

Article

Microbiological Purity of Autogenous Dental Augmentative Material After Processing with an Alkaline Ethanol Solution—In Vitro Study

Adam Jaworski ¹, Ireneusz Zawisławski ² , Magdalena Pajaczkowska ³ , Joanna Nowicka ³ , Piotr Kosior ^{4,5} , Adam Watras ^{6,*} , Maciej Dobrzyński ⁷  and Rafał J. Wiglusz ^{6,*} 

¹ SP ZOZ MSWiA w Szczecinie, ul. Jagiellońska 44, 70-382 Szczecin, Poland; adam_jaworski@onet.eu

² Department of Human Nutrition, Faculty of Biotechnology and Food Science, Wrocław University of Environmental and Life Sciences, Józefa Chełmońskiego 37, 51-630 Wrocław, Poland; ireneusz.zawislak@upwr.edu.pl

³ Department of Microbiology, Faculty of Medicine, Wrocław Medical University, Chałubińskiego 4, 50-367 Wrocław, Poland; magdalena.pajaczkowska@umw.edu.pl (M.P.); joanna.nowicka@umw.edu.pl (J.N.)

⁴ Department of Conservative Dentistry with Endodontics, Wrocław Medical University, Krakowska 26, 50-425 Wrocław, Poland; dr-kosior@o2.pl

⁵ Faculty of Health Sciences, Angelus Silesius Academy of Applied Sciences in Wałbrzych, Zamkowa 4, 58-300 Wałbrzych, Poland

⁶ Institute of Low Temperatures and Structure Research, Polish Academy of Sciences, Okólna 2, 50-422 Wrocław, Poland

⁷ Department of Pediatric Dentistry and Preclinical Dentistry, Wrocław Medical University, Krakowska 26, 50-425 Wrocław, Poland; maciej.dobrzynski@umw.edu.pl

* Correspondence: a.watras@intibs.pl (A.W.); r.wiglusz@intibs.pl (R.J.W.)

Abstract

Introduction: Teeth intended for use as autogenous augmentative material may carry microbiological contamination, which can compromise the safety of regenerative procedures in the oral cavity. Therefore, effective disinfection protocols are crucial to ensure the microbiological purity of dentin-derived graft materials. **Objective:** This study aimed to evaluate the effectiveness of a 30% alkaline ethanol solution containing 0.5 M sodium hydroxide in eliminating microorganisms from dentin material intended for autogenous augmentation. **Materials and Methods:** The study included 44 extracted teeth that were processed using the Smart Dentin Grinder procedure. The presence of microorganisms was analysed using standard microbiological methods before and after treatment with the disinfectant solution. Additionally, the potential association between tooth eruption status and the effectiveness of the disinfection process was evaluated using Fisher's exact test, with odds ratios calculated using the Haldane–Anscombe correction to address zero cell counts. **Results:** Processing dentin in a 30% alkaline ethyl alcohol solution containing 0.5 M sodium hydroxide is an effective method for eliminating microorganisms, thereby rendering the material completely microbiologically pure. **Conclusions:** The dentine processing procedure used appears to ensure the production of autogenous material free from microbiological contamination, indicating its potential safety in clinical applications.

Keywords: autogenous augmentation; dentin; microbial contamination; autografts; disinfection; dental materials



Academic Editor: Iole Vozza

Received: 7 November 2025

Revised: 22 December 2025

Accepted: 23 December 2025

Published: 25 December 2025

Copyright: © 2025 by the authors.

Licensee MDPI, Basel, Switzerland.

This article is an open access article distributed under the terms and conditions of the [Creative Commons Attribution \(CC BY\)](https://creativecommons.org/licenses/by/4.0/) license.

1. Introduction

Tooth extraction is a standard dental and maxillofacial surgical procedure, usually necessary due to advanced tooth decay, gum disease, periapical inflammation, mechanical

trauma, or overcrowding, particularly in the case of wisdom teeth [1–7]. Following tooth extraction, the alveolar socket often requires augmentation with a bone substitute to enable proper tissue regeneration before planned implantation. Various materials can be used for this purpose, including autogenous, allogeneic, xenogeneic, and alloplastic materials [8].

The first attempts to use dental material for reconstructing bone defects were made in 1967. Over the following years, the importance of biomaterials obtained from teeth increased, and they were analysed more frequently in scientific research. This led to the development of the method and the first use of demineralised dental material in allogeneic transplantation in humans in 1975. Then, in 2003, an autogenous transplantation procedure was performed. In 2015, a freshly demineralised tooth was probably used for the first time as material for autogenous transplantation [9–13]. Many technological solutions and devices for grinding and processing teeth have been developed over the years, enabling them to be used as an autogenous bone substitute [14–18]. One such device is the Smart Dentin Grinder[®], manufactured by KometaBio Inc., Cresskill, NJ, USA.

Autogenous processed dentin (autogenous dentin/tooth graft) is increasingly being used in alveolar ridge reconstruction, particularly in procedures such as socket preservation following tooth extraction or bone augmentation before implant placement. In a prospective clinical study, Kim et al. [19] used autogenous tooth bone graft material (AutoBT), made from crushed dentin, to fill post-extraction sockets in 15 patients. This allowed for implant stabilisation and minimised bone loss at the 22.5-month follow-up. A randomised clinical trial comparing autogenous whole tooth grafts (AWTG) and autogenous demineralised dental grafts (ADDG) also observed bone regeneration without signs of inflammation and found that the newly formed bone integrated with the graft [20]. In turn, the studies by Alramli et al. [21] involved sinus lift augmentation using dentin matrix combined with PRF (platelet-rich fibrin). CBCT imaging and histomorphological studies showed the formation of new bone surrounding the processed dentin particles. Pogorzelska et al. [22] presented a case study of a 42-year-old female patient for whom autogenous dentin material, prepared from her ground tooth 18 using a Smart Dentin Grinder device, was used to raise the floor of the maxillary sinus. Following a two-year observation period, the implant in the area of tooth 16 was found to be stable, with no resorption or adverse radiological changes observed at the graft site. Another clinical report used demineralised dentin from a deciduous tooth to regenerate and preserve the alveolar socket. After two years of observation, normal mineralisation and integration with the bone were observed [23]. A meta-analysis by Mahardawi et al. [24] indicates that autogenous dentin grafting is effective in preserving ridge width after tooth extraction, making it an attractive option in alveolar ridge surgery. Processed dentine therefore combines the advantages of osteoinduction and osteoconduction while using autologous material, reducing the immunological risk and eliminating the need to harvest bone from other sites.

The KometaBio Inc. protocol for obtaining autogenous dental material begins with the proper preparation of a previously extracted tooth. This involves removing fillings and prosthetic restorations and cleaning the periodontal ligament surface to remove any remnants, deposits, and tartar. Additionally, discoloured dentin and carious tissue must be removed. Once prepared, the tooth can be processed in the Smart Dentin Grinder[®], which involves grinding and sieving through a set of vibrating sieves of varying diameters and takes approximately 20 s. The resulting granulate consists of grains sized 300–1200 micrometres. To minimise material loss, this process is repeated several times. To eliminate bacteria and their toxins, the obtained granules are treated with an alkaline ethyl alcohol solution containing 30% 0.5 M sodium hydroxide. Next, the material is washed twice with sterile phosphate-buffered saline. The final, optional stage involves thermal treatment of the material by roasting it at 140 °C using an electric hot plate. The dry material obtained in

this way can be used for auto-transplantation. Demineralised dentin can be utilised for post-extraction socket augmentation, maxillary sinus floor elevation, and periodontal tissue regeneration [16,25].

In vitro studies of the Dentin Cleanser™ action and its main ingredients—sodium hydroxide and ethanol—indicate that these substances exhibit clear antimicrobial properties against certain oral bacteria. The 0.5 M NaOH solution containing 20% ethanol—the standard composition of Dentin Cleanser™—effectively inhibits the growth of periodontopathogenic and endodontic bacteria such as *Porphyromonas gingivalis* and *Enterococcus faecalis*. This has been demonstrated by the diffusion method in a study by Calvo-Guirado et al. [26], among others. In contrast, PBS lacks bactericidal properties and serves only as a neutral buffer in culture models. Kommerein et al. [27] showed the effectiveness of ethanol solutions in various dilutions (REPHA-OS® containing 69% ethanol or analytical ethanol solution (69%)), indicating that its effect on oral biofilms depends on concentration: low concentrations only cause a stress response in bacteria, while higher concentrations destroy biofilm structures. On the other hand, NaOH is a strong base that alters the pH, making the environment unfavourable for the survival of many bacterial species. However, its effect on complex oral biofilms has not yet been comprehensively studied [28]. These analyses suggest that, while the main components of Dentin Cleanser™ exhibit antimicrobial activity, extensive research evaluating their effect on the multi-species microflora of the oral cavity and biofilms characteristic of clinical conditions is lacking.

The normal range of permanent teeth in young adults is 28–32 [29–36]. Third molars often lack sufficient space to erupt fully. Due to their position and the limited available space, they usually become impacted or cause dental problems requiring their removal [5,7,37–39]. A completely impacted tooth is fully embedded in the jawbone and has not broken through the gum, meaning it has no contact with the bacteria present in the mouth or the heavy metals that may be found in food, water, or the air we breathe in. On the other hand, a partially impacted tooth has broken through the gum but is blocked or unable to erupt fully. Their difficult-to-clean position makes partially impacted teeth more susceptible to infection and decay, as it is impossible to maintain proper hygiene, thereby contributing to bacterial accumulation. Furthermore, a partially impacted tooth is in contact with the external environment, which can lead to contamination by heavy metals [40–46].

The oral microbiota of a healthy person consists of a complex community of microorganisms that naturally colonise various surfaces in the mouth, including the tongue, gums, teeth, tonsils, palate, and inner cheeks. This ecosystem consists mainly of bacteria, but also includes fungi, viruses, and archaea. The genera *Bifidobacterium*, *Lactobacillus*, *Peptococcus*, *Fusobacterium*, *Staphylococcus*, and *Streptococcus* naturally occur in the oral cavity. Maintaining the biological balance of the microbiota supports carbohydrate digestion and protects the body [47–53]. However, some of these microorganisms can become etiological factors in infection when they enter the post-extraction wound. Therefore, materials used for alveolar ridge regeneration must be free of pathogens.

The Purpose of the Study

This study aimed to evaluate the effectiveness of a 30% alkaline ethanol solution containing 0.5 M sodium hydroxide in eliminating microorganisms from dentin material intended for autogenous augmentation.

2. Materials and Methods

Before commencing the study, approval was obtained from the Bioethics Committee of the Medical University of Wrocław (No. 278/2019). The research material was received at the end of 2019/beginning of 2020 from the Independent Public Healthcare Centre of

the Ministry of Internal Affairs and Administration in Szczecin. All participants were informed of the study's purpose before providing informed consent for the use of their extracted tooth.

2.1. Research Material

The research material consisted of 44 third molars from 44 patients aged 16–47 who underwent surgical extraction. The extraction of completely impacted teeth was performed without separating them. Half of the teeth were partially affected, and the other half were completely impacted.

2.2. Preparation of the Research Material

All extracted teeth were processed using a single standardised protocol. Only teeth without any signs of carious lesions were included in the study; specimens presenting even minimal initial caries were excluded from further preparation.

Each tooth was treated as a separate sample. After extraction, the teeth were stored at -80°C for one month before processing. Each sample was then placed in the chamber of a KometaBio Smart Dentin Grinder (KometaBio Inc., Cresskill, NJ, USA) and processed according to the manufacturer's instructions (see Figures 1 and 2).



Figure 1. The tooth grinding process (duration: 30 s).

The material obtained was divided into two samples: a control and a research sample. The test samples were flooded with the KometaBio Dentin Cleanser solution (KometaBio Inc., Cresskill, NJ, USA) included in the kit for 10 min to moisten and cover all the material resulting from grinding and sorting the tooth. After 10 min, any excess solution was drained off using sterile gauze pads. Next, the dentin material was flooded with a dedicated Dulbecco's Phosphate-Buffered Saline KometaBio solution (KometaBio Inc., Cresskill, NJ, USA) for 3 min. As in the previous step, the excess fluid was absorbed using sterile gauze pads.



Figure 2. Material sorting process (duration: 10 s).

2.3. Microbiological Evaluation

Both the control and test samples were transferred to 10 mL of liquid thioglycollate broth (Biomaxima, Lublin, Poland). The samples were incubated at 37 °C for 10 days, with microorganism growth monitored every 24 h.

After incubation, the solid media were inoculated to identify the anaerobic and aerobic bacteria present. Schaedler agar containing 5% sheep blood (BD, Heidelberg, Germany) was used for anaerobic bacteria (37 °C, 48 h in the presence of the GENbag atmosphere generator, anaer (Biomereix, Marcy-l'Étoile, France)), and Columbia agar (BD, Heidelberg, Germany) for aerobic bacteria (37 °C, 48 h). Isolated colonies from Schaedler agar (incubated anaerobically) were transferred onto two sets of plates: one set incubated aerobically and the other anaerobically. Growth observed in both environments confirmed that the microorganism was a facultative anaerobe.

The analysis performed focused solely on the qualitative aspect (presence or absence of bacteria).

The cultured microorganisms were isolated and grouped based on colony morphology and Gram staining (Chempur, Piekary Slaskie, Poland).

2.4. Statistical Analysis

The obtained data were analysed statistically using Fisher's exact test due to small sample sizes. The strength of the association between sample type (control vs. research) and the presence of bacteria, analysed separately for wholly and partially impacted teeth, was expressed as an odds ratio (OR). To allow OR estimation in the presence of a zero cell, the Haldane–Anscombe correction was applied before calculating the OR and its confidence interval. Statistical analysis was performed using Statistica 13 (StatSoft, Krakow, Poland) software.

3. Results

The results of microbiological cultures showed that all microorganisms belonged to the group of facultative anaerobes. Bacterial cultures were obtained from all 22 partially retained teeth samples that had not been disinfected (control samples), indicating microbiological contamination in each case (see Table 1).

Table 1. Microbiological analysis of samples from partially retained teeth.

No. of Sample	Sample Type	Microbiological Analysis		
1	* control	1A: Gram+ cocci		
	** research	1B: Gram+ cocci		
2	control	2A1: Gram+ cocci	2A2: Gram+ cocci	2A3: Gram+ rods
	research	2B1: Gram+ cocci	2B2: Gram+ cocci	-
3	control	3A1: Gram+ cocci, stringing together	3A2: Gram+ cocci	
	research	-	3B2: Gram+ cocci	
4	control	4A1: Gram+ cocci		
	research	-		
5	control	5A1: Gram+ cocci		
	research	5B1: Gram+ cocci		
6	control	6A1: Gram+ cocci		
	research	-		
7	control	7A1: Gram+ cocci	7A2: Gram+ rods	7A3: Gram+ cocci forming clusters
	research	-	7B2: Gram+ rods	-
8	control	8A1: Gram+ cocci, stringing together	8A2: Gram+ cocci, forming clusters	
	research	-	8B2: Gram+ cocci, forming clusters	
9	control	9A1: Gram+ cocci, forming clusters		
	research	-		
10	control	10A1: Gram- rods		
	research	10B1: Gram- rods		
11	control	11A1: Gram+ rods	11A2: Gram+ cocci, forming clusters	
	research	-	11B2: Gram+ cocci, forming clusters	
12	control	12A1: Gram+ cocci, forming clusters	12A2: Gram+ cocci	
	research	12B1: Gram+ cocci, forming clusters	-	
13	control	13A1: Gram+ cocci, stringing together		
	research	-		
14	control	14A1: Gram+ cocci		
	research	14B1: Gram+ cocci		
15	control	15A1: Gram+ cocci	15A2: Gram+ cocci, stringing together	
	research	15B1: Gram+ cocci	15B2: Gram+ cocci, stringing together	
16	control	16A1: Gram+ cocci	16A2: Gram+ rods	16A3: Gram+ cocci forming clusters
	research	16B1: Gram+ cocci	-	16B3: Gram+ cocci forming clusters
17	control	17A1: Gram+ rods	17A2: Gram+ cocci	
	research	-	-	
18	control	18A1: Gram+ cocci		
	research	-		
19	control	19A1: Gram- rods	19A2: Gram+ cocci	
	research	-	19B2: Gram+ cocci	
20	control	20A1: yeast	20A2: Gram+ rods	
	research	-	-	
21	control	21A1: Gram+ cocci	21A2: Gram+ cocci	
	research	21B1: Gram+ cocci	-	
22	control	22A1: Gram+ cocci, stringing together		
	research	-		

* control—sample without disinfection, ** test—sample after disinfection, green indicates complete reduction in microorganisms after using Dentin Cleanser.

Of the 22 samples of completely impacted teeth that had not undergone disinfection, bacterial growth was found in 17, indicating a high level of microorganisms (Table 2).

Table 2. Microbiological analysis of samples from completely impacted teeth.

No. of Sample	Sample Type	Microbiological Analysis	
		23A1: Gram+ cocci	23A2: Gram+ cocci
1	* control	23A1: Gram+ cocci	23A2: Gram+ cocci
	** research	-	-
2	control	24A1: Gram+ cocci	
	research	-	
3	control	25A: Gram+ cocci stringing together	
	research	-	
4	control	26A1: Gram+ cocci, stringing together	
	research	-	
5	control	-	
	research	-	
6	control	28A1: Gram+ cocci	
	research	-	
7	control	29A1: Gram+ cocci, stringing together	
	research	-	
8	control	30A1: Gram+ cocci	
	research	-	
9	control	31A1: Gram+ cocci	
	research	-	
10	control	32A1: Gram+ cocci	
	research	-	
11	control	-	
	research	-	
12	control	-	
	research	-	
13	control	35A1: Gram+ cocci, forming clusters	
	research	35B1: Gram+ cocci	
14	control	36A1: Gram+ cocci	
	research	-	
15	control	-	-
	research	-	-
16	control	-	
	research	-	
17	control	39A1: Gram+ rods	
	research	-	
18	control	40A1: Gram+ cocci	40A2: Gram+ cocci, stringing together
	research	-	-
19	control	41A1: Gram+ cocci, forming clusters	
	research	-	
20	control	42A1: Gram+ cocci, forming clusters	
	research	-	
21	control	43A1: Gram+ cocci, stringing together	
	research	-	
22	control	44A1: cocci	
	research	-	

* control—sample without disinfection, ** test—sample after disinfection, green indicates complete reduction in microorganisms after using Dentin Cleanser.

Statistical analysis revealed a significant difference in the presence of microorganisms in the samples before and after processing. Taking tooth eruption status into account, processing was less effective in partially impacted samples than in completely impacted ones. No microbial growth was observed in any of the samples from completely impacted teeth. In partially impacted samples, no microorganism growth occurred in 8 of 22 cases (approximately 36.4%; see Table 3).

Table 3. Effect of dentin processing on the presence of microorganisms.

The Process of Tooth Eruption	Sample Type	Presence of Bacteria in the Sample		Total	<i>p</i>	OR
		Present	Absent			
Partially retained	control	22	0	22	0.002	26.4
	research	14	8	22		
	Total	36	8	44		
Completely impacted	control	17	5	22	<0.001	143.2
	research	0	22	22		
	Total	17	27	44		

The disinfection procedure was fully effective for completely impacted teeth, whereas for partially retained teeth, results varied (Table 4).

Table 4. Effectiveness of disinfection procedures depending on the condition of tooth retention.

The Process of Tooth Eruption	Presence of Bacteria in the Sample		Total	<i>p</i>	OR
	Present	Absent			
Partially retained	14	8	22	<0.001	77.0
Completely impacted	0	22	22		

4. Discussion

In recent decades, significant advances in regenerative medicine, particularly in dentistry, have been made. The occurrence of numerous bone defects resulting from trauma and disease poses an essential challenge in clinical practice, emphasising the importance of materials that facilitate bone regeneration [54].

Of the various materials available, autologous bone grafting (i.e., autotransplantation, involving the transportation of bone or tooth tissue from one site to another within the same patient) is widely accepted due to its osteoregenerative properties [55].

Using infected autogenous dentin material for bone augmentation is associated with a high risk of surgical and biological complications [56]. The presence of bacterial biofilms on the graft can lead to persistent inflammation, impaired healing, and the development of abscesses or fistulas. This is due to bacteria embedded in biofilms being resistant to antibiotics and to the host's immune response [57,58]. Furthermore, infected material can disrupt the balance between bone resorption and formation, resulting in decreased osteoconduction and potential loss of osteogenic properties. This is evident from the observation that even minor contamination can negatively impact osteoblast activity [59]. Consequently, graft integration with the bone may be delayed or prevented entirely, thereby increasing the risk of material removal. In cases of augmentation in preparation for implant placement, infected dentin can further increase the risk of early infection and subsequent peri-implantitis [60]. Insufficient graft sterility may also lead to soft tissue dehiscence and material exposure, worsening the prognosis and complicating surgical treatment [61].

When transplanting processed dentin material, it is essential that the material is decontaminated and acts as an osteoconductive scaffold. This scaffold should provide a mineral substrate during resorption, stimulate osteoinductive cell activity to generate new bone, and release growth factors, such as bone morphogenetic proteins (BMPs). BMPs play a vital part in converting undifferentiated mesenchymal cells into osteogenic cells (osteoblasts) [54].

An important issue is the impact of sterilisation methods on the preservation of dentine's biologically active components. Although decontamination procedures are necessary to eliminate microorganisms, they may also lead to partial degradation of extracellular matrix proteins, including growth factors responsible for osteoinduction. High-temperature sterilisation methods, such as autoclaving and heat treatment, effectively ensure material sterility but are associated with protein denaturation and reduced BMP activity, which may limit the regenerative potential of the graft [62]. In contrast, chemical and low-temperature methods are considered gentler, allowing for the preservation of a greater amount of dentine bioactive components [63–65]. Studies indicate that appropriately selected chemical decontamination protocols enable effective microbial elimination while maintaining the material's ability to release BMP-2 and induce an osteogenic response, which is crucial for successful bone augmentation [66].

Teeth are made up of four types of inorganic calcium phosphate material: hydroxyapatite, tricalcium phosphate, amorphous calcium phosphate, and octacalcium phosphate. These materials are known to have osteoconductive properties, making them biocompatible for bone grafting. The organic matrix of dentin is dominated by a fibrous network of type I collagen, accounting for 90% of its composition. The remaining 10% consists of non-collagenous proteins, such as osteocalcin, osteonectin, sialoprotein, and phosphoprotein, which play a role in bone calcification and growth. These growth factors include bone morphogenetic proteins (BMPs), LIM 1 mineralisation protein, and insulin-like growth factors. These properties give teeth osteoinductive capabilities [67].

Our research has demonstrated the high effectiveness of dentin processing using a 30% alkaline ethyl alcohol solution containing 0.5 M sodium hydroxide in eliminating microorganisms. This confirms its usefulness as an autogenous material for bone tissue regeneration. Other researchers have obtained similar results. For example, Calvo-Guirado et al. (2021) demonstrated that using both dentin cleanser (a 30% alkaline ethyl alcohol solution containing 0.5 M sodium hydroxide) and EDTA (ethylenediaminetetraacetic acid) inhibits the growth of bacteria such as *Escherichia coli*, *Enterococcus faecalis*, and *Porphyromonas gingivalis* [26]. Furthermore, Khanijou et al. and Dlućik et al. demonstrated that there was no bacterial growth 10 min after application of the solution [14,68].

However, Kubaszek et al. [69] demonstrated the presence of *Staphylococcus epidermidis*, *Streptococcus sanguinis*, and *Blautia producta* in material treated with a 30% alkaline solution of ethyl alcohol containing 0.5 M sodium hydroxide for 10 min. It should be noted, however, that the protocol's effectiveness may vary depending on the stage of tooth eruption. Kubaszek et al. [69] did not specify whether the teeth were entirely or partially impacted, but the presence of bacteria after disinfection may suggest partial retention. This is consistent with microorganisms' tendency to form biofilms, known as dental plaque, which adhere tightly to the complex structures of partially impacted teeth. Numerous studies have shown that microorganisms living in biofilms exhibit increased tolerance to antiseptics compared to their free-living (planktonic) counterparts [70–73].

Complete sterility is not always achieved in partially impacted teeth, underscoring the need for further in vitro and in situ research to confirm the safety of dentin materials under various clinical conditions.

The microbiological purity of processed dentin is a key factor in the safety and effectiveness of autogenous grafts. Studies show that the mechanical grinding process itself, as well as the use of dentin cleanser and Dulbecco's Phosphate-Buffered Saline (PBS) solutions in dentin processing, do not ensure sterility in an analysis by Wojtowicz et al. [74], for example, only a small percentage of samples were completely free of microorganisms, and the bacteria detected mainly originated from the natural microflora of the patient's oral cavity [75]. Mahajan et al. [76] confirmed that various sterilisation protocols, including chemical and thermal ones, effectively eliminate microorganisms. However, they may differ in their impact on the osteogenic properties of the graft by altering BMP-2 (bone morphogenetic protein-2) levels, a key protein that induces bone formation. Pelozo et al. [77] reported that autoclaving is highly effective microbiologically, but it can alter dentine's physical properties, including reducing its microhardness. In practice, this means that, while the tissue may be sterile, its structure could be less conducive to the stability and integrity of the biomaterial. The technological process of dentine processing requires careful consideration of the chosen decontamination method. The study of Minetti et al. [78] and a systematic review by Inchingolo et al. [79] confirm that properly cleaned and demineralised dentine is safe and promotes bone regeneration. Furthermore, Kubaszek et al. [69] demonstrated that appropriately prepared autogenous dentin matrix does not introduce new pathogens. This microflora is similar to the patient's, potentially reducing the risk of immune reactions. These findings suggest that ensuring the microbiological purity of autogenous dentin material hinges not on grinding alone, but on a rigorous, standardised protocol for decontaminating and processing dentin.

When properly processed and microbiologically clean, autogenous dentin material used in alveolar bone augmentation has a wide range of clinical applications. As an autogenous material, it eliminates the risk of immune reactions. It can be used in guided tissue regeneration, alveolar volume preservation, alveolar ridge augmentation, maxillary sinus bone grafts, and reconstructions following tumour resection or cyst enucleation. It can also be used in other procedures requiring bone tissue reconstruction [80].

Certain limitations of this study should be taken into account, including the absence of data for primary Dental Hygiene in patients from whom the teeth were obtained for the survey. The microbiological assessment was limited to the detection of microorganisms, without quantitative analysis or species-level identification. Furthermore, the relatively small sample size reflects the study's preliminary nature. Future studies should include a larger study group, as well as quantitative assessment and species-level identification of bacterial isolates, to more comprehensively evaluate the effectiveness of the dentin disinfection protocol.

5. Conclusions

Using a 30% ethyl alcohol solution containing 0.5 M sodium hydroxide enables the complete removal of microorganisms from dentin in cases of complete impaction, making the solution a microbiologically safe bone substitute. For partially impacted teeth, the disinfection protocol significantly reduces the presence of microorganisms, though it does not guarantee complete sterility.

Future studies should also consider the stage of mechanical biofilm removal, for example, by using a sterile swab or brush on the tooth surface during dentin preparation, as this may further reduce the number of microorganisms in material obtained from partially retained teeth.

Author Contributions: Conceptualization, A.J., M.D., and R.J.W.; methodology, A.J., I.Z., M.P., J.N., M.D., and R.J.W.; software, I.Z.; validation, A.J., I.Z., M.P., M.D., and R.J.W.; formal analysis, A.J., I.Z., M.P., and J.N.; investigation, A.J., I.Z., M.P., and J.N.; resources, A.J. and M.D.; data curation, A.J., I.Z., M.P., and J.N.; writing—original draft preparation, A.J., I.Z., M.P., J.N., P.K., A.W., M.D., and R.J.W.; writing—review and editing, M.P., J.N., A.W., M.D., and R.J.W.; visualisation, M.P., A.W., and M.D.; supervision, P.K., M.D., and R.J.W.; project administration, M.D. and R.J.W.; funding acquisition, M.D. All authors have read and agreed to the published version of the manuscript.

Funding: This work was financed by a subsidy from Wroclaw Medical University, number SUBZ.B180.25.091.

Institutional Review Board Statement: Prior to commencing the study, approval was obtained from the Bioethics Committee of the Medical University of Wroclaw (No. 278/2019). The research material was obtained at the end of 2019/beginning of 2020 from the Independent Public Healthcare Centre of the Ministry of Internal Affairs and Administration in Szczecin.

Informed Consent Statement: Informed consent was obtained from all subjects involved in the study.

Data Availability Statement: The original contributions presented in this study are included in the article. Further inquiries can be directed to the corresponding author.

Conflicts of Interest: The authors declare no conflicts of interest.

References

1. Akhter, R.; Hassan, N.M.M.; Aida, J.; Zaman, K.U.; Morita, M. Risk Indicators for Tooth Loss Due to Caries and Periodontal Disease in Recipients of Free Dental Treatment in an Adult Population in Bangladesh. *Oral Health Prev. Dent.* **2008**, *6*, 199–207. [\[PubMed\]](#)
2. AlGhamdi, A.; Almarghlani, A.; Alyafi, R.; Ibraheem, W.; Assaggaf, M.; Howait, M.; Alsofi, L.; Banjar, A.; Al-Zahrani, M.; Kayal, R. Prevalence of Periodontitis in High School Children in Saudi Arabia: A National Study. *Ann. Saudi Med.* **2020**, *40*, 7–14. [\[CrossRef\]](#) [\[PubMed\]](#)
3. Aljafar, A.; Alibrahim, H.; Alahmed, A.; AbuAli, A.; Nazir, M.; Alakel, A.; Almas, K. Reasons for Permanent Teeth Extractions and Related Factors among Adult Patients in the Eastern Province of Saudi Arabia. *Sci. World J.* **2021**, *2021*, 5534455. [\[CrossRef\]](#) [\[PubMed\]](#)
4. Almarghlani, A. Prevalence, Predictors, and Reasons for Permanent Tooth Extraction Among High School Students in Saudi Arabia: A National Cross-Sectional Study. *Cureus* **2022**, *14*, e28687. [\[CrossRef\]](#)
5. Broers, D.L.M.; Dubois, L.; de Lange, J.; Su, N.; de Jongh, A. Reasons for Tooth Removal in Adults: A Systematic Review. *Int. Dent. J.* **2022**, *72*, 52–57. [\[CrossRef\]](#)
6. Fayaz, Y.; Ahmadi, N.A.; Ahmadi, S.U.; Atiq, M.A. Common Reasons for Permanent Tooth Extraction and Its Correlation with Demographical Factors in Kabul, Afghanistan. *Clin. Cosmet. Investig. Dent.* **2024**, *16*, 25–31. [\[CrossRef\]](#)
7. Suzuki, S.; Sugihara, N.; Kamijo, H.; Morita, M.; Kawato, T.; Tsuneishi, M.; Kobayashi, K.; Hasuike, Y.; Sato, T. Reasons for Tooth Extractions in Japan: The Second Nationwide Survey. *Int. Dent. J.* **2022**, *72*, 366–372. [\[CrossRef\]](#)
8. Zakrzewski, W.; Dobrzynski, M.; Wiglusz, R.J.; Rybak, Z.; Szymonowicz, M. Selected Nanomaterials' Application Enhanced with the Use of Stem Cells in Acceleration of Alveolar Bone Regeneration during Augmentation Process. *Nanomaterials* **2020**, *10*, 1216. [\[CrossRef\]](#)
9. Kim, E.-S. Autogenous Fresh Demineralized Tooth Graft Prepared at Chairside for Dental Implant. *Maxillofac. Plast. Reconstr. Surg.* **2015**, *37*, 8. [\[CrossRef\]](#)
10. Kim, E.-S.; Lee, I.-K.; Kang, J.-Y.; Lee, E.-Y. Various Autogenous Fresh Demineralized Tooth Forms for Alveolar Socket Preservation in Anterior Tooth Extraction Sites: A Series of 4 Cases. *Maxillofac. Plast. Reconstr. Surg.* **2015**, *37*, 27. [\[CrossRef\]](#)
11. Murata, M. Autogenous Demineralized Dentin Matrix for Maxillary Sinus Augmentation in Humans: The First Clinical Report. *J. Dent. Res.* **2003**, *82*, B243.
12. Nordenram, Å.; Bang, G.; Bernhoft, C.-H. A Clinical-Radiographic Study of Allogenic Demineralized Dentin Implants in Cystic Jaw Cavities. *Int. J. Oral Surg.* **1975**, *4*, 61–64. [\[CrossRef\]](#) [\[PubMed\]](#)
13. Yeomans, J.D.; Urist, M.R. Bone Induction by Decalcified Dentine Implanted into Oral, Osseous and Muscle Tissues. *Arch. Oral Biol.* **1967**, *12*, 999–IN16, IN13–IN14, 1007–1008, IN15–IN16. [\[CrossRef\]](#) [\[PubMed\]](#)
14. Dłucik, R.; Orzechowska-Wylegała, B.; Dłucik, D.; Puzzolo, D.; Santoro, G.; Micali, A.; Testagrossa, B.; Acri, G. Comparison of Clinical Efficacy of Three Different Dentin Matrix Biomaterials Obtained from Different Devices. *Expert Rev. Med. Devices* **2023**, *20*, 313–327. [\[CrossRef\]](#)

15. Struzik, N.; Kensy, J.; Piszko, P.J.; Kiryk, J.; Wiśniewska, K.; Kiryk, S.; Korjat, L.; Horodniczy, T.; Sobierajska, P.; Matys, J.; et al. Contamination in Bone Substitute Materials: A Systematic Review. *Appl. Sci.* **2024**, *14*, 8266. [\[CrossRef\]](#)
16. Kosior, P.; Kosior, I.; Dobrzyński, M. Immediate implantation with augmentation using material obtained from the patient's own removed teeth—A case report. *Implantol. Stomatol.* **2017**, *8*, 78–81.
17. Sun, H.; Yin, X.; Yang, C.; Kuang, H.; Luo, W. Advances in Autogenous Dentin Matrix Graft as a Promising Biomaterial for Guided Bone Regeneration in Maxillofacial Region: A Review. *Medicine* **2024**, *103*, e39422. [\[CrossRef\]](#)
18. Um, I.W. Demineralized Dentin Matrix (DDM) As a Carrier for Recombinant Human Bone Morphogenetic Proteins (RhBMP-2). *Adv. Exp. Med. Biol.* **2018**, *1077*, 487–499. [\[CrossRef\]](#)
19. Kim, Y.-K.; Yun, P.-Y.; Um, I.-W.; Lee, H.-J.; Yi, Y.-J.; Bae, J.-H.; Lee, J. 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. [\[CrossRef\]](#)
20. Elfana, A.; El-Kholy, S.; Saleh, H.A.; Fawzy El-Sayed, K. Alveolar Ridge Preservation Using Autogenous Whole-tooth versus Demineralized Dentin Grafts: A Randomized Controlled Clinical Trial. *Clin. Oral Implant. Res.* **2021**, *32*, 539–548. [\[CrossRef\]](#)
21. Alrmali, A.; Saleh, M.H.A.; Mazzocco, J.; Zimmer, J.M.; Testori, T.; Wang, H. Auto-dentin Platelet-rich Fibrin Matrix Is an Alternative Biomaterial for Different Augmentation Procedures: A Retrospective Case Series Report. *Clin. Exp. Dent. Res.* **2023**, *9*, 993–1004. [\[CrossRef\]](#) [\[PubMed\]](#)
22. Pogorzelska, A.; Adamiec, M.; Kotarski, J. Autogenous Augmentation of the Maxillary Alveolar Process Using Odontogenic Material—A Case Report. *Nowa Stomatol.* **2025**, *1*, 36–40.
23. Minetti, E.; Taschieri, S.; Corbella, S. Autologous Deciduous Tooth-Derived Material for Alveolar Ridge Preservation: A Clinical and Histological Case Report. *Case. Rep. Dent.* **2020**, *2020*, 2936878. [\[CrossRef\]](#) [\[PubMed\]](#)
24. Mahardawi, B.; Jiaranuchart, S.; Phrueksotsai, C.; Panya, S.; Arunjarosuk, S.; Pimkhaokham, A. Using Autogenous Tooth Graft for Alveolar Ridge Preservation: A Systematic Review and Meta-Analysis. *J. Prosthet. Dent.* **2025**, *in press*. [\[CrossRef\]](#)
25. Bębenek, K.; Kiryk, J.; Kosior, P.; Szczygielski, T.; Mikulewicz, M.; Dobrzyński, M. The Use of Autogenous Augmentation Material Obtained from the Patient's Own Teeth. *Inżynier. Fiz. Med.* **2018**, *7*, 289–292.
26. Calvo-Guirado, J.L.; Garcés-Villalá, M.A.; Mahesh, L.; De Carlos-Villafranca, F.A. Effectiveness of Chemical Disinfection in Discarding Pathogenic Bacteria of Human Particulate Tooth Graft. *Indian J. Dent. Sci.* **2021**, *13*, 277–282. [\[CrossRef\]](#)
27. Kommerein, N.; Weigel, A.J.; Stiesch, M.; Doll, K. Plant-Based Oral Care Product Exhibits Antibacterial Effects on Different Stages of Oral Multispecies Biofilm Development in Vitro. *BMC Oral Health* **2021**, *21*, 170. [\[CrossRef\]](#)
28. Karacic, J.; Ruf, M.; Herzog, J.; Astasov-Frauenhoffer, M.; Sahrman, P. Effect of Dentifrice Ingredients on Volume and Vitality of a Simulated Periodontal Multispecies Biofilm. *Dent. J.* **2024**, *12*, 141. [\[CrossRef\]](#)
29. Bruzda-Zwiech, A. *Stomatologia Wieków Rozwojowego*; Szpringer-Nodzak, M., Wochna-Sobańska, M., Eds.; PZWL: Warsaw, Poland, 2003.
30. Cameron, A.C.; Widmer, R.P. *Stomatologia Dziecięca*; Edra Urban & Partner: Wrocław, Poland, 2012.
31. Dobrzyński, M.; Bazan, J.; Rosińczuk-Tonderys, J.; Pałka, Ł.; Parulska, O.; Całkosiński, I. Odontogenesis as a Research Model in Experimental Toxicology. *Życie Weter.* **2014**, *89*, 58–60.
32. Herman, P.; Uracz, W.; Kopański, Z.; Brukwicka, I. Selected Aspects of Temporary and Permanent Teeth Physiology—The Role of Nutrients. *J. Clin. Healthc.* **2016**, *4*, 22–24.
33. Hovorakova, M.; Lesot, H.; Peterka, M.; Peterkova, R. The Developmental Relationship between the Deciduous Dentition and the Oral Vestibule in Human Embryos. *Anat. Embryol.* **2005**, *209*, 303–313. [\[CrossRef\]](#) [\[PubMed\]](#)
34. Hovorakova, M.; Lesot, H.; Peterka, M.; Peterkova, R. Early Development of the Human Dentition Revisited. *J. Anat.* **2018**, *233*, 135–145. [\[CrossRef\]](#) [\[PubMed\]](#)
35. Hovorakova, M.; Lesot, H.; Vonesch, J.; Peterka, M.; Peterkova, R. Early Development of the Lower Deciduous Dentition and Oral Vestibule in Human Embryos. *Eur. J. Oral Sci.* **2007**, *115*, 280–287. [\[CrossRef\]](#) [\[PubMed\]](#)
36. Kaushal, A.; Kamboj, M. Odontogenesis: A Highly Complex Cell-Cell Interaction Process. In Proceedings of the BEBI'09, 2nd WSEAS International Conference on Biomedical Electronics and Biomedical Informatic, Moscow, Russia, 20–22 August 2009; pp. 143–147.
37. Chestnutt, I.G.; Binnie, V.I.; Taylor, M.M. Reasons for Tooth Extraction in Scotland. *J. Dent.* **2000**, *28*, 295–297. [\[CrossRef\]](#) [\[PubMed\]](#)
38. Lesolang, R.R.; Motloba, D.P.; Lalloo, R. Patterns and Reasons for Tooth Extraction at the Winterveldt Clinic: 1998–2002. *South Afr. Dent. J.* **2009**, *64*, 214–218.
39. McCaul, L.K.; Jenkins, W.M.M.; Kay, E.J. The Reasons for the Extraction of Various Tooth Types in Scotland: A 15-Year Follow Up. *J. Dent.* **2001**, *29*, 401–407. [\[CrossRef\]](#) [\[PubMed\]](#)
40. Alsaegh, M.A.; Abushweme, D.A.; Ahmed, K.O.; Ahmed, S.O. The Pattern of Mandibular Third Molar Impaction and Its Relationship with the Development of Distal Caries in Adjacent Second Molars among Emiratis: A Retrospective Study. *BMC Oral Health* **2022**, *22*, 306. [\[CrossRef\]](#)
41. Anwar, N.; Khan, A.R.; Narayan, K.A.; Ab Manan, A.H. A Six-Year Review of the Third Molar Cases Treated in the Dental Department of Penang Hospital in Malaysia. *Dent. Res. J.* **2008**, *5*, 53–60.

42. Dominiak, M.; Gedrange, T.; Rahnama, M. *Podstawy Chirurgii Stomatologicznej*; Dominiak, M., Gedrange, T., Rahnama, M., Eds.; Edra Urban & Partner: Wrocław, Poland, 2022.
43. Eshghpour, M.; Nezadi, A.; Moradi, A.; Shamsabadi, R.M.; Rezaei, N.; Nejat, A. Pattern of Mandibular Third Molar Impaction: A Cross-Sectional Study in Northeast of Iran. *Niger. J. Clin. Pract.* **2014**, *17*, 673. [[CrossRef](#)]
44. Kumar, V.R.; Yadav, P.; Kahsu, E.; Girkar, F.; Chakraborty, R. Prevalence and Pattern of Mandibular Third Molar Impaction in Eritrean Population: A Retrospective Study. *J. Contemp. Dent. Pract.* **2017**, *18*, 100–106. [[CrossRef](#)]
45. Padhye, M.N.; Dabir, A.V.; Girotra, C.S.; Pandhi, V.H. Pattern of Mandibular Third Molar Impaction in the Indian Population: A Retrospective Clinico-Radiographic Survey. *Oral Surg. Oral Med. Oral Pathol. Oral Radiol.* **2013**, *116*, e161–e166. [[CrossRef](#)] [[PubMed](#)]
46. Zaman, M.U.; Almutairi, N.S.; Abdulrahman Alnashwan, M.; Albogami, S.M.; Alkhamash, N.M.; Alam, M.K. Pattern of Mandibular Third Molar Impaction in Nonsyndromic 17760 Patients: A Retrospective Study among Saudi Population in Central Region, Saudi Arabia. *Biomed. Res. Int.* **2021**, *2021*, 1880750. [[CrossRef](#)] [[PubMed](#)]
47. Jaworski, A.; Dudek, K.; Jurczak, I. Structure and Functional Capacity of the Bacterial Human Gastrointestinal Microbiota in Healthy State and Variety of Disease States. *J. Health Study Med.* **2016**, *4*, 37–61.
48. Krakowiak, O.; Nowak, R. Human Digestive Tract Microflora—Significance, Development, Modification. *Postępy Fitoter.* **2015**, *3*, 193–200.
49. Swora-Cwynar, E.; Miżdał, A.; Pompecka, A.; Karczewski, J.; Dobrowolska-Zachwieja, A. Funkcje i Znaczenie Mikrobioty Jelitowej. In *Fizjologia Żywnienia*; Krauss, H., Ed.; PZWL Wydawnictwo Lekarskie: Warsaw, Poland, 2019.
50. Li, J.; Quinque, D.; Horz, H.-P.; Li, M.; Rzhetskaya, M.; Raff, J.A.; Hayes, M.G.; Stoneking, M. Comparative Analysis of the Human Saliva Microbiome from Different Climate Zones: Alaska, Germany, and Africa. *BMC Microbiol.* **2014**, *14*, 316. [[CrossRef](#)] [[PubMed](#)]
51. Nasidze, I.; Li, J.; Quinque, D.; Tang, K.; Stoneking, M. Global Diversity in the Human Salivary Microbiome. *Genome Res.* **2009**, *19*, 636–643. [[CrossRef](#)]
52. Sarkar, A.; Stoneking, M.; Nandineni, M.R. Unraveling the Human Salivary Microbiome Diversity in Indian Populations. *PLoS ONE* **2017**, *12*, e0184515. [[CrossRef](#)]
53. Takayasu, L.; Suda, W.; Takanashi, K.; Iioka, E.; Kurokawa, R.; Shindo, C.; Hattori, Y.; Yamashita, N.; Nishijima, S.; Oshima, K.; et al. Circadian Oscillations of Microbial and Functional Composition in the Human Salivary Microbiome. *DNA Res.* **2017**, *24*, 261–270. [[CrossRef](#)]
54. Minetti, E.; Dipalma, G.; Palermo, A.; Inchingolo, A.D.; Viapiano, F.; Inchingolo, A.M.; Inchingolo, F. The Most Suitable System to Grind the Whole Tooth to Use It as Graft Material. *Explor. Med.* **2024**, *5*, 1–16. [[CrossRef](#)]
55. Obulareddy, V.T.; Porwal, A.; Noor, T.; Catalano, F.; Minervini, G.; D’Amico, C.; Mancini, M.; Gorassini, F.; Fiorillo, L.; Cervino, G. Tooth as a Bone Graft Material: A Narrative Review. *Eur. J. Gen. Dent.* **2023**, *12*, 072–081. [[CrossRef](#)]
56. Nisyrios, T.; Karygianni, L.; Fretwurst, T.; Nelson, K.; Hellwig, E.; Schmelzeisen, R.; Al-Ahmad, A. High Potential of Bacterial Adhesion on Block Bone Graft Materials. *Materials* **2020**, *13*, 2102. [[CrossRef](#)] [[PubMed](#)]
57. Janjua, O.S.; Qureshi, S.M.; Shaikh, M.S.; Alnazzawi, A.; Rodriguez-Lozano, F.J.; Pecci-Lloret, M.P.; Zafar, M.S. Autogenous Tooth Bone Grafts for Repair and Regeneration of Maxillofacial Defects: A Narrative Review. *Int. J. Environ. Res. Public Health* **2022**, *19*, 3690. [[CrossRef](#)] [[PubMed](#)]
58. Całkosiński, I.; Dobrzyński, M.; Całkosińska, M.; Seweryn, E.; Bronowicka-Szydełko, A.; Dzierżba, K.; Ceremuga, I.; Gamian, A. Characterization of an Inflammatory Response. *Postępy Hig. Med. Dosw.* **2009**, *63*, 395–408.
59. Singh, S.; Sarma, D.K.; Verma, V.; Nagpal, R.; Kumar, M. From Cells to Environment: Exploring the Interplay between Factors Shaping Bone Health and Disease. *Medicina* **2023**, *59*, 1546. [[CrossRef](#)]
60. Munakata, M.; Kataoka, Y.; Yamaguchi, K.; Sanda, M. Risk Factors for Early Implant Failure and Selection of Bone Grafting Materials for Various Bone Augmentation Procedures: A Narrative Review. *Bioengineering* **2024**, *11*, 192. [[CrossRef](#)]
61. Kaddas, C.; Papamanoli, E.; Bobetsis, Y.A. Etiology and Treatment of Peri-Implant Soft Tissue Dehiscences: A Narrative Review. *Dent. J.* **2022**, *10*, 86. [[CrossRef](#)]
62. He, S.; Hu, R.; Yao, X.; Cui, J.; Liu, H.; Zhu, M.; Ning, L. The Effects of Heat and Hydrogen Peroxide Treatment on the Osteoinductivity of Demineralized Cortical Bone: A Potential Method for Preparing Tendon/Ligament Repair Scaffolds. *Regen. Biomater.* **2024**, *11*, rbac116. [[CrossRef](#)]
63. Bono, N.; Tarsini, P.; Candiani, G. BMP-2 and Type I Collagen Preservation in Human Deciduous Teeth after Demineralization. *J. Appl. Biomater. Funct. Mater.* **2018**, *17*. [[CrossRef](#)]
64. Murata, M.; Akazawa, T.; Mitsugi, M.; Um, I.-W.; Kim, K.-W.; Kim, Y.-K. Human Dentin as Novel Biomaterial for Bone Regeneration. In *Biomaterials—Physics and Chemistry*; InTech: Rijeka, Croatia, 2011.
65. Olchowoy, A.; Olchowoy, C.; Zawislak, I.; Matys, J.; Dobrzyński, M. Revolutionizing Bone Regeneration with Grinder-Based Dentin Biomaterial: A Systematic Review. *Int. J. Mol. Sci.* **2024**, *25*, 9583. [[CrossRef](#)]

66. Grawish, M.E.; Grawish, L.M.; Grawish, H.M.; Grawish, M.M.; Holiel, A.A.; Sultan, N.; El-Negoly, S.A. Demineralized Dentin Matrix for Dental and Alveolar Bone Tissues Regeneration: An Innovative Scope Review. *Tissue Eng. Regen. Med.* **2022**, *19*, 687–701. [[CrossRef](#)]
67. Gual-Vaques, P.; Polis-Yanes, C.; Estrugo-Devesa, A.; Ayuso-Montero, R.; Mari-Roig, A.; Lopez-Lopez, J. Autogenous Teeth Used for Bone Grafting: A Systematic Review. *Med. Oral Patol. Oral Cir. Bucal.* **2017**, *23*, e112. [[CrossRef](#)] [[PubMed](#)]
68. Khanijou, M.; Zhang, R.; Boonsiriset, K.; Srisatjaluk, R.L.; Suphangu, S.; Pairuchvej, V.; Wongsirichat, N.; Seriwatanachai, D. Physicochemical and Osteogenic Properties of Chairside Processed Tooth Derived Bone Substitute and Bone Graft Materials. *Dent. Mater. J.* **2021**, *40*, 173–183. [[CrossRef](#)] [[PubMed](#)]
69. Kubaszek, B.; Morawiec, T.; Mertas, A.; Wachol, K.; Nowak-Wachol, A.; Śmieszek-Wilczewska, J.; Łopaciński, M.; Cholewka, A. Radiological and Microbiological Evaluation of the Efficacy of Alveolar Bone Repair Using Autogenous Dentin Matrix—Preliminary Study. *Coatings* **2022**, *12*, 909. [[CrossRef](#)]
70. Rath, S.; Bal, S.C.B.; Dubey, D. Oral Biofilm: Development Mechanism, Multidrug Resistance, and Their Effective Management with Novel Techniques. *Rambam Maimonides Med. J.* **2021**, *12*, e0004. [[CrossRef](#)]
71. Thomas, J.G.; Nakaishi, L.A. Managing the Complexity of a Dynamic Biofilm. *J. Am. Dent. Assoc.* **2006**, *137*, S10–S15. [[CrossRef](#)]
72. Saleem, H.G.M.; Seers, C.A.; Sabri, A.N.; Reynolds, E.C. Dental Plaque Bacteria with Reduced Susceptibility to Chlorhexidine Are Multidrug Resistant. *BMC Microbiol.* **2016**, *16*, 214. [[CrossRef](#)]
73. Wang, Z.; Shen, Y.; Haapasalo, M. Dynamics of Dissolution, Killing, and Inhibition of Dental Plaque Biofilm. *Front Microbiol.* **2020**, *11*, 964. [[CrossRef](#)]
74. Wojtowicz, A.; Wychowański, P.; Osiak, M.; Kresa, I. *Microbiological Analysis of Dental Tissues in Grinding, Processing and GBR Autotransplantation Procedures*; Dental Tribune International: Leipzig, Germany, 2018.
75. Dobrzynski, M.; Pajackowska, M.; Nowicka, J.; Jaworski, A.; Kosior, P.; Szymonowicz, M.; Kuropka, P.; Rybak, Z.; Bogucki, Z.A.; Filipiak, J.; et al. Study of Surface Structure Changes for Selected Ceramics Used in the CAD/CAM System on the Degree of Microbial Colonization, In Vitro Tests. *Biomed. Res. Int.* **2019**, *2019*, 9130806. [[CrossRef](#)]
76. Mahajan, S.; Kaur, R.K.; Grover, V.; Mehta, M.; Prashar, S.; Jain, A. Efficacy of Different Sterilization Protocols and Its Impact on Osteogenic Potential of Autogenous Tooth Bone Graft: A Comparative In Vitro Study. *J. Indian Soc. Periodontol.* **2024**, *28*, 673–679. [[CrossRef](#)]
77. Pelozo, L.; Silva-Neto, R.; de Oliveira, L.; Salvador, S.; Corona, S.; Souza-Gabriel, A. Comparison of the Methods of Disinfection/Sterilization of Extracted Human Roots for Research Purposes. *Dent. Med. Probl.* **2022**, *59*, 381–387. [[CrossRef](#)]
78. Minetti, E.; Taschieri, S.; Corbella, S. A Histologic Study on the Use of Tooth as a Graft Material in Oral Surgery: Analysis of 187 Samples. *Materials* **2025**, *18*, 2518. [[CrossRef](#)]
79. Inchingolo, A.M.; Patano, A.; Di Pede, C.; Inchingolo, A.D.; Palmieri, G.; de Ruvo, E.; Campanelli, M.; Buongiorno, S.; Carpentiere, V.; Piras, F.; et al. Autologous Tooth Graft: Innovative Biomaterial for Bone Regeneration. Tooth Transformer® and the Role of Microbiota in Regenerative Dentistry. A Systematic Review. *J. Funct. Biomater.* **2023**, *14*, 132. [[CrossRef](#)]
80. Sah, A.; Shridhar, D.B. Clinical Application of Autogenous Tooth as Bone Graft Material in Extraction Socket- A Prospective Study. *Clin. Epidemiol. Glob. Health* **2022**, *16*, 101063. [[CrossRef](#)]

Disclaimer/Publisher’s Note: The statements, opinions and data contained in all publications are solely those of the individual author(s) and contributor(s) and not of MDPI and/or the editor(s). MDPI and/or the editor(s) disclaim responsibility for any injury to people or property resulting from any ideas, methods, instructions or products referred to in the content.