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Original Article

Influence of the acidity of electrolyzed water on the microhardness of inner layer dentin

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Abstract *Background/purpose:* Electrolyzed water has been identified as an effective disinfectant that could represent as an alternative to sodium hypochlorite. Unfortunately, it remains unclear whether the texture or physical properties of dentin are affected by the application of electrolyzed water of different acidities. This study was aimed to assess the influence of electrolyzed waters with differing pHs on the demineralizing of inner dentin.
Materials and methods: The coronal superficial dentin of 20 human molars was exposed and further bisected into two pieces perpendicular to the dentin surface. The samples were immersed in strongly acidic electrolyzed water (AW group), neutral electrolyzed water (NW group), 5% sodium hypochlorite (positive control, NL group), or deionized water (negative control, DW group). Microhardness of the inner layer dentin was measured at a depth of 25 and 50 μm beneath the superficial surface layer every 5 up to 60 min.
Results: At a depth of 25 μm, microhardness decreased with increasing immersion time in all but the DW group. The AW group exhibited a decreasing trend from the first 5 min that became significant after 35 min of immersion and was the most rapid decrease in the four groups. The rate of decline in the NW group was low and similar to that of the NL group. Both NW and NL groups exhibited significantly less demineralization than the AW group after 15 min of immersion. No significant microhardness change was found at a depth of 50 μm in any of the samples.

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Conclusion: AW produces a more pronounced softening of dentin than NW at a depth of 25 μm.

Introduction

Disinfection and sterilization are essential in dental and medical field. Sodium hypochlorite (NaOCl) is one of the dental disinfectants and a commonly used root canal irrigant (within 0.5%–6% concentration) in endodontic treatment due to its superior bactericidal effect.[1,2] 0.25% NaOCl in the way of oral rinse application is reported to be effective on reducing plaque formation and gingival bleeding and capable of the application in periodontal treatment.[3] However, 2.5% and 5.25% NaOCl used as root canal irrigant has been verified to cause a reduction of microhardness of dentin despite of its excellent bactericidal effect.[2,4] The use of 1% sodium hypochlorite was also verified to possess a softening ability on dentin,[5,6] however, Pascon et al. reported that dentin immersed in 1% NaOCl showed no significant change in both microhardness and roughness.[7] Aydin et al. mentioned that 0.1% NaOCl could cause a significant decrease of microhardness on enamel or dentin when stored for twelve months.[8] Studies also revealed a bond strength reduction of dentin being treated with 5% NaOCl.[9,10] Besides, NaOCl as a root canal irrigant may not effectively remove the smear layer or cause moderate to severe cytotoxicity when extruded the root apex.[11,12] Therefore, either for the purpose of root canal irrigation or mouth rinse, the extensive application of NaOCl may induce a unfavorable potentiality to dentin regardless of the concentration.

Recently, electrolyzed water (EW), a novel disinfection system, has been identified as an effective disinfectant that could represent an alternative to sodium hypochlorite.[13–15] EW is a product of electrolysis of a dilute sodium chloride solution in an electrolysis cell into acidic electrolyzed water and basic electrolyzed water.[16] EW is gaining popularity as a sanitizer in the food industry with the merits of low irritation of tissues, robust bactericidal and virucidal effects, low cost and non-polluting.[17–19] Reports have mentioned that EW is effective as a sterilizer for medical instruments in hospitals[20] and also useful for the sterilization of dental chairs pipelines,[21] impression material,[22,23] denture bases,[24] mouth rinse[25] and root canal irrigants[14,15] in dental field.

EW can be classified according to the acidity into strongly acidic EW, weakly acidic EW and neutral EW.[26] Strongly acidic EW (AW) used as drinking water has been reported to cause no harm to enamel or morphological changes in mice after eight weeks of administration and also showed significant antibacterial effects both in vitro and in vivo, which suggest that it could be applied as an effective mouthwash.[25,27] On the contrary, other studies found that AW caused a noticeable decalcification of enamel exposed for more than 60 min. After seven days of immersion in AW, exposed enamel revealed an SEM image pattern similar to that of phosphoric acid-etched enamel, while seven days of immersion in NW caused no surface texture change.[28] Qing et al. have reported that AW as a root canal irrigant caused no decrease of microhardness in dentin.[29] Ghisi et al. demonstrated that electrochemically activated water (similar to AW) reduced the microhardness of dentin as other root canal irrigants within 500–1000 μm from the root canal lumen.[30] According to our pilot study, AW has a softening effect on dentin even immersed for 5 min.

As the population ages, more tooth preservation is noted, although root exposure becomes an issue. It is also well-known that cervical abrasion is more frequently seen in the elderly than in younger age groups.[31,32] The increasing exposure of root surfaces makes them vulnerable to plaque and caries. Any mouthwash used will entail a higher frequency of contact with the dentin. Even though AW has the potential of decreasing the microhardness on the outmost layer of dentin, there is still no research reported the influence of EW of different pH values on the inner dentin in order to realize the possible application of EW in mouth rinse or root canal irrigation condition.

This study was designed to measure the Vickers microhardness of inner layer dentin at depths of 25 μm and 50 μm after immersion in EW at different pH values for a total immersion time up to 60 min for realizing the penetration ability of different pH values of EW. We hypothesized that different acidity values of EW would have no penetration effect on the microhardness of the inner dentin with increasing immersion periods.

Materials and methods

Preparation of dentin specimen

Twenty intact molars extracted within one month without caries, fillings, prosthetics, or cracks were preserved in 2% chloramine-T solution for study. Each tooth was cut at the cemento-enamel junction and the occlusal enamel surface of the crown removed to expose the superficial dentin using a low-speed diamond disc (Isomet®, Buehler, Lake Bluff, IL, USA). The sectioned tooth piece was embedded in resin (Resin 27-751®; Refine Tech Co., Ltd., Yokohama, Japan) with the occlusal dentin exposed. The dentin-resin block was further bisected perpendicular to the superficial dentin surface using the diamond disc to expose the inner dentin surface (Fig. 1a–c). In order to mimic the tooth surface and also provide a better measuring surface, both of the superficial and longitudinal surfaces of each half dentin-resin block were sequentially grounded by sandpaper grits #600, #800, #1000, #1200, #1500, and #2000 under running water and served as dentin specimens for this study. Written

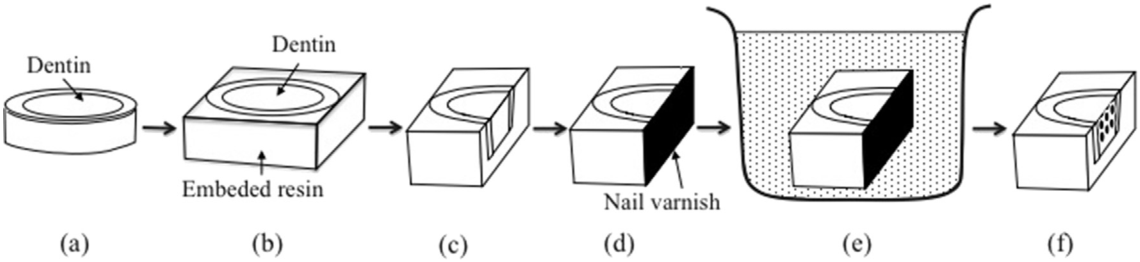

Figure 1 Diagram of the immersion processes of a dentin block from a specimen and of preparation for hardness measurements.

informed consent was obtained from all subjects prior to samples collection. Ethical approval was obtained from the Institutional Review Board at Chung-Ho Memorial Hospital, Kaohsiung Medical University (KMUHIRB—E(1)-20150083).

Preparation of immersion solutions

Four kinds of immersion media were used in this study: (1) AW ($pH = 2.4 \pm 0.04$) (Mini Super Water ED-007®, Hirata Co., Osaka, Japan) and (2) NW ($pH = 6.9 \pm 0.04$) (Asahi Pretec Aqua 21®, Asahi Pretec Co., Kobe, Japan) as the experimental ones; (3) 5% sodium hypochlorite (NL, $pH = 13.2 \pm 0.02$) (Neo Cleaner®, Neo Dental Chemical Products Co., Tokyo, Japan) as a positive control; and (4) deionized water (DW, $pH = 7.4 \pm 0.01$) as a negative control.

Microhardness measurement of the longitudinal aspect of dentin

Five dentin specimens were randomly placed into each of the four solutions. The Vickers microhardness of the inner layer dentin surface in each specimen prior to immersion was tested at depths of 25 μm and 50 μm beneath the superficial dentin surface at five points, separately, to define the baseline microhardness at each measuring depth in each group, using a microhardness tester (HMV-2T®; Shimadzu Corporation, Kyoto, Japan). Each indentation was executed at a distance of 100 μm apart from the others to avoid superimposing.

A layer of nail varnish was applied to the longitudinal surface (perpendicular to the superficial dentin surface) of each specimen to protect it from the influence of the immersion liquid before each immersion (Fig. 1-d). The specimens were immersed in a beaker filled with 500 mL immersion liquid for 5-min intervals. During the immersion periods, a magnetic stirrer was employed to create uniform specimen-solution contact in the beaker. Over the immersion intervals, the varnished inner dentin surface was removed by polishing with #2000 waterproof sandpaper under running water to expose the protected dentin. Vickers microhardness of the longitudinal dentin surface was measured the same way as performed in the baseline measurements at 25 μm and 50 μm depths. The inner dentin surface of each specimen was coated again with nail varnish for further 5-min immersion before measurement. This procedure was repeated until the total immersion time reached 60 min. The whole procedure from specimen preparation to microharness measurement of inner dentin was shown in Fig. 1.

The Vickers hardness numbers (Hv) of four groups were measured by the microhardness tester under an indentation strength of 490.3 mN (equivalent to 50 g) for 15 s. Indentations caused by the diamond indenter on each specimen were observed through a microscope eyepiece, and the lengths of two diagonal lines of the indentation were measured to calculate each microhardness value using built-in software.

Statistical analysis

The microhardness and amounts of change in each group at each time point were compared and analyzed using SPSS 19.0 version statistical software. ANOVA method and Tukey's HSD test were conducted and significance *p*-value ($p < 0.0038$) was adjusted by Bonferroni's correction. The repeat measurements method of the general linear model and Least-Significant Difference multiple comparison test were employed to assess the effects of various solutions on the dentin microhardness change over time and to analyze the trends in overall microhardness change.

Results

Microhardness of the longitudinal aspect of dentin at 25 μm

The dentin microhardness over time of each group measured at 25 μm is shown in Table 1. No significant difference was found among the four groups ($p = 0.8142$) at the baseline. The AW group presented a statistical significant difference with the NW, NL and DW groups after 15 min immersion, while no significant difference could be seen among the NW, NL and DW groups. The AW group showed a decreasing tendency from the first 5 min of immersion and presented a significant difference after 35 min immersion ($p < 0.05$), however, the NW group and NL group showed no statistical significance within the 60 min immersion period even though a decreasing tendency was detected. After 60 min of immersion, the microhardness decreased to 25% of baseline in the AW group, 66% of baseline in the NW group and 71% of baseline in the NL group. There was almost no change in the DW group. Fig. 2 reveals the time dependent microhardness values at 25-μm depth in each group.

Table 1 Microhardness (Average ± SD) of inner dentin at 25 μm and at each interval after immersion in different solutions.

Time (min)	AW	NW	NL	DW
0	59.35 ± 1.91^{Aa}	58.71 ± 4.90^{Aa}	57.87 ± 3.58^{Aa}	57.46 ± 2.24^{Aa}
5	48.54 ± 8.40^{Aa}	56.99 ± 5.15^{Aa}	55.06 ± 7.08^{Aa}	58.33 ± 0.54^{Aa}
10	42.90 ± 8.25^{Aa}	53.84 ± 7.48^{Aa}	53.56 ± 9.05^{Aa}	56.28 ± 3.76^{Aa}
15	32.61 ± 7.37^{Ba}	52.86 ± 6.02^{Aa}	51.30 ± 11.27^{Aa}	56.94 ± 3.48^{Aa}
20	31.07 ± 11.12^{Ba}	50.10 ± 6.40^{Aa}	51.26 ± 8.65^{Aa}	58.03 ± 3.24^{Aa}
25	26.61 ± 12.70^{Ba}	49.01 ± 6.75^{Aa}	50.24 ± 9.02^{Aa}	57.36 ± 1.75^{Aa}
30	23.04 ± 7.61^{Ba}	48.41 ± 6.45^{Aa}	47.66 ± 7.32^{Aa}	57.18 ± 2.83^{Aa}
35	21.54 ± 4.32^{Bb}	46.49 ± 4.24^{Aa}	48.42 ± 6.49^{Aa}	57.55 ± 1.96^{Aa}
40	16.74 ± 3.55^{Bb}	43.87 ± 4.99^{Aa}	44.23 ± 5.38^{Aa}	57.25 ± 2.81^{Aa}
45	16.80 ± 5.37^{Bb}	41.90 ± 2.66^{Aa}	42.13 ± 4.06^{Aa}	26.24 ± 2.78^{Aa}
50	16.80 ± 2.97^{Bb}	42.74 ± 2.81^{Aa}	43.90 ± 5.98^{Aa}	55.86 ± 3.90^{Aa}
55	14.48 ± 3.87^{Bb}	40.43 ± 7.43^{Aa}	42.27 ± 6.81^{Aa}	56.88 ± 3.05^{Aa}
60	14.93 ± 3.55^{Bb}	38.81 ± 5.74^{Aa}	40.81 ± 6.85^{Aa}	56.78 ± 2.40^{Aa}

Different upper letters indicate statistically significant difference ($P < 0.05$) between solutions. Different lower letters indicate statistically significant difference ($P < 0.05$) between time intervals of each solution.

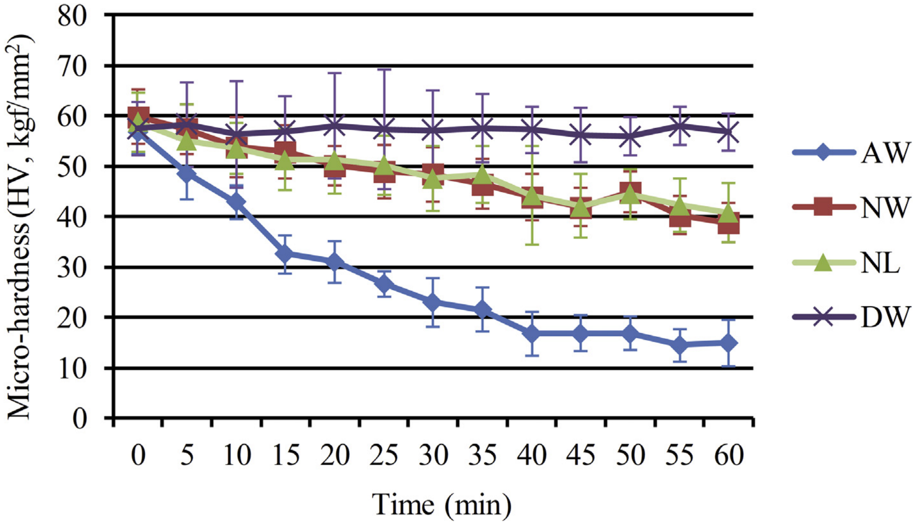

Figure 2 Average microhardness measurements at the longitudinal surface 25 μm beneath the superficial surface in each group plotted against time. AW: strongly acidic electrolyzed water, NW: neutral electrolyzed water, NL: 5% sodium hypochlorite, DW: deionized water.

Microhardness of the longitudinal aspect of dentin at 50 μm

The time-dependent microhardness of each group measured at 50 μm is shown in Table 2. The microhardness values for the longitudinal 50-μm depth showed no significant difference among the four groups ($p = 0.6307$) and were used as the baseline for the 50-μm depth microhardness. No significant difference was noted between the microhardness at 0 min and 60 min immersion in any group ($p > 0.05$). Fig. 3 reveals the microhardness changes over time in the 50-μm depth dentin in each group.

Discussion

The bactericidal effect of EW is known to be related with the residual chlorine, which converts to hypochlorous acid or hypochlorite ion.[26] The concentration of residual chlorine in AW (49 ± 1.7 ppm) is greater than that in NW (38 ± 0.0 ppm).[33] It is reported that if the residual chlorine is reduced to half of the original concentration, the bactericidal capacity is unchanged and remains effective within the pH range of 2.6–7.0.[18,34] However, EW possess different degrees of acidity, pH is one of the factors needed to take into consideration when EW is applied in the oral environment. The bactericidal effect of AW (pH = 2.4) has been verified to deteriorate rapidly but the pH still kept its acidity in three month storage period, while the acidity (pH = 6.9) and bactericidal activity of NW remains stable over the same period.[33] AW has been reported to exert a demineralization effect on enamel, raising concern over its effects on teeth when used as a mouthwash.[28] This study was focused on evaluating the influence of different pH values of EW on the microhardness of dentin at various depths.

Table 2 Microhardness (Average ± SD) of dentin at 50 μm and at each interval after immersion in different solutions.

Time (min)	AW	NW	NL	DW
0	60.13 ± 2.53	60.46 ± 4.84	61.96 ± 5.39	58.67 ± 1.75
5	60.68 ± 3.74	58.96 ± 3.69	60.24 ± 6.74	59.11 ± 4.15
10	61.92 ± 4.18	59.30 ± 3.64	59.94 ± 4.98	59.28 ± 2.52
15	60.72 ± 2.56	59.19 ± 3.85	59.94 ± 5.86	57.66 ± 2.84
20	58.89 ± 2.58	59.24 ± 3.24	59.43 ± 5.91	58.65 ± 2.56
25	61.50 ± 2.24	59.59 ± 4.85	58.66 ± 5.34	58.96 ± 1.10
30	60.38 ± 1.71	57.17 ± 5.04	57.63 ± 4.79	58.31 ± 3.56
35	61.07 ± 1.36	59.79 ± 4.27	58.74 ± 4.78	57.58 ± 2.30
40	58.90 ± 4.68	58.99 ± 4.05	52.66 ± 10.31	57.12 ± 2.86
45	59.14 ± 3.40	58.04 ± 3.07	55.36 ± 5.60	57.10 ± 1.94
50	59.10 ± 2.03	59.02 ± 3.67	54.67 ± 4.83	57.45 ± 1.48
55	59.83 ± 3.75	58.31 ± 3.29	56.55 ± 4.69	57.63 ± 2.08
60	59.33 ± 3.17	58.92 ± 3.60	55.38 ± 4.92	57.47 ± 3.19

No statistically significant difference ($P > 0.05$) appeared in either solutions or time intervals of each solution.

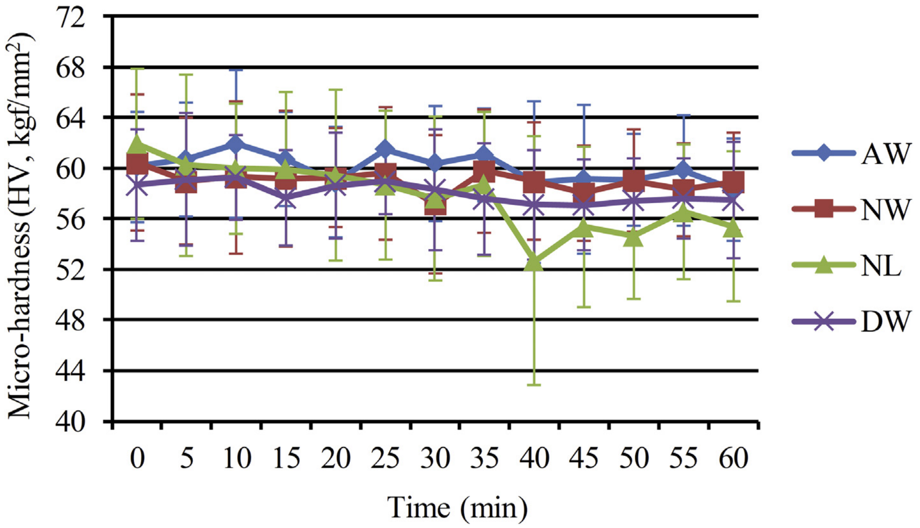

Figure 3 Average microhardness measured at the longitudinal surface 50 μm beneath the superficial surface in each group plotted against time. AW: strongly acidic electrolyzed water, NW: neutral electrolyzed water, NL: 5% sodium hypochlorite, DW: deionized water.

In order to realize the effect of each EW solution on inner layer dentin, the longitudinal side dentin was covered with protective varnish to prevent direct contact by the solution while permitting solution to contact the superficial layer of occlusal surface dentin to simulate real-life conditions. This method was similar to that adopted by Oliveira et al. who investigated the effects of root canal irrigants on dentin microhardness.[5]

The AW group showed an insignificant but distinct decrement apart from the NW, NL and DW groups after the first five minutes, and tended to reveal a significant difference beyond 15 min of immersion compared with that of the other three groups. AW, similar to NW, contains chlorine-relative substances as its main bactericidal and organic dissolution components but differs in its low pH (pH = 2.4). It is well known that the pH of AW is below the critical pH for demineralization of enamel (pH = 5.2) and dentin (pH = 6.7).[35] It has been reported to etch enamel in a manner similar to that of phosphoric acid.[28] AW has been reported to maintain its low pH even though the bactericidal effect decreased.[33] This indicates that AW, in contrast with NW, appears to exert two effects upon dentin, with the chlorine moieties dissolving the organic portion and the low pH causing demineralization.

The changes in microhardness over time of the NW and NL groups at the 25-μm depth (Fig. 1) were similar with no significant difference ($p > 0.05$). It is known that NaOCl can soften the organic portion of dentin,[36] and will shift to HOCl by the pH.[37] Neutral electrolyzed water was reported to have more HOCl and less volatile Cl_2, which are associated with superior bactericidal effect.[33] Report has mentioned that instead of the neutral pH and comparatively low oxidation-reduction potential, the sterilization effect of NW was mainly due to HOCl, one of the derivatives from NaOCl,[38] which is known to act as a deproteinizer that can also result in dentin degradation[14] and organic components within the smear layer.[38] Besides, the dentin does not demineralize unless the critical pH is below 6.7.[35]

Therefore, it is strongly indicated that the softening effect of dentin over time in NW is mainly originated from the influence of HOCl and OCl^- moieties rather than the neutral pH. AW group showed a significant decrease of microhardness than that of other three groups (Fig. 1). Marshall et al. mentioned that decreased pH of phosphoric acid increased the dentin recession depth.[39] This may explain why AW could induce a marked decrease of microhardness in dentin than NW. According to the results of this study, the hypothesis that acidity of EW had no effect on microhardness of dentin was disproven by the fact that EW caused a decrease of microhardness in dentin at 25-μm depth.

None of the four groups showed prominent microhardness change at the 50-μm depth of inner dentin after 60-min immersion. Camps et al. have proven that dentin possesses a buffering capacity to recover most of the applied phosphoric acid from the surface,[40] and the capacity is originated from the hydroxyapatite of the mineral portion of dentin.[40,41] Marshall et al. declared that the demineralization of dentin by phosphoric acid solutions at pH 2.0 and 4.0 showed a plateau soon after etching, while no plateau seen at pH 0.09 even lasting for 30 min.[39] This may indicate that even though AW as an acidic EW ($pH = 2.4$) has no capacity to demineralize the dentin at the 50-μm depth level. Accordingly, the neutral pH may lead NW to be less deteriorate the dentin even though softening effect on dentin is detected at the superficial layer. In other words, the neutrality of NW provides no synergistic deterioration effect to dentin compare to AW.

Mouthwash or root canal irrigant is basically used to contact directly with the tooth surfaces, or the root canal space to provide antimicrobial effect. The immersion of dentin in different EW caused a different softening result: (1) AW (acidic EW) induces a dissolution of organic component of dentin from the beginning of immersion and prolongs even at 60-min duration; (2) NW (neutral EW) exerts milder and unobvious slope of softening effect to dentin within the same immersion period. The former causing a significant decrease of microhardness than the latter within 25-μm depth. The structure of the demineralized inner layer dentin has not yet been elucidated, and whether the bonding ability of long-term EW-exposed dentin would be altered is not clear. Further investigation of the long-term study of acidic EW in penetration to the inner dentin, and of its potential effects on bonding strength with resin composite, are needed before decisions can be made about using it as a mouthwash or root canal irrigant application.

EW with different pH values induced different degrees of softening effect to the inner dentin. The most significant softening of dentin at 25 μm was caused by AW followed in decreasing order by NW, NL and DW. There were no significant post-immersion changes with any of the four solutions at 50-μm depth. From the standpoint of microhardness, the pH of EW plays an important role in the softening of dentin and NW is preferable to the use of mouthwash or root canal irrigation.

Declaration of Competing Interest

All authors declare no conflicts of interests.

Acknowledgements

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jds.2019.09.007.

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