Cervical wear pathobiology by robot-simulated 3-year toothbrushing – New methodological approach

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ABSTRACT

Objectives: An ex-vivo study was aimed at (i) programming clinically validated robot three-year random toothbrushing, (ii) evaluating cervical macro- and microwear patterns on all tooth groups of different functional age, (iii) documenting and codificating wear related morphological features at the cemento-enamel junction in young teeth and on roots in older teeth.

Design: Following ethical approval random toothbrushing (44 strokes per tooth horizontally, rotating, vertically; 2x/d) with manual toothbrushes and low-abrasive dentifrice was performed in an artificial oral cavity with brushing-force 3.5 N on 14 extracted human teeth. Morphological features were examined by SEM at baseline and after simulated 3 years using the replication technique. 3D-SEM analyses were carried out with a four-quadrant back scattered electron detector. Wilcoxon-Mann-Whitney-test was used for statistical analyses.

Results: 3-year random toothbrushing with horizontal, rotating and vertical brushing movements revealed morphological features classified as four enamel patterns, one dentin pattern and three cervical patterns. Negative impacts were enamel, cementum and dentin loss. Positive impact on oral health was removing dental calculus and straightening cervical traumatic and iatrogenic damages. The volume loss varied from $\bar{x} = 34.25\, \text{nl}$ to $\bar{x} = 87.75\, \text{nl}$. Wear extended apically from 100 to 1500 micrometres.

Conclusion: Robot simulated toothbrushing in an artificial oral cavity, with subsequent SEM and 3D-SEM assessment, elucidated both negative and oral health-contributing micromorphology patterns of cervical wear after simulated 3-year random toothbrushing. Cervical macro- and microwear of cementum revealed, for the first time, what we describe as overhanging enamel peninsulas and enamel islands on roots in young teeth, but no enamel islands on roots from older teeth after root cementum loss. In contrast, many older teeth exhibited enamel peninsulas.

1. Introduction

Occlusal tooth wear occurs naturally in all omnivorous and herbivorous animals due to abrasion and attrition. All mammalian teeth are adapted to withstand significant stress and wear to remain functional throughout life (Ungar, 2015). The stomatognathic system is capable of compensating occlusal tooth wear by limited continuous eruption throughout life, even in the human dentition (Ainamo & Ainamo, 1984; Gaengler, 1986; Wiedemann et al., 2021). Approximal wear is caused by tribological abrasion due to the physiological mobility and friction of teeth and is compensated by anterior migration.


In contrast to physiological wear, pathological cervical root wear occurs mainly in humans and rarely in other animals and is considered a relatively recent phenomenon due to oral hygiene and an erosive diet (Bartlett & Shah, 2006; Gaengler et al., 2005). Brushing too vigorously with abrasive dentifrice is a significant cause of gingival recession and subsequent cervical abrasion lesions combined with dentin...
human teeth. These were mounted in a plastic lower jaw to simulate the flow chart (Fig. 1) summarizes the step-by-step methodology. Replication of teeth and SEM evaluation were performed at 2 incisors – 2.5 more than the rigid neck. Consequently, the horizontal brushing in the caries/gingivitis risk area next to gum line resulted in 60 – 70% plaque removal versus 30 – 40% removal after brushing with rigid toothbrushes.

It was, therefore, hypothesised that robot simulated toothbrushing based on clinically validated programmes in an artificial oral cavity may contribute to elucidate the mechanisms of cervical wear. The primary objective was to characterise abrasion patterns of brushing lesions in young premolars and older incisors, canines and molars by scanning electron microscopy summarised in a catalogue of criteria for evaluating cervical tooth wear. The quantitative analysis of volume loss due to brushing was performed using 3D SEM and 3D surface model alignment measurement software. An improved understanding of dynamic morphological changes of tooth size over lifetime would strengthen the scientific basis of oral hygiene and support clinical diagnostic and therapeutic decisions in periodontology and restorative dentistry.

2. Materials and methods

The study protocol was submitted to IRB (Ethics Committee of the University of Witten-Herdecke), and approval was given on 3 May 2021 (application number: SR-67/2021). The amendment for an additional video observation study to determine the average number of toothbrushing strokes per single tooth and the individual brushing time of 50 subjects was approved on 8 January 2022.

A robot-simulated three-year toothbrushing cycle was performed. Replication of teeth and SEM evaluation were performed at 2 incisors (41, 42), 1 canine (43), 2 premolars (44, 45) and 2 molars (46, 47). The flow chart (Fig. 1) summarizes the step-by-step methodology.

2.1. Human teeth

Two segmented dentition models were constructed, each with seven human teeth. These were mounted in a plastic lower jaw to simulate the anatomical position of a eugenic dentition (Table 1, Appendix Fig. 1). The donated teeth were selected according to the severity of baseline cervical wear depending on years of function and the donor’s age, resulting in an overview of individual morphological features with different volume loss of enamel, cementum and root dentin. Both dentition models included two incisors and one canine of subjects from 40 to 65 years of age that had to be extracted for periodontal reasons. Two juvenile premolars of 10-15 years old adolescents with immature enamel and negligible enamel wear were extracted for orthodontic reasons. Two molars of young adults aged 18 to 35 years which had to be removed for caries or surgical reasons, were included. For collecting human teeth in local dental practices in Lower Saxony, the normative framework for using extracted teeth in research and academic education by Gross et al. (2015) was followed. Tooth donation was anonymous and voluntary. The teeth were placed in a 0.1% thymol solution and subjected to professional tooth cleaning before being incorporated into the models. The cervical tissue remnants of the periodontal ligament were removed with 1% NaOCl. The human teeth were polymerised on pins and could thus be easily detached from the model for impression taking and storage. In the terminal positions, two artificial teeth were used for

![Fig. 1. Experiment flow chart: The robot simulated 3-year toothbrushing on 14 extracted human teeth in anatomical position, SEM-coding and 3D-SEM measurement of cervical volume loss after toothbrushing with two types of manual toothbrushes (flexible ball joint neck and conventional rigid neck).](image-url)
the wisdom tooth 48 and the canine 33 to simulate the interproximal space next to the test teeth (Appendix Table 1, Fig. 1).

Two experimental groups with a representative sample of one lower jaw hemi-arch (7 teeth) were constructed: Group A for brushing with the flexible neck toothbrushes, and Group B with the rigid neck toothbrushes (Fig. 1). All experimental conditions such as random brushing programmes, brushing force and time, change of toothbrushes after simulated 3 months, dentifrice slurry flow and change in the artificial oral cavity, room temperature at day- and nighttime etc. were identical in both groups.

2.4. Cervical wear evaluation

A slurry consisted of 4 × 10−3 M H2SO4, 1 × 10−3 M NaOH, 4 × 10−4 M FeCl3, 5 × 10−4 M FeCl2, 2 × 10−3 M HCl, 2 × 10−3 M NaCl, 2 × 10−4 M Na2SO4 and 2 × 10−4 M Na2CO3. The robot test dentition was installed, and the pump speed was set to 7.50 rpm. Ten seconds in slow mode speed were used for counting the brushing strokes. Fifty subjects (32 female and 18 male) aged 19–68 participated (Appendix Table 2).

2.3. Abrasion cycling model

The six-axis robot (FS 02 N, Kawasaki Robotics, Akashi, Hyogo, Japan) was configured to simulate human toothbrushing movements. Parameters such as brushing force, brushing movement and brushing time were taken from clinical studies to ensure clinically validated and homogeneous study conditions (Lang et al., 2014). The combined toothbrushing programme alternated between the three most used toothbrushing movements: 2 x horizontal (17 s), 1 x rotating (19 s) and 1 x vertical brushing (22 s), performed with a brushing force of 3.5 N. The robot performed 44 strokes per buccal tooth site in one cycle, which was clinically confirmed by the video observation results. Assuming that teeth were brushed twice daily on average, the cycle was repeated 60x per month, and 95,040 brush strokes per tooth were performed during the simulated three years. The robot-simulated 3-year toothbrushing cycle was completed with two soft-bristle manual toothbrushes (Haleon, Weybridge, UK).

An Artificial Oral Cavity was used to rinse all human teeth to simulate continuous toothbrushing. For constant slurry irrigation, a peristaltic pump (Concept 420smd, SAIER, Gundelfingen, Germany) was installed, and the pump speed was set to 7.50 rpm = 14 ml/min. The slurry consisted of 4 × 10−3 ml Sensodyne Extra Fresh dentifrice (Haleon, Weybridge, UK) and 900 ml water (ratio 1:3) and was renewed every simulated three months (5 h 15 min laboratory cycle) with the replacement of the manual toothbrush.

2.4. Cervical wear evaluation

The buccal surfaces of teeth were moulded with A-silicone (Affinis Putty Soft and Affinis light body, Coltene Holding AG, Alstätten, Switzerland) and replicated with an epoxy resin (Easy-Mix N 5000 Epoxyd, Weicon, Münster, Germany). Epoxy resin replicas were sputtered with a 20 nm thin gold layer (Sputter CCU-010 HV, Zizers, Switzerland). The areas selected for SEM investigation were located in the cervical region of the tooth (Appendix Fig. 3). The standard magnifications used were 100x and 400x. The areas defined using image coding panels (Fig. 2; Appendix Section 2.1). Combined image boards were created for each tooth, showing examined tooth surfaces at 200x and 400x magnification (Appendix Section 2.2). In addition, backscattered electron images were used to create side views of the cervical abrasion lesions (Appendix Section 2.3). The combined image boards of the wear features of each tooth were compared with the coding panels and scored by two examiners, one non-blinded (KW) and one blinded (PG). In case of disagreement, the code was discussed to reach consistency.

SEM identified what we have termed enamel peninsulas (Fig. 2) and enamel islands (Fig. 3). These contribute to higher cervical abrasion due to their hardness and represent a new abrasion risk area. They appear after micro-wear on root surfaces and are dominant features with potential plaque accumulation around and later calculus formation underneath the peninsulas after abrasion of softer root cementum, leaving an enamel roof ridge. Peninsulas were visible after cervical wear in 11 out of 14 teeth, and even solitary enamel islands were detected in 3 out of 14 teeth. The islands were completely hidden by the normal cementum layers (pre-wear) and are visible post-wear in young teeth, whereas in older teeth, with exposed root dentin, these developmental anomalies have worn off.

The Wilcoxon signed-rank test (n = 7; α = 0.05, o.t.) demonstrated for both toothbrushes significant differences between pre- and post-brushing for micro-calculus removal (pDSC = 0.0078) (Fig. 4). The rigid toothbrush also showed significant differences in the W-test for exposed prismatic enamel (pEPE = 0.0156) and peninsula formation (pPF = 0.0313).
The cemento-enamel junction in this sample was the edge-to-edge Type 2 in 6 teeth and the gap Type 3 in 5 teeth. CEJ is rather stable, only one incisor and one molar lost the overlapping cementum due to wear and another molar kept the overlapping cementum on enamel even after wear (Fig. 4).

Quantitative measurement showed a volume loss from $x = 34.25\text{nl}$ to $x = 87.75\text{nl}$ (Fig. 5; Appendix Table 5). The wear rate did not differ significantly and both soft-bristle manual toothbrushes contributed to the very low volume loss (in the nanolitre range).

Wear extended 100–1500 µm apical from the cemento-enamel junction. The distribution of the volume loss was visualized using Reshaper 3D (Fig. 5).

4. Discussion

Since the 1970s, SEM has been recognised as the gold standard for microwear analysis, providing high-resolution images that now include high-resolution 3D point clouds (Dietz, 2012; Montag et al., 2018). Dental microwear texture analysis (DMTA) has been introduced as a promising technology (Ungar et al., 2008). It provides three-dimensional images with a lower technique-sensitive reproducibility of characteristic occlusal microwear patterns and was later extended to the buccal enamel microwear (Percher et al., 2017). The DMTA is, therefore, an excellent tool for short-living dietary articulation-dependent marks on teeth in palaeontology and recent animals and men (Berkovitz & Shellis, 2018).

Oral hygiene devices such as toothbrushes contribute to oral health mainly by plaque control but, in contrast, also contribute to wear. Because of this complex function, research tests should mirror the positive and negative effects on teeth in all age groups. We developed a new ex-vivo methodology, combining toothbrush testing with a robot simulation of three-year brushing in an Artificial Oral Cavity. Unlike conventional profilometric investigations, SEM and 3D-SEM can quantify wear effects under more realistic clinical conditions, including human teeth in an anatomic position.

Man-made cervical wear starts early in adolescence and develops over years and decades, resulting in enamel loss, cementum loss, replacement by calculus, and even heavy dentin loss. For the first time, this study compares human cervical wear in different age groups and the progression over time in the clinically validated robot simulation of 3-
year toothbrushing.

A major advantage of a robot-assisted ex-vivo method is its ability to investigate long-term tooth wear processes in short time and under standardised conditions to ensure reproducibility. In contrast, clinical trials with differences in patients’ oral hygiene habits and compliance problems have limited comparability of data (Rajwani et al., 2020).

The robot programmes in this study were applied after earlier computer-assisted planimetric plaque assessment of these new prototypes compared to a reference toothbrush (Lang et al., 2014; Acherkouk et al., 2022). The plaque control in risk areas next to the gum line and interdentally by flexible neck toothbrushes was superior to the control brush. Therefore, the cervical tooth wear study was started as a further application area for the established robot system. Furthermore, an optimised clinical simulation was ensured by using an artificial oral cavity for wet brushing with dentifrice in combination with the clinically validated robot movements. A peristaltic pump applied the slurry permanently to the teeth, and the brushing motion agitated the slurry around all teeth to simulate the typical clinical toothbrushing environment.

The robot programme also reflects the brushing behaviour demonstrated in other video observation studies (Ganss et al., 2018; Schlüeter et al., 2018). The frequently cited study by Heath and Wilson (1974) reports an average toothbrush motion speed of 4.5 Hz. Based on research data, a clinically relevant brushing force of 3.5 N was applied in this study (Ganss et al., 2009; Van der Weijden et al., 1998). Addy et al. (2002) estimated an average brushing time of 10 s for the buccal surface of a tooth. The video results of this study confirmed the common European toothbrushing standards. They resulted in 44 brushing strokes per buccal surface and 31,680 strokes in one year, and 95,040 strokes during the simulated three-year toothbrushing period. The number of toothbrushing strokes per tooth site is the only biophysical parameter that determines the risk of cervical wear. The speed of motions and the
duration of toothbrushing are individually different and do not contribute to the risk of brushing.

Acherkouk et al. (2022) showed that manual toothbrushes with ball-joint necks flexed 2.0 to 2.5 times more than reference toothbrushes with rigid necks under the same force. Therefore, the ball joint improves the biophysical brushing action. It is designed to reduce the risk of tooth wear, gum recession and dentin hypersensitivity caused by excessive brushing compared to a classic rigid neck toothbrush.

4.1. Morphological change due to toothbrushing

Results of this study concerning the very low cervical volume loss showed that manual toothbrushes and low-abrasive dentifrice could avoid heavy macrowear. The disappearance of abrasion marks can be attributed to continuous slight abrasion in contrast to clinically heavy wear by toothbrushes with hard filaments and high brushing force > 5 N. The typical feature of the perikymata is gradually lost over time and is replaced by a predominantly worn appearance of the enamel surface. Mannerberg (1961) found that among 20-year-olds, 20% of tooth surfaces are so worn that individual perikymata are flattened. Enamel infractions are very common in teeth of all age groups. They are considered a natural phenomenon (Scott et al., 1949; Zachrisson et al., 1980). With increasing age, the limited continuous eruption, which compensates for occlusal wear, does not result in the exposure of tooth roots. However, chronic periodontal diseases and higher toothbrushing forces may contribute to exposed cervical surfaces being susceptible to wear (Aw et al., 2002). According to Lussi et al. (1993), 84.6% of subjects with cervical abrasion complained of hypersensitivity. In this study, open dentin tubules have been exposed by abrasion, especially in young premolars. This is consistent with the findings that the risk of dentin hypersensitivity decreases significantly with age 60 due to remineralisation and sclerosis of the dentin (Carvalho & Lussi, 2017).

The most important result of the study is that the clinical cervical macrowear does not correspond to the SEM-microwear with a very complex morphological composite structure of enamel margins, cementum layers, dental calculus and exposed root dentin. The tooth wear also contributes to oral health by removing hidden calculus, flattening overhanging enamel margins and polishing dentin lesions.

Enamel islands on the root surface and enamel peninsulas became apparent after micro-wear and may contribute to biofilm attachment and de-novo formation of micro-calculus along the whole margin of the cemento-(dentin)-enamel junction. The mineralization of protected plaque accumulation underneath the roof ridge like worn enamel peninsulas resulted in after-wear micro-calculus.

Finally, a catalogue of micromorphological features and the coding of changes over time due to toothbrushing may contribute to the structural pathobiology of cervical wear as a basis for future research.

4.2. Cervical volume loss due to abrasion

The detail of the SEM scans allowed the data meshes to be reliably overlaid based on the natural roughness of the enamel surface and the cervical enamel margin. The reference alignment technique used in this study is known to result in significantly lower errors and more accurate measurements (O’Toole et al., 2019). The same operator (KW) performed all alignments to exclude errors between examiners. In addition, alignment and evaluation for each pair of data meshes were repeated on different days to check reproducibility. The average deviation between the individual measurements was ±0.011mm³. Measurement inaccuracies resulted in a depth of 0.6 µm, a length of 6.0 µm and a volume of 0.007 mm³ concerning the known geometric dimensions of the reference body. In contrast to a recent study using an in-vitro combined erosive/abrasive protocol on buccal molar surfaces demonstrating the most wear resistance of cementum (Al Shammari et al., 2022), all four young premolars in the present study exhibited substantial cementum loss with opening of a wear resistant enamel island in one of them (Appendix Fig. 22). Continuous monitoring of tooth wear rates is desirable to elucidate the aetiology of wear processes and improve clinical prevention. Laser scanning microscopy is an alternative to the
SEM methodology. Further research is needed to determine the exact accuracy and precision of the method by using a more extensive data set. Due to the limited number of human tooth samples in the present study, the universality of the results still needs to be validated. Nevertheless, statistical significance could be shown for some of the morphological features and there was a clear trend that the toothbrush with a flexible neck contributed to noticeably lower abrasion than the rigid brush.

5. Conclusions

According to the presented video-controlled clinical part of the study, the number of tooth brushing strokes per tooth site is the only biophysical parameter determining the brushing efficacy. The frequency of movements and the brushing time is individually different and they do not contribute to effectiveness. However, the head design of toothbrushes and the angle of the head to the tooth in manual toothbrushing may contribute to avoiding heavy cervical wear.

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**Fig. 5.** Electron microscopic view of Incisor A42 (40 year old subject), pre - baseline with calculus, post – after toothbrushing with rounded enamel edges. The colour-coded surface model of the cervical wear lesion after toothbrushing shows the amount of root dentin loss. Blue - low, Red - high loss of dentin. Overall volume loss - 43 nl on cervical mesh surface of 1.538 mm$^2$. Height scale colour coding [in $\mu$m]: Blue: 5 – 30 $\mu$m, green: 30 – 64 $\mu$m, yellow: 64 – 75 $\mu$m, red: 75 – 98 $\mu$m. (in colour!)
Cervical tooth wear is an individual dental phenomenon that varies from tooth to tooth with increasing age, is dependent on toothbrushing and erosive diets, and ultimately contributes to oral health as well as to the harmful loss of tooth structures.

Positive impact: Removing remnants of dental calculus along enamel margins masking the cemento-enamel junction (CEJ) and smoothing traumatic and iatrogenic damages.

Negative impact: Slight enamel loss over the whole smooth surface, cementum loss next to the CEJ, heavy root dentin loss because of combined wear due to bristle configuration of toothbrushes and dentifrices used and supplemented by erosive diets. Cervical wear also opens unique enamel islands and enamel peninsulas on the root surfaces and frequent enamel vertical fractures at cemento-enamel junction.

Based on exploring the dentition in adolescents, young adults and adults, the following observations were noted. In adolescents no or only negligible wear of the enamel was apparent. The root cementum was still thin in premolars of juvenile patients (10–15 years old), and the not yet fully matured enamel exhibited exposed prismatic structures after the simulated three-year toothbrushing cycle. The molars of young adult patients, aged between 18 and 35 years, were at significant risk of erosion and exposed root surfaces. Wear processes produced undermining wear of the cementum, enamel and dentine in vitro.

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Supplementary data associated with this article can be found in the online version at doi:10.1016/j.archoralbio.2024.105981.

References


CRediT authorship contribution statement

Sandor Nietzsche: Resources, Methodology, Investigation, Data curation. Katharina Wilke: Writing – review & editing, Writing – original draft, Visualization, Methodology, Investigation, Data curation. Peter Gaengler: Writing – review & editing, Writing – original draft, Supervision, Project administration, Methodology, Investigation, Funding acquisition, Conceptualization. Tomas Lang: Validation, Supervision, Funding acquisition, Data curation, Conceptualization. Roshan Varghese: Supervision, Investigation, Conceptualization. Steve Mason: Validation, Supervision, Conceptualization. Mathias Hemmle: Software, Resources.

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Declaration of Competing Interest

None.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.archoralbio.2024.105981.


