

Bioluminescent Assessment of Microbial Load in Areas of High Exposure to Humans at a Dental Operator

Aashruthaa S¹, R. Gayathri^{2*}, Jayalakshmi Somasundaram³, V. Vishnu Priya⁴, Kavitha.S⁵

¹Saveetha Dental College and Hospitals Saveetha Institute of Medical and Technical Sciences, Saveetha University, Chennai, India

^{2*}Department of Biochemistry, Saveetha Dental college and Hospitals Saveetha Institute of Medical and Technical Sciences, Saveetha University, Chennai, India

³Jayalakshmi Somasundaram White Lab - Material Research Centre Saveetha Dental college and Hospitals Saveetha Institute of Medical and Technical Sciences, Saveetha University, Chennai, India

⁴Department of Biochemistry, Saveetha Dental college and Hospitals Saveetha Institute of Medical and Technical Sciences, Saveetha University, Chennai, India

⁵Department of Biochemistry, Saveetha Dental college and Hospitals Saveetha Institute of Medical and Technical Sciences, Saveetha University, Chennai, India

Abstract

Introduction:

The oral cavity is an ideal medium for bacterial growth. Exposure to microorganisms through blood and saliva have a high risk for transmission of infectious diseases. Constant usage of dental instruments promote proliferation of microbes and pose potential risk to immunocompromised patients. Bioluminometer can be used to instantly verify the contamination.

Materials and Methods:

Samples were taken using ultrasnap swabs at areas of high exposure, especially the dental tray and dental light. The microbial load was measured using the Hygiene bio luminometer and the results were analysed statistically.

Results:

There was a significant decline in microbial load post disinfection. It can be inferred that regular disinfection protocols need to be maintained in order to protect both patients and healthcare professionals.

Conclusion:

The use of a luminometer to measure microbial load is highly useful as its fast and can verify the load instantly. Disinfection in regular intervals causes significant decrease in contamination and hence, a protocol needs to be followed after every consultation to prevent disease transmission. It can also pinpoint the high exposure areas that need regular disinfection so that the disinfection can be used with more caution.

Keywords: Dental health, infection control, decontamination, hygiene, novel method, bio luminometer, innovative technique

1. INTRODUCTION

The oral cavity is an ideal medium for bacterial growth. It harbours over 700 species of bacteria which can lead to spread infections among dental professionals and other patients (1). These are also other infection microbes present which pose equal harm if expelled into the surroundings. The working environment of dentists leaves them exposed to hazards and health concerns due to the microorganisms that reside in the oral cavity.(2)

Aerosolization of contaminants can lead to the development of several airway and respiratory diseases. Therefore, preventive strategies need to be implemented to monitor and control microbial contamination. Many culture based methods are being used to do so. But, they are highly time consuming. Alternatives that are rapid and accurate are required for effective use in the field.(3)

Exposure to microorganisms has a high risk of transmission of infectious diseases. The production of aerosols and splatter are a major concern as their pathogenic capacity is high due to the combination of water, tooth and material debris, plaque, blood and saliva mixture. (4) Aerosolized microbes are due to the active performance of high powered handpiece utilisation. Not only does it cause splatter, it can serve as space for proliferation.

Since the occurrence of viral diseases such as SARS and different variants, dental practice must be considered as a possible source of transmission.(5) Constant use of dental instruments promotes proliferation and poses a risk to immunocompromised patients.(6)

Portable ATP bio luminometers have been used in the food industry to monitor effectiveness of cleaning.(7) It has been suggested that it can be adapted and put to use as an indicator of surface soiling and cleanliness in hospital settings. (8)The

alternative of microbiological monitoring offers the advantage of quantitative results for changes in surface loads, but requires time and technical expertise. Portable bioluminometry measures ATP residues and yields quantitative results. ATP is a proxy for a broad range of contaminants as it is the energy currency. Bio Luminometers can be used to indicate different ATP levels and in the process, the microbial load. Our team has extensive knowledge and research experience that has translated into high quality publications (9–18), (19–28).

The aim of this study is to analyse the microbial load in a dental operatory, specifically the dental light and dental tray which are highly exposed to humans.

2. MATERIALS AND METHODS

Sample Collection

Swabs were taken using ultrasnap swabs at high exposure areas, here, dental tray and dental light area. Swabbing was done in a criss cross pattern. The area was swabbed in a square of about 4 by 4 sq.inches (10×10cm). Pressure was applied to pick up surface residue and penetrate any biofilm present. Swab was rotated on all sides to collect samples on all sides of the swab tip. Swabs were taken pre and post disinfection.

Bioluminometer

The load was measured using Hygiene Systemsure Plus. This model has a Pass/Caution/Fail result and programmed Pass/Fail limits that can be set by the user. The results are shown in the form of RLU(Relative Light Units), the units of measure used in bioluminescence. The device contains a natural enzyme which produces a simple bioluminescent reaction on contact with ATP. The quantity of light generated is directly proportional to the amount of ATP present in the sample.

Disinfectant

Disinfection was done using sodium hypochlorite from a branded source.

Disinfection was done for 20-30 secs per area and for each instrument. Disinfectants contain active substances containing bactericidal or virucidal activity. 400ml of liquid sodium hypochlorite is mixed with 20L of water, making a 0.1% solution.

3. RESULT AND DISCUSSION

There was a significant decline in microbial load post disinfection (Figure 1). It can be inferred that regular disinfection protocols need to be maintained in order to protect both patients and healthcare professionals.

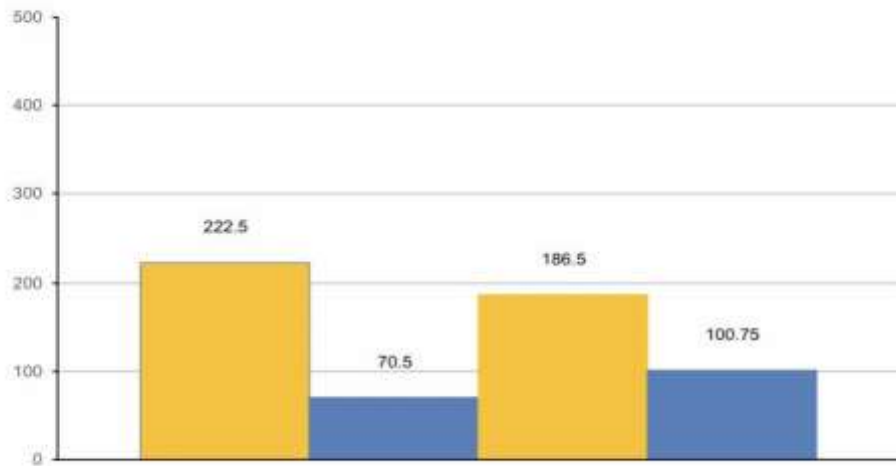


Figure 1: Graph depicting significant decline in microbial load post disinfection.

Bioluminescence is a result of a biochemical reaction. Luciferin/Luciferase+ATP+O₂→light output. The substrate combines with ATP and O₂ and results in a decay, causing photon emission. The RLU output is directly proportional to the amount of ATP collected from the sample. A high RLU reading indicates a large amount of ATP and presence of contaminants. (29). The bacterial/microbial count showed significant decline post disinfection in both tray and light areas. There was less reduction in microbial load in the tray area. That may be due to the constant usage of instruments and handling them by hand. Though the disinfection method yielded good results, other aspects such as efficiency of friction, frequency of disinfection and sterilisation need to be considered.(30)

This makes it clear that regular disinfection can aid reduction of microbial load and

prevent proliferation. The values of the ATP bioluminescence assay showed a significant difference. Our team has extensive knowledge and research experience that has translate into high quality publications (31), (32), (33), (34), (35,36), (37), (38), (39), (40), (41), (42), (43), (44).

It is highly necessary to protect not only the patients, but also the healthcare practitioners. (45).

4. CONCLUSION

Hospital borne infections are quite detrimental and are not easy to tray in comparison to other infections. Disinfection in regular intervals is highly necessary to be followed as a protocol. The cleaning and disinfection processes reduced the microbial load of the inspected surfaces. The values of the ATP bioluminescence assay showed the significant difference between pre and post disinfection.

Furthermore, the use of a luminometer in highly crowded areas such as shops and malls can decrease risk of infection. It can also pinpoint high exposure areas that require regular disinfection and hence disinfection can be made use of in a more cautious way.

Conflict of Interest:

The authors hereby declare that there is no conflict of interest in this study.

Acknowledgement:

The authors express their gratitude to Saveetha Dental College & Hospitals for supporting and for successful completion of this project.

Source of funding:

The present project is funded by

1. Saveetha Institute of Medical and Technical Sciences
2. Saveetha Dental College and Hospitals

3. Saveetha University
4. Jayachandran Infrastructure Pvt Ltd

Author Contribution:

A) Santhosh S - contributed in designing the study, execution of the project, statistical analysis, manuscript drafting.

B) Dr. Jayalakshmi - contributed in designing the study, execution of the project, statistical analysis, manuscript drafting.

C) Dr.V.Vishnupriya - contributed in study design, guiding the research work, manuscript correction.

D) Dr. Gayathri R - study design, statistical analysis, manuscript proofreading and correction.

E) Dr. Kavitha S - study design, statistical analysis, manuscript proofreading and correction.

5. REFERENCES

1. Kimmerle H, Wiedmann-Al-Ahmad M, Pelz K, Wittmer A, Hellwig E, Al-Ahmad A. Airborne microbes in different dental environments in comparison to a public area. *Arch Oral Biol.* 2012 Jun;57(6):689–96.
Kumbargere Nagraj S, Eachempati P, Paisi M, Nasser M, Sivaramakrishnan G, Verbeek JH. Interventions to reduce contaminated aerosols produced during dental procedures for preventing infectious diseases. *Cochrane Database Syst Rev.* 2020 Oct 12;10(10):CD013686.
2. Liebers V, Bachmann D, Franke G, Freundt S, Stubel H, Düser M, et al. Determination of ATP-activity as a useful tool for monitoring microbial load in aqueous humidifier samples [Internet]. Vol. 218, *International Journal of Hygiene and Environmental Health.* 2015. p. 246–53. Available from: <http://dx.doi.org/10.1016/j.ijheh.2014.11.004>
3. Koletsi D, Belibasakis GN, Eliades T. Interventions to Reduce Aerosolized Microbes in Dental Practice: A Systematic Review with Network Meta-analysis of Randomized Controlled Trials. *J Dent Res.* 2020 Oct;99(11):1228–38.
4. Spicciarelli V, Marruganti C, Viviano M, Baldini N, Franciosi G, Tortoriello M, et al. Prevention and safety in the dental office after Novel Human Coronavirus outbreak: unresolved questions and future directions. *J Osseointegr.* 2020 Apr 15;12(2):145–53.
5. Ahmed R, Mulder R. A Systematic Review on the Efficacy of Vaporized Hydrogen Peroxide as a Non-Contact Decontamination System for Pathogens Associated with the Dental Environment. *Int J Environ Res Public Health* [Internet]. 2021 Apr 29;18(9). Available from:

- <http://dx.doi.org/10.3390/ijerph18094748>
6. Website [Internet]. Available from: <https://doi.org/10.1002/j.2050-0416.2001.tb00077.x>
 7. Whiteley GS, Derry C, Glasbey T. Reliability testing for portable adenosine triphosphate bioluminometers. *Infect Control Hosp Epidemiol.* 2013 May;34(5):538–40.
 8. Sathivel A, Raghavendran HRB, Srinivasan P, Devaki T. Anti-oxidative and anti-hyperlipidemic nature of *Ulva lactuca* crude polysaccharide on D-galactosamine induced hepatitis in rats. *Food Chem Toxicol.* 2008 Oct;46(10):3262–7.
 9. Sekar D, Lakshmanan G, Mani P, Biruntha M. Methylation-dependent circulating microRNA 510 in preeclampsia patients. *Hypertens Res.* 2019 Oct;42(10):1647–8.
 10. Rajeshkumar S, Menon S, Venkat Kumar S, Tambuwala MM, Bakshi HA, Mehta M, et al. Antibacterial and antioxidant potential of biosynthesized copper nanoparticles mediated through *Cissus arnotiana* plant extract. *J Photochem Photobiol B.* 2019 Aug;197:111531.
 11. Lakshmi T, Krishnan V, Rajendran R, Madhusudhanan N. *Azadirachta indica*: A herbal panacea in dentistry - An update. *Pharmacogn Rev.* 2015 Jan;9(17):41–4.
 12. Felicita AS, Chandrasekar S, Shanthasundari KK. Determination of craniofacial relation among the subethnic Indian population: a modified approach - (Sagittal relation). *Indian J Dent Res.* 2012 May;23(3):305–12.
 13. Thejeswar EP, Thenmozhi MS. Educational research-iPad system vs textbook system. *J Adv Pharm Technol Res.* 2015;8(8):1158.
 14. Saravanan A, Senthil Kumar P, Jeevanantham S, Karishma S, Tajsabreen B, Yaashikaa PR, et al. Effective water/wastewater treatment methodologies for toxic pollutants removal: Processes and applications towards sustainable development. *Chemosphere.* 2021 Oct;280:130595.
 15. Menon A, Thenmozhi MS. Correlation between thyroid function and obesity. *J Adv Pharm Technol Res.* 2016 Oct;9(10):1568.
 16. Sahu D, Kannan GM, Vijayaraghavan R. Size-dependent effect of zinc oxide on toxicity and inflammatory potential of human monocytes. *J Toxicol Environ Health A.* 2014;77(4):177–91.
 17. Wang Y, Zhang Y, Guo Y, Lu J, Veeraraghavan VP, Mohan SK, et al. Synthesis of Zinc oxide nanoparticles from *Marsdenia tenacissima* inhibits the cell proliferation and induces apoptosis in laryngeal cancer cells (Hep-2). *J Photochem Photobiol B.* 2019 Dec;201:111624.
 18. Wadhwa R, Paudel KR, Chin LH, Hon CM, Madheswaran T, Gupta G, et al. Anti-inflammatory and anticancer activities of Naringenin-loaded liquid crystalline nanoparticles in vitro. *J Food Biochem.* 2021 Jan;45(1):e13572.
 19. Reddy P, Krithikadatta J, Srinivasan V, Raghu S, Velumurugan N. Dental Caries Profile and Associated Risk Factors Among Adolescent School Children in an Urban South-Indian City. *Oral Health Prev Dent.* 2020 Apr 1;18(1):379–86.
 20. Eapen BV, Baig MF, Avinash S. An Assessment of the Incidence of Prolonged Postoperative Bleeding After Dental Extraction Among Patients on Uninterrupted Low Dose Aspirin Therapy and to Evaluate the Need to Stop Such Medication Prior to Dental Extractions. *J Maxillofac Oral Surg.* 2017 Mar;16(1):48–52.
 21. Devarajan Y, Nagappan B, Choubey G, Vellaiyan S, Mehar K. Renewable Pathway and Twin Fueling Approach on Ignition Analysis of a Dual-

- Fuelled Compression Ignition Engine. *Energy Fuels*. 2021 Jun 17;35(12):9930–6.
22. Barabadi H, Mojab F, Vahidi H, Marashi B, Talank N, Hosseini O, et al. Green synthesis, characterization, antibacterial and biofilm inhibitory activity of silver nanoparticles compared to commercial silver nanoparticles [Internet]. Vol. 129, *Inorganic Chemistry Communications*. 2021. p. 108647. Available from: <http://dx.doi.org/10.1016/j.inoche.2021.108647>
 23. Manickam A, Devarasan E, Manogaran G, Priyan MK, Varatharajan R, Hsu CH, et al. Score level based latent fingerprint enhancement and matching using SIFT feature. *Multimed Tools Appl*. 2019 Feb 1;78(3):3065–85.
 24. Subramaniam N, Muthukrishnan A. Oral mucositis and microbial colonization in oral cancer patients undergoing radiotherapy and chemotherapy: A prospective analysis in a tertiary care dental hospital [Internet]. Vol. 10, *Journal of Investigative and Clinical Dentistry*. 2019. Available from: <http://dx.doi.org/10.1111/jicd.12454>
 25. Rohit Singh T, Ezhilarasan D. Ethanolic Extract of *Lagerstroemia Speciosa* (L.) Pers., Induces Apoptosis and Cell Cycle Arrest in HepG2 Cells. *Nutr Cancer*. 2020;72(1):146–56.
 26. Wahab PUA, Abdul Wahab PU, Senthil Nathan P, Madhulaxmi M, Muthusekhar MR, Loong SC, et al. Risk Factors for Post-operative Infection Following Single Piece Osteotomy [Internet]. Vol. 16, *Journal of Maxillofacial and Oral Surgery*. 2017. p. 328–32. Available from: <http://dx.doi.org/10.1007/s12663-016-0983-6>
 27. Krishnamurthy A, Sherlin HJ, Ramalingam K, Natesan A, Premkumar P, Ramani P, et al. Glandular odontogenic cyst: report of two cases and review of literature. *Head Neck Pathol*. 2009 Jun;3(2):153–8.
 28. Levrini L, Mangano A, Margherini S, Tenconi C, Vigetti D, Muollo R, et al. ATP Bioluminometers Analysis on the Surfaces of Removable Orthodontic Aligners after the Use of Different Cleaning Methods. *Int J Dent* [Internet]. 2016 May 8 [cited 2022 Dec 17];2016. Available from: <https://doi.org/10.1155/2016/5926941>
 29. Ferreira AM, de Andrade D, Rigotti MA, de Almeida MTG, Guerra OG, dos Santos Junior AG. Assessment of disinfection of hospital surfaces using different monitoring methods. *Rev Lat Am Enfermagem*. 2015 May-Jun;23(3):466–74.
 30. Mohan SK, Priya VV, Others. Lipid peroxidation, glutathione, ascorbic acid, vitamin E, antioxidant enzyme and serum homocysteine status in patients with polycystic ovary syndrome. *Early Pregnancy*. 2009;1(3):44–9.
 31. Ravikumar D, Gurunathan D, Gayathri R, Priya VV, Geetha RV. DNA profiling of *Streptococcus mutans* in children with and without black tooth stains: A polymerase chain reaction analysis. *Dent Res J*. 2018 Sep-Oct;15(5):334–9.
 32. Wei W, Li R, Liu Q, Devanathadesikan Seshadri V, Veeraraghavan VP, Surapaneni KM, et al. Amelioration of oxidative stress, inflammation and tumor promotion by Tin oxide-Sodium alginate-Polyethylene glycol-Allyl isothiocyanate nanocomposites on the 1,2-Dimethylhydrazine induced colon carcinogenesis in rats. *Arab J Chem*. 2021 Aug 1;14(8):103238.
 33. Saravanakumar K, Sriram B, Sathiyaseelan A, Mariadoss AVA, Hu

- X, Han KS, et al. Synthesis, characterization, and cytotoxicity of starch-encapsulated biogenic silver nanoparticle and its improved antibacterial activity. *Int J Biol Macromol.* 2021 Jul 1;182:1409–18.
34. Han X, Jiang X, Guo L, Wang Y, Veeraraghavan VP, Krishna Mohan S, et al. Anticarcinogenic potential of gold nanoparticles synthesized from *Trichosanthes kirilowii* in colon cancer cells through the induction of apoptotic pathway. *Artif Cells Nanomed Biotechnol.* 2019 Dec;47(1):3577–84.
 35. Zhang L, Chinnathambi A, Alharbi SA, Veeraraghavan VP, Mohan SK, Zhang G. Punicalagin promotes the apoptosis in human cervical cancer (ME-180) cells through mitochondrial pathway and by inhibiting the NF- κ B signaling pathway. *Saudi J Biol Sci.* 2020 Apr;27(4):1100–6.
 36. Sankari G, Krishnamoorthy E, Jayakumar S, Gunasekaran S, Priya VV, Subramaniam S, et al. Analysis of serum immunoglobulins using Fourier transform infrared spectral measurements. *Early Pregnancy.* 2010;2(3):42–8.
 37. Suresh M, Vishnu Priya V, Gayathri R. Effect of e-learning on academic performance of undergraduate students. *Drug Invention Today* [Internet]. 2018;10(9). Available from: <https://search.ebscohost.com/login.aspx?direct=true&profile=ehost&scope=site&authtype=crawler&jrnl=09757619&AN=131123673&h=D%2FAiHy4kem6euQ5kW5AgcIa%2FX5JBE BhXszfG0gF5EMGaVJYZRDVIW9 SlCtMGnOvI49I1qp6eub55fNb0U3xuEA%3D%3D&crl=c>
 38. Veeraraghavan VP, Periadurai ND, Karunakaran T, Hussain S, Surapaneni KM, Jiao X. Green synthesis of silver nanoparticles from aqueous extract of *Scutellaria barbata* and coating on the cotton fabric for antimicrobial applications and wound healing activity in fibroblast cells (L929). *Saudi J Biol Sci.* 2021 Jul;28(7):3633–40.
 39. Shah PM, Priya VV, Gayathri R. Quercetin-a flavonoid: a systematic review. *Res J Pharm Biol Chem Sci.* 2016;8(8):878.
 40. Jayaraman S, Devarajan N, Rajagopal P, Babu S, Ganesan SK, Veeraraghavan VP, et al. β -Sitosterol Circumvents Obesity Induced Inflammation and Insulin Resistance by down-Regulating IKK β /NF- κ B and JNK Signaling Pathway in Adipocytes of Type 2 Diabetic Rats. *Molecules* [Internet]. 2021 Apr 6;26(7). Available from: <http://dx.doi.org/10.3390/molecules26072101>
 41. Manohar J, Gayathri R, Vishnupriya V. Tenderisation of meat using bromelain from pineapple extract. *Int J Pharm Sci Rev Res.* 2016;39(1):81–5.
 42. Balaji V, Priya VV, Gayathri R. Awareness of risk factors for obesity among College students in Tamil Nadu: A Questionnaire based study. *Research Journal of Pharmacy and Technology; Raipur.* 2017 May;10(5):1367–9.
 43. Dave PH, Vishnupriya V, Gayathri R. Herbal remedies for anxiety and depression-a review. *Research Journal of Pharmacy and Technology.* 2016;9(8):1253–6.
 44. Nascimento EA da S, Poveda V de B, Monteiro J. Evaluation of different monitoring methods of surface cleanliness in operating rooms. *Rev Bras Enferm.* 2021 Jul 9;74(3):e20201263.