ISSN- O: 2458 - 868X, ISSN –P: 2458 - 8687

International Journal of Medical Science and Innovative Research (IJMSIR)

IJMSIR : A Medical Publication Hub

Available Online at: www.ijmsir.com

Volume – 3, Issue –1, January - 2018, Page No. : 306 - 315

Evaluation of the Effect of Storage on Sterilization and Structural Integrity of Enamel of Teeth Preserved In A Tooth Bank– An In-Vitro Study

[1]Manpreet Kaur, Reader, College of Dental sciences & Research Centre, Ahmedabad, Gujarat.

[2]Anup Panda, Professor & Head, College of Dental Sciences & Research Centre, Ahmedabad, Gujarat.

[3]Krishna Trivedi Dere, Reader, College of Dental sciences & Research Centre, Ahmedabad, Gujarat.

[4]Mira Virda, Senior Lecturer, College of Dental sciences & Research Centre, Ahmedabad, Gujarat.

[5]Natasha Shiggaon, Reader, College of Dental sciences & Research Centre, Ahmedabad, Gujarat.

Correspondence Author: Manpreet Kaur, Department of Pedodontics & Preventive dentistry, College of Dental Sciences & Research Centre, Ahmedabad-380058, Gujarat, India.

Type of Publication: Original Research Paper

Conflicts of Interest: Nil

Abstract

Objective: The aim of present study was to evaluate the effect of storage on sterilization and structural integrity of enamel of extracted teeth preserved in a Tooth Tissue Bank.

Study design: Forty five freshly extracted teeth were collected and sterilized by ultrasonic waves and 6% H_2O_2 combination. The teeth were divided into three groups (A ,B, and C) with 15 teeth in each group and were stored in Hank's Balanced Salts Solution at 4□C.The sample teeth of group A,B and C were used to measure the effect of storage on sterilization, enamel hardness and enamel calcium solubility respectively at 3,6, 12 and 18 month intervals of time.

Results: More than 50% samples of Group A showed presence of aerobic bacteria after 9 months of storage period. Enamel hardness was not significantly effected up till 3 months of storage period. Enamel solubility increased successively upon storage but increase was not significant till 3 months storage.

Conclusion: Teeth stored in a tooth tissue bank maintain their Structural integrity upto 3 months and remain sterile not beyond 6 months time period.

Keywords: Tooth bank, Enamel hardness, Enamel solubility, Flame photometry

Introduction

Natural enamel and dentin serve as the golden standard in the search for an ideal restorative material. For centuries, scientists have been searching for an ideal material, but in that quest have introduced a large diversity of materials which claim to be close to the properties of natural teeth but do not match them in all biomechanical aspects. So why not use the gold standard itself i.e. natural enamel and dentin.

Many clinicians have reported the reuse of human tooth tissue by means of adhesive re-bonding of traumatically fractured tooth parts [3, 14 ,31 ,49]. Extracted permanent teeth have been employed in removable and fixed prosthesis [26], in composite class- II restorations, reconstruction of cusps and for various other laboratory procedures [46]. However, these applications are dependent on the availability of healthy tooth tissue. The high number of extractions of healthy teeth performed for orthodontic reasons and over retained deciduous teeth can serve as precious biological tissue which has been discarded until now. To enable the large scale availability

of this precious tissue, there is an increasing interest in the preservation of teeth and concept of tooth banks .The tooth banks can be set up in the similar way as blood or bone banks.

A human tooth bank (HTB) is a non-profit institution where the human teeth are preserved, stored and supplied for research or laboratory training of students and for clinical use [39].

For the proper working of HTB it is important to control sterilization and to preserve structural integrity of stored teeth. But the aspects of sterilization procedures and stocking methods are not strictly defined. The best method for extracted tooth sterilization has not yet been defined [44] .

In the last century treating the extracted teeth with hot water or alcohol was used as a method of sterilization , but was not successful in preventing infection[1].There on variety of methods had been tried to achieve asepsis of teeth.

For example, sterilization by gamma radiations has been reported to be an effective method to prevent transmission of bacteria , fungi and viruses [36 ,58].A combination of ultrasonic waves and hydrogen peroxide was reported to be an effective method of cold sterilization possessing bactericidal , fungicidal, and sporicidal properties [1].Center for disease control and prevention [9,10] recommended that extracted teeth used for education and research purpose should be disinfected with sodium hypochlorite or liquid chemical germicides .However, sodium Hypo-chlorite can increase the porosity of human enamel by de-proteinization.

Although sterilization is an important aspect of storing tooth tissue, it is also important to maintain the structural integrity of teeth stored over a period of time in a tooth tissue bank. Various media had been proposed for the purpose of storing teeth but no strict definition of an ideal storage medium has been documented . Cryo-preservation of teeth has been suggested for preservation of tooth tissue in a tissue bank[40,41,61]. $CaCl_2$ -buffered saline solution has been advocated for storage of teeth [25].Use of Hanks Balanced Salts Solution has been recommended [51].But the effects of these media on the physical properties of enamel are still not clear.

Therefore, the present study was undertaken to evaluate the effect of storage on sterilization, enamel solubility and enamel hardness of extracted healthy teeth preserved in a tooth tissue bank.

Materials And Methods

This study was conducted on forty five freshly extracted teeth collected from the outpatient Department of Oral and Maxillofacial Surgery and the Department of Orthodontia, Punjab Govt. Dental College and Hospital, Amritsar.

Teeth free from caries, morphologic defects, previous restoration and cracks were selected as sample teeth. The collected samples were thoroughly scaled, polished and freed of soft tissues and periodontal remnants. The pulps were removed from root canals and samples were stored in de-ionized water until use. The samples were divided into three groups.

Group (A)- A sample of 15 teeth was used for bacteriological examination to study the effect of storage on sterilization of teeth

Group (B)-A sample of 15 teeth was used to study the effect of storage on enamel hardness by Vickers's hardness test.

Group (C)-A sample of 15 teeth was used to study the effect of storage on enamel solubility by determining the amount of soluble calcium ion (Ca^{++}) concentration using flame photometry.

Methodology

Sterilization of teeth.

After preparation , all the sample teeth were taken out from de-ionized water and placed in autoclaved culture tubes containing 5 ml of H_2 O_2 (6%).The tubes were then placed in the ultrasonic tank in upright position .The tank operating at 42 GHz and 100 W output , was filled with water up to the neck of the tubes .Each tooth was sonicated for 30 minutes (Fig 1).

Fig-1 Ultrasonic tank

Bacteriological examination (for group A)

The teeth sterilized by the above method were put into Robertson's cooked meat medium (RCM) and were incubated at 37^0C for 24 hours. To isolate the aerobic microorganisms, a loopful was taken from each sample with the help of standard loop (3.26mm) and was incubated on to sheep agar medium and MacConkey's agar medium. These plates were kept in aerobic environment in the incubator at 37^0C. After 24 hours, the plates were examined for bacterial growth. For anaerobic examination, a loopful from each sample was incubated anaerobically in the anaerobic jar for 48 hours (Fig 2). Gaspak system (Fig 3) was used to create anaerobiosis. When H_2So_4(25%) was added to the sachet, H_2 gas was released which combined with O_2 to form H_2O thus creating anaerobiosis. Methylene blue was used as an indicator. It remained colorless before opening the jar indicating effective anaerbiosis. In addition, a plate inoculated with pseudomonas aeruginosa was also kept in the jar to check the anaerobiosis. After 48 hours, plates were taken out and examined for any significant bacterial growth. In case of growth on culture plates, further inoculation on blood agar medium was done and incubated aerobically at 37^0C to test aero tolerance.

Identification of isolates: The cultures were identified by their colony characteristics, morphology, staining characteristics and biochemical reactions. The growth was examined by taking a smear and staining it with gram stain. The isolated bacteria were identified by studying their motility and by subjecting them to various biochemical tests.

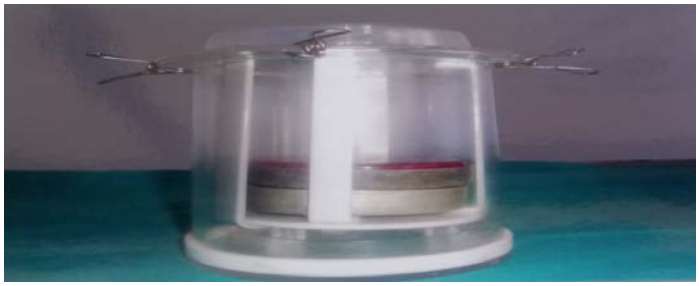

Fig-2 Anearobic jar

Fig-3 Gas Pack

Enamel hardness (for group B)

A suitable technique adopted to test the surface enamel hardness. The hardness of the enamel was tested with a Vickers's micro hardness tester equipped with 136 degree pyramidal diamond point and 1kg load.(fig-4)

Fig-4 Microhardness tester with Vicker's indenter

Enamel calcium solubility (group C)

Sample preparation: To measure the surface enamel calcium solubility a short metal tube of 3mm diameter was fixed end on to an intact enamel surface. The tooth was then completely covered by molten sticky wax and tube carefully removed, exposing an enamel window, which was then cleaned with alcohol and warm water. Each tooth was then subjected to 30 minutes of decalcification in 10 ml 0.2 N-acetic acid adjusted to pH 4 by adding 1N NaOH. The amount of soluble calcium in the acid solution is an indicator of enamel solubility, which was detected by flame photometry (Fig 5). After measuring enamel calcium solubility, the area of decalcification was painted so that new area can be exposed on next examination.

Fig-5 Flame Photometer

All the sample teeth were then cleaned and sterilized. The readings obtained by all these tests were taken as baseline values. The samples were then stored in Hank's balanced salt solution at 4□C temperature in deep freezer. Microbiological examination was done at 3, 6, 9 and 12 month intervals. Enamel hardness and enamel solubility measurements were repeated at 3, 6, 9, 12 and 18 month intervals. The results obtained were compiled, tabulated and statistically analyzed.

Result

Table 1	Presence of aerobic/anaerobic growth									
Sample no.	Time period in months									
	0		3		6		9		12	
	Aerobic	Anaerobic	Aerobic	Anaerobic	Aerobic	Anaerobic	Aerobic	Anaerobic	Aerobic	Anaerobic
1	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Yes	Nil
2	Nil	Nil	Nil	Nil	Nil	Nil	Yes	Nil	Yes	Nil
3	Nil	Nil	Nil	Nil	Nil	Nil	Yes	Nil	Yes	Nil
4	Nil	Nil	Nil	Nil	Nil	Nil	Yes	Nil	Yes	Nil
5	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil
6	Nil	Nil	Nil	Nil	Nil	Nil	Yes	Nil	Yes	Nil
7	Nil	Nil	Nil	Nil	Nil	Nil	Yes	Nil	Yes	Nil
8	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Yes	Nil
9	Nil	Nil	Nil	Nil	Nil	Nil	Yes	Nil	Yes	Nil
10	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Yes	Nil
11	Nil	Nil	Nil	Nil	Nil	Nil	Yes	Nil	Yes	Nil
12	Nil	Nil	Nil	Nil	Nil	Nil	Yes	Nil	Yes	Nil
13	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil
14	Nil	Nil	Nil	Nil	Nil	Nil	Yes	Nil	Yes	Nil
15	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Yes	Nil

Table 2	Enamel hardness of tested teeth					
Sample No.	Time period in months					
	0	3	6	9	12	18
1	332.8	301.0	300.0	288.0	279.4	264.5
2	382.1	343.3	299.2	292.1	282.9	370.0
3	289.3	366.6	370.0	320.5	301.3	280.7
4	366.3	328.0	322.0	301.0	303.7	287.4
5	409.9	359.5	320.2	310.0	300.5	283.3
6	363.7	352.4	310.0	302.0	290.3	278.4
7	381.9	380.1	303.3	270.0	260.0	250.6
8	291.4	359.0	250.4	220.3	220.1	203.1
9	332.7	328.8	330.7	303.0	299.2	279.4
10	372.3	368.2	289.3	250.1	243.0	225.8
11	315.8	304.0	301.1	278.2	201.0	178.7
12	393.0	Broken	Broken	Broken	Broken	Broken
13	289.3	275.9	256.9	250.0	218.6	185.7
14	342.1	323.2	320.1	311.0	300.2	293.1
15	334.3	327.0	301.1	300.0	295.0	256.3
Mean	346.46	336.93	305.31	285.4	271.1	259.8
Standard Deviation	37.53	29.07	28.40	27.53	34.65	48.03

Table 3: Values of student t- test for enamel hardness						
Time (in months)	0	3	6	9	12	18
Mean	346.46	336.93	305.31	285.4	271.1	259.8
Standard deviation	37.53	29.07	28.4	27.53	34.65	48.03
Computed t value	Base	0.95	4.10	6.08	7.51.	8.64
t value from table at 5% confidence level	Base	2.16	2.16	2.16	2.16	2.16
Nature of difference at 5% Confidence level	Base	NS	S	S	S	S

Table 4: Values of student t- test for enamel solubility						
Time(in months)	0	3	6	9	12	18
Mean	38.33	46.4	49.3	56.12	64.76	73.73
Standard deviation	11.75	12.65	11.96	13.78	11.83	10.89
Computed t value	Base	2.66	3.62	5.87	8.71	11.67
t value from table at 5% confidence interval	Base	2.16	2.16	2.16	2.16	2.16
Nature of difference at 5% confidence level	Base	S	S	S	S	S
t value from table at 1% confidence interval	Base	2.977	2.977	2.977	2.977	2.977
Nature of difference at 1% confidence level	Base	NS	S	S	S	S

Table 5	The amount of soluble calcium (PPM) in acetic acid solution					
Sample No.	Time period in months					
	0	3	6	9	12	18
1	50	61	60	79	75	81
2	44	54	59	77	81	92
3	52	60	63.5	66.5	70	78
4	51	59	62	68	81	86
5	48	56	48	50	60	71
6	18	30	34.5	41	49	58
7	25	32	38	42.5	52	59
8	51	58	64.5	71	83	93
9	34	40	42	49	59	67
10	21	20	26	31.5	42	57
11	42	51	54	61.5	66.5	73
12	49	57	62	60	72	78
13	36	42	46	50.5	61	70
14	28	43	44	53.5	62	69
15	26	33	36	41.0	58	74
Mean	38.33	46.4	49.3	56.12	64.76	73.73
Standard Deviation	11.75	12.65	11.96	13.78	11.83	10.89

The microbial growth, Enamel hardness and enamel solubility were measured at 0, 3 ,6 ,9 ,12 and 18 month intervals, are presented in table 1, 2, and 3 respectively. From table 1 it is clear that there is no bacteriological growth (aerobic/anaerobic) till 6 months of storage. But later (after 9 months) 60% samples showed the presence of aerobic bacteria. Bacterial growth was present in almost all the samples (86.6%) after 12 months of storage.

For enamel hardness the value of "t"(0.95)calculated from data (at 3 months) is less than value of t from statistical table , indicating that there is no statistically significant change in the measured hardness after 3 months. Since the value of "t"(4.103) calculated from data(6 months) is more than that of value of t from statistical table, it indicates that there is a statistically significant change in the measured hardness after 6 months. Further computations were made at 9, 12, and 18 month intervals as presented in table 3.

There was no statistically significant change in the measured enamel solubility after 3 months at 1% confidence level, but was present at 5% confidence level. Further computations were made at 9, 12 and 18 month intervals and presented in table 4.

Discussion

The concept of Tooth Banks has evolved from the use of extracted teeth for various purposes in the practice of dentistry. Hence large collections of extracted teeth are required. As the extracted teeth are prone to colonization by various micro-organisms proper sterilization procedure is mandatory for their storage. Teeth should also be stored in a proper medium to maintain physical properties of dental hard tissues. Hence , there is an increasing interest in the preservation of extracted teeth.

Tooth Banks are services that receive teeth from several regions and provide them for restorative , educational and research purposes following a processing protocol in

which teeth are identified, sterilized and individually stored [39]. In the present study, the effect of storage time on sterilization and structural integrity of enamel of extracted teeth stored in Hanks Balanced Salts Solution (HBSS) at 4^0C at 3 month intervals, till 18 months was seen. Teeth selected for storage were freshly extracted premolar teeth which were removed due to orthodontic reasons. It was preferred to free the teeth from pulp and periodontal ligaments to prevent any risk of antigenic reaction, as these tissues contributed to the failure of transplanted teeth due to their strong antigenic property [24,47,48,56]. The teeth were mechanically cleaned, washed and thereafter put through sterilization procedure. Mechanical cleaning reduces the bacterial load of teeth by 10^{-4} approximately which further increases the effectiveness of sterilization[1]. It was also documented that anaerobic organisms appeared to be more affected by handling and cleaning which exposed them to aeration to which they were sensitive. In the present study more than 50% of tooth samples showed the presence of only aerobic microbes (Pseudomonas aeruginosa, Acinenetobacter lwofii, E coli) after 6 month of storage. No anaerobic organisms were found (table 1).

Different methods of sterilization had been used for the extracted teeth in the past but with little success. Comfort MB [11,12] suggested use of antibiotics for prevention of contamination of stored teeth. However the sterility level of 100% could not be achieved with that method. White and Hays[59] have demonstrated the inefficiency of ethylene oxide gas against Bacillus Subtilis spores placed in the pulp chamber of extracted human molars.

Use of silver cations was also suggested. This method was sufficiently antibacterial to cause sterilization of infected dentin. The deposition of silver however modified dentin surfaces[54].

Gamma radiation is often used to sterilize commercial and non- commercial hospital items and was frequently used in scientific literature in relation to tooth sterilization. It was observed that radiation changed the colour of dental structure from white, due to denaturation of organic components of dental substrate [36].Moreover, the apparatus required is not readily available and can not be processed in hospitals. The plant would only be active when sufficient material is available to make it's use economically viable[13].

The method of sterilization by using a combination of ultrasonic waves and H_2O_2 is distinct from other techniques in its effectiveness and practicability,as ultrasonic tanks are readily available and economically viable. Additionally , H_2O_2 can be totally eliminated by washing with sterile water or catalase before clinical use. It was found that when extracted teeth were treated in 6% H_2O_2 solution in an ultrasonic bath at 35^0C for 30 min, no viable organisms could be recovered[1]. Moreover difference in the hardness and solubility of enamel before and after sonication treatment were not statistically significant [1].Various storage medium were tried to store the teeth in human tooth bank to maintain mechanical properties of dental hard tissues including deionized water,$CaCl_2$buffered saline solution,cryopreservation and Hank,s balanced salts solution (HBSS).

Hablitz [51] reported that deionized water lacks calcium and phosphate ions therefore chemical potential for dissolution of enamel and dentin is high and storage in calcium chloride also cannot prevent demineralization because of absence phosphate ions ,the major component of calcified tissue other than calcium. It was suggested that chemical potential of HBSS to dissolve the calcium phosphate is low and surface demineralization is prevented. As HBSS is slightly basic and is highly concentrated in Ca^{++} ,Mg^{++} ,Na^+ ,Po_4^{---} and Cl^-

ions, evidently the composition of HBSS is comparable to the dental mineral phases.

White J,R.V. Faller [57] found that several factors are critical before the application of any technique used to assess de or remineralization, and among them sample preparation procedures are extremely important. Any mechanical preparation was avoided in the samples ,used to measure enamel hardness. Vicker's hardness test involves a diamond pyramid indenter with a 136 degree angle between opposite faces. Because of the simplicity of surface micro hardness method and obvious implication on clinical practice, it has been used widely to measure enamel hardness [5, 32].

According to this study, human enamel showed average hardness of 346±37, prior to storage (table 2).These values were in good agreement with most of the data in the literature where micro indentation was applied to enamel to evaluate it's hardness [15,43,60]. The storage of teeth in HBSS resulted in successive decrease in the enamel hardness (table 2).Statistical description of data using t test (table 3) showed that change was not significant up to 3 months .But after 3 months enamel hardness decreased significantly in a non linear fashion. The hardness reduced up to 25% as compared to the base value, in the time span of 18 month. Likewise enamel calcium solubility increased with time (table 5). Statistical increase in solubility was insignificant (t=2.6, p=0.01) up to three months (table 4). But later the solubility had increased significantly in a non linear manner. The solubility (ppm) increased up to 60% as compared to baseline value. These changes in enamel hardness and enamel solubility thus indicate that mechanical properties of calcified tissues are sensitive to their mineral content [16,22,25,34].

Conclusion

It was clear from the results of this study that when teeth are stored in a Human tooth tissue bank at a temperature of 4^0C, their sterility can be maintained up to a period of 6 months. Moreover, no significant change in enamel solubility and hardness occurs on storage of teeth up to 3 months. However, it is suggested that role of viruses and fungal contaminants should be further investigated, which was not in the domain of this research.

References

1. Ahmed FIK and Russell C .Sterilization of teeth for homogenous transplantation. *British Journal of Oral surgery 1976*:14;143-149.

2. Amaecha BT, Higham SM,Edgar WM."(a)Efficacy of sterilization methods and their effect on enamel demineralization." *Caries Research1998* , 32, .441-446."(b) Effect of sterilization methods on structural integrity of artificial enamel caries for intra-oral cariogenicity test." *J Dent1999*,27 .4,.313-316.

3. Amte G,Sarant H. "Restoration of fractured immature maxillary central incisors using the crown fragements." *Pediatr. Den1986t*, .8,.285-288.

4. Ana Claudia Durante Ramires-Romito,Marcia Tuiolla Wanderley,Marta Dutra Machado Oliveria,Jose Carlos Tettorossi Imparato,Maria Salete Nahas Pires Correa.."Biological restoration of primary anterior teeth."Quintesssence International 2000, .31 .6,.405-411.

5. Arends J,Bosch J, J.Ten.."Demineralization and remineralization evaluation techniques."*J Dent Res1992*l.71,.924-928.

6. Bentley E.M.."The value of ultrasonic cleaners in dental practice"*BDJ1994*,.177 .2, .53-56

7. Berel Perkulis,Warren E. Engelhard, William S. Kramer.."Ultrasonic and Benzalkonium Chloride as a method of sterilizing dental instruments".*ASDC J Child 1970*,.37,.69-78.

8. Brown L R, Wachtel L W, Wheatcroft MG..”Diffusion of niacin through extracted human teeth and its effect on bacterial penetration into dentin”.*J Dent Res1962*, .41,.684-694.

9. Centers for Disease Control..”Recommended infection-control practices for dentistry”.*MMWR1986*,.35,.237-242.

10 .Centers for Disease Control..”Recommandation of prevention of HIV transmission in health care settings”.*MMWR19*87.36,.1S-18S.

11. Comfort M.B..”The prevention of contamination of teeth stroed for transplantation”.*Oral Surg Oral Med Oral Pathol*.1980.49 ,3.200-203.

12. Comfort M.B..”An investigation into the reduction of tooth contamination in delayed transplantation.”*Int.J.Oral.Surg1982*,.11 .3,.122-126.

13.Chandler N.P..”Preparation of dental enamel for use in intra-oral cariogenecity experiments.”*J.Dent*,1990.80 .1,.54-58.

14.Chosak A,Eidelman E..”Rehabilitation of a fractured incisor using the patients natural crown.”*J Dent Child1964*,.31,.19-21.

15.Craig R G.,Peyton F. A. .”The micro-hardness of enamel and dentine.”*J. Dent. Res*.1958, .37 .4,.661-668.

16.Curey J.D., Brear K..”Young’s modulus and yield stress in mammalian mineralized.”*Jour.Mat.Science1990*,.1,.14-20.

17.David K. Warfield,Scott Q. Brington..”Ultrasonic potentiation of the sporicidal activity of gluteraldehyde.” *O. Surg. O. Med. O. Path1982*.,.53 .4,.342-346.

18.Dominici J. T., Eleazer, Clark S. J, Staat R.H., Scheetz J.P..”Disinfection/Sterilization of extracted teeth for dental student use.”*J.Dent.Educ2001*..65,.1278-1280.

19.Doughlas E. parsell, Bradeley M. Stetwart, John R. Parker, Todd G. Nick, Leslie Karns, Roger B. Johnson.. “The effect of steam sterilization on the physical properties and perceived cutting characteristics of extracted teeth.” *J.Dent.Edu1998*..62 .3, .260-263.

20.Erneste Maitland Pafford, Phoenix, Ariz. .”Homogenous transplants of preserved frozen teeth.”*Oral Surgery,Oral Medicine,Oral Pathology1956*..9,.55-70.

21.Eugene A. Pantera..”Sterilization of extracted human teeth.”*J. Dent. Edu1990.* .54 .4,.283-285.

22.Feagin F., T. Koulourides,W.Pigman. .”The characterization of enamel surface demineralization ,remineralization,and associated hardness changes in human and bovine enamel.”*Archs. Oral. Bio1969* .14,.1407-1417.

23.Finn Brudevold..”Study of the phosphste solubility of the human enamel sueface.*”J Dent.Res1948*..27 3,.320-329.

24.Guranick W C. and Schulman L. B. July .”Tooth Transplantation.”*DCNA1962* .499-511.

25.Gustafson M.B., Martin R. B., Gibson V., Storms D. H., Stover S. M., Gibeling J., Griffin LK. . “Calcium buffering is required to maintain bone stiffness in saline solution .*” J. Biomech*.1996 .29, .1191-1194.

26.Helen B. . “Contaminant microbiota associated to extracted human teeth.*” Rev. de Clin . Pesq. Odonto2004*..1 .2, out/dez.

27.Herkstroter F M, Witjer M, Ruben J, Arends J. .” Time dependency of micro-hardness indentations in human and bovine dentine compared human enamel.” *Caries Res1989*, .23,.342-344.

28.Howard Martin. 1976. “Ultrasonic disinfection of the root canal.” *O.Surg. O.Med. O.Path*. .1, .92-98.

29.Ingram G. S. .”Chemical events during tooth dissolution.*” J. Dent. Res.1990* .69. .581-586.

30.Juliana Silva Moura,Lidiany Karla Azevedo Rogrigues,Altair Antoninha Del Bel Cury,Emilena Maria Xisto Lima, Renata Mathus cunha Rodrigues Garcia.”Influence of storage solution on enamel

demineralization submitted to Ph cycling." *J. Appl. Oral*. Sci.2004 12 . 3

31. Kanca J. ."Replacement of a fractured incisor fragment over pulpal exposure".*Quintessence Int.1993* .24,.81-84.

32.Kodaka, Debri, Yamada, Kuroiwa.."Correlation between microhardness and mineral content in sound human enamel." *Caries Res1992*,.26,.139-141.

33. Kumar M,Sequeira P S,Peter S, Bhat GK.."Sterilization of extracted human teeth for educational use."2005 .23 .4,.256-258.

34.Lee S. Y., Lin C. T.." Storage effect on dentine substrate and on resultant composite bond strengths."*J. Oral. Rehabiltation*.1997.24,.823-834.

35.Laird W.R.E., Walmsley A. D.."Ultrasound in dentistry.Part-1 Biophysical reaction." *J Dent.1991* .19,.14-17.

36.Lidiany Karla Azevedo Rodrigues,Jaime Aparecido Cury and marines Nobre dos Santos."The effect of gamma radiation on enamel hardness and its resistance to demineralization in vitro."

37.Miklos Cserepfalvi, Paul J. Price. ."Transplantation of a preserved human tooth to monkey.Preliminary report of case." *J. Dent. Res1968*. 47 .4,.641-643.

38.Mosovich H, Creugars.."The novel use of extracted teeth as a dental restorative material- the 'Natural inlay'." *Journal of dentistry1998*, .26 .1. .21-24.

39.Nassif A C, Tieri F, Da Ana PA, Botta SB, Imparato JC.."Structuralization of a human teeth bank."*Pesqui Odontol Bras2003*.17 .1,.70-74.

40.Politis C, Vrielinck L, Schepers S, Lambrichts I.."Cryopreservation of teeth.Organizational aspects of a tissue bank for tooth tissue."*Acta.Stomatol.Belg1995*. .92 .4..149-154.

41.Richard J. Coburn, P.L. Henriques. ."The effect of cold storage on the potential tooth transplant."*O.Surg.O.Med.O.Path1962*. .15 .1,.99-105.

42.Richborg B,Manne J,Thevsenot A. ."The YAG laser in children's conservative dentistry."*Rev Stomatol Chir.Maxillofac1986*..89,.176-180.

43.Robert C Caldwell, R. W. Gilmore.."Microhardness studies of intact surface enamel." *J. Dent. Res1957*. .36 .5..732-738.

44.Roberta Barcelos, Aline A. Neves, Laura Primo, Ivete Pomarico Ribiro de souza.."Biological restoration as an alternative treatment for primary posterior teeth."*J Clin Pediatr Den*t2003..27 .4,.305-310.

45.Sandra Kalil Bussardori, Marcos Augusto do Rego, Rogerio Junqueira Pereira, Antonio Carlos Guedes Pinto.."Human enamel venner restoration in a deciduous tooth: clinical case." *J Ciin. Pediatr Dent*2003..27 .2,.111-116.

46.Santos JFF, Bianch JR. . "Restoration of severely damaged teeth with resin bonding systems." *Quint. Int1991*. . 22. .611-615.

47.Schulman L. B., ."The transplant of tooth homografts." *J Oral Surg*,1964 . 17, .389-394.

48.Schulman L. B..." Allogenic tooth transplantation." *J Oral Surg1972*, .30 .6,.395-409.

49.Simonsen R. J. . "Traumatic fracture restoration:an alternative use of the acid etch technique." *Quint. Int*.1979 .10, .15-22.

50.Skoog, Holler and Nieman." Principles of instrumental analysis."5th edition, Harcourt college publishers.

51.Stefan Habelitz, Grayson W, Marshall Jr., Mehdi Balooch, Sally J. Marshall. ."Nano-indentation and storage of teeth."*Journal of Biomechanics2002*, .35,.995-998.

52.Sullivan HR.."The solubility of enamel surfaces."*J.Dent.Res1954*,.33 .4,.504-510.

53.Tate WH, White RR..”Disinfection of human teeth for educational purposes.”*J.Dent.Ed.1991*,.54,.583-585.

54.Thibodeau E. A., Handelman S. L., Marquis R.E. .”Inhibition and killing of oral bacteria by silver ions generated with low intensity direct current.”*J.Dent.Res1978*, .57,9,.922-926.

55.Walmsley A. D., Williams A.R. .” Measurment onic tootf cavitational activity with in ultrasonic baths.”*J. Dent1991*, .19.62-66.

56.White E., and Rogers T.E.,.”Effect of primary allogenic tooth transplants on rejection of skin allografts in rabbits.” *J. Dent. Res1967*, .46,.565-570..

57.White J,Faller RV and W.D. Bowman..” Demineralization and remineralization evaluation techniques-added considerations.” *J. Dent. R1992*. .71,.929-933.

58.White J.M., Goodies H.E, Marshalls. J, Marshall G.W..”Sterilization of teeth by gamma radiation.”*J.Dent.Res1994*,.73 .9,.1560-1567.

59.White R R, Hays GL..” Failure of ethylene oxide to sterilize extracted human teeth.” *Dent. Mater1995*..11,.321-323.

60.Xu H. H. K., Smith D. T., Jashanmir S,Romberg E., Kelly J.R., Thompson V P, Rekow E d..”Indentation damage and mechanical properties of human enamel and dentine.” *J. Dent. Res1998*. 77 .3, .472-480.

61.Young Hak Oh, Zhong Minche , Jun Chul Hong, Eun Ju Lee, Seung Jong Lee, Jin Kim..” Cryopreservation of human teeth for future organization of a tooth bank-A preliminary study.” *Cryobiology2005*, .51, .322-329.

62.Zero D T, Rahbek I., Fu J., Proskin H M, Featherstone J. D. B..” Comparison of the Iodide permeability test, the surface micro hardness test,and mineral dissolution of Bovine enamel following acid challenge.”*Caries Res1990*..24, .181-188.

63.Zu-Pyn Yang, Che-Shoa..”A 3-year follow up of a homotransplanted tooth from a tooth bank.” *J. Endo1990*. .16 .1, .34-37.