Author/s:
Cochrane, S;Burrow, MF;Parashos, P

Title:
Effect on the mechanical properties of human and bovine dentine of intracanal medicaments and irrigants

Date:
2019-03-01

Citation:
Cochrane, S., Burrow, M. F. & Parashos, P. (2019). Effect on the mechanical properties of human and bovine dentine of intracanal medicaments and irrigants. Australian Dental Journal, 64 (1), pp.35-42. https://doi.org/10.1111/adj.12655.

Persistent Link:
https://hdl.handle.net/11343/284537

Title Page

Title: Effect on the mechanical properties of human and bovine dentine of intracanal medicaments and irrigants.

Authors: Sally Cochrane, Michael F Burrow, Peter Parashos

Address: The Melbourne Dental School, Faculty of Medicine, Dentistry and Health Sciences, The University of Melbourne, Victoria, Australia

Corresponding author:
Prof. Peter Parashos
Melbourne Dental School, The University of Melbourne
Level 5, 720 Swanston Street, Carlton, VIC 3053, Australia
Email: parashos@unimelb.edu.au

Ph: +61 3 9341 1472

Fax: +61 3 9341 1595

Acknowledgements

The authors gratefully acknowledge the support of the Australian Dental Research Fund, Australian Society of Endodontology Inc and the Melbourne Dental School.

Conflict of Interest

The authors certify that they have no proprietary, financial, or other personal interest of any nature or kind in any product, service, and/or company that is presented in this article.

PROF. MICHAEL BURROW (Orcid ID : 0000-0003-2199-4270)
PROF. PETER PARASHOS (Orcid ID : 0000-0001-7191-0560)

Article type : Scientific Article

Abstract

Background: Evidence is lacking concerning the suitability of using bovine dentine in endodontic research. This study compared the mechanical properties of human and bovine root dentine using endodontic medicaments and irrigants.

Methods: Standardised human and bovine dentine bars were allocated to six experimental groups (n=15): calcium hydroxide paste (Calasept® Plus); Odontopaste®; 0.5% and 1% NaOCl gels; 1% and 4% NaOCl solutions. The bars were exposed to the materials for seven days and then immediately subjected to a three-point bend test and Vickers microhardness test. Data were analysed using analysis of variance with Fisher's pairwise comparisons, with $P < 0.05$.

Results: Human dentine displayed a significantly higher modulus of elasticity ($P = 0.001$), higher microhardness ($P < 0.001$), and lower flexural strength ($P = 0.004$) compared with bovine dentine. Calcium hydroxide and Odontopaste® did not result in a significant change to the mechanical properties of human dentine. In human dentine, 0.5% NaOCl gel caused a significant decrease in flexural strength ($P < 0.001$) and microhardness ($P = 0.02$).

Conclusions: Bovine dentine was a suitable substrate relative to human dentine. The use of a 0.5% or 1% NaOCl gel as an intracanal medicament is not supported by this study.

Introduction

Laboratory tests are necessary to determine if root canal medicaments and irrigants alter the mechanical properties of dentine, especially when new products are first released onto the market. Whilst it is preferred to use human dentine for such tests, due to ethical and logistical restrictions and difficulty in obtaining sample homogeneity, bovine dentine is commonly used. Several studies have compared the chemical and physical structure of human and bovine dentine in an attempt to verify the validity of using bovine dentine.[1-5] However, very few studies have compared the mechanical properties, with most having compared dentine microhardness, some reporting human dentine to have a higher microhardness value[6,7] and others found no significant difference.[8,9] Currently, there is insufficient evidence to determine if bovine root dentine is a suitable alternative to human root dentine when testing the effect of endodontic materials on the mechanical properties of dentine.

Calcium hydroxide displays antimicrobial[10] and tissue dissolving[11] properties and is the most commonly used intracanal medicament.[12] The hydroxyl ions released in an aqueous environment react with bacterial cells and disrupt cellular processes with effects including protein denaturation, lipid peroxidation of cell membranes, and splitting of DNA strands.[13] Odontopaste® (Australian Dental Manufacturing, Brisbane, Qld, Australia) is a contemporary steroid/antibiotic paste with antimicrobial activity[14] but with no published literature on its effects on dentine.

Sodium hypochlorite (NaOCl) is the most commonly used endodontic irrigant.[15] It displays excellent antimicrobial[16] and tissue dissolution properties[17] due to its high pH and chloramination action.[18] Consequently, NaOCl has been suggested as an intracanal medicament.[19] However, to date no study has reported the use of NaOCl as an inter-appointment dressing, although an Australian company (Dentalife, Ringwood, VIC, Australia) recently developed novel NaOCl gels to be used as intracanal medicaments.

With the organic component of dentine composed of 90% collagen, NaOCl can degrade dentine thereby affecting the mechanical properties.[20] Most laboratory studies have shown that an increase in the concentration of NaOCl has a negative effect on the flexural strength and modulus of elasticity of dentine.[21-23] However, studies with contrasting results have also been published.[17,24,25] Exposure to NaOCl has also been shown to reduce dentine microhardness.[25,26] Only one study assessed the effect of a NaOCl gel formulation on the mechanical properties of dentine,[27] reporting no significant difference in the microhardness of radicular dentine when exposed to 0.1 mL of either a 5.5% NaOCl gel, 2.5% NaOCl solution or a 6% NaOCl solution with surface modifiers for 15 minutes.

Therefore, this study aimed to compare the mechanical properties of human and bovine root dentine by testing using 0.5% and 1% NaOCl gels, calcium hydroxide paste, Odontopaste®, and 1% and 4% NaOCl solutions.

Materials and methods

Preparation of dentine specimens

Thirty-five intact human permanent maxillary and mandibular premolars, extracted for orthodontic reasons, were collected with the patients' informed consent and approval by the Ethics in Human Research Committee of The University of Melbourne (Ethics ID no. 1543824). Thirty-five bovine permanent mandibular incisors were sourced from an abattoir. All teeth were stored in 0.4% thymol solution at 3°C immediately after extraction. Each tooth provided a maximum of four dentine bars.

Dentine bars were prepared by longitudinally sectioning teeth using a Minitom precision cut-off machine (Struers, Ballerup, Denmark) with a diamond-coated cut-off wheel (Struers, Ballerup, Denmark) under continuous water flow. This resulted in rectangular dentine bars measuring 0.8 mm × 1.2 mm × 10 mm. The bars were finished with 2500 grit silicon carbide paper (Wetordry Tri-M-ite™, 3M, St. Paul, MN, USA) using a TegraPol-25 polishing machine (Struers, Ballerup, Denmark). The bars were then inspected for irregularities and cracks using a microscope at 24× magnification (Möller-Wedel International, Wedel, Germany) and then stored in 0.9% sterile unbuffered saline solution (Baxter, Old Toongabbie, NSW, Australia) at 3°C until further use.

Exposure to medicaments

A total of 105 human dentine bars and 105 bovine bars were evenly distributed to the control and experimental groups for each substrate (n = 15):

- 0.9% sterile unbuffered saline solution
- Calcium hydroxide paste (41-46%) (Calasept® Plus, Nordiska Dental AB, Ängelholm, Sweden)
- Odontopaste® - 0.5% calcium hydroxide, 1% triamcinolone acetonide and 5% clindamycin (Australian Dental Manufacturing, Kenmore Hills, QLD, Australia)
- 0.5% NaOCl gel (Dentalife, Ringwood, VIC, Australia)
- 1% NaOCl gel (Dentalife, Ringwood, VIC, Australia)
- 1% NaOCl solution (Dentalife, Ringwood, VIC, Australia)

- 4% NaOCl solution (Dentalife, Ringwood, VIC, Australia).

The dentine bars were completely immersed in 10 mL of the test medicament and were exposed to them for seven days at 37 °C and 100% humidity. Then, the bars were rinsed with distilled water and immediately subjected to a three-point bend test.

Three-point bend test

The 105 dentine bars in each substrate group were tested using an Imperial 1000 load testing machine (Mecmesin Limited, Slinfold, West Sussex, United Kingdom). The dentinal tubules were parallel to the cross-head and the bars were kept hydrated with distilled water during testing. The load testing machine was run at a cross-head speed of 1 mm min^{-1} until fracture. Data were recorded using Emperor™ software (Mecmesin Limited, Slinfold, West Sussex, United Kingdom) to calculate the load-displacement curve. Young's modulus and the flexural bend strength were calculated using standard equations.[28]

Microhardness test

Dentine microhardness was measured using a Vickers microhardness tester model 402-MVD (Wolpert Wilson® Instruments, Aachen, Germany). The bars were placed in the apparatus with the pulpal surface towards the indenting stylus. Four separate indentations were made using a 200 g load and a 10 sec dwell time with a distance of at least 500 µm between indentations. The four values were averaged to produce one Vickers hardness number (VHN) for each specimen.

Statistical analysis

The elastic modulus, flexural strength and Vickers hardness values were corrected for lack of normality using logarithmic transformation. The medicaments were compared using one-way analysis of variance (ANOVA) with Fisher's pairwise comparison. The results for human and bovine dentine were compared using two-way ANOVA with Fisher's pairwise test. Statistical significance was set at the 5% level and analyses were carried out using Minitab 17 (Minitab Inc., State College, PA, USA).

Results

Human dentine

The modulus of elasticity, flexural strength and microhardness values of the control and experimental groups for human dentine are shown in Tables 1-3 respectively. Exposure of the human root dentine bars to calcium hydroxide and Odontopaste® did not result in a significant change in the modulus of elasticity, flexural strength and microhardness compared with the control. Whilst exposure of human dentine to the 0.5% NaOCl gel did not result in a significant reduction in the modulus of elasticity (Table 1), it did result in a significant reduction in the flexural strength ($P < 0.001$) (Table 2) and microhardness value ($P = 0.02$) (Table 3). In decreasing order of the modulus of elasticity, flexural strength and microhardness values, exposure of human dentine to the 1% NaOCl gel, 1% NaOCl solution and 4% NaOCl solution resulted in a significant reduction in the mechanical properties compared with the control (Tables 1-3).

Bovine dentine

Tables 1-3 display the modulus of elasticity, flexural strength and microhardness values of the control and experimental groups for bovine dentine respectively. Following exposure to calcium hydroxide paste and Odontopaste®, only calcium hydroxide resulted in a significant change to bovine dentine's mechanical properties with a significant reduction in the flexural strength ($P = 0.02$) (Table 2). A similar trend to human dentine was seen in bovine dentine following exposure of the NaOCl experimental groups. However, exposure to the 0.5% NaOCl gel resulted in a significantly lower modulus of elasticity compared with the 1% NaOCl gel (Table 1) and the opposite was seen for microhardness values (Table 3). There was no significant difference between the flexural strength of bovine dentine following exposure to the 0.5% and 1% NaOCl gels (Table 2). The surface roughness of bovine dentine exposed to the 4% NaOCl solution prevented measurement of the microhardness and this group was excluded from microhardness testing.

Comparison of human and bovine dentine

The modulus of elasticity, flexural strength and microhardness for human and bovine dentine following exposure to the control and experimental groups are shown in Table 4. Overall, human dentine had a 1.13 times greater modulus of elasticity than bovine dentine ($P = 0.001$). However, this was not statistically significant for the control group. Human dentine displayed a significantly lower flexural strength than bovine dentine in both the control group ($P < 0.001$) and overall ($P = 0.004$). Human dentine displayed a 1.47 times greater microhardness than bovine dentine ($P < 0.001$).

Discussion

This study used dentine bars with all four surfaces exposed to relatively large volumes of the medicament or irrigant, over a seven-day period. Whilst these conditions differ from the clinical scenario, as a laboratory-based study, the protocol allowed for a controlled and standardised environment to observe and compare effects and differences. This is particularly important in preliminary studies to analyse the effects of new materials on dentine, and to add to the limited research into the differences between bovine and human dentine. When endodontic treatment is completed in two or more appointments, generally there is a period of at least seven days between appointments, hence the exposure period in the present study for the dentine bars. Whilst this time-frame was sufficient to demonstrate changes in dentine properties in some instances, longer time periods could possibly have shown more changes where none were seen after the seven days. This is a limitation of this study indicating the need for further investigation. The rectangular shape of the dentine bars allowed standardisation of the dentinal tubule orientation. This is important as the elastic modulus and flexural strength of dentine is dependent on the orientation of the mineralised collagen fibrils.[29,30] The tubules were orientated parallel to the applied force in this study.

Comparison of human and bovine dentine

Despite the lack of evidence for its use, studies have used bovine dentine[31,32] possibly on the basis that in terms of chemical composition, bovine dentine rather than other animal dentine, is the most suitable substitute for human dentine in laboratory-based studies.[5] Additionally, bovine teeth are more readily available and provide a large specimen compared with human teeth. The results of the present study show a significant difference in the mechanical properties between the two substrates.

In addition to the differing composition and microstructure of the two substrates, other factors that may contribute to these differences include orientation of dentinal tubules,[29,30] age of dentine[33,34] and the presence of sclerotic dentine.[35] Human dentine has been shown to have a higher degree of mineralisation[5] and lower tubule density than bovine dentine with no significant difference in the tubule diameter between substrates.[1,3] Therefore, human dentine is a more brittle substrate than bovine dentine, which may explain the lower flexural strength of the human dentine control group compared with the bovine control group. Further investigation, such as fracture toughness tests, may provide more information explaining the differences between these two substrates. Although the differences were statistically significant, the factors by which the two substrates differed, 1.13 for modulus of elasticity and 0.89 for flexural strength, were small.

The finding of a higher microhardness value of human dentine was supported by previous work.[6,7] Because human dentine is more mineralised than bovine dentine[5], and as microhardness is an indirect measurement of mineral content, human dentine consistently displayed a higher microhardness value than bovine dentine in this study. The age of the dentine may also be a contributing factor where aged dentine was found to have a higher hardness[34]. The age of the animals from which the teeth were obtained for the present study was around 30 months and root development was complete. The ages of the human teeth ranged from approximately 14 to 25 years and hence the root development may have been relatively further advanced. Therefore, whilst within each group the ages were similar, the age-related relative performance in the tests between bovine and human teeth is an area requiring further research. Nevertheless, overall, the present study showed similar trends in the human and bovine specimens following exposure to the medicaments.

Calcium hydroxide and Odontopaste®

The lack of significant change in the elastic modulus following exposure to calcium hydroxide supports the results of previous research using a similar study design with human dentine.[21,34] Currently there are no other studies assessing the effect of Odontopaste® on the mechanical properties of dentine, so this is the first to report no change in the elastic modulus.

Odontopaste® had no significant effect on the flexural strength of human or bovine dentine. However, whilst the calcium hydroxide paste produced no significant change in the flexural strength of human dentine, there was a significant reduction in the flexural strength of bovine dentine. The results of the current study differ from those of previously published studies. Studies using human dentine reported a significant reduction in the flexural strength following a 7-10 day exposure of calcium hydroxide[21,36] whilst a study using bovine dentine found no significant change.[37] These contrasting results are likely due to differing concentrations, exposure times and testing methods and were reflected in a systematic review analysing the effect of calcium hydroxide on the mechanical properties of radicular dentine.[38] The review included studies using human, bovine and ovine dentine and concluded that the majority of laboratory-based studies showed a reduction in the mechanical properties of dentine following exposure to calcium hydroxide for five weeks or longer, although the data were inconclusive for shorter exposure periods.[38]

Similar to the flexural strength and the results of a systematic review,[38] there are conflicting results regarding the effect of calcium hydroxide on dentine microhardness. Yassen et al.[39] and Yilmaz et al.[40] reported no significant difference after a 1-2 week exposure whilst Yoldaş et al.[41] found a significant reduction after a three-day exposure to calcium hydroxide. However, Yassen et al.[39] and Yilmaz et al.[40] used NaOCl during mechanical preparation and Yoldaş et al.[41] used saline, which may account for the different findings.

Sodium Hypochlorite

Most studies using a three-point bend test indicate that NaOCl solutions cause a concentration- and time-dependent reduction of elastic modulus in human root dentine bars.[21,22] Exposure to 3-9% NaOCl for 1-2 hours has been reported to result in a significant reduction in the elastic modulus of dentine.[21,22] However, a one-hour exposure to 1% NaOCl and a two-hour exposure to 0.5% NaOCl produced no significant reduction in elastic modulus compared with the saline control.[22]

Similarly, previous studies showed a concentration dependent reduction in the flexural strength of dentine following exposure to NaOCl solutions.[21,22] It is difficult to compare the present results with these studies due to differing exposure times and concentrations, nevertheless, a similar concentration-dependent trend was found in the present study.

Several studies reported that exposure to NaOCl resulted in no significant reduction in the modulus of elasticity and flexural strength of human dentine bars using a three-point bend test.[17,24,25] The varying methodologies may account for these contrasting results. Machnick et al.[24] and Cullen et al.[17] stored the extracted teeth in chloramine-T which is an N-chloromide compound that contains active chlorine.[42] Whilst it is a weaker chlorine-based oxidant than hypochlorous acid from which NaOCl is derived,[43] it will still degrade collagen. Further, chloramine-T has been shown to leach out of teeth for a period of four weeks or more after being transferred to distilled water.[44] Therefore, the chloramine-T storage solution may account for the lack of differences between the control and experimental groups. The short five-minute exposure time to only one surface in Marcelino et al.[25] explains the absence of negative effects of NaOCl on flexural strength.

The effect of the NaOCl experimental groups on the modulus of elasticity and flexural strength of bovine dentine differed slightly from those in human dentine. It was hoped that bovine dentine might improve the standardisation of the samples by using dentine of a similar age from intact teeth. However, the variation in the microstructure of dentine and variables such as the orientation of dentinal tubules may negate the possibly improved homogeneity in the bovine samples and account for these contrasting results. Few studies have investigated the effect of NaOCl on bovine dentine. Soares et al.[32] reported no significant difference to the flexural strength of endodontically treated bovine teeth using 1% NaOCl solution compared with non-endodontically treated teeth. However, the exposure time was not standardised and the dentine bars were prepared after exposure.

In the present study, in decreasing order of microhardness values, the 0.5% NaOCl gel, 1% NaOCl gel, 1% NaOCl solution and 4% NaOCl solution resulted in a significant reduction in microhardness value for human and bovine dentine compared with both the saline control and between these NaOCl groups. Sodium hypochlorite degrades the collagen network in dentine[45] and so the hydroxyapatite crystals are not as well supported, which may account for the reduction in microhardness following exposure to NaOCl. The results from the present study are in line with the reported concentration-dependent effect of NaOCl on bovine root dentine microhardness.[31] The majority of studies assessing the effect of NaOCl solutions on the microhardness of human root dentine have short exposure times of 5-15 minutes and have shown that concentrations of 1-6 % significantly reduce the microhardness values of dentine compared with saline.[25,26]

Garcia et al.[27] assessed the effect of three NaOCl formulations on the microhardness of human radicular dentine. Dentine was exposed to a 2.5% NaOCl solution, 6% NaOCl solution with surface modifiers and a 5.5% NaOCl gel intended for use during mechanical instrumentation. All three formulations reduced the microhardness, however, in contrast to the findings of this study no statistically significant differences between the groups were observed. This may be due to the small volume of medicaments used, 0.1 mL, a short exposure time of 15 minutes and only one dentine surface being exposed to the medicaments.

Owing to the size of the indentation area, the indentations of the current study and previous studies have included both intertubular dentine (ITD) and peritubular dentine (PTD). Therefore, the hardness values are a composite average. As ITD matrix governs the elastic behaviour of dentine,[46] nano-indentations of ITD using an atomic force microscope may have the potential to provide a greater understanding of the effects of intracanal medicaments on the mechanical properties of human and bovine dentine.[47]

Although a lower concentration of the NaOCl gel may not display a significant alteration to the mechanical properties of dentine, it will have a negative impact on its antimicrobial properties. Any significant change to the mechanical properties of dentine may result in an increased risk of tooth fracture. With reported high healing and functional retention rates of 94-95% following endodontic treatment,[48] the use of a material that may increase the risk of fracture should be used with caution.

Conclusions

Whilst the mechanical properties of human dentine differed from those of bovine dentine following exposure to varying medicaments with the actual values differing, the two substrates showed similar trends. Therefore, the results of this study support the use of bovine dentine in laboratory-based studies assessing the mechanical properties of dentine, but care must be taken when interpreting the results. Within the limitations of this laboratory-based study, the 0.5% and 1% NaOCl gels seem not to be suitable for use as intracanal medicaments.

References

1. Schilke R, Lisson JA, Bauß O, Geurtsen W. Comparison of the number and diameter of dentinal tubules in human and bovine dentine by scanning electron microscopic investigation. Arch Oral Biol 2000;45:355-361.

2. Schmalz G, Hiller K-A, Nunez LJ, Stoll J, Weis K. Permeability characteristics of bovine and human dentin under different pretreatment conditions. J Endod 2001;27:23-30.

3. Camargo CH, Siviero M, Camargo SE, de Oliveira SH, Carvalho CA, Valera MC. Topographical, diametral, and quantitative analysis of dentin tubules in the root canals of human and bovine teeth. J Endod 2007;33:422-426.

4. Lopes MB, Sinhoreti MA, Gonini Junior A, Consani S, McCabe JF. Comparative study of tubular diameter and quantity for human and bovine dentin at different depths. Braz Dent J 2009;20:279-283.

5. Teruel JDD, Alcolea A, Hernandez A, Ruiz AJ. Comparison of chemical composition of enamel and dentine in human, bovine, porcine and ovine teeth. Arch Oral Biol 2015;60:768-775.

6. Hara AT, Queiroz CS, Paes Leme AF, Serra MC, Cury JA. Caries progression and inhibition in human and bovine root dentine in situ. Caries Res 2003;37:339-344.

7. Castanho GM, Marques MM, Marques JB, Camargo MA, De Cara AA. Micromorphological and hardness analyses of human and bovine sclerotic dentin: a comparative study. Braz Oral Res 2011;25:274-279.

8. Fonseca RB, Haiter-Neto F, Carlo HL, et al. Radiodensity and hardness of enamel and dentin of human and bovine teeth, varying bovine teeth age. Arch Oral Biol 2008;53:1023-1029.

9. Turssi CP, Messias DF, Corona SM, Serra MC. Viability of using enamel and dentin from bovine origin as a substitute for human counterparts in an intraoral erosion model. Braz Dent J 2010;21:332-336.

10. Byström A, Claesson R, Sundqvist G. The antibacterial effect of camphorated paramonochlorophenol, camphorated phenol and calcium hydroxide in the treatment of infected root canals. Endod Dent Traumatol 1985;1:170-175.

11. Hasselgren G, Olsson B, Cvek M. Effects of calcium hydroxide and sodium hypochlorite on the dissolution of necrotic porcine muscle tissue. J Endod 1988;14:125-127.

12. Lee M, Winkler J, Hartwell G, Stewart J, Caine R. Current trends in endodontic practice: emergency treatments and technological armamentarium. J Endod 2009;35:35-39.

13. Siqueira JF, Lopes HP. Mechanisms of antimicrobial activity of calcium hydroxide: a critical review. Int Endod J 1999;32:361-369.

14. Plutzer B, Zilm P, Ratnayake J, Cathro P. Comparative efficacy of endodontic medicaments and sodium hypochlorite against Enterococcus faecalis biofilms. Aust Dent J 2018;63:208-216.

15. Clarkson RM, Podlich HM, Savage NW, Moule AJ. A survey of sodium hypochlorite use by general dental practitioners and endodontists in Australia. Aust Dent J 2003;48:20-26.

16. McDonnell G, Russell AD. Antiseptics and disinfectants: activity, action, and resistance. Clin Microbiol Rev 1999;12:147-179.

17. Cullen JK, Wealleans JA, Kirkpatrick TC, Yaccino JM. The effect of 8.25% sodium hypochlorite on dental pulp dissolution and dentin flexural strength and modulus. J Endod 2015;41:920-924.

18. Estrela C, Estrela CR, Barbin EL, Spano JC, Marchesan MA, Pecora JD. Mechanism of action of sodium hypochlorite. Braz Dent J 2002;13:113-117.

19. Ragnarsson KT, Rechenberg DK, Attin T, Zehnder M. Available chlorine consumption from NaOCl solutions passively placed in instrumented human root canals. Int Endod J 2015;48:435-440.

20 Pascon FM, Kantovitz KR, Sacramento PA, Nobre-dos-Santos M, Puppin-Rontani RM. Effect of sodium hypochlorite on dentine mechanical properties. A review. J Dent 2009;37:903-908.

21 Grigoratos D, Knowles J, Ng YL, Gulabivala K. Effect of exposing dentine to sodium hypochlorite and calcium hydroxide on its flexural strength and elastic modulus. Int Endod J 2001;34:113-119.

22. Sim TPC, Knowles JC, Ng YL, Shelton J, Gulabivala K. Effect of sodium hypochlorite on mechanical properties of dentine and tooth surface strain. Int Endod J 2001;34:120-132.

23. Cecchin D, Farina AP, Souza MA, et al. Evaluation of antimicrobial effectiveness and dentine mechanical properties after use of chemical and natural auxiliary irrigants. J Dent 2015;43:695-702.

24. Machnick TK, Torabinejad M, Munoz CA, Shabahang S. Effect of MTAD on flexural strength and modulus of elasticity of dentin. J Endod 2003;29:747-750.

25. Marcelino AP, Bruniera JF, Rached FA, Silva SR, Messias DC. Impact of chemical agents for surface treatments on microhardness and flexural strength of root dentin. Braz Oral Res 2014;28:1-6.

26. Aslantas EE, Buzoglu HD, Altundasar E, Serper A. Effect of EDTA, sodium hypochlorite, and chlorhexidine gluconate with or without surface modifiers on dentin microhardness. J Endod 2014;40:876-879.

27. Garcia AJ, Kuga MC, Palma-Dibb RG, et al. Effect of sodium hypochlorite under several formulations on root canal dentin microhardness. J Investig Clin Dent 2013;4:229-232.

28. ASTM, American Society for Testing and Materials. Standard test methods for flexural properties of un-reinforced and reinforced plastics and electrical insulating materials. 2015;D790-15e2.

29. Arola DD, Reprogel RK. Tubule orientation and the fatigue strength of human dentin. Biomater 2006;27:2131-2140.

30. Pongprueksa P, Senawongse P, Vongphan N. Effect of dentinal tubule orientation on the modulus of elasticity of resin-infiltrated demineralized dentin. Dent Mater J 2014;33:54-58.

31. Slutzky-Goldberg I, Maree M, Liberman R, Heling I. Effect of sodium hypochlorite on dentin microhardness. J Endod 2004;30:880-882.

32. Soares CJ, Santana FR, Silva NR, Preira JC, Pereira CA. Influence of the endodontic treatment on mechanical properties of root dentin. J Endod 2007;33:603-606.

33. Eldarrat AH, High AS, Kale GM. Age-related changes in ac-impedance spectroscopy studies of normal human dentine: further investigations. J Mater Sci Mater Med 2010;21:45-51.

34. Montoya C, Arango-Santander S, Pelaez-Vargas A, Arola D, Ossa EA. Effect of aging on the microstructure, hardness and chemical composition of dentin. Arch Oral Biol 2015;60:1811-1820.

35. Senawongse P, Otsuki M, Tagami J, Mjör IA. Morphological characterization and permeability of attrited human dentine. Arch Oral Biol 2008;53:14-19.

36. Marending M, Stark WJ, Brunner TJ, Fischer J, Zehnder M. Comparative assessment of time-related bioactive glass and calcium hydroxide effects on mechanical properties of human root dentin. Dent Traumatol 2009;25:126-129.

37. Moazami F, Sahebi S, Jamshidi D, Alavi A. The long-term effect of calcium hydroxide, calcium-enriched mixture cement and mineral trioxide aggregate on dentin strength. Iran Endod J 2014;9:185-189.

38. Yassen GH, Platt JA. The effect of nonsetting calcium hydroxide on root fracture and mechanical properties of radicular dentine: a systematic review. Int Endod J 2013;46:112-118.

39. Yassen GH, Vail MM, Chu TG, Platt JA. The effect of medicaments used in endodontic regeneration on root fracture and microhardness of radicular dentine. Int Endod J 2013;46:688-695.

40. Yilmaz S, Dumani A, Yoldas O. The effect of antibiotic pastes on microhardness of dentin. DentTraumatol 2016;32:27-31.

41. Yoldaş O, Doğan C, Seydaoğlu G. The effect of two different calcium hydroxide combinations on root dentine microhardness. Int Endod J 2004;37:828-831.

42. Vaz N, Manjunatha AS, Puttaswamy. Kinetic and mechanistic studies on the oxidative decolourisation of orange-II dye with alkaline chloramine-T. J Chem Res 2015;39:363-367.

43. Arnitz R, Nagl M, Gottardi W. Microbicidal activity of monochloramine and chloramine T compared. J Hosp Inf 2009;73:164-170.

44. Rolland SL, Carrick TE, Walls AW, McCabe JF. Dentin decontamination using chloramine T prior to experiments involving bacteria. Dent Mater 2007;23:1468-1472.

45. Davies JMS, Horwitz DA, Davies KJA. Potential roles of hypochlorous acid and N-chloroamines in collagen breakdown by phagocytic cells in synovitis. Free Rad Biol Med 1993;15:637-643.

46. Kinney JH, Balooch M, Marshall GW, Marshall SJ. A micromechanics model of the elastic properties of human dentine. Arch Oral Biol 1999;44:813-822.

47. Inoue T, Saito M, Yamamoto M et al. Comparison of nanohardness between coronal and radicular intertubular dentin. Dent Mater J 2009;28:295-300.

48. Ng YL, Mann V, Gulabivala K. A prospective study of the factors affecting outcomes of nonsurgical root canal treatment: part 1: periapical health. Int Endod J 2011;44:583-609.

Table 1. Modulus of elasticity of human and bovine dentine after seven-day medicament exposure – means and confidence intervals (CI).

	Human Dentine			Bovine Dentine		
Treatment	Mean (GPa)[†]	95% CI	Stats[‡]	Mean (GPa)[†]	95% CI	Stats[‡]
Saline	13.8	(12.3, 15.6)	A	12.6	(11.2, 14.1)	A
Calcium hydroxide	14.4	(12.8, 16.3)	A	13.9	(12.4, 15.6)	A
Odontopaste®	14.4	(12.8, 16.3)	A	13.8	(12.3, 15.5)	A
0.5% NaOCl gel	13.5	(11.9, 15.2)	A	8.0	(7.1, 9)	C

1% NaOCl gel	9.6	(8.5, 10.8)	B	9.9	(8.8, 11.1)	B
1% NaOCl solution	7.5	(6.6, 8.5)	C	5.0	(4.5, 5.6)	D
4% NaOCl solution	2.4	(2.2, 2.8)	D	3.1	(2.8, 3.5)	E

†Back transformed means (gigapascals).

‡Statistical differences – means that do not share a letter vertically are significantly different.

Table 2. Flexural strength of human and bovine dentine after seven-day medicament exposure – means and confidence intervals (CI).

	Human Dentine			Bovine Dentine		
Treatment	Mean (MPa)†	95% CI	Stats‡	Mean (MPa)†	95% CI	Stats‡
Saline	227.8	(199.8, 259.7)	A	311.4	(274.1, 353.7)	A
Calcium hydroxide	202.8	(177.8, 231.2)	A	250.1	(220.1, 284.1)	B
Odontopaste®	224.5	(196.9, 256)	A	333.1	(293.2, 378.5)	A
0.5% NaOCl gel	161.0	(141.3, 183.6)	B	133.4	(117.4, 151.6)	C, D
1% NaOCl gel	128.1	(112.4, 146.1)	C	143.9	(126.7, 163.5)	C
1% NaOCl solution	88.0	(77.2, 100.3)	D	115.5	(101.7, 131.3)	D
4% NaOCl solution	37.8	(33.1, 43.1)	E	27.4	(24.1, 31.1)	E

†Back transformed means (megapascals).

‡Statistical differences – means that do not share a letter vertically are significantly different.

Table 3. Microhardness of human and bovine dentine after seven-day medicament exposure – means and confidence intervals (CI).

	Human Dentine			Bovine Dentine		
Treatment	Mean (VHN) †	95% CI	Stats‡	Mean (VHN) †	95% CI	Stats‡
Saline	47.5	(43.4, 52.1)	A	35.5	(31.5, 39.5)	A
Calcium hydroxide	53.1	(48.5, 58.2)	A	36.1	(32.3, 40.5)	A
Odontopaste®	51.5	(47, 56.4)	A	36.0	(32.2, 40.3)	A
0.5% NaOCl gel	41.0	(37.4, 44.9)	B	23.8	(21.3, 26.6)	B
1% NaOCl gel	21.9	(20, 24)	C	18.7	(16.7, 21)	C
1% NaOCl solution	12.1	(11.1, 13.3)	D	6.7	(6, 7.5)	D
4% NaOCl solution§	11.8	(10.7, 12.9)	D			

†VHN, Vickers Hardness Number – back transformed means.

‡Statistical differences – means that do not share a letter vertically are significantly different.

§ Bovine group exposed to 4% NaOCl solution excluded from microhardness test due to excessive surface roughness preventing measurement.

Table 4. Comparison of the modulus of elasticity, flexural strength and microhardness for human (H) and bovine (B) dentine after seven-day medicament exposure.

	Modulus of Elasticity			Flexural Strength			Microhardness		
Treatment	Ratio (H/B)[†]	95% CI	P	Ratio (H/B)[†]	95% CI	P	Ratio (H/B)[†]	95% CI	P
Saline	1.10	(0.93, 1.30)	0.26	0.73	(0.61, 0.88)	< 0.001	1.35	(1.17, 1.56)	< 0.001
Calcium hydroxide	1.04	(0.88, 1.23)	0.65	0.81	(0.68, 0.97)	0.02	1.47	(1.27, 1.70)	< 0.001
Odontopaste®	1.04	(0.88, 1.23)	0.62	0.67	(0.56, 0.81)	< 0.001	1.43	(1.24, 1.65)	< 0.001
0.5% NaOCl gel	1.69	(1.43, 1.99)	< 0.001	1.21	(1.01, 1.45)	0.04	1.72	(1.49, 1.99)	< 0.001
1% NaOCl gel	0.97	(0.82, 1.14)	0.71	0.89	(0.74, 1.07)	0.21	1.17	(1.01, 1.35)	0.03
1% NaOCl solution	1.50	(1.27, 1.77)	< 0.001	0.76	(0.63, 0.91)	0.004	1.80	(1.56, 2.08)	< 0.001
4% NaOCl solution[‡]	0.78	(0.66, 0.92)	0.004	1.38	(1.15, 1.65)	0.001			
Overall	1.13	(1.05, 1.21)	0.001	0.89	(0.83, 0.96)	0.004	1.47	(1.39, 1.57)	< 0.001

[†]Back transformed ratios

[‡] Bovine group exposed to 4% NaOCl solution excluded from microhardness test due to surface roughness preventing measurement