Toothbrushes and jetting devices and their gingival injury potential

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1. Introduction

Good oral hygiene is one of the most inevitable tools to prevent oral diseases. However, intensive oral hygiene can possibly lead to gingival injury. It was, therefore, the aim (i) to standardize the in-vitro pig gum test and (ii) to evaluate the gingival injury potential of two popular powered toothbrushes: Oral B 6500 (Procter & Gamble, Cincinnati, Ohio, United States) and Philips Sonicare Diamond Clean (Philips, Amsterdam, Netherlands), two jetting devices: Sonicare AirFloss Pro (Philips, Amsterdam, Netherlands) and Waterpik WP 560 (Water Pik, Inc. Fort Collins, Colorado) and a manual toothbrush (Ormed, D).

Therefore, an *in-vitro* study was carried out on pig jaws. All powered and manual toothbrushes were tested using different combinations of brushing time, force and oral region, whereas the variable in testing jetting devices was oral region only. All tests were carried out by one calibrated dental professional Anete Liepina-Busch (ALB). The process quality of this investigator-initiated study (IIS) was ensured by ORMED laboratory rules. The samples were histopathologically controlled, to measure the extent of injury.

The main goal of this IIS was to investigate a possible correlation of extended brushing time and increased force and the respective injury potential of manual and powered toothbrushes and jetting devices. Additionally, it was the intention to compare the injury potential of the mentioned devices, using different time and force combinations.

2. Literature review

Many studies have been conducted over the past decades on different oral care products such as manual and powered toothbrushes and air jetting devices to assess their effects on both dental plaque removal and oral hygiene improvement. The methodology of these studies has become similar over the years of research, so that they are easily comparable. As oral hygiene of the population improves, new issues appear. The safety of different toothbrushes and their injury or gingival abrasion potential is slowly becoming a subject of several studies. So far, there is inconsistency of how to record and measure gingival injury. The inconsistency in methods and results of research into gingival injury due to tooth

brushing with different oral hygiene devices has made it difficult to compare these studies in their methodology and outcomes.
The following chapter will give a brief literature overview of the design of different toothbrushes and their first injury assessments.

2.1 Injury potential of oral care products

2.1.1 Manual toothbrushes

Design

The bristle toothbrush as we know it today was first invented around the year 1600, in China. The first patent of the toothbrush was granted in America in 1857, and since then its design has undergone very little changes.

Manual toothbrushes are a subject of 'General requirements of manual toothbrushes' (**DIN EN ISO 20126**, developed in 1973 and amended 2005). According to these requirements, the definition of a manual toothbrush is: a 'hand-powered device, the working end of which carries filaments primarily for cleaning surfaces within the oral cavity'. Moreover, several physical properties, including impact resistance, need to be met. A manual toothbrush has to have a brush head, which is the working end of the toothbrush and to which the filaments are attached. A filament stands for a single strand within the brush head. Filaments are supposed to be composed in a tuft that is a group of these filaments. Some resistance criteria as, for example, a tuft removal force, which is the force, required to remove one tuft from the brush head, need to be met as well.

Most toothbrushes on the market earlier were made entirely out of plastic and have thick multi-tufted synthetic bristles (Golding et al. 1982). Some variation was found in elasticity and form of the brush head. Today, manual toothbrushes come in a large number of shapes and sizes and are usually made of plastic moulded handles and nylon bristles. Toothbrush bristles are mostly synthetic and range from 'very soft' to 'soft' in texture, although 'medium' and 'hard' bristle versions are available as well. Bristle designs can be considered based on tuft patterns. A regular toothbrush has bristles in block patterns with equal bristle heights that are spread evenly. According to Beals et al. (2000), different multileveled patterns with mixed tuft heights claim to be most effective in reaching difficult areas of the teeth. The zigzag pattern is designed to sweep up plaque more effectively. Toothbrushes with bristles and a polishing-cup in the middle are supposed to clean surface stains effectively.

There have been few studies that search for the correlation between toothbrush design and certain oral conditions. Cifcibasi et al. (2014) contend that the toothbrush design has no influence on gingival recession ($p>0.05$). However, a recent in-vitro study of Kumar et al (2014) showed that the average surface roughness (Ra) was the lowest with flat trim bristle toothbrushes, therefore it produces the least enamel surface abrasion ($p=0.032$).

First injury assessments

There are comparatively few studies that deal with gingival injuries caused by oral hygiene. Most of them focus on efficiency of certain oral hygiene products, efficiency to remove plaque and possible adverse effects on hard tooth tissue. In order to understand gingival trauma, a short overview of classification, assessment methods and categorising of gingival injury is required.

A new 'Classification of Periodontal and Peri - implant Diseases and Conditions' was recently published by Caton et al. (2018), American Academy of Periodontology (AAP) and the European Federation of Periodontology (EFP), updating the 1999 classification of periodontal diseases. The gingival injury described in this proposal belongs to the group of 'non-dental biofilm induced gingival diseases and more precisely- traumatic lesions', which are primary caused by mechanical trauma. This classification proposal does not mention diagnostic criteria for gingival traumatic lesions.

However, several methods have been described so far in literature to assess gingival injury, inter alia: visual, histology, sonography (thickness measurement) and laboratory-chemical (evidence of blood cells, protein DNA). The categorisation of the severity of gingival injury has not been established yet. To the best of our knowledge, there is no 'gold standard' in whether recognizing, categorising or recording gingival injuries. Nevertheless, there have been few attempts to classify gingival injuries caused by tooth brushing.

In 1974 Anneroth et al. did an experiment on 15 dogs, to see if the material of a toothbrush has an effect on gingiva. Nylon brushes with a bristle diameter of ~0.25 mm and polytene brushes with a bristle diameter thicker at the base and thinner at the end of the bristle (0.7

mm to 0.25 mm), were compared in their potential to cause gingival damage. Different brushing time (from 10-60 seconds) and the same force (approx. 2.5 – 2.9 N) was used. Histological examination was done after brushing. The results of histology were collected by grading the damage in a relative scale from 0-3, where 0 stands for no damage, 1 for superficial epithelial damage, 2 for epithelium damage including half the thickness of the epithelium and 3 - damages that include subepithelial connective tissue. There were injury findings of all grades in most of the samples with both toothbrushes. Altogether the nylon brush caused 62 gingival damages and the polytene, 39. Wilcoxon's statistical test showed that both brushes had different potential to cause gingival damage and that the polytene brush appeared to cause less gingival damage than the nylon brush (p=0.05).

A few years later, in 1977, Alexander et al. tested the effect of manual toothbrushes on soft tissue abrasion which was measured using hamster cheek pouch tissue brushed mechanically for various intervals. A sensitive method for estimating proteins in solutions containing DNA was used for this purpose. Even with a small presence of DNA, proteins can be estimated. They found that with increasing brushing pressure (0.98 N up to 1.96 N) and time (200 up to 1000 strokes), there was a corresponding rise in the amount of protein removed. For instance, at the point of lowest pressure and time (0.98 N and 200 strokes), 0.118 mg protein adsorbance was measured, whereas at the highest pressure and time point (1.96 N and 1000 strokes), 0.327 mg protein adsorbance was observed. The effect of increased brushing time and pressure in tissue abrasion was proved to be highly significant (p=0.001). Similar tendencies were observed when comparing hardness of toothbrushes. Medium toothbrushes removed significantly more protein (p=0.001) than soft ones when compared directly to each other at all evaluation points (100-600 strokes). Additionally, the cut bristle toothbrushes were more abrasive at all stroke levels than round-end toothbrushes, and at the 600 stroke brush level the difference in protein removed between these two toothbrushes raised to 54 % (0.148 mg with round-end toothbrushes and 0.319 mg with cut bristle toothbrushes). The use or absence of dentifrice did not play a significant part in tissue abrasion.

Most studies using disclosing solutions deal with disclosing plaque to measure the efficiency of different toothbrushes. According to Datta et al. (2017), disclosing solutions change the colour of dental plaque to make it contrast with the tooth surface. Dental plaque can retain a large amount of dye substances so it can be used for disclosing purposes. There is a polarity difference between the plaque and the dye. Due to the interaction, the particles of the dye are bound to the plaque surface by electrostatic interaction (proteins) and hydrogen bonds (polysaccharides). For instance, Gallagher et al. (1977) carried out *in-vivo* and *in-vitro* tests to estimate the mechanism of the differential staining phenomenon of the two-tone disclosing agent. It was noted that the differential staining was dependent upon the thickness of the plaque. New, thin plaque was stained red or pink, whereas older and thicker plaque was stained blue or blue-purple. It was not associated with the type of bacterial flora or other biochemical factors. Just a few years later, the same method was used to stain gingiva and reveal gingival injuries.

Plaque disclosing solutions have been used in dentistry for a long time and have undergone major changes since being introduced in 1914 by Skinner. The spectrum of the brands and methods to stain gingiva using plaque disclosing agent varies widely. However, there are two main ingredients in most disclosing solutions currently used that are relevant to present study. These are Phloxine B and Patent blue. Phloxine B stains newer plaque and superficial injuries red or pink, whereas Patent blue stains older plaque and deeper injuries blue or blue-purple.

The pioneers of assessing gingival abrasion caused by brushing and a study group who standardised one of the most important gingival injury assessment methods today- staining gingiva, is Breitenmoser et al. (1979). The test objective was to determine- under substantially standardised clinical conditions, whether the bristle end form of a manual toothbrush influences the gingival lesion potential. Thirty subjects with clinically healthy gingiva took part in the test. Brushing was performed in circular fashion using a modified Bass method. The brush force was set at 4.9 N, a special test set-up and apparatus was used which enabled the subject to control the desired force continuously. The lesions of the gingival surface were stained with a plaque disclosing solution Dis-Plaque (Pacemaker Corporation, Portland, USA). The area of gingiva was photographed and compared using Germann's (1971) quasi standardisation method after 30 seconds of brushing with a cut bristle end form and round bristle end form, in 14 day intervals. The results showed cut bristle ends caused significantly greater (by 30 %) gingival lesions (mm^2) than rounded bristle ends ($p<0.01$).

Sandholm et al. (1982) classified visual signs of trauma caused to gingival tissues by standardised toothbrushing into three simplified types: 'type 1 as a positive lesion (erosion of epithelial surface)’, type 2 as an uncertain lesion (an epithelial flap leaves the underlying tissue uncovered)’ and ‘type 3 as a non- traumatized gingival unit (laceration of the surface epithelium on otherwise intact gingival surface). He also compared the plausibility of these visual findings with scanning electron microscopy results. The results showed close to 90 % of findings matched correctly.

A research group in Finland conducted three different studies. In 1984 Niemi et al. investigated whether stiffness of the toothbrush and the use of dentifrice influence the amount of brushing injuries. Teeth and gums were stained with a basic fuchsine solution before brushing to reveal pre-existing injuries. Obvious visual gingival lacerations and ulcerations were recorded as a brushing injury. The plausibility of these findings had been proven before in a separate study of Sandholm et al. (1982). The results indicated that the hard bristle toothbrush and use of abrasive dentifrice caused more frequent injuries than soft toothbrushes and an absence of dentifrice. The highest average number of new lesions was 9.2 after using a hard toothbrush and abrasive dentifrice. The lowest average number: 1.2 lesions per person were found after using a soft toothbrush and no dentifrice. Statistically significant differences were found only when the abrasive powder was compared to no dentifrice while using the soft brush ($p<0.05$). Later in 1986, Niemi et al. in the same manner recorded the number of gingival lesions after brushing with a V-shaped manual toothbrush, a multi-tufted manual and a powered toothbrush. Manual toothbrushes in this study were found to have caused significantly more gingival abrasion (number of lesions) than the powered toothbrush. The mean difference between the powered and V-shaped manual toothbrush was 4.32 lesions ($p<0.005$). This is significantly more damage than in comparison between the powered toothbrush and the multi-tufted manual toothbrushes (mean difference=2.18 lesions; $p<0.05$). The third study from Niemi et al. (1987) found that the type of toothbrush grip affects gingival injury during brushing as well. The methodology was similar to that in the previous study. Significantly more gingival injuries were found when the toothbrush was held with the palm grip than with the pen grip (mean=2.12; $p<0.01$).

A research group from the Netherlands, Danser et al. (1998), did an extensive study to evaluate the incidence of gingival abrasions as a result of tooth brushing with manual and powered toothbrushes. Gingival abrasion was assessed and recorded using the method adapted from Breitenmoser et al. (1979). Two experiments were carried out. In the first experiment 50 subjects brushed for three weeks every second day with either a manual or powered toothbrush. Teeth and gums were disclosed with Mira-2-Tone (Hager & Werken, GmbH & Co. Duisburg, Germany) solution. Abrasion sites were scored as small (≤ 5 mm) or large sites (> 5 mm). Next, the participants brushed in the random split-mouth order. After brushing and a second disclosing, plaque and abrasion were re-assessed. It is interesting to note, that all of the participants had small abrasion sites pre-brushing. The mean number of small sites (≤ 5 mm) was 3.85 pre-brushing and 4.46 post-brushing for the powered toothbrush and 3.31 and 3.84 with the manual toothbrush. The mean number with large sites (> 5 mm) was 1.67 pre-brushing and 1.89 post-brushing with the powered toothbrush and 1.71 and 2.22 with the manual toothbrush. More small sites of injured gingiva than large sites were found at both pre and post brushing stages. The results showed similar injury potential for both powered and manual toothbrushes. In experiment number 2, a new group of 47 subjects brushed with two powered toothbrushes, from which one was used to evaluate the effects of toothbrush bristle end-rounding and the other to evaluate the effects of brush-handle speed on brushing force. At first the participants brushed in a split-mouth order with two different types of end-rounding. Plaque and abrasion were assessed in a similar manner as in the first experiment. Immediately after, the subjects re-brushed with a different (2800 rpm and 3600 rpm) speed powered toothbrush during which brushing force was measured. The results of this experiment showed that end-rounding does affect the incidence of gingival abrasion. No increase was found for the amount of larger sites with gingival abrasion before and after brushing. More small sites were found after brushing with a “gothic” end-rounding toothbrush compared to the “roman” end-rounding (p= 0.02). The mean force of brushing with the 2800 rpm powered toothbrush was 1.68 N and with the 3600 rpm, 1.66 N. There was no relationship between tooth brushing force and the incidence of gingival abrasion.

Staining of gingiva was also used later by Imfeld et al. (2000). Gingival injury potential of several popular manual toothbrushes in Switzerland was tested on pig gums. Paro Plak 2 Colour plaque revelator (ESRO AG, Thalwil, Switzerland) was used to stain gingiva pre- and post-brushing. It contains erythrosine, which stains cells that have lost their membrane stability. This is similar to Dis-Plaque (Pacemaker Corporation, Portland, USA) which was used in the study of Breitenmoser et al. (1979) and Mira-2-Tone (Hager&Werken, Duisburg, Germany), which was then also used later by Danser et al. (1998). Injured areas were then digitised and expressed in absolute values in percentage. Gingival injury potential was measured after 30, 60, 120 and 240 seconds and ranked from 1-15, where 1 was the lowest and 15 the highest injury potential. For instance, after 4 minutes of brushing the highest injury potential showed Dentalux Flexible soft-medium manual toothbrush with 32.4 % injured area. The lowest injury potential at this point showed Mentadent C Contact soft with 13.9 %. Only 2 of the 15 toothbrushes were rated as ‘not harmful to the gingiva’.

Versteeg et al. (2005) used the same methodology as Danser et al. (1998) to assess the impact of dentifrice on gingival injury. Small abrasion sites were 5.86 sites with and 5.75 without dentifrice. These findings were not statistically significant.

Comparative analysis between hard and soft filament toothbrushes related to gingival abrasion of Carvalho et al. (2007) is warning that the use of hard-filament toothbrushes resulted in a significantly higher (p=0.018) mean number of lesions: 11.6, when compared to the soft-filament toothbrushes (mean=7.9). A more recent study of Zanatta et al. (2011) concludes that medium toothbrushes have a greater ability to remove biofilm but also cause more gingival abrasion than soft toothbrushes (p<0.01). The assessment of gingival injuries was made by simply taking pictures before and after brushing. These results validate previous findings (Breitenmoser et al, 1979; Sandholm et al, 1982 Niemi et al, 1984; Danser et al, 1998). A similar study from Zimmer et al. (2011) confirms that manual toothbrushes with hard bristles may better remove plaque, but also cause significantly more (p <0.01) soft tissue trauma compared to brushes with medium and soft bristles. Danser gingival abrasion index was used in this study. A recent study of Caporossi et al (2016) also emphases that round-ended bristles remove plaque more effectively without causing a higher incidence of gingival abrasion when compared with tapered/cut bristles. Additionally, according to the results of this study, the use of dentifrice significantly increases gingival injury risk (p<0.001). Gingival abrasion was evaluated using 2-tone disclosing solution to visualize the injured areas. Mean percentage of sites with gingival abrasion with tapered bristles and use of dentifrice was increasing from 5.5 to 14.3 whereas with water and round-ended filament 7.0 to 13.5.

The newest systematic review from Ranzan et al. (2018) is claiming to be the first one assessing potential adverse effects of both manual toothbrush bristle stiffness and end-shape on gingiva. Only 13 studies met the criteria of this review presenting safety as primary outcome. Most of the studies reported no adverse effects after brushing with soft and extrasoft toothbrushes. However, gingival injuries seem to be similar for both tapered and round-ended bristles.

De Nutte et al (2018) used the gingiva staining method to reveal gingival injury on the human palate after brushing with a manual toothbrush. Mira-2-Tone (Hager&Werken, Duisburg, Germany) disclosing solution was used in his study. However, the purpose of his study was to observe the healing progress of the injury.

2.1.2 Powered toothbrushes

Design

According to Kulkarni et al. (2017), a Swedish watchmaker, Fredrick Wilhelm Tornberg is credited with designing the first mechanical toothbrush in 1885. The first powered toothbrush was introduced at the American Dental Association Convention in St. Louis in 1938. It was in the 1960s when powered toothbrushes appeared on the market. Since then, the use of powered toothbrushes has widely spread and testing of different powered brushes in controlling plaque, gingivitis and staining was initiated. According to the patent of the powered toothbrush that belongs to Blaustein, Lawrence A. (Moreland Hills, OH, US, United States Patent Application No. 20020017474, 2002), it consists of a handle with a motor and a moving portion or the head which rotates, oscillates and/or reciprocates.

As stated in the Cochrane review from Yaacob et al. (2014) which is an update of a similar review in 2005, of powered vs. manual tooth brushing for oral health, powered toothbrushes can be divided into several groups according to their movement type: side to side action, counter oscillation, rotation oscillation, circular, ultrasonic and ionic. However, the clinical importance and potential to cause gingival injury of the different actions remains unclear.

First injury assessments

Although powered toothbrushes appeared on the market in 1960s, research of their potential to cause gingival injury began considerably later. Some of the studies mentioned in the previous chapter (2.1.1.) compare injury potential between manual and powered toothbrushes (Niemi et al. 1986; Danser et al. 1988). Methodology and findings of both mentioned studies were slightly different. Niemi et al. (1986) did not stain gingiva for visual examination of brushing injuries. The mean difference in the number of brushing lesions between powered and V-shaped manual toothbrushes was 4.32 ($p<0.005$) whereas with multitufted manual toothbrush – 2.18 ($p<0.05$). Results of this study suggested manual toothbrushes have significantly higher gingival injury potential than powered toothbrushes.

Danser et al. (1988) used plaque disclosing agent to stain gingiva before and after brushing. This method was adapted from Breitenmoser et al. (1974). The mean number with large sites of gingival injury (> 5 mm) was 1.67 lesions pre-brushing and 1.89 lesions post-brushing with a powered toothbrush and 1.71 lesions pre-brushing and 2.22 lesions post-brushing with a manual toothbrush. As seen before and after brushing, there were more small sites of injured gingiva than large sites. The results showed similar injury potential for both powered and manual toothbrushes.

A crossover study of Mantokoudis et al. (2001) compared two different powered toothbrushes and a manual toothbrush with respect to their clinical efficacy and gingival abrasion. 26 dental student volunteers took part in this study. The extent and severity of gingival abrasions were assessed by disclosing gingiva with a modified method of Breitenmoser et al. (1979) and adapted by Danser et al. (1998). The number of sites with gingival abrasions was registered as small (≤ 5 mm) and large (> 5 mm) sites, similar to the study of Danser et al. (1998). The baseline mean value with number of small abrasions was 8.71. Gingival abrasion values increased with all three toothbrushes after two weeks of brushing. The mean number of injuries after brushing with Braun Oral B Plaque Control 3D powered toothbrush increased to 10.75; manual toothbrush to 12.17 and the other powered toothbrush (Braun Oral B Plaque Control Ultra) to 12.54. Statistically, the results of this study showed no difference between both powered toothbrushes and a manual brush in their potential to cause gingival injury.

Dentino et al. (2002) tested the safety of manual and oscillating-rotating powered toothbrushes where safety data was provided by clinical attachment levels (probing depth in mm) and recession (mm) measurements. The mean values of probing depth and recession at baseline and after 6 months were 1.65 and 0.17 mm, 1.75 mm and 0.14 mm respectively with the powered toothbrush and 1.66 mm and 0.15 mm, 1.68 mm and 0.15 mm respectively with the manual toothbrush. The mean values of probing depth and recession between both groups were not significantly different. A concern that powered toothbrushes may induce gingival recession has been rejected by a study of Dorfer et al.

(2009). In fact, his study proves that after 6 month of use, overall recession was reduced by both manual and powered toothbrushes.

A systematic review of Van der Weijden et al. (2011) was summarizing *in-vivo* and *in-vitro* studies of the preceding two decades. A search of trials through May 2010 was conducted to identify appropriate studies that evaluated the effects of an oscillating-rotating power toothbrush compared to a manual tooth-brush with respect to soft and/or hard tissue safety. Eligible trials incorporated a safety evaluation as a primary or secondary outcome parameter (i.e., gingival recession, observed/reported adverse events, and hard tissue effects) or used a surrogate parameter (i.e., stained gingival abrasion and brushing force) to assess safety. Results of *in-vivo* studies consistently showed post-brushing increases in the mean number of gingival abrasions: 0.2 to 4.3 in the powered toothbrush group and 0.5 to 5.6 in the manual toothbrush group. According to the statistics these changes were not significantly different in both groups. In two studies where the brushing force was an outcome, the manual toothbrush showed significantly higher ($p \leq 0.0001$) force used than powered toothbrushes. Overall results show no clinically relevant concern to hard or soft tissues after brushing with oscillating-rotating toothbrushes.

A meta-analysis comparing manual and powered toothbrushes published by Vibhute et al. (2012) includes three randomized controlled trials from 2002-2005. All three studies had a soft tissue trauma as a secondary outcome parameter. All of these studies report soft tissue trauma. However, none of the adverse effects reported were a major cause for concern, and were not investigated further.

An observational study by Rosema et al. (2014) determines gingival recession in manual and power toothbrush users and evaluates the relationship between gingival recession and gingival abrasion scores. Gingiva staining pre- and post- brushing with Mira-2-Tone (Hager&Werken, Duisburg, Germany) plaque disclosing agent was used to reveal gingival injuries. The average pre-brushing scores of gingival abrasion were similar. Both, manual and powered toothbrush groups showed a significant increase in gingival abrasions post-brushing ($p<0.001$). The manual toothbrushing group had a median increase of 12.5 abrasions; the powered toothbrushing group showed a median score of 10 more abrasions post-brushing. The difference between the groups was also statistically significant ($p=0.004$). As seen in previous studies, small abrasion sites were more common than large ones. Both small and large abrasion sites increased more in the manual toothbrushing group (p=0.005). Overall results showed lower post-brushing gingival abrasion levels in the powered toothbrushing group. However, there was no correlation with gingival recession as a result of small abrasions.

2.1.3 Oral douches and first jetting devices

Design

Even if dental floss and interdental brushes remain the first choice for dental cleaning, the effectiveness depends on proper use and technique; and as shown in research from Staehle at al. (2004) about active oral health behaviour in Germany and Switzerland, cleaning difficult-to-reach areas can be a challenge that leads to lack of motivation and avoiding interdental cleaning. Jetting devices also known as water flossers or oral douches are supposed to be an easy and more effective alternative to string floss and interdental brushes. Over the years jetting devices have developed from other powered interdental cleaners.

Braun Oral B Interclean was one of the first powered devices for interdental cleaning. A handle, similar to a regular powered toothbrush with a tip, containing special cleaning filament and a centrifugal cleaning action, is supposed to clean interdental spaces more effectively and easily than regular string floss. Cronin et al. (1996, 1997) in his two studies suggests that it has an equivalent efficacy to dental floss for the reduction of interproximal plaque and gingivitis. No significant soft tissues alteration was observed in either of the studies. Similar studies with the same outcome were carried out by Gordon et al. (1996) and Isaacs et al. (1999). Additionally, Gordons study questionnaire for participants showed that 69.5% would prefer the powered device to conventional flossing.

Later, in 2005 Cronin et al. researched one of the simplest powered interdental devices- Oral B Hummingbird, basically a small, electric device, that vibrates, fitted with either a single-use prepared string floss attachment (‘flosette’) or tooth pick attachment. It was compared to regular string floss. All interdental cleaning methods were described as safe, with no evidence of oral hard or soft tissue trauma.

According to Lyle (2012), oral irrigation has been on the market for about 50 years. The first oral irrigator (also called a jetting device, water flosser or water pick) was introduced in 1962, around 20 years later than the powered toothbrush. This device has a completely different approach to cleaning interdental spaces. The water comes out of the device under pulsating pressure in order to clean each interdental space, regardless of its size and shape. This has been a challenge for the patients and dental professionals when choosing interdental brushes. The benefits in her study are the removal of biofilm from tooth surface and bacteria from periodontal pockets.

Few other studies have tested jetting devices and proved them to be effective. For instance, Sharma et al. (2008) showed that the dental water jet with a special orthodontic tip is more effective to remove plaque and reduce gingival bleeding than a manual toothbrush and dental floss for people with orthodontic appliances. In his historical review, Jahn (2010) included all the studies about oral irrigators from the year 1962 to 2009. The findings supported the dental water jet as an important tool for reducing bleeding and gingivitis, however more research is needed to prove its ability to remove bacterial biofilm. There has been a recent study from Sharma et al. (2012b) that suggests Waterpik is 80% more effective than Sonicare® Air Floss and significantly more ($p = 0.02$) effective than Sonicare® Air Floss Pro to reduce gingivitis and therefore improve gum health. According to another similar study from Sharma et al. (2012a), the use of the Waterpik removes significantly more plaque (74.9% vs. 57.5%) from tooth surfaces than the Sonicare Air Floss when used with a manual toothbrush. Though one must remember that industry sponsored studies may report more favourable outcomes.

In contrast, a meta-analysis from Salzer et al. (2015) showed that so far, oral irrigators have failed to show significant plaque reduction but seem to be effective in reducing gingivitis. This confirms previous findings of Husseini et al. (2008). Tawakoli et al. (2015) investigated biofilm removal efficacy of the oral irrigator (Waterpik Sensonic WP-100E) and the sonic toothbrush (Waterpik Sensonic SR-3000E). The oral irrigator showed a better performance than the sonic toothbrush in removing biofilm, however no toothpaste was used with the sonic toothbrush which would have possibly lead to more accurate results. A recent study from Stauff et al. (2018) showed that both dental floss and Airfloss jetting devices reduce gingivitis and plaque but jetting devices remove significantly more plaque ($p=0.003$) than dental floss. Altogether jetting devices offer a good alternative to dental flossing for patients.

First injury assessments

So far, there is very little evidence about the safety of jetting devices and studies of their impact on gingiva. For instance, Vogel et al. (2014) in their *in-vitro* study evaluated the interdental cleaning and gingival injury potential of the powered sonic interdental toothbrush Waterpik sensonic Professional SR 1000E (Water Pik, Inc., Fort Collins, USA) and two manual interdental brushes: Curaprox 1009 single tuft (Curaden AG, Kriens, Switzerland) and Lactona Double Single Tuft (Lactona Europe BV, Bergen op Zoom, Netherlands). Before and after brushing, the porcine gingiva was stained with Paro Plak 2-tone (ESRO AG, Thalwil, Switzerland) disclosing pellets. According to this study, it contains 10 parts erythrosine and three parts patent blue. Both stains are supposed to mark cells that have lost membrane integrity (Krause et al. 1984, Walker et al. 1984) and therefore reveal gingival injury. Evaluations were each made after 15, 30, 60, 120 seconds of brushing. The powered interdental toothbrush showed the highest injury potential. After 120 seconds of brushing, the median value of the injured area with the powered toothbrush was 54.4%. A significant difference between the brushes was noticed only between Waterpik powered interdental toothbrush and Lactona manual interdental brush at the brushing interval of 15 seconds ($p<0.05$). However, a powered interdental toothbrush has a different cleaning approach to interdental space than a jetting device (contact vs. non-contact) and is therefore hard to compare.

Most of the studies on jetting devices have been published by the research teams of two leading companies that produce jetting devices, Waterpik and Philips. For instance, Jolkovsky et al. (2016) summarised in their literature review of 50 years of research that using a water flosser 'is safe and effective'. Definitive conclusions are unclear as this assumption is based only on 'decades of use by the public and a body of evidence that has not reported any adverse effects'. However, in this review there is no evidence of detrimental effects on the attachment or junctional epithelium.

A recent study by Mwatha et al. (2017) reported that Philip Airfloss Pro provides similar reduction in gingivitis and plaque to string floss. In their view, all study regimens were safe on oral tissues. The investigation methods of safety remain unclear. It is important to mention that authors have rendered a 'conflict of interests' statement, as they are employees of Philips, the manufacturers of the Airfloss Pro that was tested in this study.

2.2 *Injury assessment methods in-vitro vs. in-vivo*

2.2.1 *In-vivo* trials

Most of the studies including safety as an outcome found in the literature from 1977 to 2011 have been clinical *in-vivo* trials on humans (Breitenmoser et al. 1979, Sandholm et al. 1982, Niemi et al. 1984, Danser et al. 1998, Warren et al. 2001, Carvalho et al. 2007, Zanatta et al. 2011, Zimmer et al. 2011). Few *in-vivo* studies on dogs (Anneroth et al. 1975 and Tomofuji et al 2007) and one study on hamster cheek pouch tissue (Alexander et al. 1977) have been done so far. Randomised clinical trial has been a ‘gold standard’ in dentistry to measure the effectiveness and safety of different treatments and oral hygiene products. The benefits of clinical trials are assessing one variable whilst controlling all other factors.

Another suitable study design is an observational cross-sectional study. The assessment is focused on the distribution of a certain condition among the population. Cross-sectional studies investigate on the relationship between an outcome – for instance, reduction of gingivitis and plaque due to tooth brushing and correlating risk factors - gingival abrasion and gingival recession (Mantokoudis et al. 2001, Rosema et al. 2014, Mwatha et al. 2017).

2.2.2. *In-vitro* trials

In comparison with *in-vivo* trials, very few *in-vitro* studies have been published so far where safety is a primary outcome. To the best of our knowledge, there has been only one *in-vitro* study on porcine gingiva that states safety as one of the primary outcomes. Vogel et al. (2014) in their *in-vitro* study evaluates the interdental cleaning and gingival injury potential of a powered sonic interdental toothbrush Waterpik sensonic Professional SR 1000E (Water Pik, Inc., Fort Collins, USA) and two manual interdental brushes: Curaprox 1009 single tuft (Curaden AG, Kriens, Switzerland) and Lactona Double Single Tuft (Lactona Europe BV, Bergen op Zoom, Netherlands). The brushes were tested in a similar manner to some of the previously mentioned *in-vivo* studies. The soft tissue injury potential was evaluated using front teeth and gingiva of pigs. Test brushes were mounted in a brushing device. Before and after each brushing cycle, pig gums were stained with a plaque disclosing agent to highlight injured areas. These were digitized and evaluated planimetrically later.

There are few aspects that must be mentioned in choosing the *in-vitro* study design. With regard to the gingival injury, ethical issues of using human gingiva as a substrate must be recognised. Additionally, to assess gingival injury, fresh pig cadaver jaws can be fixed firmly to provide standardised brushing. Moreover, they can be positioned in a way that is easy to stain before and after brushing and to take standardised photographs.

Unfortunately, there are no direct reference values from other *in-vitro* studies, with similar objectives and methodology, where safety is the primary outcome. However, the clinical relevance of the findings from *in-vitro* studies remains questionable, as the oral situation differs from the conditions in laboratory.

3. Material and Methods

This study was approved by the Ethics Committee of the Witten- Herdecke University. Ethical or data protection concerns were excluded and the application was accepted and authorised to proceed (s. Annex).

3.1 Study workflow

Ethical approval

↓

Sample access from slaughter house, 24 h *post mortem*

↓

Storage in refridgerator at 5°C

↓

Transfer to the laboratory

↓

Calibration of the investigator (ALB), concerning brushing time, movement, force and tooth areas

↓

Staining for pre-existing injuries

↓

Brushing test, strictly according to manufacturers recommendation (§3.3) and brushing protokol (Table 3)

↓

2. staining to reveal injuries

↓

Photo documentation

↓

Selection of histological biopsies

↓

Ex-vivo biopsies

↓

Histological procession

↓

Planimetrical assessment of ex-vivo clinical results

↓

Assessment of the histological results

↓

Selection of the appropriate statistics

3.2 Selection of pig jaws

3.2.1 Slaughtering time and test time

In order to achieve a homogeneous group of gingiva samples, testing time had to be no later than 48 h after slaughtering. Fresh pig jaws were collected from the butchery Jedowski Unna GmbH (Germany), 24 hours after slaughtering. Jaws were kept moist and at constant temperature of 5°C during transportation and testing. The jaws collected, were tested the same day and brought back to the slaughterhouse for appropriate disposal.

3.2.2 Selection process of the pig jaws

The pig jaws used for the study had the following underlying two conditions: the gingiva was visually intact and there was no visible tooth exfoliation (most of the pigs had mixed dentition at the test point).

Damaged jaws or damaged areas of jaws were sorted out. Only intact areas of gingiva were used for testing. Few incisor-regions were intact. Most of the testing was executed around premolar and molar areas.

3.2.3 Number of samples

To achieve statistically significant results, it was the aim to use at least six samples with each force and time combination of different toothbrushes. 90 gingiva samples were used altogether for the statistical evaluation (Table 1). The majority of the samples included premolar and molar regions. However, two intact incisor regions were used as well.

Table 1: Samples, their number (1-90), origin (jaw number), side of the oral cavity (L - lingual; B - buccal) and tooth region (P - premolars; M - molars; I - incisors).

Sample	Jaw	Side	Region		Sample	Jaw	Side	Region		Sample	Jaw	Side	Region
1	1	B	M		31	15	B	M		61	30	L	P
2	1	B	P		32	15	L	M		62	31	L	P
3	1	L	M		33	15	B	P		63	31	L	I
4	1	L	P		34	19	B	M		64	32	L	P
5	2	L	M		35	19	B	P		65	32	L	I
6	3	L	M		36	19	L	M		66	33	L	M
7	3	L	P		37	19	L	P		67	34	L	M
8	5	L	M		38	20	L	M		68	35	L	M
9	6	B	M		39	20	L	P		69	35	B	M
10	6	L	P		40	21	L	M		70	36	B	P
11	6	L	M		41	22	B	M		71	36	L	P
12	7	B	M		42	22	B	P		72	37	B	M
13	7	L	M		43	22	L	P		73	37	B	P
14	7	L	P		44	22	L	M		74	37	L	M
15	8	B	P		45	23	L	P		75	38	L	M
16	8	L	P		46	24	L	M		76	38	L	P
17	9	L	M		47	24	L	P		77	39	B	P
18	10	L	M		48	25	L	P		78	39	L	P
19	10	L	P		49	25	L	M		79	40	B	M
20	11	B	M		50	26	L	P		80	40	L	P
21	11	L	M		51	26	L	M		81	40	L	M
22	11	L	P		52	27	B	M		82	41	L	P
23	12	B	M		53	27	L	M		83	41	B	M
24	12	L	M		54	28	B	P		84	41	B	P
25	12	L	P		55	28	L	P		85	42	B	M
26	13	L	M		56	29	B	P		86	42	L	M
27	13	L	P		57	29	L	P		87	43	L	P
28	14	B	M		58	30	B	M		88	43	L	M
29	14	B	P		59	30	B	P		89	43	B	M
30	14	L	M		60	30	L	M		90	44	L	P

3.3 Study devices, brushing movement and regions

Figure 1: Tested brushes (from left to right): ORMED manual brush, Oral B 6500 powered toothbrush, Philips Sonicare Diamond Clean powered toothbrush, Waterpik WP 560 jetting device, Philips AirFloss Pro jetting device.

3.3.1 Manual toothbrush Ormed

Ormed manual toothbrush is a simple manual toothbrush with a plastic handle, soft nylon bristles and a flat trim. Brushing was performed in circular mode using the modified Bass method. The toothbrush was held at a 45 degree angle to gingival margin and gently moved forward with small, oval circular strokes brushing the buccal and lingual regions of incisors, premolars and molars. The brushing movement included the tooth surface and the gingival areas next to the gumline and interdentally. The hidden gingival col was not reached because of the anatomical position.

3.3.2 Oral B 6500 powered toothbrush

An Oral B 6500 powered brush (P&G, US) is a hand held, battery operated, rechargeable device, that has a plastic handle and a removable brush head. It has three basic movements: it oscillates, rotates, and pulsates. It has 6 brushing modes: Daily Clean, Deep Clean, Gum Care, Sensitive, Whitening and Tongue Cleaning. The device was used in a 'daily clean' mode together with a 'sensitive' brush head (P&G, US). 'Daily clean' mode works with 45,000 pulsations and 9,900 oscillations per minute and is a standard mode. The sensitive brush head has soft, ultrathin bristles that are gentle on gingiva, combined with regular bristles to remove plaque. The build-in pressure sensor lights up if one brushes too hard to prevent gingival damage. A timer helps to brush for recommended two minutes. The investigator (ALB) followed the manufacturer's instructions. The brush was held at a 45-degree angle towards gingival margin, then turned on and moved from tooth to tooth brushing the buccal and lingual regions of incisors, premolars and molars. The brushing movement included the tooth surface and the gingival areas next to the gumline and interdentally. The hidden gingival col was not reached because of the anatomical position.

3.3.3 Philips Sonicare Diamond Clean powered toothbrush

Philips Sonicare Diamond Clean powered brush (Philips, Germany) is a battery operated powered toothbrush with a plastic handle and removable brush. The brush head of a Philips Sonicare toothbrush sweeps (side to side action) at a frequency of 31,000 brush strokes per minute. It has features that prompt the dental professional-recommended two-minute period. It was used in the standard 'Clean' Mode together with a Sonicare Diamond Clean Standard sonic toothbrush head. (Philips, Germany). The brush head has densely-packed diamond-shaped bristles, that are supposed to remove plaque more effectively. The investigator (ALB) followed the manufacturer's instructions. The brush head was placed at a 45-degree angle towards gingival margin, then turned on and glided across the gingival margin from tooth to tooth brushing the buccal and lingual regions of premolars and molars. The brushing movement included the tooth surface and the gingival areas next to the gumline and interdentally. The hidden gingival col was not reached because of the anatomical position.

3.3.4 Waterpik WP560 High Pressure mode jetting device

Waterpik W560 (WF; Water Pik, Inc., Fort Collins, USA) is a powered jetting device that has a water reservoir, pressure control button and a removable interdental tip. It delivers a pulsating stream of water at 100 PSI through the tip. The device was used in a 'high pressure' mode. The investigator (ALB) followed the manufacturer's instructions and

directed the tip at the interdental area, turned on, held it for manufacturer's recommended three seconds and then moved forward to the next interdental space following the pattern of gingival margin from tooth to tooth on buccal and lingual regions of premolars and molars.
The cleaned area included the gingival areas next to the gumline and interdentally. The hidden gingival col was not reached because of the anatomical position.

3.3.5 Philips AirFloss Pro jetting device

Sonicare® Air Floss (AF; Philips Healthcare, Bothell, USA) is a hand-held rechargeable device that utilises air under pressure through interdental tip to deliver micro droplets of water and air to the interdental area. The small reservoir holds approximately two teaspoons of water. The pressure of the water was not disclosed by manufacturer and is therefore unknown.
The investigator (ALB) filled the reservoir to capacity with lukewarm water and followed manufacturer's instructions, placing the tip at interdental spaces, activating the device by pushing the activation button. The activation time is set by manufacturer and lasts five seconds per interdental space. Same was done at each interdental space, on buccal and lingual regions of premolars and molars. The cleaned area included the gingival areas next to the gumline and interdentally. The hidden gingival col was not reached because of the anatomical position.

3.4 Evaluation principles

3.4.1 Staining of the gingiva

Paro Plak 2-tone Disclosing Tablets (ESRO AG, Thalwil, Switzerland) were used in this study to stain gingiva. The Paro Plak disclosing tablet is a lactose-based coated tablet, containing Patent blue and Phloxin B dye making bacterial plaque visible.
Phloxin B, commonly known as Phloxin, is a water soluble red dye used in cosmetics, medicine and food. For example, it is used in hematoxylin-phloxine-saffron (HPS) staining to colour the cytoplasm and connective tissue in shades of red. Used as a plaque discloser, it stains immature plaque red, mature purple and acidic plaque blue. Patent blue is a dark blue synthetic tri-phenyl methane dye, mainly used as food colouring and contains E Number E131. The colour of the dye depends on the pH. As an oral hygiene tool it would indicate new plaque as red and older plaque as blue.

In the present study, gingiva was stained once before brushing- to reveal pre-existing injures which may have occurred during feeding and slaughtering; and once again after brushing- to reveal injuries that resulted from trauma of the epithelium during brushing (Figure 2, 3 and 4). For this purpose, plaque disclosing tablets Paro Plak 2-tone were used. The exact composition according to manufacturer can be seen in Table 2.

Table 2: Qualitative Composition (according to manufacturer).

No.	Trade names	INCI EC	EINECS No.	CAS No.
1.	Microcrystaline Cellulose	Cellulose	-	-
2.	Patent blue E131 (C.I. 42090)	C.I. 42090	-	-
3.	Phloxin B (C.I. 45410)	C.I. 45410	-	-
4.	Flavor Rasperry 84293-31	Flavor	-	-
5.	Lactose Monohydrate	Lactose	200-559-2	64044-51-5
6.	Xylitol	Xylitol	201-788-0	87-99-0
7.	Talcum	Talcum (E553b)	238-877-9, 215-285-9	14807-96-9, 1318-59-8
8.	Magnesium stearate	Magnesium stearate	292-967-2	91031-63-9, C16-18-

As one tablet is supposed to be used in one human oral cavity, one tablet was also used to stain 4 halves of pig jaw. Tablets were smashed and mixed with a few drops of water and then carefully applied with a silicone brush with rounded bristle ends to avoid injuries. Afterwards, it was rinsed with tap water.

Figure 2: Pig jaw (test sample) before staining with Paro Plak 2-tone.

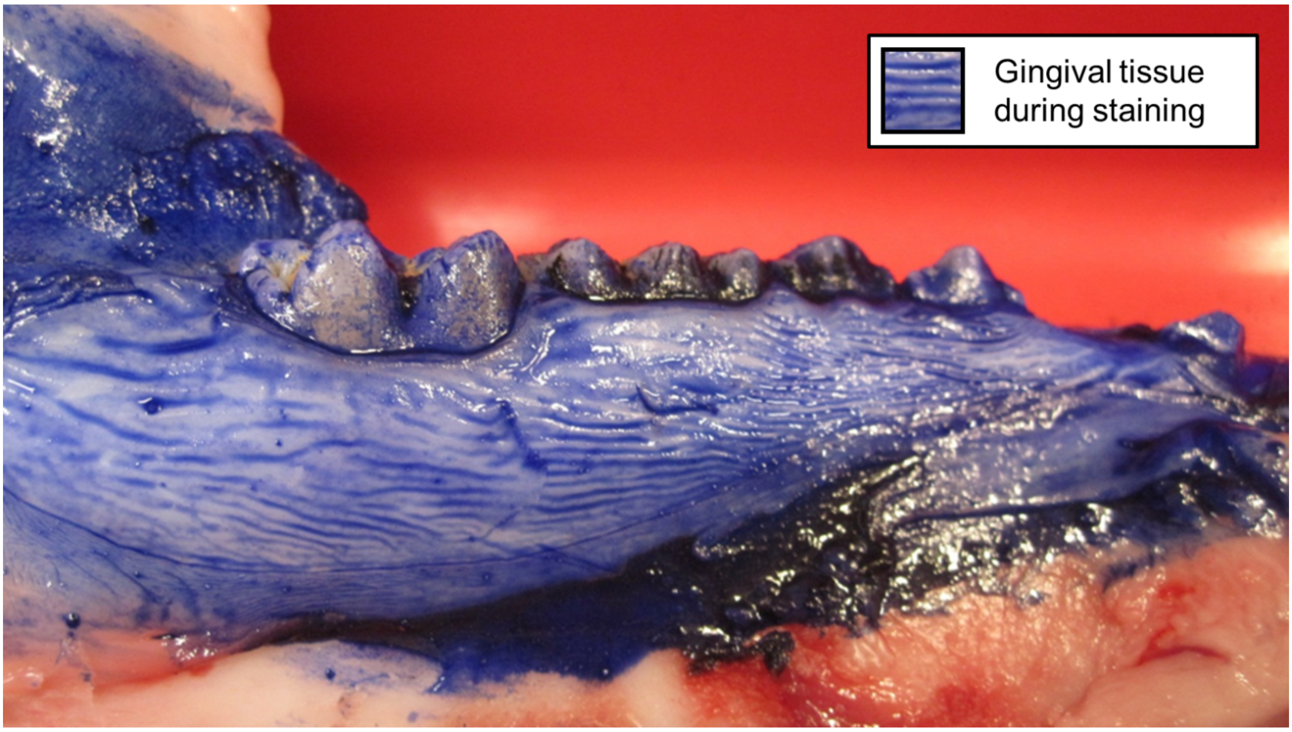

Figure 3: Pig jaw (test sample, same jaw from Fig. 2) during the staining with Paro Plak 2-tone.

Figure 4: Pig jaw (test sample, same jaw from Fig. 2 and 3) after rinsing with tap water. Injury revelation: visualization of injured gingival areas; deep transepithelial injuries stained bluish.

3.4.2 Brushing force, time and regions

The brushing force for manual and powered toothbrushes was calibrated with the tested brush for one minute before each run. Postage scales (Soehnle, Germany) were used for this purpose and the brush was moved over the scales applying constant pressure until the right amount of force was achieved and then continued for one minute. The brushing force was 2.0 N (approx. 200g on the scales), 3.0 N (approx. 300g on the scales), 3.5 N (approx. 350g on the scales) and 5.0 N (approx. 500g on the scales) to simulate different clinical situations . Both jetting devices were used at the manufacturers set pressure. Waterpik was used at 100 PSI (pound-force per square inch). PSI is an imperial and US unit which is equivalent to 6.9 bar (metric unit of pressure). Airfloss Pro was used at 'x' PSI, as the manufacturer did not disclose the pressure of Airfloss Pro application.

The brushing time was chosen 10, 20 or 30 seconds with manual and two powered toothbrushes, to observe possible changes in injuries by increasing the brushing time. Both jetting devices were used as suggested by manufacturer. Air Floss was applied for 5 seconds per interdental space, Waterpik was applied for 3 seconds per interdental space.

Finally, different pressure and time combinations were used to achieve results not only on gingival damage but also to assess the influence of time and pressure of brushing to simulate clinically different brushing manners. In the table below (Table 3), an exact brushing protocol is presented.

Table 3: Brushing protocol.

Device (name)	Brushing force (N or PSI with jetting devices)	Time (sec)	Number of samples per pressure/time combination (n)	Number of samples per toothbrush (n)
Ormed	3	10	7	21
	3	20	8	
	5	30	6	
Oral B	2	10	6	20
	2	20	7	
	3,5	30	6	
Oral B	5	30	1	
Sonicare	2	10	6	23
	2	20	6	
	5	30	8	
Sonicare	2	30	3	
Waterpik	100 (PSI)	3	11	11
AirFlossPro	X (unknown) (PSI)	5	13	13

All devices, manual and powered toothbrushes, as well as both jetting devices were used on buccal and lingual regions of premolars, molars and incisors. The brushing movement included the tooth surface and the gingival areas next to the gumline and interdentally. The hidden gingival col was not reached because of the anatomical position.

Samples were kept moist using distilled water and a spray bottle as a premature dehydration of the samples could anticipate falsified damage. Dentifrice was deliberately not used during the brushing, as the abrasives in the dentifrice could generate falsified data.

3.4.3 Photographic documentation

Each jaw was digitised before staining to record the jaw number and arrange pictures. Another two photographs were taken: after first staining (pre-brushing) and after second staining (post-brushing). A digital camera Canon DS126211 on a tripod and natural daylight were used to take photos.

3.4.4 Evaluation with Photoshop

Photoshop Version C6 (Adobe Inc., San Jose, US) was used to evaluate Photos.
At first, the brushed area was marked as seen in Figure 5 using following criteria:

- Left-side border: vertical line from gingival margin of beginning of premolars/molars (interdental space) to mucogingival line
- Lower border: mucogingival line
- Right-side border: vertical line from gingival margin of the ending of premolars/molars (interdental space) to mucogingival line
- Upper border: along gingival margin of premolar/molar

The size of the brushed area was then expressed in pixel amount (px) by the programme.

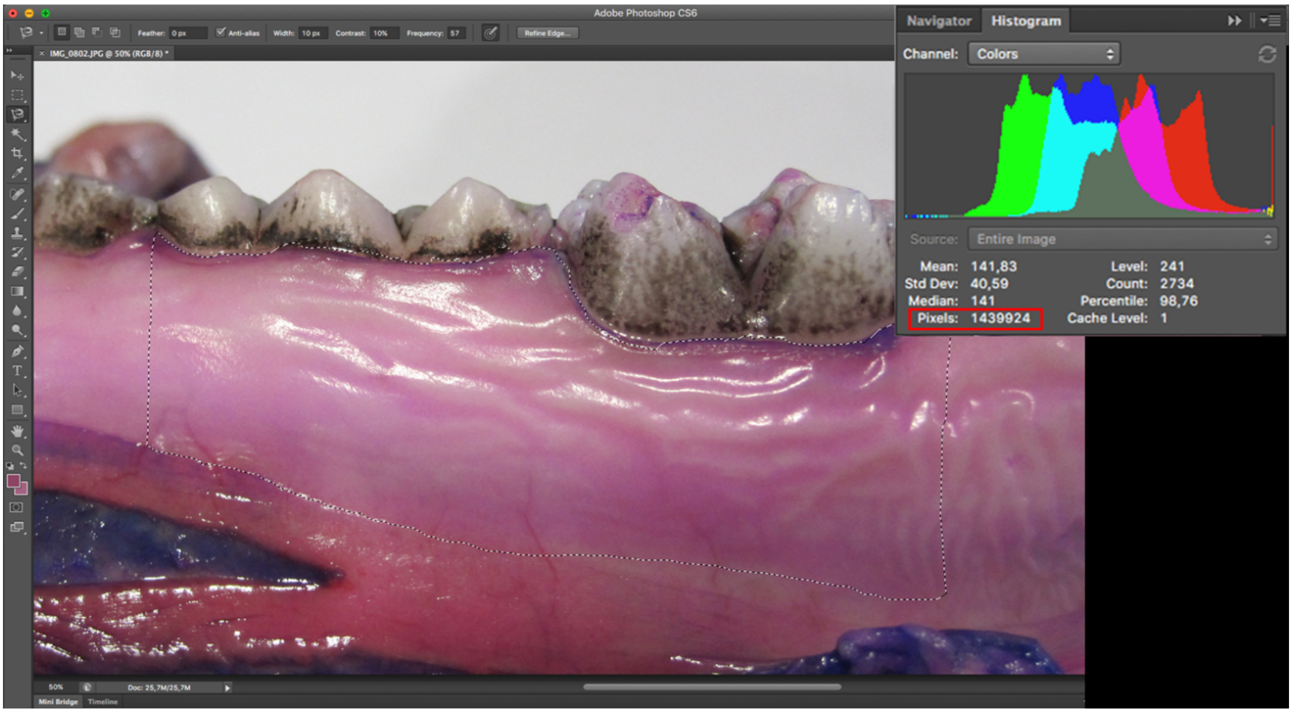

Figure 5: Brushed area marked with broken black and white line and measured in pixel (px) by programme, jaw 7L, after brushed with Oral B powered toothbrush for 30 seconds, force 2 N (Source: screenshot of computer iMac; 21.5-inch; Serial No. C17KC5XXDNCR; Software Mac OS 10.14.5).

Second, the injured area was marked by Photoshop's tool 'colour range' (Figure 6). This tool is based on the programme's ability to recognise a hand-picked colour on the picture and mark it elsewhere on the same picture. Stained gingival areas were picked visually by ALB and the program marked areas of similar colours elsewhere inside the borders of the brushed area defined previously. Again, the size of this area was expressed in pixels by the programme and named later in collected data 'by colour range'.

The colour range of the stained gingiva appeared to be very wide hence a lot of different shades were recognised by the programme's tool 'colour range', from light pink to dark blue-purple. According to the developer, the tool can recognise up to 40 tints and shades of each hue. As all of the shades needed to be included in injured area, a second approach was developed. The injured area was detected visually directly from photographs and marked as an area manually by **ALB** (Figure 7). The size of this area was expressed in pixels by the programme and named **'by hand'**.

At this point there was a decision made to use only results from the second measurements for the statistical evaluation, hence ‘by hand’. Following aspects led to this decision. The photo evaluation showed clearly that healthy pig gingiva can have wide colour range as well, which could be possibly recognized as injury, when appears slightly darker. Additionally, with the first method, the colour of injured gingiva had to be visually recognised and hand-picked as well, so it was a logical decision to choose a visual examination of the photos by a trained professional (ALB) and use ‘by hand’ measurements for the statistical evaluation. Finally, all measurements were expressed as absolute values to extract the percentage of injured areas of each sample (calculated using Microsoft Excel).

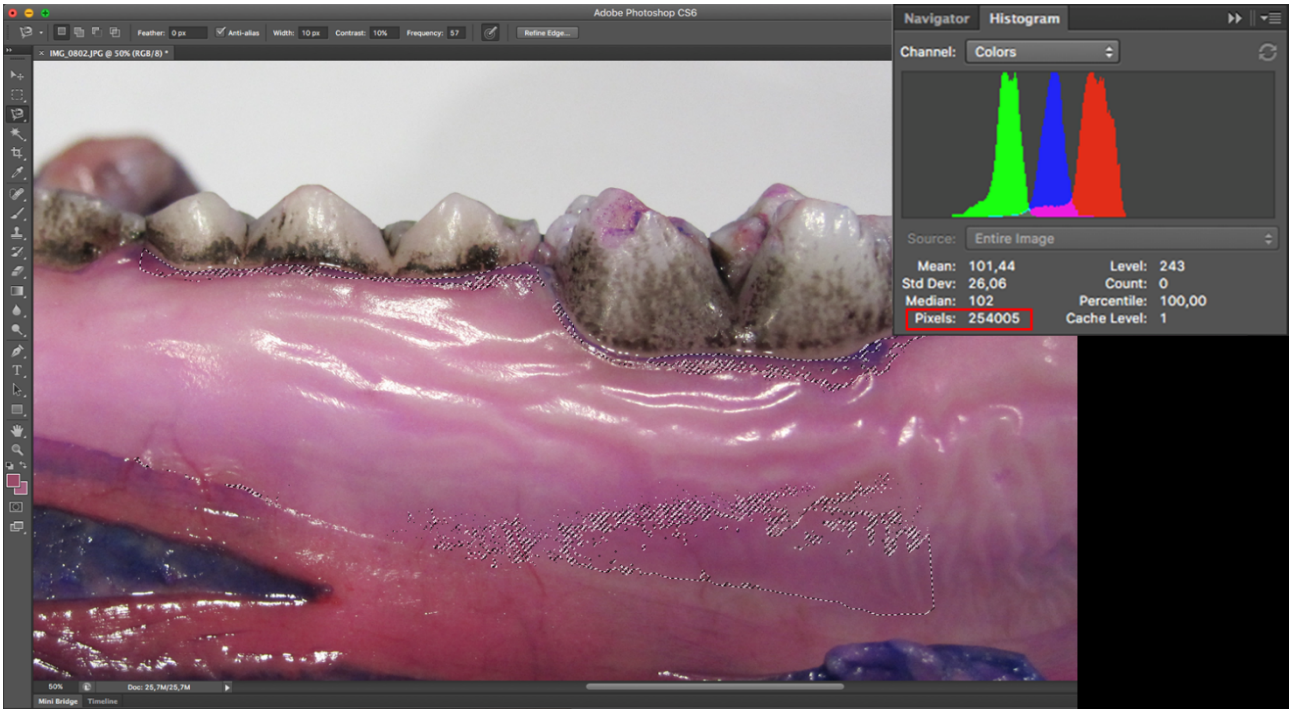

Figure 6: Injured area by ‘colour range’ marked with broken black and white line and measured in pixel (px) by program, same jaw as in Fig. 5 after brushed with Oral B powered toothbrush for 30 seconds, force 2 N (Source: screenshot of computer iMac; 21.5-inch; Serial No. C17KC5XXDNCR; Software Mac OS 10.14.5).

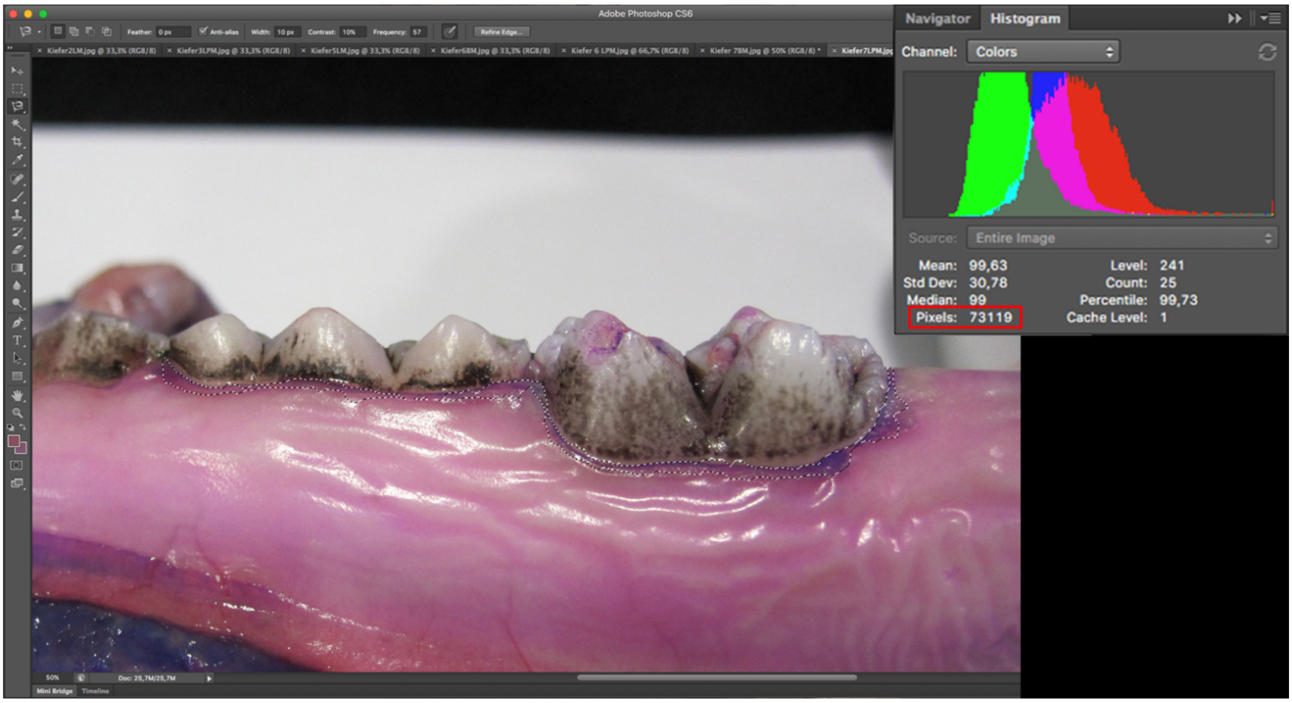

Figure 7: Injured area 'by hand' marked with broken black and white line and measured in pixel (px) by program, same jaw as in Fig. 5 and 6 after brushed with Oral B powered toothbrush for 30 seconds, force 2 N (Source: screenshot of computer iMac; 21.5-inch; Serial No. C17KC5XXDNCR; Software Mac OS 10.14.5).

3.4.5 Histological evaluation

Only a small time frame was available to perform all steps of this study with the pig jaws before the histological examination. After just few hours, cells in the epithelium and connective tissue start to decompose, so that histological outcome can easily be falsified through the process of autolysis. Therefore, one sample per region and per toothbrush was surgically removed directly after digitising the outcome (Figure 8). It was immediately placed in labelled embedding cassettes in 3.7% formaldehyde solution to preserve tissue.

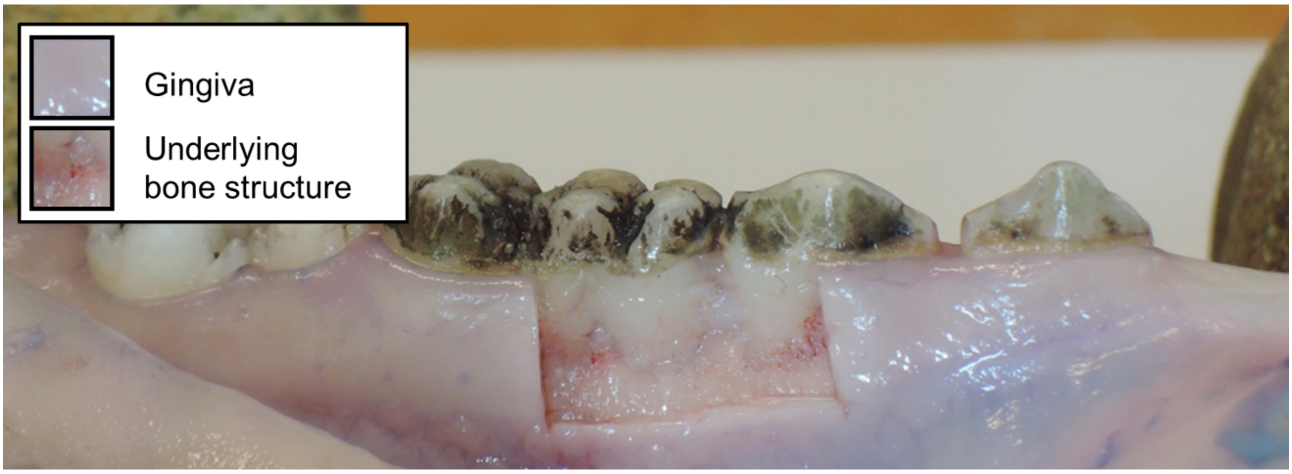

Figure 8: Incision site of molar/premolar lingual region with surgically removed gingiva and exposed underlying bone structure (Jaw 29 MPL).

Eighteen samples were selected out of total 90 samples and were prepared at the pathological practice of Dr. med. Renate Weskamp (Unna, Germany). The sample choice was based on the best suitability for the histologic procedures (incision site, thickness of the tissue etc.). However, every device was represented at least twice. Every sample had a code (according to Table 1) to blind the affiliation of the sample to a certain device. To assess the architecture and detect the pathological changes in the tissue, haematoxylin and eosin (HE) staining was applied. HE staining is a routine procedure in histology. Haematoxylin stains cell nuclei and the parts of the cytoplasm that are rich in rough endoplasmic reticulum a blue-violet. Eosin stains the other parts of cytoplasm and some fibrous extracellular structures red (Sobotta et al. 2006). A microbiological evaluation was carried out in collaboration and consultation between the clinical operator (ALB) and a trained professional pathologist Dr. Renate Weskamp (Unna, Germany). The most meaningful samples were digitised using microscope camera. Below, digitized histological samples of a normal structure of porcine gingiva and different types of injury found are shown (Figure 9, 10 and 11). Histological outcome was later compared to the results of the planimetrical assessment. However histological results were not included in the statistics.

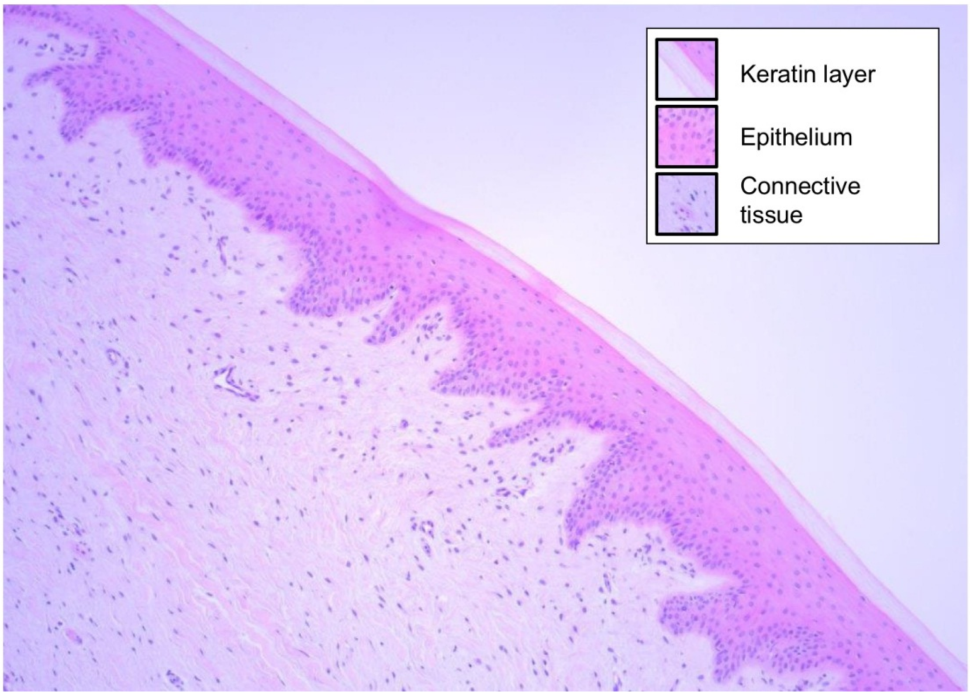

Figure 9: Normal structure of porcine gingiva after brushing with a manual toothbrush for 20 seconds, force 3.0 N; jaw 1 ML; original magnification x100; step section; 2 µm, HE Staining.

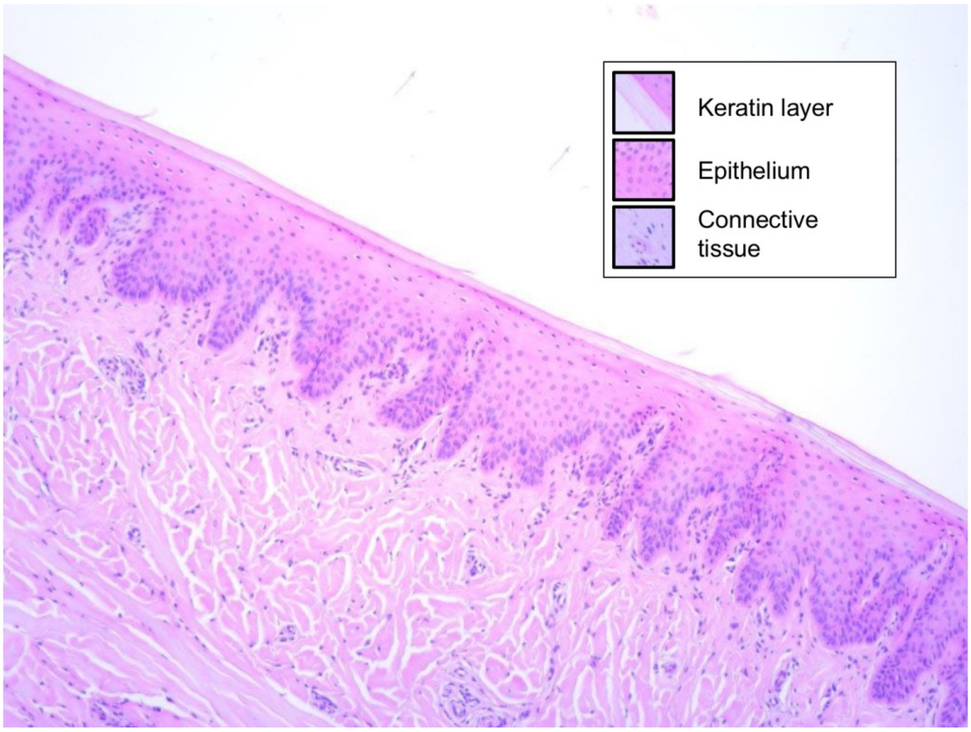

Figure 10: Reduction of keratin layer after brushing with a manual toothbrush for 20 seconds, force 3.0 N; jaw 32 iL, ; original magnification x100; step section; 2 µm, HE Staining.

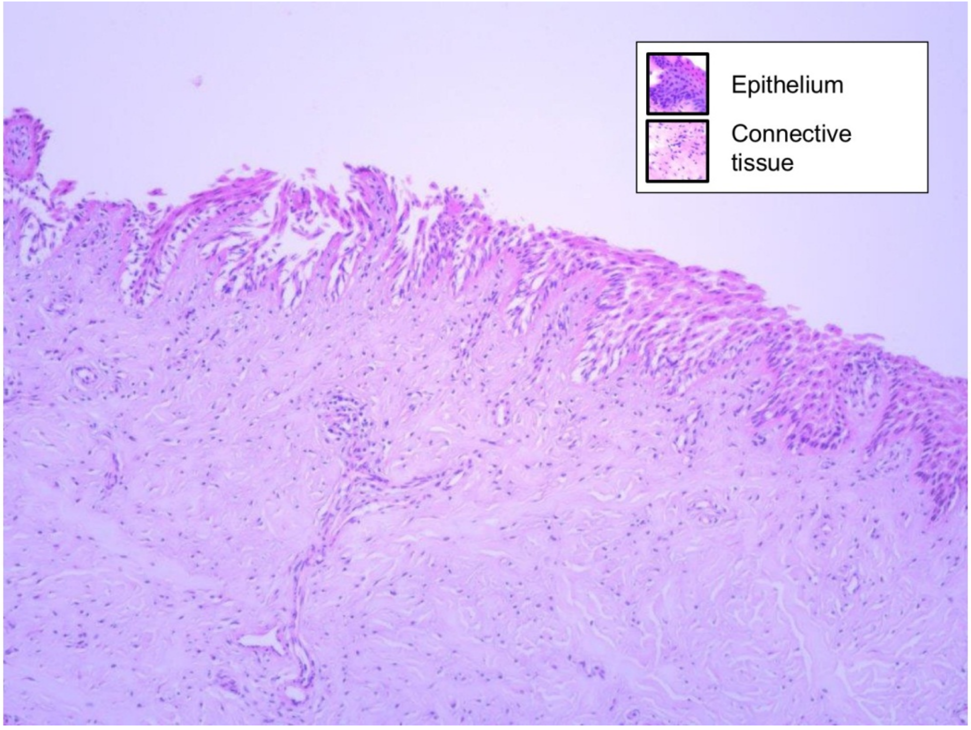

Figure 11: Transepithelial injury with partial loss of epithelium after Waterpik application; jaw 44 PL; original magnification x200; step section; 2 µm, HE Staining.

3.5 Statistical evaluation

The statistical evaluation was carried out in collaboration with Dr. Karl W. Weich (ORMED, Germany). Most of the studies, including manual and powered toothbrush safety as a primary outcome, use Wilcoxon-Mann-Whitney test to perform the statistical evaluation (Anneroth et al. 1975, Breitenmoser et al. 1979, Danser et al. 1998, Carvalho et al. 2007, Zanatta et al. 2011). This statistical method has proved to be the most suitable to compare safety or injury potential between different toothbrushes.

3.5.1. Test setup

Objectives

Manual and powered toothbrushes have been often tested for efficiency to reduce plaque and therefore contribute towards dental hygiene. However, there is very little evidence of the potential of powered toothbrush to induce gum abrasions. Moreover, less is known concerning abrasion risk of high pressure jetting devices. It was, therefore, the aim (i) to standardise the in-vitro pig gum test and (ii) to test the gingival injury potential of manual toothbrush, two different powered toothbrushes and two jetting devices.

Target variable / Criterion

Percentage of injured area ('by hand').
The analysis was carried out using the applied statistical software package IBM SPSS Statistics Premium, release 24.0, 64-Bit-Version (IBM, Armonk, USA).

3.5.2. Statistical rationale

The present paragraph describes the statistical analysis carried out followed by a short justification of the methods applied. The Kolmogorov-Smirnov-test (KS-test; one sample test) was used to test the target variable, percentage of injured area ('by hand') – for a normal distribution (N=90). A significance level of $\alpha = 0.10$ (10%) was applied to test this assumption of a parametric t-test. Expected and desired result of the KS-test was to accept the H_0 and to keep it. The results indicated that the null hypothesis (H_0) of normality was clearly rejected respectively not accepted for the parameter of injured area (%) and consequently that in this study the null hypothesis is equivalent to the working hypothesis/alternative hypothesis.

Therefore, in a next step, the target variable was analysed by the non-parametric Wilcoxon-Mann-Whitney-U-test (WMW-test or U-test). The WMW-test can be applied on data characterised by ordinal or also unknown distributions – contrary to the t-test – while being nearly as efficient as the parametric t-test in terms of power efficiency (pe) (WMW-test: 95% > pe > 90%: t-test). To illustrate the distribution of injured area values (%) for the 11 tested objects, a box plot diagram is provided (Figure 12).

For all two-tailed tests investigating differences in the injured area between the objects, the significance level was set at the basic p-value of $\alpha=0.05$ (5%) (‚significant') (*). Also higher significance values are reported, following the standard significance level classification: $\alpha=0.01$ (1%) (‚very significant') (**) and $\alpha=0.001$ (1‰) (‚highly significant') (***). All the statistical analyses of the differences between the objects/combinations, with regard to the target variable, have kept the basic p-value of $\alpha = 0.05$ (5%) (10% alternatively in terms of exploratory research). However, also higher significance values (1% and 1%) are mentioned, following common practice in most medical studies.

This study used an exploratory design to compare the most different parameters of oral hygiene devices (manual and two powered toothbrushes and two jetting devices). Therefore, the adjustment of most important results did not follow stringent, specific hypothesis. Because of the non-orthogonal contrasts, the Bonferroni correction is not possible.

4. Results

4.1. Gingival injury

All collected data was noted, gathered and calculated using Microsoft Excel 97-2004 Workbook by ALB. There were injury findings in every sample. The gingiva was injured most markedly by Oral B powered toothbrush after brushing for 10 seconds with 2 N force. The least damage was produced by the Sonicare powered toothbrush after brushing for 10 seconds with 2 N force (median values 13.14% and 3.06 % of injured area per brushed surface). In the following table, mean and median values are presented as well as standard deviations and interquartile range (IQR). IQR is the difference between the values of the third and the first quartile of a variable distribution.

Table 4: Findings regarding gingival injury.

Means (M), standard deviations (SD), medians (Med) and interquartile ranges (IQR) of injured area 'by hand' (%) per brushed surface for all objects/devices. Number (n) of observations for manual and powered toothbrushes = 6 - 8 samples. Waterpik: n=11, AirFloss: n=13 samples

Object/Device	Percentage injured area			
	M	SD	Med	IQR
ORMED 3.0 N 10 sec	8.18	4.81	7.88	7.84
ORMED 3.0 N 20 sec	10.99	11.10	4.68	18.86
ORMED 5.0 N 30 sec	11.72	9.04	9.66	15.27
Oral-B 2.0 N 10 sec	14.89	8.48	13.14	17.06
Oral-B 2.0 N 20 sec	7.43	7.43	3.38	12.02
Oral-B 3.5 N 30 sec	8.56	5.28	8.02	10.48
Sonicare 2.0 N 10 sec	5.22	4.00	3.06	7.01
Sonicare 2.0 N 20 sec	8.65	4.50	8.03	4.49
Sonicare 5.0 N 30 sec	21.80	27.46	8.97	22.26
Waterpik 100 PSI 3 sec	5.88	3.84	5.02	4.26
AirFloss X PSI 5 sec	9.22	6.55	9.66	7.95

The following 15 comparison pairs were selected for the Wilcoxon-Mann-Whitney-Test (Table 5).

Table 5: A-priori-test-contrasts of objects / combinations of non-orthogonal contrasts.

Oral-B 2.0 N 10 sec	vs.	Oral-B 2.0 N 20 sec
Oral-B 2.0 N 10 sec	vs.	Oral-B 3.5 N 30 sec
Oral-B 2.0 N 20 sec	vs.	Oral-B 3.5 N 30 sec
ORMED 3.0 N 10 sec	vs.	ORMED 3.0 N 20 sec
Oral-B 3.5 N 30 sec	vs.	ORMED 5.0 N 30 sec
Oral-B 2.0 N 10 sec	vs.	Sonicare 2.0 N 10 sec
Oral-B 2.0 N 20 sec	vs.	Sonicare 2.0 N 10 sec
ORMED 5.0 N 30 sec	vs.	Sonicare 5.0 N 30 sec
Waterpik 100 PSI 3 sec	vs.	AirFloss X PSI 5 sec
Oral-B 3.5 N 30 sec	vs.	Waterpik 100 PSI 3 sec
Oral-B 3.5 N 30 sec	vs.	AirFloss X PSI 5 sec
Sonicare 5.0 N 30 sec	vs.	Waterpik 100 PSI 3 sec
Sonicare 5.0 N 30 sec	vs.	AirFloss X PSI 5 sec
ORMED 5.0 N 30 sec	vs.	Waterpik 100 PSI 3 sec
ORMED 5.0 N 30 sec	vs.	AirFloss X PSI 5 sec

It was the aim of the statistical analysis to compare as many different force and time parameters of the devices as possible, because of initially measured similarities and differences in the injuries. Therefore, non-orthogonal contrasts of objects/combinations were used.

4.1.1. Comparisons between test objects, descriptive statistics

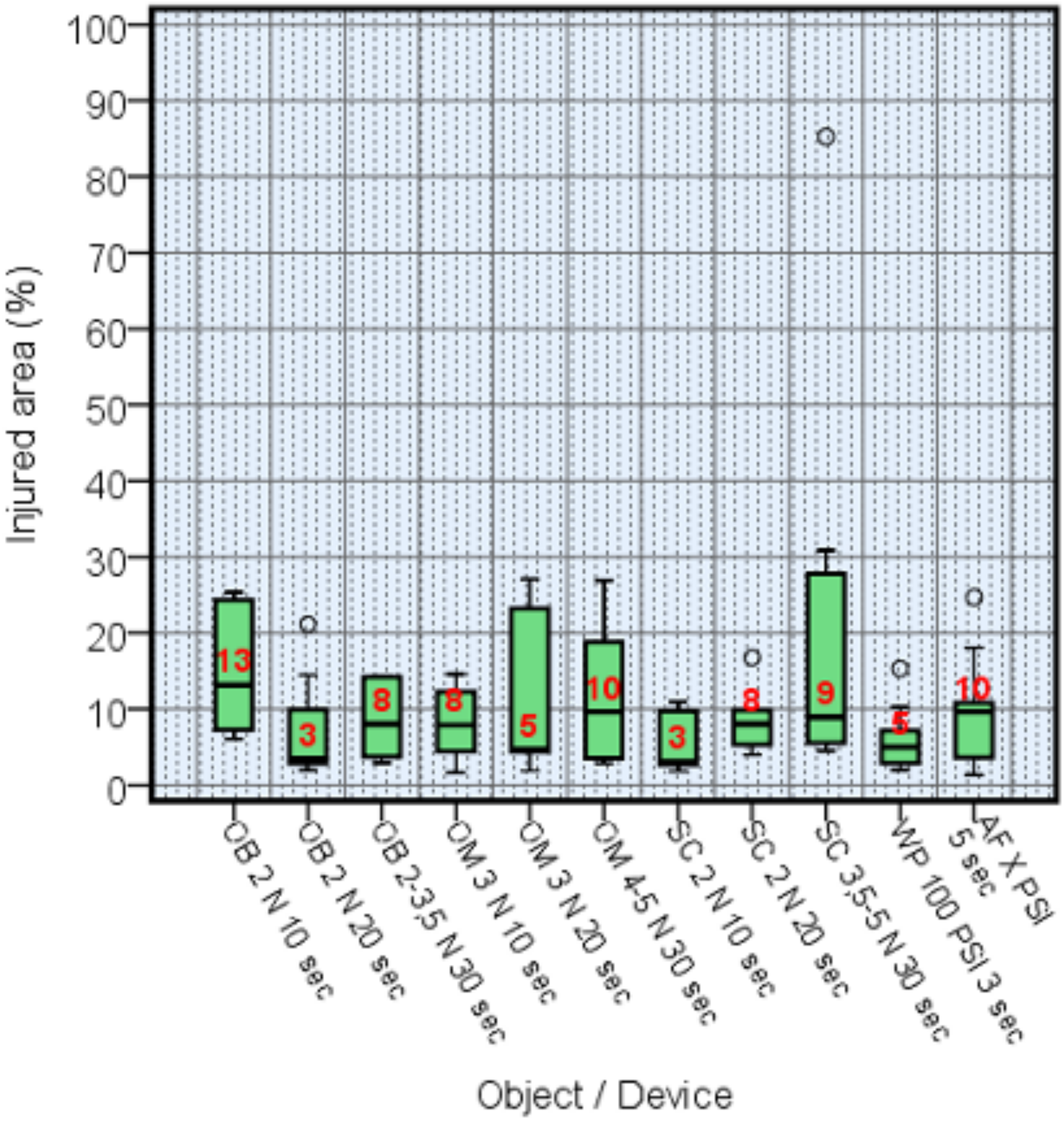

Figure 12: Box Plots of injured area (%) for all test objects.

Abbreviations: OM = ORMED; OB = Oral-B; SC = Sonicare; WP = Waterpik; AF = AirFloss

Number of observations: 6 - 8 samples (Waterpik: n=11, AirFloss: n=13 samples)

Explanation: The graphic above shows box plots of all devices with the corresponding force (N) and time (sec) setting underneath. The median is drawn as a line through the centre of the box (median values see Table 4). The box represents the middle 50% of the data values (= interquartile range). It is connected at both sides with the last data point within the 1,5*interquartile range from the first resp. third quartile. Data points outside are defined as outliers (○) (outside the 1.5*interquartile range) or extreme values (*) (outside the 3*interquartile range). One extreme value was excluded from the database.

4.1.2. Results of Wilcoxon-Mann-Whitney-Test

The results from the Wilcoxon-Mann-Whitney-Test are gathered in three following tables. They represent analysis of all data (Table 6), analysis of high score (Table 7) and residual analysis (Table 8). Significant results between the comparison pairs are marked red.

Table 6: Wilcoxon-Mann-Whitney-Test: Multiple contrasts of objects/devices. Analysis of all scores/data.

n of observations = 6 - 8 samples (Waterpik: n=11, AirFloss: n=13 samples); U = Test statistic of Mann-Whitney-Test; red marked cells = significant results; p = Significance value; ** = very significant (p ≤ 0.01)

Object/Device	Statistic	Waterpik 100 PSI 3 sec	AirFloss X PSI 5 sec
ORMED 3.0 N 10 sec	U	36.0	50.0
	p	0.355	0.855
ORMED 3.0 N 20 sec	U	31.0	37.0
	p	0.640	0.861
ORMED 5.0 N 30 sec	U	29.0	45.0
	P	0.143	0.612
Oral-B 2.0 N 10 sec	U	9.0**	23.0
	p	0.010	0.161
Oral-B 2.0 N 20 sec	U	38.0	32.0
	p	0.735	0.285
Oral-B 3.5 N 30 sec	U	26.0	36.0
	p	0.349	0.792
Sonicare 2.0 N 10 sec	U	31.0	21.0
	p	0.640	0.114
Sonicare 2.0 N 20 sec	U	19.0	39.0
	p	0.111	1.000
Sonicare 5.0 N 30 sec	U	21.0*	36.0
	p	0.037	0.247
Waterpik 100 PSI 3 sec	U		53.0
	p		0.174

Results:

The working hypothesis of unequal means of the tested objects respectively combinations of device/pressure/moving time can be accepted for 2 of 15 comparisons:

Oral-B 2.0 N 10 sec vs. Waterpik 100 PSI 3 sec. (p=0.010)
Sonicare 5.0 N 30 sec vs. Waterpik 100 PSI 3 sec. (p=0.037)

In terms of descriptive statistics, ‚Oral-B 2.0 N 10 sec‘ and ‘Sonicare 5.0 N 30 sec’ score substantially higher and have therefore higher injury potential than ‘Waterpik 100 PSI 3 sec’ in the target variable, injured area (%). There was no significant difference between other 13 device/pressure/time combinations. These numbers provide no indication of any difference between the same devices and their injury potential when pressure or time is increased. Increased brushing pressure and time seems to have no influence on the injury potential of Oral B powered toothbrush and ORMED manual toothbrush. There is also no difference in injury potential between several other device combinations.

Table 7: Wilcoxon-Mann-Whitney-Test: Multiple contrasts of objects/devices. Analysis of high score (50. Percentile – 100. Percentile).

n of observations = 3 - 4 samples (Waterpik: n=6, AirFloss: n=6 samples); U = Test statistic of Mann-Whitney-Test; red marked cells = significant results; p = Significance value; * = significant ($p \leq 0.05$)

Object/Device	Statistic	Waterpik 100 PSI 3 sec	AirFloss X PSI 5 sec
ORMED 3.0 N 10 sec	U	5.0	11.0
	p	0.136	0.831
ORMED 3.0 N 20 sec	U	6.0	7.0
	p	0.439	0.606
ORMED 5.0 N 30 sec	U	1.0*	4.0
	P	0.019	0.088
Oral-B 2.0 N 10 sec	U	0.0*	3.0
	p	0.020	0.121
Oral-B 2.0 N 20 sec	U	7.0	9.0
	p	0,606	1.000
Oral-B 3.5 N 30 sec	U	3.0	7.0
	p	0.121	0.606
Sonicare 2.0 N 10 sec	U	9.0	3.0
	p	0.100	0.121
Sonicare 2.0 N 20 sec	U	4.0	5.0
	p	0.197	0.302
Sonicare 5.0 N 30 sec	U	1.0*	4.0
	p	0.019	0.088
Waterpik 100 PSI 3 sec	U		6.0
	p		0.055

Results:

The ‘high score analysis’ presents the data of injured area per device that are over the median and therefore more ‘risky’. Furthermore, it presents the only dispersion of the data that is resistant against outliers. The working hypothesis of unequal means of the tested objects resp. combinations of device/pressure/moving time can be accepted for 3 of 15 comparisons:

Oral-B 2.0 N 10 sec	vs.	Waterpik 100 PSI 3 sec ($p=0.020$)
ORMED 5.0 N 30 sec	vs.	Waterpik 100 PSI 3 sec. ($p=0.019$)
Sonicare 5.0 N 30 sec	vs.	Waterpik 100 PSI 3 sec. ($p=0.019$)

In terms of descriptive statistics ‚Oral-B 2.0 N 10 sec‘, ORMED 5.0 N 30 sec‘ and ‘Sonicare 5.0 N 30 sec’ score substantially higher than ‚Waterpik 100 PSI 3 sec‘ in the target variable ‚injured area (%)’ and have therefore higher potential to cause gingival injury. There was no significant difference between other 12 device/pressure/time combinations.

Lastly, there was also a significant correlation noticed between the size of the brushed surface and established injured area of $r = -0.22^*$ ($p=0.042$) (Pearson correlation coefficient). The higher the size of brushed surface measured, the lower the size of injured area measured respectively. On this basis, a third analysis was made. The influence of the correlation between brushed surface and injured area was partialled out and the rest of the values were saved. These adjusted values were then used for the third residual analysis with residual variable as a dependant variable.

Table 8: Wilcoxon-Mann-Whitney-Test: Multiple contrasts of objects/devices. Residual Analysis.

n of observations = 6 - 8 samples (Waterpik: n=12, AirFloss: n=13 samples); U = Test statistic of Mann-Whitney-Test; red marked cells = significant results; p = Significance value; * = significant ($p \leq 0.05$); ** = very significant ($p \leq 0.01$)

Object/Device	Statistic	Waterpik 100 PSI 3 sec	AirFloss X PSI 5 sec
ORMED 3.0 N 10 sec	U	45.0	46.0
	p	0.817	0.664
ORMED 3.0 N 20 sec	U	30.0	31.0
	p	0.574	0.483
ORMED 5.0 N 30 sec	U	16.0*	35.0
	P	0.014	0.218
Oral-B 2.0 N 10 sec	U	12.0*	30.0
	p	0.025	0.430
Oral-B 2.0 N 20 sec	U	29.0	28.0
	p	0.272	0.166
Oral-B 3.5 N 30 sec	U	29.0	38.0
	p	0.512	0.930
Sonicare 2.0 N 10 sec	U	19.0	13.0*
	p	0.111	0.023
Sonicare 2.0 N 20 sec	U	28.0	33.0
	p	0.454	0.599
Sonicare 5.0 N 30 sec	U	8.0**	30.0
	p	0.002	0.111
Waterpik 100 PSI 3 sec	U		50.0
	p		0.128

Results:

The residual analysis delivers according to the values of the brushed surfaces, the estimated values for the injured area. The residuals present positive and negative deviations from the original values of injury and their estimated values. The working hypothesis of unequal means of the tested objects resp. combinations of device/pressure/moving time can be accepted for 4 of 15 comparisons:

Oral-B 2.0 N 10 sec	vs.	Waterpik 100 PSI 3 sec (p= 0.025)
ORMED 5.0 N 30 sec	vs.	Waterpik 100 PSI 3 sec (p=0.014)
Sonicare 5.0 N 30 sec	vs.	Waterpik 100 PSI 3 sec (p=0.002)
Sonicare 2.0 N 10 sec	vs.	AirFloss X PSI 5 sec. (p=0.023)

In terms of descriptive statistics ‚Oral-B 2.0 N 10 sec‘, ‚ORMED 5.0 N 30 sec‘ and ‚Sonicare 5.0 N 30 sec‘ score substantially higher than ‚Waterpik 100 PSI 3 sec‘ in the target variable ‚injured area (%)‘ and have therefore higher injury potential estimated. Additionally ‘AirFloss X PSI 5 sec’ scores higher than ‘Sonicare 2.0 N 10 sec’ and have therefore higher injury potential estimated. There was no significant difference between other 11 device/pressure/time combinations.

4.2 Gingival injury potential

4.2.1. Powered and manual toothbrushes

Altogether 44 pig jaws met the criteria and were selected for this study. Out of a total of 90 samples, 65 samples were brushed with powered and manual toothbrushes. There were injury findings at every evaluation point. Visible injury findings were found on each sample ranging from 1.3% to 26.9% injured area ('by hand') per brushed surface. The mean values of injured area ('by hand') had a range from 5.2% to 21.8% per brushed surface. The overview of 65 assessed samples that were brushed with powered and manual toothbrushes, seems to demonstrate a statistically non-significant individual susceptibility to injuries in buccal and lingual areas around premolars and molars as well as incisors.

4.2.2 Jetting devices

Altogether 44 pig jaws met the criteria and were selected for this study. Out of a total of 90 samples, 25 samples were cleaned with jetting devices. Visible injury findings were found on each sample in a range from 1.3 – 41.3 % injured area ('by hand') per brushed surface for Waterpik and Airfloss. The mean values of injured area ('by hand') were 5.8% for Waterpik and 9.2 % for Airfloss per brushed surface. The overview of 25 assessed samples that were cleaned with jetting devices, seems to demonstrate a statistically non-significant individual susceptibility to injuries in buccal and lingual areas around premolars and molars.

4.3 Histological outcome

Altogether 18 samples including each device- and region- combination were surgically removed by ALB and used for histological evaluation (HE Staining). Every sample was coded so that the pathologist (RW) did not have any information about any affiliation to a certain device. This was done to reduce or eliminate bias, until after a trial outcome was concluded.

After histological examination in collaboration with pathologist (RW), several peculiarities were discovered. Overall, samples show a range from ‚no injury' or 'without defects' to 'transepithelial abrasion' or 'ulceration' in both powered and manual toothbrushes as well as Waterpik and Airflow. Additionally, there were noticeable signs of marginal gingivitis of different grade in 13 out of 18 histologically evaluated samples. This diagnosis was pre-existing and did not result from brushing manipulations. The following table (Table 9) shows a correlation between histological diagnosis of the sample, the corresponding injured area in percentage (‘by hand’) per brushed surface and the macroscopic appearance.

Table 9: Comparison between samples, their histological diagnosis, injured area (%) and macroscopic appearance.

Sample: device, force, time	**Histological diagnosis**	**Injured area (%) ‚by hand’ of brushed surface**	**Injured area in macroscopic appearance**
Jaw 1, molars, lingual: Ormed 3.0 N 10 sec	Epithelium of oral mucosa without defects	5.86	Slightly stained thin strip along gingival margin of molars
Jaw 44, premolars, lingual: Waterpik 100 PSI	Broad and profound erosion of the squamous epithelium, spreading over at least a half of the width of epithelium, partially reaching in to connective tissue, equivalent to an ulceration.	41.33	Remarkably blue-purple stained area, close to half of the brushed area, reaching from marginal gingiva down to muco-gingival border
Jaw 8, premolars, buccal: Sonicare 5.0 N 30 sec	Epithelium of oral mucosa without defects. Mild chronic marginal gingivitis	24.72	Light blue-purple stained stripe-like area along the whole marginal gingiva of the premolars, masked by gingivitis
Jaw 32, incisors lingual: Ormed 3.0 N 20 sec	Superficial erosion in oral squamous epithelium, not more than ¼ from full length of whole specimen. Mild chronic marginal gingivitis.	1.87	Light blue-purple stained stripe-like area along the marginal gingiva of the incisors, masked by gingivitis

Jaw 15, premolars and molars lingual: Ormed 5.0 N 30 sec	Erosion in the epithelium of oral mucosa and deep complete defects, reaching until the basal membrane (Ulcer)	4.05 (molar area)	Dark blue-purple stained stripe-like area along the whole marginal gingiva of the molars. In the premolar area, brushed surface stained in dark blue-purple tone (excluded from measurements due to poor picture quality)
Jaw 31, incisors lingual: Oral B 2.0 N 10 sec	epithelium of oral mucosa without defects	16.27	Light blue-purple stained stripe-like area along the marginal gingiva of the incisors
Jaw 36 premolars buccal: Airfloss X PSI	Epithelium of oral mucosa without defects. Mild chronic marginal gingivitis	24.7	Light pink stripe-like area around the marginal gingiva of premolars, masked by gingivitis
Jaw 26 molars lingual: Sonicare 2.0 N 20 sec	Epithelium of oral mucosa without defects. Significant chronic marginal gingivitis	5.33	Light pink stripe-like area around the marginal gingiva of molars getting darker towards the distal border of molars, masked by gingivitis
Jaw 22 molars and premolars buccal: Oral B 2.0 N 20 sec	Epithelium of oral mucosa without defects. Chronic marginal gingivitis	21.15 (molar area) and 14.48 (premolar area)	Light pink stripe-like area around the marginal gingiva of premolars and

			molars, masked by gingivitis
Jaw 5 molars buccal: Oral B 5.0 N 30 sec	Outlined profound erosion and focal ulceration of epithelium of oral mucosa. Significant chronic marginal gingivitis	Excluded due to poor picture quality	-
Jaw 40 molars lingual: Waterpik 100 PSI	Epithelium of oral mucosa without defects. Profound chronic marginal gingivitis	2.95	Light pink, slightly interrupted area around the marginal gingiva of molars, masked by gingivitis
Jaw 30 molars and premolars buccal: Oral B 2.0 N 10 sec	Epithelium of oral mucosa without defects. Significant chronic marginal gingivitis	25.34 (molar area) and 24.34 (premolar area)	Light purple stripe-like area around the marginal gingiva of premolars and molars, masked by gingivitis
Jaw 16 molars buccal: Oral B 2.0 N 10 sec	Epithelium of oral mucosa without defects	Excluded due to poor picture quality	-
Jaw 37 molars buccal: Airfloss X PSI	Epithelium of oral mucosa without defects. Chronic marginal gingivitis	9.66	Light pink stripe-like area around the marginal gingiva of the first molar, masked by gingivitis
Jaw 28 premolars buccal: Ormed 3.0 N 20 sec	Profound erosion of epithelium of oral mucosa. Chronic marginal gingivitis	27.1	Light purple stripe-like area around the marginal gingiva of premolars, masked by gingivitis
Jaw 19 molars and premolars buccal: Sonicare 2.0 N 10 sec	Epithelium of oral mucosa without defects. Significant chronic marginal gingivitis	10.94 (molar area) and 9.72 (premolar area)	Light pink stripe-like area around the marginal gingiva of premolars and molars, getting

			darker, purple towards molars, masked by gingivitis
Jaw 5 premolars buccal: Oral B 5.0 N 30 sec	Various profound erosions and one small ulceration of epithelium of oral mucosa. Chronic marginal gingivitis.	Excluded due to poor picture quality	-
Jaw 25 premolars lingual: Sonicare 2.0 N 20 sec	Squamous epithelium with partially loosened keratin layer, without profound erosions or ulcerations. Significant chronic marginal gingivitis	8.55	Light pink, slightly interrupted area around the marginal gingiva of premolars, masked by gingivitis

Some of the histological samples were digitised to show different grades of gingival injury (Figure 13-20). Absolute values as percentage of injured area per field of application have been attached under every diagnosis.

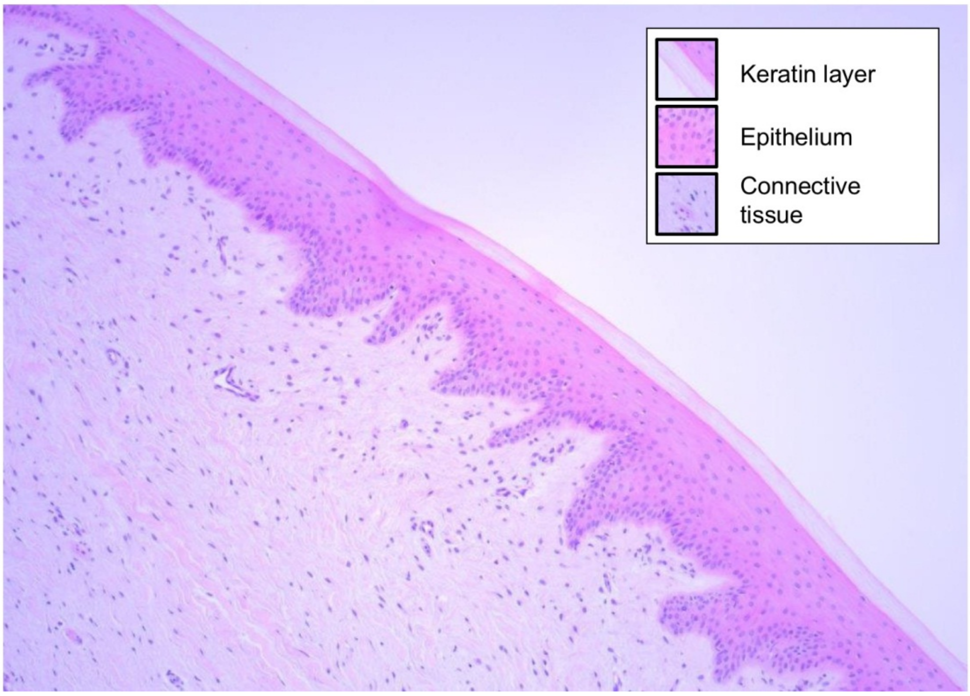

Figure 13: Normal structure of porcine gingiva after brushing with a manual toothbrush for 10 seconds, force 3.0 N; jaw 1 ML, original magnification x100; step section; 2 µm, HE Staining.

A tissue sample of 1.3 x 0.7 x 0.15 cm was cut in maximum 2 mm strong slats, completely embedded and cut into layers. Microscopically on both sides of the specimen, there is epithelium of medium width with a thin nucleated keratin layer. The epithelium is building long, narrow rete pegs.

1ML diagnosis: Epithelium of oral mucosa without defects.

Injured area per brushed surface (by hand): 5.86 %

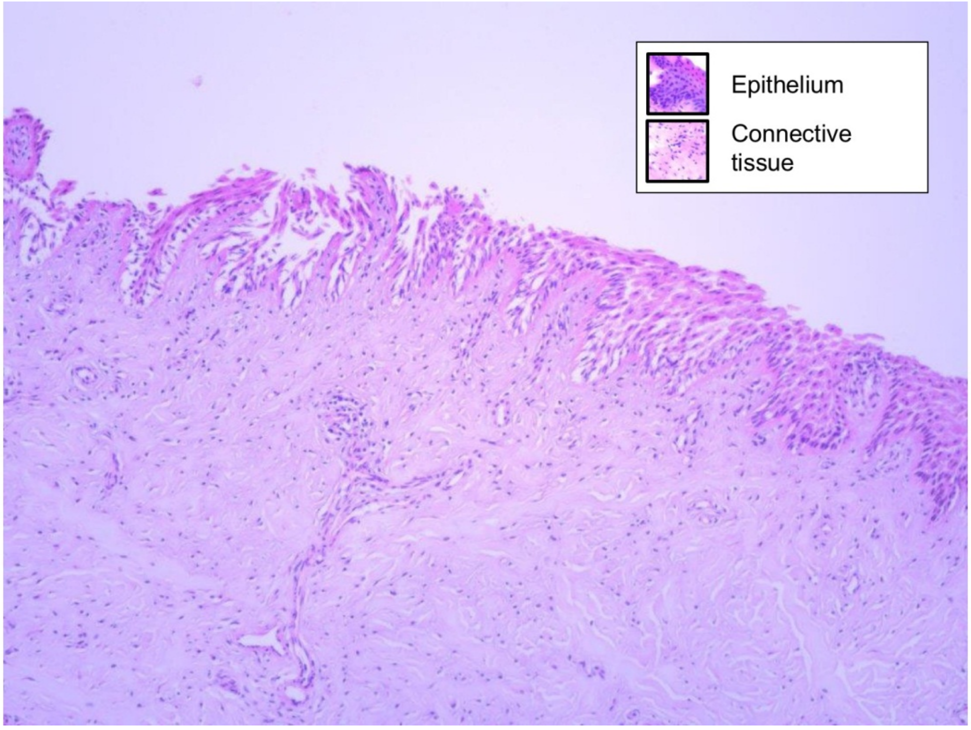

Figure 14: Transepithelial injury with partial loss of epithelium after Waterpik application with 100 PSI force for 3 seconds; jaw 44 PL; original magnification x200; step section; 2 µm, HE staining.

A white tissue sample of 0.8 x 0.5 x 0.2 cm was cut in maximum 2 mm strong slats and completely embedded. The slats were cut into layers. Microscopically one of the slats shows a bony structure on the bottom. One side of the slat does not have any epithelium. The other side shows loosened squamous epithelium. At some areas it is completely missing, revealing naked papillae of connective tissue on the surface. In other areas, epithelium is missing only till papillae of connective tissue; in the spaces between papillae epithelium is preserved. Minor lymphocyte infiltration around blood vessels can be observed in the subepithelial connective tissue.

44PL diagnosis: broad and profound erosion of the squamous epithelium, spreading over at least a half of the width of epithelium, partially reaching in to connective tissue, equivalent to an ulceration.

Injured area per brushed surface (by hand): 41.33 %

8PB: Sonicare 5.0 N 30 sec. Step section; 2 µm, HE Staining.

A white tissue sample of 1.1 x 0.5 x 0.2 cm was cut in maximum 2 mm strong slats and completely embedded and cut into layers. On the one side of the slat normally layered squamous epithelium of varying thickness is visible. It has a thin nucleated keratin layer. On the other side, narrow junctional epithelium with thicker lymphocyte infiltration around blood vessels can be observed in the subepithelial connective tissue.

8PB diagnosis: Epithelium of oral mucosa without defects. Mild chronic marginal gingivitis

Injured area per brushed surface (by hand): 24.72 %

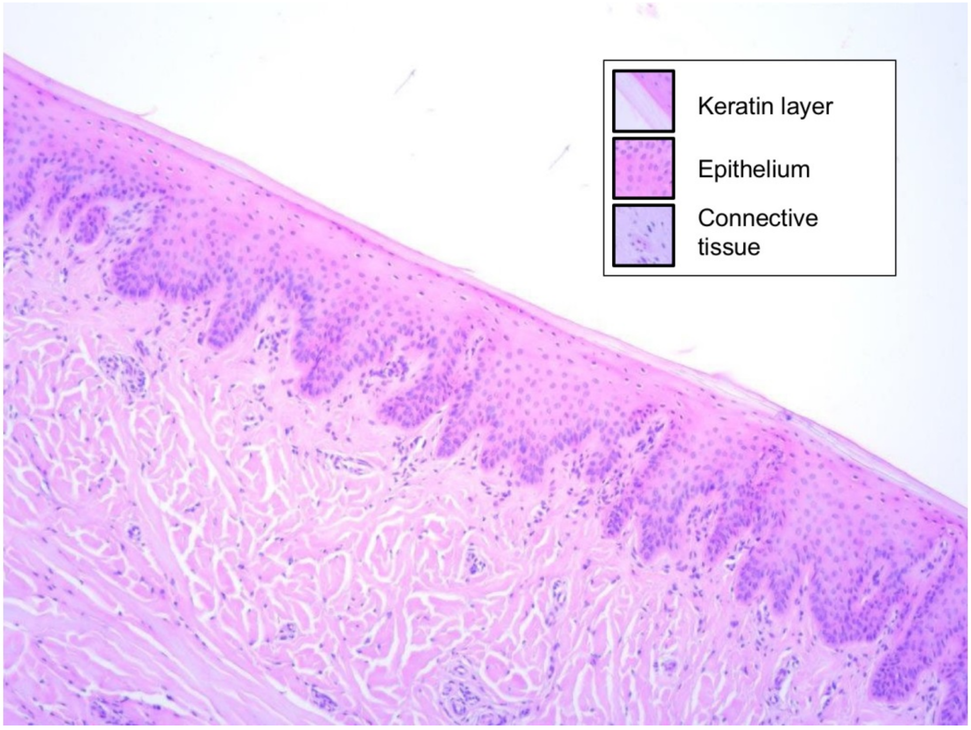

Figure 15: Reduction of keratin layer after brushing with Ormed manual toothbrush for 20 seconds, force 3.0 N; Jaw 32 iL; original magnification x100; step section; 2 µm, HE Staining.

A white tissue sample of 0.4 x 0.5 x 0.2 cm was cut in maximum 2 mm strong slats and completely embedded and cut into layers. Microscopically on the one side there is wide normally layered intact squamous epithelium with a thin nucleated keratin layer. In a short stretch, not more than ¼ from full length, a flat defect in epithelium was observed. In a fold towards tooth surface, some lymphocytes and plasma cells are visible around blood vessels.

32iL diagnosis: superficial erosion in oral squamous epithelium, not more than ¼ from full length of whole specimen. Mild chronic marginal gingivitis.

Injured area per brushed surface (by hand): 1.87 %

15 PML: Ormed 5.0 N 30 sec. Step section; 2 µm, HE Staining.

A white tissue sample of 2.5 x 0.5 x 0.2 cm was cut in maximum 2 mm strong slats and completely embedded and cut into layers. Microscopically on the one side there is normally layered intact squamous epithelium of medium width with a thin nucleated keratin layer. In some slats superficial defects of epithelium are visible, partially reaching into the connective tissue. In the area towards the tooth lymphocytes are visible around blood vessels.

15PML diagnosis: Erosion in the epithelium of oral mucosa and deep complete defects, reaching until the basal membrane (Ulcer)

Injured area per brushed surface (by hand): 4.05 % (molar region)

31iL: Oral B 2.0 N 10 sec. Step section; 2 µm, HE Staining.

A white tissue sample of 1 x 1 x 0.3 cm was cut in maximum 2 mm strong slats, completely embedded and cut into layers. Microscopically on the one side of the specimen there is wide, normally layered squamous epithelium with a thin slightly nucleated keratin layer. On the other side next to the border with connective tissue some small bars of nucleated bone tissue are embedded. Epithelium shows no traces of defects.

31iL diagnosis: epithelium of oral mucosa without defects

Injured area per brushed surface (by hand): 16.27 %

36PB: Airfloss X PSI. Step section; 2 µm, HE Staining.

A white tissue sample of 1.8 x 0.5 x 0.2 cm was cut in maximum 2 mm strong slats, completely embedded and cut into layers. On the one side there is epithelium of medium width with thin nucleated keratin layer. In the transition area towards tooth surface there are infiltrates made out of lymphocytes around blood vessels and one small follicle without reactive center.

36PB diagnosis: Epithelium of oral mucosa without defects. Mild chronic marginal gingivitis

Injured area per brushed surface (by hand): 24.7 %

26ML: Sonicare 2.0 N 20 sec. Step section; 2 µm, HE Staining.

A tissue sample of 1.1 x 0.7 x 0.3 cm was cut in maximum 2 mm strong slats, completely embedded and cut into layers. On the one side there is epithelium of different width and a thin nucleated keratin layer. In the transition area, epithelium gets wider and superficially loosened. In the subepithelial area some infiltrates made out of lymphocytes and plasma cells are visible.

26ML diagnosis: Epithelium of oral mucosa without defects. Significant chronic marginal gingivitis

Injured area per brushed surface (by hand): 5.33 %

22MPB: Oral B 2.0 N 20 sec. Step section; 2 µm, HE Staining.

A tissue sample of 1.1 x 0.6 x 0.2 cm was cut in maximum 2 mm strong slats, completely embedded and cut into layers. Microscopically on the one side of the specimen there is a normally layered squamous epithelium of different width and with a thin slightly nucleated keratin layer. Towards the tooth surface, squamous epithelium is building slightly irregular, but sharply defined cones. In the connective tissue lie some denser infiltrates of lymphocytes.

22MPB diagnosis: Epithelium of oral mucosa without defects. Chronic marginal gingivitis

Injured area per brushed surface (by hand): 21.15 % (molar area) and 14.48 % (premolar area)

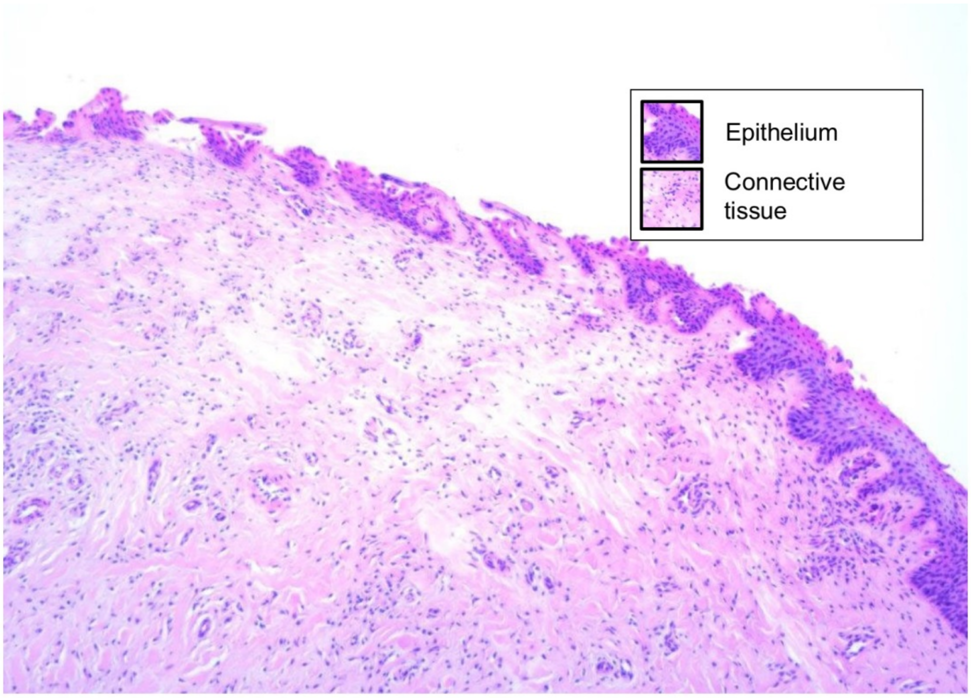

Figure 16: Transepithelial injury with partial loss of epithelium after brushing with Oral B powered toothbrush for 30 seconds, force 5.0 N; jaw 5MB, original magnification x100; step section; 2 µm, HE Staining.

A tissue sample of 1 x 0.4 x 0.2 cm was cut in maximum 2 mm strong slats, completely embedded and cut into layers. On the one side there is epithelium of medium width with a thin nucleated keratin layer. In some specimens the width of epithelium gets narrower towards the tooth surface, in other specimens it is completely missing. The surface is damaged down to the basal membrane. Under basal membrane there are some dense infiltrates of lymphocytes and towards marginal epithelium a slightly bigger follicle without reactive center.

5MB diagnosis: outlined profound erosion and focal ulceration of epithelium of oral mucosa. Significant chronic marginal gingivitis

Injured area per brushed surface (by hand): excluded due to poor picture quality

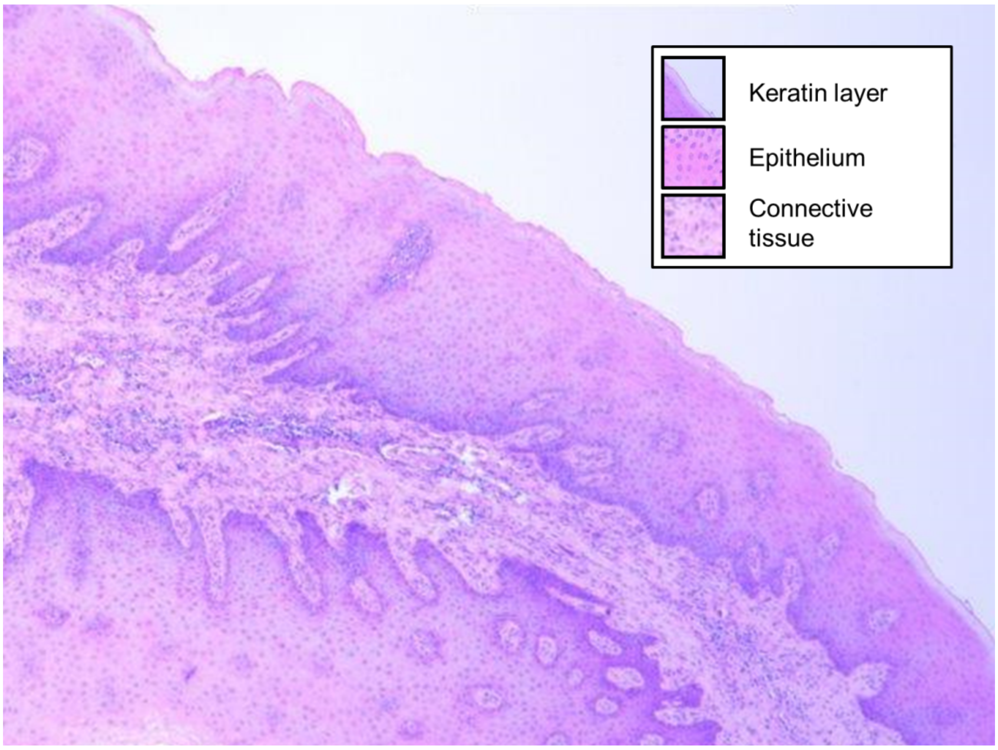

Figure 17: Normal structure of porcine gingiva after Waterpik application with 100 PSI force for 3 seconds ; jaw 40 ML, original magnification x100; step section; 2 µm, HE Staining.

A tissue sample of 2 x 0.8 x 0.3 cm was cut in maximum 2 mm strong slats, completely embedded and cut into layers. Microscopically on the one side of the specimen there is wide squamous epithelium, with a thin nucleated keratin layer. This epithelium is building thin extended cones that partially join in the area towards the tooth. In the subepithelial area, there are increased amounts of blood vessels with dense infiltrates of lymphocytes around. In some areas there are some cone building in marginal epithelium as well.

40 ML diagnosis: Epithelium of oral mucosa without defects. Profound chronic marginal gingivitis

Injured area per brushed surface (by hand): 2.95 %

30MPB: Oral B 2.0 N 10 sec. Step section; 2 µm, HE Staining.

A tissue sample of 1.5 x 0.5 x 0.25 cm was cut in maximum 2 mm strong slats, completely embedded and cut into layers. Microscopically on the one side of the specimen there is wide squamous epithelium, with a thin nucleated keratin layer. The epithelium is building narrow, long bars that partially join in the area towards the tooth. Here there are some blood vessels lying with dense infiltrates of lymphocytes around them.

30MPB diagnosis: Epithelium of oral mucosa without defects. Significant chronic marginal gingivitis

Injured area per brushed surface (by hand): 25.34% (molar area) and 24.34% (premolar area)

16MB: Oral B 2.0 N 10 sec. Step section; 2 µm, HE Staining.

A tissue sample of 1.6 x 0.7 x 0.2 cm was cut in maximum 2 mm strong slats, completely embedded and cut into layers. Microscopically on the one side of the specimen there is normally layered squamous epithelium, of different width and with a thin slightly nucleated keratin layer. The epithelium is building some longer bars in the area towards the tooth. Between increased amount of blood capillaries lymphocytes can be observed. On the other side, in connective tissue, some loosely spread lymphocytes are capturing some structure of bone.

16MB diagnosis: Epithelium of oral mucosa without defects

Injured area per brushed surface (by hand): excluded due to poor picture quality

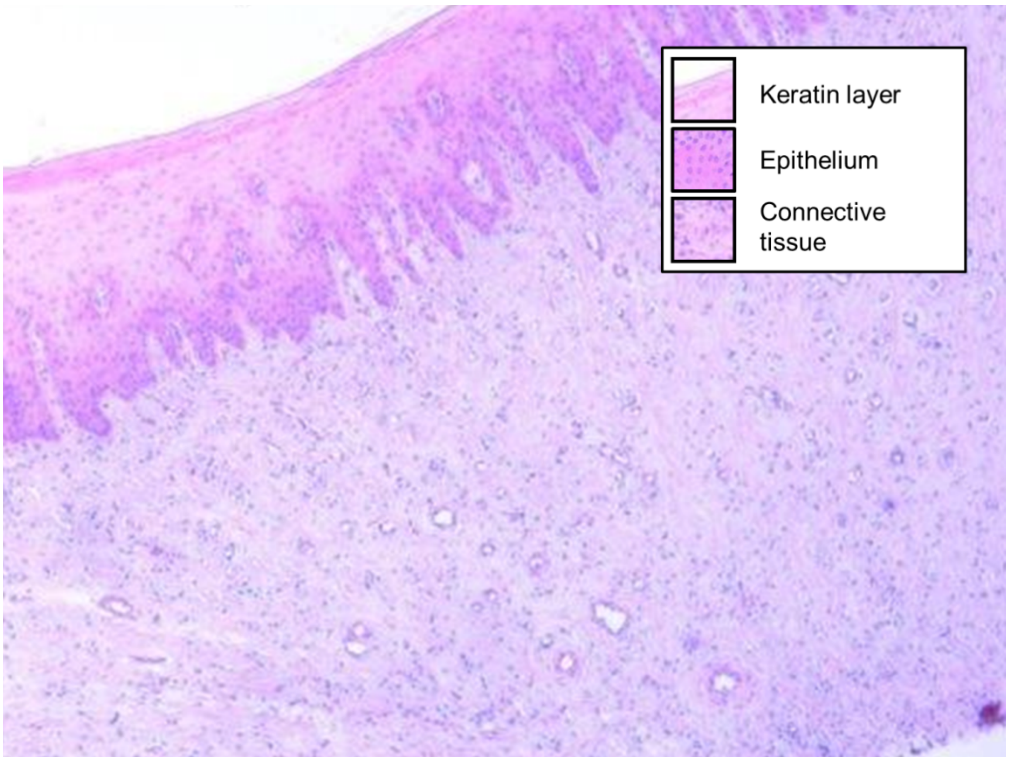

Figure 18: Normal structure of porcine gingiva after Sonicare Airfloss application for 5 seconds, X PSI ; jaw 37 MB, original magnification x40; step section; 2 µm, HE Staining.

A tissue sample of 1.8 x 0.6 x 0.2 cm was cut in maximum 2 mm strong slats, completely embedded and cut into layers. Microscopically on both sides of the specimen there is epithelium of medium to broad width with a thin, slightly nucleated keratin layer. The epithelium gets wider towards gingival margin. Subepithelial increased amount of blood capillaries are visible. Partly, some dense infiltrates of lymphocytes are lying here. At the bottom of some specimens, small complexes of bone structure are present.

37MB diagnosis: Epithelium of oral mucosa without defects. Chronic marginal gingivitis

Injured area per brushed surface (by hand): 9.66 %

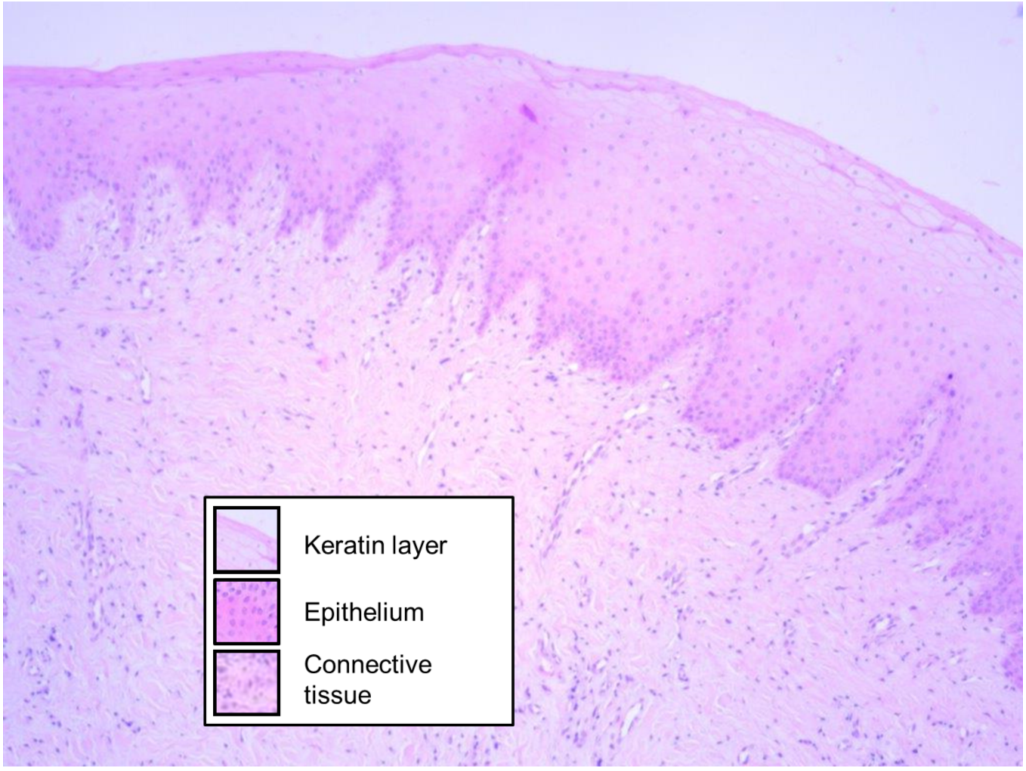

Figure 19: Transepithelial injury with partial loss of epithelium after brushing with manual toothbrush for 20 seconds, force 3.0 N; jaw 28 PB, original magnification x100; step section; 2 µm, HE Staining.

Microscopically on both sides there is epithelium of different width and with a thin slightly nucleated keratin layer. In some areas the epithelium is building some thin cones. In the area towards the tooth surface it is getting wider and is slightly loosened. At one point towards the bottom, epithelium is missing; just some small remains of epithelium are left between papillae of connective tissue.

28PB diagnosis: outlined profound erosion of epithelium of oral mucosa. Chronic marginal gingivitis

Injured area per brushed surface (by hand): 27.1 %

19MPB: Sonicare 2.0 N 10 sec. Step section; 2 µm, HE Staining.

A tissue sample of 2.6 x 0.8 x 0.2 cm was cut in maximum 2 mm strong slats, completely embedded and cut into layers. On the one side, the specimen shows epithelium of slightly different width and a nucleated keratin layer which is building sharp, prolonged bars and anastomose in the crest area. Between those, some dense infiltrates of lymphocytes around blood capillaries are lying together with one lymph follicle.

19MPB diagnosis: Epithelium of oral mucosa without defects. Significant chronic marginal gingivitis

Injured area per brushed surface (by hand): 10.94 % (molar area), 9.72 % (premolar area)

5PB: Oral B 5.0 N 30 sec. Step section; 2 µm, HE Staining.

A tissue sample of 1 x 0.4 x 0.2 cm was cut in maximum 2 mm strong slats, completely embedded and cut into layers. Microscopically, on the one side of specimen, an epithelium is slightly intensified in color and has no smooth surface. The epithelium is missing partially towards the basal membrane. In the connective tissue, some lymphocytes are lying around blood vessels and there is one follicle without a reactive center.

5PB diagnosis: various profound erosions and one small ulceration of epithelium of oral mucosa. Chronic marginal gingivitis.

Injured area per brushed surface (by hand): excluded due to poor picture quality

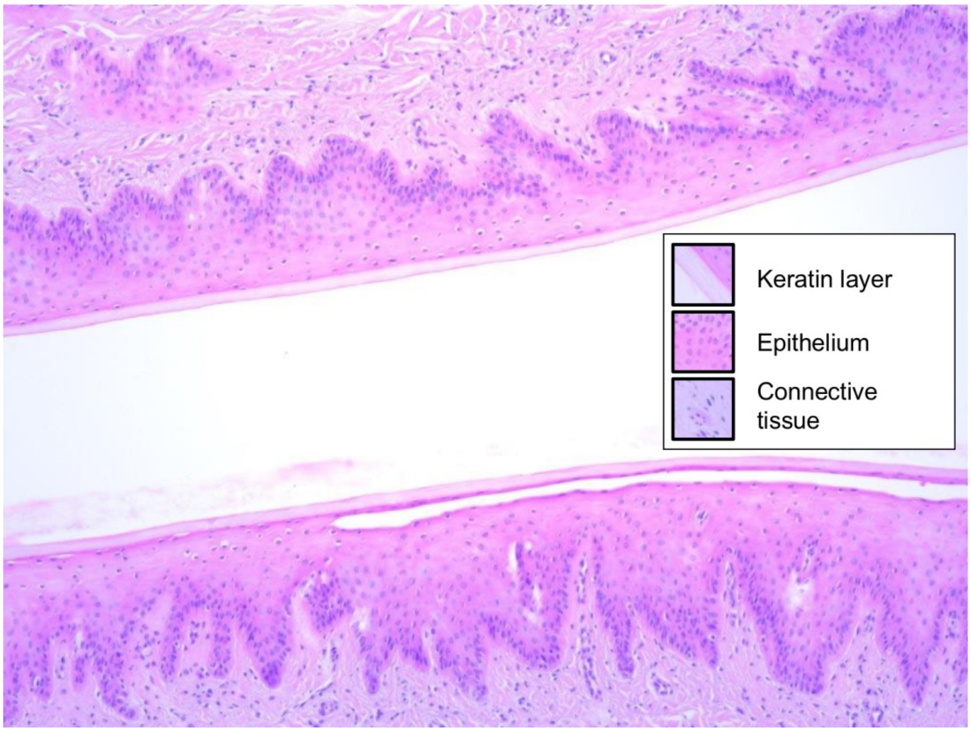

Figure 20: Reduction of keratin layer after brushing with Philips Sonicare powered toothbrush for 20 seconds, force 2.0 N; jaw 25 PL; original magnification x100; step section; 2 µm, HE staining.

A tissue sample of 1 x 0.6 x 0.15 cm was cut in maximum 2 mm strong slats, completely embedded and cut into layers. Microscopically, on the upper and lower sections of specimen there is squamous epithelium of medium width, with a thin nucleated keratin layer. The keratin layer is loosened and even lifted off in some areas. In the crestal area, epithelium is building connecting cones, between lay some lymphocyte infiltrates and some follicles.

25PL diagnosis: Squamous epithelium with partially loosened keratin layer, without profound erosions or ulcerations. Significant chronic marginal gingivitis

Injured area per brushed surface (by hand): 8.55%

5. Discussion

Dental hygiene has improved remarkably in recent years. This is related to education and motivation through healthcare personal. However, the question arises whether excessive practice of oral hygiene supported by the industry with continuously new manual and powered oral care products lead to better dental health. It could be that exactly this trend leads to increasing traumatic appearances in form of gingival injury and gingival recession. The aim of this study was to test a manual, two powered toothbrushes and two jetting devices in respect to their gingival injury potential. In the following chapter the protocol of the study, the results and the need for future studies will be discussed.

5.1 Protocol of study

5.1.1 Study design

This was an *in-vitro* study on pig gums. To evaluate the effect of different oral hygiene products like manual and powered toothbrushes and jetting devices on gingival tissue, several study designs are acceptable. Short and long-term studies as well as *in-vitro* and *in-vivo* studies can be used for this purpose. There were several reasons to choose the *in-vitro* study design. On the one side, the majority of research with *in-vivo* human or animal participants requires appropriate consent. To carry out such study *in-vivo* would have been very difficult for several reasons: It is acceptable to disclose injuries caused by brushing in human or animal oral cavity. However, it would not be possible to surgically remove the injured area for a histological examination as it was done in present study.

With this kind of trial an inevitable question arises, whether the results would not be clinically more significant if human test persons would be used instead of pig gums. In such *in-vivo* study few requirements need to be met, among other things, the same steady pressure during brushing and the same brushing technique. Another important element is always a consistent motivation of the test person. Already in some earlier studies toothbrushes were connected to pressure sensors, which showed the exact pressure applied (Fraleigh et al. 1967, Breitenmoser et al. 1979). To avoid such errors, a trained, highly calibrated professional (ALB) undertook this task, thereby standardising the different variables (brushing time, force and technique).

Furthermore, injuries were digitised with standardized photography. To take such photographs inside human oral cavity would have been very difficult if not impossible as all the surrounding tissue, buccal and lingual mucosa, the tongue and saliva would have seriously impaired access to the injured area. The question arises- how clinically relevant are studies using pigs as human substitutes? Imfeld in 2001 tested whether bovine teeth can be used as a substitute to human teeth. The results showed that bovine mandibular front teeth can be used for *in-vitro* studies of dental abrasion. It could be assumed that the soft tissue of human and bovine gingiva is similar as well.

However, later in 2011 Yassen et al. did a review of *in-vitro* and *in-situ* studies that directly compared the use of bovine teeth as a substitute for human teeth in dental experiments. The results were inconsistent, whether bovine teeth can be considered an appropriate substitute for human teeth in dental research. The study needed to be highly standardised and this was done by the use of pigs. At the fattening piggery, the pigs are kept in large groups where they are organised according to their size and gender. Pigs of the same size and gender are placed into sties together. This enables feeding to be regulated in accordance with the group's requirements. Assuming that all pigs have the same environment, feeding cycles and the same race, there is a large possibility that anatomical characteristics are at least very similar. And according to Cai et al. (2017), the dento-gingival environments of porcine and bovine jaws were similar to those of human jaws, and no significant difference was detected between these two animal models (p=0.178). It is important to keep in mind that difference in morphology, chemical composition and physical properties between human and bovine oral tissue must be considered, when interpreting results obtained from any experiment using bovine tissue.
Finally, the present study was an *in-vitro* study and ethical approval was obtained.

5.1.2 The number of samples

The number of samples was established during the study. The main aim was to have enough samples in each device/area/pressure/time- combination for a sufficient statistical evaluation. Altogether, 90 samples were evaluated for this study, from 11 to 23 samples per toothbrush. This difference in sample amount is due to the fact that the valuable samples, without pre-existing injury, were detected just in the process of staining. Moreover, the amount of the jaws available per day was limited and they had to be utilised quickly. The exact distribution of samples can be extracted from Table 1 Paragraph 3.1.3. A comparison with other studies is difficult, as both material and methodology are not exactly the same as in studies mentioned in the literature review. However, in a similar study from Vogel et al. (2014), only 4 samples per toothbrush were used.

5.1.3 Selection of samples

Injuries

Selection of samples was the most challenging part of the study. Only 1/3 of the collected material was eligible for the analysis of gingival injury potential. Approximately 2/3 of the pig jaws collected were injured through feeding. Some injuries were visually noticeable by the operator (ALB) and were sorted out. However other injuries appeared just after first staining and were sorted out at this stage. Very few samples were sorted out even later in the study process, as injuries became visible only through digitisation and enlargement. The particular distribution of damages along the samples begs the question which characteristics of the gingival material could possibly have an influence on the outcome?

There seems to be some variability in individual trauma susceptibility of gingival tissue among the pigs. It is well documented that individual susceptibility maybe different from region to region, from age to age in human as well as in animal studies. Moreover, the influence of the chosen examined regions and tooth exfoliation of the deciduous teeth of pigs have to be discussed.

Individual Trauma Susceptibility

Mierau and Spindler (1984) name many factors that can cause gingival recession: condition and characteristics of the toothbrush, brushing technique and frequency, however they also point out the multi-causal connections in development of gingival recession. Besides the combination of higher brushing force and longer brushing time, anatomy and physiology of the individual, such as thin vestibular alveolar bone, seems to play a significant role in development of gingival recession.

Assuming that the anatomy of gingiva and underlying tissue has a high influence on the trauma susceptibility, there is a need for a closer look at studies dealing with gingival biotype. According to the gingival biotype review of Esfahrood et al. (2013), two main types of gingival anatomy can be divided - flat and highly scalloped. Today they are classified as thin and thick gingival biotype and have high clinical relevance. The authors also suggest that the bone contour is very similar to the gingival contour above it. However, in a study by Fu et al. (2010), computed tomography measurements showed that the thickness of the labial gingiva had only a moderate association with the underlying bone radiographically.

In their systematic review, Zweers et al. (2014) summarized previous studies about gingival biotype and came to conclusion that based on the available literature, it can be confidently categorised in three biotypes: thin scalloped; thick flat and thick scalloped. In their view, the dental, gingival and osseous dimensions have a weak to moderate association. Uniformly, positive associations between gingival thickness, keratinized tissue and bone morphotype were found.

It has been proven that gingival biotype influences tissue resorption after a traumatic experience such as tooth extraction or implantation. In 2013, Abraham et al. reported that although tissue biotypes have different gingival and osseous architectures, they exhibit different pathological responses when subjected to inflammatory, traumatic or surgical insults and therefore needs appropriate tissue management. According to Abrahamsson et al. (1996), thick tissues (that is ≥2.5 mm) can avoid significant crestal bone recession, whereas response of gingiva with thin gingival biotype (≤2,5 mm) can lead to gingival recession. Further investigation is needed to prove whether thin gingival biotype would be more susceptible to gingival abrasion caused by brushing.

Examined Regions

The conception of the study was to include all three jaw regions, incisors, premolars and molars respectively. This would enable more reliable interpretation and transfer of results to human oral cavity. Unfortunately, close to 100 % of the jaws used had injured front teeth area and were sorted out. This was due to the slaughtering process as the pig jaws are always halved in the middle, between the incisors. In a similar study from Vogel et al. 2014, front sextants of pig jaws were used to assess gingival injury potential of manual and powered interdental toothbrushes. These were obtained from the animal hospital and the front teeth area was intact.

It remains unclear whether the results of Vogel's study would have been different if all three jaw regions were used. According to Jati et al. (2016), decreased alveolar bone crest thickness, combined with delicate gingival margin, commonly found in maxillary canines and mandibular incisors are one of the predisposing factors for gingival recession. Taking into account the anatomy of these regions, it could be speculated that they are more susceptible to gingival injury as well. In most of the studies on human gingiva, all three gingival areas, incisors, premolars and molars (Danser et al. 1998, Mantokoudis et al. 2001) or at least two gingival areas: canine and premolar region (Breitenmoser et al. 1979) were used to assess gingival injury potential of different toothbrushes. In the present study premolar and molar region appeared to be suitable for investigating gingival injuries.

Tooth exfoliation

According to the slaughterer, most of the pigs were around 12 months old. This is the time were exfoliation of deciduous teeth takes place. Samples with signs of tooth exfoliation were sorted out, as these areas would be more susceptible to traumatic brushing injuries.

Pari et al. (2014), in their study on gingival diseases in childhood, described the prevalence of various soft tissue diseases and importance of long term overall oral health maintenance in childhood. Among that, clinical and histological correlations in childhood gingiva have been enlightened. For example, reddish colour of gingiva appears histologically as thinner epithelium, a lesser degree of cornification and greater vascularity. Such characteristics could make gingiva more susceptible to damage.

5.1.4 Gingiva staining

To assess clinically the incidence and severity of gingival injuries, different methods can be used. One can be a visual inspection supported by standardised examination methods and indexes (Niemi et al. 1984, 1986, 1987; Carvalho et al. 2007; Zanatta et al. 2011). In 2005, Sharma et al. investigated gingival injury by using a more precise visual examination. In their study, different parameters were used as a sign of gingival injury: colour, oedema, bleeding and hard tissue damage, like abrasions. Experience in these studies has shown that visual examination of gingival injury does not seem to provide significant results to evaluate damage of gingival epithelium. Moreover, the quality of these examinations is largely dependent on the quality and experience of the examining professional.

Histological examination (Anneroth et al. 1974), scaning electron microscopy (Sandholm et al. 1982) and measuring DNA protein (Alexander 1977) have been used previously. These methods are quite complex and costly and require a trained professional’s attention.

Staining of gingiva by the use of plaque disclosing solution to reveal, record and measure gingival injury has been implemented by Breitenmoser et al. (1979) and modified later by Danser et al. (1998). Several other research groups have later used it and it has been established as a valid and precise method to investigate gingival injury potential (Imfeld 2000, Mantoukoudis et al. 2001, Versteeg et al. 2005, Carvalho et al. 2007, Zanatta et al. 2011, Zimmer et al. 2011, Vogel at al. 2014, Caporossi et al. 2016, De Nutte et al. 2018).

5.1.5 Brushing duration, force and technique

There are three characteristics in tooth brushing that differs between the patients and can remarkably influence the outcome: brushing duration, force and technique. In this study, all of these parameters were set. Already in 1989 Mierau et al. proved that tooth brushing with scrubbing and rotating movements and force over 2 N can lead to gingival recession and V-shaped defects of gingiva.

Brushing duration

Vehkalahti (1989) emphasises the significance of frequent tooth brushing on gingival recessions. Frequent tooth brushers in this study had more surfaces with recession than had those brushing their teeth infrequently. This was confirmed later by a clinical study of Khocht et al. (1993). In this comparative study, the effect of higher brushing time, or rather, more strokes per brushing time on gingival recession, was addressed. However, already in 1998, Saxer et al. proved that that there is a consistent difference in the time that patients believe they brush and the time they actually spend brushing. The actual tooth brushing means in this study were between 72.8 and 83.5 seconds. The time subjects estimated they had brushed ranged between 134.1 to 148.1 seconds. Claydon et al. in 2008 reported that the average time patients spend on their oral hygiene is 60 seconds. This tendency was confirmed later by Ganss et al (2009). In this study appropriate brushing habits were defined: brushing at least twice daily for 120 s with a brushing force of less than 3 N and with circling or vertical sweeping movements. Still, only 25.2% of the participants fulfilled all criteria, emphasising the ongoing need for oral hygiene education. There have been several studies that address the effect of increased brushing time to the consensus minimum of 2 minutes as it increases plaque removal to an extent likely to provide clinically significant oral health benefits (Creeth et al. 2009, 2016).

Brushing duration in the present study was chosen 10, 20 or 30 seconds with manual and two powered toothbrushes, to observe possible changes in injuries by increasing the brushing time. Both jetting devices were used as suggested by manufacturer. Air Floss was applied for 5 seconds per interdental space, Waterpik was applied for 3 seconds per interdental space.

One of the hypotheses of the study was that gingival injuries get larger/deeper by brushing longer. In their study about gingival injury potential of interdental brushes Vogel et al. (2014) observed that the extent of gingival injuries in the region of attached mucosa increased with brushing time. The outcome showed that some interdental brushes clean more effectively with less injury potential. However, the effect of brushing time on the occurrence and extent of injuries was not investigated.

One could easily speculate that the longer the brushing time of a manual or powered toothbrush the higher the prevalence and extent of gingival injuries. This hypothesis was proven by a research group from Belgium in 2018 (De Nutte et al.). The palate of patients was brushed with a manual toothbrush continuously for 2 Minutes. The lesion was stained, digitalised and observed clinically every 24 hours until complete healing. The longer the exposure time of gingiva to trauma (hence the brushing time) the more lesions were visible, the larger the abrasions were and the longer time they needed to heal completely. However, in the present study, several combinations of different force and different brushing duration between toothbrushes and jetting devices were compared, in order to evaluate their potential altogether to cause gingival injury and to see if there are significant differences between these devices.

Brushing force

As well as time, brushing force can be jointly responsible for the traumatic injury of gingiva. Already in 1962, Phaneuf et al. reported that a powered toothbrush is pressed more gently towards tooth and gingival tissue than a manual toothbrush. The average brushing force for hand brushing was estimated approximately at 3.0 N, whereas powered brushing was only 1.0 N. This was confirmed later in a study of Boyd et al. (1997). The outcome of both studies showed higher injury potential with the use of manual toothbrush. In his view, this outcome was related to higher brushing force with a manual toothbrush.

A number of studies suggest that less force is applied to the tissue while using a powered toothbrush in comparison to a manual toothbrush (Phaneuf et al. 1962, Niemi et al. 1986, 1987). The results from these studies showed that patients used the powered toothbrush with just 31% of the brushing force used with a manual toothbrush. According to Mierau et al. (1989), epithelial damage occurs by increasing the force over 2.0 N. Already in 1979, Breitenmoser et al. in their study about the damaging effects of toothbrush bristle end form on gingiva standardized the force applied during brushing. This was set at 4.9 N force. A brushing device, designed especially for his study, lit a light to indicate when the desired brushing force 4.9 N was applied. The clear outcome their study suggested that cut bristle ends of a toothbrush cause significantly greater gingival abrasions than rounded bristle ends. However, the greater extent of lesions caused by cut bristle ends could not be explained by accidentally higher brushing force.

Later in 1998, Danser et al. observed in their study that brushing force is not influenced by the speed of the brush head and has no correlation with the incidence of gingival abrasion. A more recent study from Van der Weijden et al. (2004) investigated whether a high brushing force induces more gingival abrasion than a low (regular) brushing force. The results of their study could not show the difference between high and low brushing force and the incidence of gingival abrasion.

In the present study, different forces with manual and powered toothbrushes in a range from 2 to 5 N were applied. The calibration of the force applied was carried out before each run. It was the aim to set up a hypothesis- that greater brushing force would cause more or/and deeper injuries. Both jetting devices were not manipulated and were applied at the manufacturer's set pressure. A Waterpik interdental cleaner was applied in a 'high pressure' mode at 100 PSI (pound force per square inch), which is widely used unit in USA. This is equivalent to 6.9 bar. A high pressure mode was used deliberately to simulate a ‘worst case’ scenario. Sonicare Airfloss was the only device where it was not possible to find out the exact water pressure applied. According to the customer service of Philips: ‘Philips Sonicare AirFloss Pro has been designed to the *optimal* pressure to ensure it is effective in removing plaque while remaining gentle on gums and dental work’. Therefore, it was very difficult to compare both jetting devices, as the water pressure of Sonicare AirFloss is unknown. The hypothesis that higher brushing force induces more gingival injuries was not confirmed in the present study.

Brushing technique

In the present study, a highly calibrated professional (ALB) brushed each segment of pig gingiva. Brushing with a manual toothbrush was performed in circular mode using the modified Bass method (Breitenmoser et al. 1979). All other devices were used strictly according to manufacturer’s instructions.

In a similar study by Vogel et al. (2014), brushes were evaluated using a specially manufactured brushing device and a standardised horizontal movement. This is supposed to represent the way an average patient uses interdental brushes. It could be speculated, that this brushing method induced more injuries as the modified Bass method in the present study.

5.2. Gingival injury

The overview of 65 assessed samples that were brushed with powered and manual toothbrushes, seems to demonstrate a statistically non-significant individual susceptibility to injuries in buccal and lingual areas around premolars and molars as well as incisors. The overview of 25 assessed samples that were cleaned with jetting devices, seems to demonstrate a statistically non-significant individual susceptibility to injuries in buccal and lingual areas around premolars and molars as well.

The size of the injured area had a range from 0.0008 % to 31.94 % per brushed surface with manual and powered toothbrushes and from 2-15% with Waterpik and 1-25% with Airfloss jetting devices. However, the staining method revealed injury in every single sample. This has been done earlier by several research groups (Breitenmoser et al. 1979; Danser et al.

1998; Mantoukoudis et al. 2001 and Vogel et al. 2014). Notably, higher brushing force or/and brushing time did not show, as expected, a higher risk of gingival injury.

Generally, it is clear that every use of toothbrush causes changes in gingival epithelium. According to Amano et al. (2007) brushing disrupts cell plasma membrane barriers in the oral cavity and activates gene expression events that may lead to local adaptive changes in tissue architecture beneficial to gingival health. These changes can be as harmless as increased desquamation of the keratin layer up to different crack and fissure formation.

Correlation between stained injuries and gingivitis

In the present study, histological evaluation showed a range of different outcomes after tooth brushing: normal structure of porcine gingiva, as well as intraepithelial alteration with reduction of the keratin layer up to transepithelial injury reaching into connective tissue with partial loss of epithelium. Interestingly, a closer look at the results of gingival injury measured planimetrically and their corresponding histological outcome shows that histological diagnosis does not necessarily match the corresponding measurement of injured area. For instance, there were few histological samples without histologically visible signs of injury, but then corresponding injured area measured after staining in a range from 2.95 – 25.34 % per brushing field. It was also noticed that in histological results, which were done in collaboration with a trained pathologist, many of the samples showed mild up to significant chronic gingivitis, which was pre-existing and not caused by brushing manipulations. This matches the macroscopic evidence in pictures.

The histological appearance of gingivitis has been well described (Klinge et al. 1983, Page et al. 1986). Gingivitis with a chronic component as described in the histological report shows histologically infiltration with B and T lymphocytes and a capillary proliferation forming a granulomatous response. Despite extensive research, clinically healthy gingiva can still show histologically mentioned changes, so it is very difficult to differentiate between normal gingival tissue and initial gingivitis (Loe et al. 1965).
In the present study, gingivitis was not revealed by the first staining of gingiva, but it could be speculated that the second staining revealed gingivitis and finally simulated injury which was masked by gingivitis. To the best of our knowledge, so far there have been no studies to confirm that gingivitis can be stained and revealed by plaque disclosing agent.

Clinical relevance

The critical question which needs to be asked in order to assess the clinical relevance of this study, is- what is the scenario of repeated traumatization of gingiva during daily tooth brushing? In the present study there were injury findings in every sample.

Gingival lesion as a result of extensive tooth brushing has been described earlier. There has been evidence that patients with intensive oral hygiene more frequently show gingival recessions, V-shaped gingival defects and Stillmann clefts (Gorman et al. 1967, Sangnes et al. 1976, Breitenmoser et al. 1979).
Moreover, there is positive association between gingival abrasion and recession. The characteristics of abrasive gingival lesion are loss of epithelium and exposure of connective tissue (Breitenmoser et al. 1979). This could be a possible demonstration of how gingival abrasions leads to gingival recession. The aetiology of gingival recession was long the subject of controversial debate. Different factors have been discussed as possible causes of recession. According to Sangnes et al. (1976), recessions appear in the areas where alveolar bone is thinner and spongious bone is missing, therefore more susceptible to traumatic forces. Mieler et al. (1985) suggests that correcting misalignments of the teeth, reduced bone substance, high frenula and parafunctional habits can be responsible for gingival recession. Wennström et al. (1987), in their study, addressed the effect of accelerated orthodontic forces on gingival recession. In a recent study by Zanatta et al. (2011), it was demonstrated that the cervical region of gingiva exhibited a higher prevalence of gingival abrasion, regardless of the type of toothbrush, with a higher number of abrasions in the medium toothbrush group. The evaluation of gingiva was done on a purely visual basis.

All of the mentioned factors have possible effects on gingival recession. Nowadays, the aetiology of gingival recession is seen as more multicausal. However, there is one condition that is crucial in the development of gingival recession: dehiscence between bone and gingival tissue.

Summarising the results from short- and long-term studies about the correlation between tooth brushing, gingival trauma and gingival recession, Litonjua et al. (2003) found that in short-term studies, gingival trauma and gingival abrasion may result from tooth brushing, but the direct relationship between traumatic oral hygiene and gingival recession is inconclusive. Long-term studies remain debatable or do not defend the hypothesis that recession could be caused by tooth brushing.

A hypothesis that excessive tooth brushing could lead to non-inflammatory gingival recession has been extensively researched later. For example, a recent systematic review from Rajpakse (2007) states there is no evidence to support an association between tooth brushing and gingival recession, due to a lack of studies that satisfy all inclusion quality criteria.

5.3 Histological Evaluation

One more long established method to determine and evaluate gingival injury is histological evaluation. Haematoxylin and eosin stain is one of the principal stains in histology (Fischer et al. 2008). According to the protocol of their study, haematoxylin has a deep blue-purple colour and stains nucleic acids by a complex reaction. For a contrast, eosin is pink and stains proteins non-specifically. In a typical tissue, nuclei are stained blue, whereas the cytoplasm and extracellular matrix have varying degrees of pink staining. This was done in the present study on 18 samples, including each device and region. It has been done earlier in several studies on animals only.

Anneroth et al. (1975) did an experimental study on dogs. The aim of this study was to evaluate gingival damage cause by tooth brushing histologically. The conclusion was that the two different types of brushes most likely cause different damages on the gingival tissue and that the polytene brush appeared to cause less tissue damage than the nylon brush. Interestingly, marginal gingivitis was discovered in some dogs but they were still included in the study.

In a study by Plagmann et al. (1978) miniature pigs were used to test 2 different tufted toothbrushes (multi tufted and space tufted). After 4 weeks of brushing (three times a week, for 20 seconds) gingival tissue was histologically evaluated. Both toothbrushes showed structure roughening and damaged surface of epithelium with detached cells on the alveolar mucosa. A similar outcome was found on free gingiva.

Histological evaluation is a valuable and reliable method to assess the grade of gingival injury and it did present meaningful results. The correlation between injury findings, histological outcome and macroscopic presence has been discussed in previous chapters.

5.4 Future Studies

All of the tested oral care devices, manual and two powered toothbrushes as well as two jetting devices, caused changes in gingival epithelium leading to gingival injury and possible gingival recession. It would be interesting to see how pre-existing gingivitis influences the potential of the brush to cause gingival injury and whether gingivitis can be stained with plaque disclosing agents. It is important to note, that clinically, not only the size but also the profoundness of the injury will most likely have an impact on the healing. This hypothesis remains open to being either refuted or confirmed in bigger studies with higher amount of samples and supported by thorough histological examination.

6. Summary and Conclusions

The aim of the present study was to standardise the in-vitro pig gum test and to evaluate the gingival injury potential of different oral care devices, manual and two powered toothbrushes as well as two jetting devices. Moreover, it was questioned whether the brushing time and force has any effect on the incidence and severity of gingival injury.

All five test devices were applied at altogether 90 fresh gingival areas of pig jaws around premolars, molars and incisors buccally and lingually, directly after slaughtering. The brushing pressure was calibrated before each run and the brushing time was recorded. Gingival injuries were revealed with plaque disclosing agent before and after application. Injured areas were digitised, planimetrically recorded and expressed as absolute values and percentage per field of application. Finally, some samples were histopathologically controlled (HE staining). Statistics included t-Test and Mann-Whitney-Test.

The results showed injuries in every sample. All means of injured areas due to brushing range from 5.2-21.8 %. Total range of injured area for all devices was 1.3% - 41.3%. There was no indication of an increased injury potential when pressure or time was increased.

Histological evaluation showed a wide range of outcomes: from normal structure of pig gingiva (hence no injury) to mild intraepithelial alterations and even deep transepithelial injuries. Also this outcome is concerning, it was not included in the statistical analysis and therefore cannot be interpreted to have a statistical relevance.

In-vitro pig gum tests of oral hygiene devices are recommended for gingival injury risk assessment. All devices showed low injury potential with no statistically significant differences from area to area.

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9. Curriculum Vitae

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1989 - 1993	Primary school in Riga
1993 - 2000	Secondary school in Riga. High School Degree.
2000 - 2006	Study of dentistry at Riga Stradina University in Riga, Latvia Graduation: Dentist Degree
18.01.2008.	Dental Practice Allowance (Latvia)
16.07.2008.	Temporary professional permit according to §13 ZHG (Germany)
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<u>Occupation:</u>

01.09.2000 - 30.06.2006	Dental assistant at the dental practice „A-Serviss“(Riga, Latvia)
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08.10.2007 - 09.11.2007	Trainee at the practice Dr. W. Ross (Obstmarschenweg 297, Stade, Germany)
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04.12.2013 – today	Dentist at Zahnklinik Unna (Frankfurter Str. 69, 59425 Unna)

10. Annex

10.1. Approval by the Ethics Committee:

(e-mail communication, cited)

Von: Gängler, Peter
Gesendet: Dienstag, 23. August 2016 12:54
An: Pleger, Andrea
Betreff: Promotionsprojekt mit Schweinekiefern

Liebe Frau Pleger, lieber Herr Kollege Gaidzik,

ich betreue ein Promotionsprojekt zu Abrasionsdefekten durch Zahnbürsten an der Mundschleimhaut und Gingiva, die an Schweinekiefern ausgelöst werden sollen. Schweinekiefer sind grundsätzlich ein traditionelles Modell für chirurugische Übungen in der Lehre und für vorklinische Testungen. Es handelt sich um Schlachtabfälle, die wir kostenlos von einem Unnaer Schlachtbetrieb beziehen können und nach der Biostoffverordnung korrekt im Labor bearbeiten können und gleichentags auch wieder über den Schlachtbetrieb entsorgen werden.
Ist dazu ene ethische Freigabe erforderlich?

Danke für Ihre Bemühungen und herzliche Grüße, Ihr Peter Gängler

Von: Pleger, Andrea
Gesendet: Mittwoch, 24. August 2016 07:56
An: Gängler, Peter
Betreff: WG: Promotionsprojekt mit Schweinekiefern

Lieber Herr Professor Gängler,

Herr Gaidzik hat schon geantwortet (s. u.). Es ist keine ethische Freigabe erforderlich.

Gruß A. P.

Von: Prof. Dr. Gaidzik [mailto:pwgaidzik@yahoo.de]
Gesendet: Dienstag, 23. August 2016 20:02
An: Pleger, Andrea
Betreff: AW: Promotionsprojekt mit Schweinekiefern

Nein, nicht erforderlich!

10.2. All Data

Sample	Jaw	Side	Region	Brushed surface (px)	Injured surface by Color Ra	Percentage of injured area	Injured Surface by Hand (px	Percentage of injured area	Pressure (N)	Time (sec)	Toothbrush	Pathology	Comment
1	1	B	M	188436	9817	5.2097263792	6891	3.6569445329	3	10	Ormed		Jaw 4, 16, 17,
2	1	B	P	101147	19094	18.8774753576	5283	5.2230911446	3	10	Ormed		
3	1	L	M	890976	387255	43.464133714	52239	5.8631208921	3	10	Ormed	No damage	
4	1	L	P	252883	20920	8.2726003725	35707	14.119968523	3	10	Ormed		
5	2	L	M	1364174	63646	4.6655338688	135069	9.9011563041	3	10	Ormed		
6	3	L	M	1343078	428924	31.9358965004	196165	14.6056297549	3	10	Ormed		
7	3	L	P	500208	55373	11.0699948821	52212	10.438057768	3	10	Ormed		
8	5	L	M	2403785	221666	9.221540196	87073	3.622328952	5	30	Oral B		
9	6	B	M	900009	119177	13.2417564713	98633	10.9591126311	3.5	30	Oral B		
10	6	L	P	386282	7255	1.8781615504	14502	3.7542520749	3.5	30	Oral B		
11	6	L	M	386282	7255	1.8781615504	54959	14.2276885798	3.5	30	Oral B		
12	7	B	M	947489	141908	14.9772715039	136725	14.4302466836	2	30	Oral B		
13	7	L	M	1439924	254005	17.6401671199	73119	5.0779763376	2	30	Oral B		
14	7	L	P	428948	11873	2.7679345748	12497	2.9134067533	2	30	Oral B		
15	8	B	P	689189	145304	21.0833312778	170336	24.7154263925	5	30	Sonicare	No damage	
16	8	L	P	963068	41200	4.2779949079	71657	7.4404922602	5	30	Sonicare		
17	9	L	M	2960272	373728	12.6247858305	133051	4.4945532032	5	30	Sonicare		
18	10	L	M	2016506	60308	2.9907176076	115859	5.7455321234	5	30	Sonicare		
19	10	L	P	669463	41569	6.2093050699	70236	10.4913938485	5	30	Sonicare		
20	11	B	M	690180	216214	31.3271900084	213119	30.8787562665	3.5	30	Sonicare		
21	11	L	M	1886002	353148	18.724688521	100793	5.3442679276	3.5	30	Sonicare		
22	11	L	P	517033	130656	25.2703405779	440808	85.257227295	3.5	30	Sonicare		
23	12	B	M	1113185	141858	12.7434343797	138365	12.4296500582	2	30	Sonicare		
24	12	L	M	1882334	421898	22.4135567864	149245	7.9287204077	2	30	Sonicare		
25	12	L	P	748706	122199	16.3213597861	33569	4.4836023753	2	30	Sonicare		
26	13	L	M	1839036	363284	19.754045054	55708	3.0291957308	4	30	Ormed		
27	13	L	P	599993	55504	9.2507745924	39640	6.6067437453	4	30	Ormed		
28	14	B	M	1021409	113342	11.0966322012	190145	18.6159511028	4	30	Ormed		
29	14	B	P	612722	141532	23.0988931359	116434	19.0027451275	4	30	Ormed		
30	14	L	M	2357476	503834	21.3717552162	67287	2.8541966069	4	30	Ormed		
31	15	B	M	718949	135963	18.9113553256	91437	12.718148297	5	30	Ormed		
32	15	L	M	2493923	123325	4.9450203555	101105	4.0540545959	5	30	Ormed	Erosion	
33	15	B	P	504803	114304	22.64328857	135863	26.914063506	5	30	Ormed		
34	19	B	M	562451	58879	10.4682896821	61509	10.9358859705	2	10	Sonicare	No damage	
35	19	B	P	335508	24144	7.1962516542	32606	9.7183971768	2	10	Sonicare	No damage	
36	19	L	M	698225	14125	2.0229868595	18954	2.71459773	2	10	Sonicare		
37	19	L	P	287842	2889	1.0036756276	8780	3.0502845311	2	10	Sonicare		
38	20	L	M	776529	45037	5.7997833951	14066	1.8113940368	2	10	Sonicare		
39	20	L	P	277687	1568	0.5646645324	8544	3.0768455131	2	10	Sonicare		
40	21	L	M	999880	4537	0.4537544505	16270	1.6271952634	3	10	Ormed		
41	22	B	M	336585	12517	3.7188228828	71179	21.1474070443	2	20	Oral B	No damage	
42	22	B	P	199216	2706	1.3583246326	28853	14.4832744358	2	20	Oral B	No damage	
43	22	L	P	415843	5472	1.315881234	14043	3.376995645	2	20	Oral B		
44	22	L	M	1053395	29336	2.7849002511	56123	5.3278209978	2	20	Oral B		
45	23	L	P	395834	13918	3.5161203939	12815	3.2374682316	2	20	Oral B		
46	24	L	M	860234	7	0.0008137321	21192	2.4635157411	2	20	Oral B		
47	24	L	P	346045	2217	0.6406681212	6748	1.9500354	2	20	Oral B		
48	25	L	P	361923	26119	7.2167284201	30946	8.550437524	2	20	Sonicare	Keratin layer detached	
49	25	L	M	977500	6369	0.6515601023	38668	3.9558056266	2	20	Sonicare		
50	26	L	P	415623	37089	8.9237121141	40819	9.8211600417	2	20	Sonicare		
51	26	L	M	1136525	49022	4.3133235081	60523	5.325267812	2	20	Sonicare	No damage	
52	27	B	M	561030	45358	8.0847726503	93983	16.7518671016	2	20	Sonicare		
53	27	L	M	901032	55318	6.1394045938	67681	7.5114979268	2	20	Sonicare		
54	28	B	P	388096	95104	24.5052770449	105180	27.1015418865	3	20	Ormed	Deep Erosion	
55	28	L	P	382617	34246	8.9504648251	18378	4.803236657	3	20	Ormed		
56	29	B	P	448711	80623	17.9676896711	104221	23.2267539686	3	20	Ormed		
57	29	L	P	577702	22133	3.8312140169	26305	4.5533856556	3	20	Ormed		
58	30	B	M	576347	102148	17.7233506898	146062	25.3427188829	2	10	Oral B	No damage	
59	30	B	P	370862	50824	13.7042889269	90274	24.3416688687	2	10	Oral B	No damage	
60	30	L	M	1341895	100683	7.5030460655	97749	7.2844000462	2	10	Oral B		
61	30	L	P	601784	49427	8.2134121213	60216	10.006248089	2	10	Oral B		
62	31	L	P	411649	38072	9.2486560152	24994	6.07167757	2	10	Oral B		
63	31	L	I	122043	8170	6.6943618233	19861	16.2737723589	2	10	Oral B	No damage	
64	32	L	P	432669	19387	4.4807924765	18929	4.3749378855	3	20	Ormed		
65	32	L	I	532697	11899	2.2337276163	9969	1.8714203384	3	20	Ormed	Superficial Erosion	
66	33	L	M	1170515	83863	7.1646241184	116506	9.9533965818	?	5	Airfloss		
67	34	L	M	1605128	33790	2.1051280646	56044	3.4915595516	?	5	Airfloss		
68	35	L	M	1131214	69707	6.1621408504	110270	9.7479345199	?	5	Airfloss		
69	35	B	M	1280304	119161	9.3072426549	108543	8.4779083718	?	5	Airfloss		
70	36	B	P	653905	119784	18.3182572392	161516	24.7002240387	?	5	Airfloss	No damage	
71	36	L	P	983636	25819	2.6248530961	106339	10.8108080631	?	5	Airfloss		
72	37	B	M	798983	40426	5.0596821209	77166	9.6580277678	?	5	Airfloss	No damage	
73	37	B	P	589412	37173	6.3067938895	72100	12.2325300469	?	5	Airfloss		
74	37	L	M	1016545	7336	0.7216601331	36661	3.6064315894	?	5	Airfloss		
75	38	L	M	899348	96410	10.7199882582	38329	4.2618652624	?	5	Airfloss		
76	38	L	P	404341	4409	1.0904162576	14311	3.53933932	?	5	Airfloss		
77	39	B	P	1281677	225963	17.6302609784	231583	18.0687489906	?	5	Airfloss		
78	39	L	P	934428	7870	0.8422264744	12402	1.3272290642	?	5	Airfloss		
79	40	B	M	1284024	28334	2.2066565734	25095	1.9544027214	100 (PSI)	3	Waterpik		
80	40	L	P	564109	38772	6.8731397655	21859	3.8749603357	100 (PSI)	3	Waterpik		
81	40	L	M	1216395	94351	7.7566086674	35939	2.9545501256	100 (PSI)	3	Waterpik	No damage	
82	41	L	P	873978	74401	8.5129145127	56319	6.4439837158	100 (PSI)	3	Waterpik		
83	41	B	M	1001506	27214	2.7173077346	25992	2.595291491	100 (PSI)	3	Waterpik		
84	41	B	P	543392	33278	6.124124021	15408	2.8355220541	100 (PSI)	3	Waterpik		
85	42	B	M	515212	10859	2.1076760634	52925	10.2724703617	100 (PSI)	3	Waterpik		
86	42	L	M	2053322	167950	8.1794282631	93218	4.53986272	100 (PSI)	3	Waterpik		
87	43	L	P	312153	2089	0.6692231053	47807	15.3152460492	100 (PSI)	3	Waterpik		
88	43	L	M	698769	214276	30.6647833547	50350	7.2055285795	100 (PSI)	3	Waterpik		
89	43	B	M	1202360	41301	3.4349945108	85540	7.1143417945	100 (PSI)	3	Waterpik		
90	44	L	P	1303416	250696	19.23376727	538647	41.3257931466	100 (PSI)	3	Waterpik	Ulceration	

11. Law statement

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Statutory Declaration

I declare that I have authored this thesis independently, that I have not used other than the declared sources / resources, and that I have explicitly marked all material which has been quoted either literally or by content from the used sources. I have not submitted this thesis in this or in a similar form in any other universities.

Date, place

Signature

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Eidesstattliche Erklärung

Ich versichere (an Eides statt), dass ich die zur Erlangung des Doktorgrades der Zahnheilkunde oder Medizin vorgelegte Dissertationsschrift mit dem Thema „Zahnbürsten und Mundduschen und deren Verletzungspotenzial der Gingiva“ selbständig und ohne fremde Hilfe angefertigt und die in der Arbeit verwendete Literatur vollständig zitiert habe.
Ich habe diese Dissertation weder in dieser noch in einer ähnlichen Form an einer anderen Hochschule eingereicht.

Ort, Datum

Unterschrift