

Wear, mechanical and chemical properties of castor oil toothbrush bristles

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ABSTRACT

Manual toothbrushes with polyamide bristles are used for daily oral hygiene. Toothbrush bristles made from alternative raw materials like castor oil are increasingly produced but scarcely investigated. Medium hardness toothbrushes with bristles made of castor oil (AlterraBambus (ALT), Alverde (ALV), Dr. BestGreenClean (DRB), HydrophilBambus (HYD), ProkudentRecycling (PRO)) and one control toothbrush (ADAcontrol (ADA)) (n = 8) were investigated for wear, dentin-surface-roughness, elastic modulus and chemical composition. Toothbrushes were subjected to 12.5k, 25k, 37.5k and 50k cycles (toothbrush-simulator) simulating 6 months of toothbrushing. Macroscopic and microscopic (50 × magnification, SEM/micro-CT) images of bristle-ends, surface and overall quality were evaluated before and after mentioned intervals according to DIN EN ISO 20126. Data were statistically analyzed (Friedman-Test; ANOVA). No obvious wear was visible in macroscopic images. SEM-images showed acceptable bristle-ends in ADA (100 %) and DRB (100 %), PRO (96 %), ALV (87 %), ALT (82 %) and HYD (73 %), while bristle-surfaces were unacceptable only in HYD at 0 and 12.5k cycles. Overall evaluation was acceptable in ADA and DRB (100 %), PRO (96 %) ALV (84 %), ALT (82 %) and HYD (51 %) with significant difference in ALV and HYD at different intervals. Dentin-surface-roughness ranged from 3.4 to 3.8 µm (HYD-ALT), dentin-abrasion ranged from 60 to 95 µm (ALV-ALT) and elastic modulus ranged from 1.14 to 1.81 GPa (PRO-ALT) at baseline and from 0.61 to 1.11 GPa (PRO-ADA) after 50 k cycles. Bristles had similar elemental compositions: carbon (54.6–62.7 %), nitrogen (19.4–24.3 %) and oxygen (16.0–21.1 %), in agreement with ADA. Bristles of toothbrushes except HYD had acceptable bristle ends and surfaces. Dentin-surface-roughness, mechanical and chemical properties of castor oil bristles were similar to those of conventional polyamide bristles.

1. Introduction

Toothbrushes have been used for centuries [1] and are still the most effective means of oral hygiene at home [2–8]. With the development of the first fully synthetic bristle fiber by the US company DuPont in 1938, natural fibers were gradually replaced with polyamide (trade name nylon) [9]. The continuous development in manufacturing technology up to the mass production of toothbrushes made manual toothbrushes affordable and accessible to the general population for the first time. The continuous expansion of manufacturers' product ranges with new designs is necessary to remain commercially competitive in the market, but also reflects the high consumer purchasing power for toothbrushes

worldwide [2,10].

With the multitude of toothbrush models that exist today, consumers are faced with a major challenge when choosing a suitable model [1]; the decision for a toothbrush model is oftentimes random. A manual toothbrush with polyamide fiber bristles is the most widely used instrument for daily oral hygiene [2,10] and are usually produced on the basis of crude oil and/or natural gas [11].

The finite nature of fossil resources such as crude oil and the increasing desire of consumers to be more environmentally conscious have prompted the dental industry to rethink its approach. Toothbrushes with bristles made from alternative raw materials such as castor oil are increasingly being produced. However, the production of

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alternative toothbrush bristles that are equivalent in their properties to conventional polyamide bristles is still a major challenge, and declarations on toothbrush packaging can also be confusing for consumers. With statements such as “bio-based bristles made from castor oil” or “medium bristle hardness”, very few consumers are actually aware of the exact meaning of these claims. For most toothbrushes and most users, it is not fully clear when significant wear is expected and replacement for a new brush recommended.

Particularly in considering the fact that toothbrushes play an important role in the prevention of oral diseases, new types of toothbrushes based on alternative raw materials such as castor oil should be investigated to assess whether they meet the requirements such as sufficient bristle end rounding and comply with current standards. Therefore, the aim of this study is to investigate toothbrushes with castor oil bristles regarding their wear based on the bristle end rounding and bristle surface in addition to the associated dentin surface roughness, their chemical composition and their modulus of elasticity. The null hypothesis states that there is no difference against a control toothbrush with polyamide bristles (ADA control).

2. Materials and methods

2.1. Groups

A total of five manual toothbrush models with castor oil-based bristles were tested: „Alterra Bambus Zahnbürste“ (ALT, Dirk Rossmann, Burgwedel, Deutschland), „Alverde Zahnbürste“ (ALV, dm-drogerie markt, Karlsruhe, Deutschland), „Dr. Best Green Clean Zahnbürste“ (DRB, GlaxoSmithKline Consumer Healthcare, München, Germany), „Hydrophil Bambus-Zahnbürste“ (HYD, wasserneutral, Hamburg, Deutschland) and „Prokudent Recycling Zahnbürste“ (PRO, Dirk Rossmann) (Fig. 1). The standard toothbrush of the American Dental Association “ADA Control” (ADA, Oral-B, Procter & Gamble, Schwalbach, Germany) with polyamide bristles served as a reference group (Fig. 1). According to the manufacturers, all toothbrushes correspond to the “medium” degree of hardness. Details of the technical dimensions of the toothbrushes tested with the caliper are listed in Table 1.

2.2. Sample preparation

A total of 48 dentin specimens (6 groups with $n = 8$) were prepared. These were obtained from extracted bovine maxillary teeth in the form of thin dentin rods measuring $2 \text{ mm} \times 3 \text{ mm} \times 15 \text{ mm}$ and embedded in the provisional material Luxatemp Automix Plus (DMG Chemisch-Pharmazeutische Fabrik, Hamburg, Germany). The specimen surfaces were polished with the “SS-200 Grinder/Polisher” (LECO Corporation, Michigan, USA) using the finest grit sandpaper (800) to create a coplanar plane between the resin and dentin, remove resin overhangs from the dentin surface and obtain a uniformly smooth dentin surface. The surface of the dentin specimen simulated a closed row of teeth in the mouth with three semicircular protrusions in the middle of which the dentin rod was embedded [12]. The dentin specimens were stored in tap

water at 37° . Two vertical lines were engraved to mark the reference areas at the left and right ends of the dentin rod and the reference areas were covered with adhesive tape for protection prior to toothbrushing simulation (Fig. 1).

2.3. Toothbrush simulator

The toothbrushes had a contact weight of 150 g in the toothbrush simulator “Zahnbürst-Prüfmaschine-linear LR 1” (SyndiCAD, Munich, Germany) (Fig. 2) and lay evenly flat on the dentine specimen (Fig. 2). The toothpaste “Colgate Total” (Colgate Palmolive, Hamburg, Germany) was prepared for the brushing simulation as a toothpaste slurry with water in a ratio of 1:1 and poured into each individual sample chamber. The toothbrush simulator performed 70 cycles per minute back and forth movement of the toothbrush against the specimen. The number of cycles 12.5 k simulated the use of the manual toothbrush over a period of approximately 1.5 months, with twice daily use for 2 min brushing time each. In total, the toothbrushing simulation was carried out in four intervals of 12.5 k cycles each. Thus, simulating a period of 6 months in total.

At the end of each interval, the specimens, and toothbrushes ($n = 3$) were removed from the toothbrushing simulator and cleaned in an ultrasonic bath for 3 min. Macroscopic images of the toothbrushes were taken and the bristles of the toothbrushes were examined under a scanning electron microscope (SEM). A new simulation of 12.5 k cycles was then started. The remaining toothbrushes ($n = 5$) were simulated for 50 k cycles without intermediate examination of the bristles. This examination was used for the subsequent dentin surface analysis regarding surface roughness. The workflow of this study is presented in Fig. 2.

2.4. Macroscopic images of toothbrushes

After the toothbrushing simulation as described above, the condition of the toothbrushes ($n = 3$) was recorded macroscopically with a “D7200” SLR camera (Nikon, Tokyo, Japan) from the front and the side. The extent to which the bristles changed macroscopically during the toothbrushing simulation was evaluated. The focus was on signs of wear such as bending, splaying, or fraying of the bristles. The wear was measured using the Conforti index based on van Leeuwen [13].

2.5. Microscopic images of bristles

After the toothbrushing simulation, some bristles on a lateral bristle tuft in the center of the bristle head of the toothbrush were cut off with a scalpel, sputtered with gold-palladium and examined via SEM (GEMINI FESEM SUPRATM55VP, Carl Zeiss SMT, Oberkochen, Germany) at $50 \times$ magnification. Images were taken to observe three individual bristles of 3 toothbrushes on each SEM image in the initial situation and after four further intervals ($n = 9$). The SEM images were used to assess the bristle end rounding and the bristle surface texture.

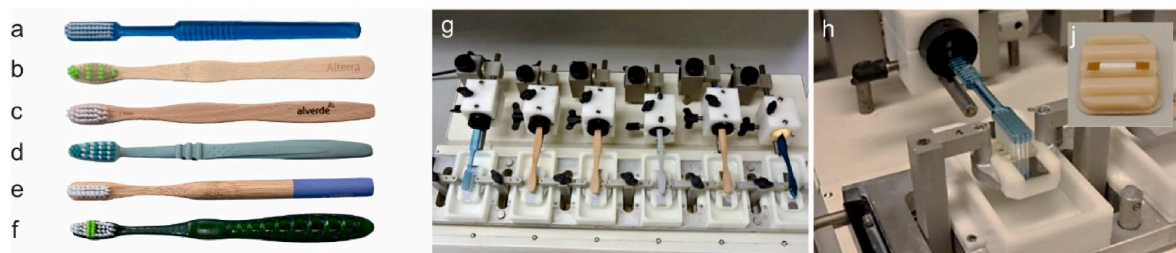


Fig. 1. The ADA Control toothbrush (a) and the five manual toothbrushes with castor oil bristles: Alterra (b), Alverde (c), Dr. Best (d), Hydrophil (e), and Prokudent (f), the toothbrush simulator with six clamped toothbrushes (g) and the toothbrush ADA lying flat and parallel on the specimen (h) consisting of the bovine dentin rod embedded in a temporary restorative material (j).

Table 1
Technical dimensions of the toothbrushes.

Toothbrush	ADA Control	Alterra	Alverde	Dr. Best	Hydrophil	Prokudent
Toothbrush length	180 mm	191 mm	190 mm	192 mm	189 mm	194 mm
Toothbrush head length	29.5 mm	30.5 mm	26.5 mm	29.0 mm	27.0 mm	29.0 mm
Toothbrush head width	11.8 mm	12.5 mm	13.5 mm	13 mm	11.5 mm	13 mm
Number of tufts	47	38	31	37	34	37
Number of bristles (filaments) per tuft white/green	42/-	36/36	38/-	42/42	42/-	54/54
Bristle diameter	0.20 mm	0.20 mm	0.19 mm	0.20 mm	0.18 mm	0.17 mm
Bristle length	11.0 mm	11.0 mm	11.0 mm	11.0 mm	11.0 mm	11.0 mm
	long					
	short	9.0 mm	9.5 mm	9.5 mm	–	9.0 mm

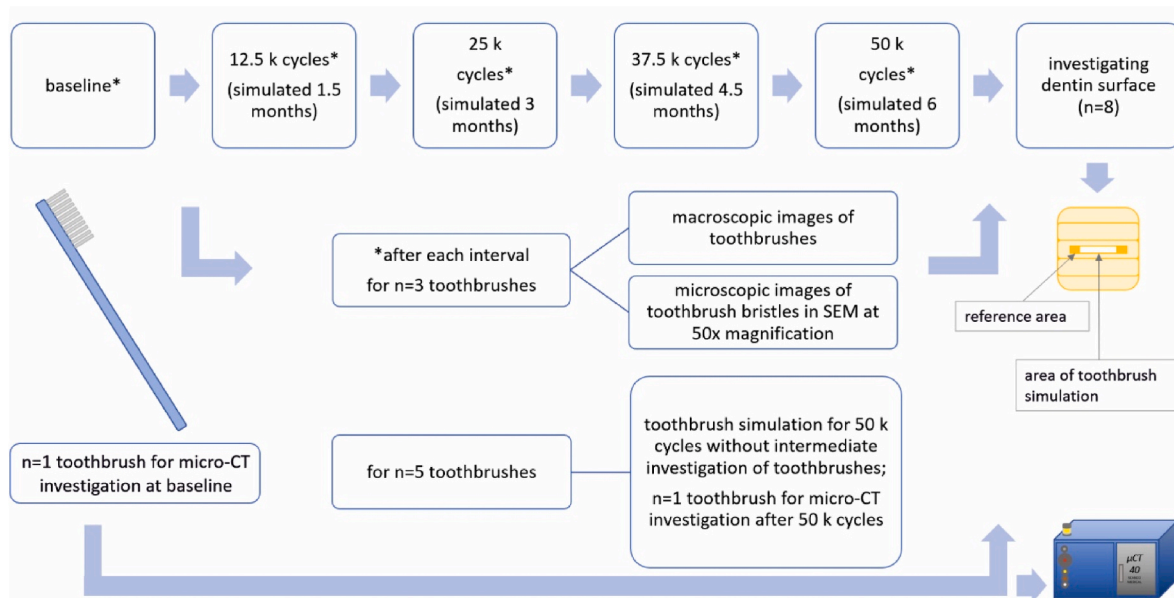


Fig. 2. Workflow of this study.

2.5.1. Bristle end rounding

A transparent mask was created in Microsoft PowerPoint (Microsoft 365 MSO, version 2211, Redmond, United States) for the evaluation of the bristle end rounding, which was superimposed over the individual bristles of the SEM images (Fig. 3). This mask was based on possible acceptable bristle end rounding listed in "DIN EN ISO 20126 Dentistry - Manual toothbrushes - General requirements and test methods 2021" [14]. The mask contained 5 different bristle end roundings, two of which were mirrored due to their asymmetry, resulting in a total of 7 possible outlines. The transparent mask could be placed over each individual bristle in an SEM image and was both rotatable and scalable, as the bristles in the SEM images were not all parallel to each other. This was used to check whether the outline of the respective bristle matched that of the transparent mask. If the contour of the bristle end rounding corresponded to one of the seven contours of the mask, the tested bristle was classified as acceptable regarding its end rounding and was awarded grade 1 (Fig. 3). If the bristle end rounding did not follow any of the possible contours of the mask, the bristle was considered unacceptable in terms of its end rounding and was given a grade of 0 (Fig. 3).

2.5.2. Bristle surface

The bristle surface was evaluated using a classification based on roughness, unevenness and material overhangs in grades I and II. In this part of the study, the focus was on assessing the surface both at the bristle tip and laterally along the bristle. Bristles that reached grade I were labeled as acceptable, whereas bristles that were categorized as grade II were unacceptable (Fig. 3).

2.5.3. Overall bristle evaluation

The evaluation criteria of bristle end rounding according to "ISO 20126" and the bristle surface resulted in an overall bristle evaluation. Bristles with an acceptable bristle end rounding 1 and a grade I bristle surface passed the test. Bristles that had an unacceptable bristle end rounding 0, regardless of their bristle surface, were considered to have failed, Table 2.

2.6. Microcomputed tomography (micro-CT)

The brush head was separated from a toothbrush in the initial state (before) and after 50 k cycles (after) of toothbrushing simulation, placed horizontally in the sample holder ($\varnothing = 37$ mm) of the microcomputed tomography (Micro-CT 40, Scanco Medical AG, Switzerland) and 695 slices were scanned with high resolution ($18 \mu\text{m}$) so that the entire length of the bristles was recorded. The acceleration voltage was 70 kV, the cathode current $114 \mu\text{A}$ and the integration time 600 ms. The micro-CT images were converted into a binary image using the Fiji software and evaluated two- and three-dimensionally (2D and 3D) [15]. The bristles in the initial state were compared with the bristles after 50 k cycles of tooth brushing simulation. For the 2D evaluation, a slice in the upper one third of the brush head in the before scan was compared with the corresponding slice in the after scan and evaluated qualitatively. Moreover, the 3D volume of bristles was evaluated and screenshots from different views were taken.

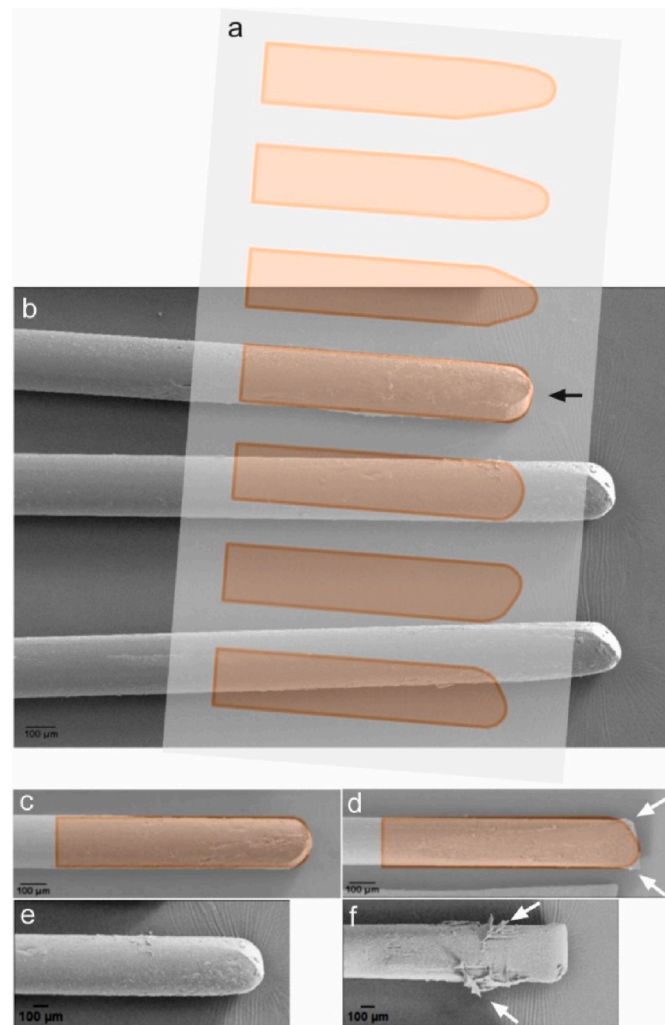


Fig. 3. Transparent mask (a) for assessing the bristle end rounding according to DIN 20126 with the various forms of acceptable end rounding (modified according [14]) being superimposed on an SEM image at 50x magnification (b) with the black arrow pointing at the mask superimposed over a bristle. An example of acceptable bristle end rounding grade 1 (c) and unacceptable bristle end rounding grade 0 (d) with white arrows pointing at the bristle parts that are not included within the boundaries of the mask. SEM images of bristle surface texture at 50x magnification: Grade I has a smooth and even surface and is acceptable (e), Grade II has severe material overhang and roughness and is unacceptable (white arrows) (f).

Table 2
Evaluation and grading of bristle ends and surfaces and the overall bristle evaluation.

Bristle end	Bristle surface	Overall bristle evaluation
Grade 1 = acceptable	Grade I = acceptable	acceptable
Grade 1 = acceptable	Grade II = unacceptable	unacceptable
Grade 0 = unacceptable	Grade I = acceptable	unacceptable
Grade 0 = unacceptable	Grade II = unacceptable	unacceptable

2.7. Chemical elemental analysis

The chemical composition of the elements of the toothbrush bristles (n = 3) was determined in the SEM using the “Genesis Spectrum EDAX” software (AMETEK, Mahwah, USA). For the material characterization, the generated X-rays were used, which were analyzed using an energy dispersive X-ray spectrometer (EDX).

2.8. Mechanical evaluation

To determine the mechanical properties of toothbrush bristles, the modulus of elasticity of the toothbrush bristles (n = 30) was determined in a tensile test using the TC 550 micro tensile tester (SyndiCAD, Munich, Germany) at baseline and after 50 k cycles. Using a scalpel, individual bristles were removed from a tuft of bristles in the central area of the toothbrush head from each type of toothbrush.

The diameter of the bristle was measured and then the bristle was screwed into both sample holder clamping devices using a screwdriver. The free length of the bristle between the sample holder clamping devices was determined using the calipers and noted in the software. During the stress-strain measurement, a tensile force with a maximum force of 100 N was applied at a speed of 2 mm/min. Since the software was able to record the parameter of the sample cross-section, it was possible in this test to calculate the modulus of elasticity by measuring the applied force using a calibrated force sensor.

2.9. Dentin surface roughness

The VHX-970F digital microscope (KEYENCE, Neu-Isenburg, Germany) was used to determine the dentin surface roughness Sa. For each dentin specimen (n = 8), a total of eight measurements were taken each in toothbrush simulation and in the reference area.

2.10. Dentin abrasion

The dentin rods (n = 8) were powdered (MET-L-CHECK developer D70, Helling GmbH, Heidgraben, Germany) and scanned using a chromatic confocal laser scanner (hardware: KF-30, SyndiCAD, Munich, Germany; software: Certiga, Unterhaching, Germany). The scans were used to evaluate the dentin abrasion after 50 k cycles using the software “match 3D” (Gloger, Weilheim, Deutschland, 1998) [16].

2.11. Statistical analysis

A Friedman test followed by a Dunn-Bonferroni post-hoc test was carried out for the statistical evaluation of bristle end rounding, bristle surface area and overall bristle evaluation. Data of the dentin surface roughness, dentin abrasion and modulus of elasticity were tested for normality using the Shapiro-Wilk test. Data of dentin surface roughness and dentin abrasion were subjected to the one-way ANOVA test with the Bonferroni post-hoc test. Data of the modulus of elasticity were analyzed using the paired samples t-test. The data of the chemical elemental analysis were presented descriptively. Statistical analysis was carried out using the IBM SPSS Statistics 28 (Armonk, United States) at a significance level of $\alpha = 0.05$.

3. Results

3.1. Macroscopic images of toothbrushes

The macroscopic images of the toothbrushes were taken in the initial situation and after each interval of 12.5 k cycles. After 50 k cycles, only minor changes in the toothbrushes were visible, such as flattening of the bristles and slight spreading and splaying of the bristles (Figs. 4–5). The Conforti index based on van Leeuwen was obtained as follows: ADA = 0, ALT = 1, ALV = 1, DRB = 1, HYD = 1 and PRO = 1.

3.2. Microscopic images of bristles

3.2.1. Bristle end rounding

The bristle end rounding of all the toothbrushes tested was acceptable and did not change significantly over the course of the simulation time points, except for ALV (Fig. 6). The toothbrushes from ADA and DRB showed acceptable bristle end rounding on 100 % of the bristles.

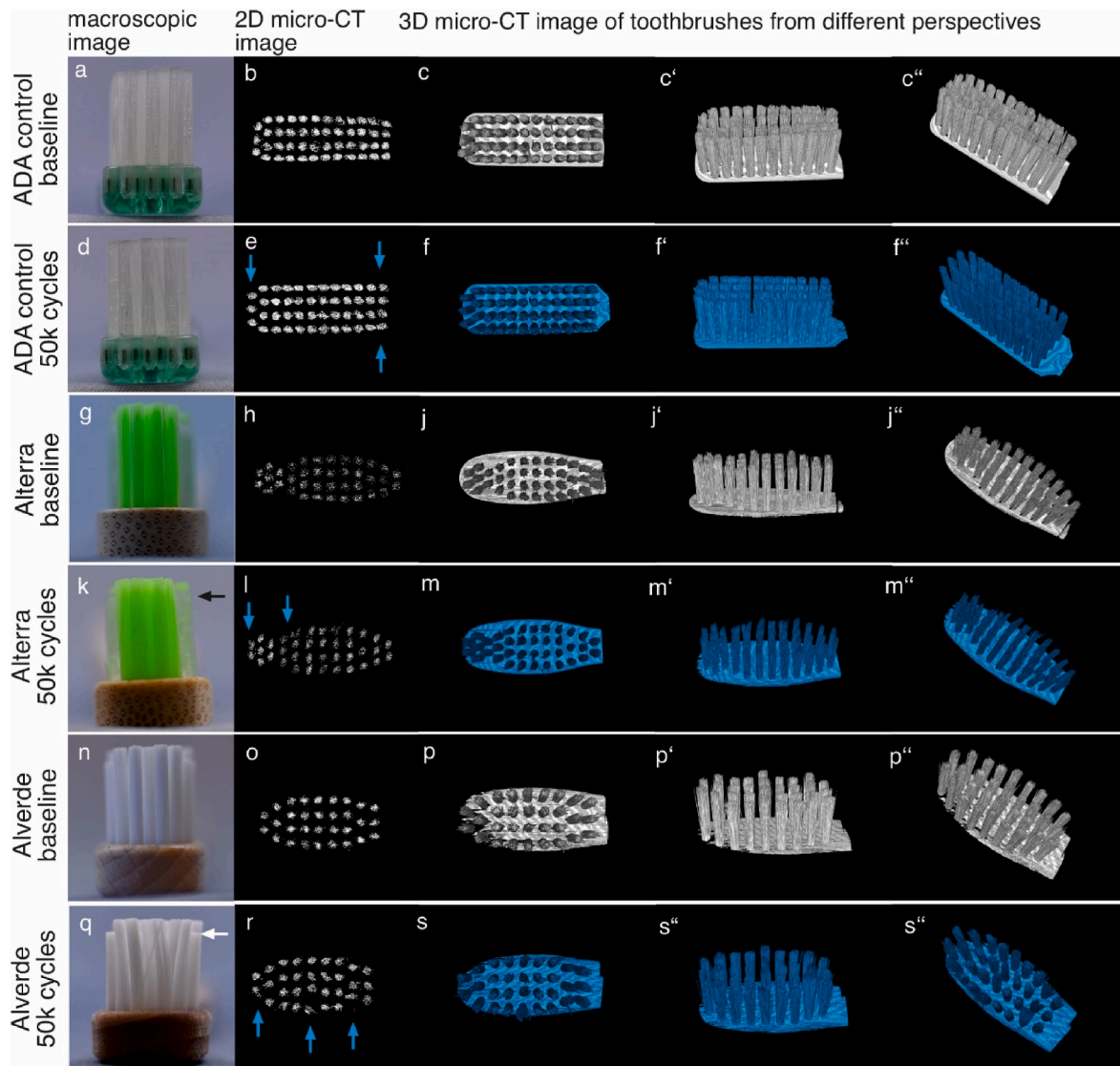


Fig. 4. Macroscopic images (first column), 2D (second column) and 3D micro-CT images (remaining columns) of the toothbrushes ADA, Alterra and Alverde at baseline and after 50k cycles. Minor changes are visible in the macroscopic images of toothbrushes such as the flattening of the bristles (black arrow) in ALT (k) and slight spreading of the bristles (white arrow) in ALV (q). The blue arrows in the 2D micro-CT images point at the bristle changes: in ADA slight spreading of the bristle tufts are visible in the front and rear (right) areas of the brush head (e), in Alterra bristles are slightly closer together in the upper area (l), in Alverde bristle tufts are irregularly displaced in the upper third. These changes in bristle positions are also observed in the 3D micro-CT images after 50 k cycles from different perspectives.

The toothbrushes from PRO (96 %), ALV (87 %), ALT (82 %) and HYD (73 %) also achieved high percentages of acceptable bristle end rounding. For ALV, there were differences between the measurement times in the Friedman test ($p = 0.002$), but no significant differences between the different time points in the pairwise comparison of the Dunn-Bonferroni post-hoc test ($p > 0.05$). There was no significant difference for all other toothbrushes (Table 3).

3.2.2. Bristle surface

Except for HYD, the toothbrushes had acceptable bristle surfaces at all intervals (Fig. 6). The bristle surface of the HYD toothbrush was unacceptable at baseline and after 12.5 k cycles and differed significantly from the other cycles ($p < 0.001$) (Table 3).

3.2.3. Overall bristle evaluation

The overall bristle evaluation, which is made up of bristle end rounding and bristle surface, showed in this study that these criteria were acceptable for most toothbrushes (ADA 100 %, DRB 100 %, PRO 96 %, ALV 84 %, ALT 82 %). Only HYD (51 %) achieved an acceptable

overall bristle rating for only half of all the bristles tested. There were significant differences between the time points for ALV compared to 50 k cycles ($p < 0.001$). For HYD, the overall acceptable bristle rating was lower at baseline, after 12.5 k and 25 k cycles than at the later time points ($p < 0.001$) (Table 3).

3.3. Microcomputed tomography (micro-CT)

The bristles of the toothbrushes were clearly visible in the micro-CT images. In the 2D and 3D comparisons, there were hardly any differences between the ADA (Fig. 4) and PRO (Fig. 5), the bristles of ALT (Fig. 4) and DRB (Fig. 5) were closer together especially in the front tufts, and the bristle tufts of ALV (Fig. 4) and HYD (Fig. 5) were clearly shifted and less concentric.

3.4. Chemical elemental analysis

All bristles had a similar composition of elements: carbon (54.6–62.7 %), nitrogen (19.4–24.3 %) and oxygen (16.0–21.1 %), consistent with

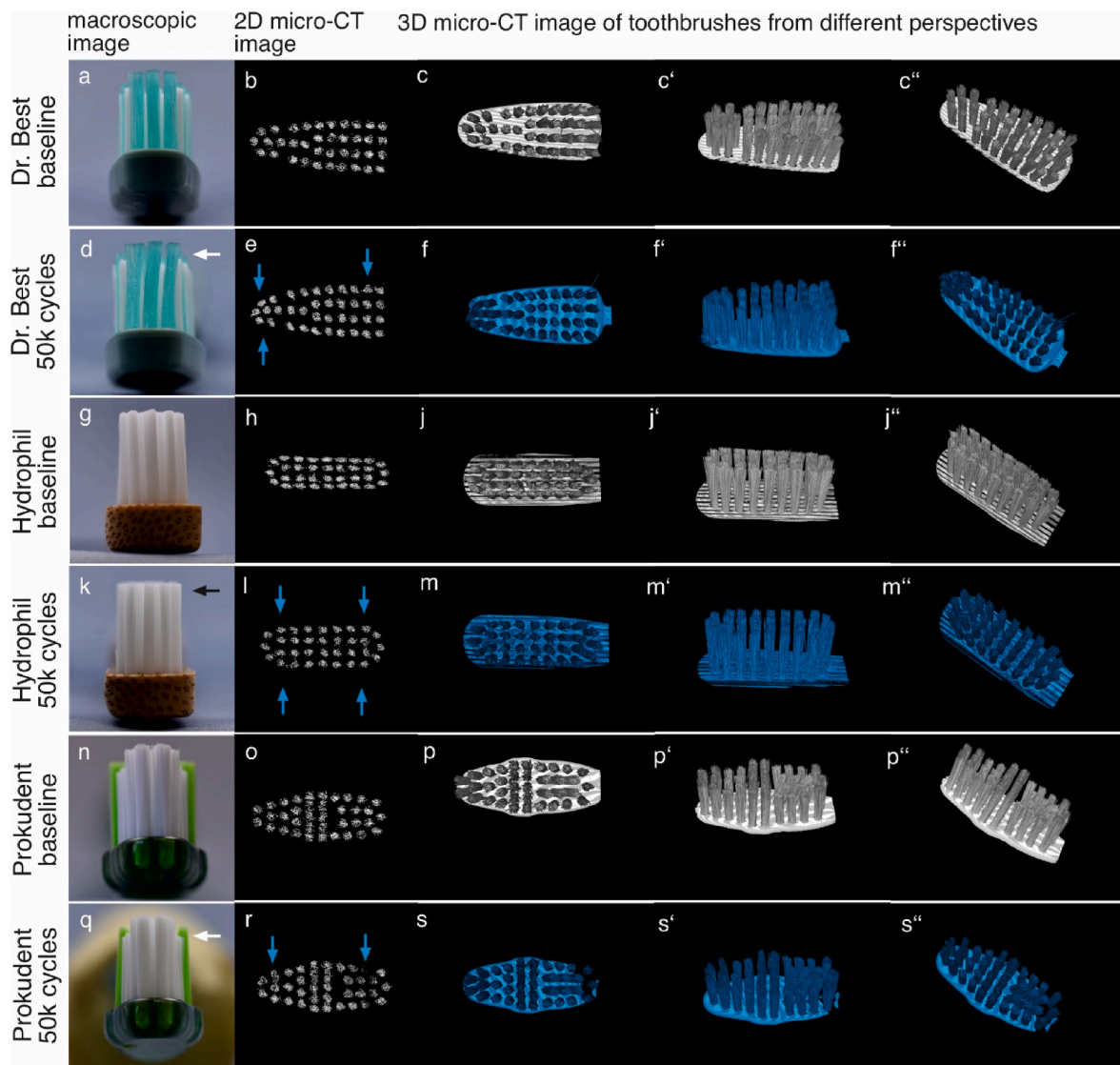


Fig. 5. Macroscopic images (first column), 2D (second column) and 3D micro-CT images (remaining columns) of the toothbrushes Dr. Best, Hydrophil and Prokudent at baseline and after 50k cycles. Minor changes are visible in the macroscopic images of toothbrushes such as the flattening of the bristles (black arrow) in HYD (k) and slight spreading of the bristles (white arrow) in DRB (d) and PRO (q). The blue arrows in the 2D micro-CT images point at the bristle changes: in DRB the tufts in the front are squeezed closer together, while in the rear they are irregularly arranged (e), HYD bristle tufts are spreaded further apart and shifted outwards (l), in PRO have hardly any visible changes in the bristles (r). These changes in bristle positions are also observed in the 3D micro-CT images after 50 k cycles from different perspectives.

the control toothbrush ADA.

3.5. Mechanical evaluation

The values of the modulus of elasticity of the toothbrush bristles ranged at baseline from 1.14 GPa (PRO green) with the lowest value to 1.81 GPa (ALT white) with the highest value, ADA had a value of 1.55 GPa (Table 4). Data were normally distributed ($p > 0.05$) except for ALT green ($p < 0.001$) and DRB white bristles ($p = 0.001$) but the ANOVA is robust against deviations from normality [17]. After 50 k cycles of toothbrush simulation, the modulus of elasticity was statistically significantly lower in all groups with the lowest value 0.61 GPa (PRO green) and the highest value 1.11 GPa (ADA). There was a statistically significant difference between the baseline measurement and measurement after 50 k cycles in all toothbrushes (Table 4).

3.6. Dentin surface roughness

Data of the dentin surface roughness Sa were normally distributed ($p > 0.05$) and ranged from 3.4 to 3.8 μm in the simulation and reference areas (HYD-ALT). The data for Sa showed no significant differences between the groups and areas ($F = 0.964$; $Df = 11, 84$; $p = 0.485$) (Table 5).

3.7. Dentin abrasion

Data of the dentin abrasion were normally distributed ($p = 0.720$), the one-way ANOVA showed a statistically significant difference between the groups ($p = 0.044$) but the post-hoc Bonferroni test showed no significant difference between the groups ($p > 0.05$). Greatest value of dentin abrasion was observed in ALT ($-95 \mu\text{m} \pm 23$) and lowest in ALV ($-60 \mu\text{m} \pm 28$) (Table 5).

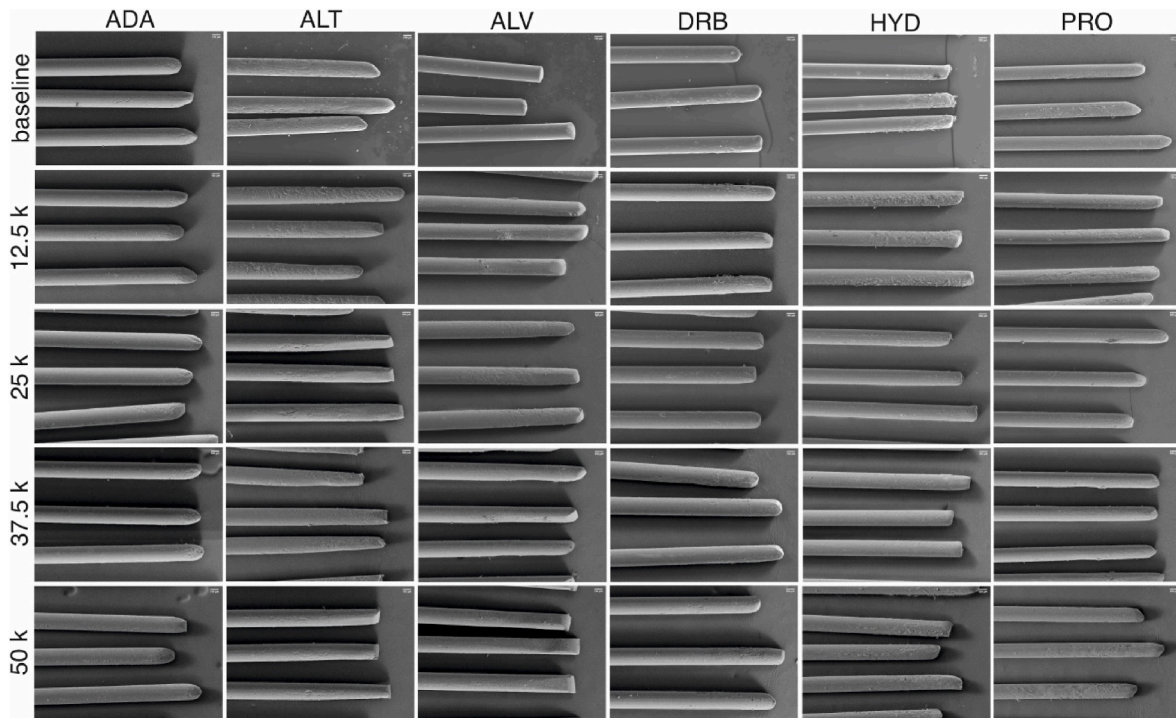


Fig. 6. Representative SEM images of bristles of all toothbrushes at 50 × magnification at different simulation intervals (baseline, 12.5 k, 25 k, 37.5 k and 50 k cycles).

Table 3

Bristle end rounding, bristle surface and overall bristle evaluation of the different toothbrushes: number (n) of acceptable bristles at the different intervals.

Cycles							
Toothbrush	0 k (n)	12,5 k (n)	25 k (n)	37,5 k (n)	50 k (n)	Σ acceptable bristles (%)	<i>p</i> – value ^e
Bristle end rounding							
ADA	9	9	9	9	9	100.0	1.000
Control							
Alterra	9	7	8	8	5	82.2	0.132
Alverde	9	9	9	8	4	86.7	0.002 ^a
Dr. Best	9	9	9	9	9	100.0	1.000
Hydrophil	6	6	5	8	8	73.3	0.406
Prokudent	9	9	9	8	8	95.6	0.406
Bristle surface							
ADA	9	9	9	9	9	100	1.000
Control							
Alterra	9	9	9	9	9	100	1.000
Alverde	9	9	9	9	8	97.7	0.406
Dr. Best	9	9	9	9	9	100	1.000
Hydrophil	3	2	9	9	9	71.1	<0.001 ^b
Prokudent	9	9	9	9	9	100	1.000
Overall bristle evaluation							
ADA	9	9	9	9	9	100.0	1.000
Control							
Alterra	9	7	8	8	5	82.2	0.132
Alverde	9	9	9	8	3	84.4	0.001 ^c
Dr. Best	9	9	9	9	9	100.0	1.000
Hydrophil	0	2	5	8	8	51.1	0.001 ^d
Prokudent	9	9	9	8	8	95.6	0.406

^a No significant differences between the time points ($p > 0.05$).

^b Significant differences between the following points in time ($p < 0.05$): 0 k – 25 k ($p = 0.025$); 0 k – 37.5 k ($p = 0.025$); 0 k – 50 k ($p = 0.025$); 12.5 k – 25 k ($p = 0.009$); 12.5 k – 37.5 k ($p = 0.009$); 12.5 k – 50 k ($p = 0.009$).

^c 0 k – 50 k ($p = 0.025$); 12.5 k – 50 k ($p = 0.025$); 25 k – 50 k ($p = 0.025$).

^d 0 k – 37.5 k ($p = 0.03$); 0 k – 50 k ($p = 0.03$); 12.5 k – 37.5 k ($p = 0.025$); 12.5 k – 50 k ($p = 0.025$).

^e Level of significance is $\alpha = 0.05$.

Table 4

Mean values \pm standard deviation (SD) of the modulus of elasticity (GPa) at baseline and after 50 k cycles.

Toothbrush	Modulus of elasticity (GPa) \pm SD at baseline	Modulus of elasticity (GPa) \pm SD after 50 k cycles	Mean of paired differences (GPa) \pm SD	<i>p</i> -value
ADA	1.55 \pm 0.13	1.11 \pm 0.07	0.44 \pm 0.08	<0.001
Control				
Alterra white	1.81 \pm 0.28	1.05 \pm 0.15	0.76 \pm 0.17	<0.001
Alterra green	1.45 \pm 0.24	1.04 \pm 0.11	0.41 \pm 0.16	<0.001
Alverde	1.47 \pm 0.19	0.92 \pm 0.10	0.55 \pm 0.12	<0.001
Dr. Best white	1.40 \pm 0.12	0.96 \pm 0.06	0.44 \pm 0.08	<0.001
Dr. Best green	1.45 \pm 0.13	0.90 \pm 0.08	0.55 \pm 0.06	<0.001
Hydrophil	1.43 \pm 0.13	0.89 \pm 0.07	0.55 \pm 0.07	<0.001
Prokudent white	1.63 \pm 0.16	0.77 \pm 0.08	0.87 \pm 0.11	<0.001
Prokudent green	1.14 \pm 0.13	0.61 \pm 0.06	0.53 \pm 0.13	<0.001

4. Discussion

The null hypothesis can be accepted for toothbrushes with bristles made of castor oil regarding macroscopic images, the chemical composition and surface roughness. However, it can be rejected due to varying percentage of acceptable bristle ends and bristle surfaces among toothbrushes, changes observed in the micro-CT images and the mechanical properties in terms of elastic modulus that varied significantly yet in the range between 1.1 and 1.8 GPa.

After simulating 6 months of toothbrushing, none of the toothbrushes showed significant flattening or spreading of the bristles. This wear corresponded to score 1 (scale score 0 – score 4) according to the Conforti index based on van Leeuwen et al., 2019 regarding the wear of the bristles [13]. The minor wear after the toothbrushing simulation of

Table 5

Mean values and standard deviation (SD) of the dentin surface roughness Sa (μm) and the dentin abrasion (μm).

Toothbrush	Mean Sa \pm SD (μm) in the simulation area ^a	Mean Sa \pm SD (μm) in the reference area ^b	Mean dentin abrasion \pm SD (μm)
ADA	3.5 \pm 0.2	3.6 \pm 0.4	−69.7 \pm 17.1
Control			
Alterra	3.8 \pm 0.5	3.8 \pm 0.3	−95.4 \pm 22.8
Alverde	3.4 \pm 0.4	3.5 \pm 0.3	−60.1 \pm 27.9
Dr. Best	3.7 \pm 0.4	3.6 \pm 0.5	−92.6 \pm 29.8
Hydrophil	3.6 \pm 0.3	3.4 \pm 0.4	−80.0 \pm 27.0
Prokudent	3.5 \pm 0.3	3.5 \pm 0.3	−69.6 \pm 23.2

^a Surface position Simulation area: Area of brushing movement, exposed dentin surface.

^b Surface position Reference area: unbrushed protected dentin surface.

up to 6 months could be attributed to the low contact weight of 150 g, among other things. Different contact weights, varying between 100 and 350 g, were used in various toothbrushing simulation studies [18–22]. For electric toothbrushes, the integrated testing of the force exerted when brushing teeth is set to the range 0.8–2.5 N, which corresponds approximately to a contact weight in the range 80–250 g [23].

All toothbrushes were loaded with 150 g in the toothbrush simulator. Each toothbrush was meticulously checked for correct adjustment in the toothbrush simulator to make sure the load is equal in all toothbrushes. The variation in tuft composition in terms of number of bristles and bristle length is assumed to affect the cleaning efficiency of the toothbrush, however, this was out of the scope of this study.

The micro-CT images were able to confirm the macroscopic appearance of the bristles on some toothbrushes, such as ADA and Prokudent. Although only a flattening of the bristles was macroscopically visible in Alterra, the micro-CT images showed more tightly packed bristles after the toothbrushing simulation. The spreading of the bristles was clearly visible both macroscopically and in the micro-CT images of the Alverde toothbrush. A clear deviation from the macroscopic appearance was seen in the bristles of the Hydrophil toothbrush, where the bristles were more widely spaced than in the initial situation. This allowed the bristle arrangement in the micro-CT images before and after the toothbrushing simulation to be examined in more detail than in the macroscopic images alone.

An advantage of micro-CT over macroscopic images is more display of details, moreover, the 3D-model can be rotated, enlarged and viewed from different perspectives for better viewing. Fine differences in bristle positions could be well identified in the micro-CT images which was not the case in the macroscopic images.

The microscopic appearance of the bristle end rounding was examined in accordance with the DIN standard “DIN EN ISO 20126” [14]. The bristle end rounding was acceptable for most toothbrushes, even after 37.5 k cycles, which corresponds to 4.5 months of use. The bristle surface quality, on the other hand, was acceptable on all but one toothbrush. This exception was Hydrophil in the initial situation and after 12.5 k cycles. With further use and wear, the bristle surface quality became acceptable.

Until now, the DIN standard has only considered the bristle end rounding, but our study has shown that the bristle surface quality is also an important criterion for assessing bristles. Consideration of the bristle surface plays a significant role, among other things, regarding possible increased bacterial adhesion to bristles and the risk of injury to soft and hard tissue from bristles. Although the bristle surface quality of most of the toothbrushes in this study was acceptable, this criterion for the quality of a toothbrush should be considered and included in the DIN standard.

In our study, the continuous contour and the overlapping of the bristles with one of the outlines of the transparent mask were important for assessing bristle end rounding. The overall bristle evaluation was

acceptable in most cases. Only with Hydrophil was the overall acceptable rating of the bristles up to 25 k cycles, i.e., a simulation of 3 months, significantly lower than at the later time points. This observation could be attributed to wear upon toothbrushing which rendered the bristle ends and surfaces more regular than at baseline.

In the current study the number of cycles up to 50 k cycles corresponds to a toothbrushing simulation of 6 months because it relates to the period of use of the toothbrush. In an earlier study, 12.5 k cycles corresponded to a simulation period of two years. These considerations are based on the work of Sorensen et al. from 2002, in which this number of cycles refers to a single tooth surface when abrasion of the brushed surface, i.e. tooth or restorative material, is examined [12]. Furthermore, the number of cycles varies from one study to another [18–22]. In the current study, however, the focus was on the wear of the toothbrush bristles and not the abrasion of the dentin, which is why the extrapolations of the toothbrushing cycles were based on the duration of toothbrush use.

There was no difference in dentin surface roughness between the area of toothbrush simulation and the reference area. It can be concluded that the toothbrushing motion did not alter the surface structure with neither type of bristles. The dentin roughness was measured in a small area only. Therefore, several measurements have been performed on each sample. We have added the dentin abrasion for a better understanding. Nevertheless, most toothbrushes had a high percentage of acceptable bristle ends even after 50 k cycles despite the performed dentin abrasion. Only two toothbrushes (ADA and HYD) had a regular bristle profile but this did not result in neither especially high nor low dentin abrasion.

The duration of toothbrush use cannot have a generalized recommendation, as it is influenced by several individual factors [24,25]. However, there are various reasons for replacing toothbrushes. These include inadequate cleaning of teeth from plaque, excessive microbial contamination of the toothbrush and a possible risk of injury from kinked or twisted bristles over time [26]. In this study, there was no indication of extraordinary wear after a simulated use of 6 months. Possibly this could be attributed to the specimen which consisted of an embedded dentin rod that has a lower hardness than sound enamel [27]. Furthermore, the flat dentin surface does not represent the normal anatomical form of human teeth covered with enamel.

In contrast to the clinical situation, this in vitro study took place under standardized conditions without exposing the toothbrushes to biochemical or microbial stress. In addition, uncontrollable variables such as the individual contact force and brushing technique as well as the oral microflora and presence of biofilm also play a role in a clinical setting. Therefore, no generalized recommendation can be made about replacing the toothbrush after a certain period. Manufacturers recommend replacing a manual toothbrush after 2–3 months, which could not be verified in our study. In the current investigation, most of the toothbrushes examined continued to have acceptable bristle end rounding and bristle surfaces after 4.5–6 months of toothbrushing simulation and showed hardly any macroscopic wear or splaying of the bristles. However, the definition of the simulation duration primarily served the purpose of comparability between different toothbrushes with different running times but the transfer to clinical application can vary.

Since the modulus of elasticity indicates the relative stiffness or rigidity of a material in the elastic range and is a material constant, it is suitable for comparing the brush stiffness of different toothbrushes [28, 29]. The modulus of elasticity of all the toothbrushes tested was comparable and ranged from 1.1 to 1.8 GPa, which is consistent with the manufacturers' stated degree of hardness. Stiffness of a toothbrush depends not only on the bristle material used, but also on the number of bristles and tufts, the number of bristles per tuft hole per unit area and the dimensions of the individual monofilaments [1,2].

The measurement of the modulus of elasticity after toothbrush simulation of 50 k cycles revealed an interesting finding: mean values of

all groups have significantly decreased compared to measurements at baseline. Thus, despite showing only minimal macroscopic changes and displaying mainly acceptable bristle end rounding, the mechanical properties have decreased which could not be detected by other tests.

Similarly, the chemical elemental analysis showed comparable composition of the bristles, which was evident from the equal distribution of elements in the spectra diagrams of the bristle materials examined. Since only small percentage differences were found between the toothbrush models, it can be assumed that toothbrush bristles based on castor oil have a very similar composition to the bristles of the reference toothbrush ADA Control, which are made of polyamide 6.6.

The polymer obtained from the raw material “castor oil” chemically belongs to the group of polyamides and the synthesis pathway is described in the literature [30,31]. The background to our approach was a chemical elemental analysis (EDX) to evaluate the possible presence of impurities. FTIR was not used in this study because of the color of the bristles as dyes can interfere with the measurement in FTIR. Some bristles were colored in three toothbrushes, so FTIR could not be applied.

The use of renewable raw materials to produce toothbrush bristles and handles meets the increased environmental awareness of many consumers. Environmentally friendly production includes the use of electricity from renewable energies, the use of production facilities available for conventional production and the use of renewable raw materials instead of fossil raw materials.

The manufacturing process for toothbrushes with castor oil-based bristles provides information about the composition. Castor oil can be obtained from the seeds of the *Ricinus communis* tree, also known as the miracle tree, by cold pressing. Castor oil contains 80–85 % ricinoleic acid, which is the raw material for polyamide production [31]. By chemically treating castor oil, a polyamide can be created as the end product in a complex multi-stage process [30,31]. Like conventional polyamide bristles, this end product is therefore not biodegradable, which is not specifically pointed out by the manufacturers. This study has shown that the bristles are ultimately chemically very similar to classic polyamide bristles, which is not explicitly mentioned on the packaging and the consumer is probably not aware of.

5. Conclusions

Considering the limitations of this study, the following can be concluded:

- Most of the toothbrush models tested with castor oil bristles were acceptable in terms of bristle end rounding and bristle surface texture when new and after use. After six months of simulated toothbrushing, microscopic and macroscopic signs of wear were very minimal.
- Castor oil bristles had similar behavior and properties to polyamide bristles, with a few exceptions.
- The dentin surface roughness did not differ between toothbrushes.
- Castor oil bristles had a similar chemical composition to polyamide bristles.
- The modulus of elasticity of castor oil bristles did not differ from polyamide bristles.
- The modulus of elasticity of all toothbrushes significantly decreased after toothbrush simulation.
- DIN EN ISO 20126 for manual toothbrushes only considers the bristle end rounding, but should also include the bristle surface texture for more comprehensive evaluation of toothbrush bristles.

CRediT authorship contribution statement

Dalia Kaisarly: Writing – review & editing, Writing – original draft, Supervision, Software, Project administration, Methodology, Formal analysis, Conceptualization. **Lilli Heusinger:** Writing – review &

editing, Writing – original draft, Software, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Gisela Dachs:** Writing – review & editing, Data curation, Conceptualization. **Moataz El Gezawi:** Writing – review & editing, Supervision, Methodology, Formal analysis, Conceptualization. **Anja Liebermann:** Writing – review & editing, Methodology. **Kurt Erdelt:** Writing – review & editing, Methodology. **Ludwig Czibere:** Writing – review & editing. **Falk Schwen-dicke:** Writing – review & editing. **Katrin Heck:** Writing – review & editing, Methodology. **Miriam Draenert:** Writing – review & editing, Supervision, Methodology, Formal analysis, Conceptualization.

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Data availability

Data will be made available on request.

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