

1	Retrospective study of the effectiveness of different treatment methods for equine cheek
2	teeth periodontal disease.
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19	Key words: Horse, Dentistry, Periodontal Disease, Metronidazole, Polyvinyl Siloxane,
20	Diastema burr.
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22	The study was a clinical improvement retrospective record audit and the guidelines of the
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Summary

27 Reasons for performing the stud	27	Reasons for	performing t	he stud
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- Equine cheek teeth periodontal disease is a common, often painful dental condition that
 may lead to premature tooth loss if left untreated^{1,2,3}. All cases of periodontal disease in this
 study were associated with diastemata, with the majority of cases being secondary or senile
 diastemata. At present limited objective information is available comparing different
- 32 treatment methods for the condition to assist clinicians in making evidence-based
- 33 treatment decisions.

Objectives

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- 35 To compare the effectiveness of four commonly used treatments for equine periodontal
- disease (additional to routine dental treatment and equilibration) to assess their merit in
- 37 reducing periodontal pocket depth.

38 Study Design

39 Retrospective case series.

Methods

- 41 Four commonly used equine periodontal treatments were compared: Removing feed
- 42 material and lavaging the periodontal pocket with dilute chlorhexidine and rinsing the
- 43 mouth with chlorhexidine based mouthwash ('Hexarinse^A'), 'clean and antiseptic lavage'
- 44 (CL); CL plus the addition of metronidazole antibiotics into the periodontal pockets (M); M
- 45 plus the addition of a polyvinyl siloxane temporary filling over the diastema (PVS); and
- diastema widening to open the diastema and increase the interdental space between



- 47 adjacent teeth, then PVS (DW). Periodontal pocket depth measurements were compared
- 48 before and after treatment at treatment intervals between two and six months.

49 Results

Treatment groups CL, M and PVS showed statistically significant reductions in pocket depth 50 following treatment. Mean pocket depth reduction was greatest in the DW group (and this 51 52 was the only group with no cases involving an increase in pocket depth), but this was based on a small sample size and was not statistically significant. Additional analysis to compare 53 effectiveness revealed a strong confounding effect of initial pocket depth. After taking this 54 55 into account, there was some evidence that DW was associated with smaller improvements 56 than the other treatments, however this result was also based on a small sample size, and influenced by a small number of cases with particularly large improvements. Among the 57 other treatments, no statistically significant differences in effectiveness were found. 58

Conclusion

- This study has shown that treatment methods CL, M and PVS are associated with
- statistically significant reductions in pocket depth. Due to the confounding effect of initial
- 62 pocket depth, no clear differences in effectiveness were found between treatment
- 63 methods.



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Introduction

Equine cheek teeth periodontal disease is a very common, often debilitating disease, clinically affecting around 60% of horses over 15 years of age¹. The disease is often secondary to diastemata, or the presence of a space between adjacent teeth where they should normally be in contact⁴. This space can then allow the entrapment of feed, which may be forced deeper into the interdental spaces by the forces of mastication. It is the combination of these foreign bodies stretching, inflaming and destroying the gingiva and later the periodontal ligament, combined with the secondary bacterial infection which create painful periodontal pockets.⁵ Left untreated, this destruction can progress to dental pain, dysmastication and premature tooth loss as well as periapical infection, oromaxillary fistulas with secondary sinusitis or osteomyelitis in severe cases^{2,5,6,7}. There are many causes of equine diastemata which are often described as primary, secondary or senile diastemata. Primary diastemata occur when there is either insufficient caudal angulation of the first cheek teeth (06°) or rostral angulation of the caudal cheek teeth (10° and 11°) to cause the normal compression of the cheek teeth arcades into a single functioning unit, hence creating interdental spaces. Alternatively, primary diastemata may be caused by teeth with adequate angulation but where the tooth buds have developed too far apart, also creating diastemata 5. Secondary diastemata occur when there is overcrowding in the arcade, causing displacement of the cheek teeth during eruption and the formation of interdental spaces around the displaced teeth. Diastemata can also develop secondary to cheek teeth overgrowths such as tall upper 6° or lower 11°, or around supernumerary teeth. In these cases the tall teeth become displaced either rostrally (tall upper 6s) or caudally (tall lower



11s) by the teeth in the opposing arcade, creating interdental spaces. The drift of adjacent teeth following a cheek tooth extraction can also lead to diastemata⁵. 88 Senile diastemata occurs when the narrower tooth apices erupt into the clinical crown, at 89 which point the dental arcade can no longer achieve compression due to the smaller size of 90 the apical aspect of the teeth, leaving interdental spaces^{5,8}. However the diastemata are 91 92 caused, these interdental spaces can then allow feed stasis and may lead to the development of periodontal disease⁵. 93 Due to the continuous eruption of equine cheek teeth, it has been suggested that 94 95 periodontal disease may be a reversible process in this species with Cox, Dixon and Smith 96 (2012)³ finding no histological features suggestive of irreversible change. As such, investigation into the most effective treatment methods to prevent further progression of 97 98 the condition, if not reverse the process are vital to improving equine dental health. A number of studies have investigated the aetiology and pathogenesis of the condition^{1,2,3,9} 99 and individual treatment methods^{10,11}, with Dixon et al. (2014)¹² showing a complete 100 remission of clinical signs in 72.6% of cases treated with diastema widening. Several studies 101 have also discussed other treatment options such as inserting perioceutics into the 102 periodontal pockets or using dental impression material to cover the periodontal pocket and 103 protect it from further insult. 1,5,7,11 However, there is limited objective information available 104 105 comparing the effectiveness of these different treatment methods^{7, 13}. As such, decisions on treatment methods may be based on recommendations from colleagues or personal 106 preference rather than on scientific evidence. 107

The aim of this study was to compare four commonly used treatment methods for equine periodontal disease to assess their effectiveness in reducing the depth of the periodontal pocket. The four methods assessed were: Removing the feed and lavaging the pocket with dilute chlorhexidine and rinsing with chlorhexidine based mouthwash ('Hexarinse^A'), 'clean and antiseptic lavage' (CL); CL plus placing small pieces of metronidazole antibiotic tablets as a perioceutic into the periodontal pockets after cleaning (M); M plus placing a polyvinyl siloxane (PVS) temporary 'filling' (also impregnated with crushed metronidazole) over the diastema as a temporary cover to protect the healing gingiva (PVS); and diastema widening with a burr and then placing metronidazole and a PVS temporary filling (DW). In all cases the teeth were 'floated' and 'equilibrated' as much as could safely be done in one treatment to remove or reduce enamel points and overgrowths. The horses were reviewed two to six months later and the pocket depths were remeasured to assess the effectiveness of the treatment method used.

Materials and Methods

A retrospective record audit was carried out on the clinical records of patients seen for routine equine dental examinations and treatments. During these dental visits, horses were sedated with a combination of xylazine^B 0.5-0.6mg/kg, butorphanol^D 0.01mg/kg plus or minus detomidine^E 2-4mcg/kg depending on the horse's temperament. The horses were then placed within portable stocks with the head elevated to allow a thorough examination. The external masticatory system was evaluated as well as excursion to cheek teeth contact and the mandible's rostrocaudal mobility assessed. The incisors were evaluated and treated if required before a full mouth speculum was placed on the horse. The mouth was then



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cleaned and thoroughly examined with a speculum light, a head light and a dental mirror and pathological changes were recorded on a dental chart (Figure 1). If periodontal disease was noted, the feed was removed and the pocket depth was then measured with a periodontal probe^G. The remaining teeth were also evaluated and treated as necessary; sharp enamel points were removed and other abnormalities such as excessive transverse ridges, hooks, displaced teeth or other overgrowths were also treated. Similar treatments were performed on all cases, reducing the possibility of confounding by the effects of these additional treatments. However, these additional treatments were not included in the statistical analysis. The treatment options for the periodontal disease were then discussed with the client and a decision was made based on periodontal pocket depth, financial considerations and owner's preference or concerns. The criterion for inclusion in the study was that the horse must have had at least one cheek tooth periodontal pocket with a depth of 5mm or more. In addition, the horses must have been examined again for a second treatment within 6 months of the initial consultation as it was considered longer intervals may not reflect the true outcome of the treatment used. All cases reviewed (100% sample) between the 1st January 2014 and the 1st September 2014 fitting the above criteria were included in the study. The initial consultations for some horses were before this period. All treatments and measurements were completed by the one operator to eliminate any interoperator variation. Statistical analysis was carried out using the statistical programming language R¹⁴, together with packages 'nlme' and 'multcomp' (see Supplementary Information for details).



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Application of treatments

Clean and antiseptic lavage: The feed was removed from the diastemata using a combination of elongated alligator forceps, dental picks, H files (30mm, size 55) grasped in a pair of haemostats to pull the feed out and water irrigation/ water picks with dilute chlorhexidine to flush out the pockets. The mouth was also rinsed with chlorhexidine based mouthwash ('Hexarinse^{A'}), which contains 1.4g/L chlorhexidine gluconate as well as zinc gluconate and cetylpyridinium in a palatable liquid. It was also recommended that the client flush the horse's mouth out with a hose, and clients were offered the option of continuing with the chlorhexidine based mouthwash ('Hexarinse'^A). It was recommended that they flush the mouth thoroughly with a hose for around one minute to remove the feed material, then administer the chlorhexidine based mouthwash ('Hexarinse'A) at a dose of 30mL up each side of the mouth, administered in a syringe. They were asked to do this daily for 2 weeks, then on alternate days for 2 weeks, and subsequently 2-3 times per week as a maintenance. This was offered and recommended in all cases of significant periodontal disease but was not always accepted by the client, usually for financial reasons. The study did not record owner compliance. Metronidazole antibiotics: Once the feed in the diastema had been removed and the pocket irrigated with dilute chlorhexidine, 200mg metronidazole antibiotic tablets were then crushed or cut into small pieces, around 1-2mm diameter and these pieces were then placed digitally into the periodontal pockets. The size of the periodontal pockets determined the number of pieces of metronidazole placed, as they were placed until they began to protrude from the gingival margin. They were always placed lingually but larger pockets could also be placed buccally. The mouth was then rinsed with chlorhexidine based

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mouthwash ('Hexarinse^{A'}). This treatment was only used on mandibular pockets as the author has found it virtually impossible to prevent antibiotics in maxillary periodontal pockets from falling out due to the effects of gravity. The pockets were then inspected with a light and mirror to ensure that the antibiotics had been placed correctly. Polyvinyl siloxane: This treatment method involved the same as for metronidazole antibiotics (metronidazole antibiotics were initially placed into the periodontal pockets), and a PVS temporary filling was then placed over them. A small amount (enough to make around a 1cm ball, more for larger pockets such as after diastema widening) of the two parts of the PVS were mixed digitally and some crushed metronidazole was also mixed through the PVS until a consistent colour was obtained. The putty was then pushed into the diastema, as far down as possible and scalloped out slightly digitally on the lingual, occlusal and buccal borders. It was found to be important not to leave 'tags' overhanging from the diastema as these temporary fillings appeared to be more easily removed by the horse's tongue. The putty would usually set within 1-2 minutes and this was checked on the excess putty that had been removed to ensure the catalyst reaction had been achieved. The placed temporary filling was then examined digitally and visually to ensure a good seal to the gingival margin had been achieved and with minimal or no overhanging tags. The same method was used for both maxillary and mandibular teeth. Diastema widening with a mechanical bur: Diastema widening was performed with a solid carbide diastema burr (Powerfloat^H) in the affected interdental space. The diastema was first carefully examined to assess the location of the pulp cavities on the bordering teeth, so any pulps particularly close to the diastema could be carefully monitored to avoid pulp exposure. The orientation of the diastema was also noted as in some instances, such as the



mandibular 10/11 interproximal space, it can often be quite oblique. Initially a tapered burr was passed vertically through the diastema, stopping at least every 10 seconds to flush the mouth and reassess visually to avoid thermal damage and reduce the risk of pulp exposure. Once the tapered burr had been successfully passed, a 4.54mm conical burr was then also passed vertically through (again stopping every 10 seconds to cool the teeth and visually reassess) to complete the diastema. The adjacent teeth were then carefully examined for any sign of pulp exposure and any remaining feed material was removed before metronidazole antibiotics and PVS were placed in/ over the diastema respectively.

Results

Initial data set: A total of 295 cases (in 47 horses) were recorded, where one case represents a treatment performed for one diastema on one horse. Extractions were recorded as two cases if two diastemata were affected (rostral and caudal to the extracted tooth). In some cases, repeated treatments were performed on the same horse within the study period. 51 cases (12 horses) were second treatments on a given horse within the study period, and 3 cases (1 horse) were third treatments. Treatment outcomes were measured by the improvement (in millimetres) in periodontal pocket depth between visits. Eliminated cases: The data set included two extractions, which were represented by four cases (two horses with one extraction each; one rostral and one caudal periodontal pocket for each extracted tooth). The teeth were extracted due to excessive mobility (greater than 3mm) in the teeth from deep periodontal disease, and both were older horses (>21 years

old). These cases were removed from the data set used for the statistical analysis, due to 219 220 the qualitatively different nature of extractions compared to the other treatment methods. Final data set: After the removal of the extraction cases from the data set, 291 cases 221 remained (47 horses). 222 223 In 86 out of the 291 cases, periodontal pockets were fully healed by the time of the next visit, implying that the response values (pocket depth improvement) were limited or 224 truncated at the maximum depth of the initial pocket. However, the measured 225 improvements in these cases were still found to be relatively high (5.47±1.69 mm) 226 227 compared to the rest of the data set (Table 1, Figure 2). 228 One-sample t-tests were calculated to test whether the measured improvements in pocket depth for each of the four treatment methods were significantly different from zero 229 230 (Table 2). P-values and confidence intervals were adjusted for multiple testing by applying the Bonferroni correction to achieve a family-wise error rate of 5% across the four tests. In 231 232 summary, the improvements in treatment groups CL, M and PVS were strongly statistically significantly different from zero (P < 0.001), with mean reductions in pocket depth of 1.75 233 mm (95% CI 0.84–2.66 mm), 2.81 mm (95% CI 2.24–3.39 mm), and 3.64 mm (95% CI 2.47– 234 4.81 mm) respectively (Tables 1 and 2). The improvement in treatment group DW (mean 235 reduction 4.00 mm, 95% CI -1.24-9.24 mm, P = 0.2) was not statistically significant after 236 237 adjusting for multiple testing. However, this conclusion is based on a relatively small sample size (there were 9 cases across 5 horses in the DW treatment group). 238 A linear mixed-effects model was fitted to compare the effectiveness of the different 239 treatment methods, while controlling for possible confounding variables and correlations 240



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among measurements from each horse (see Supplementary Information for further details on the model). The model results showed that the previous pocket depth prior to treatment was the strongest predictor of the subsequent amount of improvement (Supplementary Table S1). The estimated coefficient for this term is positive and highly significant, implying that deeper pockets tended to experience greater improvement following treatment. Further analysis of the data revealed that the choice of treatment method was strongly dependent on the initial pocket depth, with more intensive treatment methods generally chosen for deeper pockets. This is unsurprising, since more serious cases of periodontal disease with deeper pockets will clearly tend to require more intensive treatment. However, this meant that there was a strong confounding effect of previous pocket depth prior to treatment on the amount of improvement following treatment. This confounding effect made it difficult for the model to reliably estimate the separate effect of the choice of treatment method. After taking into account the effect of previous pocket depth, pairwise comparisons between treatment methods revealed that DW was in fact associated with significantly smaller magnitude improvements than each of the other methods. However, this result was based on a small sample size and was strongly influenced by a single influential data point, so should not be interpreted as strong evidence. No other statistically significant pairwise differences were found among the other treatment methods (see Supplementary Information for additional details). In summary, the analysis found that the treatment groups for cleaning and antiseptic lavaging of pockets, instilling metronidazole, and the polyvinyl siloxane temporary fillings each showed strongly statistically significant reductions in periodontal pocket depth. The



treatment group for diastema widening did not show a significant reduction after adjusting for multiple testing. Comparisons between the treatment methods revealed a strong confounding effect of previous pocket depth prior to treatment, and some evidence that diastema widening was associated with smaller reductions than the other treatments.

In addition, it was noted that the provision of additional chlorhexidine based mouthwash ('Hexarinse^A') to the client following treatment was found not to have a statistically significant association with the improvement in pocket depth. However this simply recorded whether chlorhexidine based mouthwash ('Hexarinse^A') was dispensed to the client, and did not measure client compliance, making it difficult to interpret this result.

Discussion

With advances in equine dental education and examination techniques, veterinary practitioners are becoming more aware of the pain and morbidity associated with periodontal disease and therefore the importance of a scientific approach to its treatment. This study found that cases treated with metronidazole antibiotics, polyvinyl siloxane temporary fillings, and/ or diastema widening showed greater mean reductions in pocket depth than that achieved with dental equilibration and cleaning and antiseptic lavaging the pockets alone. However, it was not possible to conclusively link the differences to the choice of the different procedures. After accounting for the confounding effect of initial pocket depth, the differences between the treatment methods in this study were found not to be statistically significant, except for some evidence for smaller reductions for diastema widening. A randomised choice of treatment method could potentially avoid these issues in



a future study, however this would be difficult to justify to clients if it involves additional expense or risk.

The results point to the key finding that the treatment methods used were quite strongly influenced by the initial pocket depth; shallower pockets were often just cleaned and lavaged, whereas diastema widening was performed only for deeper pockets. When the potential risks involved in diastema widening (opening a pulp cavity, or causing thermal pulpar damage) were explained to the client, the treatment method was rarely chosen for milder cases. Vice versa, with deeper pockets, the clients were more likely to accept more aggressive (and expensive) treatment methods. This did affect the possible improvement with these treatment methods as with the milder cases, the possible improvement was of course much less.

Diastemata were present to some degree in all cases in this study. The majority of the diastemata, particularly those in horses with multiple diastemata, were present in horses with concurrent peripheral dental caries, which is very prevalent within the Western Australian horse population (62% prevalence in treated horses, K. Jackson, unpublished data, 2014). In these cases the interproximal spaces (as well as buccal and lingual/ palatal aspects of the teeth) were missing a significant proportion of the peripheral cementum, creating diastemata in the clinical crown. There were also numerous cases of senile diastemata and secondary diastemata from displaced, tall teeth or hooks. No cases of primary diastemata were noted, however this may have been a contributing factor in some cases with peripheral dental caries.

It should be noted that in some cases where dental overgrowths were treated, these could not be completely reduced in one treatment; either for risks of exposing the pulp cavity or

in the mouths of elderly horses where reduction could take the cheek teeth permanently 309 out of occlusion. In these cases the abnormal forces on the teeth from the overgrowths may 310 have still been contributing to the development of diastemata and may have influenced the 311 effectiveness of the treatments performed. 312 The longevity of the polyvinyl siloxane temporary fillings was also difficult to assess. Without 313 314 sedating and checking daily it is impossible to know how long the fillings remained in place, 315 but the author has certainly had many still present up to 18 weeks after placement. In cases 316 where they were still present (usually wider diastemata with very deep infection), once they were removed, the underlying gingiva certainly appeared much less inflamed and as stated 317 above, the pocket depth had significantly reduced in most cases. However in other cases, 318 particularly in narrower diastemata, and horses with very agile tongues, it is difficult to 319 know how long they remained in place. 320 Furthermore, it was difficult to accurately assess the effectiveness of providing additional 321 chlorhexidine based mouthwash ('Hexarinse^{A'}) in this study, as it was only recorded whether 322 323 the product was purchased by the client but not how often it was used. This study only assessed metronidazole antibiotics as a perioceutic, future studies 324 comparing the effects of instilling different antibiotics such as doxycycline or even other 325 perioceutics such as platelet derived growth factor¹⁷ into the periodontal pockets could be 326 327 useful to compare their effectiveness in our equine patients. Studies examining the bacterial populations present in periodontal pockets and culture and sensitivity on these bacterial 328 populations may also lead to other antibiotic perioceutic options being used and tested in 329 the future. 330



An important point from the research is that conservative therapy should always be considered before resorting to extraction. While of course not all teeth can be saved, it will never be known whether a tooth could have been saved if it is extracted. There were cases during this study of horses over 20 years old with pocket depths of 25-30mm, and in one case there was even slight mobility of the teeth. Extraction was discussed but it was decided to try conservative therapy, and in most cases there was significant reduction in pocket depth, if not almost complete resolution of the periodontal disease.

In summary, this study found that cleaning and lavaging, adding metronidazole antibiotics and adding a polyvinyl siloxane temporary filling were all associated with significant reductions in pocket depth. After accounting for the confounding effect of initial pocket depth, the effectiveness of the different treatment methods could not be statistically differentiated, except for some evidence that diastema widening showed smaller reductions than the other treatments. Diastema widening has been shown to be clinically effective in previous studies^{11,12}; and while this study did not find a statistically significant improvement in pocket depth in diastema widening cases, the result is difficult to judge due to the small sample size in this group.



Conflict of Interest Statement

None of the authors of this paper have any financial or personal relationship with other 349 people or organisations which could bias or inappropriately influence the contents of this 350 351 paper. 352 Manufacturers' details: A'Hexarinse': Virbac Animal Health, Milperra, Australia. 353 ^B'Xylazil': Troy Ilium, Glendenning, Australia. 354 355 ^D'Butorgesic': Troy Ilium, Glendenning, Australia. E'Calmant': Ranvet, Banksmeadow, Australia. 356 ^GPeriodontal Probe: EVDS Dental Instruments (periodontal dental probe with 4mm 357 358 increments), South Grafton, Australia. ^H'Powerfloat': EVDS Dental Instruments, South Grafton, Australia. 359 360 Statistical software: R statistical programming language, version 3.1.1; 'nlme' R package [8], version 3.1-118; 'multcomp' R package [9], version 1.3-8. 361



Supplementary Information Items

- 363 Additional information on the statistical analysis can be found in the Supplementary
- 364 Information (text and Supplementary Table S1).
- 365 Code and Data Availability
- R code and a spreadsheet with anonymised data to reproduce the statistical analysis in this
- 367 study are available at: https://github.com/lmweber/Jackson-periodontal-disease



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Table 1. Summary statistics (mean, median, standard deviation, minimum, maximum, and number of cases) for measured improvements in periodontal pocket depth between visits for each of the four treatment methods. CL = cleaning and antiseptic lavaging; M = additional use of metronidazole antibiotic tablets; PVS = additional use of polyvinyl siloxane temporary filling; DW = additional diastema widening. Total number of cases = 291.

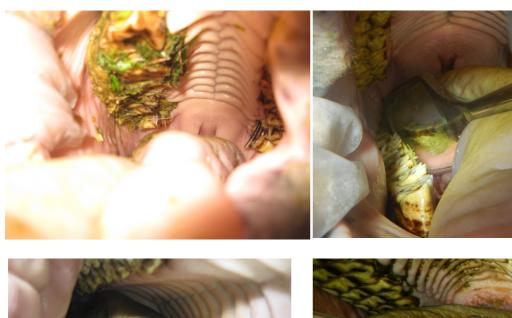
	Mean (mm)	Median	Std. dev.	Min (mm)	Max (mm)	N
		(mm)	(mm)			
CL	1.75	3.00	2.82	-5.0	6.0	64
М	2.81	2.00	2.73	-3.0	12.0	145
PVS	3.64	3.00	3.90	-4.0	26.0	73
DW	4.00	3.00	4.90	0.0	16.0	9

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Table 2. Test results evaluating whether measured improvements in pocket depth for each treatment method are significantly different from zero (one-sample Student's t-tests, alternative hypothesis of zero effect, two-sided). Confidence intervals and p-values are adjusted for multiple testing using the Bonferroni method to achieve a family-wise error rate of 5% across four tests. CL = cleaning and antiseptic lavaging; M = additional use of metronidazole antibiotic tablets; PVS = additional use of polyvinyl siloxane temporary filling; DW = additional diastema widening; df = degrees of freedom; CI = confidence interval; * indicates Bonferroni adjusted values.

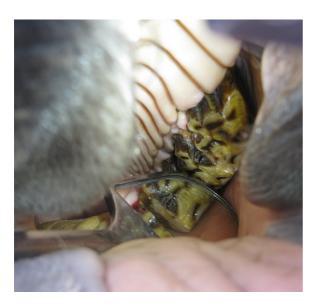
	t-value	df	95% CI*	p-value*
CL	4.97	63	[0.84, 2.66]	<0.001
М	12.43	144	[2.24, 3.39]	<0.001
PVS	7.98	72	[2.47, 4.81]	<0.001
DW	2.45	8	[-1.24, 9.24]	0.2

- 424 Figure 1. Feed packing in diastemata causing periodontal disease (top). PVS temporary filling
- after placement (middle left and right). After diastema widening but before PVS filling was
- 426 placed (bottom).









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Figure 2. Distributions of measured improvements in periodontal pocket depth between visits for each of the four treatment methods. The box plots show the median, first and third quartiles, and extreme values. CL = cleaning and antiseptic lavaging; M = additional use of metronidazole antibiotic tablets; PVS = additional use of polyvinyl siloxane temporary filling; DW = additional diastema widening.

Improvement by treatment method

