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DOI:
10.1016/j.jdent.2021.103830

Document Version
Peer reviewed version

Citation for published version (Harvard):
Grimaldi, R, Yonel, Z, Chapple, I, Butler, A, Hall, C, Reinbold, K & Mantzourani, M 2021, 'Randomised methodology development study to investigate plaque removal efficacy of manual toothbrushes', *Journal of Dentistry*, vol. 116, 103830. https://doi.org/10.1016/j.jdent.2021.103830

Randomised methodology development study to investigate plaque removal efficacy of manual toothbrushes

Short title: Plaque removal efficacy of manual toothbrushes

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Keywords: Dental plaque control, Dental plaque index, Toothbrushing efficacy, *In vivo*

Declaration of Interest statement: This study was sponsored by GSK Consumer Health, of whom RG, AB, KR and MM are employees. ZY and IC are employees of the Institute of Clinical Sciences and CH is the Director of Raven Lane Consulting Ltd, which were funded by GSK Consumer Healthcare.

Abstract (250 words max, currently 250)

Objectives: *In vivo* toothbrush studies differ widely in design, plaque indices, plaque accumulation period, brushing duration and regimen. This study aimed to evaluate plaque removal efficacy of toothbrushes to guide development of clinical models.

Methods: This was a single-centre, randomised, controlled, examiner-blind, method development study in 80 healthy participants. Over 28 days, participants brushed twice-daily for 1 minute using a fluoride toothpaste and one of four marketed toothbrushes: Toothbrushes A (compact head) and B (regular head): medium-hard; flat trim; end-rounded bristles; Toothbrush C: medium-hard; end-rounded bristles; silky, tapered filaments; compact head; Toothbrush D: soft; tapered filaments; compact head. Supra-gingival plaque removal was evaluated immediately after single brushing events (Days 0, 7, 28) and following 7- and 28-days' use via Rustogi modified Navy Plaque Index (RPI) and Turesky modified Quigley Hein Plaque Index (TPI).

Results: All toothbrushes provided significant plaque removal after single-brushing events ($p<.0001$, both indices). Toothbrushes A and B showed significant ($p<.05$) pre-brushing RPI/TPI plaque reductions after 7- and 28-days use compared to Day 0 pre-brushing (except Toothbrush B, Day 28, non-significant TPI). There were no significant differences with Toothbrush C. Toothbrush D TPI was significantly lower at both timepoints compared to Day 0 pre-brushing ($p<.05$). Study toothbrushes were generally well-tolerated.

Conclusion: All toothbrushes showed plaque-removal efficacy after single-brushing events and small but increasing reductions in pre-brushing plaque levels over 28 days. Similar trends were observed irrespective of the plaque index employed. These data will inform the design of future clinical studies of plaque removal efficacy using manual toothbrushes.

Clinical Significance Statement (50 of 50 words): Regular effective oral hygiene can help prevent and treat gingivitis, principally via twice-daily mechanical cleaning with a toothbrush. Data generated from this methodology development study provides useful information for the design of future clinical studies to assess plaque removal efficacy of manual toothbrushes and generate results to inform clinical recommendations.

Introduction

Dental plaque is a diverse and organised community of microorganisms on the tooth surface that forms a biofilm and is recognised as the causative agent of gingivitis [Kinane et al., 2017]. While gingivitis is a reversible condition, if left untreated, it can progress to periodontitis. As such, maintenance of good gingival health is important in the primary prevention of periodontitis [Chapple et al., 2018]. Gingivitis can be treated and prevented with regular effective oral hygiene, principally via twice-daily mechanical cleaning with a toothbrush [Chapple et al., 2015; Ower, 2003].

Since the introduction of simple toothbrushes with nylon bristles and straight plastic handles in the 1930s, the modern toothbrush has evolved to include new materials, different bristle shapes and stiffness, complex bristle tuft arrangements and novel handle designs, all with the aim of improving plaque removal, particularly from ‘hard to reach’ areas of the mouth such as interproximal regions and third molars. A systematic review of manual toothbrush studies (including 59 publications with a total of 212 brushing exercises) reported that bristle tuft arrangement and duration of use contributed to the plaque removal efficacy of a toothbrush (30–53% plaque removal after a single brushing occasion). Furthermore, the review indicated that modifications to brush design could lead to better cleaning, for instance a change from vertical to angled bristle tuft arrangements could improve interproximal plaque removal [Slot et al., 2012].

In vitro brushing models have long been used to evaluate the cleaning capability of a toothbrush typically by measuring removal of artificial stain from the tooth surface [Stookey et al., 1982]. Such models are useful but limited in their ability to mimic the tenacity/adherent properties of dental plaque *in vivo* and the variation in brushing techniques within the general population. Clinical evaluation of self-performed plaque removal provides a more realistic assessment of the in-use performance of a toothbrush.

Single-use brushing studies are often used as screening tools to give an initial indication of cleaning performance. It is not unreasonable to expect toothbrushes that perform well after a single brushing to show greater plaque removal with continued use [Cronin et al., 2001; Slot et al., 2012]. Short- (1–4 weeks) and longer- (>4 weeks) term clinical studies evaluating plaque removal efficacy of manual toothbrushes are also reported [Cronin et al., 2000; Nathoo et al., 2004; Singh et al., 2001]. A longer period of home use allows study participants to familiarise themselves with a new toothbrush and provides a more representative picture of product performance over time. Longer-term toothbrushing studies

(≥4 weeks) are also required to determine the impact of a toothbrush on gingival health [Association, 2016; Robinson et al., 2006].

Overall, manual toothbrush studies evaluating self-performed plaque removal efficacy differ widely in design, plaque index used, period of plaque accumulation, brushing duration and regimens [Robinson et al., 2006; Slot et al., 2012]. Many clinical indices have been developed to assess supra-gingival plaque accumulation; however, depending on the index used, a wide range of percentage differences in plaque removal are reported [Slot et al., 2012]. The two most commonly employed in toothbrush evaluation studies [Robinson et al., 2006; Slot et al., 2012] are the six-site Turesky modification of the Quigley and Hein Plaque Index (TPI) [Lobene et al., 1982; Turesky et al., 1970], which focusses on plaque accumulation on the lower gingival third of the tooth surface, and the Rustogi modification of the Navy Plaque Index (RPI) [Rustogi et al., 1992], which focusses on plaque accumulation along the gingival margin and interproximal areas. Both indices are descriptive, based on the examiner's subjective assessment of surface plaque coverage, and are strongly correlated with each other [Cugini et al., 2006].

In the absence of professional or industry-accepted guidelines to establish equivalence or superiority, this clinical method development study sought to develop a reproducible clinical model to assess the plaque removal efficacy of manual toothbrushes. The study aimed to evaluate plaque removal efficacy of four marketed manual toothbrushes with differing bristle types and brush head design in healthy, dentate participants after single brushing events (Days 0, 7 and 28) and with continued use after twice daily brushing for 7 and 28 days.

Materials and methods

This was a randomised, controlled, four treatment, examiner-blind, parallel design, method development study (ClinicalTrials.gov Identifier: NCT04025684). It was carried out at a UK research facility in full compliance with the International Council for Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use, the ethical principles of the Declaration of Helsinki and all applicable local good clinical practice regulations and participant privacy requirements. The final study protocol, informed consent form and all other participant-facing information were reviewed and approved by an independent institutional review board (London-Bromley Research Ethics Committee; REF no: 19/LO/1173l; ID: 266363; 2nd August 2019). Anonymised individual participant data and

study documents can be requested for further research from www.clinicalstudydatarequest.com.

Study procedures

Participants, recruited from the general population, were in good general and oral health, were aged 18–65 years, routinely used a manual toothbrush and had ≥20 natural, permanent teeth with ≥40 gradable tooth surfaces (at least two-thirds of the tooth surface assessable for RPI). The gradable surface count did not include fully crowned/extensively restored, grossly carious, orthodontically-banded/bonded or abutment teeth or tooth surfaces with calculus deposits which, in the clinical examiner's opinion, would interfere with plaque assessment. Third molars could be included if, due to tooth loss, they were functioning as second molars. At Screening (Visit 1), participants gave written informed consent prior to any study procedures taking place.

Exclusion criteria included: any medical condition that could increase study participation risk or impact outcomes; taking a medication/having a treatment that could interfere with study outcomes; pregnancy; breastfeeding; intolerance/hypersensitivity to study materials; moderate-severe gingivitis; gross periodontal disease/active periodontitis; active caries that could impact study outcomes; gross intra-oral neglect; restorations in a poor state of repair; high extrinsic dental stain or calculus; tongue or lip piecing; multiple dental implants; a fixed bridge or removable partial dentures. Also excluded were participants who had taken antibiotics within 2 weeks of screening, had undergone routine prophylaxis within 4 weeks of screening, had undergone tooth bleaching within 8 weeks of screening or had had treatment for periodontal disease treatment in the 12 months prior to screening. Non-emergency, elective dental treatments (including prophylaxis) were delayed until study completion.

To standardise oral hygiene practice, eligible participants were provided with a regular, flat-trim toothbrush (Oral B® Sensi-Soft; ultra-soft; Procter & Gamble, Cincinnati, US marketplace) and a 1450 parts per million (ppm) fluoride toothpaste (Aquafresh® Triple protection; GSK Consumer Healthcare; Brentford, UK marketplace) and brushed twice daily for at least 5 days between screening and baseline visits (lead-in period). Participants were instructed to refrain from all oral hygiene procedures for 12–18 hours and from eating or drinking for ≥1 hour before their visit and until all visit procedures were completed. Safety was monitored at all visits and across the whole study period.

At the baseline visit, eligible participants with Day 0 pre-brushing mean RPI gingival/interproximal means ≥0.6 were stratified (lower stratum: ≥0.6 to ≤0.8; higher

stratum: >0.8 to 1.0) and randomised to one of the following manual toothbrushes according to a schedule generated by an approved vendor.

- Toothbrush A: Standard, medium hard toothbrush; compact head; flat trim, nylon (PA6.12) bristles; designed to clean tooth surfaces (Oral B Indicator 123; Procter & Gamble; UK marketplace);
- Toothbrush B: Standard, medium hard toothbrush; regular head; flat trim nylon (PA6.12) bristles; designed to clean tooth surfaces (Dr Best Original; GSK Consumer Healthcare; German marketplace);
- Toothbrush C: Medium hard toothbrush; compact head; end-rounded, co-extruded polybutylene terephthalate (PBT) bristles, 'silky' nylon (PA6.12) bristles and 'tapered' PBT bristles; designed to clean tooth surfaces, interproximal areas and along the gingival margin (Dr Best Multi Expert; GSK Consumer Healthcare; German marketplace);
- Toothbrush D: Soft toothbrush; compact head; 'tapered' PBT bristles; designed to clean inter-proximal areas (parodontax Interdental; GSK Consumer Healthcare; German marketplace).

Participants were instructed to apply a full brush head strip of toothpaste (Aquafresh® Triple protection; GSK Consumer Healthcare; Brentford, UK marketplace; 1450 ppm fluoride toothpaste) then to brush the entire dentition for 1 timed minute twice daily (morning and evening). On-site, participants rinsed with 10 mL tap water post-brushing for 5 seconds; at home, they could rinse with tap water post-brushing according to their usual habit. To monitor compliance, participants recorded each brushing occasion in the provided diary.

Plaque levels were evaluated using the RPI and the TPI on the facial and lingual surfaces (7-7 in each arch) of all gradable teeth prior to and post each brushing at the study centre on Days 0, 7 and 28. Prior to assessment, plaque was disclosed according to the disclosing solution manufacturer's instructions (Trace® solution; Young™ Dental Co., Earth City, MO, USA).

- RPI (Figure 1A): Each facial and lingual tooth surface was divided into nine zones (A–I) with disclosed plaque scored as present (1) or absent (0) in each zone. Mean RPI was calculated overall (all sites), for the gingival margin sites (A–C) and for the interproximal sites (D, F) [Rustogi et al., 1992].
- TPI (Figure 1B): Each tooth surface was divided into three areas, with three scores recorded facially (mesiofacial, facial, distofacial) and three scores lingually (mesiolingual,

lingual, distolingual). Disclosed plaque was scored from 0 (no plaque) to 5 (plaque covering two-thirds or more of the crown of the tooth) in each area. Mean TPI was calculated overall (all sites) and for the interproximal sites (mesiofacial/lingual and distofacial/lingual) [Quigley and Hein, 1962; Turesky et al., 1970].

Clinical efficacy assessments were performed by a single dental examiner to minimize inter-examiner variability. Intra-examiner variability was monitored by performing repeat clinical assessments for both plaque indices across the study period in randomly selected participants.

Statistical analysis

No formal sample size was produced; however, based on similar studies evaluating the plaque removal efficacy of manual toothbrushes [He et al., 2009; Nathoo et al., 2004], approximately 20 evaluable participants per product group was considered sufficient to provide reliable estimates of treatment effect for the purpose of this method development study and to aid in future clinical study design. It was planned to screen approximately 200 participants to ensure approximately 100 entered into the lead-in period and approximately 80 were randomised to study product.

A modified intent-to-treat population (mITT) was used for efficacy analyses, comprising all randomised participants who had a post-baseline plaque assessment. Primary outcome variables were Day 0 changes from pre- to post-brushing in overall (all sites) mean RPI and TPI. For each participant these were calculated as total score for all tooth sites assessed and then divided by total number of tooth sites assessed according to each index. Change from baseline in mean RPI or TPI overall was analysed using analysis of covariance (ANCOVA). The model included product group as a fixed effect and baseline mean RPI or TPI overall value, respectively, as a covariate. For TPI overall only, the model also included RPI stratification level as a factor; this was not included for RPI analysis as the actual value was used as a covariate.

Secondary outcome variables were: pre- and post-brushing mean RPI and TPI overall (Day 7, Day 28), interproximal (RPI and TPI) and gingival margin (RPI) sites (Day 0, Day 7, Day 28) and change from baseline (Day 0 pre-brushing mean) to post-brushing mean in RPI and TPI overall (Day 7, Day 28), interproximal (RPI and TPI) and gingival margin (RPI) sites (Day 0, Day 7, Day 28), and change from baseline for these sites to Day 7 and Day 28 pre-brushing.

Adjusted mean change was derived from the ANCOVA model. These outcomes were analysed as for the primary outcome variables with product group as a fixed effect and the appropriate baseline value as a covariate.

Tests for normality and homogeneity of variance were carried out for the ANCOVA models and were considered satisfactory for both primary and secondary variables.

Percent change from mean baseline was derived as 100*(adjusted mean change/mean baseline value).

During the study, eight participants were randomised to incorrect stratification groups due to misinterpretation of stratification groups by the site team. As this affected the primary and secondary outcome endpoint analyses, corrective actions were taken for handling incorrect data. Stratification groups used in statistical analyses were recalculated based on the actual data for the RPI. All analyses described are based on actual stratification groups.

All safety data were reported for the Safety Population as per actual study toothbrush participants used. The safety profiles of the study products were assessed with respect to adverse events, oral soft tissue examinations, oral hard tissue examinations and incidents (for plaque disclosure dye).

The first and repeat plaque assessments on each tooth site were cross-tabulated. A weighted kappa coefficient (κ), along with the 95% CI was calculated to assess the intra-examiner reliability. Fleiss-Cohen weighted kappa was calculated for the repeatability analysis. Reliability was deemed excellent if $\kappa > 0.75$; fair to good if $0.4 \leq \kappa \leq 0.75$; poor if $\kappa < 0.4$. A $\kappa \geq 0.6$ was considered indicative of good repeatability across the study period.

Results

A total of 125 participants were screened for entry into the study, of whom 100 were enrolled. After completing the lead-in period, 80 participants were randomised to one of four study toothbrush groups (Figure 2). There were more female (64.6%) than male participants. Mean age across the study groups was similar (overall mean of 34.6 [standard deviation 10.58] years; range 19–65 years). Day 0 pre-brushing RPI gingival/interproximal mean was lower (≥0.6 to ≤0.8) for 43 participants (54.4%) and higher (>0.8 to 1.0) for 36 participants

(45.6%). Distribution of participants with lower or higher baseline RPI gingival/interproximal mean was well balanced across the study product groups (Table 1).

The mean number of additional and missed brushings was <1 for all study groups with no participant reporting more than four missed or additional brushings. There was excellent intra-examiner repeatability for RPI (κ=0.92; 95% CI 0.91, 0.92) and TPI (κ=0.84; 95% CI 0.83, 0.85) assessments.

Primary endpoints

Mean RPI and TPI overall for the mITT population are shown in Figures 3A and 3B, respectively. Baseline values were similar across toothbrush groups for both indexes. Statistical analysis of adjusted mean change from baseline (Day 0, pre-brushing) to Day 0 post-brushing (Tables 2 and 3, respectively) showed a significant plaque reduction for all toothbrushes in both indices ($p<.0001$ for all). Percentage change from baseline at this timepoint showed similar decreases between RPI and TPI for each toothbrush group (Figure 4).

Secondary endpoints

Overall mean TPI and RPI after single brushing events

Similar pre- to post-brushing results were seen at Days 7 and 28 (Figures 3A and 3B). There was a significant plaque reduction between baseline and respective post-brushing RPI (Table 2) and TPI (Table 3) means ($p<.0001$ for all) and similar percentage decreases (Figure 4) at all timepoints, for both indices.

At all timepoints, Toothbrush B showed the highest percentage change from baseline (Figure 4) with no other toothbrush showing persistently lower percentage changes from another.

Overall mean TPI and RPI over time (twice-daily brushing)

Comparing pre-brushing means over time, at Days 7 and 28 there were decreases compared to Day 0 pre-brushing means for both RPI (Figure 3A) and TPI (Figure 3B). For RPI, significant decreases from baseline were shown at Day 7 ($p<0.05$ for Toothbrushes A, B, D) and Day 28 ($p<0.05$ for Toothbrushes A, B, D) for all but Toothbrush C (Table 2). For TPI, significant changes from baseline were shown for Toothbrushes A and B at Day 7 ($p<0.05$ for both) and Toothbrush A at Day 28 ($p=0.0059$) (Table 3).

Gingival margin and interproximal mean TPI and RPI

Figure 5A shows means for RPI gingival margin and interproximal sites with percentage decreases from baseline shown in Table 2. For RPI gingival margin means, compared to Day 0 baseline pre-brushing, statistically significant decreases were observed in post-brushing means for all toothbrushes at Day 0 ($p<.05$ for all), Day 7 ($p<.0001$ for all) and Day 28 ($p<.05$ for all). Small percentage changes were observed when comparing Day 0 pre-brushing means to twice-daily pre-brushing means at Days 7 and 28 (Table 2); significant decreases for Toothbrushes A and B at Day 7 ($p<.05$ for both) and Toothbrush A at Day 28 were shown ($p=0.0103$).

For RPI interproximal sites, there were larger decreases in pre- to post- brushing means (Figure 5A), with around 50% plaque decreases when post-brushing means at every time point were compared to baseline (Day 0) pre-brushing means ($p<.0001$ for all) (Table 2). Comparison of Day 0 pre-brushing means to Day 7 pre-brushing means following twice-daily brushing showed significant decreases for Toothbrushes A, B and D ($p<.05$ for all). At Day 28, interproximal RPI means were significantly decreased for Toothbrushes A and D ($p<.05$).

TPI interproximal means were, on average, slightly lower than the overall means (Figure 5B, Table 3). Significant differences were found in change from baseline means (Day 0 pre-brushing) to Days 0, 7 and 28 post-brushing ($p<.0001$ for all) means. Comparing twice-daily pre-brushing means to Day 0 pre-brushing (change over time), at Day 7, there were significant differences for Toothbrushes A and B ($p<.05$) and at Day 28, there were significant differences for Toothbrushes A, B and C ($p<.05$).

Safety

Five treatment emergent AEs (TEAEs) were reported by three participants: two for Toothbrush B (‘pain in jaw’ in one participant, ‘pharyngitis’ in one participant); two for Toothbrush D (‘gingival pain’ and ‘tooth fracture’ in a single participant); one for Toothbrush C (‘tooth injury’). Four were mild with one, the tooth injury, classified as moderate. By the end of the study, ‘pain in jaw’ and ‘tooth injury’ were ongoing with the other three resolved. There were no treatment-related or serious AEs. One TEAE (‘pharyngitis’) required treatment with an inadmissible medication (penicillin), which led to discontinuation of the study product and withdrawal of the participant from the study. The event was resolved prior to withdrawal of the participant from the study.

Discussion

This method development study was designed to evaluate plaque removal efficacy of four different manual toothbrushes. While numerous toothbrush studies evaluating self-performed plaque removal efficacy are reported, many aspects of protocol design differ between studies [Cronin et al., 2001; Cronin et al., 2000; He et al., 2009; Nathoo et al., 2004; Robinson et al., 2006; Sharma et al., 2000; Singh et al., 2001; Slot et al., 2012]. As such, the findings of this current study will be used to develop more robust clinical models for future toothbrush evaluation studies.

The plaque removal efficacy of a manual toothbrush is determined by three main factors: frequency and duration of use, toothbrush design, and the skill of the individual using the toothbrush [Cugini et al., 2006; Frandsen, 1986]. The user needs time to adapt to using a different toothbrush; therefore, including a period of 'home use' in the clinical study design allows study participants to familiarise themselves with a new toothbrush and may provide a more representative picture of product performance when frequency and duration of use are controlled. As such, a 'home-use' period of 28 days was included in this study, not only to allow study participants to fully familiarise themselves with their new toothbrush but also to inform the design of future plaque and gingivitis studies where at least 4 weeks' intervention is required to see an effect on gingival health [Association, 2016]

In this study, adjusted mean change from baseline after a single brushing event (Days 0, 7 and 28) was consistent and statistically significant for all toothbrushes and both plaque assessment indices. Overall percentage change from baseline in mean RPI and TPI after single brushing events at Day 28 was similar for all toothbrushes (20%–35% range). Plaque removal efficacy also significantly increased over time for all toothbrushes post-brushing compared to Day 0 pre-brushing following 7 and 28 days' use. The single-use and 28-day results are in line with the published literature where clinical studies have demonstrated plaque removal efficacy for manual toothbrushes after a single-use and in the short-term (1–4 weeks) [Cronin et al., 2000; Nathoo et al., 2004; Sharma et al., 2000; Singh et al., 2001].

Modifications to brush design may lead to better cleaning, especially in harder to reach places; for example, a change from vertical to angled bristle tuft arrangements can improve interproximal plaque removal [Slot et al., 2012]. The toothbrushes used in this study were selected to help investigate how differing designs might impact plaque removal. All study toothbrushes were designed to clean tooth surfaces; Toothbrushes C and D were also designed to clean interproximal areas and Toothbrush C to clean along the gingival margin. The indices used in this study helped elucidate plaque removal at different sites including

those considered harder to reach. Overall, results showed that percentage change from mean baseline for RPI was higher at interproximal sites compared to gingival margin sites, where only a small percentage change was observed after both single brushing events (post-brushing) and over time. Given their particular designs, Toothbrushes C and D might have been expected to show different plaque reduction scores in interproximal and gingival margin regions. However, while this study did not aim to compare toothbrushes, and no statistical comparisons were performed, no differences in rank order were observed and plaque removal (pre- and post-brushing scores) was similar for all four toothbrushes at all tooth sites.

Another way a study can be influenced is by factors affecting the skill of the individual using the toothbrush. The potential impact of study participation on clinical study participants is well documented [Benedetti et al., 2016]. In this study, we wanted to maintain participants' typical oral health habits and assess toothbrush cleaning efficacy under near normal use conditions; therefore, no professional oral hygiene instruction was given. However, the different brushing styles of individual participants could also have impacted the results. Providing specific brushing instructions, while taking the study away from a more 'real world' setting, would help standardise oral hygiene practice and may provide a better understanding of the impact of different design features on the cleaning performance of manual toothbrushes. A systemic review and meta-analysis showed evidence in favour of the modified Bass technique against normal toothbrushing techniques (Scrub technique) [Janakiram et al., 2018]. Future toothbrush studies could involve demonstration of this technique to help ensure participants brush in a similar manner during the study. Conversely, the similarities in performance observed here between toothbrushes may have resulted from participants brushing all areas of their teeth more carefully than they would in their usual practice; hence, even the toothbrushes not specifically designed to access the gingival margin/interproximal regions had an effect there. Indeed, the general reduction in pre- and post-brushing plaque levels over the study course may be evidence of heightened participant motivation across the study population.

Other important aspects of toothbrush use are frequency and duration. Dental professionals typically recommend ≥2 minutes brushing at least twice daily for good oral hygiene; however, studies reported in the scientific literature indicate actual brushing duration to be much shorter (working estimate 45 seconds) (Gallagher et al. 2009). As such, a 1-minute brushing time was selected for this study as representative of consumer behaviour and will likely continue to be included in future study designs.

A final, key aspect of study design is the selection of clinical indices. This choice is influenced by several factors such as study objectives, study duration, toothbrush design and target population. For this study, RPI and TPI were used to assess plaque removal efficacy. Both scales are descriptive, based on the examiner's subjective assessment of surface plaque coverage, and are strongly correlated with each other [Cugini et al., 2006]. RPI is the more demanding of the two to score, requiring the examiner to make nine assessments per tooth surface, compared to three for TPI. American Dental Association (ADA) guidelines [Association, 2016] and a systematic review of powered toothbrush studies [Robinson et al., 2006] recommend use of the TPI; however, RPI is reported to yield numerically greater pre- to post-brushing differences [Slot et al., 2012]. This was observed in our study where RPI detected greater plaque removal than the TPI, especially at interproximal sites. This observation does not call into question the validity of the TPI, in fact the two indices showed strong correlation, but provides useful information when selecting the more appropriate index for future studies.

In conclusion, the observations from this study support a more customised approach to toothbrush study design rather than a single, standard method. Study design is dependent upon the questions asked. For instance, if there is a need to assess cleaning performance at interproximal sites, our results indicate RPI to be more sensitive than TPI; if overall cleaning performance is under evaluation, TPI would be the more suitable choice of index, being recommended by ADA guidelines for assessment of the cleaning performance [Association, 2016] and easier to use in the clinical setting.

Acknowledgements: Help with manuscript preparation was provided by Dr Eleanor Roberts, Director of Beeline Science Communications, Ltd.

References (to be styled once content finalised)

Association AD: Toothbrushes; in Affairs ACoS (ed): Acceptance Program Requirements. American Dental Association, 2016.

Benedetti F, Carlino E, Piedimonte A: Increasing uncertainty in CNS clinical trials: the role of placebo, nocebo, and Hawthorne effects. The Lancet Neurology 2016;15:736-747.

Chapple IL, Van der Weijden F, Doerfer C, Herrera D, Shapira L, Polak D, Madianos P, Louropoulou A, Machtei E, Donos N, Greenwell H, Van Winkelhoff AJ, Eren Kuru B, Arweiler N, Teughels W, Aimetti M, Molina A, Montero E, Graziani F: Primary prevention of periodontitis: managing gingivitis. J Clin Periodontol 2015;42 Suppl 16:S71-76.

Chapple ILC, Mealey BL, Van Dyke TE, Bartold PM, Dommisch H, Eickholz P, Geisinger ML, Genco RJ, Glogauer M, Goldstein M, Griffin TJ, Holmstrup P, Johnson GK, Kapila Y, Lang NP, Meyle J, Murakami S, Plemons J, Romito GA, Shapira L, Tatakis DN, Teughels W, Trombelli L, Walter C, Wimmer G, Xenoudi P, Yoshie H: Periodontal health and gingival diseases and conditions on an intact and a reduced periodontium: Consensus report of workgroup 1 of the 2017 World Workshop on the Classification of Periodontal and Peri-Implant Diseases and Conditions. J Clin Periodontol 2018;45 Suppl 20:S68-S77.

Cronin MJ, Dembling WZ, Jacobs DM, Low MA, Warren PR: A comparative single-use clinical study of the efficacy of two manual toothbrushes with angled bristles. Am J Dent 2001;14:263-266.

Cronin MJ, Dembling WZ, Low MA, Jacobs DM, Weber DA: A comparative clinical investigation of a novel toothbrush designed to enhance plaque removal efficacy. Am J Dent 2000;13:21a-26a.

Cugini M, Thompson M, Warren PR: Correlations between two plaque indices in assessment of toothbrush effectiveness. J Contemp Dent Pract 2006;7:1-9.

Frandsen A: Mechanical oral hygiene practices; in Löe H, Kleinman DV (eds): Dental plaque control measures and oral hygiene practices: Proceedings from a State-of-the-Science Workshop. USA, Oxford University Press, 1986, vol 1, pp 93-116.

He T, Li S, Sun L: Clinical comparison of the plaque removal efficacy of a manual toothbrush with criss-cross bristle design. Am J Dent 2009;22:200-202.

Janakiram C, Taha F, Joe J: The Efficacy of Plaque Control by Various Toothbrushing Techniques-A Systematic Review and Meta-Analysis. Journal of Clinical and Diagnostic Research 2018;12.

Kinane DF, Stathopoulou PG, Papapanou PN: Periodontal diseases. Nat Rev Dis Primers 2017;3:17038.

Lobene RR, Soparkar PM, Newman MB: Use of dental floss. Effect on plaque and gingivitis. Clin Prev Dent 1982;4:5-8.

Nathoo S, Chaknis P, Petrone M, DeVizio W, Volpe AR: A clinical comparison of the gingivitis reduction and plaque-removal efficacy of a new manual toothbrush. Compend Contin Educ Dent 2004;25:37-45.

Ower P: The role of self-administered plaque control in the management of periodontal diseases: I. A review of the evidence. Dent Update 2003;30:60-64, 66, 68.

Quigley GA, Hein JW: Comparative cleansing efficiency of manual and power brushing. The Journal of the American Dental Association 1962;65:26-29.

Robinson PG, Damien Walmsley A, Heanue M, Deacon S, Deery C, Glenny AM, Worthington H, Shaw W: Quality of trials in a systematic review of powered toothbrushes: suggestions for future clinical trials. J Periodontol 2006;77:1944-1953.

Rustogi KN, Curtis JP, Volpe AR, Kemp JH, McCool JJ, Korn LR: Refinement of the Modified Navy Plaque Index to increase plaque scoring efficiency in gumline and interproximal tooth areas. J Clin Dent 1992;3:C9-12.

Sharma NC, Qaqish JG, Galustians HJ, King DW, Low MA, Jacobs DM, Weber DA: An advanced toothbrush with improved plaque removal efficacy. Am J Dent 2000;13:15a-19a.

Singh SM, Battista GW, Rustogi KN, DeVizio W, Volpe AR, Petrone ME, Proskin HM: The comparative plaque removal efficacy of two advanced manual toothbrush designs in two independent clinical studies. J Clin Dent 2001;12:83-86.

Slot DE, Wiggelinkhuizen L, Rosema NA, Van der Weijden GA: The efficacy of manual toothbrushes following a brushing exercise: a systematic review. Int J Dent Hyg 2012;10:187-197.

Stookey G, Burkhard T, Schemehorn B: In vitro removal of stain with dentifrices. Journal of dental research 1982;61:1236-1239.

Turesky S, Gilmore ND, Glickman I: Reduced plaque formation by the chloromethyl analogue of victamine C. J Periodontol 1970;41:41-43.

Table 1: Demographic and baseline characteristics (mITT population)

		Toothbrush A (n=20)	Toothbrush B (n=19)	Toothbrush C (n=20)	Toothbrush D (n=20)
Sex (n, %)					
	Male	6 (30.0)	7 (36.8)	10 (50.0)	5 (25.0)
	Female	14 (70.0)	12 (63.2)	10 (50.0)	15 (75.0)
Ethnicity					
	African*	3 (15.0)	1 (5.3)	4 (20.0)	1 (5.0)
	Asian**	5 (25.0)	2 (5.3)	1 (5.0)	1 (5.0)
	White***	12 (60.0)	16 (84.2)	15 (75.0)	18 (90.0)
Age					
	Mean (SD)	34.2 (10.17)	33.6 (11.0)	31.6 (9.16)	39.2 (11.16)
	Min–Max	22–58	21–56	19–51	22–65
Day 0 mean pre-brushing gingival/interproximal RPI					
	Lower	11 (55.0)	10 (52.6)	11 (55.0)	43 (54.4)
	Higher	9 (45.0)	9 (47.4)	9 (45.0)	36 (45.6)

*African American/African heritage; **Central/South Asian, East Asian, South East Asian heritage; *** Arabic/North African, White, Caucasian, European heritage

n: number; SD: standard deviation

Table 2: Change from Day 0 pre-brushing in adjusted mean RPI (mITT population)

	Toothbrush A	Toothbrush B	Toothbrush C	Toothbrush D
	Overall Mean change (SE) [95%CI] p-value			
	Gingival margin/interproximal % change from mean baseline			
Day 0	-0.10 (0.010)	-0.12 (0.010)	-0.10 (0.010)	-0.10 (0.010)
Post	[-0.11, -0.08] <.0001	[-0.14, -0.10) <.0001	[-0.12, -0.08] <.0001	[-0.12, -0.08] <.0001
	-5.0%*/-49.2%**	-9.1%**/-56.5%**	-5.5%*/-50.2%**	-7.2%** /-51.9%**
Day 7	-0.04 (0.012)	-0.06 (0.013)	-0.02 (0.012)	-0.03 (0.012)
Pre	[-0.07, -0.02]	[-0.08, -0.03]	[-0.04, 0.00]	[-0.05, 0.00]
	0.0006 (-9.1%)	<.0001 (-11.6%)	0.1113 (-5.0%)	0.0361 (-5.5%)
	-2.3%*/-24.7%*	-3.7%*/-27.8%*	-1.4% (NS)/-10.6% (NS)	-1.3% (NS)/-15.7%*
Day 7	-0.13 (0.014)	-0.16 (0.015)	-0.10 (0.014)	-0.12 (0.014)
Post	[-0.16, -0.11] <.0001	[-0.19, -0.13] <.0001	[-0.12, -0.07] <.0001	[-0.15, -0.10] <.0001
	-12.7%**/-56.9%**	-17.2%**/-61.4%**	-9.8%**/-41.8%**	-12.1%**/-57.3%**
Day 28	-0.04 (0.015)	-0.03 (0.016)	-0.02 (0.014)	-0.04 (0.014)
Pre	[-0.07, -0.01]	[-0.07, 0.00]	[-0.05, 0.01]	[-0.07, -0.01]
	0.0041 (-9.0%)	0.0476 (-6.5%)	0.1432 (-4.3%)	0.0050 (-8.7%)
	-4.3%*/-17.5%*	-1.3% (NS)/-16.2% (NS)	-0.7% (NS)/-5.5% (NS)	-2.1% (NS)/-21.1%*
Day 28	-0.15 (0.017)	-0.16 (0.018)	-0.13 (0.016)	-0.12 (0.016)
Post	[-0.18, -0.11] <.0001	[-0.20, -0.12] <.0001	[-0.16, -0.10] <.0001	[-0.15, -0.09] <.0001
	-17.6%**/-55.3%**	-16.5%**/-57.0%**	-12.4%**/-53.1%**	-10.4%*/-58.6%**

*p<0.05; **p<0.0001; NS: Not significant; % change from baseline mean defined as: 100 x (adjusted mean change/mean baseline value)

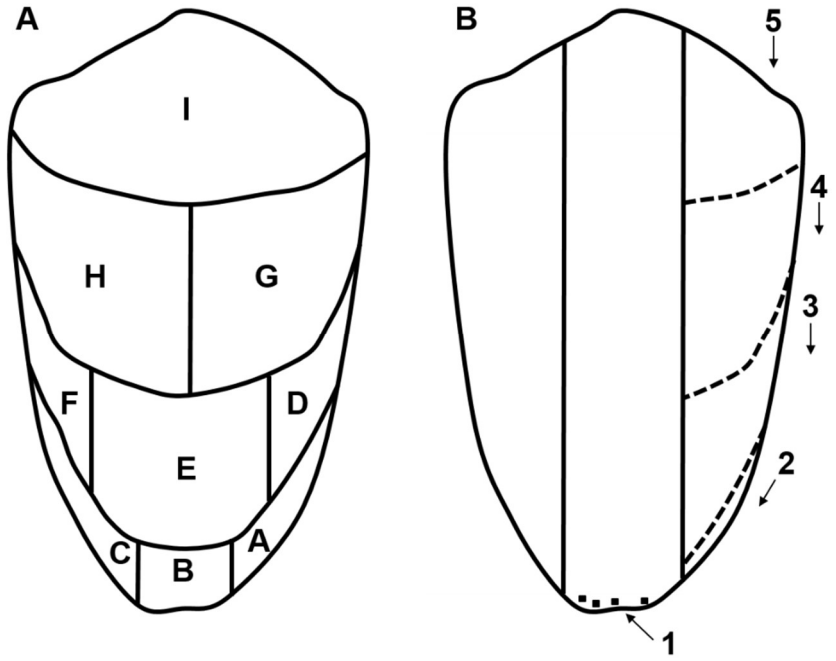
Table 3: Change from Day 0 pre-brushing in adjusted mean TPI (mITT population)

	Toothbrush A	Toothbrush B	Toothbrush C	Toothbrush D
	Overall Mean change (SE) [95%CI] p-value Interproximal % change from mean baseline			
Day 0 Post	-0.50 (0.065) [-0.62, -0.36] <.0001 -18.9%**	-0.62 (0.066) [-0.75, -0.49) <.0001 -23.0%**	-0.55 (0.066) [-0.68, -0.42] <.0001 -19.9%**	-0.57 (0.066) [-0.70, -0.44] <.0001 -21.2%**
Day 7 Pre	-0.16 (0.071) [-0.30, -0.18] 0.0280 (-6.3%) -6.7%*	-0.20 (0.077) [-0.35, -0.041] 0.0137 (-7.7%) -7.7%*	-0.05 (0.070) [-0.19, 0.09] 0.4597 (-2.0%) -2.8% (NS)	-0.01 (0.070) [-0.15, 0.12] 0.8527 (-0.05%) -0.3% (NS)
Day 7 Post	-0.72 (0.082) [-0.88, -0.56] <.0001 -27.2%**	-0.89 (0.090) [-1.1, -0.71] <.0001 -32.6%**	-0.61 (0.081) [-0.77, -0.45] <.0001 -21.8%**	-0.66 (0.081) [-0.82, -0.49] <.0001 -23.8%**
Day 28 Pre	-0.20 (0.070) [-0.34, -0.06] 0.0059 (-7.9%) -9.5%*	-0.11 (0.077) [-0.26, 0.04] 0.1564 (-4.2%) -5.7%*	-0.11 (0.067) [-0.24, 0.24] 0.1071 (-4.2%) -5.3%*	-0.12 (0.067) [-0.25, 0.01] 0.0767 (-5.0%) -5.6% (NS)
Day 28 Post	-0.76 (0.086) [-0.9, -0.59] <.0001 -29.8%**	-0.79 (0.094) [-0.98, -0.60] <.0001 -29.4%**	-0.69 (0.083) [-0.86, -0.53] <.0001 -25.8%**	-0.63 (0.083) [-0.79, -0.46] <.0001 -24.1%**

*p<0.05; **p<0.0001; NS: Not significant; % change from baseline mean defined as: 100 x (adjusted mean change/mean baseline value)

Figures

Figure 1: A) Rustogi modification of the Navy Plaque Index (RPI) (adapted from [Rustogi et al., 1992]) and B) Turesky modification of the Quigley and Hein Plaque Index (TPI) (adapted from [Cugini et al., 2006])



RPI assessment areas are denoted by letters; scored as (1) present or (0) absent of plaque in each zone. TPI assessment areas (mesiofacial/lingual, facial/lingual, distofacial/lingual) shown by vertical lines; scored as (approximate areas represented by dashed lines): 0 = No plaque; 1 = Separate flecks of plaque at the cervical margin; 2 = A thin continuous band of plaque (up to 1 mm) at the cervical margin; 3 = A band of plaque wider than 1 mm but covering less than 1/3rd of the crown; 4 = Plaque covering at least 1/3rd but less than 2/3^{rds} of the crown; 5 = Plaque covering 2/3^{rds} or more of the crown.

Figure 2: Study flow

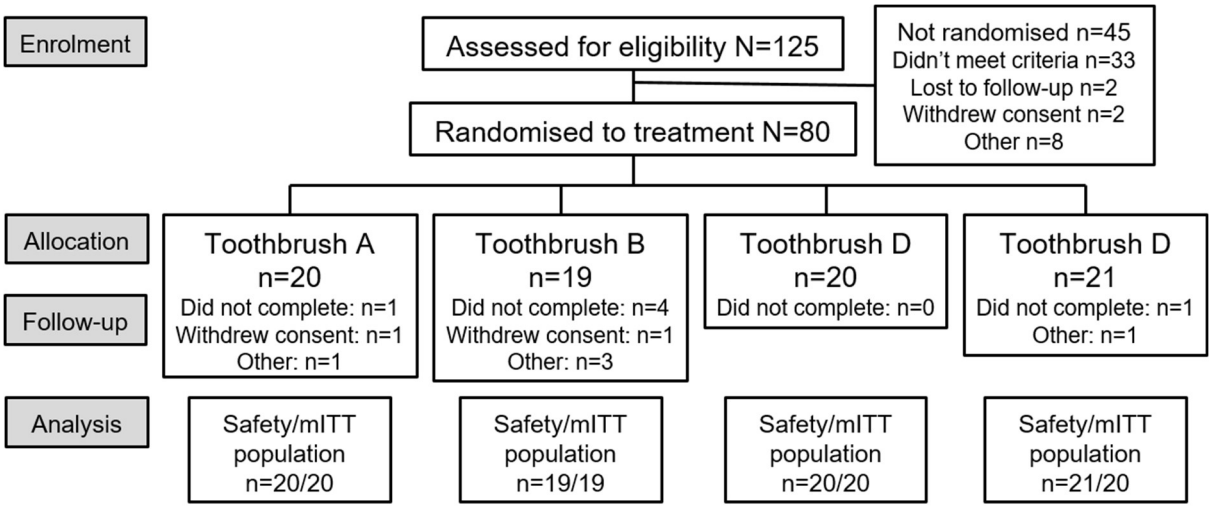
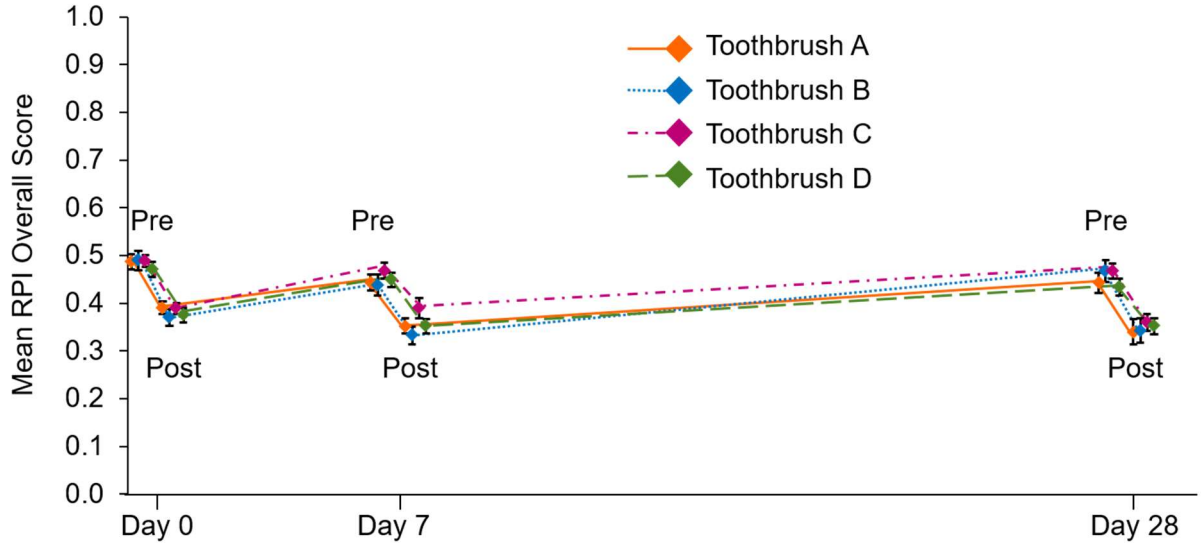


Figure 3: Raw means overall (SE) over time for A) RPI and B) TPI (mITT population)

A



B

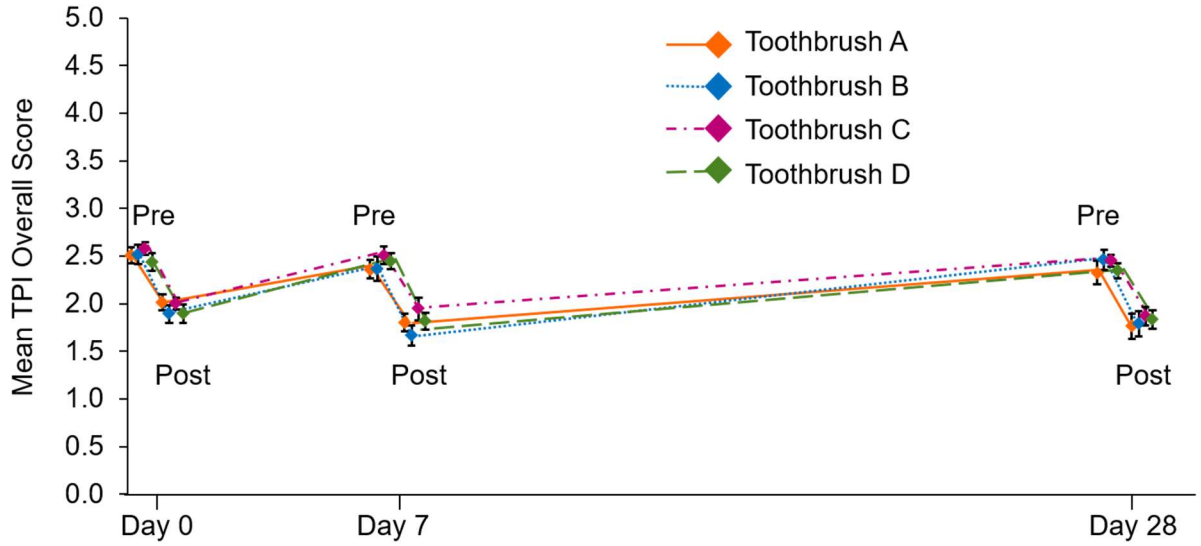


Figure 4: Percentage mean change from baseline after a single brushing event for RPI and TPI (mITT population)

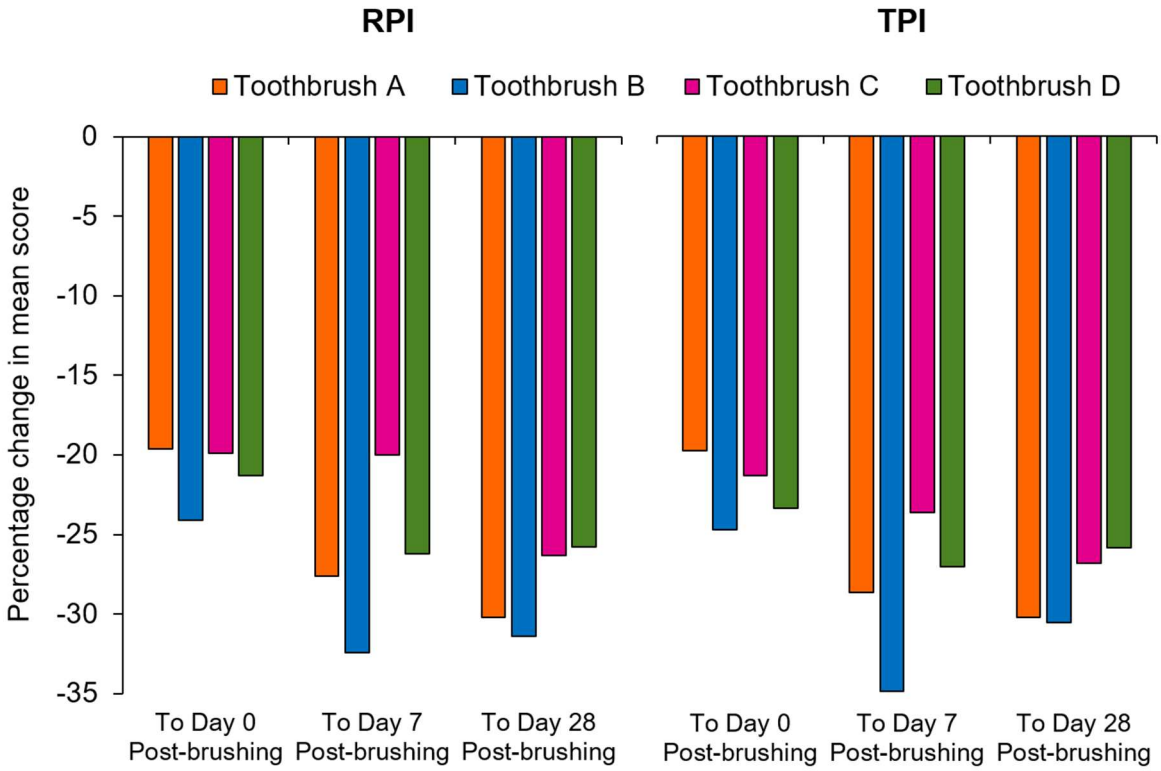
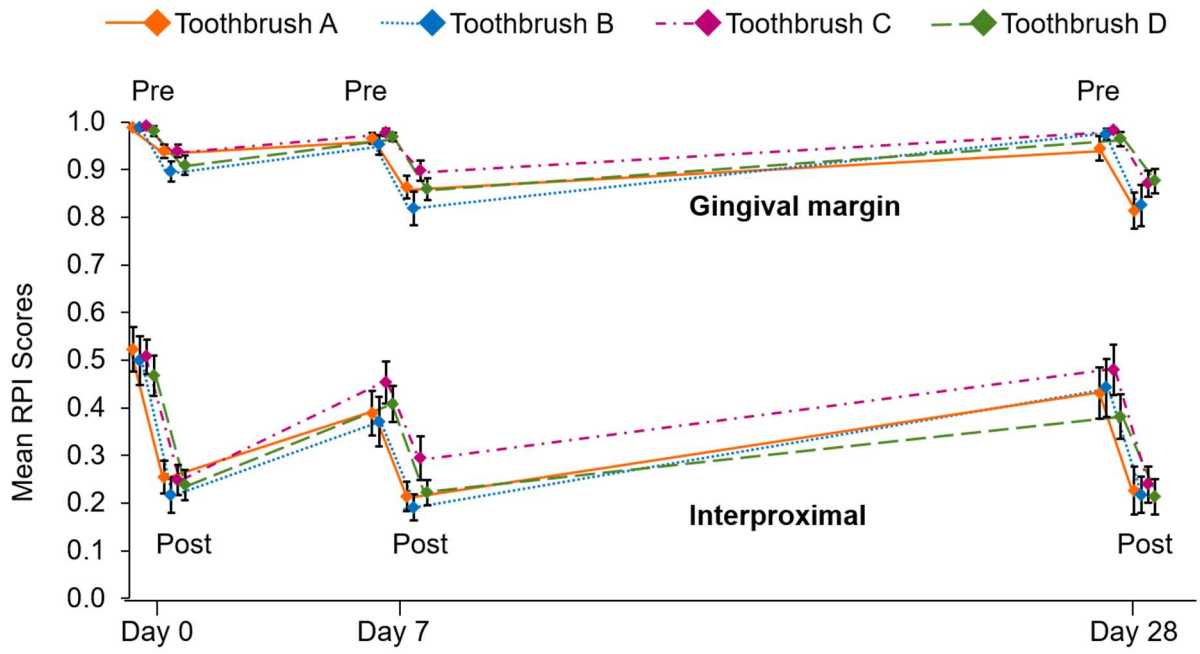


Figure 5: Raw means overall (SE) over time for A) RPI gingival margin and interproximal sites and B) TPI interproximal sites (mITT population)

A



B