**Tooth Bio-Banking**

1. **Introduction**
2. **Need of Tooth Bio-Banking System as resource material**
* **Stem Cell Therapies**
* **Education and Research**
* **Biological Restorations**
* **Bone Graft Material**
1. **Biosafety and ethical concerns for Tooth Bio-Banking**
2. **Consideration For Tooth Bio-Banking Set Up**
3. **Conclusion**

**Author**

**Dr. Mohit.K.Gunwal**

**Associate Professor (BDS, MDS)**

**Department Of Conservative Dentistry and Endodontics**

**VSPMs Dental College and Research Institute**

**Nagpur - 440019 (MH).**

**BIOBANK**

‘A collection of biological material and the associated data and information stored in an organized system for a population or a large subset of a population’

***Organisation for Economic Cooperation and Development (OECD) (2006)***

**Introduction**

 In recent times, both government and private sector organizations have become interested in the banking of human tissues for future therapeutic uses because it provides real-time solutions to complex health-related challenges involving various human body tissues.

Currently, stem cell banks and blood banking systems are established practices in the field of modern medicine. Blood banking systems are an age-old, established practice that provides life-saving advantages to patients as an affordable, safe, effective, and easily available solution. Blood banks aim to provide blood and its related components to patients in times of urgency. Blood banks are a very well-established system, and various organizations like the World Health Organisation (WHO) and the US Food and Drug Administration (FDA) have constantly regulated their function and safety issues in the public interest.

Advancements made in regenerative medical science and its clinical applications have led to exponential growth and increased awareness of stem cell banking. Currently, stem cell collections from tissues like bone marrow, blood, foetal material, and the umbilical cord are established practices with practical and ethical concerns. The prospective study on postnatal cell populations produced from dental pulp tissue conducted by ***Gronthos and Shi*** two decades ago opened new avenues for researchers in field of stem cells and gave dentistry a direction towards regenerative medical science.

Today, it is critically important to set up tooth banking systems (TBS) with suitable protocols and facilities not just for stem cell extraction but also to meet the demand for extracted teeth used as teaching and learning resources in dentistry schools, research projects, and clinical restorative needs.

**Need of Tooth Bio-Banking System**

Over the past decades, Dentistry has evolved in terms of better clinical techniques, dental material formulations, regenerative therapies, and explorative research. This has enhanced the utility and need for extracted human teeth. Therefore, with the substantial increase in demand for extracted teeth, setting up a tooth bio-banking system becomes essential.

 The basic concept of human tooth banking was developed in 1981 as there was a continuously growing demand for extracted teeth for research proposes and also to abolish illegal and hazardous procurement.

Human tooth bank (HTB) is a nonprofit organization affiliated with a college, university, or any other organization aimed at satisfying academic requirements by donating human teeth for research activities and preclinical laboratory training of students.

*Nassif AC, Tieri F, Ana PA, et al.* 2003



**Indications for Human tooth Banking (Tooth Bio-Banking)**



**Stem Cell Therapies**

Oral tissues provide potential sites for the collection of stem cells. Dental stem cells (DSCs) include adult dental pulp stem cells (DPSC), human exfoliated deciduous teeth or stem cells from exfoliated deciduous teeth (SHED), periodontal ligament stem cells (PDLSC), alveolar bone stem cells (ABSC), stem cells from the apical papilla (SCAP), tooth germ progenitor cells (TGPC), Dental follicle stem cells (DFSC), oral epithelial tissue, gingiva, and salivary glands. Amongst the above mentioned types of stem cells, those from human exfoliated deciduous teeth or stem cells from exfoliated deciduous teeth (SHED) are the most preferred, as they are easily available and safe. Dental stem cells (DSCs) have the potential to differentiate into adipocytes, neural cells, osteocytes, chondrocytes, and myocytes. Also, they can be utilised for regeneration of dentin, periodontal ligament, cementum, pulp, salivary gland tissues, the temporomandibular joint, and whole tooth regeneration. India is one of the few nations with official regulations governing research using stem cell products and derivatives (SCPDs). National Guidelines for Stem Cell Research were recently published in 2017, after ICMR and DBT modified the current regulations jointly. The document's primary guiding principle aimed to stop the rampant commercialization of untested stem-cell therapies and to generate new information based on good scientific reasoning while taking all ethical issues into account.

In the past, stem cell banks have collected bone marrow and placental cord blood-derived stem cells, but recent years have seen exponential growth in dental stem cell banking, particularly in India, the United States, and the United Kingdom. Currently, there are no proper guidelines, particularly for tooth banking systems utilised for stem cell therapies, as most of the regulations focus on stem cell banking. Also, there is a lack of public funded tooth banking systems, whereas the private sector focuses primarily on the preservation of stem cells. Therefore, establishing properly structured tooth banks would help in a safe, ethical, and legal way of stem cell procurement, storage, and utilisation in a wide range of systemic diseases and regenerative research.

**Education and Research**

Utilisation ofextracted human teeth (EHT) is an integral part of undergraduate and postgraduate dental educational training and learning programmes worldwide. The basic utilisation involves understanding the internal anatomy and histology of the tooth, tooth preparations for restorative purposes, endodontic procedures, research related to dental materials and procedures, and the restorative material itself. In spite of the availability of artificial teeth and simulators, the use of EHT for teaching, learning, and research is irreplaceable because of their cost effective availability. Also, artificial teeth or simulator systems fail in the exact replication of required biomechanical properties and tactile perception, similar to a real clinical case situation.

EHT used for research purposes cannot be replaced by artificial and expensive methods like finite element analysis or simulators, and they still remain the gold standard choice for laboratory research. Development and In-vitro or ex-vivo testing of various properties of newer restorative material formulations and techniques like evaluation of fracture strength, bonding, microleakege etc. carried out on EHT provide more realistic and clinically relatable observations. The major concerns related to the use of EHT for research purposes include illegal and unethical procurement, cross contamination, availability, and improper storage, which may affect the quality of the research outcome. A properly established tooth banking system can address all these concerns and provide researchers with good quality EHT.

**Biological Restorations**

 Restorative dentistry focuses on the idealistic rehabilitation of the biomechanical functions and properties of diseased or injured teeth. Though there is exponential growth and improvement in the restorative material formulations, all the contemporary restorative materials cannot match the exact bio-mechanical property of a natural tooth. Numerous researchers have suggested the use of natural enamel and dentine for restorative purposes. The choice of using natural tooth substances for restorative purposes presents as a gold standard option when compared to synthetic restorative materials, and it is called ‘Biological Restorations’.

‘Biological Restorations’ can be defined as the procedures in which a sterile natural enamel and/or dentin tissue is used to reinstate the carious, fractured, missing and defective structures of the tooth, so as to re-establish the Bio-functional requirements, and maintain a healthy stomatognathic system.

 *Gunwal, M. K. et.al. (2022)*

Clinical application of biological restorations presents a wide range of treatment alternatives for deciduous and permanent dentitions, including restoration of carious teeth, defects, and fractured teeth. Also, treatment alternatives like inlays, veneers, post and core, full crowns and abutments can be obtained. The availability of natural teeth in the appropriate shape and shade is a prerequisite for biological restorations. Also, the patients' and/or parents' consent for the procedure is of prime importance.

Biological restorations provide a natural, conservative, time saving, cost-effective, and aesthetic restorative solution. In spite of its biological and economical benefits, awareness and clinical applications of ‘Biological Restorations’ are limited due to a lack of knowledge regarding the procedure and also due to the effortless and safe unavailability of matching natural teeth. Clinically, it is always not possible to use the patient’s own fractured fragment or donor tooth for the reattachment or biological restoration procedure. Therefore, establishing a tooth banking system for proper collection, cleaning, sterilisation, storage, and distribution of natural tooth substance is pivotal. Establishment of a tooth banking system will encourage clinicians to perform biological restorations in routine clinical practise as there will be ethical availability of extracted teeth without concerns of cross-contamination. Also, it will provide a properly stored, wide range of extracted teeth of different forms and shades, matching the clinical requirements.

**Bone Graft Material**

The field of medical sciences has been using bone graft materials for decades, with the earliest noted evidence in the year 1688, when a human cranial defect was corrected with the use of canine bone. In the field of dentistry has seen an exponential boost in the use of bone graft materials due to the increased need for implants and the repair of a range of defects arising from traumatic injuries, infections, and cancers. Various types of grafts are available, including autograft, allograft, xenograft, phytogenic material, synthetic bone substitute materials, hydroxyapattite, tricalcium phosphate ceramics, biphasic calcium phosphate ceramics, bioactive glass, calcium phosphate cements, calcium sulphate, polymers, metals (magnesium, strontium, zinc, and silicon), composite bone substitute material, growth factor-based bone substitutes, and bone substitutes with infused living osteogenic cells. Despite the emergence of various bone replacements in recent years, autografts continue to be the gold standard for grafting materials because they possess all four essential biological qualities needed: osseointegration, osteogenesis, osteoconduction, and osteoinduction. However, autografts present some disadvantages, including the requirement of a secondary surgical site, increased postoperative visits, injury and morbidity at the donor site, scarring, a high cost, and limited availability.

Researchers are working restlessly to overcome these limitations, and the pathbreaking research presented by Yeomans and Urist on human dentin’s bone-inducing properties has opened up a completely new avenue in the field of bone grafting materials.

Tooth is a complex composite structure containing inorganic calcium phosphates, organic collage type I, water, growth factors like bone morphogenic proteins (BMPs), and other proteins with the capability to induce bone resorption and generation processes. The chemical composition of dentin tissue is similar to that of bone, where dentin contains 70–75% inorganic, 20% organic, and 10% water, while bone contains 65% inorganic and 35% organic content. Dentin contains 70% hydroxyappetite, which is low crystalline in nature and similar to that of bone appetite. Research has shown that when compared to bone, teeth present with properties like osetoconduction, osteoinduction, and osteogenesis and similar histological properties, which make them a potential alternative to conventional bone graft material. In 2009, the Korean Tooth Bank (KTB) in Seoul, Korea, developed noval bone graft material from patients own extracted teeth. The hospital tooth bank (HTB) in Seoul National University Bundang Hospital 2010 stored and used tooth-developed bone graft material following the guidelines established in accordance with the tooth bank’s definition.

Clinical research on extracted tooth powder or block as bone graft material for dentoalveolar repair concludes that they are effective bone graft material and provide successful clinical, radiographical, and histological outcomes. This development also highlights the dire need for the establishment of a proper tooth banking system for the safe and economical use of extracted teeth.

**Biosafety and ethical concerns for Tooth Bio-Banking**

Extracted human teeth are mostly procured from dental clinics, hospitals, institutes, and, in some countries, also from cemeteries. All of these practises raise serious ethical issues. The unknown origin of EHT acquired for educational, research, or restorative purposes leads to illegal marketing and an increased risk of cross contamination. The establishment of a proper tooth banking system can eliminate the risk of cross contamination and illicit trade in EHT.

 Concerns are raised regarding the use and handling of extracted tooth as there is potential contact with the blood born microbial/pathological agents. This concern is not only associated with the clinicians but also with the individuals collecting the EHT, including students and researchers. In particular, it is important to consider pathogens like the hepatitis B and C viruses (HBV and HCV) and the human immunodeficiency virus (HIV).

International agencies like the Occupational Safety and Health Administration (OSHA) and the Centre for Disease Control and Prevention (CDC) have considered and regulated the proper handling and storage of EHT for the prevention of cross contamination. Human teeth that have been extracted are viewed as a potential source of blood-borne infections by OSHA, and the CDC made suggestions about suitable approaches to preparing and storing extracted human teeth for ex vivo use. Apart from cross-contamination, there is one more concern related to the consent of the patient or Donor of teeth regarding its use after the extraction process.

Sterilizing the extracted tooth appropriately provides the best solution to get rid of the concern related to biosecurity. Various methods are recommended for the purpose of sterilization and disinfection which includes use of autoclave, boiling, iodine-based solution, hydrogen peroxide, quaternary ammonium compounds, glutaraldehyde, formaldehyde, thymol, sodium hypochlorite (NaoCl), ethylene oxide and gamma radiations. According to the literature, the most efficient ways to sterilize an extracted tooth are with 10% formalin, 5.25% sodium hypochlorite, and use of autoclave from 20-40 minutes.

One more concern regarding the utilization of EHT is illegal procurement at a high cost and its unknown origin. Establishing the practice of tooth banking system can completely eliminate the process of illegal and unsafe procurement of extracted teeth and, at the same time, provide critical information to both the donor and the individual procuring them.

**Consideration For Tooth Bio-Banking Set Up**

* Designated collection centres should be established for the safe collection of extracted human teeth to eliminate unethical and illegal utilisation. The initial disinfection procedure should be performed at the collection centre using personal protection equipment.
* Mandatory written informed consent should be obtained from the Donor (Patient) with a detailed medical history to avoid the chances of cross contamination with highly transmissible diseases.
* Proper transportation of EHT to the banking facility should be attained in safe containers while maintaining biosafety and special consideration should be given to extracted teeth to use for stem cell banking.
* EHT should be evaluated microscopically and segregated according to need. Also, sample processing should be done according to the required use of ETH.
* All the discarded extracted teeth should be handled and disposed of according to biosafety norms.

**Conclusion**

Tooth banking is a promising and innovative approach for preserving extracted dental teeth and related tissues for the purpose of dental stem cell preservation and as a resource material in dental teaching, in vitro research, biological restorations, and developing bone graft materials. The establishment of a tooth banking system can potentially unlock the various medical and therapeutic benefits of extracted teeth and pulp stem cells. However, there is an absolute lack of awareness in clinicians and patients regarding safe and effective utilization of extracted human teeth. Also there is no established guidelines form the regulators regarding proper and uniform formation and functioning of tooth bank.

**Reference**

1. Creation and Governance of Human Genetic Research Databases, OECD Publishing, 2006 October 25 ISBN-92-64-02852-8.
2. Hess JR. Conventional blood banking and blood component storage regulation: opportunities for improvement. Blood Transfus. 2010;8 Suppl 3 (Suppl 3):s9-15.
3. Epstein J, Seitz R, Dhingra N, et al. Role of regulatory agencies. Biologicals. 2009;37(2):94–102.
4. Master Z, Crowley AP, Smith C, Wigle D, Terzic A, Sharp RR. Stem cell preservation for regenerative therapies: ethical and governance considerations for the health care sector. NPJ Regen Med. 2020;5(1):23.
5. Zeitlin BD. Banking on teeth - Stem cells and the dental office. Biomed J. 2020 Apr;43(2):124-133.
6. Rosemann A., Luo H.Y. Attitudes towards the donation of human embryos for stem cell research among Chinese IVF patients and students. J Bioethic Inq. 2018;15:441–457.
7. Sivaraman M.A.F. Using surplus embryos and research embryos in stem cell research: ethical viewpoints of buddhist, hindu and catholic leaders in Malaysia on the permissibility of research. Sci Eng Ethics. 2018;24:129–149.
8. Gronthos S., Mankani M., Brahim J., Robey P.G., Shi S. Postnatal human dental pulp stem cells (DPSCs) in vitro and in vivo. Proc Natl Acad Sci U S A. 2000;97:13625–13630.
9. Shi S., Robey P.G., Gronthos S. Comparison of human dental pulp and bone marrow stromal stem cells by cDNA microarray analysis. Bone. 2001;29:532–539.
10. Vishwanathaiah S, Eshaq RH, Maganur PC, Hakami MM, Manqari AI, Mawkili LK, Alhazmi AE, Alsam SM, Jafer AA, Mohammed TI, Jafer MA. Knowledge and Attitude of Undergraduate Students and Interns about Human Tooth Bank: A Cross-sectional Study. Int J Clin Pediatr Dent. 2022;15(1):60-64.
11. Nassif AC, Tieri F, Ana PA, et al. Structuralization of a human teeth bank. Pesqui Odontol Bras. 2003;17(01):70–74.
12. Farias FF. Human tooth bank. Project Bank of Human Teeth by Flávio Furta do de Farias. 2011 June (Accessed on 04/08/23). http://bancodedenteshumanos.blogspot.com/2011/06/projeto-banco-de-dentes-humanos-por\_27.html.
13. Desai AV, Kariya PB, Bansal B. Knowledge, attitude and practice regarding dental stem cells and tooth banking among dental professionals: A questionnaire-based cross-sectional study. Journal of Clinical and Diagnostic Research 2022;16(11):ZC36–ZC42.
14. Katge F, Shetty AJ, Rusawat B, Vamsi KC. Knowledge and attitude of Indian dentists regarding dental stem cells: A cross-sectional descriptive survey. Indian J Dent Res. 2017;28(4):367-374.
15. Arora V, Arora P, Munshi AK. Banking stem cells from human exfoliated deciduous teeth (SHED): saving for the future. J Clin Pediatr Dent. 2009;33(4):289-94.
16. Saber SE. Tissue engineering in endodontics. J Oral Sci. 2009;51(4):495-507.
17. Lahiry S, Choudhury S, Sinha R, Chatterjee S. The National Guidelines for Stem Cell Research (2017): What academicians need to know? Perspect Clin Res. 2019 Oct-Dec;10(4):148-154.
18. Indian Council of Medical Research. National Guidelines for Stem Cell Research. 2017. [Last accessed on 2023 July 17] Available from: <http://www.icmr.nic.in/guidelines/Guidelines_for_stem_cell_research_2017.pdf> .
19. Shaik PS, Yaddanapalli SC, Ravoori S, Pachava S, Lodagala A, Pavani NP. Management of extracted human teeth by dental students in East Coastal Region of India. J Indian Assoc Public Health Dent 2021;19:206-11.
20. Gunwal, M. K., Bagda, K. P., & Mistry, K. Biological restorations: Literature review with proposed definition & classification. International Journal of Health Sciences 2022; 6(S8): 1725–1730.
21. Nawrocka A, Łukomska-Szymańska M. Extracted human teeth and their utility in dental research. Recommendations on proper preservation: A literature review. Dent Med Probl. 2019;56(2):185-190.
22. Albrecht L, Ferreria E, Passos M. Teeth processing in human teeth bank proposal of protocol. RSBO 2013;10(4):386-93.
23. Meza, MS, Michel, IM, Rivas, RA, et al. Obtaining human teeth for dental education: A cross-sectional study to create ethical and transparent processes. J Dent Educ. 2023; 87: 50– 59.
24. Smitha D, Tijare MS, Amith HV, Gujjar KR, Sharma R. Knowledge, attitude and practice regarding handling of extracted human teeth among students of a dental college in Bhopal. J Indian Assoc Public Health Dent. 2014;12:276–82
25. Kim,Y.;System,B.;Kim,Y.;Um,I.;Murata,M.ClinicalReportToothBankSystemforBoneRegeneration-SafetyReport.J.Hard TissueBiol.2014,23,371–376.
26. Cenicante,J.;Botelho,J.;Machado,V.;Mendes,J.J.;Mascarenhas,P.;Alcoforado,G.;Santos,.The Use of Autogenous Teeth for Alveolar Ridge Preservation : A Literature Review. Appl Sci 2021;11:1853.
27. Kim YK, Lee J, Um IW, Kim KW, Murata M, Akazawa T, Mitsugi M. Tooth-derived bone graft material. J Korean Assoc Oral Maxillofac Surg. 2013;39(3):103-11.
28. Gunwal MK, Bagda K, Gupta S, Oak AM. Knowledge, awareness and perception amongst dental practitioners towards natural tooth fragment reattachment procedures in clinical practice-A cross-sectional survey. Dent Traumatol. 2021;37(6):779-785.
29. Gunwal MK, Bagda K, Mistry K. Knowledge, awareness, and practice of postgraduate students towards tooth fragment reattachment procedure and biological restorations - A cross-sectional survey. Eur. Chem. Bull. 2023;12(4):3128-3133.
30. Bhaskar SN Orban BJ. Orban's Oral Histology and Embryology. 11th ed. St. Louis: Mosby Year Book; 1991.
31. Nanci A Ten Cate AR. Ten Cate's Oral Histology : Development Structure and Function. 8th ed. St. Louis Mo: Elsevier; 2013.
32. Zhao R, Yang R, Cooper PR, Khurshid Z, Shavandi A, Ratnayake J. Bone Grafts and Substitutes in Dentistry: A Review of Current Trends and Developments. Molecules. 2021. 18;26(10):3007.
33. Sanan A, Haines SJ. Repairing holes in the head: a history of cranioplasty. Neurosurgery. 1997;40(3):588-603
34. Yeomans, J.D.; Urist, M.R. Bone Induction by Decalcified Dentine Implanted into Oral, Osseous and Muscle Tissues. Arch. Oral Biol. 1967, 12, 999–1008.
35. Bang, G.; Urist, M.R. Bone Induction in Excavation Chambers in Matrix of Decalcified Dentin. Arch. Surg 1967, 94, 781–789
36. Kim, Y.-K.; Lee, J.K.; Kim, K.-W.; Um, I.-W.; Murata, M. Healing Mechanism and Clinical Application of Autogenous Tooth Bone Graft Material; Pignatello, R., Ed.; IntechOpen: London, UK, 2013; Volume 395, ISBN 978-953-51-1051-4
37. Um IW and Murata M. Technical Manual for Korea Tooth Bank. CharmYun Publishing Inc., Seoul, Korea 2011.
38. Sandhu SV, Tiwari R, Bhullar RK, Bansal H, Bhandari R, Kakkar T, Bhusri R. Sterilization of extracted human teeth: A comparative analysis. J Oral Biol Craniofac Res. 2012;2(3):170-5.
39. Chandki R, Maru R, Gunwal M, Garg A, Mishra A. A comparison of different methods for difinfection or sterilization of extracted human teeth to be used for dental education purposes. World J Dent 2013;4(1):29-31.
40. Boyce R, Mull J. Complying with the occupational safety and health administration: Guidelines for the dental office. Dent Clin North Am. 2008;52(3):653–668.
41. Rutala WA, Weber DJ. Disinfection and sterilization: An overview. Am J Infect Control. 2013;41(5 Suppl):S2–S5.
42. Kohn WG, Collins AS, Cleveland JL, Harte JA, Eklund KJ, Malvitz DM; Centers for Disease Control and Prevention. Guidelines for infection control in dental health-care settings: 2003. MMWR Recomm Rep. 2003;52(RR-17):1–61.
43. Holden A. & Dracopoulos S. Owning the tooth: Exploring the ethical and legal issues relating to the use of extracted human teeth in dental education in Australia. Aust. Dent. J. 2017, 62, 146–151.