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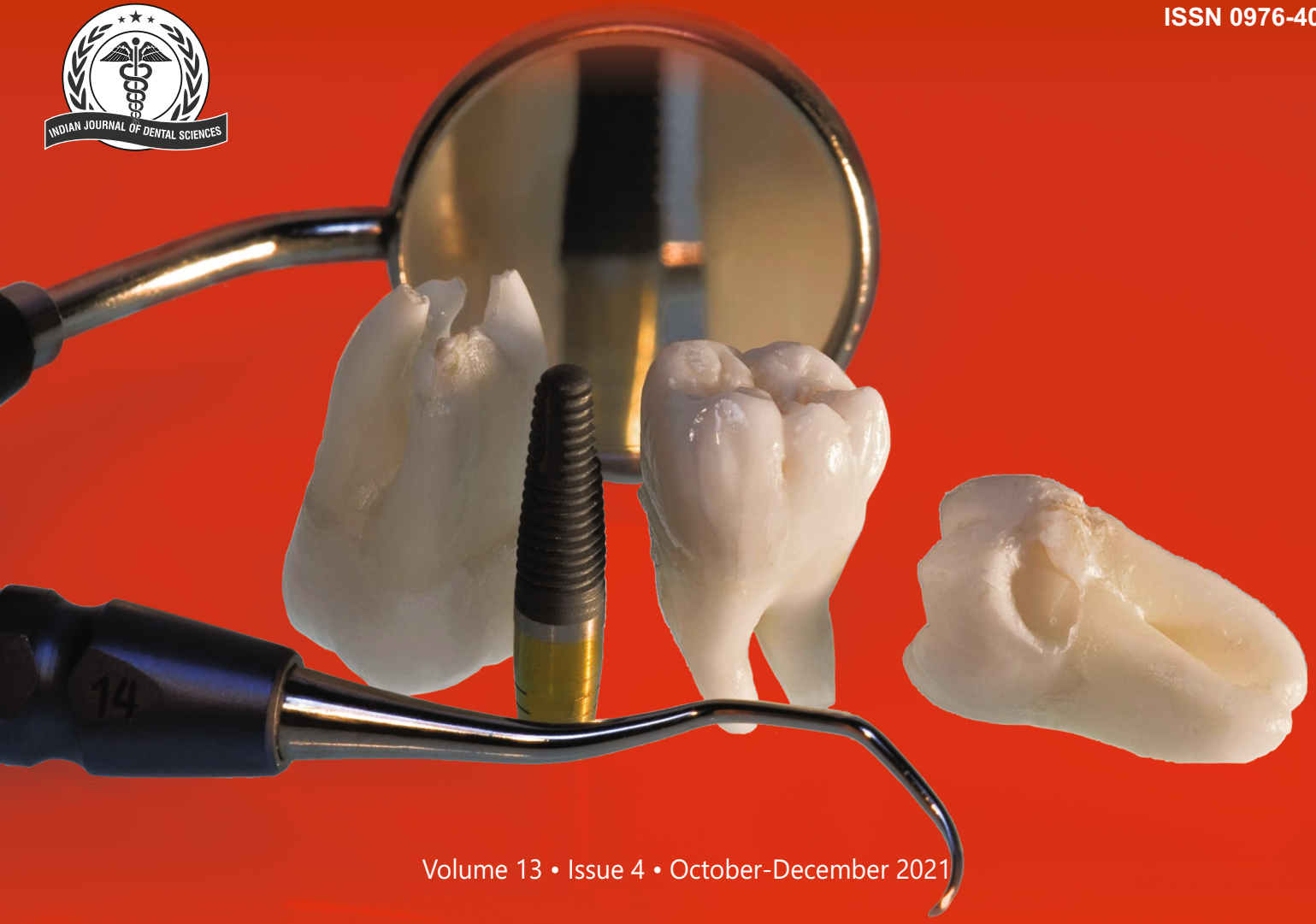

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Original Article

Effectiveness of Chemical Disinfection in Discarding Pathogenic Bacteria of Human Particulate Tooth Graft: An *In vitro* Study

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Abstract

Introduction: Extracted teeth are utilized in dentistry as particulate autologous dentin for immediate grafting of the extraction site after mechanical cleaning and chemical disinfection. The objective of this study was to determine the effectiveness of 0.5M sodium hydroxide in 20% ethanol (Dentin Cleanser™) in eliminating three different types of pathogenic bacteria in comparison to ethylenediaminetetraacetic acid (EDTA) or citric acid. Seven naive extracted teeth were mechanically cleaned, dried, and sectioned to separate the crown from the roots. Each tooth was separately crushed using the Smart Dentin Grinder® device. The sterile particles of crown or root were subdivided into three equal-size groups where each was then contaminated and incubated in an oven at 37°C under low pressure and oxygen flow over 48 h for *Escherichia coli* (Group A) and *Enterococcus faecalis* (Group B) and over 72 h for *Porphyromonas gingivalis* (Group C), respectively. On each agar Petri dish, four paper discs, each loaded with one of the following solutions: Dentin Cleanser (sodium hydroxide plus ethanol), 10% EDTA, phosphate-buffered saline (PBS), or 10% citric acid, were placed in the safe distance for not interfering with disinfectant agent activity. All pathogenic bacteria were highly sensitive to Dentin Cleanser and EDTA disinfectant activity while citric acid or PBS exhibited low or no sensitivity. No difference in sensitivity was found between crown and root particulate or particle size. Our findings show that Dentin Cleanser is most effective in eliminating those pathogenic bacteria without demineralizing the particulate. **Context:** The experiment was done in the University Laboratory. **Aims:** The objective of this study was to determine the effectiveness of 0.5M sodium hydroxide in 20% ethanol (Dentin Cleanser™) in eliminating three different types of pathogenic bacteria in comparison to EDTA or citric acid, before tooth graft will be used as a biomaterial. **Settings and Design:** The study protocol was approved by the Catholic University of Murcia Ethics Committee (UCAM; registration number 6781; July 21, 2017). Seven human teeth were extracted from a 60-year-old patient due to advanced periodontal disease (two central upper incisors, one upper canine, one upper premolar, two lower molars, and one lower canine). The patient received no financial compensation for participating in this study. **Materials and Methods:** Seven naïve extracted teeth were mechanically cleaned, dried, and sectioned to separate the crown from the roots. Each tooth was separately crushed using the Smart Dentin Grinder® device (KometaBio Inc., Cresskill, NJ, USA). The particles were sieved to obtain particles ranging from 400 to 600 um and 800–1200 um in size, all sterilized using an autoclave. The sterile particles of crown or root were subdivided into three equal-size groups where each was then contaminated and incubated in an oven at 37°C under low pressure and oxygen flow over 48 h for *E. coli* (Group A) and *E. faecalis* (Group B) and over 72 h for *P. gingivalis* (Group C), respectively. Then, each subgroup was immersed in 15 agar Petri dishes and again each was inoculated with the same bacteria allowing full growth of bacteria. On each agar Petri dish, four paper discs, each loaded with one of the following solutions: Dentin Cleanser (sodium hydroxide plus ethanol), 10% EDTA, PBS, or 10% citric acid, were placed in the safe distance for not interfering with disinfectant agent activity. **Statistical Analysis Used:** Statistical analysis was performed using PASW Statistics v. 18.0.0 software (SPSS). One-way analysis of variance was applied for the comparison of the means for halos, assuming a level of significance of 95% ($P < 0.05$). Kolmogorov–Smirnov and Shapiro–Wilk tests were applied

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for normality. **Results:** The results of the present study show that the 0.5M sodium hydroxide with 20% ethanol also termed as Dentin Cleanser presented the widest diameter halo of free bacterial growth, most effective against *P. gingivalis* (15.76 ± 0.28), less effective against *E. faecalis* (13.21 ± 0.72) and *E. coli* (12.14 ± 0.12). Similarly, EDTA was effective in eliminating the same strains of bacteria, while citric acid was not effective according to the Duraffourd halo inhibition scale. PBS had no effect in inhibiting bacterial growth. Both the Dentin Cleanser and EDTA were significantly effective in inhibiting bacterial growth in comparison to PBS and citric acid. No significant differences were observed between the dentin particulate and the dentin–enamel mix particulate or human bone marrow related to the level of sensitivity measured for each of the disinfectants tested. **Conclusions:** The use of the Dentin Cleanser (sodium hydroxide plus ethanol) or EDTA 10% appears to be an effective disinfectant method of tooth particulate prior to use of such particulate as autologous grafting material. Although *E. coli, P. gingivalis,* and *E. faecalis* were found to be sensitive to the presence of these disinfectants, additional bacterial types should be further investigated.

Keywords: Autologous graft, bone grafts, dentin graft, dentin grinder disinfection, graft, ground teeth, human teeth, particulate dentin graft, tooth extraction

Introduction

Utilizing extracted human teeth as an immediate source for autologous graft to preserve and augment the alveolar ridge is becoming a standard clinical procedure due to the availability of efficient chairside tooth processing technology.[1-6] In principle, autologous dentin tissue has inherent qualities of autologous bone and presents a negligible risk of exposing patients to disease transfer or contaminants. The material has the potential of becoming the gold standard for socket preservation. However, some concern is evident regarding bacteria that already reside in the biofilm on the extracted tooth. It is still prudent to attempt to clean as much of the bacteria as possible by mechanical cleaning of the tooth following the extraction using a bur or an ultrasonic scaler.[7,8] In addition to mechanical removal of the biofilm and contaminants, some authors proposed to further disinfect the dentin graft using different chemical agents that are employed in root canal disinfection therapies.[9-13] Kim *et al*. reported that root blocks that were disinfected with 1% of chlorhexidine for 10 min enabled their safe use in grafting bone defects, while others used ethanol.[14] Some authors used acid treatment for desmineralizing denting graft also used for bacteria reduction.[3,4] Binderman *et al*. suggested to cleanse and disinfect the autologous dentin particulate before grafting by chemical sterilization using sodium hydroxide 0.5M plus 20% ethanol (Dentin Cleanser, KometaBio Inc., NJ, USA) for 5 min followed by two rinses of the particulate in PBS. This 8-min process is said to render the tooth particulate bacteria free.[6] Calvo-Guirado *et al*. followed Binderman technique in order to use those disinfected teeth as bone graft in postextraction sockets.[15-17]

The objective of this study was to determine the antibacterial activity of dental disinfectant agents during processing of extracted teeth for immediate grafting of the extraction sites. We shall determine the antibacterial activity of (a) Dentin Cleanser 0.5M NaOH and 20% ethanol, (b) 10% of ethylenediaminetetraacetic acid (EDTA), (c) 10% citric acid, and (d) phosphate-buffered saline (PBS) when applied to bacterially contaminated particulate dentin.

Materials and Methods

The study protocol was approved by the Catholic University of Murcia Ethics Committee (UCAM; registration number 6781; July 21, 2017). Seven human teeth were extracted from a 60-year-old patient due to advanced periodontal disease (two central upper incisors, one upper canine, one upper premolar, two lower molars, and one lower canine). The patient received no financial compensation for participating in this study. The patient signed an informed consent form to donate his teeth for use in this *in vitro* study. The teeth were cleaned using straight fissure carbide burs with water irrigation, removing soft tissue, calculus debris, and biofilm and then they were dried with an air syringe. After being cleaned and dried, the teeth were decoronated using a carbide tungsten bur in order to separate the crown from their root [Figure 1]. Then, roots and crowns were pulverized separately using the Smart Dentin Grinder® device (KometaBio Inc., Cresskill, NJ, USA), sieved into specific size ranges, and deposited into four separate glass containers. In this study, we have used two tooth particle sizes of 400–600 and of 800–1200 microns. The separation of crown particulate from root particulate was done in order to examine the effect of disinfectants on dentin versus dentin and enamel mix.

The root and crown particulate source materials were then divided into four different groups, and in addition, a sample of human bone marrow representing cellular soft tissue was added for comparison. The groups are:

- Group A: 800–1200 μm enamel + dentin
- Group B: 400–600 μm enamel + dentin
- Group C: human marrow tissue
- Group D: 800–1200 μm dentin
- Group E: 400–600 μm dentin.

All the particulate dentin and dentin–enamel mix were then autoclave sterilized for 15 min at 121°C. A sample from each group was put into brain–heart infusion broth and incubated for 24 h at 37°C to confirm sterility. Each of the five groups, ground teeth ($n = 4$) and marrow ($n = 1$) were further divided into three subgroups [Figure 2]. At that time, all 15 samples were inoculated, each group with 10 ml of culture medium that included one of the three types of bacteria, *Escherichia Coli*, *Porphyromonas gingivalis,* and *Enterococcus faecalis* for 1 h

at room temperature and then incubated for 24 h in 370º C [Figure 3]. The kits of bacteria were KWIK-STIK™ 2 Pack Catalog No. 0495P of *E. coli* derived from ATCC® 35218, KWIK-STIK™ 2 Pack Catalog No. 0912P of *P. gingivalis* derived from ATCC® 33277™*, and KWIK-STIK™ 2 Pack Catalog No. 0366P *E. faecalis* derived from ATCC® 29212™*. Each KWIK-STIK unit contains a lyophilized microorganism pellet, an ampoule of hydrating fluid, and an inoculating swab. By putting the fluid into lyophilized bacterial pellet, the specific bacterial strain is dissolved and ready for inoculation. After incubation, the infected particulate dentin, dentin–enamel mix, and marrow tissue were transferred into plates with nutrient agar, Columbia blood agar (VWR International Eurolab, SL, Llinars del Vallés, Barcelona, Spain), and Schaedler agar with sheep blood (Remel, Lenexa, KS, USA) + Hemin Vitamin K plates. Each of 15 agar plates was manually infected a second time with one of the three bacteria [Figures 3 and 4]. The inoculation of each of bacteria onto the agar plate was performed by using the inoculating swab. After inoculating, all 15 plates were incubated in an oven at 37°C under low pressure and oxygen flow, for 48 h for the *E. coli* and *E. faecalis* and 72 h for the *P. gingivalis,* respectively [Figure 4].

The tip of the pipette was replaced after each disc impregnation. The following solutions were used for each of the discs [Figure 5]:

- T1: KometaBio Dentin Cleanser – 0.5N sodium hydroxide and 20% ethanol solution [Figure 5a]
- T2: EDTA [Figure 5b]
- T3: PBS [Figure 5c]
- T4: Citric acid 10% [Figure 5d].

After an incubation period, paper discs impregnated with each of the cleansing agents were placed on the infected particulate [Figure 6]. Each of the blank sterile paper discs was impregnated with one of the cleansing agents using a sterile pipette in the amount of 5 ul.

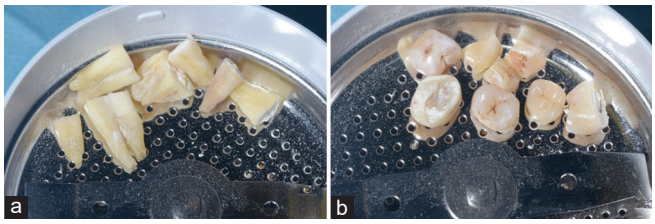

Figure 1: Human roots (a) and crowns (b) inside the Smart Dentin Grinder chamber

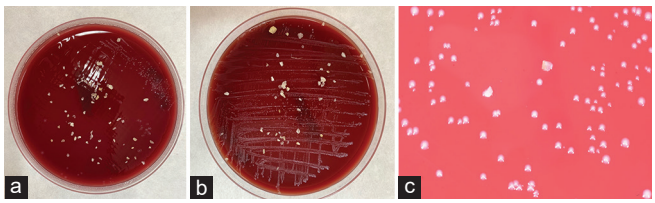

Figure 3: Petri dishes with tooth particles (a); Teeth particles contaminated (b); Big tooth particles surrounded by bacteria (c)

The inhibition halo method was used to assess the sensitivity of the cleansing product, as shown in Figure 6. The measurement of inhibition halos is a technique used in antibiogram to determine the susceptibility of the bacterium against antimicrobial agents such as oral antiseptics.[18,19]

The halo inhibition samples were measured after 24, 48, and 72 h of incubation (37°C), with the help of a millimeter gauge provided by the Laboratory of Bacteriology and Mycology of the Catholic University of Murcia. The samples were observed with the naked eye at 30 cm away from the eyes. To measure the inhibition halos, the Petri dishes were placed on a white grid paper background with a millimeter interval grid. The grid was then aligned with the center of each blank disc so that the diameter of each halo could be easily measured through the center of the disc. Measurement was taken from one side of the ingestion zone to the other side of it [Table 1]. The bigger the halo effect, the more sensitive the bacteria is to the cleansing product, or the more effective the disinfectant is [Figure 7]. We measured the bacterial halo inhibition diameters at 24, 48, and 72 h and correlated these measurements to bacterial sensitivity by following the Duraffourd scale. For the interpretation of qualitative results, the guidelines by Duraffourd 1983 were taken as a reference.[20] These are:

- Negative (−) halo less than or equal to 8 mm in diameter

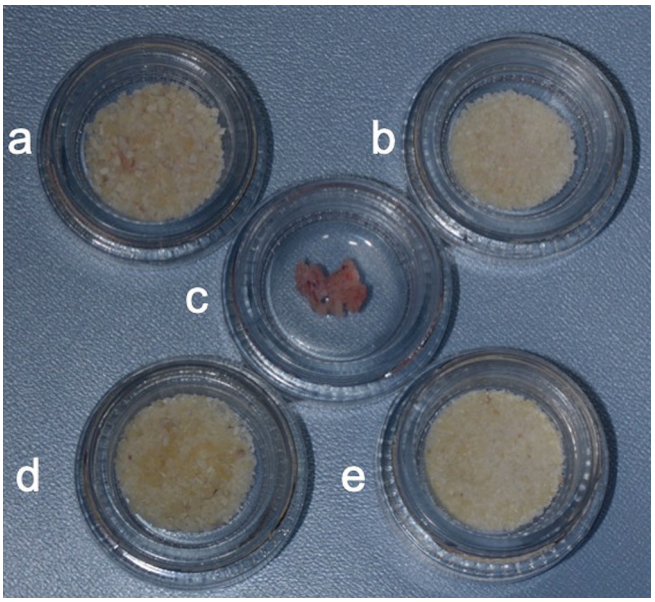

Figure 2: (a) 800–1200 um enamel particulate; (b) 400–600 um enamel particulate; (c) Marrow human bone pieces; (d) 800–1200 um dentin particulate; (e) 400–600 um dentin particulate

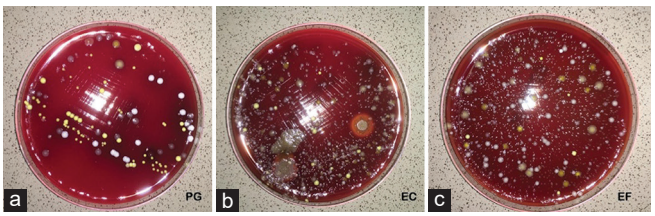

Figure 4: Dentin particles contaminated with *Porphyromonas gingivalis*, *Escherichia coli,* and *Enterococcus faecalis*

Figure 5: The following solutions were used for each of the discs: (a) KometaBio Dentin Cleanser – 0.5N Sodium hydroxide and 20% ethanol solution; (b) Ethylenediaminetetraacetic acid; (c) Phosphate-buffered saline; (d) Citric acid 10%

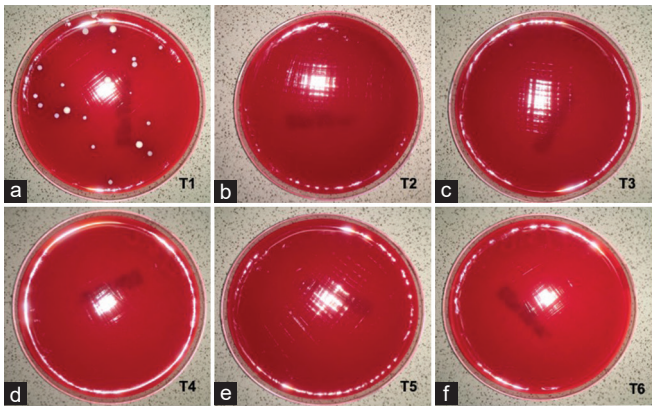

Figure 6: Three bacteria used in the study, (a and b) Germ 1 *Escherichia coli*; (c and d) Germ 2 *Enterococcus faecalis*; (e and f) Germ 3 *Porphyromonas gingivalis*

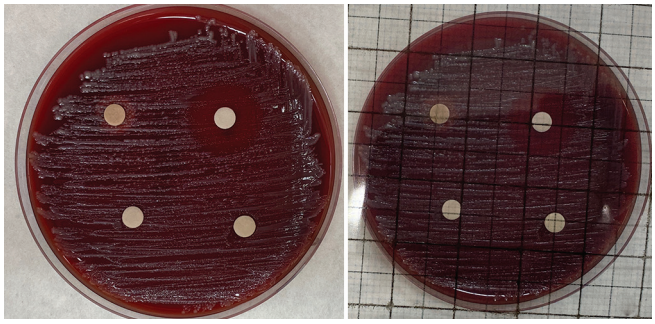

Figure 7: Method of measuring the size of the halos

- Sensitive (sensitive = +) halo from 9 to 14 mm
- Very sensitive (very sensitive = ++) halo from 15 to 19 mm
- Highly sensitive or positive (H. S. = +++) if it was equal to or >20 mm.

Statistical analysis

Statistical analysis was performed using PASW Statistics v. 18.0.0 software (SPSS)(Armonk, New York 10504-1722 United States of América). One-way analysis of variance was applied for the comparison of the means for halos, assuming a level of significance of 95% ($P < 0.05$). Kolmogorov–Smirnov and Shapiro–Wilk tests were applied for normality.

Results

The results of the present study show that the 0.5M sodium hydroxide with 20% ethanol also termed as Dentin Cleanser presented the widest diameter halo of free bacterial growth, most effective against *P. gingivalis* (15.76 ± 0.28), less effective against *E. faecalis* (13.21 ± 0.72) and *E. coli* (12.14 ± 0.12). Similarly, EDTA was effective in eliminating the same strains of bacteria [Table 1], while citric acid was not effective according to the Duraffourd[20] halo inhibition scale. PBS had no effect in inhibiting bacterial growth. Both the Dentin Cleanser and EDTA were significantly effective in inhibiting bacterial growth in comparison to PBS and citric acid [Table 2].

No significant differences were observed between the dentin particulate and the dentin–enamel mix particulate or human bone marrow related to the level of sensitivity measured for each of the disinfectants tested [Table 3].

Discussion

The present study reveals that the Dentin Cleanser that consists of sodium hydroxide and ethanol is most effective in eliminating the pathogenic bacteria of the biofilm of tooth surfaces. Sodium hydroxide solution is being used as a cleansing agent because of its defatting properties in many medical applications. It is also effective in degrading proteins and nucleic acids as well as inactivating most viruses, bacteria, yeasts, fungi, and endotoxins. It is, therefore, considered a potent disinfection agent, as documented in several studies.[21-23] Thalhimer and Palmer reported that sodium hydroxide showed marked germicidal activity even in a dilution of 1: 2500,[21] while Olitsky and Boëz reported that sodium hydroxide was

Table 1: Bacterial inhibition halos diameter (mean±standard deviation)

Samples	Microorganism (mean±SD)			
	Escherichia coli (mm)	*Porphyromonas gingivalis* (mm)	*Enterococcus faecalis* (mm)	*P*
Dentin Cleanser	12.14±0.12	15.76±0.28	13.21±0.72	0.002*
EDTA	10.11±0.34	14.72±0.91	12.19±0.24	0.037*
Citric acid	5.32±0.17	4.67±0.11	5.21±0.72	3.287
PBS	NIH	NIH	NIH	7.892

*Significant P<0.05 in comparison to PBS. NIH: No inhibition halos detected, PB: Phosphate buffered, SD: Standard deviation, PBS: Phosphate-buffered saline, EDTA: Ethylenediaminetetraacetic acid

Table 2: Kolmogorov-Smirnov and Shapiro-Wilk tests

	Normality test					
	Kolmogorov-Smirnov			Shapiro-Wilk		
	Statistic	Sample	Significance	Statistic	Time	Significance
Dentin Cleanser	0.164	90	0.002*	0.562	90	0.003*
EDTA	0.211	90	0.006*	0.645	90	0.041*
Citric acid	0.445	90	0.641	0.891	90	0.621
PBS	0.872	90	0.976	0.988	90	0.922

*Significance P<0.05. Data showed that Dentin Cleanser and EDTA acid were the most effective in tooth particle disinfection.
EDTA: Ethylenediaminetetraacetic acid, PBS: Phosphate-buffered saline

Table 3: Different cleansers for crushed teeth in order to disinfect the particles

Sample	T1	T2	T3	T4
Dentin graft contaminated with *Porphyromonas gingivalis, Enterococcus faecalis*, and *Escherichia coli*	Dentin Cleanser ethanol 20%	EDTA 10%	PBS saline	Citric acid 10%
800-1200 um enamel	N	N	P	P
400-600 um enamel	N	N	P	P
Marrow human bone	N	N	P	P
800-1200 um dentin	N	N	P	P
400-600 um dentin	N	N	P	P

N: Negative for bacteria, P: Positive for bacteria, EDTA: Ethylenediaminetetraacetic acid, PBS: Phosphate-buffered saline

efficient against viruses.[24] The second active ingredient in the Dentin Cleanser solution, ethanol, is known for its denaturation action of proteins, effectiveness against a wide spectrum of microbial species. It is safer than other disinfectants because ethanol easily evaporates, and the human body can also metabolize ethanol. Altogether, the Dentin Cleanser is both a strong cleansing and disinfecting agent being able to penetrate and discard the biomass in the dentin tubules, exposing the clean surface of mineralized dentin matrix.

On the other hand, EDTA is a chelating agent that has been shown to remove bacteria by binding Mg2+ and Ca2+ ions from the outer cell wall of bacteria and eliminate biofilm colonization and proliferation by reducing their adhesion to particulate surfaces.[25,26] The antimicrobial effects of EDTA have been demonstrated for a range of clinical microorganisms that include Gram-negative and Gram-positive bacteria, yeasts, ameba, and fungi. Furthermore, EDTA as a cation chelator of calcium, magnesium, and zinc is inactivating metabolic and energy functions of bacteria, thus inhibiting their growth and function. Although these two agents are both effective in eliminating bacteria, as we show in this *in vitro* study, we believe that in the context of using these agents on dentin particulate, the two work differently. The Dentin Cleanser (sodium hydroxide plus ethanol) exposes the dentin to a high alkaline environment that breaks down proteins and removes nucleic acids as well as cleanses the biomass from dentin particulate surfaces. EDTA, on the other hand, as a chelating agent reduces the binding of bacteria to dentin surface and their growth. Moreover, EDTA is known to dissolve hydroxyapatite dentin mineral by chelating its calcium, thus exposing its organic collagenous matrix. In contrast, 10% of citric acid, that is able to dissolve the dentin mineral, was not effective in eliminating the pathogenic bacteria.

Conclusions

The use of the Dentin Cleanser (sodium hydroxide plus ethanol) or EDTA 10% appears to be an effective disinfectant method of tooth particulate prior to the use of such particulate as autologous grafting material. Although *E. coli, P. gingivalis,* and *E. faecalis* were found to be sensitive to the presence of these disinfectants, additional bacterial types should be further investigated.

Ethical clearance

The Ethical Comitee 7212/2018. San Antonio Catholic University of Murcia.

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Nil.

Conflicts of interest

There are no conflicts of interest.

References

1. Kim YK, Yun PY, Um IW, Lee HJ, Yi YJ, Bae JH, *et al*. Alveolar ridge preservation of an extraction socket using autogenous tooth bone graft material for implant site development: Prospective case series. J Adv Prosthodont 2014;6:521-7.
2. Pang KM, Um IW, Kim YK, Woo JM, Kim SM, Lee JH. Autogenous demineralized dentin matrix from extracted tooth for the augmentation of alveolar bone defect: A prospective randomized clinical trial in comparison with anorganic bovine bone. Clin Oral Implants Res 2017;28:809-15.
3. Gomes MF, Abreu PP, Morosolli AR, Araújo MM, Goulart Md. Densitometric analysis of the autogenous demineralized dentin matrix on the dental socket wound healing process in humans. Braz Oral Res 2006;20:324-30.
4. Gomes MF, dos Anjos MJ, Nogueira TO, Guimarães SA. Histologic evaluation of the osteoinductive property of autogenous demineralized dentin matrix on surgical bone defects in rabbit skulls using human amniotic membrane for guided bone regeneration. Int J Oral Maxillofac Implants 2001;16:563-71.
5. Mazor Z, Horowitz R, Prasad H, Kotsakis G. Healing dynamics following alveolar ridge preservation with autologous tooth structure. Int J Periodontics Restorative Dent 2019;39:697-702.
6. Binderman I, Gideon H, Sapoznikov L. A novel procedure to process extracted teeth for immediate grafting of autogenous dentin. J Interdiscip Med Dent Sci 2014;2:6.
7. Joshi CP, D'Lima CB, Samat UC, Karde PA, Patil AG, Dani NH. Comparative alveolar ridge preservation using allogenous tooth graft versus free-dried bone allograft: A randomized, controlled, prospective, clinical pilot study. Contemp Clin Dent 2017;8:211-7.
8. Valdec S, Pasic P, Soltermann A, Thoma D, Stadlinger B, Rücker M. Alveolar ridge preservation with autologous particulated dentin – A case series. Int J Implant Dent 2017;3:12.
9. Tsesis I, Elbahary S, Venezia NB, Rosen E. Bacterial colonization in the apical part of extracted human teeth following root-end resection and filling: A confocal laser scanning microscopy study. Clin Oral Investig 2018;22:267-74.
10. Stauffacher S, Lussi A, Nietzsche S, Neuhaus KW, Eick S. Bacterial invasion into radicular dentine – An *in vitro* study. Clin Oral Investig 2017;21:1743-52.
11. Soares JA, Leonardo MR, Da Silva LA, Tanomaru Filho M, Ito IY. Histomicrobiologic aspects of the root canal system and periapical lesions in dogs' teeth after rotary instrumentation and intracanal dressing with Ca (OH) 2 pastes. J Appl Oral Sci 2006;14:355-64.
12. Gomes BP, Pinheiro ET, Gadê-Neto CR, Sousa EL, Ferraz CC, Zaia AA, *et al.* Microbiological examination of infected dental root canals. Oral Microbiol Immunol 2004;19:71-6.
13. Sousa BC, Gomes FD, Ferreira CM, Rocha MM, Barros EB, Albuquerque DS. Persistent extra-radicular bacterial biofilm in endodontically treated human teeth: Scanning electron microscopy analysis after apical surgery, Microsc Res Tech 2017;80:662-7.
14. Kim YK, Pang KM, Yun PY, Leem DH, Um IW. Long-term follow-up of autogenous tooth bone graft blocks with dental implants. Clin Case Rep 2017;5:108-18.
15. Calvo-Guirado JL, Ballester-Montilla A, De Aza PN, Fernández-Domínguez M, Alexandre Gehrke S, Cegarra-Del Pino P, *et al*. Extracted human teeth characterization by SEM - EDX evaluation as a biomaterial for socket preservation: An *in vitro* study. Materials (Basel) 2019;25:12.
16. Calvo-Guirado JL, Maté-Sánchez de Val JE, Ramos-Oltra ML, Pérez-Albacete Martínez C, Ramírez-Fernández MP, Maiquez-Gosálvez M, *et al*. The use of tooth particles as a biomaterial in post-extraction sockets. Experimental Study in Dogs. Dent J (Basel) 2018;6:6.
17. Calvo-Guirado JL, Cegarra Del Pino P, Sapoznikov L, Delgado Ruiz RA, Fernández-Domínguez M, Gehrke SA. A new procedure for processing extracted teeth for immediate grafting in post-extraction sockets. An experimental study in American Fox Hound dogs. Ann Anat 2018;217:14-23.
18. Clinical Laboratory Standards Institute. Performance Standards for Antimicrobial Disk Susceptibility Tests; Approved Standard – 9th ed. CLSI document M2-A9. Vol. 26. Wayne, PA: Clinical Laboratory Standards Institute; 2006. p. 1.
19. Bauer AW, Perry DM, Kirby WM. Single disc antibiotic sensitivity testing of Staphylococci. AMA Arch Intern Med 1959;104:208-16.
20. Duraffourd C, D' Hervicourt L, La Praz JC. Cuaderno de Fitoterapia Clínica. France: Editorial Masson; 1983.
21. Thalhimer W, Palmer B. A comparison of the bactericidal action of quinone with that of some of the commoner disinfectants. J Infect Dis 1911;9:181-9.
22. Ernest C. McCulloch. The germicidal efficiency of sodium hydroxide. J Bacteriol 1933;25:469-93.
23. Myers RP. The effect of hydroxyl ion concentration on the thermal death rate of bacterium coli. J Bacteriol 1928;15:341-56.
24. Olitsky PK, Boëz L. Studies on the physical and chemical properties of the virus of foot-and-mouth disease: IV. Cultivation experiments. J Exp Med 1927;45:833-48.
25. Finnegan S, Percival SL. EDTA: An antimicrobial and antibiofilm agent for use in wound care. Adv Wound Care (New Rochelle) 2015;4:415-21.
26. Gray GW, Wilkinson SG. The effect of ethylenediaminetetraacetic acid on the cell walls of some gram-negative bacteria. J Gen Microbiol 1965;39:385-99.