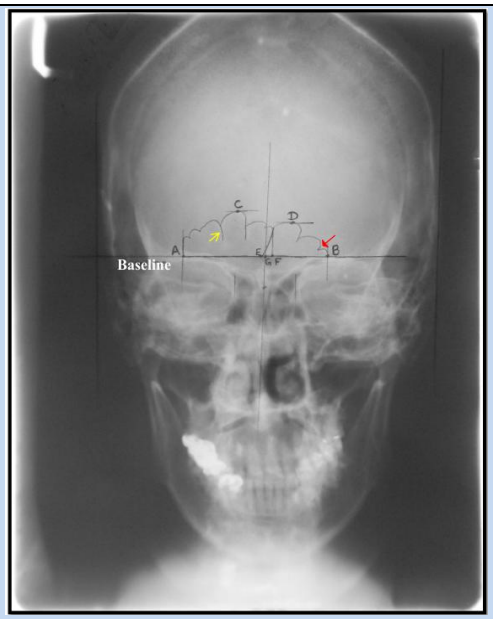


Fig.1: Traced PA skull radiograph showing borders of sinus and metric variables. Red arrow – indicates scalloped arcade. Yellow arrow indicates-partial sinus line on left side. Point A to B- maximum width of frontal sinus. Baseline to point C - maximum height of frontal sinus.

RESULTS

In this study 109 PA skull radiographs were captured of which 9 were excluded from the study. 6 were excluded because of the absence of frontal sinuses and 3 were excluded because of the unilateral presence of a single sinus.

Thus prevalence of absence of the frontal sinuses in this study was 5.50% and the prevalence of a single unilateral sinus was 2.75%. The study sample comprised 60 males and 40 females. Measurement of all of the metric variables was carried by one researcher and one observer. To check inter-observer variability an independent sample t- test was applied. Results of the independent sample t- test were non-significant for all of the metric variables confirming no significant inter-observer variability. The maximum height and the maximum width of the frontal sinus for the given sample varied between 0.2cm to 3.1cm and between 2.5cm to 9.3cm respectively. Both the maximum height and maximum width of the frontal sinus was found to be a unique feature for each individual. The discrete frequency distribution for both maximum height and the maximum width were prepared separately. The different values of height and width occurred with much less frequency but still uniqueness was maintained by considering each value separately. The number of partial sinus lines also showed a great deal of variation ranging from 0 to 12. The number of scalloped arcades on both the right and left side varied from 0 to 7. To evaluate the forensic significance of radiographic images of the frontal sinus a "traced" radiograph was superimposed upon another "traced" radiograph from the same study. An assessment of any patterns of similarity and meaningful relationship that may exist between the two "traced" radiographs was made by both the researcher and the observer The superimposition procedure relied on the outline of sinus and on various other parameters including the height and width of the sinus, the number of partial sinus lines and number of scalloped arcades. It was found that not all of the parameters of either of the two superimposed radiographs matched exactly i.e. 100% matching was not observed. Against this background it

can be convincingly stated that, the radiographic appearance of the frontal sinus in each individual is different and unique. Accordingly the radiographic appearance of the frontal sinus can be therefore used as a feature of uniqueness in forensic science in cases of human identification.

DISCUSSION

Forensic personal identification, using appropriate techniques, is a fundamental scientific discipline used in the identification of the living, recently deceased and compromised human remains. It is often used as a tool in crime scene investigations.

Scientific identification of human remains can be accomplished by a variety of methods including fingerprint, dental, anthropological, genetic and radiological examinations.[4] DNA analysis is well recognized as a method of verification of identity of an unknown person but is expensive and not always suitable in cases of mass disaster.[9] The use of radiographs in cases of both routine and mass disaster eventualities is well recognized and has shown to be effective, swift and relatively easy to implement.[6] The first recorded use of radiographic techniques for the purposes of identification was reported by Schuller in 1921. Radiographically assisted dental identification may be either comparative or reconstructive.[10] It has been reported that some 72% of positive identifications in modern forensic science have been obtained by comparing ante mortem and post-mortem radiographs.[4]

Anatomically, the frontal sinuses can be defined as pneumatic cavities covered by mucosa, located between the internal and external cortical bones of the frontal bone.[11] Sinuses remain stable throughout life after age of 20 years. As a consequence the age at which the ante mortem radiograph was captured does not matter provided the individual was at least 20 years of age at the time.[6] The thick bone of the anterior wall of the sinus and its curved convexity are the first barrier to the effects of cranial trauma and the ability to resist fracture. Considerable force of up to 1600 foot pounds of impact is required to fracture the anterior wall of the frontal sinus. This is almost twice as much as it takes to fracture the parasymphyseal area of the mandible and 50 % more than required to fracture the malar eminence of the zygoma.[7] In consideration the present study was designed to evaluate the role of the radiographic image of the frontal sinus for purposes of personal identification and explore the possibility of its uniqueness in studied population group.

A total 109 PA skull radiographs were taken in this study. Thus prevalence of absence of the frontal sinuses was 5.50% and the prevalence of a single unilateral sinus was 2.75%.

Results from other similar studies are shown in table-1; there is close correlation with the results of this present study.

Table 1 – Bilateral absence and unilateral agenesis of frontal sinus in different studies

Other similar studies	Bilateral absence	Unilateral agenesis
Aydinlioğlu et al.[12] (2003 in Turkish population	3.8%	4.8%
Çakur B et al[13] (2011)	0.73%	1.22%
Fatu et al[14] (2006)	5%	1.6%
Danesh-Sani SA[15] (2011)	8.32%	5.66%
Present study	5.50%	2.75%

The outline of the frontal sinus was traced and metric variables were measured on all of the PA skull radiographs. The data relating to the metric variables included

the total number of right & left partial sinus lines, the deviation of the septum, the maximum height and width of the sinus on both sides, the number of scalloped arcades on the right and left hand sides and the maximum width from the tangent was recorded by one researcher and one observer to check inter-observer variability. To evaluate the forensic significance of radiographic images of the frontal sinus a "traced" radiograph was superimposed upon another "traced" radiograph from the same study. An assessment of any patterns of similarity and meaningful relationship that may exist between the two "traced" radiographs was made by both the researcher and the observer The superimposition procedure relied on the outline of sinus and on various other parameters including the height and width of the sinus, the number of partial sinus lines and number of scalloped arcades. It was found that not all of the parameters of either of the two superimposed radiographs matched exactly i.e. 100% matching was not observed. Against this background it can be convincingly stated that, the radiographic appearance of the frontal sinus in each individual is different and unique. Accordingly the radiographic appearance of the frontal sinus can be therefore used as a feature of uniqueness in forensic science in cases of human identification. Application of this technique could be particularly relevant in cases of mass disaster or in cases where the skull is available and other methods of identification are either inappropriate or unavailable.

Similar results were obtained in previous studies by Marlin DC et al.[16], 1991; Reichs KJ[17], 1993; Quatrehomme G et al.[5], 1996; Nambiar P et al.[18], 1999; Kirk NJ et al.[19], 2002. Marlin DC et al demonstrated the use of frontal sinus morphology in the identification of 4 human remains.[16] Kirk NJ et al demonstrated the validity of frontal sinus pattern matching by comparing ante-mortem and post-mortem radiographs in 39 cases for forensic identification. The uniqueness of the morphology of the frontal sinus offers an opportunity to use this feature for personal identification in forensic medicine.[19]

CONCLUSION

This study shows that the frontal sinus of each individual is unique and, as a consequence, frontal sinus pattern matching can be useful as a technique in some cases of forensic identification.

A future development could be the establishment of a numerical classification system akin to that used in fingerprint analysis.

This would facilitate the adoption of a standardized approach using the metric variables of the frontal sinus for human identification.

REFERENCES

1. Paiva LAS and Segre M. Sexing the human skull through the mastoid process. *Rev Hosp Clin Fac Med Sao Paulo* 2003 Jan-Feb; 58(1): 15-20.
2. Carvalho SPM, Silva RHA, Lopes Jr C, Sales-Peres A. Use of images for human identification in forensic dentistry. *Radiol Bras* 2009; 42(2):125-30.
3. Cameriere R, Ferrante L, Molleson T, Brown B. Frontal Sinus Accuracy in Identification as Measured by False Positives in Kin Groups. *J Forensic Sci* 2008 Nov; 53(6):1280-2.
4. Kahana T, Hiss J. Identification of human remains: forensic radiology. *J Clin Forensic Med* 1997 Mar; 4(1):7-15.
5. Quatrehomme G, Fronty P, Sapanet M, Grévin G, Bailet P, Ollier A. Identification by frontal sinus pattern in forensic anthropology. *Forensic Sci Int* 1996 Dec 2; 83(2):147-53.
6. Bernstein ML. The application of photography in forensic dentistry. *Dent Clin North Am* 1983 Jan; 27(1): 151-70.
7. Nahum AM. The biomechanics of maxillofacial trauma. *Clin Plast Surg* 1975 Jan; 2(1):59-64.
8. Riepert T, Ulmcke D, Schweden F, Nafe B. Identification of unknown dead bodies by X-ray image comparison of the skull using the X-ray simulation program FoXSIS. *Forensic Sci Int* 2001 Mar; 117(1-2): 89-98.
9. Ertugrul T, Gulgun Y, Asim A, Mahmut A, Yildiray Z, Serdar T. Identification of unknown bodies by using CT images of frontal sinus. *Forensic Sci Int* 2007 Feb 14; 166(1):42-8.

10. RE Wood. Forensic aspects of maxillofacial radiology. *Forensic Sci Int* 2006; 159S: S47–S55.
11. da Silva RF, Prado FB, Caputo IG, Devito KL, Botelho Tde L, Daruge Júnior E. The forensic importance of frontal sinus radiographs. *J Forensic Leg Med* 2009 Jan; 16(1):18-23.
12. Aydinlioglu A, Kavakli A, Erdem S. Absence of Frontal Sinus in Turkish Individuals. *Yonsei Med J* 2003 Apr 30; 44(2):215-8.
13. Cakur B, Sumbullu MA, Durna NB. Aplasia and Agenesis of the Frontal Sinus in Turkish Individuals: A Retrospective Study Using Dental Volumetric Tomography. *Int J Med Sci* 2011; 8(3):278-82.
14. Fatu C, Puisoru M, Rotaru M, Truta AM. Morphometric evaluation of the frontal sinus in relation to age. *Ann Anat* 2006 May; 188(3):275-80.
15. Danesh-Sani SA, Bavandi R, Esmaili M. Frontal sinus agenesis using computed tomography. *J Craniofac Surg* 2011 Nov; 22(6):e48-51.
16. Marlin DC, Clark MA, Standish SM. Identification of Human Remains by Comparison of Frontal Sinus Radiographs: A Series of Four Cases. *J Forensic Sci* 1991 Nov;36(6):1765-72
17. Reichs KJ. Quantified comparison of frontal sinus patterns by means of computed tomography. *Forensic Sci Int* 1993 Oct; 61(2-3):141-68.
18. Nambiar P, Naidu MD, Subramaniam K. Anatomical variability of the frontal sinuses and their application in forensic identification. *Clin Anat* 1999; 12(1):16-9.
19. Kirk NJ, Wood RE, Goldstein M. Skeletal identification using the frontal sinus region: A retrospective study of 39 cases. *J Forensic Sci* 2002 Mar; 47:318-23.

*****

JOURNAL of FORENSIC ODONTO-STOMATOLOGY
VOLUME 33 Number 1 July 2015

SECTION IDENTIFICATION

In Vitro Description of Macroscopic Changes of Dental Amalgam Discs Subject to High Temperatures to Forensic Purposes

Carlos Arcos[1], Juan-David Díaz[1], Kenny Canencio[1], Diana Rodríguez[1], Carlos Viveros[1], Jonathan Vega[1], Juliana Lores[2], Gustavo Sinisterra[1], Wilmer Sepúlveda[1], Freddy Moreno[3]

[1]*School of Dentistry at Universidad del Valle, Cali, Colombia*
[2]*Pontificia Universidad Javeriana, Cali, Colombia*
[3]*Department of Basic Sciences of Health, Faculty of Health Sciences, Pontificia Universidad del Valle, Cali, Colombia; Faculty of Health, Universidad del Valle, Cali, Colombia*

Corresponding author: fmorenog@javerianacali.edu.co; freddy.a.moreno@correounivalle.edu.co

The authors declare that they have no conflict of interest.

ABSTRACT
Objective: To describe the behavior of 45 discs of dental amalgam of known dimension prepared from three commercially available brands of dental amalgam (Contour® Kerr®–USA, Admix® SDI®–Australia and Nu Alloy® Newstethic®–Colombia) when subjected to the action of high temperatures (200°C, 400°C, 600°C, 800°C, 1000°C). It was hoped to establish parameters that could be used for human dental identification in cases of charred, burned or incinerated human remains.
Materials and methods: A pseudo-experimental descriptive in-vitro study was designed to describe the macroscopic physical changes to the surface of 45 discs of pre-prepared amalgam of three commercially available brands exposed to a range of high temperatures.
Results: Characteristic and repetitive physical changes were a noticeable feature of the discs of amalgam of each brand of amalgam subjected to the different temperature ranges. These physical changes included changes in dimensional stability, changes in texture, changes in colour, changes in the appearance of fissures and cracks and changes in the fracture and fragmentation of the sample.
Conclusions: The characteristics of dental amalgam may be of assistance in cases of human identification where charred, burned or incinerated human remains are a feature and where fingerprints or other soft tissue features are unavailable.

KEYWORDS**:** Forensic sciences, forensic dentistry, dental identification, dental amalgam, high temperatures.

INTRODUCTION

The importance of identification of the deceased has been embraced and accepted across society since time immemorial. Identification of the deceased is paramount for social, cultural, religious, legal and financial reasons.

Post mortem investigations are often multi-disciplinary. The variety of disciplines involved in an investigation may include data collection, analysis and interpretation. Input from various forensic experts, including forensic odontologists, is usually routine. Input from the appropriate legal authority is, in the majority of cases, mandatory.

Post mortem examination of both hard and soft tissues of the stomatognathic system is appropriate in the process to establish definitive identity of the deceased. Positive identification is dependant upon reliable coincidence between ante-mortem and post-mortem dental records.

In cases of human identification involving charred, burned or incinerated human remains where visual identification is problematic and where fingerprints or other soft tissue features are unavailable the stomatognathic system may provide distinctive and circumstantial evidence to achieve a positive identification using dental, anthropological and DNA analysis [(1)].

In circumstances such as those described above the forensic odontologist relies on comparison of the ante-mortem and post-mortem records of the deceased [(2, 3)].

It is well recognized that the resilience of dental tissues and that of dental restorative materials, including amalgam, both play a key part in human identification [(4, 5)].

The American Board of Forensic Odontology [(6)], with national and international support, has listed four categories of certainty of identity when comparing ante- mortem and post-mortem dental records; positive identification (total coincidence), possible identification (compatibility), insufficient evidence (inadequate information available) and exclusion (incoherence and incompatibility) [(7)].

Dental amalgam is characterized by being a low cost biocompatible material, with good physical and mechanical properties. These properties include good thermal and electrical conductivity, together with good compressive and flexural strength and a low capacity of permanent deformation. However dental amalgam is non-adhesive to tooth substrate, is opaque, of low aesthetic specification and is not anti-cariogenic. Amalgam is used frequently to restore Class I and II cavities where restoration of the structure of the tooth is of a higher priority than any aesthetic consideration [(8-10)]. Globally, dental caries is the most prevalent disease presenting in adults.

Over the last 150 years dental amalgam has been the material of choice in restorative dentistry because of its durability and cost-effectiveness, despite current trends toward the development and use of polymeric materials with superior aesthetic properties [(11-15)].

The purpose of this study is to establish in vitro parameters regarding the behavior of dental amalgam when subjected to high temperatures. Forensic experts could then use this information to determine the range of temperature in which the changes occurred. It is hoped that this may contribute to the process of forensic dental identification in case of burned, charred or incinerated human remains.

MATERIALS AND METHODS

This is a pseudo-experimental descriptive transversal observational study that analyzed, through stereomicroscopy, the

behavior of 45 discs of dental amalgam of known dimension prepared from three commercially available brands (Contour® Kerr®–USA, Admix® SDI®–Australia and Nu Alloy® Newstethic®–Colombia) when subjected to the action of high temperatures (200°C, 400°C, 600°C, 800°C, 1000°C). In the interests of comparison and standardisation, three dental amalgam systems with similar proportional composition were used in the study, Two of the brands are used commonly on a global basis (Contour® Kerr®–USA and Admix® SDI®–Australia) whilst the other is used commonly in Latin America (Nu Alloy® Newstethic®–Colombia).

PRODUCTION OF THE DISCS OF DENTAL AMALGAM USED IN THE STUDY

Aluminum matrix, acetate sheets and glass slabs were used to simultaneously prepare 5 discs of amalgam, each one 10 millimeters in diameter and 4 millimeters thick. Four mono-dose dental amalgam capsules were used for each disc. Each capsule was triturated in a Variamix® Dentsply® amalgamator for 12 seconds. The discs of amalgam were prepared by the conventional technique of packing (placement of the amalgam in the matrix), condensation (compaction of the amalgam in the matrix) and polishing (adaptation of the amalgam with the edge of the matrix) [16, 17]. The surfaces of the discs were not polished (with the aim of not altering the composition of the surface relative to the center of the disc). Once the crystallization phase was complete, the discs were removed from the matrix (Figure 1).

HANDLING AND CONSERVATION OF THE SAMPLE

Once the discs of dental amalgam had been produced, each specimen was stored individually in a plastic opaque container and maintained at a relative humidity and ambient temperature. Before the application of high temperatures, a digital photography was taken to each of the specimens through a Leuchtturm® digital stereomicroscopy of 1.3 mega pixels at 15X and 50X. The discs were distributed randomly on each of the six groups (one control group –ambient temperature– and five intervention groups) according to temperature ranges (Table 1).

APPLICATION OF HIGH TEMPERATURES

This procedure was performed based on the scientific and technical protocol established by the Odontostomatology Department of University of Pavia (Italy) [18] and based on studies carried out by the School of Dentistry of Universidad del Valle (Colombia) [19]. The in vitro model as proposed in this study was carried out in an oven and not on direct flame. From previous studies it was noted that the highest temperature reached had been 1000°C that was achieved within 25 or 30 minutes and later maintained at approximately 500°C until all of the oxygen was depleted or all the organic contents reduced to coal (carbonization) or to calcium compounds, phosphates, silica or other trace elements (incineration) [20]. This "muffle effect" in situ, was performed to simulate the effect of perioral tissues, facial musculature, bone tissue and periodontal and dental tissues[3].

The intervention group specimens corresponding to each range of temperature were placed in individual trays of refractory coating (Cera-Fina® Whipmix®) to facilitate their handling. They were then subjected to direct heat inside a muffle type oven (Thermolyne®) previously calibrated to five different temperature ranges (200°C, 400°C, 600°C, 800°C, 1.000°C) with a climbing rate of 10°C per minute from an initial

temperature of 28°C until each of the proposed temperatures were reached. For example, nine specimens were introduced (three discs of every brand) according to the 200°C group, each one on their respective tray, at a temperature range starting at 28°C to 200°C, The oven was then left cooling to ambient temperature before removal of the trays with the specimens. So on for 600°C, 800°C and 1.000°C group specimens.

By being subjected to high temperatures, the experimental discs of dental amalgam may be found to demonstrate changes in color, texture, fissures and cracks, fractures, dimensional stability and explosion[20].

SAMPLE OBSERVATION

Before describing the changes in the characteristics of the pre-prepared discs of dental amalgam subjected to high temperatures, two of the authors underwent training regarding the observation of these changes (variables) in the control group (color, texture, fissures and cracks, fractures, dimensional stability and fragmentation), and had to unify the criteria for observation. In order to estimate the intraobserver and interobserver agreement, the Kappa test was performed in the Stata® software ver. 6.0.

STATISTICAL ANALYSIS

IBM SPSS Statistics® Ver. 22.0 software was used to calculate the prevalence (%) of macroscopic changes in the sample. The variables that were considered were temperature, color, texture, fissures and cracks, fractures, dimensional stability and explosion.

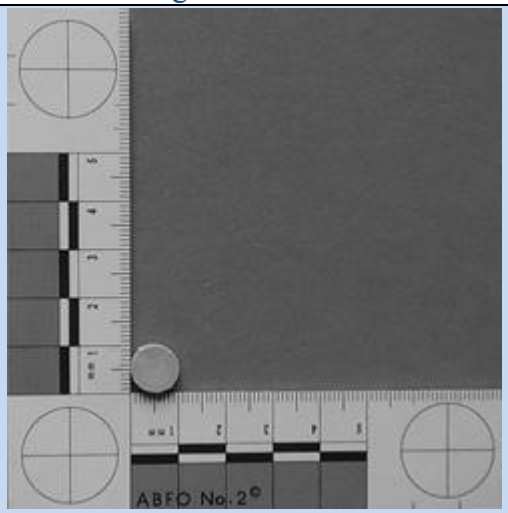


Fig.1: Disc elaborated in dental amalgam.

RESULTS

Respect standardization of observers, Kappa test determined the intraobserver standardization (0.88 and 0.86) and interobserver (0.89 and 0.90) of the two observers, respectively.

The three commercial brands (Contour® Kerr®–USA, Admix® SDI®–Australia and Nu Alloy® Newstethic®–Colombia) of dental amalgam had a different behavior in each temperature range (Table 2). At 15X (Figure 2) and at 50X (Figure 3) changes in dimensional stability, texture, color, fissures and cracks, fracture and explosion changes were seen in the dental amalgam discs that were subjected to the action of high temperatures. In general, at 200°C the dental amalgam discs of the three commercial brands lost brightness

and the surface seemed rough, much more in the Admix® SDI®–Australia sample discs. At 400°C numerous rounded nodules with a porous appearance together with fissures and cracks appeared (these nodules in the Admix® SDI®–Australia discs appeared at 200°C). At 600°C the dental amalgam discs of the three commercial brands turned opaque and had a dark grey color. Deep fracture lines were observed in the places where the nodules had been previously present and there was evidence of loss of dimensional stability that appeared as a convex shape to the surface of the disc; at 800°C there was fragmentation of each disc where the appearance of rough, opaque and fragile pieces were observed. They tended to pulverize by touch. Shiny, bright and compact fragments grouped into rounded structures. The same changes at 800^0C were seen at 1000^0C.

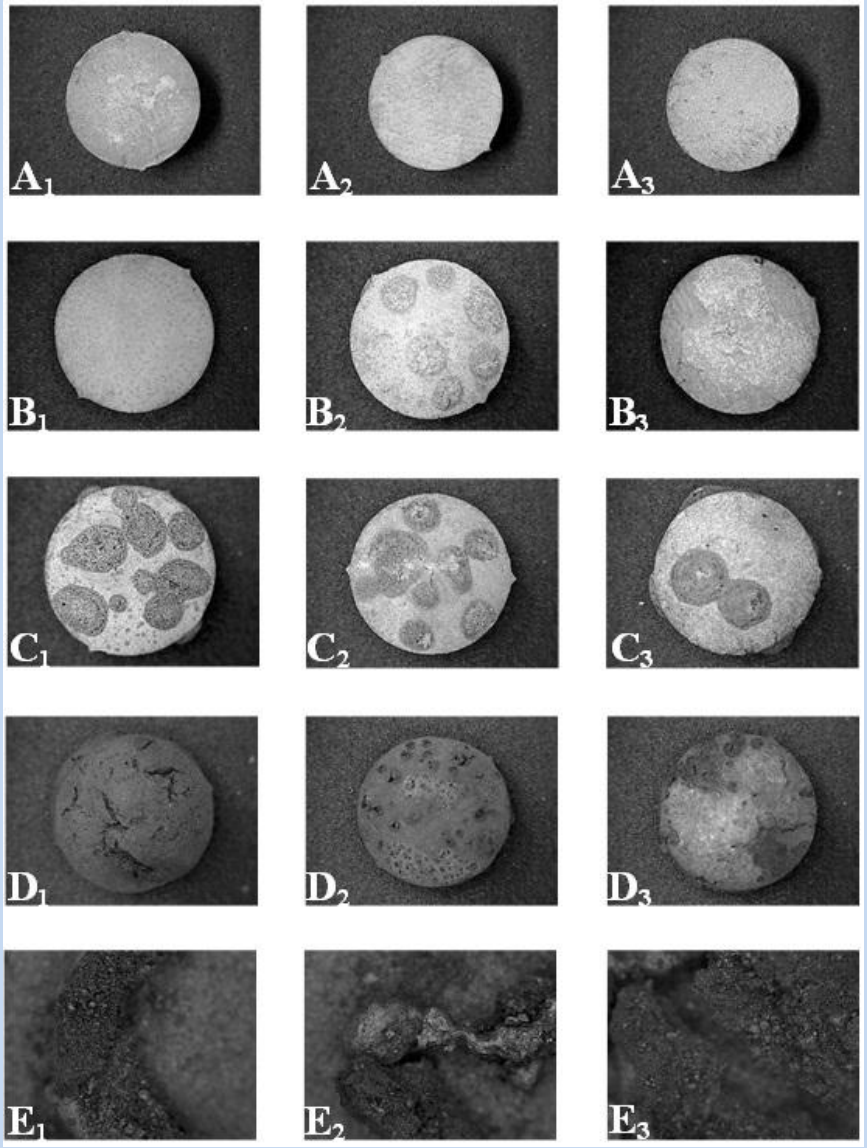


Fig. 2: Amalgam discs observed at 15X. A1 Contour® Kerr®–USA amalgam disc, A2 Admix® SDI®–Australia amalgam disc and A3 Newstethic®–Colombia amalgam disc. B1 Contour® Kerr® amalgam disc, B2 Admix® SDI®–Australia amalgam disc in which silver nodules were observed and B3 Newstethic®–Colombia amalgam disc subjected to a temperature of 200°C. C1 Contour® Kerr®–USA amalgam disc, C2

Admix® SDI®–Australia amalgam disc and C3 Newstethic®–Colombia amalgam disc subjected to a temperature of 400°C in which silver nodules were observed. D1 Contour® Kerr®–USA amalgam disc, D2 Admix® SDI®–Australia amalgam disc and D3 Newstethic®–Colombia amalgam disc subjected to a temperature of 600°C in which the silver nodules disappear. E1 Contour® Kerr®–USA amalgam disc, E2 Admix® SDI®–Australia amalgam disc and E3 Nu Alloy® Newsthetic®–Colombia amalgam disc subjected to a temperature of 800°C in which the conformation of silver spheres and bands was observed.

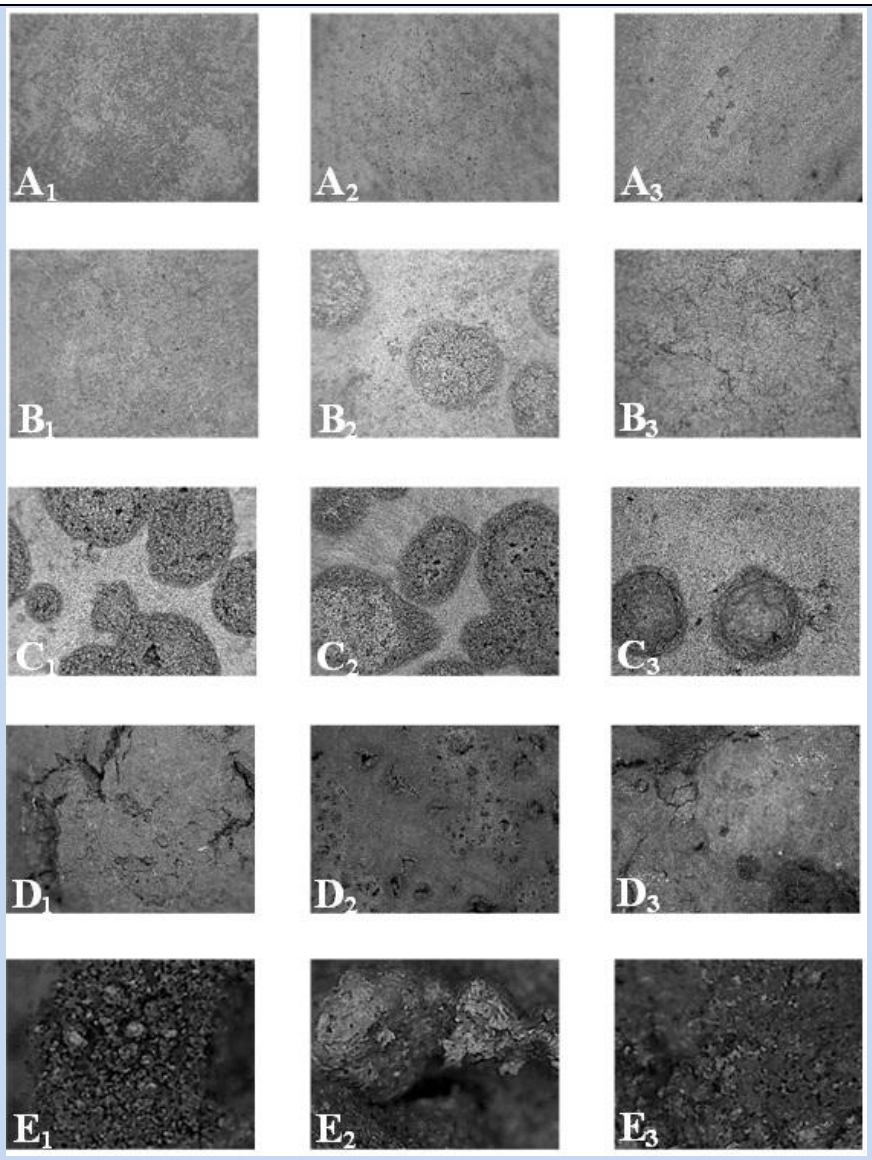


Fig. 3: Amalgam discs at 50X in which the appearance of silver nodules was notice between 200° and 400°C, the disappearance of the same at 800°C, and the conformation of silver spheres (E1 and E3) and bands (E2) at 1000°C.

DISCUSSION

Before discussing the behavior of the three commercial brands of dental amalgam

used in this study it should be noted that they are "latest generation" dental materials with different sizes of mixed particles and contain a high copper content to eliminate the "gamma 2 phase". The components of Contour® Kerr®–USA are 41% silver, 31% tin, 28% copper and 41% mercury; those for Admix® SDI®–Australia are 40% silver, 31% tin, 29% copper and 47,4% mercury; and for Nu Alloy® Newsthetic®–Colombia are 45% silver, 31% tin, 24% copper and 50% mercury. It is important to notice the proportions of silver, copper and tin that are used in the overall composition the alloy[17].

There are many publications in the forensic literature in respect of the behavior of dental materials when subject to the action of high temperatures. Merlati et al[18], subjected teeth restored with amalgam to high temperatures. The study showed that bubbles appeared in the surface of the amalgam at 200°C and mercury evaporated at 600^0C. The same authors in other study, subjected 75 teeth (25 class I amalgam restored teeth and 25 class V amalgam restored teeth) to high temperatures, observing that in each temperature range there were repeated changes in the samples and at 1000°C the amalgam disintegrated[22].

Moreno et al[19], performed a study where 200 teeth were subjected to high temperatures. 50 teeth were filled with dental amalgam (GS-80 SDI®). The purpose of the study was to describe the subsequent behavior of the amalgam fillings whilst being subjected to the action of high temperatures and to establish parameters that could be used in cases of forensic dental identification where burned, charred or incinerated human remains were a feature of the case. The authors described that at 200°C and at 400°C the amalgam suffered loss of brightness and nodule formation on the surface. At 600°C the amalgam developed an opaque black color together with the loss of morphological features. At 800°C the texture became roughened and some specimens were fragmented. Finally between 1000°C and 1200°C the amalgam cracked into fragments.

Patidar et al[23], subjected teeth filled with different materials, including dental amalgam, to the action of high temperatures. His study found that after 200°C the appearance of surface of the amalgam fillings presented as a rough and fissured. These features became more evident with increasing temperature: at 800°C the appearance of globular structures in the restorations surface was noted.

Aramburo et al[24], performed an in vitro pseudo-experimental study in order to observe the micro structural physical changes in dental tissues (enamel, dentin and cementum) and in dental materials commonly used in endodontics in 124 human teeth. Forty two teeth were restored with dental amalgam (GS-80® SDI®), and then subjected to high temperatures. The authors found that at 200°C the amalgams turned opaque and rough by forming surface nodules. At 400°C the amalgam suffered loss of brightness and fissures were also observed. At 600°C the amalgam turned black. At 800°C the black colour was still present together with rounded nodules and internal fissures. At 1000°C the amalgam fragmented and pulverized.

Moreno and Mejia[25] carried out a pilot study, using scanning electron microscopy, to standardize the technique used to observe teeth subjected to high temperatures. In the case of the dental amalgam at 200°C the superficial texture and structure changes are related to the melting points of the metals that form the

alloy. At 400°C the amalgam presented with roughness in the occlusal surface associated with the development of nodules that arise when the mercury evaporates through gas bubbles. When the temperature lowers by action of the environmental pressure, the other elements of the alloy are grouped and driven by the mercury to form these nodules.

All of these in vitro studies described the changes that occurred in dental amalgam restorations by subjecting extracted restored teeth to the action of high temperatures. All of the studies found the formation of rounded or globular structures in the surface of the amalgam, a fact that was first described by Günther and Schmidt –and referenced by Rötzscher et al[27]– as "silver bullets" that develop at 800°C due to mercury evaporation.

The formation of "rounded or globular structures" and "silver bullets" is now well recognized in the literature. This in vitro study was designed to further investigate and to progress the process of the formation of these "rounded or globular structures" and "silver bullets" in dental amalgam.

Against this background the study was based on the use of pre-prepared discs of amalgam rather than the use of extracted teeth that had been previously been restored with amalgam.

Dental amalgam subjected to high temperatures presents with superficial texture and structural changes related to melting points of the metals that form the alloy. Between 200°C and 400°C the amalgam presents with roughness apparent on the occlusal surface related to the development of nodules that arise when the mercury evaporates through gas bubbles. These gas bubbles drive with them silver particles to the amalgam surface and because of decreases in pressure and temperature the gas bubbles and silver particles coalesce to form the so called "silver bullets". Temperature affects the gamma 1 phase so that the mercury as it passes from solid to liquid state sweeps silver particles to the surface and evaporates and leaves the silver particles to form a superficial nodule. At temperatures between 800°C and 1.000°C, the silver particles reach melting point and evaporate, resulting in the disappearance of the nodules. As the temperature decreases little spheres or (betas) are formed composed of either copper or tin. They are fragmented and pulverized as a result of the oxidation of the copper (from 600°C) and due to the early fusion of tin (since 200°C)[28-30]. These changes result in dimensional instability, fractures and further disintegration (fragmentation and pulverization) of the dental amalgam[21].

It is recognized that this study has certain limitations associated with the choice of using pre-prepared "in vitro" discs of amalgam rather than the choice of using "in vivo" extracted teeth that had been previously been restored with dental amalgam. The behavior of amalgam restorations in extracted teeth subjected to high temperatures has been reported in previous studies. However in this study it was decided to use pre-prepared discs of amalgam to standardize the macroscopic changes that occur when amalgam is free from the dimensional changes associated with the dental tissues.

Table 1. Sample distribution						
Amalgam brands	**Temperature**					
	Control group	**Intervention group**				
	28ºC	**200ºC**	**400ºC**	**600ºC**	**800ºC**	**1000ºC**
Contour® Kerr®	3	3	3	3	3	3
Admix® SDI®	3	3	3	3	3	3
Nu Alloy® Newstethic®	3	3	3	3	3	3

CONCLUSIONS

Dental amalgam when subjected to high temperatures suffers changes in color, texture, the development of fissures and cracks, fractures, dimensional stability and explosion. The most distinctive change is the development of silver nodules in the surface of the amalgam from 200°C to 400°C and the subsequent disappearance of these silver nodules to form spheres and bands at temperatures of up to 1000°C.

The development of silver nodules, and their subsequent disappearance, is a regular feature of the "latest generation" of current commercially available brands of dental amalgam (rich in copper) when they are subjected to high temperatures. This paper provides further evidence of how changes in the appearance of the surface of dental amalgam restorations subject to high temperatures may facilitate determination of the maximum temperature to which the burned, charred or incinerated human remains were subjected.

Further studies are recommended including a wider range of commercially available brands of dental amalgam. These additional studies should incorporate data obtained from scanning electron microscopy, mass spectrometry and atomic force microscopy to identify the presence (qualitative analysis) and the quantity (quantitative analysis) of the components of the dental amalgam. The use of extracted teeth containing dental amalgam of a known brand would be beneficial.

ACKNOWLEDGMENTS

This research was financed by the internship grant of the Vice-President of Research at Universidad del Valle.

Table 2. Frequency of the changes of the discs of dental amalgam subjected to high temperatures			
Characteristics	***Contour® Kerr®***	***Admix® SDI®***	***Nu Alloy® Newstethic®***
200ºC			
Color	Bright gray color remains (100%)	Bright gray color remains (100%)	Bright gray color remains (100%)
Texture	The surface appearance is porous (100%)	The surface appearance is porous (100%) Superficial nodules appear (100%)	The surface appearance is rough and porous (100%)
Fissures and cracks	Are not presented (None)	Are not presented	Are not presented
Fracture	It's not presented (None)	It's not presented	It's not presented
Dimensional stability	It's not observed	It's not observed	It's not observed
Fragmentation	It's not observed	It's not observed	It's not observed
400ºC			
Color	Gray color remains but not as bright (100%)	Gray color remains but not as bright (100%)	Gray color remains but not as bright (100%)
Texture	The surface appearance is porous (100%) Superficial nodules appear (100%)	The surface appearance is compact (100%) Superficial nodules appear (100%)	The surface appearance is porous (100%) Superficial nodules appear (100%)
Fissures and cracks	Pores and cracks are observed on the nodules (100%)	Pores and cracks are observed on the nodules (100%)	Pores and cracks are observed on the nodules (100%)
Fracture	It's not presented	It's not presented	It's not presented

Dimensional stability	It's not observed	It's not observed	It's not observed
Fragmentation	It's not observed	It's not observed	It's not observed
600ºC			
Color	Changes to a dark opaque gray (100%)	Changes to a dark opaque gray (100%)	Changes to a dark opaque gray (100%)
Texture	The surface appearance is compact (100%) and the nodules disappear (100%)	The surface appearance is compact (100%) and there's traces of nodules (100%)	The surface appearance is compact (100%) and the nodules disappear (100%)
Fissures and cracks	There are fissures and cracks where the nodules were (100%)	There are fissures and cracks where the nodules were (100%)	There are fissures and cracks where the nodules were (100%)
Fracture	Deep fracture lines are observed (66.6%)	It's not presented	Deep fracture lines are observed (33.3%)
Dimensional stability	Thermic expansion is observed –shape alteration– (100%)	Thermic expansion is observed –shape alteration– (66.6%)	Thermic expansion is observed –shape alteration– (33.3%)
Fragmentation	It's not observed	It's not observed	It's not observed
800ºC and 1.000ºC			
Color	Changes to black opaque with bright gray spheres (100%)	Changes to black opaque with bright gray spheres (100%)	Changes to black opaque with bright gray spheres (100%)
Texture	A pulverized phase and a compact phase shaped like little spheres are observed	A pulverized phase and a compact phase shaped like little spheres and bands are observed	A pulverized phase and a compact phase shaped like little spheres are observed
Fissures and cracks	Fragmentation and pulverization are observed (100%)	Fragmentation and pulverization are observed (100%)	Fragmentation and pulverization are observed (100%)
Fracture	Fragmentation is observed (100%)	Fragmentation is observed (100%)	Fragmentation is observed (100%)
Dimensional stability	Fragmentation and pulverization by thermic expansion are observed– shape alteration– (100%)	Fragmentation and pulverization by thermic expansion are observed –shape alteration– (100%)	Fragmentation and pulverization by thermic expansion are observed– shape alteration– (100%)
Fragmentation	Fragmentation and destruction of the disc is observed (100%)	Fragmentation and destruction of the disc is observed (100%)	Fragmentation and destruction of the disc is observed (100%)

REFERENCES

1. Rothwell BR. Principles of dental identification. Dent Clin North Am 2001; 45:253-70.
2. Norrlander AL. Burned and incinerated remains, In Bowers CM, Bell GL (ed.). Manual of Forensic Odontology, 3th edition. Colorado Springs. American Society of Forensic Odontology; 1997.
3. Delattre VF. Burned beyond recognition: Systematic approach to the dental identification of charred human remains. J Forensic Sci 2000; 45(3):589-596.
4. Sweet D. Why a dentist for the identification? Dent Clin North Am 2001; 45:237-251.
5. Pretty IA, Sweet D. A look at forensic dentistry. Part 1: The role of teeth in the determination of human identity. Br Dental J 2001; 190:359-66.
6. American Board of Forensic Odontology ABFO. Body identification guidelines. J Am Dent Assoc 1994; 125(9):1244-1254.
7. Marín L, Moreno F. Forensic dentistry: dental identification of burned corpses. Report of two cases. Revista Estomatología 2004: 12(2):57-70.
8. Chain MC, Baratieri LN. Aesthetic restorations with composite resins in posterior teeth. Editora Artes Médicas: San Pablo; 2001.
9. Guzmán H. Dental biomaterials in clinical use. 3th edition, Bogotá. Ediciones ECOE; 2003.
10. Nocchi C. Restorative dentistry: Health and Aesthetic, 2nd edition. Buenos Aires. Editorial Médica Panamericana; 2008.
11. Cook A. Amalgam: Dead or alive? Dent Update. 2006; 33:94-8.
12. Yengopal V, Harneker SY, Patel N, Siegfried N. Dental fillings for the treatment of caries in the primary dentition. Cochrane Database Syst Rev 2009; (2):CD004483.
13. Maserejian NN, Hauser R, Tavares M, Trachtenberg FL, Shrader P, McKinlay S. Dental composites and amalgam and physical development in children. J Dent Res 2012; 91(11):1019-25.
14. Phillips RW. Skinner's Science of Dental Materials, 9th Edition. Philadelphia WB. Saunders Company; 1992.

15. Craig RG, Powers JM, Wataha JC. Dental Materials: Properties and Manipulation, 7th Edition, Mosby St Louis; 2000.
16. Stratis S, Bryant RW. The influence of modified cavity design and finishing techniques on the clinical performance of amalgam restorations: a 2-year clinical study. J Oral Rehabil 1998; 25(4):269-78.
17. Gómez CA, Arismendi JA. Preclinical and clinical evaluation of a commercial dental amalgam. Rev Fac Odontol Univ Antioq 2010; 22(1):63-71.
18. Merlati G, Danesino P, Savio C, Fassina G, Osculati A, Menghini P. Observations of dental prostheses and restorations subjected to high temperatures: experimental studies to aid identification processes. J Forensic Odontostomatol 2002; 20(2):17-24.
19. Moreno S, León M, Marín L, Moreno F. In vitro behavior of the dental tissues and some dental materials subjected to high temperatures with forensic purposes. Colomb Med 2008; 39 Supl 1:28-46.
20. Symes SA, Dirkmaat DC, Ousley S, Chapman E, Cabo L. Recovery and interpretation of burned human remains. US Department of Justice Office of Justice Programs United States of America: Rockville; 2012 [Update 2012 March; cited 2014 April 29]. https://www.ncjrs.gov/pdffiles1/nij/grants/237966.pdf
21. Moreno S, Merlati G, Marín L, Savio C, Moreno F. Effects of high temperatures on different dental restorative systems: Experimental study to aid identification processes. J Forensic Dent Sci 2009; 1(1):17-23.
22. Merlati G, Savio C, Danesino P, Fassina G, Menghini P. Further Study of restored and unrestored teeth subjected to high temperatures. J Forensic Odontostomatology 2004; 22(2):17-24.
23. Patidar KA1, Parwani R, Wanjari S. Effects of high temperature on different restorations in forensic identification: Dental samples and mandible. J Forensic Dent Sci 2010;2(1):37-43.
24. Aramburo J, Zapata A, Zúñiga S, Moreno F. Stereomicroscopic analysis of dental materials employed in endodontic exposed to high temperatures. Rev Estomat 2011; 19(2):8-15.
25. Moreno F, Mejía C. Scanning electron microscopy analysis of two endodontically treated teeth subjected to high temperatures. A pilot study. Rev Fac Odontol Univ Antioq 2011; 23(19):22-36.
26. Vásquez L, Rodríguez P, Moreno F. In vitro behavior of dental tissues and some dental materials of endodontics use, submitted to high temperatures with forensic applications. Rev Odont Mex 2012; 16(3):171-181.
27. Rötzscher K, Grundmann C, Benthaus S. The effects of high temperatures on human teeth and dentures. Int Poster J Dent Oral Med 2004; 6: Poster 213.
28. Odanov Z, Djurdjev M. Investigation of the mechanism of mercury removal from a silver dental amalgam alloy. J Serb Chem Soc 2004; 69(12):1111-20.
29. Mrowec S, Stokosa A. Oxidation of copper at high temperatures. Oxidation of Metals 1971; 3:291-311.
30. Kerl B, Forbeck F. Mercurio. En: Stohmann F, Editor. Great Encyclopedia of Industrial Chemistry Tomo X. Barcelona F. Soix; 1956.

*****

JOURNAL of FORENSIC ODONTO-STOMATOLOGY
VOLUME 33 Number 1 July 2015

SECTION IDENTIFICATION

Histological assessment of cellular changes in postmortem gingival specimens for estimation of time since death

Achla Bharti Yadav[1], Punnya V Angadi[2], Alka D Kale[2], Sumit Kumar Yadav[3]

[1]*Department of Oral Pathology, Post Graduate Institute of Dental Sciences, Pt. B. D Sharma University of Health Sciences, Rohtak, Haryana, India*

[2]*Department of Oral Pathology and Microbiology, KLE VK Institute of Dental Sciences and Hospital, Belgaum, Karnataka, India*

[3]*Department of Orthodontics & Dentofacial Orthopedics, Mithila Minority Dental College & Hospital, Darbhanga, Bihar, India*

Corresponding author: drachlabharti@gmail.com

The authors declare that they have no conflict of interest.

ABSTRACT

Estimating the time after death is an important aspect of the role of a forensic expert. After death, the body undergoes substantial changes in its chemical and physical composition which can prove useful in providing an indication of the post-mortem interval. The most accurate estimate of the time of death is best achieved early in the post-mortem interval before the many environmental variables are able to affect the result. Whilst dependence on macroscopic observations was the foundation of the past practice, the application of histological techniques is proving to be an increasingly valuable tool in forensic research. The present study was conducted to evaluate the histologic post-mortem changes that take place in human gingival tissues and to correlate these changes with the time interval after death. Thirty one samples of post-mortem human gingival tissues were obtained from a pool of decedents at varied post-mortem intervals (0-8hrs, 8-16hrs, 16-24 hrs). Ante-mortem samples of gingival tissues for comparison were obtained from patients undergoing crown lengthening procedure. Histological changes in the epithelium (cytoplasmic and nuclear) and connective tissue were assessed. The initial epithelial changes observed were homogenization and eosinophilia while cytoplasmic vacuolation and other alterations, including shredding of the epithelium, ballooning, loss of nuclei and suprabasilar split were noticed in late post-mortem interval (16-24 hrs). Nuclear changes such as vacuolation, karyorrhexis, pyknosis and karyolysis became increasingly apparent with lengthening post–mortem intervals. Homogenizations of collagen and fibroblast vacuolation were also observed. To conclude; the initiation of decomposition at cellular level appeared within 24 hours of death and other features of decomposition were observed subsequently. Against this background, histological changes in the gingival tissues may be useful in estimating the time of death in the early post-mortem period.

KEYWORDS: Post-mortem Interval, Histologic Changes, Gingiva, Eosinophilia, Homogenization, Vacuolation.

JFOS. July 2015, Vol.33, No.1 Pag 19-26
ISSN :2219-6749

INTRODUCTION

The period of time before death is known as the ante-mortem period whilst that after death is called the post-mortem period. After death, the body undergoes dramatic changes in its chemical and physical composition, which are termed as post-mortem changes. These changes can provide an indication of the post-mortem interval (PMI).[1] No topic in forensic medicine has been investigated as thoroughly as that of determining the time of death on the basis of post mortem findings.[2] There have been many different methods used at autopsy in an attempt to accurately and systematically determine the post-mortem interval and exact time of death. They include examination of the external physical characteristics of the body[3] such as autolysis, algor mortis, rigor mortis, livor mortis, post-mortem clotting, putrefaction and the appearance of adipocere[4], chemical changes detected in body fluids, analysis of stomach contents, determination of internal temperatures, and scene markers.[3] Some of these methods are relatively accurate under specific and controlled circumstances. However, the unpredictability of various environmental factors has prevented the development of a single reliable predictor. The most accurate estimates of the time of death are best achieved early in the post-mortem period before the many environmental variables can have any significant effect on the result.[5]

Whilst dependence on macroscopic observations together with a sense of ingenuity and "lateral thinking" were the former keystones of past forensic practice the application of both histological and molecular diagnostic techniques is becoming increasingly important as an essential part of the armamentarium of tools used in modern forensic pathology.[6] Using light microscopy cells are recognized as being dead only after they have undergone a sequence of morphological changes.[7] These changes occur as a result of two cellular processes - *apoptosis*, a programmed, orderly form of cell death, and *necrosis*, an disorderly and unpredictable form of cell death.[4] The necrotic cells show increased eosinophilia, and may have a more glassy homogenous appearance than normal cells.[7] Nuclear changes may appear in the form of one of three patterns, the basophilia of the chromatin may fade (karyolysis), nuclear shrinkage (pyknosis) may occur or the pyknotic or partially pyknotic nucleus may undergo fragmentation (karyorrhexis).[7] Studies are available relevant to the gross and histologic changes that occur in the skin after death[5], However, there have been no systemic studies published in the English literature that relate to the histological changes that occur in human gingival tissues after death.. The aim of the present study was to evaluate post-mortem epithelial (cytoplasmic & nuclear) and connective tissue degenerative changes observed in the gingival tissues obtained from 31 non-refrigerated bodies (experimental group). It was postulated that there may be a specific pattern of histological changes observed that may prove useful to determine the time interval after death. Standardization was performed using 10 samples of gingival tissues obtained from clinically healthy individuals following crown lengthening procedure (control group).

MATERIALS AND METHODS

The investigation was conducted on samples of labial gingival tissues obtained at autopsy by the Department of Forensic Medicine in accordance with the declaration of Helsinki. Ethical clearance was in accordance with the institution (IRB approval #559). General information, including the age, sex, date of death, cause of death, and the time elapsed after death and arrival at the mortuary was determined. The bodies were not subject to

refrigeration during this period between death and arrival at the mortuary. On arrival at the mortuary the cadavers were placed in a dry shaded area in temperatures that varied from cool to temperate (38^0F-77^0F). Biopsies from the labial gingival tissues were taken for analysis.

The study comprised of 2 groups:

I. Experimental group: this consisted of 31 samples from the labial gingival tissues obtained from un-refrigerated decedents. The group was further subdivided into 3 sub-groups;
   Sub- group A: Tissues obtained with <8 hrs of death (n=10),
   Sub- group B: Tissues obtained within 8-16 hrs of death (n=10)
   Sub- group C: Tissues obtained within 16-24 hrs of death (n=11).

   For sampling purposes it was deemed appropriate to divide this experimental group into three sub-divisions based on an eight hour difference. This decision was based on the fact that the process of cellular decomposition commences 10 hours post-mortem and other decomposition changes occur subsequently.[4]

II. Control group: this consisted of 10 gingival samples from clinically healthy individuals following crown - lengthening procedure. Firmly attached gingival tissue with no loss of clinical attachment and devoid of clinical signs of inflammation were included in the sample.

The specimens were fixed immediately in 10% formalin, processed, sectioned and stained with haematoxylin and eosin. The specimens were examined under a light microscope by 3 independent observers who were unaware of the time of death. The changes in the epithelium (cytoplasmic & nuclear) and connective tissue were assessed. Sequential analysis of the following histological changes of the biopsies of all individuals was carried out.

A. Epithelial changes

- Cytoplasmic features: eosinophila, homogenization/ presence of distinct cellular outline and vacuolation.
- Nuclear features: presence/absence of distinct nuclear outline, karyolysis, pyknosis, karyorrhexis and vacuolation.
- Presence of intercellular bridging/ junctions.

B. Separation of epithelium and connective tissue

C. Connective tissue changes

- distribution of collagen fibres,
- fibroblasts vacuolation,
- type and distribution of inflammatory component

The degree of histological (epithelial and connective tissue) changes in the post-mortem samples was correlated to the time interval since death. Comparison with ante-mortem samples was undertaken.

RESULTS

Epithelial changes

The initial changes observed in the epithelium of the early post-mortem period (0-8hrs) were homogenization and eosinophilia, predominantly seen in the superficial and spinous epithelial cell layers. This spread progressively to include the entire thickness of the epithelium. The changes became more apparent with increasing length of post-mortem interval (8-16 and 16-24 hrs). Cytoplasmic vacuolation in the superficial and the spinous cell layers of the epithelium was evident after a post-mortem period of 8 hrs and was limited to the spinous layer solely in sub group C. Nuclear changes such as vacuolation, karyorrhexis, pyknosis and karyolysis were present early after death (sub group A) in the superficial layers and were seen extending throughout the epithelium in the late post-mortem intervals (sub group B and C). Other

epithelial changes noticed were shredding of epithelium in the superficial layers, ballooning, loss of nuclei and suprabasilar split. All of these changes became progressively more apparent toward the end of the late post-mortem interval (sub group C). An exceptional biphasic pattern of staining was also evident in samples taken from sub group C. None of the features described above were present in the control group (group II). Both the groups including sub groups A, B and C did not demonstrate any split at the junction of epithelium and connective tissue interface (Table 1, Figure 1-3).

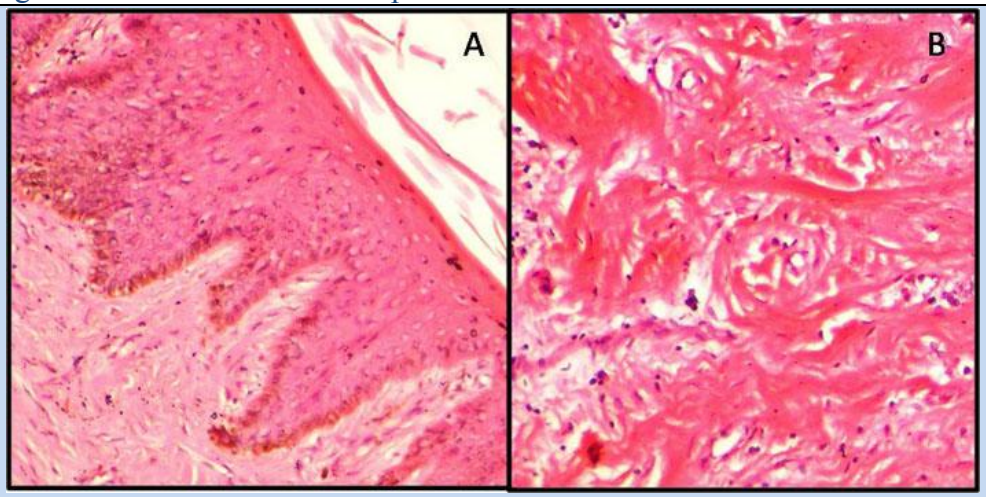


Fig.1: Early postmortem changes in the gingiva (0-8hrs). (A) Eosinophilia, homogenization and nuclear changes in superficial layers of epithelium (H&E,X100). (B) Homogenization of collagen bundles in the connective tissue stroma.

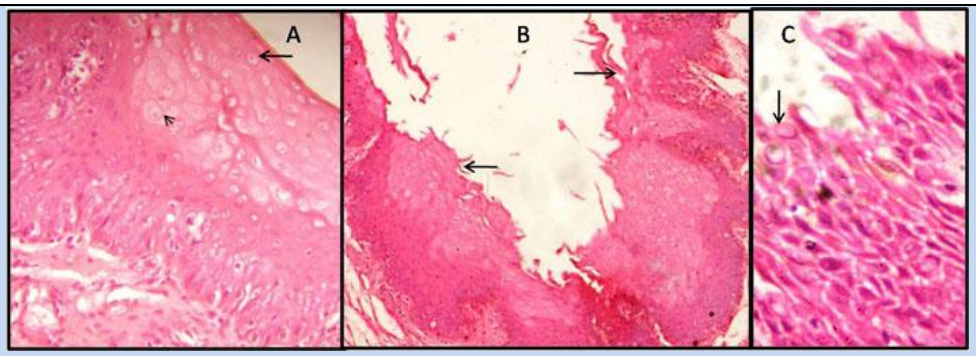


Fig. 2: Late postmortem changes in the gingiva (8-16hrs, 16-24hrs). (A) Cytoplasimic vacuolation (arrow) and ballooning of cells (arrow head) evident in the superficial and spinous layer of epithelium in 8-16 hrs of PMI (H&E,X400). (B) Shredding (arrow) and other epithelial changes are observed in the whole epithelium in subgroup C (H&E,X100). (C) Disruptive epithelium showing nuclear degenerative changes (arrow) in subgroup C (H&E,X400).

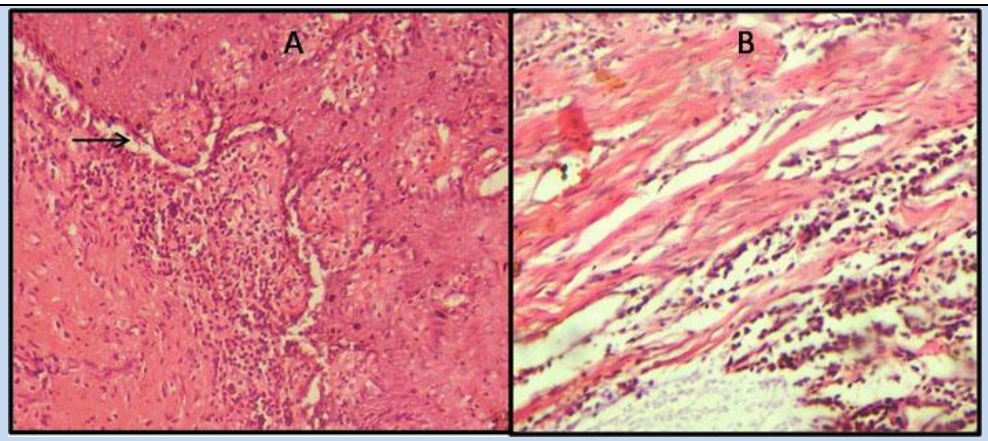


Fig. 3: (A) Suprabasilar split (arrow) observed in late postmortem period (16-24hrs) (H&E,X100). (B) Connective tissue stroma shows homogenization of collagen bundles with fibroblast vacuolation and moderate inflammatory infiltrate (16-24hrs) (H&E,X100).

Connective tissue changes

An intact parallel arrangement of collagen fibres were seen in the control group while homogenization of collagen bundles was evident in the experimental group with formation of clumps in the later period of PMI (sub group B and C). Fibroblast vacuolation commenced early after death and was most prominent in sub group C.

The inflammatory component was diffusely spread in the early samples taken in the PMI (0-8 hrs). In later samples taken from subgroups B and C (8-16hrs and 16-24hrs) the inflammatory component was focal to diffuse and composed predominantly of lymphocytes. Sub groups B and C also showed evidence of plasma cells (Table 1, Figure 1,3).

Table 1 - Histologic postmortem changes in the gingival tissue at different PMI

Histologic postmortem features	PMI <8 hrs (n=10)	PMI 8-16 hrs (n=10)	PMI 16-24 hrs (n=11)	Control (n=10)
Homogenization	In superficial & spinous layer (100%)	Throughout the epithelium (100%)	Throughout the epithelium (100%)	Absent (100%)
Eosinophilia	In superficial & spinous layer (100%)	Throughout the epithelium (100%)	Throughout the epithelium (100%)	Absent (100%)
Cytoplasmic vacuolation	Absent (100%)	In superficial & spinous layer (70%)	In superficial & spinous layer (82%)	Absent (100%)
Karyolysis	In superficial layer (40%)	In superficial & spinous layer (70%)	In superficial & spinous layer (82%)	Absent (100%)
Pyknosis	In superficial & spinous layer (90%)	Throughout the epithelium (100%)	Throughout the epithelium (100%)	Absent (100%)
Karyorrhexis	In superficial layer (90%)	In superficial & spinous layer (100%)	Throughout the epithelium (100%)	Absent (100%)

Nuclear vacuolation	In superficial & spinous layer (90%)	Throughout the epithelium (100%)	Throughout the epithelium (100%)	Absent (100%)
Epithelium (shredding)	In few (30%)	Present (90%)	Present (100%)	Absent (100%)
Epithelium (ballooning)	In one sample (10%)	Present (50%)	Present (70%)	Absent (100%)
Epithelium (disruption)	Absent (100%)	Absent (100%)	Present (60%)	Absent (100%)
Suprabasilar sepration	Absent (100%)	In few (20%)	Present (70%)	Absent (100%)
Sepration b/w Epi & CT	Absent (100%)	Absent (100%)	Absent (100%)	Absent (100%)
Distribution of collagen	Homogenized (70%)	Homogenized (clumps in few) (90%)	Homogenized (clumps in few) (82%)	Intact, Parallel arrangement (100%)
Distribution of inflammation	Diffuse (100%)	Focal & diffuse (100%)	Focal & diffuse (100%)	Absent (100%)
Type of inflammation	Lymphocytes (100%)	Lymphocytes & plasma cells (100%)	Lymphocytes & plasma cells (100%)	Absent (100%)
Fibroblasts vacuolation	Present in few (50%)	Present (80%)	Present in all (100%)	Absent (100%)
Biphasic staining pattern	Absent (100%)	Absent (100%)	Present (100%)	Absent (100%)
PM: post-mortem interval				

DISCUSSION

Whilst traditionally established techniques are commonly used to estimate the time of death during routine medico legal autopsies there is a trend toward the development and introduction of newer techniques. It is established that the reliance on a single technique can produce erroneous outcomes.[8] Following death many physico-chemical changes begin to take place in the body in an orderly manner and continue until the body eventually decomposes. Similarly, ongoing cellular changes also occur after death that depends on the time interval post-mortem and the circumstances of the death. At cellular level, in the first instance, respiration ceases and glycolysis proceeds. This results in the production of lactic acid and a corresponding decline in the pH of the cellular contents. Ultimately all activities of cell metabolism terminate and the subsequent predominance of lytic enzymes results in autolysis. Post-mortem changes are associated with tissue degradation and the release of proteolytic lysosomal enzymes from the cells.[9,10] Like other body tissue cells, oral mucosa loses its normal morphology as a result of post-mortem autolysis and putrefaction. These cellular changes can be a useful criterion and marker for estimating the post-mortem interval. Currently there are no publications in the literature that correlate the degree of histological changes observed in post-mortem human gingival tissues with the time since death. The present study was aimed to assess the early post-mortem changes in the human

gingival tissues and emphasizes the need for further research.

The aim of this study was to observe and assess the various sequential changes seen in the microscopic appearance of human gingival tissues (epithelium and connective tissue) at different PMI. The initial changes observed in the early post-mortem period (0-8hrs) in the epithelium included homogenization and eosinphilia predominantly in the superficial and spinous layers. This spread progressively to include the entire thickness of the epithelium. The changes became more apparent with increasing length of post-mortem interval (8-16 and 16-24 hrs).

Homogenization could be attributed to a decline in the level of cellular glycogen content. Eosinophilia could be attributed to both depletion of normal basophilia transmitted by RNA in cytoplasm and also in part due to augmentation of eosin binding to denatured intra-cytoplasmic proteins. Cell death results in a decline of intra-cellular pH which activates the release of DNAase. This, in turn, results in fading of the chromatin (karyolysis)[6,7]. Karolysis was evident in the superficial layers of all of the experimental samples. Pyknosis (result of nuclear shrinkage) and karyorrhexis (fragmentation of the nucleus)[7,11] was predominantly evident in the superficial layers in the early post-mortem interval samples. In the other later post-mortem samples all of these changes were apparent throughout the entire thickness of the epithelium.

With increasing post-mortem intervals nuclei may undergo complete dissolution. This phenomenon was observed in later post-mortem samples from sub-groups B and C (16-24 hrs). Furthermore, vacoluation of the cells due to digestion of the organelles has also been reported after death.[7,10-12]. In the present study, the nuclear vacoulation was observed as early as 0-8 hrs after death and at this post-mortem interval vacuolation was limited to the superficial layers of epithelium. With increasing post-mortem intervals (8-16, 16-24hrs) vacuolation progressively increased to include the entire thickness of the epithelium.

Paradoxically, cytoplasmic vacoulation was observed only in the late post-mortem samples (8-16, 16-24 hrs) and was utterly absent in earlier samples (0-8 hrs). This observation could suggest that nuclear degenerative changes usually precede cytoplasmic degenerative changes after death. Ballooning of the cells that becomes apparent just after death was also seen in the later post-mortem intervals (8-16,16-24 hrs). This may represent a loss of potential of the cell to actively remove the influx of ions from the plasma membrame.[7] Additionally, loss of architecture of the epithelium in the late post-mortem interval as a result of shredding of the superficial layers and disruption may be caused by severance of the cells and denaturation of the proteins due to the lytic activity.[4]

All of the gingival samples exhibited an intact epithelial - connective tissue junction without any severance/ breach. This could signify that the separation of the epithelium and the lamina propria could be a change that could occur in later post-mortem intervals i.e. after 24 hrs. It is significant that Kovarik et al observed that the initial cleavage in the dermo-epidermal junction of skin occurred after 24 hrs post-mortem.[5]

In relation to the connective tissue component of the control group a parallel arrangement of collagen fibres was recognised as normal. However clumping of collagen fibres was observed within a few samples of sub-group B and within almost all of the samples within sub-group C.

As a degenerative change, fibroblasts revealed nuclear vacoulation. This nuclear

vacuolation increased proportionately with the passage of time post-mortem. It was more pronounced in samples taken 16–24after death than in the samples obtained less than 8hrs after death.

These changes could be due to hypoxia resulting from the severance from the vascular network and the start of inflammation.[10-12]. Inflammatory cells, predominantly lymphocytes and plasma cells, (in subgroup B and C) were seen distributed focally and in diffuse patterns prominently in the sub-epithelial connective tissue.

In comparing the histological findings of the experimental and control groups none of the changes seen in gingival tissues of the samples obtained post-mortem were observed in the ante-mortem samples.

CONCLUSION

There is a paucity of information in the literature in relation to the decomposition of oral tissues. As a consequence this can place restraints on the role of forensic pathologists to estimate the time of death. Although there are many methods available to estimate the time of death, none is reliable enough by itself as a "stand alone" method because of the inevitable influence of unpredictable external factors. In this context the histological changes that occur post-mortem in human gingival tissues would appear to be useful method to estimate the time of death in the early PMI (0-24 hrs). However, more research is needed to verify, refine and expand these initial results. Despite some constraints, such as the limited number of subjects and the relatively short time span of the post-mortem period, the present study demonstrated the potential of this method as a tool for use in forensic practice. Further research using larger sample sizes and an extended time range could assist forensic practitioners in estimating a precise time of death.

.

REFERENCES

1. *Gunn A. The decay, discovery and recovery of human bodies. In: Essential forensic biology, 2nd ed. Chichester, UK: Willey, 2009, p 11-13.*
2. *Becker, Ronald F. Becker, Aric W. Dutelle. Time of death determination. In: Criminal investigation, 4th ed. Jones & Bartlett Publishers, 2013, p 239.*
3. *Di Maio VJ, Di Maio D. Time of death. In: Forensic Pathology, 2nd ed. Boca Raton, FL: CRC Press, 2001, p 21– 41.*
4. *Pradeep GL, Uma K, Sharada P, Prakash N. Histological assessment of cellular changes in gingival epithelium in ante-mortem and post-mortem specimens. J Forensic Dental Sci 2009; 1(2):61-5.*
5. *Kovarik C, Stewart D, Cockerell C. Gross and histologic postmortem changes of the skin. Am J Forensic Med Pathol 2005; 26 (4): 305-8.*
6. *Gilbert L, Siang LH. Forensic histopathology. In: Tsokos M, Forensic Pathology Reviews, 5th ed. New Jersey: Springer, 2008, p 239-65.*
7. *Kumar V, Cotran RS, Robbins SL. Textbook of basic pathology, 5th ed. Elsevier, India, 1992, p 22-5.*
8. *Sheikh NA. Estimation of postmortem interval according to time course of potassium ion activity in cadaveric synovial fluid. Indian J Forensic Med Toxicol. 2007;1. Accessed 1 January 2015. Available at http://www.indmedica.com*
9. *Parikh CK. Textbook of medical jurisprudence, forensic medicine and toxicology. 6th ed, New Delhi: CBS publishers and distributors, 2004. p. 3.1.*
10. *Henssge C, Madea B. Estimation of time since death. Forensic Sci Int. 2007;165(2–3):182–4.*
11. *Cobb JP, Hotchkiss RS, Karl IE, Buchman TG. Mechanism of cell injury and death. Brit J Anaesthesia, 1996;77:3-10.*
12. *Babapulle CJ, Jayasundera NP. Cellular changes and time since death. J Med Sci Law. 1993;33(3):213-22.*

*****

JOURNAL of FORENSIC ODONTO-STOMATOLOGY
VOLUME 33 Number 1 July 2015

SECTION JURISPRUDENCE/LITIGATION

Thoughts on donation of a tooth to science, in the course of dental care

Alix Le Breton[1,2,3], Catherine Chaussain[2,3,4], Christian Herve[1], Philippe Pirnay[1,2,3*]

[1] *Medical Ethics and Legal Medicine Laboratory EA 4569, Medical School, University Paris Descartes, Sorbonne Paris Cité - 75006, Paris, France*
[2] *Dental Surgery School, Paris Descartes University, Sorbonne Paris Cité, Montrouge, France*
[3] *AP-HP, Odontology Department, Bretonneau and Albert Chenevier hospitals, Paris, France*
[4] *EA 2496 Paris Descartes University, Sorbonne Paris Cité, Montrouge, France*
Corresponding author: *philippe.pirnay@aphp.fr*

The authors declare that they have no conflict of interest.

ABSTRACT
Introduction: Research on biological samples, including dental pulp stem cells (DPSC), has expanded considerably in recent years and is now seen as a way forward toward the possibilities of new therapies, such as craniofacial bone and tooth repair. The extraction of healthy teeth and their donation for scientific research is now well accepted by both patients and researchers alike. The present situation, as described above, presents a timely opportunity to reflect on the ethical and moral obligations of all of the stakeholders involved in this methodology.
Method: Twenty-two patients who received dental treatment between November 2013 and February 2014 in the dental department of Louis Mourier Hospital in Colombes, France, completed a questionnaire. The questionnaire was designed to gather data in respect of giving patients optimal information necessary to acquire informed consent for extraction of teeth to be used for odontological biomedical research.
Results: When patients agree to donate their teeth for purposes of scientific research it is vital that they are properly informed and enabled so that they are able to give consent freely
Conclusions: The risks to patients during dental extractions are minimal. However despite the growing need for a supply of extracted teeth for dental pulp stem cell research it is imperative that any ethical questions that may be raised by potential donors guarantee the security, integrity, and respect of the intentions and aspirations of the donor. To enable the acquisition of true informed consent, this article explores how the dissemination of information relating to biomedical research in the field of dental care must remain as a duty of care and professional ethics.

KEYWORDS**:** Bioethics; dental pulp stem cells; informed consent; patient rights; biological samples

JFOS. July 2015, Vol.33, No.1 Pag 27-37
ISSN :2219-6749

INTRODUCTION

Internationally, research on stem cells must meet regulatory guidelines for using human biological material. French law (Article L.1235-2 and L.1245-2 in the Public Health Code) allows surgical residues, collected during surgery, to be used for scientific purposes. This complies with the position shared by the international bioethical community.[1]

Bio-banks thus represent an important resource for determining the causes and mechanisms of many diseases.

The craniofacial area (bone and tooth) is particularly exposed to trauma, congenital malformations or acquired diseases; tissue loss often requires difficult reconstructions and causes aesthetic and functional disabilities that often deeply affect the patient's quality of life. The evidence of cells endowed with stem cell properties in adult dental pulp has prompted research on the development of alternative therapeutic approaches to craniofacial lesions. The properties of the mesenchymal cells derived from dental pulp (or dental pulp stem cells, DPSC) have been shown very similar, although not identical, to those of bone marrow mesenchymal stem cells.[2,3,4,5] Mesenchymal stem cells have now been isolated from many other tissues, potentially interesting for therapeutic use—in particular, the adipose tissue and the umbilical cord blood. However, as DPSC have a neural crest origin,[6] it is conceivable that they might be more efficient in repairing craniofacial lesions than mesenchymal stem cells from a purely mesenchymal origin. In addition, sub-populations of DPSCs also possess adipogenic, chondrogenic, neurogenic, myogenic, and endothelial differentiation capacities *in vitro,*[7] and the capacity of these cells to interact with endothelial cells has been actively studied.[8,9] Several research teams worldwide are presently studying possible new therapies for lesions through tissue engineering using DPSC. This necessitates the frequent collection of human pulp at dental clinics to isolate DPSC for the various, ongoing, experimental programmes. Healthy dental pulp can be easily collected from shedding deciduous teeth or following extraction of deciduous teeth, premolars, or third molars performed as part of an orthodontic treatment plan.

Against this background, the question of knowing how to collect teeth in an ethical manner is posed. On the one hand, the procedure itself is non-invasive way but on the other hand, the patient is in a vulnerable situation during extraction process. To date in most European countries, a tooth extracted in the context of a treatment plan corresponds to clinical waste, and, if used for research, it is considered a biological sample.[10] In this context, this study aims to define the requirements necessary in terms of information and consent to preserve the rights of the donor while ensuring their status as key players engaged in odontological biomedical research.

The research methodology used to compile this article consisted of inviting patients to complete a closed-ended questionnaire and subsequent examination of the outcomes. From this data the article progresses to explore the ethical considerations involved in obtaining the consent of the patient so that their extracted teeth can be used for odontological biomedical research. The feelings of the patient in making this decision are also considered by analysis of specific information provided in the questionnaire that was unrelated to patient protection issues.

MATERIALS AND METHODS

A study involving 22 patients monitored for all-around dental care at Louis Mourier Hospital (Colombes, France) was carried out between November 2013 and February 2014. Twelve questions, prepared in association with the medical ethics laboratory (Table 1), were asked by the

same pollster under specific conditions regarding

- The meaning, donation, and ownership of an extracted tooth
- Information, consent to research after-dental care
- Resulting feedback

This study was carried out to understand the feelings of the patient in respect of their sense of attachment to the tooth designated to be extracted, their understanding in terms of ownership of the tooth (before and after extraction), and their expectations in respect the information provided in order to make informed consent. The study also was also useful in determining the level of trust that existed between patients and their dental surgeon.

Included in the study were French-speaking adult patients, both male and female who were treated in the dental department at Louis Mourier Hospital, Colombes, France between November 2013 and February 2014, and had agreed to take part in a medical experiment. Juvenile, non-French-speaking patients and patients presenting for emergency treatment were not included.

Table 1 - Questionnaire given to patients

Questions	Answers
What does your tooth mean to you?	- Nothing - A part of me - Another answer
Do you consider that your tooth still belongs to you after its extraction?	- Yes - No
Do you look ahead to the possibility of experimentation on your extracted tooth?	- Yes - No
What would you like to do with your tooth after extraction?	- I prefer to keep it - I prefer to leave it - Donate it to science
Would you like to know if any research is conducted on your extracted tooth? If yes, when?	- Yes - No - Consultation before extraction - The day of surgery, just before - After surgery
Would you like the practitioner to ask for your consent to conduct researches on your tooth? If yes, when?	- Yes - No - Before surgery - After surgery
Is there any research that you would not want to be conducted on your tooth?	- Yes - No

Would you like to be informed about the research outcome? If yes, how?	- Yes - No - On the phone - Email - By letter
If you give your tooth to science do you consider that it still belongs to you or that it belongs to the researcher?	- It will always be mine - Il belongs to the researcher
who should inform you about the possibility of donating your tooth to science?	- The surgeon - Other member of the health-care team
Would you be afraid to have your tooth extracted for research instead of medical reasons?	- Yes - No
Do you think that in future (a few months or years) you could change your mind and ask for the end of medical research on your tooth?	- Yes - No

RESULTS

Twenty-two patients were included in this study. It had been determined beforehand that all 22 patients understood all of the questions. Patients were apt to visualize how a research team would use their extracted tooth. The answers of each patient were collated after every interview. From the answers to each question it was possible to quantify the patients into defined sub-groups

Eighty-two percent of the patients wished to be informed if their tooth was to be used for research and were willing to consent. Eighty-nine per cent of this group wished to be informed if their tooth was to be used for research and were willing to consent after the extraction had been carried out. All of the patients declared that they wished to be informed about the odontological biomedical research by the dental surgeon.

The results of the questionnaire are presented in Table 2.

DISCUSSION:

This study was designed to gain insight into the status of ownership of a tooth once it has been removed from the patient by a dental surgeon. It also explores with the meaning of tooth donation and the potential loss of belonging to the donor related to the information provided to make informed consent.

What is the status of the extracted tooth?

For more than a half of the patients in this study a tooth represents a part of them integral in the context of eating, smiling or speaking. Once items or products have been taken from the body, they become "special things".[11] According to Professor Poughon[12], parts detached from a human body keep traces of the personality of the soul that once inhabited the body. As such, the extracted teeth in this study become a detached part of the human body intended for research.

In legal terms in all of the countries studied, extracted teeth are similar to clinical waste in that they have no further use. However, if they are used for research, the status of extracted teeth changes as demonstrated in Table 3. Ethically the tooth remains a detached part of the human body and, as such, deserves respect and dignity because physical traces of the donor, for example, DNA remain present. As such repatriation to the donor is possible.

Table 2 - Results

Questions	Answers	Results
What does your tooth mean to you?	- Nothing - A part of me - Another answer	7 15 0
Do you consider that your tooth still belongs to you after its extraction?	- Yes - No	12 10
Do you look ahead to the possibility of us making experimentations on your extracted tooth?	- Yes - No	13 9
What would you like to do with your tooth after extraction?	- I prefer to keep it - I prefer to leave it - Donate it to science	1 0 21
Would you like to know if any research is conducted on your extracted tooth?	- Yes - No	18 4
If yes, when?	- Consultation before extraction - The day of surgery, just before - After surgery	0 2 16
Would you like the practitioner to ask for your consent to conduct research on your tooth?	- Yes - No	18 4
If yes, when?	- Before surgery - After surgery	2 16
Is there any research that you would not want to be realized on your tooth?	- Yes - No	0 22
Would you like to be informed about the research's outcomes?	- Yes - No	7 15
If yes, how?	- On the phone - Email - By letter	0 6 1
If you give your tooth to science do you consider that it still belongs to you or that it belongs to the researcher?	- It will always be mine - It belongs to the researcher	0 22
Who should inform you about the possibility of donating your tooth to science?	- The surgeon - Other member of the health-care team	22 0
Would you be afraid to have your tooth extracted for research instead of medical reasons?	- Yes - No	0 22
Do you think that in future (a few months or years) you could change your mind and ask for the end of medical research on your tooth?	- Yes - No	0 22

Who owns the extracted tooth?

Barely more than half of the patients considered that the extracted tooth still belonged to them.

In legal terms in some countries, for example, England, there is no law granting right of ownership in respect of the human body, its parts, and products.[13] In France, the civil code reaffirms that the human body, its parts and products cannot be subject to laws of property right. In America in the case of John Moore, the Supreme Court of California held that a patient could not claim right of ownership over human tissue designated for destruction. However, in terms of ethics, the French National Ethics Committee[14] confirmed that the human body, its elements, and its extracts cannot be subject to property rights. The medical or research team is, therefore, only the depository and guardian of biological samples. Against this background the extracted tooth belongs to the patient as long as he or she has not expressed a desire to release it from his or her possession. This ensures that the consent of the person, and source of the tooth, is respected at all times when the tooth is transferred or reused.

The reasons outlined above could probably explain why all the patients interviewed in this study considered that the tooth belonged to the researcher as soon as it was donated to science.

Table 3 - Examples of legal status of extracted teeth

Countries	Legal basis	Extracted tooth not used for research	Extracted tooth used for research
France	CSP Article L.1245-2	Clinical waste	Biological sample
England	Nuffield Council on Bioethics, "Human Tissue: Ethical and Legal Issues", April 1995	Clinical waste	Tissue removed in the course of medical treatment and used for research
Switzerland	Swiss Academy of the Medical Sciences. Biobanks, taking, conservation and use of biologic human sample.	Clinical waste	Surplus of human biological sample used for research
Germany	Nationaler Ethikrat, "Biobanks for research", Notice, 2004	Clinical waste	Human tissue removed for the purposes of treatment and subsequently used for research
Belgium	Law Article 20 of December 19th, 2008 concerns the obtainment and use of human physical samples intended for human medical applications or for scientific research. Bioethics Advisory Committee, Notice N°45 of January 19th, 2009 features the banking of human samples for research.	Clinical waste	Residual human tissue used for research
Council of Europe	Recommendation (Article 12, 2006) of the Committee of Ministers to EU member states about research involving human biological samples. .	Clinical waste	Residual human tissue used for research

Canada	Tri-council policy statement, "Ethical Conduct in Research involving Humans", 1998	Clinical waste	Tissue already removed in the course of medical treatment and used for research
Singapore	Bioethics Advisory Committee, "Human Tissue Research", 2012	Clinical waste	Human tissue removed for the purposes of treatment and subsequently used for research

Is this a donation?

Following extraction of the tooth, three possibilities were available to the patient for consideration in respect of the future of the extracted tooth: (1) reclamation of the tooth by the patient (2) collection of the tooth by an external party for research purposes (3) No preference by the patient and, in this eventuality, the tooth would be destroyed.

The first possibility is very specific to the tooth because of its symbolism from early childhood. The majority of the patients interviewed agreed to donate the extracted tooth to science, although nine of the twenty two patients had not considered this eventuality before the extraction.

When patients were asked if they wished to keep hold onto the extracted tooth in the absence of any information regarding the ultimate destination of the tooth (incineration or research) and where the patient declined, this was considered as an abandonment of ownership of the extracted tooth by the patient. However, it became apparent that these patients did not envisage that the tooth may have a fate other than incineration. **The refusal of these patients to keep hold onto their extracted teeth does not permit the presumption that they are opposed to research on the cellular component of their extracted teeth.**

In terms of ethics, when a patient was asked if they would consent, or at least if they were not opposed, to their teeth being re-used for purposes of research, then a positive answer to either of these two questions was considered to be a positive response for donation. Patients became aware of the future intentions in respect of the re-use of their teeth and, against this background, any decision was deemed to be truly voluntary.

Criteria for the donation for extracted teeth took on full meaning once patients were empowered with information provided by the dental surgeon that enabled them to make decisions based on freedom of choice. Additionally this survey demonstrated the high level of trust that exists between patient and dentist.

Which information should be given to the patient?

In legal terms in most countries, for example Canada, England, France, and Germany, the information given to the patient must be exhaustive (purpose of the research, duration of the study, risk vs. benefit, etc.). In ethical terms, the broadest possible information ensures the validity of consent.

Only four patients taking part in this study did not wish to be informed about the use of their teeth for purposes of odontological biomedical research. Following extraction these four patients felt that it did not concern them because they perceived that the extracted teeth no longer belonged to them.

However, the majority of the patients taking part in this study expected clear, honest and realistic information in respect

of the future use of their tooth. Most of them expressed the view that they would rather be informed after the tooth had been extracted because it was difficult for them to plan ahead of the extraction. Seven patients wished to be informed "out of curiosity to learn more about the scientific advances in which they would participate". Article 10 of the International Declaration on Human Genetic Data states that "the patient has the right to decide whether to be informed of research results in which he or she has participated.[15] This requirement necessitates the capture and retention of logistical patient details, including names and addresses of patients, and thus precludes patient anonymity. Article 10 of the International Declaration on Human Genetic Data also requires that this information to this effect be recorded in the medical file of the patient.

Who has to inform the patient?

In terms of legality key research personnel in some countries, such as Canada, are responsible for compliance with the consent process.[16] In France, Parliament (Article L.1122-1 of the Public Health Code) has stipulated that either the investigator of the research study or a doctor may transmit equivalent information. From a practical point of view it would be not be unreasonable to expect that the best person to transmit this information to the patient would be the person able to answer all of the potential questions likely to be raised. Against this background a member of the research team would seem to be the most appropriate person.

The Council of Europe does not require that information be provided to participants by a particular person.[17] Information such as this can be given either by the dental surgeon or by another health care team member. In most circumstances the dental surgeon is usually well familiarised with his/her patient and, in this role, is probably best placed to divulge the information. However it may be considered that the dental surgeon is in a sub-optimal position to provide the information in that he/she is not really familiar with ongoing research and, consequently, may not be the best person to properly inform the patient.

Against this background, it is not surprising that one hundred per cent of the patients interviewed in this study declared that they wanted to be informed about the research by their dental surgeon. However it is probable that their dental surgeon may not be privy to and familiar with ongoing specialist research. Consequently they may not be the best person to properly inform the patient.

By introducing a two part process, where the dentist ultimately responsible for the extraction of the tooth is not the same person charged with the obtaining the necessary consent, the patient could be given the opportunity to refuse the extraction without any fear of subsequent reprisal. In these circumstances the consent process could then be seen not to have been be influenced by any perceived patient/practitioner imbalance. Despite this consideration, one hundred per cent of the patients participating in in this study did not consider that their tooth would be extracted for purposes of research rather than for medical reasons. Accordingly there was no conflict of interest between the patient and the dental surgeon who remained independent from the odontological biomedical research.

What features in the consent for tooth donation?

Article 32 of the Declaration of Helsinki, provides for the gathering of informed consent for the use of human tissue for medical research. Article 22 of the Oviedo Convention states the consent is valid only

if the patient is fully informed of the final purpose of the conservation and use of biological elements are removed.

It is therefore necessary to ensure that the patient has not expressed any opposition to the subsequent use of the specimen. Under French law (Article L.1211-2 of the Public Health Code) relevant information must be transmitted to the patient should there be a change in the research programme for which the sample was originally provided.

However it is difficult to imagine how the person from whom a sample was taken could express their compliance to change should there be a change in the research programme for which the sample was originally provided. German[18] and Canadian[16] legislation have established derogation between the information and consent process with the result that it is impossible to contact the person who provided the original biological sample.

One hundred percent of the patients taking part in the study declared that they would not change their mind if asked to take part in a future research project and would not ask for the final results of the project in which their tooth was included. It is significant that all of the patients were aware that any change in the research project for which their tooth was originally intended could possibly result in discovery of genomic and specific information in respect of their present and future health status as well as that of their family. French law (Article L1131-1 of the Public Health Code) stipulates that it is a mandatory requirement to obtain written consent of the patient in cases of genetic research being carried out on the sample provided.

An opinion from the German Ethics Council[18] proposed that consent for the use of any biological sample to be used for research should be "broad brush" and include the possibility of the sample being used for genetic research. Consent would be revocable at any time. This opinion, if exercised, would exclude patient anonymity. Should data become irretrievably dissociated from the human source of the biological sample, sample destruction would be deemed innapropriate[19]. Against this background the introduction of consent for the recovery of surgical residues for research purposes is a matter of concern for some scientists. Nonetheless it does allow for fundamental ethical principles to be respected and could be seen as a means to protect the interests of both the medical and research communities from possible scandal and litigation.

In disputed cases where informed consent is an issue a written signed document is best evidence that the appropriate information was given at the time of consent. However it is recognised that it is more difficult to obtain informed consent than it is to recognise dissent. An expression of consent should be seen as recognition that the patient maintains control over the sample they have provided. By reference to this principle the patient is respected both as a patient and as a human being by virtue of their participation and contribution to a research project contributing to the progress of science[20].

It should be noted that publication of research projects in International Scientific Journals, such as the project described above, require compliance with the rules of ethics, including the dissemination of information and obtaining consent when human body parts are used.[21]

CONCLUSIONS

This study demonstrates that the process of consent for tooth extraction in the context of routine dental care should be separated from that of the extraction of the teeth for purposes of research. The question of the "balance of risk" to the patient associated

with the process of tooth extraction is minimal compared to the potential "greater good" benefits associated with the need of stem cell researchers to have access to dental pulp tissue.[22] The low risk associated with tooth extraction together with the high degree of patient - dentist trust relationship would infer an increased burden of ethical responsibility on the part of the dentist. Currently the dental practitioner is required to safeguard the autonomy of the patient by providing specific information in respect the destination of the tooth for the purposes of research, to obtain informed consent and to establish traceable retrospective pathways in order to reconcile the entire process.

It is suggested that the existence of a continuing climate of mutual trust that currently exists between patients, dentists and researchers will be necessary to ensure that patients will continue to participate in future research projects.

REFERENCES

1. UNESCO. Report of international Bioethics Committee on the principle of non-discrimination and non-stigmatization (internet). (updated 2014; cited 2015); Available from: http://unesdoc.unesco.org/images/0022/002211/221196E.pdf
2. Shi S, Gronthos S. Perivascular niche of postnatal mesenchymal stem cells in human bone marrow and dental pulp. *J Bone Miner Res* 2003;18:696-704.
3. Mao JJ, Giannobile WV, Helms JA et al. Craniofacial tissue engineering by stem cells. *J Dent Res* 2006;85(11):966-79.
4. Kerkis I, Caplan AI. Stem cells in dental pulp of deciduous teeth. *Tissue Eng Part B-Rev* 2012;18(2):29-38.
5. Yang X, van den Dolder J, Walboomers XF et al. The odontogenic potential of STRO-1 sorted rat dental pulp stem cells in vitro. *J Tissue Eng Regen Med* 2007;1(1):66-73.
6. Le Douarin NM, Calloni GW, Dupin E. The stem cells of the neural crest. *Cell Cycle* 2008;7:1013-9.
7. Huang GT, Gronthos S, Shi S. Mesenchymal stem cells derived from dental tissues vs. those from other sources: their biology and role in regenerative medicine. *J Dent Res* 2009 Sep;88(9):792-806.
8. Iohara K, Zheng L, Wake H et al. A novel stem cell source for vasculogenesis in ischemia: subfraction of side population cells from dental pulp. *Stem Cells* 2008;26:2408-18.
9. Janebodin K, Zeng Y, Buranaphatthana W, Ieronimakis N, Reyes M. VEGFR2-dependent angiogenic capacity of pericyte-like dental pulp stem cells. *J Dent Res* 2013 Jun;92(6):524-31.
10. Council of Europe. Recommendation of the Committee of Ministers to member states on research on biological materials of human origin. (Internet). (updated 2006; cited 2013 Mar); Available from:. http://www.coe.int/t/dg3/healthbioethic/texts_and_documents/Rec_2006_4.pdf
11. De Montgolfier S, Moutel G, Duchange N et al. Evalutation of bio bank constitution and use: multicentre analysis in France and propositions for formalising the activities of research ethics committees. *Eur J Med Genet* 2006;49(2):159-67.
12. Poughon J.M. L'individu, propriétaire de son corps? Une réponse entre scolastique juridique et réalisme économique. *L'Europe des libertés* 2003;11:3-11.
13. Nuffield Council on Bioethics. Human tissue: ethical and legal issues (internet). (updated 1995 April; cited 2013 Jul); Available from: http://nuffieldbioethics.org/wp-content/uploads/2014/07/Human-tissue.pdf
14. CCNE pour les sciences de la vie et de la santé. Avis n°93 Commercialisation des cellules souches humaines et autres lignées cellulaires. 2006:7.
15. UNESCO. The International Declaration on Human Genetic Data (internet). (updated 2003; cited 2013 Mar); Available from: http://portal.unesco.org/en/ev.php-URL_ID=17720&URL_DO=DO_TOPIC&URL_SECTION=201.html
16. Conseil de recherches en science humaines du Canada, Conseil de recherches en sciences naturelles et en génie du Canada, Instituts de recherche en santé du Canada. Enonce de politique des trois conseils: éthique de la recherche avec des êtres humains (internet). (updated 2010; cited 2013 Mar); Available from: http://www.ger.ethique.gc.ca/pdf/fra/eptc2/EPTC_2_FINALE_Web.pdf
17. Conseil de l'Europe. Rapport explicatif au protocole additionnel a la convention sur les droits de l'homme et la biomédecine relatif à la recherche biomédicale (internet). (updated 2009; cited 2013 jul); Available from: http://conventions.coe.int/Treaty/FR/Treaties/Html/195.htm
18. German National Ethics Council. Nationaler Ethikrat: Biobanks for research (internet). (updated 2004; cited 2013 jul); Available from: http://www.ethikrat.org/files/ner_opinion_biobanks.pdf
19. UNESCO. International Declaration on Human Genetic Data (Internet). (updated 2003; Cited 2013 Mar);

Available from: http://www.unesco.org/new/fr/social-and-human-sciences/themes/bioethics/human-genetic-data/

20. Pirnay P. *L'éthique en médecine bucco-dentaire*. Paris. Espace ID; 2012. p 125.
21. Pascal P, Bensiam F, Auxenfans C et al. Legal framework relating to human tissues used for research ends. *Pathol Biol* 2005; 53(10):571-575.
22. Le Breton A, Hervé C, Pirnay P. Information and consent in dental care associated with biomedical research. *Revue Santé Publique* 2013;6:803-812.

*****