applied sciences

MDPI

Article

In Vitro Analysis of Organic Ester Functional Groups in Carious Dentine

Mohammed Alturki [1,2], Ulrica Almhöjd [1,3], Garrit Koller [4,5], Fiona Warburton [6] and Avijit Banerjee [1,*]

1 Centre of Oral Clinical Translational Sciences, Faculty of Dentistry, Oral & Craniofacial Sciences, King's College London, London SE1 9RT, UK; mohammed.alturki@kcl.ac.uk (M.A.); ulrica.almhojd@gu.se (U.A.)
2 Department of Restorative Dental Sciences, College of Dentistry, King Saud University, Riyadh 11451, Saudi Arabia
3 Department of Cariology, Institute of Odontology, The Sahlgrenska Academy, University of Gothenburg, Box 450, SE-405 30 Gothenburg, Sweden
4 Centre for Host Microbiome Interactions, Faculty of Dentistry, Oral & Craniofacial Sciences, King's College London, London SE1 9RT, UK; garrit.koller@kcl.ac.uk
5 LCN—London Centre for Nanotechnology, 19 Gordon St, Bloomsbury, London WC1H 0AH, UK
6 Faculty of Dentistry, Oral & Craniofacial Sciences, King's College London, London SE1 9RT, UK; fiona.warburton14@btinternet.com
* Correspondence: avijit.banerjee@kcl.ac.uk

Abstract: Background: With the implementation of minimally invasive selective caries removal protocols to treat cavitated, deep carious dentine lesions, there is a need to investigate specific biochemical moiety distributions to help characterise and distinguish between infected (contaminated) and affected (demineralised) zones within the dentine lesion. The present in vitro investigation aimed to compare the distribution of ester functional groups (1740 cm^{-1}) within carious dentine tissue (infected and affected dentine). The null hypothesis stipulated that there are no differences in ester function intensity/distribution within carious dentine lesions. Materials and Methods: From a total of five extracted human molar teeth with carious dentine lesions, 246 points from 10 sections of carious dentine were examined using high-resolution Raman spectroscopy and characterised into infected, affected and sound dentine. The peak intensity of the characteristic vibration mode of the ester function was calculated from sample scans. Results: Analyses indicated a statistically significant difference in the spectroscopic vibration bands of esters between the infected and affected dentine zones. Conclusion: The ester functional group is higher in intensity in the caries-infected dentine zone compared to the affected tissue. This finding could be used to develop an objective indicator for the selective operative management of carious dentine.

Keywords: caries-infected; caries-affected; dentine; Raman spectroscopy; lucifer yellow; amide I; ester; minimally invasive dentistry; caries

check for updates

Citation: Alturki, M.; Almhöjd, U.; Koller, G.; Warburton, F.; Banerjee, A. In Vitro Analysis of Organic Ester Functional Groups in Carious Dentine. *Appl. Sci.* **2022**, *12*, 1088. https://doi.org/10.3390/app12031088

Academic Editor: Mary Anne Melo

Received: 30 November 2021
Accepted: 18 January 2022
Published: 20 January 2022

Publisher's Note: MDPI stays neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Copyright: © 2022 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) license (https://creativecommons.org/licenses/by/4.0/).

1. Introduction

Characterising and discriminating between denatured, necrotic, bacterially contaminated and demineralised tissue in a cavitated carious lesion is of clinical relevance when performing minimally invasive operative interventions [1,2]. Carious dentine consists of two main histological zones [3,4]. The outer, superficial, highly bacterially contaminated and denatured zone of tissue is clinically wet, soft and sticky—the caries-infected (bacterially contaminated) dentine. The remaining, deeper caries-affected (demineralised) dentine can be healed and repaired by the dentine–pulp complex and, therefore, can be retained and sealed off using biointeractive restorative materials [1,5–7]. This selective, minimally invasive operative approach preserves more tissue and has been shown to improve the long-term survival of the dentine–pulp complex [1,5,7,8]. The histological transition between these two zones of tissue, however, is diffuse and difficult to identify

clinically as well as in the laboratory, which leads to subjectivity and variations between operators/researchers and, therefore, often unnecessary excessive removal of tooth tissue by clinicians. Therefore, there is an unmet need to objectively delineate between caries-infected and affected dentine, marking the clinical endpoint of carious dentine excavation in both laboratory investigations and the clinic [5,9,10].

In vitro studies have predominantly characterised carious dentine tissues mechanically. Ogawa and co-workers used microhardness to divide carious dentine into outer (superficial) and inner lesion layers [11]. Banerjee et al. (2010) further classified the carious dentine lesion according to its Knoop hardness value and its intrinsic autofluorescent characteristics, with caries-infected dentine (CID) <25 KHN and caries-affected dentine (CAD) between 25 and 40 KHN. However, the hardness measurement only indirectly represents the basic mechanical mineral integrity of the tissue, without a direct correlation with the biochemical characteristics of the lesion [12,13], the latter having a role in tissue repair and biomaterial adhesion.

Biochemically, carious dentine contains high levels of organic compounds, proportionate with a relatively lower inorganic content, compared to sound dentine [4,14,15]. Alturki et al. (2021) characterised the carious dentine lesion using the Raman spectroscopic protein/mineral ratio (amide I/phosphate) according to the tissue microhardness. That study concluded the amide I/phosphate Raman spectral ratio could be used as a biochemical method to characterise carious dentine with a direct association with lesion histology.

In addition to amide I (-CO-NH-), which represents the main organic component in carious dentine, Almhöjd et al. (2014) showed that the protein ester functional group (-CO-OR-) can be considered a unique functional group in carious dentine, as it is not observed in sound dentine. This is due to the fact that esters are formed in an environment that contains less water and a low pH compared to that found in carious dentine [16]. However, its distribution has yet to be mapped through the histological zones of infected and affected dentine. Determining any difference in intensity, and therefore distribution, of the ester functional groups between infected and affected zones of carious dentine could help develop clinical indicators to aid practitioners with selective, minimally invasive operative carious dentine removal technologies and develop research towards developing novel chemical adhesion mechanisms for biointeractive restorative materials and tissue repair mechanisms.

Therefore, this in vitro study aimed to compare the distribution of ester functional groups within carious dentine tissue (infected and affected dentine). The null hypothesis was that there were no differences in the Raman spectroscopic peak vibrational intensities of ester functional groups between infected and affected carious dentine tissues. In addition, lucifer yellow (LY) was used to subjectively visualise any colour changes in the carious dentine zones, as it has the chemical ability to react with such ester functional groups, thus acting as a biochemical label [14].

2. Materials and Methods

2.1. Ethics and Sample Collection

Extracted human teeth with cavitated carious dentine lesions were collected under an ethics protocol reviewed and approved by NHS Health Research Authority (16/SW/0220).

2.2. Study Design

Five extracted human molar teeth with carious dentine lesions having an international caries detection and assessment system (ICDAS) lesion score >4 [17] were collected (ethics protocol reviewed and approved by NHS Health Research Authority (16/SW/0220)) and stored for no longer than three months in distilled water in a cold cabinet (+4 °C) before sectioning. Samples were sectioned longitudinally through the lesions using a slow-speed (200 rpm) water-cooled diamond blade (diamond wafering blade XL 12205, Benetec Ltd., London, UK), and digital images of the cut surfaces were captured (lens: Nikkor AF-S

Micro 60mm f/2.8G ED, D800E, Nikon, Tokyo, Japan). To obtain a flat surface for accurate measurements, the cut surfaces were polished (MetaServ 3000, Buehler, Lake Bluff, IL, USA) sequentially with silicon waterproof abrasive papers (P1200 for 10 s, P2500 for 10 s and P4000 for 4 min). The samples were first analysed using Raman spectroscopy, and then lucifer yellow was added as a biochemical ester functional group label.

2.3. Raman Spectroscopy

A high-resolution Raman spectroscope (inVia, Renishaw Plc, Wotton-under-Edge, Gloucestershire, UK) running in Streamline scanning mode was used to scan the flat tooth surfaces, and a 5/0.12 NA air objective was used. A total of 246 point scans were captured from 10 carious dentine sections. In each section, an average of 18 point scans were captured along 3 line scans starting from the outermost part of the lesion in the direction from the enamel–dentine junction towards the pulp. A further 6 line point scans were captured in clinically sound dentine in the same section. The vibrational intensity of ester functional groups was calculated for each point scan (measured at 1740 cm^{-1}). Spectrum acquisition was conducted using a 785 nm laser (10 mW for mapping mode and 0.5 mW for point scanning) using a 600 line/mm grate across the spectral bandwidth of 4000–400 cm^{-1} for each sample. Baseline correction was performed by Raman processing software (WiRe, Renishaw, UK).

2.4. Molecular Label Preparation

Lucifer yellow (carbazide; LY) was used to verify the Raman results in the carious dentine zones (infected and affected dentine), as it reacts specifically with ester groups in carious dentine tissues [18]. All tooth sections were initially exposed to 0.5 M $NaBH_4$ for one hour in ethanol to remove any unwanted reactions between the lucifer yellow and any ketones or aldehydes present in the tissues. This step left only the organic ester functional groups able to react with lucifer yellow [14]. Subsequently, all sections were then immersed in a 15 mM ethanol solution of lucifer yellow for 24 h. To reduce the risk of any unwanted electrostatic or hydrogen bonding, sections were placed in a saline solution (NaCl, 1 M) for another 24 h. In order to ensure that only covalently bound complexes were retained within the carious tissue, a final step was introduced by immersing the specimens in alkaline solution (0.5 M NaOH) for a final 24 h period.

2.5. Data Collection and Analysis

Statistical analysis and spectral correlation processing.

The ratio of Raman vibrational intensity peaks at 1650 cm^{-1}/960 cm^{-1} (amide I/phosphate) has been reported as a biochemical method to distinguish between the histological carious dentine zones [19]. The peak ratio at 1740 cm^{-1}/960 cm^{-1} was calculated in each point scan to characterise the ester (1740 cm^{-1}) group distribution in infected, affected and sound dentine [19]. All peaks were normalised to the phosphate peak (960 cm^{-1}), as this is the most intense and consistent Raman peak in dental hard tissues [20]. A descriptive analysis was performed. All analyses were carried out using IBM SPSS, version 25.0.

3. Results

Two hundred and forty-six examined points from 10 sections of carious dentine were analysed using Raman spectroscopy and characterised into infected, affected and sound dentine zones. The results showed a significant difference in the ester functional group intensities between the three histological dentine zones (Table 1, Figure 1). The descriptive analysis showed that the mean intensity of ester functional groups in the caries-infected dentine was almost three times higher than that in the caries-affected dentine (mean intensities: 3.3, 0.8 and 0.04 in infected, affected and sound, respectively).

Table 1. A descriptive analysis was used to measure the differences in ester functional group intensities at 1740 cm^{-1} between infected, affected and sound zones (characterised according to [19]), which shows a significant difference between the three zones as the confidence intervals do not overlap between the zones.

Infected Dentine **	Mean		3.39 *
	95% Confidence Interval for Mean	Lower	2.95
		Upper	3.84
	Std. Deviation		2.11
Affected Dentine **	Mean		0.87
	95% Confidence Interval for Mean	Lower	0.76
		Upper	0.98
	Std. Deviation		0.58
Sound Dentine **	Mean		0.04
	95% Confidence Interval for Mean	Lower	0.02
		Upper	0.06
	Std. Deviation		0.07

* Peak ratio (ester/phosphate). ** These zones were characterised by using the amide I/phosphate ratio: infected > 1.76, affected < 1.76 [19]. The sound dentine readings were taken from pure clinically dentine sound areas.

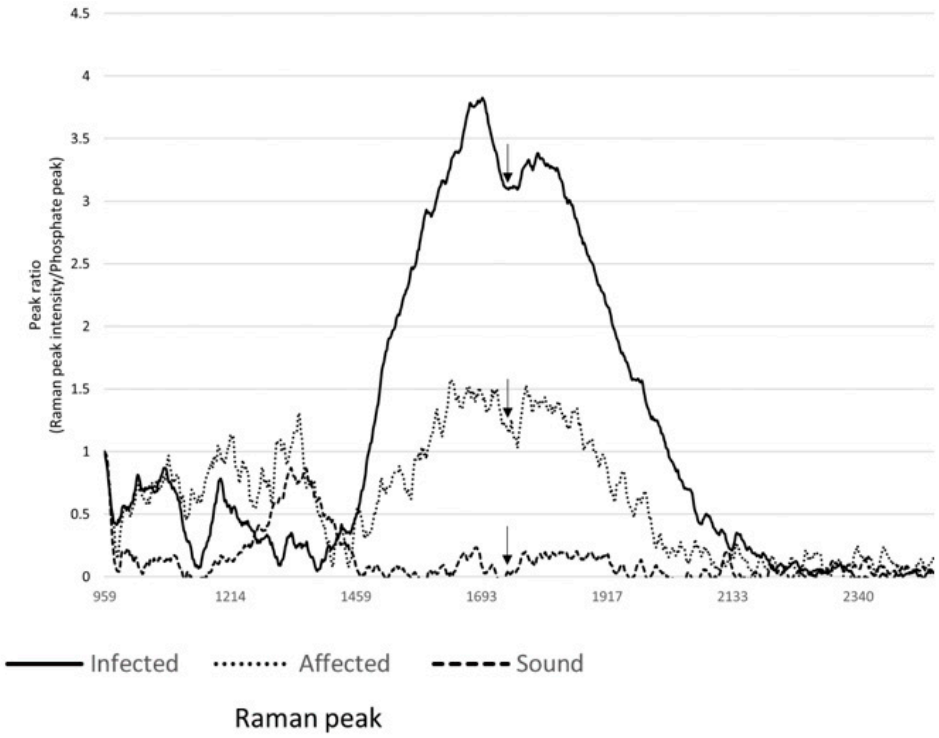

Figure 1. One of the sections showing three Raman spectra: infected, affected and sound dentine after normalising the intensities to the phosphate peak, showing differences in the ester functional group peak ratio (1740/960 cm^{-1}) between the three zones (indicated by the arrows).

Lucifer yellow (LY) was observed on the whole tooth section surface after the initial 24 h immersion in LY solution, with more noticeable discolouration found within the carious dentine lesion (Figure 2). However, after immersion in the salt and alkaline solutions, the LY staining was observed only in the carious dentine lesion (covalently bound).

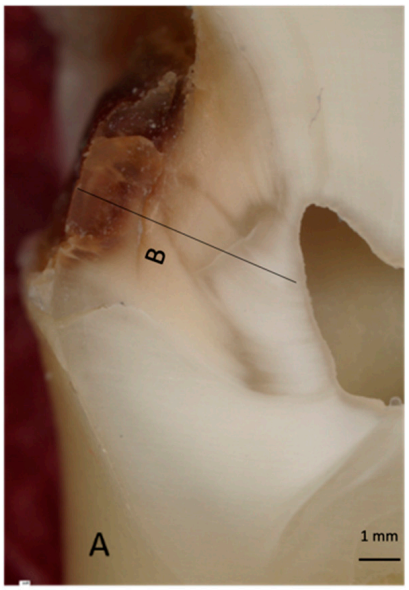

Figure 2. (A) A photomicrograph of one of the samples after sectioning and (B) the direction line of the Raman point scans that were taken to calculate the intensity of ester functional groups.

4. Discussion

Contemporary carious dentine excavation should be selective, preserving the remineralisable carious tissues and removing the infected and necrotic tissue only [1,6]. Thus, clinicians and researchers are continually trying to develop and optimise technologies that can objectively discriminate between these histological carious dentine zones. Biochemical alterations have been shown to play a significant role in carious dentine, specifically the organic components, with regard to tissue repair [14,21,22]. In vitro studies have used the organic components to distinguish carious dentine, as they become more exposed after demineralisation [14,20,23,24]. Specifically, the ester functional group has been isolated in carious dentine but not in sound dentine [18,25]. However, its distribution has not been characterised histologically to differentiate between carious dentine zones.

Esters are naturally formed from reactions between structures with alcohol functions, such as carbohydrates and carboxylic acids, from protonated proteins and are catalysed by acidic agents, described as Fischer esterification. This process is influenced by water levels and changes in acidity, which means that it increases in an environment that contains low water content and low pH [16]. The present study demonstrated that the increased relative intensity of ester functional groups was higher in caries-infected dentine compared to the adjacent caries-affected dentine. This finding is consistent with the established knowledge that there are more proteins in infected dentine compared to sound tissue [14,21]. In addition, it is in agreement with the nature of ester formation, as it forms in a more acidic environment, such as that found in caries-infected dentine. However, in the present study, there was a trace amount of ester function found in sound dentine (Table 1). This could be explained by the fact that the spectral data of the vibration bands of ester functional groups do not differentiate between types of esters, which also include glycerol-based phospholipids such as cholesterol- and phosphatidyl esters, a normal component of cell membranes [26,27]. Not all types of esters in carious dentine zones have been characterised. Only carbonyl ester functions (COOR) have been identified in carious tissues (Almhöjd et al., 2014, 2017) [14,18]. Besides these carbonyl ester functions (COOR), there might also be phosphor esters from lipids [28,29], trans- or diesters and from sugar acetals [30] or cyclic esters that can arise from lactones (lactic acid esters) originating from cariogenic bacteria [31,32]. In the present study, Raman microscopy detected all vibrational bands of ester functional groups. Therefore, a detailed analysis of ester functional groups in each carious dentine zone could be investigated further in the future.

LY was used in this study because it can react covalently with all ester groups [18]. In the present study, a change in the colour of the carious dentine was subjectively observed, which is explained by the fact that significant amounts of ester functional groups in infected and affected dentine visibly reacted with LY, but no changes in sound tissues were perceived.

To the authors' knowledge, the present study is the first to objectively show the concentration and distribution of ester functional groups within carious dentine tissues, highlighting this important biochemical alteration and correlating it to the histological zones of caries-infected and caries-affected dentine. This, in turn, could pave the way for the investigation of objective clinical markers to delineate these zones of carious dentine to aid the clinician in their selective removal as part of the minimally invasive operative approach to deep cavitated lesions. New selective operative technologies might use the ester functional group distribution to target caries-infected dentine for selective removal, and innovations in biointeractive restorative material development might explore the further use of organic components for tissue repair and adhesion. Further analysis of the biochemical nature of ester functional groups in each of the carious dentine zones would contribute to further characterisation of the tissues in this regard.

5. Conclusions

This in vitro study concluded that ester functional groups are distributed differently within carious dentine lesions, with a higher concentration in the caries-infected zone compared to the adjacent caries-affected dentine, which is detectable using Raman spectroscopy. Thus, the null hypothesis was rejected.

Author Contributions: M.A., G.K., U.A. and A.B. contributed to the conception, design, data acquisition, analysis and interpretation, as well as drafting and critical revision the manuscript. F.W. advised on the statistical analysis, interpretation of results and critical revision of the manuscript. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by College of Dentistry, King Saud University, Riyadh, Saudi Arabia, for grant sponsorship of the first author (grant no. 4/52/141590). The funder had no role in the study design, data collection and analysis, decision to publish or preparation of the manuscript.

Institutional Review Board Statement: The study was conducted in accordance with the Declaration of Helsinki, and approved by the Health Research Authority (16/SW/0220).

Informed Consent Statement: Not applicable.

Data Availability Statement: The data presented in this study are stored in a digital secured place of King's College London and available upon reasonable request.

Acknowledgments: The authors gratefully acknowledge the assistance provided by Peter Pilecki and the College of Dentistry, King Saud University, Riyadh, Saudi Arabia, for grant sponsorship of the first author (grant no. 4/52/141590). The funder had no role in the study design, data collection and analysis, decision to publish or preparation of the manuscript.

Conflicts of Interest: The authors declare no conflict of interest in relation to this study.

References

1. Banerjee, A. Selective Removal of Carious Dentin. In *Management of Deep Carious Lesions*; Schwendicke, F., Ed.; Springer International Publishing: Cham, Switzerland, 2018; pp. 55–70, ISBN 978-3-319-61370-3.
2. Slimani, A.; Terrer, E.; Manton, D.J.; Tassery, H. Carious Lesion Detection Technologies: Factual Clinical Approaches. *Br. Dent. J.* **2020**, *229*, 432–442. [CrossRef]
3. Ogushi, K.; Fusayama, T. Electron Microscopic Structure of the Two Layers of Carious Dentin. *J. Dent. Res.* **1975**, *54*, 1019–1026. [CrossRef]
4. Fusayama, T. Two Layers of Carious Dentin; Diagnosis and Treatment. *Oper Dent.* **1979**, *4*, 63–70.
5. Kidd, E.A.M. How "clean" Must a Cavity Be before Restoration? *Caries Res.* **2004**, *38*, 305–313. [CrossRef] [PubMed]
6. Schwendicke, F.; Dörfer, C.E.; Paris, S. Incomplete Caries Removal: A Systematic Review and Meta-Analysis. *J. Dent. Res.* **2013**, *92*, 306–314. [CrossRef] [PubMed]

7. Innes, N.P.T.; Frencken, J.E.; Bjørndal, L.; Maltz, M.; Manton, D.J.; Ricketts, D.; Van Landuyt, K.; Banerjee, A.; Campus, G.; Doméjean, S.; et al. Managing Carious Lesions: Consensus Recommendations on Terminology. *Adv. Dent. Res.* **2016**, *28*, 49–57. [CrossRef] [PubMed]
8. Schwendicke, F. Removing Carious Tissue: Why and How? *Monogr. Oral. Sci.* **2018**, *27*, 56–67. [CrossRef] [PubMed]
9. Banerjee, A.; Kidd, E.A.; Watson, T.F. In Vitro Evaluation of Five Alternative Methods of Carious Dentine Excavation. *Caries Res.* **2000**, *34*, 144–150. [CrossRef] [PubMed]
10. Isolan, C.P.; Sarkis-Onofre, R.; Lima, G.S.; Moraes, R.R. Bonding to Sound and Caries-Affected Dentin: A Systematic Review and Meta-Analysis. *J. Adhes. Dent.* **2018**, *20*, 7–18. [CrossRef]
11. Ogawa, K.; Yamashita, Y.; Ichijo, T.; Fusayama, T. The Ultrastructure and Hardness of the Transparent Layer of Human Carious Dentin. *J. Dent. Res.* **1983**, *62*, 7–10. [CrossRef]
12. Banerjee, A.; Cook, R.; Kellow, S.; Shah, K.; Festy, F.; Sherriff, M.; Watson, T. A Confocal Micro-Endoscopic Investigation of the Relationship between the Microhardness of Carious Dentine and Its Autofluorescence. *Eur. J. Oral Sci.* **2010**, *118*, 75–79. [CrossRef] [PubMed]
13. Lippert, F.; Lynch, R.J.M. Comparison of Knoop and Vickers Surface Microhardness and Transverse Microradiography for the Study of Early Caries Lesion Formation in Human and Bovine Enamel. *Arch. Oral Biol.* **2014**, *59*, 704–710. [CrossRef]
14. Almhöjd, U.S.; Norén, J.G.; Arvidsson, A.; Nilsson, Å.; Lingström, P. Analysis of Carious Dentine Using FTIR and ToF-SIMS. *Oral. Health Dent. Manag.* **2014**, *13*, 735–744.
15. Alturki, M.; Koller, G.; Almhöjd, U.; Banerjee, A. Chemo-Mechanical Characterization of Carious Dentine Using Raman Microscopy and Knoop Microhardness. *R. Soc. Open Sci.* **2020**, *7*, 200404. [CrossRef] [PubMed]
16. Carey, F.A.; Sundberg, R.J. *Advanced Organic Chemistry*, 5th ed.; Springer: New York, NY, USA, 2007; ISBN 978-0-387-44897-8.
17. Pitts, N.B.; Ekstrand, K.R. International Caries Detection and Assessment System (ICDAS) and Its International Caries Classification and Management System (ICCMS)—Methods for Staging of the Caries Process and Enabling Dentists to Manage Caries. *Community Dent. Oral Epidemiol.* **2013**, *41*, e41–e52. [CrossRef]
18. Almhöjd, U.S.; Lingström, P.; Nilsson, Å.; Norén, J.G.; Siljeström, S.; Östlund, Å.; Bernin, D. Molecular Insights into Covalently Stained Carious Dentine Using Solid-State NMR and ToF-SIMS. *Caries Res.* **2017**, *51*, 255–263. [CrossRef] [PubMed]
19. Alturki, M.; Koller, G.; Warburton, F.; Almhöjd, U.; Banerjee, A. Biochemical Characterisation of Carious Dentine Zones Using Raman Spectroscopy. *J. Dent.* **2021**, *105*, 103558. [CrossRef]
20. Wang, Y.; Spencer, P.; Walker, M.P. Chemical Profile of Adhesive/Caries-Affected Dentin Interfaces Using Raman Microspectroscopy. *J. Biomed. Mater. Res. A* **2007**, *81*, 279–286. [CrossRef]
21. Liu, Y.; Yao, X.; Liu, Y.W.; Wang, Y. A Fourier Transform Infrared Spectroscopy Analysis of Carious Dentin from Transparent Zone to Normal Zone. *Caries Res.* **2014**, *48*, 320–329. [CrossRef]
22. Seredin, P.; Goloshchapov, D.; Prutskij, T.; Ippolitov, Y. Phase Transformations in a Human Tooth Tissue at the Initial Stage of Caries. *PLoS ONE* **2015**, *10*, e0124008. [CrossRef]
23. Maske, T.T.; Isolan, C.P.; van de Sande, F.H.; Peixoto, A.C.; Faria-E-Silva, A.L.; Cenci, M.S.; Moraes, R.R. A Biofilm Cariogenic Challenge Model for Dentin Demineralization and Dentin Bonding Analysis. *Clin. Oral. Investig.* **2015**, *19*, 1047–1053. [CrossRef]
24. Lopes, C.D.C.A.; Limirio, P.H.J.O.; Novais, V.R.; Dechichi, P. Fourier Transform Infrared Spectroscopy (FTIR) Application Chemical Characterization of Enamel, Dentin and Bone. *Appl. Spectrosc. Rev.* **2018**, *53*, 747–769. [CrossRef]
25. Almhöjd, U.S.; Lingström, P.; Melin, L.; Nilsson, Å.; Norén, J.G. Staining of Carious Dentine Using Dyes with Covalent and Electrostatic Binding Properties—An in-Vitro Study. *Oral. Health Dent. Manag.* **2015**, *14*, 7.
26. Rostand, K.S.; Esko, J.D. Cholesterol and Cholesterol Esters: Host Receptors for Pseudomonas Aeruginosa Adherence. *J. Biol. Chem.* **1993**, *268*, 24053–24059. [CrossRef]
27. Voet, D.; Voet, J.G. Biochemistry (Second Edition). *Biochem. Educ.* **1995**, *23*, 104–105. [CrossRef]
28. Gotliv, B.-A.; Veis, A. Peritubular Dentin, a Vertebrate Apatitic Mineralized Tissue without Collagen: Role of a Phospholipid-Proteolipid Complex. *Calcif. Tissue Int.* **2007**, *81*, 191–205. [CrossRef] [PubMed]
29. Gotliv, B.A.; Veis, A. The Composition of Bovine Peritubular Dentin: Matching TOF-SIMS, Scanning Electron Microscopy and Biochemical Component Distributions. New Light on Peritubular Dentin Function. *Cells Tissues Organs* **2009**, *189*, 12–19. [CrossRef]
30. Tipson, R.S.S.; Horton, D. *Advances in Carbohydrate Chemistry and Biochemistry*; Academic Press: San Diego, CA, USA, 1995; Volume 51, ISBN 978-0-12-007251-4.
31. Goh, S.Y.; Tan, W.-S.; Khan, S.A.; Chew, H.P.; Kasim, N.H.A.; Yin, W.-F.; Chan, K.-G. Unusual Multiple Production of N-Acylhomoserine Lactones a by Burkholderia Sp. Strain C10B Isolated from Dentine Caries. *Sensors* **2014**, *14*, 8940–8949. [CrossRef]
32. Muras, A.; Mayer, C.; Otero-Casal, P.; Exterkate, R.A.M.; Brandt, B.W.; Crielaard, W.; Otero, A.; Krom, B.P. Short-Chain N-Acylhomoserine Lactone Quorum-Sensing Molecules Promote Periodontal Pathogens in In Vitro Oral Biofilms. *Appl. Environ. Microbiol.* **2020**, *86*, e01941-19. [CrossRef]