Research Article

Quality of salivary samples collected from porous and non-porous surfaces for dna extraction – A forensic evaluation

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ABSTRACT

Introduction: Saliva found on victims can be potentially recovered and typed from bite marks, cigarette butts, postage stamps, envelopes, edibles, and other objects. The detected saliva is recognized as one of the primary source of DNA by the forensic serologist. **Aim:** In this study, we have evaluated the quality of the salivary samples collected from porous (cloth and mud) and non-porous (cement and ceramic tile) surface for the number of cells obtained and their viability for the use of DNA extraction. **Materials and Methods:** Sixty samples of saliva were collected on non-porous tile and cement surface and porous cloth surface and saliva was collected at the interval of 2 and 5 days. The samples were collected, centrifuged, and analyzed with trypan blue staining. **Results:** There was a strong positive correlation between number of cells and viability of cells and the surface, which was statistically significant, $r_s = 0.726$. **Conclusion:** Saliva is found to be an emerging forensic tool. It has found to be of great help in forensic by monitoring drug abuse, help in sex determination, and bite marks analysis which can be human and non-human. Saliva provides genetic material which can be used even if it is stored suboptimal.

KEY WORDS: DNA extraction, Forensics, Non-porous, Porous, Saliva

INTRODUCTION

Saliva is one of the vital fluids secreted in human beings, which is deposited on the human skin through various actions such as biting and sucking[1,2] and hence, saliva stands as a source for identification of accused.[3]

Saliva found on victims can be potentially recovered and typed from bite marks, cigarette butts, postage stamps, envelopes, edibles, and other objects.[4] The detected saliva is recognized as one of the primary source of DNA by the forensic serologist.[5]

The oral cavity is an excellent source of easy-to-access biological material for studies of genetics, genotoxicity, epigenetics, proteomics, metabolomics, and microbiomes.[6,7] This is due to the quick, non-invasive, and low-cost collection compared to tissues such as blood.[8,9]

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The most popular sources of oral samples are saliva samples (collected by passive drool or swab) and buccal samples (collected by swabs or brushes).[8]

The saliva deposited is used for age and sex identification of the person or animal that has deposited the sample. In this study, we have evaluated the quality of the salivary samples collected from porous (cloth and mud) and non-porous (cement and ceramic tile) surface for the number of cells obtained and their viability for the use of DNA extraction.

MATERIALS AND METHODS

Sixty samples of saliva were deposited on non-porous tile and cement surface and porous cloth surface and saliva was collected at the interval of 2 and 5 days. The saliva samples were collected using the double swab technique which has been proven to be an advantageous over the single swab technique.

Double Swab Technique

This technique is similar to single swab technique here a single wet cotton swab or wet filter paper

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helps to recover saliva from the surface on which the saliva was deposited. This was followed by second application of dry cotton swab.[10]

Checking the Viability of Cells

Dye exclusion is a simple and rapid technique measuring cell viability but it is subject to the problem that viability is being determined indirectly from cell membrane integrity. The cell suspension was centrifuged to test the viability of the cells; the aliquot is centrifuged for 5 min at ×100 g and the supernatant is discarded. The size of the aliquot depends on the approximate number of cells present.

The aliquot should contain a convenient number of cells to count in a hemacytometer when suspended in 1 ml phosphate buffered saline (PBS) and then diluted again by mixing with 0.4% trypan blue (e.g., 5 × 105 cells/ml). The cell pellet was resuspend in 1 ml PBS. 1 part of 0.4% trypan blue is mixed with 1 part cell suspension (dilution of cells).

The mixture was allowed to be incubated ~3 min at room temperature. Cells were counted within 3–5 min of mixing with trypan blue, as longer incubation periods will lead to cell death and reduced viability counts.

A drop of the trypan blue/cell mixture was applied to a hemacytometer. Moreover, the unstained (viable) and stained (nonviable) cells were counted separately in the hemacytometer.

The total number of viable cells per ml of aliquot was obtained by, multiplying the total number of viable cells by 2 (the dilution factor for trypan blue). To obtain the total number of cells per ml of aliquot, add up the total number of viable and nonviable cells and multiply by 2. The percentage of viable cells was counted as follows:

Viable cells (%) = Total number of viable cells per ml of aliquot/total number of cells per ml of aliquot × 100.

RESULTS

It was found that the number of viable cells counted from the porous surface was greater than the number of viable cells counted from the non-porous surfaces. The mean of the cells collected and viable cells of the three surfaces and day 2 and 5 is represented in Figures 1 and 2, respectively. The Pearson's correlation test was done to find the correlation between the viability and number of cells to the surfaces over which the sample was found, there was a strong positive correlation between number of cells and viability of cells and the surface, which was statistically significant, $r_s = 0.726$.

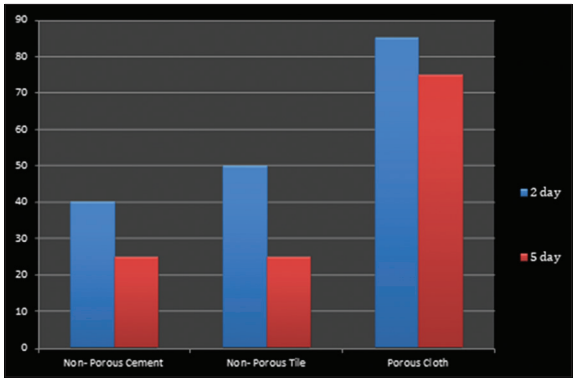

Figure 1: The mean number of cells present in the three surfaces

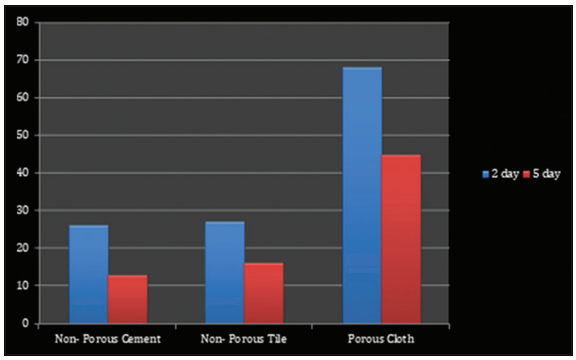

Figure 2: The mean number of viable cells obtained from the three surfaces

DISCUSSION

The average turnover of epithelial cells in the mouth is every 2.7 h and the mean number of epithelial cells per 1 mL of saliva is about 4.3×10^5 suggesting that there is higher probability of intact genomic DNA in saliva samples. Saliva can be used even when it is stored in different conditions.[11] Recent studies have shown that human genomic DNA can be reliably obtained from saliva.[12]

Biological fluids, which are more commonly found at crime scenes, cannot be analyzed with morphological techniques.[13] However, these fluids can be used for other proteomic and genetic studies. Saliva provides genetic material which can be used even if it is stored suboptimal.[10]

The cellular content is given importance because these cellular contents can be used for studies of epigenomics,[7,14] gene expression,[15,16] and proteomics.[17]

In our study, we used the double swab technique to obtain the saliva samples from porous and non-porous surfaces. It is believed that this is due to the fact that the moisture present in the first swab rehydrates and loosens the majority of the epithelial cells dried in the saliva and causes them to adhere to the cotton fibers of the swab. When the second

(dry) swab is applied to the site, the cells in the saliva are able to adhere to the fibers more easily because they are rehydrated after the application of moisture from the first swab and the time elapsed since the first swabbing. Hence, the double swab technique for recovering saliva permits collection of a larger amount of DNA evidence than the classical methods studied.[1]

We were also able to prove that the saliva samples obtained from porous surfaces found to have more viable cells than those obtained from non-porous surfaces. This is due to the fact that porous objects or surfaces aid in the retention of cells and also provide a suitable niche for the cells to stay viable for longer. The number of cells reduced between the 2nd and 5th day. The non-porous surfaces used were tile and cement; they proved to be disadvantageous when compared to the porous surfaces. This can be due to the fact that the cells on non-porous surfaces are often exposed to the activity of heat, dust, and other microorganisms at a higher effect when compared to the cells in the porous surfaces.

In our study, we were able to prove that the viable cellular yield from a porous surface was more beneficial than the yield from non-porous surfaces. However, there are a few problems associated with the dye uptake which can be assessed subjectively; small amounts of dye uptake indicative of cell injury may go unnoticed.

CONCLUSION

Saliva is one of the vital fluids secreted in the body, which has an advantage of non-invasive method of collection, and is found to be an emerging forensic tool. It has found to be of great help in forensic by monitoring drug abuse, help in sex determination, and bite marks analysis which can be human and non-human. Saliva provides genetic material which can be used even if it is stored suboptimal. DNA extraction from saliva has been used as one of the common method but many advanced techniques and from our study we have found that the salivary samples collected from the porous surfaces is found to be more advantageous than the non-porous surfaces.

REFERENCES

1. Sweet DJ, Lorente MB, Lorente JA, Valenzuela A, Villanueva EL. An improved method to recover saliva from human skin: The double swab technique. J Forensic Sci 1997;42:320-2.
2. Schenkels LC, Veerman EC, NieuwAmerongen AV. Biochemical composition of human saliva in relation to other mucosal fluids. Crit Rev Oral Biol Med 1995;6:161-75.
3. Raj CK, Garlapati K, Karunakar P, Badam R, Soni P, Lavanya R. Saliva as forensic evidence using fluorescent spectroscopy: A pilot study. J Clin Diagn Res 2018;12:ZC27-9.
4. Anzai-Kanto E, Hirata MH, Hirata RD, Nunes FD, Melani RF, Oliveira R. DNA extraction from human saliva deposited on skin and its use in forensic identification procedures. Braz Oral Res 2005;19:216-22.
5. Kirk PL. Crime investigation; Physical evidence and the police laboratory. New York: Interscience Publishers Inc.; 1953. p. 784.
6. Proia NK, Paszkiewicz GM, Nasca MA, Franke GE, Pauly JL. Smoking and smokeless tobacco-associated human buccal cell mutations and their association with oral cancer--a review. Cancer Epidemiol Biomark Prev 2006;15:1061-77.
7. Langie SA, Szic KS, Declerck K, Traen S, Koppen G, Van Camp G, *et al*. Whole-genome saliva and blood DNA methylation profiling in individuals with a respiratory allergy. PLOS One 2016;11:e0151109.
8. Theda C, Hwang SH, Czajko A, Loke YJ, Leong P, Craig JM. Quantitation of the cellular content of saliva and buccal swab samples. Sci Rep 2018;8:6944.
9. Jurez-Cedillo T, Snchez-Garca S, Mould-Quevedo JF, Garca-Pea C, Gallo JJ, Wagner FA, *et al*. Cost-effective analysis of genotyping using oral cells in the geriatric population. Genet Mol Res 2010;9:1886-95.
10. Mago J. Saliva in forensics. World J Pharm Med Res 2016;4:196-8.
11. Quinque D, Kittler R, Kayser M, Stoneking M, Nasidze I. Evaluation of saliva as a source of human DNA for population and association studies. Anal Biochem 2006;353:272-7.
12. da Silva RH, Sales-Peres A, de Oliveira RN, de Oliveira FT, Sales-Peres SH. Use of DNA technology in forensic dentistry. J Appl Oral Sci 2007;15:156-61.
13. Hamano Y, Manabe S, Morimoto C, Fujimoto S, Tamaki K. Forensic age prediction for saliva samples using methylation-sensitive high resolution melting: Exploratory application for cigarette butts. Sci Rep 2017;7.
14. Liang L, Cookson WO. Grasping nettles: Cellular heterogeneity and other confounders in epigenome-wide association studies. Hum Mol Genet 2014;23:R83-8.
15. Boyle JO, Gümüs ZH, Kacker A, Choksi VL, Bocker JM, Zhou XK, *et al*. Effects of cigarette smoke on the human oral mucosal transcriptome. Cancer Prev Res (Phila) 2010;3:266-78.
16. Kupfer DM, White VL, Jenkins MC, Burian D. Examining smoking-induced differential gene expression changes in buccal mucosa. BMC Med Genomics 2010;3:24.
17. Saliva Proteome Research: Current Status and Future Outlook. Available from: https://www.researchgate.net/publication/225051614_Saliva_proteome_research_Current_status_and_future_outlook. [Last accessed on 2019 Apr 24].

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