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Minimally invasive therapy of a late diagnosed Dentinogenesis imperfecta

MTAD: Is it the right "solution"? – An overview

Detection of Matrix Metalloproteinases (MMPs) in the root dentin of human teeth

This journal is regularly listed in CCMED / LIVIVO.

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PRACTICE

RESEARCH

Title picture hint: From case report of Basel Kharbot and Falk Schwendicke, here (upper picture) figure 1a: Initial situation of the patient at the first consultation. The grayish to amber-colored staining of the teeth was particularly remarkable, although they were affected to differing degrees. The anterior mandibular teeth were greatly affected and also had enamel fractures, while, for example, teeth 11 and 21 were clinically unaffected. The patient had a slight midline shift to the right and (picture below) figure 7a: Frontal view 12 months after treating the patient, p. 137–143

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Does "CIOTIPlus" only mean "brushing twice"?

Caries and periodontitis are biofilm associated diseases with multifactorial causes (etiology). They are still among the most common diseases affecting the general population. A major factor in the development of oral disease is related to biofilm; this is why the efficient removal of biofilm, in addition to regular dental check-ups and nutritional guidance, plays a major role in the prevention of oral disease. The removal of biofilm is not just up to dental professionals [4], but rather, the principal responsibility of the patient through regular home-based oral hygiene [6].

Nevertheless, the quality of home-based plaque removal can be described as inadequate for large parts of the population. Many dental professionals recommend the "Modified Bass Technique" for the mechanical removal of plaque using the toothbrush. However, this technique is difficult to learn. In literature, for example, no evidence can be found indicating that this technique is superior to the "horizontal scrubbing technique" with respect to plaque removal [5, 19, 25]. When employing manual as well as electric toothbrushes, it is agreed that compliance with a system of brushing is more important than adherence to a particular technique [5]. The regular implementation of a certain brushing system prevents that teeth, or tooth surfaces, are not accounted for during home-based oral hygiene [20].

As early as 1948, Bass recommended a systematic approach for brushing teeth [3]. Especially because the oral surfaces of mandibular teeth often display more hard and soft deposits than other tooth surfaces and are evidently neglected during home-based oral hygiene [17], cleaning should begin with the inner tooth surfaces during tooth brushing [17, 18]. Yet, our observations [8, 9] together with the findings from other studies have shown that patients primarily clean the vestibular surfaces first [8, 12]. Van der Sluijs et al. (2018) could determine that, in terms of plaque reduction in young patients with periodontally healthy dentitions, there was no significant difference whether or not the patients cleaned the oral or vestibular surfaces first [23].

To date, there is no clear data in literature with respect to the duration and frequency of tooth brushing [2, 6]. However, a "twice-daily, two-minute" brushing is generally recommended. Studies have shown that brushing twice daily with fluoride-containing toothpaste has a higher caries-preventive effect and reduces caries incidence more than brushing once daily [6, 10, 13]. Additionally, it has been shown that more plaque removal occurs by increasing brushing duration and employing manual as well as electric toothbrushes [15, 24, 26]. It has been observed that tooth brushing for one and two minutes achieves an average plaque reduction of 27 % and 41 %, respectively [21].

The Department of Conservative Dentistry, Periodontology and Preventive Dentistry of the Hannover Medical School introduced the "IOCTIPlus" brushing system in 2007. However, based on clinical observations, this system was changed to "CIOTIPlus" in 2009. Using this system, the patient first brushes the **c**hewing, followed by the **i**nside and the **o**utside surfaces of the teeth with a toothbrush. Afterwards, the **t**ongue and the **i**nterdental spaces are cleaned with interdental hygiene tools. Following this cleaning procedure, the patient systematically brushes the tooth surfaces using circular/rotation movements again ("**plus**") with the same (pea-sized) amount of fluoride-containing toothpaste (Fig. 1). This brushing system is not a "double" brushing in the literal sense because the entire cleaning process is not repeated in the same manner. By reapplying fluoride-containing toothpaste, the tooth surfaces are mechanically cleaned once again on the one hand, while an additional fluoride dose is supplied on the other hand; indeed, the effect of fluoride is higher on clean, plaque-free tooth hard substance [11].

The individual steps of the brushing system and technique for dental and oral hygiene are explained in detail below:

Before beginning tooth cleaning, the patient should first rinse his/her mouth vigorously with water. This ensures that coarse, non-adherent food particles are already removed from the mouth. Patients also have very different saliva qualities. Thus,

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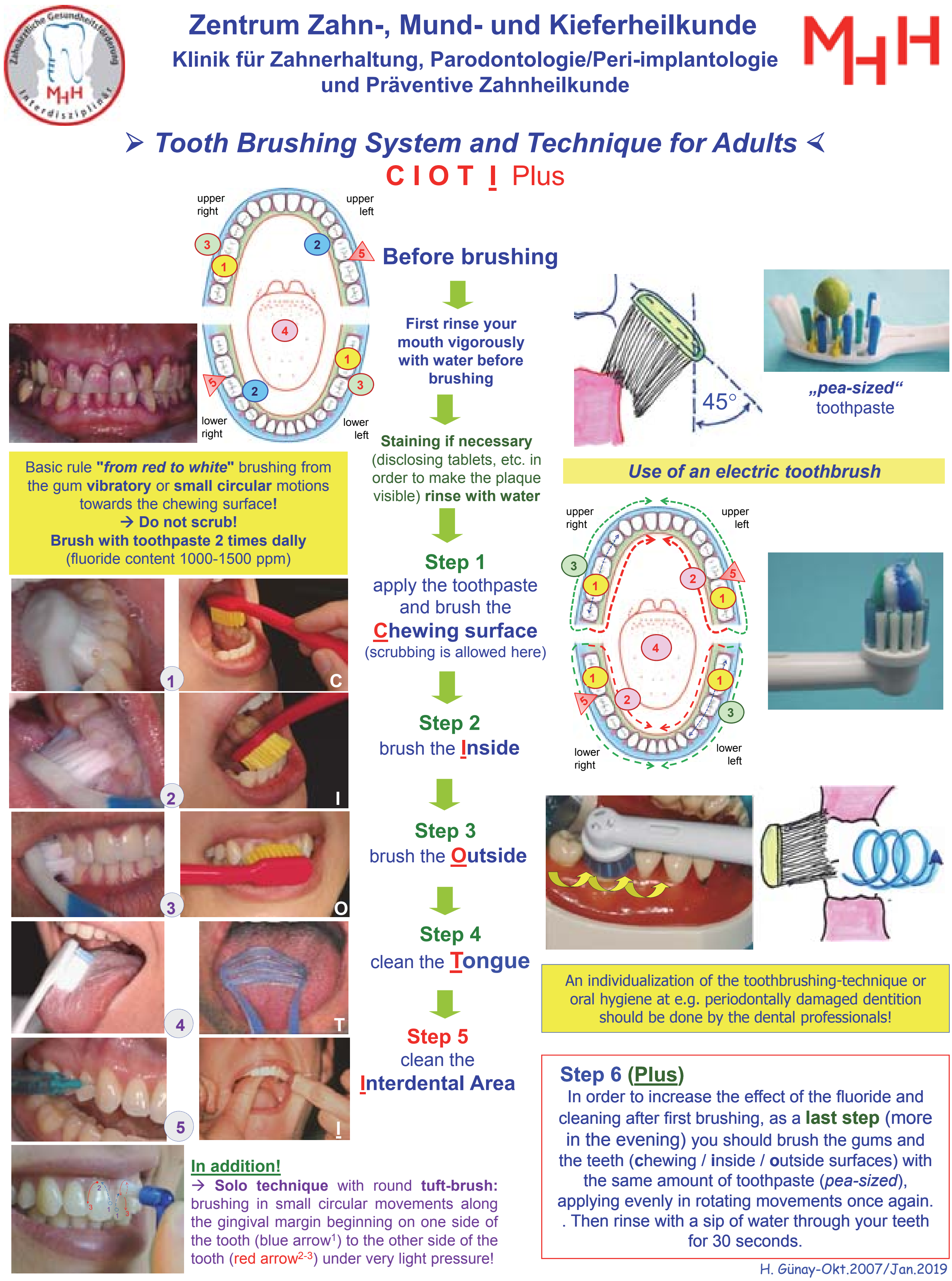
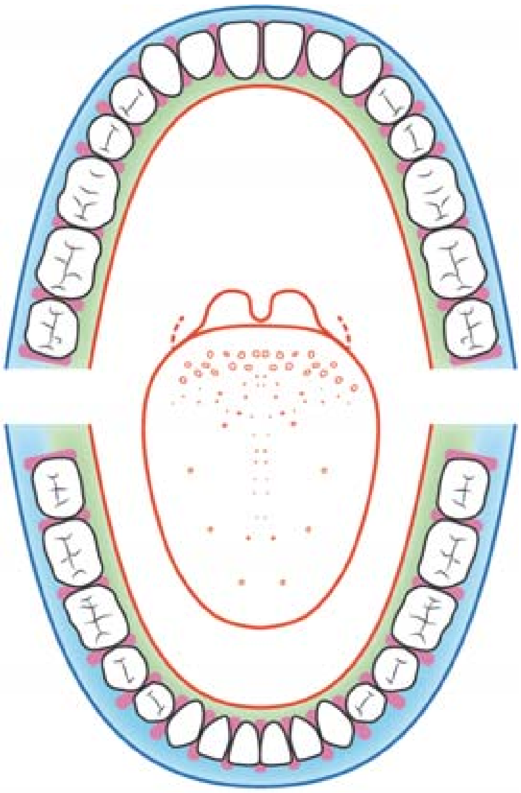


Figure 1 Tooth brushing system and technique for adults (CIOTI Plus).

Tooth Brushing System and Technique for Adults

C I O T I Plus

> Please treat the marked areas with special attention, clean them carefully and effectively! → → → →



We recommend the use of the following articles*

O Multitufted toothbrush (e.g. ………………………………………………………………………………………………)

O Single toothbrush (e.g. ………………………………………………………………………………………………)

O Unwaxed / waxed dental floss (e.g.…………………………………………………………………………………………)

O Superfloss-dental floss (e.g. ………………………………………………………………………………………………)

O Interdental brushes (e.g. ………………………………………………………………………………………………)

O Soft Picks (e.g. ………………………………………………………………………………………………)

O Tongue cleaner/scraper (e.g. ……………………………………………………… ……………………………………………)

O Electric toothbrush (e.g. ………………………………………………………………………………………………)

O Fluoride-containing toothpaste (e.g. ………………………………………………………………………………………)

O Toothpaste for sensitive tooth (e.g. ………………………………………………………………………………………)

O Fluoride gel (e.g. ………………………………………………………………………………………………)

O Fluoride rinsing solution (e.g. ………………………………………………………………………………………………)

O Chlorhexidine rinse solution (e.g. ………………………………………………………………………………………………)

O Plaque disclosing tablets (e.g. ………………………………………………………………………………………………)

O Mouth healing ointment (e.g. ………………………………………………………………………………………………)

O Others (…… ………………………………………………………………………………………………)

**All these items can be purchased in drugstores and / or pharmacies.*

Kindly yours

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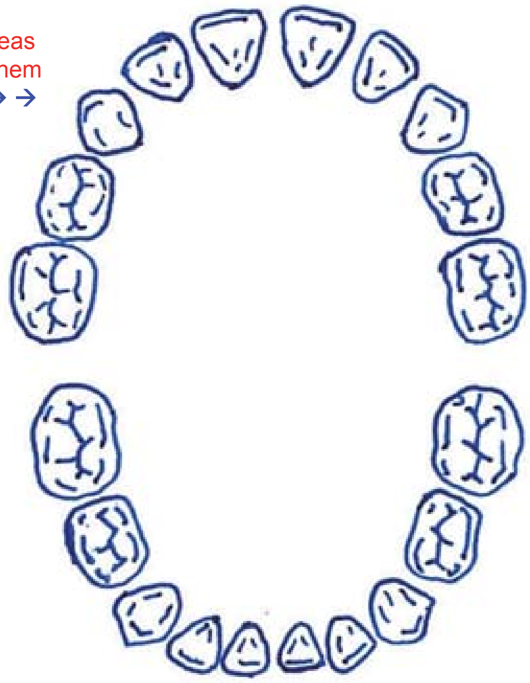
Figure 2 Tooth brushing system and technique for children (COI Plus).

Tooth Brushing System and technique for Kids - C O I Plus

Chewing surface – Outside – Inside – re-brushing

➢ Please treat the marked areas with special attention, clean them carefully and effectively! → →



We recommend the use of the following articles*

O Toothbrush	(e.g ....)
- training toothbrush	(e.g. ...)
- for Babys	(e.g. ...)
- for small children	(e.g. ...)
- for preschool children	(e.g. ...)
O electric toothbrush	(e.g. ...)
O Dental floss for children	(e.g. ...)
O Children's toothpaste	(e.g. ...)
O fluoride gel	(e.g. ...)
O Chlorhexidine rinse solution	(e.g. ...)
O plaque disclosing Tablets	(e.g. ...)
O Mouth healing ointment	(e.g. ...)
O Others	(...... ...)

*All these items can be purchased in drugstores and / or pharmacies.

Kindly yours

Department of Conservative Dentistry, Periodontology/Perio-Implantology and Preventive Dentistry
Carl-Neuberg-Str. 1, D-30625 Hannover, www.mh-hannover.de/269.html
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this step makes it easier for patients with very viscous saliva or dry mouth to brush their teeth afterwards.

In the **first step**, the patient spreads fluoride-containing toothpaste (fluoride content approx. 1450 ppm) on the chewing surfaces. These surfaces are cleaned simultaneously, as scrubbing (short back and forth movement) is explicitly permitted. The amount of toothpaste applied should be "pea-sized" (equivalent to at least 1 g toothpaste) [16]. The chewing surfaces are brushed before the inside surfaces because patients find it easier to begin brushing with the chewing surfaces, on the one hand, while on the other hand, the toothpaste at the same time gets distributed in the mouth as the chewing surfaces are brushed. Moreover, it has been shown that techniques and behaviors learned in childhood are often carried over into adult life according to the field of behavioral science [22]. Thus, a health-related behavioral change is difficult to achieve in adults [1]. Children are taught the "COI system" (brushing sequence: first chewing, followed by the outside and inside surfaces) at an early age, which likewise begins with the brushing of the chewing surfaces; this is why it is perhaps easier for adult patients to implement the system recommended here later in life.

In the **second step**, the brushing of the tooth inside surfaces is performed. The "basic rule" is to brush "from red to white". The patient performs brushing posteriorly either using vibratory (analogous to the "modified Bass Technique") or small circular movements (analogous to the "Fones technique"). However the "Bass Technique" is difficult for patients to learn, and not every toothbrush is suitable for the application of this technique, we recommend the use of small circular movements for our patients. In the area of the anterior teeth, the head of the brush is held vertically and a wiping and small circular movement (short back and forth movement) is performed. Both other studies [17] as well as our own observations [8, 9] have shown that the oral areas of the mandibular posterior teeth are especially neglected. Thus, in the lower jaw, patients should begin by brushing the inside surfaces of teeth from the last tooth on one side and then continue systematically, tooth by tooth, until they reach the last tooth on the opposite side. Afterwards, the inside surfaces of the upper jaw are brushed in a similar manner. In order to achieve the best possible result, an individualized, observation-based oral hygiene consultation should take place beforehand. By doing this, dental professionals can track patients' dental and oral hygiene using their own oral hygiene products. "Problematic areas" can in this process be identified and the patients can thereby be individually informed, motivated and instructed accordingly (iIMI).

In the **third step**, the outside surfaces are brushed. The basic rule "from red to white" applies here as well. The patient can either brush the tooth surfaces using vibratory motion based on the "modified Bass Technique" or use small circular movements based on the "Fones Technique". Here too, small circular movements are preferred. The patient

Figure 3a 63-year-old patient – 6 weeks after a supportive periodontitis therapy (SPT) – before visualization of the plaque

Figure 3b View after plaque staining (Mira-2-Ton, Hager & Werken, D-Duisburg) (t0: QHI = 3.32 and API = 100 %).

Figure 3c View after tooth brushing using CIOTI with a manual toothbrush without staining the plaque again.

Figure 3d View after brushing using CIOTI with a manual toothbrush and plaque re-staining (t01: QHI = 2.89 and API = 100 %).

63 year old patient	QHI-all	API% (mAPI)	UJ-vest	UJ-pal	LJ-vest	LJ-ling
t0	3.32	**100**	4.07	2.21	3.36	3.64
t01	2.89	**100**	3.07	2.07	3.14	3.28
t02	1.46	**86.54**	0.17	1.43	1.28	2.43
t1 after 10 days	1.02	**78.85**	0.71	1.57	0.50	1.28
t2 after 3 months	1.39	**90.38**	1.07	1.57	1.07	1.39
t3 after 6 months	1.35	**80.76**	0.71	1.42	1.43	1.71
t4 after 12 months	1.57	**90.40**	0.71	1.57	1.71	2.28
t5 after 18 months	1.08	**82.69**	0.35	0.43	1.64	1.93
t6 after 24 months	1.14	**56.25 (1.54)**	0.14	1.28	1.14	2.00
t7 after 30 months	1.28	**60.42 (1.77)**	0.07	1.43	1.21	2.42
t8 after 36 months	1.12	**52.08 (1.62)**	0.00	0.71	0.93	2.21

Table 1 Oral hygiene status (OHY) over 3 years: OHY was performed from t0 to t4 with a manual toothbrush, then with an electric toothbrush. Average QHI and API (mAPI = mod QH-API) at times t0, t01 and t02, and reduction of plaque index values at times t1–t8.
(Tab. 1: H. Günay and K. Meyer-Wübbold)

should begin to brush from the last tooth on one side of the lower jaw and then continue systematically, tooth by tooth, until the last tooth on the opposite side of the jaw is reached. Following this, the teeth of the upper jaw are brushed in the same manner.

After the smooth surfaces have been brushed, the tongue is cleaned in the **fourth step**. Depending on the amount of plaque and nature of the tongue surface, the patient can either use a special tongue cleaner/scraper or the same toothbrush. At least two (forward and backward) pulling strokes from dorsally to ventrally along the median sulcus and the lateral borders of the tongue can be employed to clean the tongue with a cleaner/scraper. With a toothbrush, the tongue can be brushed using three circular movements at the same area of tongue.

In the **fifth step**, interdental cleaning takes place at the end after the smooth surfaces and tongue have been cleaned. It should be clear to the patient that the cleaning of the interdental spaces must be carried out separately from brushing; it requires time and concentration as well as special hygiene tools. There exist various tools for cleaning the interdental spaces such as dental floss, interdental brushes, and Soft-Picks

Figure 4a View after brushing the teeth (CIOTI) and step "Plus" with a hand brush without re-staining the plaque.

Figure 4b View after brushing (CIOTI) and step "plus" with a manual toothbrush and re-staining the plaque (t02: QHI = 1.46 and API = 86.54 %).

Figure 5a Control after 10 days (t1: QHI = 1.02 and API = 78.85 %)

Figure 5b Control at 6 months before the SPT session (t3: QHI = 1.35 and API = 80.76 %).

Figure 5c Control after 12 months before the SPT session (t4: QHI = 1.57 and API = 90.40 %).

Figure 5d Control at 36 months prior to the UPT meeting (t8: QHI = 1.12 and API = 52.08 %).

(Fig. 1–5: H. Günay and K. Meyer-Wübbold)

for example. Not every hygiene tool is suitable for all of the interdental spaces. Within a dentition, interdental spaces vary in terms of width and shape. This implies that for an effective cleaning of the interdental spaces to take place, hygiene tools should be individually selected; consideration should not only be given to shape and size of the proximal spaces and the periodontal state, but also to user skill and acceptance. The recommended interdental tools should be demonstrated by dental professionals for proper use. For example, both approximal surfaces should be cleaned with two up and down movements using dental floss. The floss should be then removed as a loop out of the approximal space. When the interdental brushes and soft picks should be employed, after their insertion into the approximal space, each approximal surface should be cleaned using two horizontal brushing movements (according to the "X-Technique").

In a recent study, it was found that the cleaning of interdental spaces with dental floss before smooth surface brushing leads to more plaque reduction and fluoride concentration in the interdental spaces than when interdental cleaning was performed after the brushing of smooth surfaces [14]. Nevertheless, this aspect plays a rather minor role in the system described here. This is because another step ensues after the cleaning of the interdental spaces, whereby fluoridated toothpaste is once again applied, thus leading to a similar effect.

In the **sixth and last step** (usually in the evenings), in order to enhance the effect of fluoride and cleaning, the patient should again apply in circular/rotating movements a same amount (pea-sized) of fluoride-containing toothpaste evenly systematically (CIO) on all tooth surfaces using a toothbrush (about 1 minute). By applying fluoride-containing toothpaste once again, additional fluoride is supplied to teeth and the tooth surfaces are mechanically cleaned again. After this procedure, by taking a sip of water, the patient should dilute the toothpaste-saliva mixture (foam) in order to distribute this mixture throughout the mouth for 30 seconds, especially interdentally, and then spit it out.

"COIPlus System" for children – What does the "plus" mean here?

Analogous to the system described above for adults, we recommend the "COIPlus System" for children (Fig. 2). Firstly, the fluoridated toothpaste (fluoride content depends on the age of the child) is applied on the **c**hewing surfaces, whereby "scrubbing" is allowed in order to distribute the toothpaste in the oral cavity and at the same time to brush the chewing surfaces. The amount of toothpaste varies depending on the age of the child (e.g., rice grain, lentil, or pea size). Afterwards, the outside surfaces of the teeth are brushed; the child paints "circles on the **o**utside surfaces", corresponding to the "Fones technique". Subsequently, the tooth **i**nside surfaces are brushed with a wiping movement. "**Plus**" means that the parents make sure that the teeth are properly cleaned and re-brush the gums and teeth (chewing/outside/inside surfaces) with rotating movements and an age-appropriate amount of fluoridated toothpaste.

The goals of the "CIOTIPlus" and "COIPlus" systems are a more effective plaque reduction as well as improved fluoride supply to the tooth surface. The effectiveness of the CIOTIPlus system has already been proven in studies [7, 8]. Increased plaque removal on smooth and proximal surfaces in older patients with periodontally rehabilitated dentitions was attained using the CIOTIPlus system [8, 9]. Furthermore, in a long-term investigation on older patients it could be shown that root surface and crown margin caries formation could be minimized and periodontal conditions could be stabilized or improved through the use of this system in combination with efficient follow-up care after periodontal therapy [7]. The effectiveness of the CIOTIPlus system is shown in Figures 3 to 5 and Table 1 in relation to a patient's case.

Conclusion

A significantly improved plaque control/reduction is achieved using the described systems "CIOTIPlus" and "COIPlus." Yet, in order to recognize "problem areas" related to plaque control, and thus be able to successfully prevent caries and periodontal disease, it is absolutely necessary that each patient receives an initial individualized and observation-oriented dental and oral hygiene advice, together with information and instructions, as well as regular follow-up instructions and motivation.

Implementing a rigid time schedule for dental and oral hygiene is counterproductive. The generally recommended 2 minutes needed to carry out sufficient dental and oral hygiene is in most cases inadequate. Especially for patients with complete as well as those with periodontally compromised dentitions and extensive prosthetic restorations or other difficult dental situations (for example, crowding, fixed orthodontic appliances) more time is required. Rather than timing how long brushing should take place, we recommend that our patients perform oral and dental hygiene until all teeth, tooth surfaces, and tongue have been cleaned. Only in step 6 (plus), we advise our patients to brush for no longer than a minute. Moreover, we recommend that patients perform dental and oral hygiene twice daily. Since many patients are often under time pressure in the morning, dental and oral hygiene according to the system described above should be carried out particularly in the evening.

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PROF. DR. HÜSAMETTIN GÜNAY
Department of Conservative Dentistry, Periodontology and Preventive Dentistry, Hannover Medical School
Carl-Neuberg-Str. 1, 30625 Hanover, Germany
Guenay.H@mh-hannover.de

(Photos: Hannover Medical School)

DR. KAREN MEYER-WÜBBOLD
Department of Conservative Dentistry, Periodontology and Preventive Dentistry, Hannover Medical School
Carl-Neuberg-Str. 1, 30625 Hanover, Germany
Meyer-Wuebbold.Karen@mh-hannover.de

Basel Kharbot, Falk Schwendicke

Minimally invasive therapy of a late diagnosed Dentinogenesis imperfecta

Introduction: Hereditary dentin diseases are challenging in terms of both diagnostics and therapy, largely because the clinical presentation is highly diverse and all available treatment strategies lack supporting evidence. In the present paper, we report on a 25-year-old female patient with late-diagnosed dentinogenesis imperfecta (DI) in which the hard tissue defects were treated minimally invasively using direct adhesive restorations. Based on this case, the currently established classification of DI is reviewed critically and the selected treatment approach contrasted with alternative treatment strategies.

Methods: Hard tissue defects were restored using directly placed composite. The patient was followed up over 12 months.

Results: The direct and minimally invasive strategy that was selected allowed the restoration of teeth without additional hard tissue loss in a short time period and with limited costs. The functional and esthetic results were satisfactory.

Conclusion: A range of treatment strategies is available for managing DI. Nevertheless, all lack supporting evidence. For this specific case, the chosen strategy offered advantages over indirect restorations. The long-term prognosis remains unclear.

Keywords: Dentinogenesis imperfecta; minimally invasive therapy; composite restoration; Shields classification; interdisciplinary diagnostics

Department for Operative and Preventive Dentistry, Charité – Universitätsmedizin Berlin: Basel Kharbot, PD Dr. Falk Schwendicke
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Figure 1a Initial situation of the patient at the first consultation. The grayish to amber-colored staining of the teeth was particularly remarkable, although they were affected to differing degrees. The anterior mandibular teeth were greatly affected and also had enamel fractures, while, for example, teeth 11 and 21 were clinically unaffected. The patient had a slight midline shift to the right.

Figure 1b Intraoral view of the initial situation of 45. On the distal and occlusal surfaces the fracture gap can be seen in the mesiodistal direction.

Figure 1c Intraoral view of the initial situation of 47. The enamel fracture along the temporary filling can be seen distally. The cervical fracture margin was overgrown by gingiva.

Figure 1d Mandibular anterior teeth with considerable abrasions and enamel fractures of the central incisors.

Introduction

Dentinogenesis imperfecta (DI) represents one of the autosomal-dominant inherited dentin disorders and is characterized by clinically manifested pathological dentin structures. DI is rather rare with an estimated prevalence of 1:6,000 to 1:8,000 [2, 8, 6]. In the classification which was developed by Shields in 1973 and is still in use today, DI is differentiated from dentin dysplasia and divided into 3 types [2, 14]:

1. Type I is based on a mutation of the genes (COL1A1 and COL1A2) encoding collagen I. Type I is associated with osteogenesis imperfecta [10] whereby the dental anomalies are considered more of an accompanying disorder. The dentin is hypomineralized, resulting in frequent fractures of the enamel which is inadequately supported.
2. Type II and type III only affect the dentin whereby type III depicts the more severe form and its occurrence appears to be regionally very restricted (Maryland and Washington, DC, USA) [2]. Types II and III are not based on mutations in the collagen genes (as is the case with type I) but are instead based on genetic mutations in chromosome 4q22. The dentin sialophosphoprotein (DSPP) gene, which encodes the noncollagenous dentin matrix protein of the same name, is located on this chromosome [1, 2]. DSPP is primarily involved in the formation and organization of the dentin structures [9, 14]. DSPP can also be detected in the tissues of other organs such as bones, kidneys, liver, or lungs although its expression in the dentin is about one hundred times higher [8].

Clinically, the types of DI are characterized by a number of common characteristics which nevertheless vary across the types. The teeth have an amber to blue or gray color and are often significantly worn or have multiple fractures since the enamel easily detaches from the dentin [8, 12, 14]. This can be associated with a loss of the vertical dimension. The crowns also tend to have a rather bulbous anatomy.

Radiographs show partly or completely obliterated pulp chambers along with roots that are shortened and/or very tapered. These findings can involve both the primary and permanent dentition (DI–I). Sometimes, the primary teeth can be more severely affected than the permanent teeth (DI–II) [2, 9, 14]. Patients often complain about pain due to apical infections with no discernable cause.

Figure 2 Panoramic radiograph after the initial treatment of the patient. The lack of pulp chambers and canals, the bulbous crown morphology of the molars, and the pointed root anatomy of most teeth were particularly noticeable. Further (oral medical, periodontal) pathological findings were not detected.

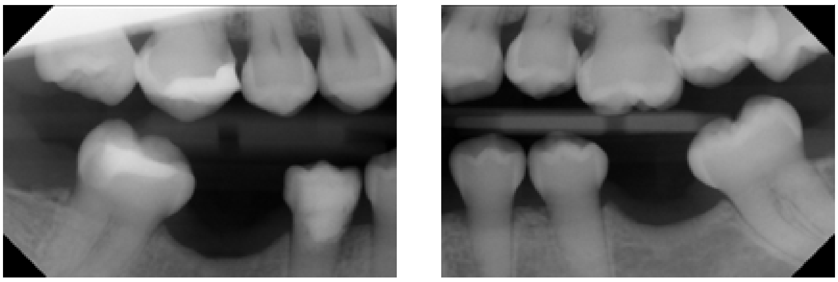

Figure 3a/b Bitewing images after the initial treatment of the patient. Along with radiopacity due to coronal restorations, the bulbous crown shape and extensive obliteration of the pulp chambers were noticeable. No carious lesions were detected. The dentin structures were homogeneous.

An established therapeutic concept is often recommended for the treatment of DI. This involves the insertion of preformed steel crowns of the first affected primary teeth. This aims to protect the teeth from occlusal stresses because of their susceptibility to fracture and to preserve the vertical dimension [2, 7]. Occasionally, the first permanent molars are treated with steel crowns until all permanent teeth have erupted to ensure that bite elevation develops properly. Ultimately, all affected teeth and (in most cases all teeth) are crowned in young adulthood to preserve or restore the vertical dimension and to protect against fractures [2]. In the present paper, we report on a female patient with late-diagnosed DI in whom the existing defects in the hard tissue were not treated in accordance with this concept, but instead using a minimally invasive technique.

Medical history

The 25-year-old patient presented initially due to acute pain in region of the lower right 5 (LR5, tooth 45). In her medical history, the patient stated “back problems”; the orthopedic diagnoses were hyperkyphosis, scoliosis, osteochondritis, and osteoporosis. In her dental history, the patient mentioned a “special situation” regarding her teeth which had not been precisely assessed to date by her attending doctor. In her childhood she had experienced traumatizing serial extractions of primary teeth. The lower 6-year molars were extracted shortly after eruption. Recently, when biting hard food, a tooth in her right mandible cracked. Since then the patient has had pain in region 45 but occasionally also “further back”. There are no similar cases in the family.

Findings

The patient did not have any pathological extraoral findings. The intraoral mucous membranes were also normal. During a visual-tactile examination the exceptional coloring of the dentition as well as two enamel fractures in the 4th quadrant were noticed (Fig. 1a). Tooth 45 showed a fracture gap in the mesiodistal direction with a loosened vestibular enamel fragment (Fig. 1b). Tooth 47 had been temporarily treated alieno loco with a glass ionomer cement filling and had a distolingual fracture

Figure 4a Status after removal of the vestibular fragment 45. A deep subgingival fracture margin was revealed. Soft dentin was surrounded by sound enamel.

Figure 4b Status after gingivectomy and initial removal of the soft tissue.

Figure 4c Status after restoration of 45 using composite. A retraction cord was used for relative moisture control.

Figure 5a Initial situation and fracture of tooth 47. The fracture margins were already overgrown by gingiva. The tooth had been treated alieno loco with glass ionomer cement.

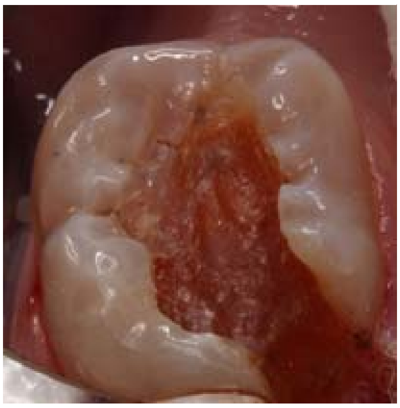

Figure 5b Removal of the temporary restoration revealed a deep subgingival fracture margin. Like for tooth 45, the dentin was rust-colored and softened.

Figure 5c Status after restoration with composite. The anatomical structure of the tooth could be restored.

(Fig. 1c). The cervical fracture margin could initially not be assessed. At this point, the mandibular anterior teeth were already affected by abrasion and the typical enamel fractures (Fig. 1d).

Sensitivity tests (using cold spray) in the molar region were uniformly negative, while in the premolar and anterior regions the tests were irregularly positive. Teeth 15, 11, and 21 reacted reproducibly positive. The PSI index was 1, 1, 1, 1*, 2, 1 and the BEWE index was 0, 0, 0, 0, 0, 0.

The patient's caries risk was assessed as low because of her good oral hygiene and, apart from 16, all existing restorations having been attributed to fractures (and not caries). Furthermore, the risk factor profile analysis was favorable (limited amount and frequency of consumption of fermentable carbohydrates, good oral hygiene, use of fluoride toothpaste, etc.). The reason for the extractions of the now missing teeth in the mandible was no longer known. The vertical dimension was preserved.

For the radiographic examination a panoramic radiograph (Fig. 2) and bitewing images (Fig. 3) were used. The characteristic features of DI such as bulbous crowns, pointed roots in some cases, and obliterated pulp chambers and canals were apparent; however, not all teeth were equally affected. Teeth 15 to 25 showed normal anatomy, for example, with clearly visible root canals. No apical translucencies often described in DI patients were detected.

The bitewing images also revealed the characteristic bulbous crown shape (Fig. 3). While the molars and premolars in the mandible did not have any radiographically visible root canals, the pulp chambers of the premolars in the 1st quadrant were properly visible and those in the 2nd quadrant were only partly obliterated. No carious lesions were detected.

Diagnosis and therapy planning

The patient was first informed about the tentative DI diagnosis. During the consultation possible complications and consequences for dental treatment were clarified and a preliminary treatment concept was prepared together with the patient. The issue of tooth preservation was a clear priority for the patient. The prognosis for tooth 45, for which a crownroot fracture with no pulp involvement was initially diagnosed, could only be assessed after removal of the fractured fragment. Likewise, the extent of the loss of dental hard tissue on tooth 47 was also only assessed during therapy. If the teeth

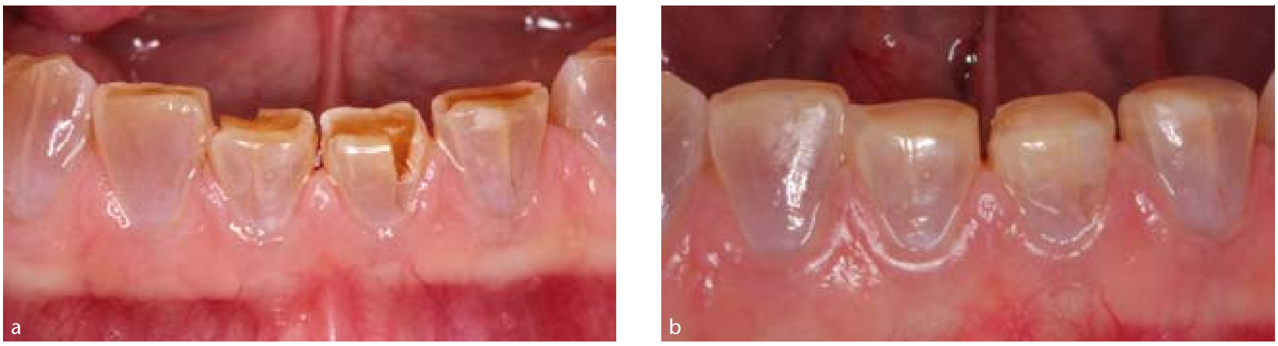

Figure 6 The mandibular anterior teeth initially showed abrasions and minor enamel fractures (**a**). These were treated restoratively with composite (**b**).

Figure 7a Frontal view 12 months after treating the patient

Figure 7b Lateral view 12 months after treating the patient

Figure 7c Maxillary image 12 months after treating the patient

Figure 7d Mandibular image 12 months after treating the patient

(Fig. 1–7: B. Kharbot)

were retainable, they were to be restored adhesively using composite.

The therapeutic goal defined with the patient was to preserve all existing teeth using non-invasive or minimally invasive procedures as far as possible. The very limited financial resources of the patient and her desire for prompt treatment were important criteria here. Further loss of hard tissue should also be avoided. The patient did not want to close the existing gaps in mandibular regio 36/46 that had been present for about 20 years. Despite the negative sensitivity tests, endodontic therapies were not initiated, particularly in light of the obliterations and the absence of radiographic abnormalities or symptoms.

Therapy

During the first treatment session, the vestibularly fractured fragment on tooth 45 was removed, revealing a deep subgingival crownroot fracture (Fig. 4a). The rust-colored appearance of the dentin, which was similar to that of carious dentin, was remarkable. Also, the hardness of the dentin was considerably lower than that of the healthy dentin, resembling the “leathery dentin” of carious lesions.

A gingivectomy was carried out vestibularly to expose and subsequently manage the fracture margins. Subsequently, the softened dentin on the visible surface was carefully removed (Fig. 4b). An effective adhesive bond could only be expected to a limited degree in the dentin area due to the structural defects. Hence, the aim was to achieve strong adhesion to the remaining enamel margins. On the mesial side, a transparent matrix was positioned with a wooden wedge for shaping the proximal areas. For the adhesive bond, 3-step etch-and-rinse system (Optibond FL, Kerr, Bioggio, Switzerland) was used and the restoration (SDR and ceram.x universal, Dentsply Sirona, Constance) was subsequently placed using a multi-layer technique. All restorations were cured for 20 sec for each increment using a polymerization lamp with a light intensity of 1,500 mW/cm^2. Figure 4c shows the restoration of 45 after completion.

On tooth 47 the gingiva was likewise removed to expose the fracture margin on the distolingual side. Again, a deep subgingival defect was revealed, this time in an area difficult to access. As was the case with tooth 45, this was not just an enamel fracture but rather an enamel dentin fracture (Fig. 5a). After removing the glass ionomer cement filling (Fig. 5b), the new restoration could be placed under relative moisture control (Fig. 5c). The procedure was analogous to the restoration of tooth 45. The exposed dentin again showed a rust-colored to brown appearance and reduced hardness.

A proper anatomical build-up in the anterior mandible was not possible, particularly for the central incisors, due to existing static and dynamic occlusal contacts. Therefore, the missing enamel areas were compensated within the now existing restorative extent (Fig. 6). In the approximal areas a transparent matrix was used where necessary. The incisal edges and surfaces were built up with a composite (ceram.x universal) after using the etch-and-rinse two-bottle adhesive system (Optibond FL).

The patient was then transferred to supportive therapy. Fluoridation with a 5 % NaF suspension (fluoridin N5, VOCO, Cuxhaven) and a visual-tactile examination of the restorations and all teeth were carried out every 3 months.

The obliterated or even missing pulp chambers and canals with the associated negative sensitivity tests are a known symptom of DI [2, 4, 9]. The affected teeth were regularly examined radiographically. Figures 7a–d show the situation 12 months after the restorative treatment of the patient.

Discussion

Both the diagnostic difficulties and the therapeutic approach that was selected will be discussed on the basis of the case presented.

The diagnosis of DI appears initially clear in this case. Despite a negative family history, the gray-brown tooth color and the presence of fractures of the hard tissue are typical features of DI. However, not all teeth were uniformly affected by the disorder. With an anomaly of the dentin caused by genetic mutation such as DI, it should be assumed that the entire dentition would be affected. Indeed, with the most common type DI–II all teeth are structurally changed without exception [2].

The radiographic diagnostics confirmed the symptoms of DI such as the bulbous crowns, shortened roots in some teeth, and obliterated pulp chambers and canals. Teeth 15 to 25 were not affected, however, and had clear pulp chambers and root canals.

In principle, hereditary dentin disorders such as DI or dentin dysplasia display considerable phenotypic variations, which can make diagnosis based on the clinically oriented Shields classification from 1973 difficult [2, 12]. As a consequence, clear differentiation of the mild DI type II from other dentin defects (dentin dysplasia I, II) is not always possible. Since mutations only in a single gene (DSPP) have been identified for all 4 isolated hereditary dentin disorders (DI II, II, DD I, II) to date, it may as well be the same disorder with varying clinical presentation. Hence, differentiating these 4 disorders on the basis of the phenotype is only limitedly possible and useful, in part because it remains unclear what therapeutic consequences would result from such a classification.

For the patient presented in this case report, the medical history (osteochondritis, osteoporosis) suggests the presence of DI in association with osteogenesis imperfecta (DI type I). Since autosomal recessive inheritance has been documented for mild cases of osteogenesis imperfecta (e.g., type IV), a negative family history does not necessarily allow to exclude this subtype of DI–I, which is the most serious symptom of the disorder [3, 12, 15]. Treatment of the DI-osteogenesis imperfecta combination should take place in close consultation with an internist.

Differential diagnoses must rule out hypocalcified forms of amelogenesis imperfecta, intrinsic discolorations (e.g., due to tetracycline exposure), or other dental development disorders such as rachitic defects [2]. Amelogenesis imperfecta could be ruled out, however, because the enamel overlaying the dentin defects was developed normally (see Fig. 4a). Similarly, tetracycline defects only affect the enamel and are characterized by horizontal bands, the localization and extent of which depend on the time of exposure and the corresponding status of the amelogenesis. Such typical involvement of the enamel was not encountered in this case. Symptoms of ongoing systemic disorders (rickets) were also not confirmed.

The therapeutic approach used here deviates considerably from the “conventional” therapeutic concept, in part because in most other cases an early diagnosis is made (these cases are often more severely affected and require a different therapeutic strategy than that selected for the patient presented here). Additionally, even the tissue-preserving preparation for modern ceramic crown restorations inevitably causes loss of healthy tooth structure, particularly enamel (which represents the only healthy tooth structure here). Furthermore, complications such as unwanted chipping of the existing enamel during cementation of indirect restorations has been reported, which would then further complicate restoration of the teeth [6].

A minimally invasive therapeutic approach using direct adhesive restoration was instead selected for this patient because the DI, which was also only recently diagnosed, was a mild form. The selected approach is characterized by its reversibility (preserves fall-back options, crowns remain possible), its tissue preservation, and the low costs and short time required (the latter were important aspects for the patient).

However, this concept also involves risks and an unclear prognosis: DI dentin has a considerably lower hardness than healthy dentin [11]; the microstructure of the dentin is also pathologically changed (the tubules and collagen network do not develop normally). DI dentin is also less densely mineralized than healthy dentin. The adhesive bond is therefore largely reduced by a restricted hybrid layer; the adhesion values for conventional adhesives are considerably lower for DI dentin than for healthy dentin [6]. Since the enamel structure remains unchanged, however, reliable adhesive bonding to the enamel margins is possible for defects surrounded by enamel (as was the case with this patient, at least in the coronal area). Overall, both the adhesive bond and the mechanical support of the adhesive restorations, however, were likely to be limited. The restorative treatment of the deep subgingival fracture margin was also difficult. Both moisture control and adaptation of the composite material on these margins proved to be challenging. Should there be a need for further treatment in these areas, e.g., due to a fracture or loss of the restoration or secondary caries, an indirect restoration or further pre-restorative surgical measures may be unavoidable.

Conclusion

Diagnosis and treatment of inherited dentin defects represent a challenge. Firstly, because these disorders are rare and consequently individual dentists have little daily experience with them. Second, the etiology and pathogenesis are little understood and accordingly, causal or biologically based therapies are not possible. Lastly, there are only limited studies supporting any diagnostic and therapy concepts. For the patient presented here, the DI was treated minimally invasively. The long-term prognosis of the placed restorations and the unrestored "DI teeth" is unclear and must be considered to be moderate at best since both the adhesion and the support of the restorations and the enamel coating by the underlying dentin structure were compromised. As part of a continuous supportive therapy, however, the selected therapy concept may represent a useful alternative to more elaborate and invasive treatments.

Conflicts of interest:

The authors declare that there is no conflict of interest within the meaning of the guidelines of the International Committee of Medical Journal Editors.

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BASEL KHARBOT
Charité – Universitätsmedizin Berlin, corporate member of Freie Universität Berlin, Humboldt-Universität zu Berlin, and Berlin Institute of Health
Department for Operative and Preventive Dentistry
Aßmannshauser Str. 4–6
14197 Berlin Germany
basel.kharbot@charite.de

(Photo: private)

PRIV.-DOZ. DR. FALK SCHWENDICKE DDS PHD MDPH
Charité – Universitätsmedizin Berlin, corporate member of Freie Universität Berlin, Humboldt-Universität zu Berlin, and Berlin Institute of Health
Department for Operative and Preventive Dentistry
Aßmannshauser Str. 4–6
14197 Berlin Germany
falk.schwendicke@charite.de

Maria Dede, Julia Timpel, Jasmin Kirsch, Christian Hannig, Marie-Theres Weber

MTAD: Is it the right “solution”? – An overview

Introduction: A plethora of chemical irrigants exists that is used for the elimination of residual microbes and endotoxins in root canals accompanied by mechanical instrumentation. However, due to complex root canal systems, the mechanical instrumentation and established irrigants still reach their limits and pathogenic microorganisms remain. Therefore, new irrigants with a wider spectrum are required.

Materials/Methods: The aim of this article was to summarize and discuss the available information concerning the irrigant MTAD (mixture of tetracycline isomer, acid and detergent) introduced in 2003 with its wider spectrum regarding antibacterial properties and the ability to remove the smear layer. A literature search was performed from English-language papers published until September of 2018.

Results: This review of literature focused on particular advantages of MTAD such as the antimicrobial efficacy, the ability of removing the smear layer and the effect on dentine structure. Also disadvantages were found, regarding the effect of MTAD on the dentine degeneration and the sealing properties of root-end filling materials.

Conclusion: MTAD could represent an effective complementary irrigant, specifically in combination with NaOCl. Further research is required to establish MTAD as a potent irrigant against more bacterial species, not only against Enterococcus faecalis.

Keywords: endodontic final irrigant; E. faecalis; MTAD; smear layer

Introduction

The main aim of the root canal treatment is to disinfect the root canal system, which requires the elimination of microorganisms and microbial components by instrumentation, irrigation and obturation of the root canal as well as the prevention of its re-infection during and after root canal treatment [9].

Many chemical irrigants have been used for the elimination of residual microbes in root canal systems accompanied by mechanical instrumentation [8]. NaOCl has been demonstrated to be an effective disinfectant and is widely accepted as the gold-standard irrigation solution [8, 11]. An irrigant should be able to disinfect, penetrate the dentine and its dentinal tubules, offer long-term antibacterial effects (substantivity), remove the smear layer, neutralize the endotoxins derived from the bacterial lysis [40, 65]. At the same time, an irrigant needs to be non-antigenic, non-toxic and non-carcinogenic [65]. However, at present, no single irrigant combines all these ideal characteristics, not even NaOCl. Considering the limitations of the well-established irrigants in Endodontics, new irrigants have been introduced that could possibly fulfill the ideal requirements. MTAD as an antibacterial irrigation solution showed promising results in several studies and has the potential to be an effective endodontic irrigant [51].

Methods

A comprehensive English literature search by Medline and by hand search was performed with the clos-

Policlinic of Operative and Pediatric Dentistry, Medical Faculty Carl Gustav Carus, TU Dresden: Maria Dede, D.M.D., Julia Timpel, D.M.D., Dr. Jasmin Kirsch, Prof. Dr. Christian Hannig, Dr. Marie-Theres Weber

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ing date of September 11, 2018. The "free text" search string [MTAD] AND [root canal treatment] was used for the survey by hand search in the internet and by Medline using PubMed. In addition, a reference survey of all the relevant papers, 82 articles in total, was conducted. No relevant articles were rejected for the present review of literature.

ELEMENT	CONTENT
Doxycycline	3 %
Citric acid	4,25 %
Polysorbate 80 (Tween 80)	0,5 %

Table 1 Composition of MTAD according to Torabinejad et al. [58]

Properties of MTAD

MTAD (Mixture of Tetracycline isomer, Acid, Detergent) is an irrigant introduced in 2003 by Mahmoud Torabinejad of Loma Linda University (California, U.S.A). MTAD is a mixture of doxycycline, citric acid and polysorbate 80 detergent [56] (Tab. 1).

The combination of a broad-spectrum antibiotic and the demineralizing agent citric acid as a chelating factor ensures inhibition of bacterial growth and removal of the smear layer. In addition, a polysorbate detergent decreases surface tension thus improving the likelihood of reaching bacteria in the ramifications of the main root canal [58]. MTAD is commercially available as a 2-part mixture (Bio pure MTAD; Dentsply, USA), consisting of powder and liquid (Fig. 1).

1. Antimicrobial activity

Doxycycline is the main component of MTAD. It does not show bactericidal properties, but it prevents the multiplication of susceptible bacteria [5]. This bacteriostatic property may be advantageous as there are no antigenic products (endotoxins) released due to the absence of bacterial cell lysis [58]. The antimicrobial effect of MTAD has been largely attributed to the presence of doxycycline. However, the substitution of doxycycline by chlorhexidine significantly reduces the efficacy of the irrigant [50].

Based on the results of the *in vitro* study of Torabinejad et al., MTAD has a strong antimicrobial efficacy against *E. faecalis* compared to 5.25 % NaOCl and 17 % EDTA, without affecting the dentine structure of human teeth [38]. These results seem important as they demonstrate the efficacy of an irrigant to inhibit this bacterial strain, which has been shown to be resistant to many commonly used intracanal irrigants and dressings [38]. *E. faecalis* is present in many failed root canal treatments [15] and is the most commonly reported bacterial species present within the canals of non-healing cases and asymptomatic infected roots [42, 52, 54]. This bacterium has a high capability to form biofilms [28]. Furthermore, *E. faecalis* seems to be less sensitive than other bacteria to the exposure of NaOCl [44]. Thereby, the time of exposure and concentration of NaOCl play an important role to inhibit the growth of *E. faecalis*. The longer the time of exposure and the higher the concentration of NaOCl, the better the disinfection abilities of NaOCl regarding *E. faecalis*. These findings seem to correlate with the resistance of the *E. faecalis* to NaOCl [44].

Several in vitro studies have demonstrated the antibacterial action of MTAD against *E. faecalis*, both in biofilm and in the planktonic phase, when used as the only irrigant in root canals of human teeth [13, 38]. Specifically, an in vitro study stated that MTAD, as the only irrigant, had a wider range of microbial inhibition against *E. faecalis* when compared with 2 % CHX and 5.25 % NaOCl [13]. Furthermore, another study showed that MTAD with maximum antibacterial activity is more effective against *E. faecalis* than 3 % NaOCl, 2 % CHX and 0.12 % CHX [31].

Two research groups, Torabinejad et al. (surface disinfection) and Siren

Properties	NaOCl	EDTA	CHX	MTAD
Biocompatibility	-	+	+	+
Ability to dissolve pulp tissue	+	-	-	-
Antimicrobial activity	++	-	+	+
Ability to remove smear layer	-	+	-	+
Dentine degeneration	+	+/-	-	+/-
Inhibition of *E. faecalis*	+	-	+	+

Table 1 Properties of the irrigants in endodontics in comparison to MTAD (CHX = chlorhexidine; NaOCl = sodium hypochlorite; EDTA = Ethylenediaminetetraacetic acid)
(Tab. 1 and 2: M. Dede)

et al. (dentine block disinfection) documented that *E. faecalis* can be eliminated from the dentine by following a protocol using MTAD in combination with NaOCl [49, 58]. Different in vitro studies showed that MTAD as a final irrigant in human teeth was effective in completely inhibiting the growth of *E. faecalis* [1, 38, 49].

In an *in vivo* study with human primary teeth, 2.5 % NaOCl and BioPure MTAD, both irrigants were equally efficient against *E. faecalis* [60]. The aim of a present *in vivo* study was to evaluate the reduction in bacterial loading with the sterile paper point method using MTAD as an irrigating solution in pulpectomized primary teeth. The results showed that MTAD could serve as an alternative irrigant for pulpectomy of necrotic teeth compared to 1 % NaOCl [18].

Unfortunately, not many studies exist which examined the effectiveness of MTAD against other bacteria except for *E. faecalis*. According to Misuriya et al., the MTAD solution showed maximum antibacterial activity and was effective against *E. faecalis*, *F. nucleatum* and *P. anaerobicus*. In case of *C. albicans*, MTAD was less effective than 5 % NaOCl, 3 % NaOCl, 2 % CHX and 0.12 % CHX [31]. After mechanical treatment and irrigation of root canals with MTAD solution, *P. intermedia*, *P. gingivalis* and *T. forsythensis* were not found in an *in vitro* study with mixed infection in root canals [32]. Regarding the antifungal activity of MTAD against *C. albicans in vitro*, 1.3 % NaOCl and 2 % CHX were equally effective and significantly superior to MTAD and Tetraclean [35].

In 2004, Luciano Giardino patented an irrigant prototype, also antibiotic-based, called Tetraclean. It is similar to MTAD, although with differences in the components. It also contains citric acid and doxycycline, although the amount of doxycycline is reduced to a third (50 mg/5 ml vs. 150 mg/5 ml of MTAD). Additionally, Tetraclean contains Polypropylene glycol instead of Polysorbate 80 and the antiseptic cetrimide [22].

Finally, it is worth mentioning that MTAD and CHX have a residual antimicrobial activity with various lasting times. This effect duration of residual antimicrobial activity is better in MTAD [60]. The substantivity of MTAD *in vitro* was significantly greater than that of 2 % CHX and 2.6 % NaOCl. The substantivity of 100 % MTAD was significantly higher against bacterial biofilms than lower concentrations (10 % and 1 %) of MTAD [33, 34].

2. Ability to remove smear layer

It is widely known and proven that the presence of organic tissue and smear layer reduces the effectiveness of antimicrobial protocols [23, 43]. The lack of increased antimicrobial efficacy may be due to the inability of NaOCl to remove the smear layer and to penetrate into dentinal tubules once the smear layer has been removed. Findings from studies of Torabinejad et al. have shown that the removal of the smear layer with a chelating solution (EDTA) with no antibacterial effect does not enhance the antibacterial effect of NaOCl. Otherwise, the combination of 1.3 % NaOCl as a root canal irrigant and MTAD as a final rinse was significantly more effective than the combination of 1.3 % NaOCl with 17 % EDTA [50, 58].

Using the scanning electronic microscope (SEM) Torabinejad et al. demonstrated that MTAD is a valid solution for the removal of the smear layer, which does not significantly change the structure of the dentinal tubules when used as a final irrigant in conjunction with 5.25 % NaOCl.

The changes in temperature at 4°C, 25°C, and 37°C play a specific role regarding the efficacy of MTAD for removing the smear layer. In a recent study, it was concluded that using 17 % EDTA and MTAD at 25°C and 37°C was more potent than using the solutions at 4°C temperature, even in the apical level of the root canal [12].

Using MTAD with ultrasonic agitation reduced the smear layer [26]. Based on the results of the SEM data in a recent study, 1.3 % NaOCl and MTAD combined with endosonic irrigation, acted synergistically to remove the smear layer and debris, while causing less erosion on the dentine surface [26]. In comparison to other solutions, MTAD has demonstrated improved results by removal of the smear layer on primary root dentine [7].

MTAD as a final rinse after the entire instrumentation with 3 % NaOCl provided the best cleaning ability in all parts of the root canal system and had good results particularly in cases of teeth with established infection in the apical part of the root canal [25]. In this study, the dentinal wall of the cervical, middle and apical thirds were observed at magnifications of up to ×1000 for the presence/absence of smear layer under SEM [25]. On the contrary, 2 studies summarized that the efficacy of 17 % EDTA as final rinse for the removal of the smear layer was better than MTAD [27, 61]. In addition, based on the outcomes and limitations of an *ex vivo* investigation, SmearClear (Sybron Endo; Orange, CA), a 17 % EDTA solution containing a cationic (cetrimide) and an anionic surfactant, was found most efficient in removing the smear layer in all regions of the root canal followed by MTAD [63].

Additionally, another present study demonstrated that chitosan (natural polysaccharide, derived from the deacetylation of chitin) was more effective in smear layer removal than MTAD, especially in the apical third of the root canal [67].

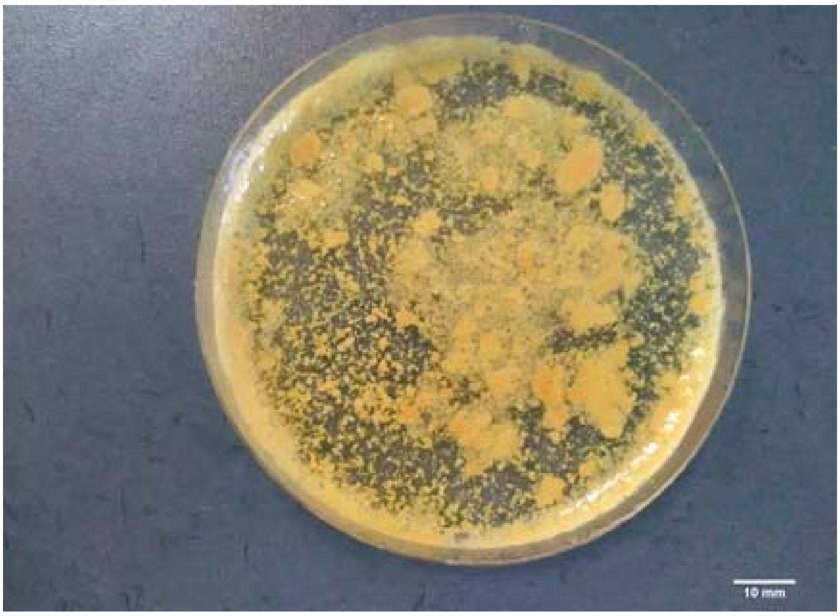
3. Effect on dentine structure

Chemicals used during endodontic treatment play an important role as they may lead to alterations of the root dentine and changes its chemical and physical properties. Pashley et al. stated that the tubular density affects the microhardness of the dentine surface – as the tubular density increases the dentine microhardness decreases, respectively [14, 41].

De Deus et al. and Sayin et al. examined the effect of endodontic irrigation solutions on root canal dentine surface and stated that there is a significant decrease of the microhardness in the dentine of the root canal after the application of EDTA [14, 48].

In accordance with Torabinejad et al. 1 % NaOCl preceding MTAD can dissolve the organic portion of the smear layer that covers the dentinal tubules after instrumentation. Due to the citric acid, MTAD is an acidic solution (pH 2.15) and is capable of removing inorganic substances. This allows MTAD to dissolve the inorganic portion of the smear layer, penetrating into dentinal tubules, and decalcifying them without affecting the structure of dentine [58]. An *in vitro* study by Pappen et al. concluded that the detergent of MTAD can additionally decrease the surface tension and increase the penetrating ability of MTAD, without affecting the structure of dentine, too. The samples were observed by confocal laser scanning microscopy (CLSM) after bacterial viability staining [40]. In an investigation of Machnick et al., there was no significant difference regarding the flexural strength and modulus of elasticity between the dentine bars exposed to saline or to MTAD [29]. Aranda et al. revealed significant dentine microhardness reduction promoted by (Biopure) MTAD and 17 % EDTA. Only the use of 17 % EDTA promoted dentinal tubules erosion *in vitro* [3].

The study of Machnick et al. compared the effect of MTAD and phosphoric acid on the bond strength to enamel and dentin using a conventional OptiBond Solo Plus dentine adhesive system. They reported that teeth treated with a MTAD protocol (20 min 1.3 % NaOCl/5 min MTAD) might not need any additional dentine conditioning before the application of the adhesive system [29]. Regarding the effect of MTAD on dentine bonding, some studies concluded that MTAD can significantly improve the resin-dentine bond stability due to its broad-spectrum matrix metalloproteinase-inhibitory effect [20, 29, 36]. Furthermore, studies stated that MTAD as *final rinse* influences the sealing properties of root-end filling materials negatively, like other chelator factors (EDTA) do as well. Leakage of root-end filling materials increased when 17 % EDTA or MTAD were used for irrigation [16, 37, 46]. A recent study concluded that the highest leakage rate was observed in the 17 % EDTA and MTAD groups when MTA, Portland cement, and Bioaggregate (BA) root-end filling materials were used [10]. Saghiri et al. showed in an *in vitro* study that MTAD as final irrigation lowered this push-out bond strength of MTA-dentine contact surfaces and thus affects it negatively [47].



(picture in laboratory, Faculty of Medicine Carl Gustav Carus, Dresden, Germany)

Figure 1 Viscosity of MTAD-fluid in a plate, a mixture of powder and liquid

4. Biocompatibility

Focusing on biocompatibility, MTAD as a final rinse for the removal of the smear layer did not cause postoperative discomfort after root canal treatment in 73 patients, according to a randomized clinical trial [59]. In this study, 1.3 % NaOCl and MTAD as endodontic irrigants were compared with irrigation using 5.25 % NaOCl and 17 % EDTA [59].

Regarding the cytotoxicity of MTAD, a study by Zhang et al. evaluated the viability of cultured fibroblasts after storage in MTAD and other standard irrigants. MTAD is less cytotoxic than eugenol, H_2O_2, Ca $(OH)_2$ paste, 5.25 % NaOCl, Peridex (CHX mouth rinse with additives) and 17 % EDTA, but more cytotoxic than 2.63 %, 1.31 % and 0.66 % NaOCl [66]. Bajrami et al. demonstrated that irrigating solutions should be used at lower concentrations to enhance cell viability. In this study incubation of rat ligamental fibroblasts in 0.1 ml/L concentrations of 3 % NaOCl and MTAD was only moderately cytotoxic, whereas 2 % CHX was highly deleterious to cell viability at this concentration. At 100 ml/L (high concentration) MTAD as well as 3 % NaOCl generated high levels of cytotoxicity to the fibroblasts [6]. Another present study also demonstrated that MTAD was less cytotoxic compared to NaOCl, CHX, QMix and EDTA [24]. This finding is in accordance with the results separately reported by Yasuda et al. and Zhang et al. that stated higher biocompatibility of MTAD in comparison with NaOCl 5.25 % and EDTA 17 % [64, 66].

Doxycycline (tetracycline) as a part of MTAD could cause permanent tooth discoloration when treatment occurs during teeth development. Tetracycline products should be avoided by young children with evolving teeth or by pregnant women, respectively [30]. NaOCl has been proven to react with MTAD thus triggering light brown discoloration by oxidizing the doxycycline component of MTAD [53, 55, 58]. This reaction may be affected by the release of doxycycline, which will be exposed to NaOCl if it is used as a final rinse after MTAD [58]. Clinically, this can be avoided by neutralizing the oxidizing action of NaOCl with a rinse of ascorbic acid before MTAD is applied into the root canal [55]. It is also worth noting that the chemical reaction between NaOCl and citric acid leads to the formation of white precipitates [2]. These interactions between NaOCl and MTAD require further investigations to validate the safety and usefulness of this combination [2].

Some studies were performed focusing on the regenerative aspect of MTAD and other irrigating solutions. MTAD was used for the regeneration of immature permanent non-vital teeth in dogs [4]. EDTA showed significantly more growing tissue in the pulp and less inflammatory cells than MTAD. The use of collagen as a scaffold material and MTAD as a surface modifier did not improve the quality of the regenerative process [4]. Considering the cytotoxicity of different irrigants on the survival of stem cells of the human apical papilla, MTAD showed the highest cytotoxicity to stem cells compared to 17 % EDTA, QMix, 5.25 % NaOCl, 2 % CHX and sterile saline [17]. The table summarizes the properties of the different irrigants in comparison to MTAD (Tab. 2).

Discussion

Since its introduction by Torabinejad et al., MTAD has been in the focus of attention as an alternative root canal irrigant. Therefore, the publications of Torabinejad et al. should be taken into consideration carefully. The presence of doxycycline in MTAD suggests that MTAD may have some substantive antimicrobial action [58]. Doxycycline, as a hydroxyl derivative of tetracycline, is the most potent anticollagenase antibiotic among commercially available tetracyclines [22]. However, the exact antimicrobial action of MTAD has not been fully understood.

The initial studies of Torabinehad et al. conducted on MTAD showed good antimicrobial activity especially against *E. faecalis* [58, 38], but *E. faecalis* is not the only dominant bacteria. Findings of the study of Rôças et al. confirm the strong association between persistent intraradicular infection and posttreatment apical periodontitis [45]. Most cases harbored a mixed infection and an increasing concern arose that the cooperative behavior of a multi-species biofilm facilitates persistence after exposure to an antimicrobial agent [21, 45]. It has been indicated that MTAD is somewhat effective against bacterial biofilms. However, it cannot disrupt or dissolve biofilms completely [33]. Actually, not many studies examined the effect of MTAD against other bacteria species than *E. faecalis*. However, the difference of the studies in methodology and microbial sampling procedures play an important role, too. Most of the studies were based on the paper point sampling method for the analysis and quantification of bacteria, which is controversially discussed by scientists.

The bactericidal activity of MTAD as the only intracanal irrigant requires improvement. Attempts have been made to enhance the effectiveness of MTAD. In 2013, a new ingredient was added to MTAD. Nisin, an antibacterial peptide, was investigated in an *in vitro* study to show whether it increased the antibacterial and antibiofilm activities of MTAD against clinical isolates of *E. faecalis*. The results clarified that MTADN (Mixture of Tetracycline isomer, an Acid, an Detergent and Nicin) effectively inhibited both the growth of *E. faecalis* root canal isolates and the biofilm isolates [56, 57].

As another important aspect, MTAD showed improved results in combination with NaOCl in the removal of the smear layer during the root canal treatment when compared to EDTA and NaOCl, respectively [25, 38, 50]. Furthermore, recent studies for MTAD showed good disinfection results when used as a final rinse in combination with NaOCl and agitated with ultrasonic [25, 26]. However, regarding the clinical relevance, the cost of the material, the availability and the clinical feasibility as a 2-part mixture, MTAD seems to be disadvantageous compared to EDTA. Clinical studies are required to establish the solution as a more practicable material in clinical practice.

Biocompatibility and the effect of MTAD on bond strength to enamel and dentine are widely accepted, but discussed controversially [19, 51]. Regarding the effect of MTAD on stem cells as well as endodontic tissue regeneration, further research may potentially lead to unforeseen results. However, MTAD as a surface modifier did not improve the quality of the regenerative process in root canal [4, 17].

Conclusion

In conclusion, MTAD represents an effective final irrigant for the removal of the smear layer in combination with NaOCl. Promising data considering the antimicrobial activity of MTAD especially against *E. faecalis* exist, but further research is required to establish MTAD as a potent irrigant against other bacterial species and biofilms. Furthermore, the effects of MTAD on the sealing properties of root-end filling materials as well as its effect on dental stem cells in regenerative endodontics are still controversially discussed.

In general, MTAD seems to be a promising additional irrigant within an irrigation protocol. No solution exists that is able to ensure complete disinfection, yet. Therefore, the combination of irrigants along with proper instrumentation of the root canal has probably a much higher impact on the treatment outcome than one single irrigant.

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Conflicts of interest:
The authors declare that there is no conflict of interest within the meaning of the guidelines of the International Committee of Medical Journal Editors.

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(Photo: Carl Gustav Carus Dresden)

MARIA DEDE, D.M.D.
Policlinic of Operative and Pediatric Dentistry, Medical Faculty Carl Gustav Carus, TU Dresden
Fetscherstrasse 74; 01307 Dresden
Maria.Dede@uniklinikum-dresden.de

Karen Meyer-Wübbold, Hüsamettin Günay

Effectiveness of CIOTIPlus depending on different toothbrushes on approximal cleaning

Introduction: Oral health awareness has increased significantly in recent years and the self-responsible home-based oral hygiene has been given a high priority in the prevention of tooth decay and periodontitis. A large number of different toothbrush systems for plaque removal are available to the patients. However, since many patients are still affected by caries and inflammatory periodontal diseases, the quality of home-based plaque removal appears to be rather inadequate. The aim of the present pilot study with cross-over design was to investigate the cleaning effect on the approximal areas by brushing twice in accordance with the CIOTIPlus system using different toothbrushes.

Methods: Fifteen participants (7 female, 8 male, mean age 50.1 ± 6.5 years) were included in this study with split-mouth design. Four toothbrushes (electric toothbrush [ETB], sonic toothbrush [STB], manual toothbrush1 [MTB1] and manual toothbrush2 [MTB2]) were each evaluated in combination with dental floss and interdental brushes in 4 separate appointments, following a plaque accumulation phase of 72 h. The participants were instructed to brush their teeth according to the „CIOTIPlus" system. The Quigley-Hein index (QHI) and the modified Approximal Plaque Index (QH-API) were determined 3 times after plaque staining to assess the plaque reduction: before first brushing (t0), after first brushing (t1), and after second brushing (t2).

Results: At t1, a significant reduction of the QHI and QH-API was observed in all groups compared to t0. The highest reduction of QHI was found in group „MTB1 and interdental brush" (Bm1IB) (ΔmQHI-t0-t1: 1.7 ± 0.3) and the lowest reduction was found in group „STB and dental floss" (BsFB) (ΔmQHI -t0-t1: 1.3 ± 0.3). The highest reduction of the QH-API was detected in group „ETB and interdental brushes" (BeIB) (ΔQH-API-t0-t1: 1.9 ± 0.5) and the lowest reduction of QH-API was found in group BsFB (ΔmQH-API-t0 -t1: 1.3 ± 0.3). After the second brushing (t2), the QHI and QH-API were significantly reduced further in all groups (QHI: 0.6 ± 0.4, QH-API: 1.1 ± 0.4) ($p < 0.0001$). The highest reduction of QHI was found in group BeIB (ΔmQHI-t0-t2: 2.5 ± 0.3) and the lowest reduction of QHI was found in the groups „MTB2 and interdental brush" (Bm2IB) (ΔmQHI-t0-t2: 2.2 ± 0.4) and „MTB2 and dental floss" (Bm2FB) (ΔmQHI-t0-t2: 2.3 ± 0.4). The highest reduction of the QH-API was detected in group BeIB (ΔQH-API-t0-t2: 3.0 ± 0.5) and the lowest reduction was found in group „MTB1 and dental floss" (Bm1FB) (ΔmQH-API-t0 -t2: 2.1 ± 0.5).

Department of ConservativeDentistry, Periodontology and Preventive Dentistry, Hannover: Dr. Karen Meyer-Wübbold, Prof. Dr. Hüsamettin Günay
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Conclusion: The second brushing using the systematic oral hygiene approach "COITIPlus" leads to a higher plaque reduction on smooth and interproximal surfaces compared to the one-time brushing, regardless of what kind of toothbrush is used.

Keywords: CIOTIPlus system; plaque reduction; interdental cleaning; two time brushing

1. Introduction

Oral health awareness in the German population has increased significantly over the last few years. In the Fifth German Oral Health Study (DMS V), 85.5 % of questioned younger adults were convinced that they could contribute "much" or "a lot" in order to maintain or improve their oral health [13]. The patients seem to be aware that plaque-/biofilm removal as part of their home-based oral hygiene routine is of great importance in caries and periodontitis prevention. However, since a number of patients are still affected by caries and inflammatory periodontal diseases, the quality of home-based plaque removal seems still to be insufficient.

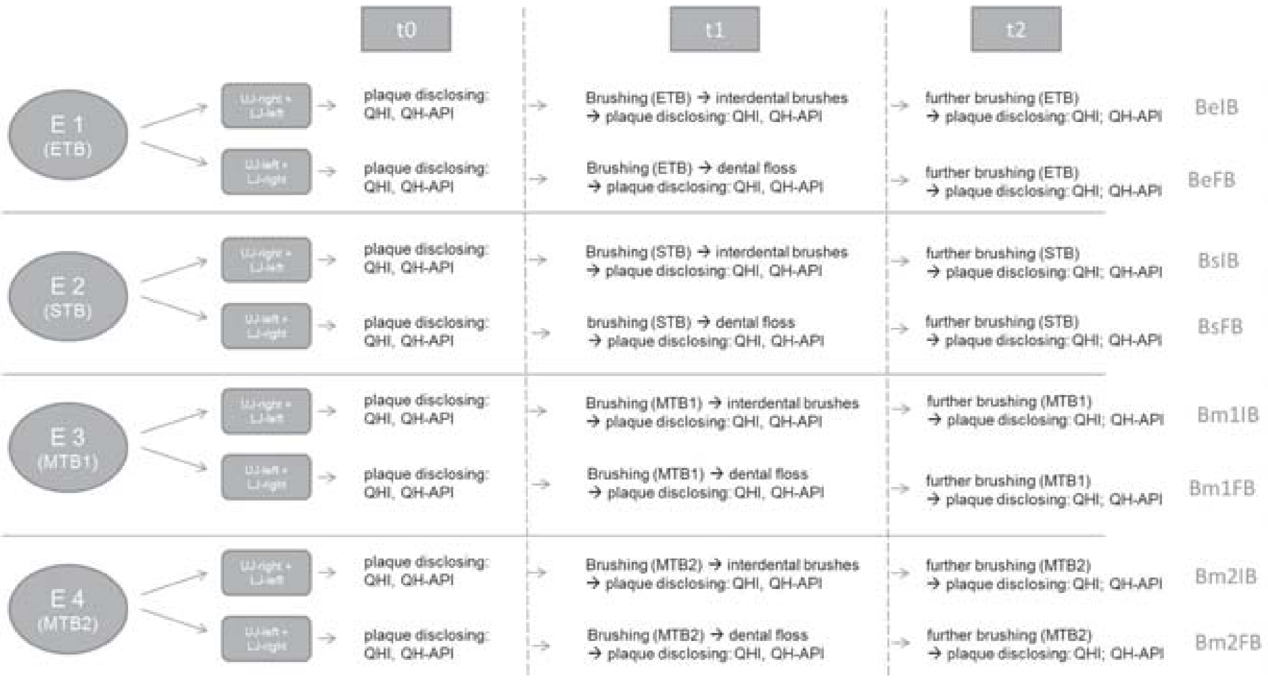
For plaque removal on smooth surfaces, a number of different toothbrush systems are available to patients in their home-based oral hygiene routine. Generally, a distinction between manual and electric toothbrushes is made. Zimmer and Lieding (2014) determined in a representative sample of the Republic of Germany, that 53 % of respondents use a manual toothbrush as part of their home-based oral hygiene routine [32]. 38 % stated, that they only used an electric toothbrush [32]. A reliable cleaning of smooth surfaces can be achieved when using a manual or an electric toothbrush correctly [27]. However, there is some indication that electric toothbrushes are superior to manual toothbrushes regarding plaque removal and gingivitis prophylaxis. In a review article it was summarized that 11 % (observation period 1–3 months), or 21 % (observation period > 3 months) more plaque can be removed and 6 % (observation period 1–3 months) or 11 % (observation period > 3 months) of gingivitis can be reduced using electric toothbrushes in comparison to manual toothbrushes [27]. Electric toothbrushes are distinguished between brushes with rotating-oscillating or sonically activated motion patterns. In literature, there is more evidence regarding the efficacy of rotating-oscillating brushes [26, 27].

The toothbrushing technique recommended most by dentists is the "bass technique", or its modification [28]. However, investigations have shown that this technique is rarely implemented by patients as part of their oral hygiene routine [4]. Also, there are no published study results that show the superiority of the bass technique in comparison to other techniques. There is a common understanding that when using manual or electric toothbrushes, the compliance with the system of plaque removal is more important than the technique itself [5, 8, 17].

Not only the cleaning of smooth surfaces, but also the effective cleaning of interdental space is important in gingivitis and caries prophylaxis, because the tooth surfaces below interproximal contact is a predilection site for caries and gingivitis [18]. Because these areas are insufficiently reached [22] when using a manual or electric toothbrush, and biofilm or food residues can oftentimes not be removed adequately, further tools are recommended [7, 21]. The cleaning of approximal area is often neglected during home-based oral hygiene. According to DMS V, 61.3 % of women and only 35.5 % of men stated, that they clean interdental area with floss [13]. Zimmer and Lieding (2014) reported similar results [32]. Out of 1025 respondents, 59 % stated that they used certain tools to clean interdental spaces, with the women's value being higher at 67 % than the men's at 51 % [32]. Most respondents stated, that they used floss followed by interdental brushes for interdental cleaning [32].

In previous examinations it was shown, that a simple modification of home-based oral hygiene in the form of brushing twice while complying to the "CIOTIPlus" system significantly reduced the formation of root surface and crown margin caries in older people and improved, or rather stabilized the periodontal conditions [9, 10]. The second brushing achieves a larger reduction in the plaque index value of smooth and interproximal surfaces in comparison to brushing once [9–11]. The choice of additional tools used for interdental cleaning is less important. This gives the "CIOTIPlus" system the potential to balance out possible deficits in interproximal cleaning [11]. When using this system the patient cleans the **c**hewing surfaces first, followed by **i**nside and **o**utside surfaces. Next the **t**ongue and **i**nterdental areas are cleaned using the respective tools. Following the cleaning process, the patient cleaned all tooth surfaces systematically for around 1 minute in circulating/rotating motions using a same amount (pea-sized) of flouride toothpaste ("**plus**") [9].

The aim of the present "crossover" pilot study was to investigate the cleaning effect of different toothbrushes in combination with tools for approximal cleaning (interdental brushes and floss) when brushing



(Fig. 1, Tab. 1–9: K. Meyer-Wübbold, H. Günay)

Figure 1 Clinical approach
ETB: electric toothbrush; STB: sonic toothbrush; MTB1: manual toothbrush 1; MTB2: manual toothbrush 2
BeIB: brushing (electric toothbrush) – interdental brushes – brushing (electric toothbrush); BeFB: brushing (electric toothbrush) – floss – brushing (electric toothbrush); BsIB: brushing (sonic toothbrush) – interdental brushes – brushing (sonic toothbrush); BsFB: brushing (sonic toothbrush) – floss – brushing (sonic toothbrush); Bm1IB: brushing (manual toothbrush 1) – interdental brushes – brushing (manual toothbrush 1); Bm1FB: brushing (manual toothbrush 1) – floss – brushing (manual toothbrush 1); Bm2IB: brushing (manual toothbrush 2) – interdental brushes – brushing (manual toothbrush 2); Bm2FB: brushing (manual toothbrush 2) – floss – brushing (manual toothbrush 2)

twice according to the CIOTIPlus system.

2. Methods

2.1 Participants

The participants were randomly selected patients from the Department of Conservative Dentistry, Periodontology and Preventive Dentistry of the Hannover Medical School.

Inclusion criteria for the participation in this project was a remaining dentition of 20 teeth in the absence of any crown restaurations, a patient age between 35–64 years and a Periodontal Screening Index (PSI) < 2. Exclusion criteria included any physical disabilities that make adequate oral hygiene more difficult, a history of radiotherapy in the head and neck region, heavy smokers (> 10 cigarettes per day) and drug intake that could result in false clinical values (e.g. anticoagulants).

Project participation was voluntary and could have been revoked at any time without stating reasons. A positive vote was issued by the ethics committee of the Hannover Medical School (vote number: 1054–2011).

2.2 Study design and collected parameters

All investigations were carried out by a practitioner with the support of an assistant. In the initial examination (E0), the anamnesis and periodontal screening index (PSI) was gathered from all participants. The dental plaque was visualized with the aid of a plaque disclosing solution (Mira-2-Ton, Hager & Werken, Duisburg). Afterwards, the Turesky's modified Quigley-Hein plaque index (QHI) [24] and a modified plaque index similar to the Quigley-Hein-Index, in order to assess the extent of approximal plaque (modified QH-API) [10], were determined using a magnifying loupe (2.5-fold, Orascoptic Lupensysteme, Sigma Dental, D-Handewitt). The tooth brushing system "CIOTIPlus" was explained, demonstrated and practiced. The chewing, inside and outside surfaces of the teeth are cleaned first for 2 minutes, which is then followed by the cleaning of the tongue and the approximal area (CIOTI). At the end, the previously cleaned surfaces and gums are cleaned again for 1 minute with circular/rotating motions using a same (pea-sized) amount of fluoride containing toothpaste (Plus).

Additionally, the sizes of the interdental brushes used during the examinations that followed were determined for every respective approximal area for every participant (IAP-probe, Fa. Curaprox). During the initial examination, the different toothbrushes were shown to all participants, and their use was demonstrated and practiced. In order to create uniform starting conditions, all participants received a professional tooth cleaning that included the cleaning and polishing of the smooth and approximal tooth surfaces.

Four follow-up examinations (E1-E4) ensued, 6each of which was preced by a 72-hour plaque accumulation phase (no home-based oral hy-

	QHI								
	total	BeIB	BeFB	BsIB	BsFB	Bm1IB	Bm1FB	Bm2IB	Bm2FB
t0	3.0 ± 0.4	3.0 ± 0.3	3.0 ± 0.3	3.0 ± 0.4	3.1 ± 0.4	3.0 ± 0.3	3.0 ± 0.3	2.8 ± 0.4	2.8 ± 0.5
t1	1.5 ± 0.5	1.4 ± 0.5	1.6 ± 0.4	1.6 ± 0.6	1.8 ± 0.5	1.3 ± 0.4	1.4 ± 0.4	1.2 ± 0.5	1.3 ± 0.5
t2	0.6 ± 0.4	0.5 ± 0.3	0.6 ± 0.4	0.6 ± 0.4	0.7 ± 0.4	0.5 ± 0.3	0.6 ± 0.2	0.6 ± 0.4	0.5 ± 0.3
t0-t1	1.5 ± 0.4	1.6 ± 0.4	1.5 ± 0.4	1.4 ± 0.4 [a]	1.3 ± 0.3 [b]	1.7 ± 0.3 [a]	1.6 ± 0.2 [b]	1.6 ± 0.4	1.5 ± 0.4
t0-t2	2.4 ± 0.4	2.5 ± 0.3 [a]	2.5 ± 0.4	2.3 ± 0.5	2.3 ± 0.3	2.5 ± 0.4	2.4 ± 0.3	2.2 ± 0.4 [a]	2.3 ± 0.4

Table 1 QHI of all groups at different times (t0, t1, t2), as well as QHI differences t0-t1 and t0-t2. Values with the same letters are significant between them in the horizontal direction.
BeIB: brushing (electric toothbrush) – interdental brushes – brushing (electric toothbrush); BeFB: brushing (electric toothbrush) – floss – brushing (electric toothbrush); BsIB: brushing (sonic toothbrush) – interdental brushes – brushing (sonic toothbrush); BsFB: brushing (sonic toothbrush) – floss – brushing (sonic toothbrush); Bm1IB: brushing (manual toothbrush 1) – interdental brushes – brushing (manual toothbrush 1); Bm1FB: brushing (manual toothbrush 1) – floss – brushing (manual toothbrush 1); Bm2IB: brushing (manual toothbrush 2) – interdental brushes – brushing (manual toothbrush 2); Bm2FB brushing (manual toothbrush 2) – floss – brushing (manual toothbrush 2)

0	QH-API								
	total	BeIB	BeFB	BsIB	BsFB	Bm1IB	Bm1FB	Bm2IB	Bm2FB
t0	3.7 ± 0.4	3.7 ± 0.3	3.7 ± 0.2	3.6 ± 0.4	3.6 ± 0.3	3.6 ± 0.3	3.6 ± 0.3	3.9 ± 0.4	3.9 ± 0.46
t1	2.1 ± 0.5	1.8 ± 0.4	2.2 ± 0.3	1.8 ± 0.5	2.3 ± 0.4	1.8 ± 0.2	2.3 ± 0.3	2.0 ± 0.4	2.5 ± 0.6
t2	1.1 ± 0.4	0.7 ± 0.35	1.0 ± 0.3	0.9 ± 0.3	1.3 ± 0.4	1.0 ± 0.2	1.5 ± 0.3	1.1 ± 0.4	1.5 ± 0.5
t0-t1	1.6 ± 0.5	1.9 ± 0.5	1.5 ± 0.3 [ab]	1.8 ± 0.4	1.3 ± 0.3 [a]	1.8 ± 0.3	1.3 ± 0.3 [a]	1.9 ± 0.7	1.4 ± 0.6
t0-t2	2.6 ± 0.5	3.0 ± 0.5 [ab]	2.7 ± 0.5 [cd]	2.7 ± 0.4 [a]	2.3 ± 0.4 [c]	2.6 ± 0.4 [b]	2.1 ± 0.5 [d]	2.8 ± 0.6	2.4 ± 0.6

Table 2 QH-API of all groups at different times (t0, t1, t2), as well as QH-API differences t0-t1 and t0-t2. Values with the same letters are significant between them in the horizontal direction.
BeIB: brushing (electric toothbrush) – interdental brushes – brushing (electric toothbrush); BeFB: brushing (electric toothbrush) – floss – brushing (electric toothbrush); BsIB brushing (sonic toothbrush) – interdental brushes – brushing (sonic toothbrush); BsFB: brushing (sonic toothbrush) – floss – brushing (sonic toothbrush); Bm1IB: brushing (manual toothbrush 1) – interdental brushes – brushing (manual toothbrush 1); Bm1FB: brushing (manual toothbrush 1) – floss – brushing (manual toothbrush 1); Bm2IB: brushing (manual toothbrush 2) – interdental brushes – brushing (manual toothbrush 2); Bm2FB: brushing (manual toothbrush 2) – floss – brushing (manual toothbrush 2)

giene, no use of oral or dental hygiene products, or sweets or chewing gum containing menthol). Every examination appointment was followed by a "wash-out phase" of at least 2 days, where participants went back to using their regular dental hygiene products. After this, the next 72-hours plaque accumulation phase began. At every examination appointment the dental plaque was visualized and the Turesky's modified QHI [24] and the modified QH-API [10] were determined (t0). For each participant, the entire dentition was not assessed as a whole. Instead, the upper right jaw and lower left jaw were combined together and evaluated separately from the upper left jaw and the lower right jaw in a split-mouth design.

After this the patients were instructed to brush their teeth systemically for at least 2 minutes. The cleaning of the smooth surfaces was always carried out following the same system (first the chewing surfaces, then the inside and finally the outside surfaces and the tongue – system: CIOTI). A different toothbrush was used at every appointment. All participants once again received a demonstration and instruction regarding the use of every respective toothbrush. At E1, an electric toothbrush with rotating-oscillating motion patterns was used (ETB) (Braun/OralB Pro 6000, head: Oral-B Precision Clean Sensitive, Fa. Braun/OralB). At E2, a sonic toothbrush was used (STB) (Hydrosonic CHS 100 with brush head Hydrosonic smart BIW 259, level „Intensive“ with 32.000 motions per minute, Fa. Curaprox), and at E3 a manual toothbrush (MTB1) (Curaprox 5460, Fa. Curaprox). At E4, also a manual toothbrush was used (MTB2) (1–2–3 Classic Care, Fa. OralB). Additionally, a toothpaste with medium abrasiveness (Elmex Sensitive Professional Repair & Prevent, CP-GABA GmbH) was used. The cleaning of the approximal area was done using interdental brushes (CPS prime, Fa. Curaprox) in the right maxilla and left mandible, and using floss (EssentialFloss, waxed, Fa. OralB) in the left maxilla and right mandible.

After this 1. cleaning process, plaque was visualized using disclosing solution and the QH and QH-API was determined (t1). Afterwards, the participants were instructed to brush the previously cleaned tooth surfaces and gums with systematic circular/rotation motions for at least 1 minute using a same (pea-sized) amount of flouride containing toothpaste and the same toothbrushes that were used during the first brushing (System: CIOTIPlus). After this 2. cleaning process, the QHI and QH-API was determined after visualizing the plaque with a disclosing solution (t2).

A "cross-over" design was chosen for this study. Due to the "cross-over-split-mouth design", 2 tools for approximal cleaning could be evaluated together per examination appointment, resulting in a total of 8 groups (Fig. 1).

After every examination appointment, the smooth and approximal surfaces of every participant were cleaned and polished professionally. At every appointment, the cleaning of all tooth surfaces was carried out by the participants themselves and controlled by the examiner ("hands-on-brushing"). The tools to cleaning approximal areas were handled by the investigator himself/herself ("hands-on-flossing/brushing").

Every tool was used on every participant in the same way, a total of 5 times per interproximal space. Both interproximal surfaces were cleaned using floss in 2 up-and-down movements, followed by removing the floss formed to a loop towards the vestibular. A new piece of floss was used for every approximal area. When using interdental brushes, both interproximal surfaces were cleaned with two horizontal brushing motions after insertion to the interproximal space (X-Technique). The brushes were then removed obliquely out of the approximal space in an occlusal and vestibular direction.

2.3 Statistical Analysis

The data analysis was performed using the statistical analysis program SPSS/PC Version 23.0 for Windows (SPSS Incorporation, Chicago, IL, USA). All collected data were evaluated pseudoanonymously. In descriptive statistics, the median, standard deviations and statistical frequencies were calculated. The variation analysis of the collected data was used for repeated measurements within the group of paired t-tests. The means comparison of ΔQHI and QH-API between the two groups was done using unpaired t-tests. If plaque values before toothbrushing differed significantly within a group, an analysis of covariance was conducted. The statistical level of significance was determined to be $p < 0.05$.

3. Results

Fifteen participants (7 female, 8 male) with a median age of 50.1 ± 6.5 years were included in the study. During the initial examination (E0) the participants showed an average QHI of 2.1 ± 0.5 and an average QH-API of 3.6 ± 0.4. 53.33 % of the participants previously used a manual toothbrush and 46.67 % previously used an electric toothbrush as part of their home-based dental hygiene. No participant had previously used a sonic toothbrush for home-based oral hygiene.

Before the first brushing (t0) all groups showed an average QHI of 3.0 ± 0.4 and an average QH-API of 3.7 ± 0.4 (Table 1 and 2).

After the 1. brushing (t1) the QHI as well as the QH-API was decreased significantly in all groups (QHI: 1.5 ± 0.5; QH-API: 2.1 ± 0.5) ($p < 0.0001$). The largest reduction of the QHI was seen in the group "brushing (brushing (MTB1) – interdental brushes – brushing (MTB1)" (Bm1IB) (ΔmQHI-t0-t1: 1.7 ± 0.3) and the lowest reduction was seen in the group "brushing (STB) – floss – brushing (STB)" (BsFB) (ΔmQHI-t0-t1: 1.3 ± 0.3). The largest reduction of the QH-API was seen in the group "brushing (ETB) – interdental brushes – brushing (ETB)" (BeIB) (ΔQH-API-t0-t1: 1.9 ± 0.5) and the lowest reduction was seen in the group BsFB (ΔmQH-API-t0-t1: 1.3 ± 0.3) (Tab. 1 and 2).

After the second brushing (t2) the QHI and QH-API was further reduced significantly in all groups (QHI:

	Average cleaning time (minutes)	
	1. cleaning process	2. cleaning process
ETB	2.4 ± 0.2	1.7 ± 0.3
STB	2.4 ± 0.1	1.6 ± 0.3
MTB1	2.3 ± 0.2	1.5 ± 0.3
MTB2	2.2 ± 0.1	1.3 ± 0.2

Table 3 Average tooth brushing time of the subjects with the different toothbrushes

	QHI	
	interdental brush	floss
t0	2.9 ± 0.4	3.0 ± 0.4
t1	1.4 ± 0.5	1.5 ± 0.5
t2	0.5 ± 0.3	0.6 ± 0.4
t0-t1	1.5 ± 0.4	1.5 ± 0.4
t0-t2	2.4 ± 0.4	2.4 ± 0.4

Table 4 QHI at different times (t0, t1, t2), as well as QHI differences t0-t1 and t0-t2 of the groups "interdental brushes" (BeIB, BsIB, Bm1IB, Bm2IB) and "dental floss" (BeFB, BsFB, Bm1FB, Bm2FB)

	QH-API	
	interdental brush	floss
t0	3.7 ± 0.4	3.7 ± 0.5
t1	1.9 ± 0.5	2.3 ± 0.4
t2	0.9 ± 0.3	1.3 ± 0.4
t0-t1	1.8 ± 0.5	1.4 ± 0.4
t0-t2	2.8 ± 0.5	2.4 ± 0.5

Table 5 QH-API at different times (t0, t1, t2), as well as QH-API differences t0-t1 and t0-t2 of the groups "interdental brushes" (BeIB, BsIB, Bm1IB, Bm2IB) and "dental floss" (BeFB, BsFB, Bm1FB, Bm2FB)

0.6 ± 0.4; QH-API: 1.1 ± 0.4) ($p < 0.0001$). The largest reduction of the QHI was recorded in the group BeIB (ΔmQHI-t0-t2: 2.5 ± 0.3) and the lowest reduction of the QHI was recorded in the groups "brushing (MTB2) – interdental brush – brushing (MTB2)" (Bm2FB) (ΔmQHI-t0-t2: 2.3 ± 0.4). The largest reduction of the QH-API was seen in the group BeIB (ΔQH-API-t0-t2: 3.0 ± 0.5) and the lowest reduction in the group "brushing (MTB1) – floss- brushing (MTB1)" (Bm1FB) (ΔmQH-API-t0-t2: 2.1 ± 0.5) (Tab. 1 and 2). The average brushing time for the first and second cleaning process for the respective toothbrushes can be gathered from table 3.

3.1 Comparison between floss and interdental brushes

In the area of smooth surfaces, a higher reduction of the plaque index was shown in the group "interdental brushes" (BeIB, BsIB, Bm1IB, Bm2IB) after the first as well as the second brushing when compared to the "floss" group (BeFB, BsFB, Bm1FB, Bm2FB), which emerged as not statistically significant (Table 4).

In the approximal area, a higher significant reduction of the plaque index value ($p < 0.0001$) was seen in the group "interdental brushes" (BeIB, BsIB, Bm1IB, Bm2IB) compared to the "floss" group (BeFB, BsFB, Bm1FB, Bm2FB) after the first and second brushing (Table 5).

3.2 Comparison between toothbrushes

In the groups that used interdental brushes, it was shown that after the first brushing the smooth surfaces of group Bm1IB showed the largest reduction and the group BsIB the lowest reduction of plaque index (Table 1). In approximal areas after the first brushing, the largest reduction of plaque index was seen in the group BeIB and the lowest reduction was seen in the group Bm1IB (Table 2). After the second brushing, the group BeIB showed the largest and the group Bm2IB showed the lowest reduction of plaque index on smooth surfaces (Table 1). In the approximal areas the largest reduction of plaque index was seen in group BeIB and the lowest reduction in group Bm1IB after the second brushing (Table 2).

In the groups that used floss, it was shown that after the first brushing, the smooth surfaces of the group Bm1FB showed the largest reduction and the group BsFB the lowest reduction of plaque index (Table 1). In approximal areas after the first brushing, the largest reduction of plaque index was seen in the group BeFB and the lowest reduction was seen in the group BsFB (Table 2). After the second brushing, the group BeFB showed the largest and the group Bm2FB showed the lowest reduction of plaque index on smooth surfaces (Table 1). In the approximal areas the largest reduction of plaque index was seen in group BeFB and the lowest reduction in group Bm1FB after the second brushing (Table 2).

Tables 6–9 show the results of the QH-API and QHI of all groups divided into oral and vestibular surfaces of maxilla and mandible. In total, higher reduction of plaque index values were found after the first and second brushing on the vestibular smooth surfaces and vestibular approximal area when compared to the oral regions ($p < 0.0001$), where a reduction in vestibular plaque index values was higher in the maxilla than in the mandible ($p < 0.0001$). At both points in time, a higher reduction of plaque index values was found on the oral surfaces of the mandible compared to the oral surfaces of the maxilla ($p > 0.0001$).

4. Discussion

The results of this study show, that two-time brushing in combination with approximal cleaning using interdental brushes and floss according to a system can lead to significantly lower plaque index values on smooth and approximal surfaces in instructed patients when compared to a single cleaning. In literature, electric toothbrushes are described as more effective than manual toothbrushes [26, 27]. This could not be fully confirmed in the present investigation. It was shown, that the tested "manual toothbrush 1 – MTB1" led to the largest reduction of the plaque index value on smooth surfaces after the first brushing in comparison to all other toothbrushes. The second largest reduction of plaque index values was seen in the electric toothbrush with rotating-oscillating motion pattern (ETB), followed by the tested "manual toothbrush 2 – MTB2" and the sonic toothbrush (STB). After the second brushing, the result changed slightly. The ETB showed the largest reduction of plaque index value, followed by MTB1, STB, and MTB2. In approximal spaces, the largest reduction in plaque index values was seen in ETB, followed by MTB2, STB and MTB1 after the first and second brushing. The larger reduction of plaque index value was statistically significant in comparison to STB and MTB1.

The manufacturer of MTB1 did not use nylon brushes, but rather "curen filaments". Specific details on these components could not be found, neither from the manufacturer nor in literature. According to the manufacturer, the material absorbs less water than nylon and remains stable even in wet conditions, which is why filaments with a finer thickness are usually used for the head of toothbrushes. Because of this, the total amount of filaments per brush head can be maximized. In MTB1, more than 5400 individual fibres with rounded ends and a 0.1 mm diameter, arranged in a classic flat "multi-tufted", densely packed 5 rows of 39 tufts. The results of this investigation suggests, that the large number of fibres in classic flat, multitufted, densely packed tufts are advantageous in cleaning smooth surfaces. However, a lower reduction of the plaque index value was registered in approximal spaces using this manual toothbrush. This could possibly be because of the flat bristle field. It could be seen that an optimal alignment to the surface of the tooth can only be achieved when the flat bristles are angled at around 45° [3]. If the bristles are placed vertically onto the tooth surface, like in the "scrubbing- or fones technique", the bristles contact only the prominent area of the oral and vestibular smooth surfaces of the tooth surface [3].

Furthermore, a so-called "blocking effect" is observed in flat bristles

	Difference QHI (interdental brush)							
	BeIB		BsIB		Bm1IB		Bm2IB	
	t0-t1	t0-t2	t0-t1	t0-t2	t0-t1	t0-t2	t0-t1	t0-t2
UJ vest.	2.8 ± 0.8	3.7 ± 0.6	2.3 ± 0.6	3.4 ± 0.6	2.9 ± 0.7	3.4 ± 0.6	2.7 ± 0.8	3.1 ± 0.8
UJ oral	0.6 ± 0.4	1.4 ± 0.7	0.7 ± 0.5	1.4 ± 0.7	0.6 ± 0.3	1.4 ± 0.6	0.6 ± 0.5	1.4 ± 0.5
LJ vest.	2.1 ± 0.6	2.9 ± 0.6	1.8 ± 0.8	2.7 ± 0.7	2.1 ± 0.7	2.9 ± 0.5	2.1 ± 0.6	2.7 ± 0.5
LJ oral	1.0 ± 0.7	2.3 ± 0.7	0.9 ± 0.6	2.2 ± 0.7	1.2 ± 0.7	2.2 ± 0.7	1.0 ± 0.8	1.9 ± 0.7

Table 6 QHI differences t0-t1 and t0-t2 of the group "interdental brushes" (BeIB, BsIB, Bm1IB, Bm2IB) divided into the vestibular and oral surfaces of the upper jaw (UJ) and lower jaw (LJ)

	Difference QH-API (interdental brush)							
	BeIB		BsIB		Bm1IB		Bm2IB	
	t0-t1	t0-t2	t0-t1	t0-t2	t0-t1	t0-t2	t0-t1	t0-t2
UJ vest.	3.2 ± 0.8	4.1 ± 0.7	3.0 ± 0.8	4.1 ± 0.7	3.1 ± 0.7	3.8 ± 0.6	2.9 ± 1.2	3.7 ± 1.0
UJ oral	0.8 ± 0.4	1.6 ± 0.7	0.6 ± 0.4	1.2 ± 0.6	0.6 ± 0.4	1.2 ± 0.5	1.0 ± 0.7	1.9 ± 0.8
LJ vest.	2.7 ± 0.7	3.5 ± 0.4	2.5 ± 0.7	3.3 ± 0.7	2.4 ± 0.7	3.1 ± 0.5	2.3 ± 1.0	3.2 ± 0.8
LJ oral	1.2 ± 0.6	2.8 ± 0.7	0.9 ± 0.6	2.2 ± 0.6	1.0 ± 0.3	2.3 ± 0.5	1.1 ± 0.9	2.4 ± 1.0

Table 7 QH-API differences t0-t1 and t0-t2 of the group "interdental brushes" (BeIB, BsIB, Bm1IB, Bm2IB) divided into the vestibular and oral surfaces of the upper jaw (UJ) and lower jaw (LJ)

with filaments arranged parallely, which describes that filaments impede each other when advancing into tapered down areas, such as interdental spaces [3]. The disadvantages of flat bristles can be avoided with the bristle's angle to the tooth surface of around 45°, as described in the "bass technique". The manufacturer of the "manual toothbrush 1" recommends to place the bristles slightly diagonally in a 45° angle to the gingiva and to clean the tooth surface in small circulating movements. The "bass technique" is most recommended by dentists, however, it is difficult to learn and is hardly implemented by patients [4]. Rather, during examinations more circulating or horizontal brushing motions were found [4]. All patients were instructed on the correct application of each toothbrush defined by the respective manufacturers. It was observed that many participants fell back into familiar technique during the cleaning process, which could explain the lower reduction of plaque index values when using MTB1 in approximal spaces.

The sonic toothbrush used in the present investigations achieved lower reduction of plaque index values in smooth and interproximal areas than the manual toothbrushes tested. This contradicts other examinations, in which effective plaque removal with sonic toothbrushes when compared to manual toothbrushes were observed [1, 16, 31]. The manufacturer of the sonic toothbrush used in the present study recommends an angle of 45° to the tooth surface for optimal cleaning of the gingival margin, similar to MTB1. The bristles should be placed onto the tooth surface lightly and without pressure. The user should remain 2–3 seconds per tooth and then carry out a tilting motion without pressure [source: manual and instruction video Hydrosonic, Curaprox]. As previously mentioned, the patients of the present investigation were instructed on the application of each respective toothbrush in the beginning. It cannot be excluded that the manufacturer's predetermined technique was not fully implemented. The technique provided by the manufacturer is very similar to the "bass technique" and was therefore possibly difficult to implement by participants. Additionally, it was being seen that the short motionless remaining of the brush head on the tooth was difficult to do for the patients. The examined patients grew impatient quickly, which was possibly connected to the feeling of being "under surveillance". Patients using a STB often fall back into the motion pattern of a manual toothbrush during its use. They were also not used to clean their teeth with as little pressure as possible. The sonic toothbrushes used in this study did not come with pressure control and it cannot be excluded that patients applied too much pressure, which possibly lowered the cleaning performance of the sonic toothbrush. Different experiences with different toothbrushes should also be considered when interpreting the results.

	Difference QHI (floss)							
	BeFB		BsFB		Bm1FB		Bm2FB	
	t0-t1	t0-t2	t0-t1	t0-t2	t0-t1	t0-t2	t0-t1	t0-t2
UJ vest.	2.5 ± 0.6	3.5 ± 0.6	2.1 ± 0.7	3.2 ± 0.6	2.7 ± 0.4	3.4 ± 0.5	2.7 ± 0.8	3.3 ± 0.7
UJ oral	0.7 ± 0.4	1.7 ± 0.5	0.5 ± 0.3	1.5 ± 0.4	0.4 ± 0.3	1.2 ± 0.4	0.4 ± 0.3	1.2 ± 0.5
LJ vest.	2.0 ± 0.7	3.0 ± 0.5	1.7 ± 0.6	2.8 ± 0.3	2.4 ± 0.6	3.0 ± 0.5	1.9 ± 0.9	2.8 ± 0.7
LJ oral	0.9 ± 0.5	2.2 ± 0.7	0.8 ± 0.4	2.0 ± 0.8	0.9 ± 0.5	2.2 ± 0.6	0.8 ± 0.6	1.7 ± 0.7

Table 8 QHI differences t0-t1 and t0-t2 of the group "dental floss" (BeFB, BsFB, Bm1FB, Bm2FB) divided into the vestibular and oral surfaces of the upper jaw (UJ) and lower jaw (LJ)

	Difference QH-API (floss)							
	BeFB		BsFB		Bm1FB		Bm2FB	
	t0-t1	t0-t2	t0-t1	t0-t2	t0-t1	t0-t2	t0-t1	t0-t2
UJ vest.	2.4 ± 0.5	3.6 ± 0.5	2.0 ± 0.6	3.0 ± 0.8	2.1 ± 0.6	2.7 ± 0.8	2.4 ± 0.9	3.6 ± 0.6
UJ oral	0.8 ± 0.5	1.9 ± 0.7	0.4 ± 0.3	1.3 ± 0.6	0.4 ± 0.4	1.1 ± 0.6	0.6 ± 0.5	1.5 ± 0.7
LJ vest.	2.1 ± 0.7	3.2 ± 0.6	1.8 ± 0.7	2.8 ± 0.5	1.9 ± 0.5	2.6 ± 0.7	1.6 ± 0.9	2.7 ± 0.7
LJ oral	1.0 ± 0.5	2.3 ± 0.9	0.8 ± 0.3	1.8 ± 0.5	0.8 ± 0.4	2.1 ± 0.7	1.0 ± 0.7	2.1 ± 0.9

Table 9 QH-API differences t0-t1 and t0-t2 of the group "dental floss" (BeFB, BsFB, Bm1FB, Bm2FB) divided into the vestibular and oral surfaces of the upper jaw (UJ) and lower jaw (LJ)

The participants used different toothbrushes as part of their home-based oral hygiene routine. 53.3 % of participants stated that they used a manual toothbrush and 46.67 % stated they used an electric toothbrush with rotating-oscillating motion patterns. No participant had used a sonic toothbrush in their home-based oral hygiene routine. This could also explain the lower reduction of plaque index values for the group STB.

Ganss et al. (2018) observed participants during the brushing process with an electric and manual toothbrush in a video [6]. Regardless of the type of toothbrush used, the authors found that vestibular surfaces were cleaned sufficiently, however, oral surfaces were reached insufficiently [6]. This was confirmed in the present study, where regardless of the toothbrush used, plaque index value reduction was higher on vestibular compared to oral surfaces. Furthermore, Ganss et al. (2018) noticed numerous changes between areas when using electric and manual toothbrushes during the cleaning process [6]. Identical motion patterns (horizontal and circulating cleaning motions) were registered with both toothbrushes. Only 50.5 % of participants allowed "passive motions" (positioning of brush head on the tooth with less than 2 motions) when using the electric toothbrush. This "passive brushing" took less than 10 % of total brushing time [6]. In order for electric toothbrushes to achieve optimal cleaning performance a "passive motion" is quite useful. The electric toothbrushes with rotating-oscillating motion patterns used in this study were not found to be superior compared to other toothbrushes, as previously described in literature. This could also be attributed to possible incorrect application despite previous instruction.

In the present study, the cleaning time for the first brushing was not limited. In the second cleaning process, it was made sure that patients do not exceed a 2 minute brushing time. However, the average cleaning processes did not differ in duration with different brushes in the first and second process from one another (Table 3), so that the differences of plaque index value reduction cannot be attributed to varying cleaning processes. The total cleaning duration is increased by brushing twice. In the present investigation, the participants brushed the smooth surfaces with different toothbrushes for an average of 2.3 ± 0.1 minutes in the first cleaning process and 1.5 ± 0.1 minutes in the second cleaning process. In total, an average cleaning time of 3.9 ± 0.2 minutes results. By increasing brushing time, more plaque can be reduced with manual and electric toothbrushes [15, 25, 29]. It was observed that 27 % of plaque reduction can be achieved with one-minute brushing and 41 % with two-minute brushing. In a survey of a representative sample of the Republic of Germany, 75 % stated to brush for 2–3 minutes (44 % 2 minutes; 32 % 3 minutes) [32].

There is often a discrepancy between estimated and real cleaning period [20]. An examination showed that the real cleaning process averaged 68.8 seconds, but was perceived by patients to be more than twice as long (148.1 seconds) [20]. Because of that it seems expedient to recommend that patients brush twice following a specific system (e.g. CIOTI-Plus) and increase brushing time indirectly, instead of solely increasing brushing time.

Studies have shown that more plaque can be removed in the approximal area when using certain tools for approximal cleaning in addition to brushing teeth with a toothbrush [14, 22]. Interdental brushes seem to be more effective than floss regarding interproximal cleaning [2]. This was confirmed by the results of this study. Regardless of the type of toothbrush, the groups that used interdental brushes shows significantly larger plaque reduction than groups that used floss in approximal surfaces after the first and second brushing process.

In different investigations, it could be determined that floss is often not adequately used and a sufficient cleaning of approximal area can therefore not occur [19, 30]. In the present study, not all participants used additional tools for approximal cleaning in their home-based oral hygiene process, and were therefore not equally practiced in their application. It was shown in preliminary investigations that there are big differences between individuals when applying tools for approximal cleaning, and that not all participants were able to reach and clean all approximal spaces themselves. A standardized application of these tools could not have been possible by self-application, which is why a distortion of results would have occurred during evaluation of the cleaning performance. In order to avoid these disadvantages and create similar conditions, the investigator took charge of cleaning the approximal spaces himself, applying the tools in the same way for every participants.

In the present investigation a „split-mouth-design“ was used. This design was chosen to maintain a low number of examination appointments. The commonly described disadvantage of the „carry-across“-effect [12] does not apply according to the results of the study, because solely the mechanical cleaning was carried out evaluated through collecting plaque indices. The one-time mechanical cleaning has no systemic effect on how a “carry-across-effect” could be formed. Another disadvantage in the “split-mouth-design” is the missing barrier between the jaw segments. In this study, mesial approximal surfaces for middle incisors were not included in the evaluation, which means that this disadvantage was also not relevant in this study. All participants were right-handed. In general it is assumed that the right half of the jaw is more difficult to clean for right-handed people than the left half of the jaw. In order to avoid possible distortion of the results, the „cross-over-split-mouth-design“ was chosen on purpose. For the test subjects, the right maxilla and left mandible, as well as left maxilla and right mandible, were summarized and valued respectively.

It was not evaluated in the present study if the differences in reduction of plaque index value are of clinical relevance in respect to caries and periodontitis prevention when using different toothbrushes. Further long-term investigations are necessary, where participants use different toothbrushes over a longer period of time in their home-based oral hygiene routine and data on caries and periodontitis prevalence are collected.

5. Conclusion

A higher reduction of plaque index value can be achieved by brushing the smooth and approximal surfaces twice than with single brushing, regardless the type of toothbrush used. The usage of interdental brushes for approximal plaque reduction seems more effective than floss in the present patient population. Electric toothbrushes do not necessarily lead to a higher reduction of the plaque-index-value when compared to manual toothbrushes. In order to achieve optimal plaque reduction when using manual or electric toothbrushes, a thorough instruction and an intensive training by dental professionals should have occurred.

Conflicts of interest:
The authors state, that there is no conflict of interest in terms of the guidelines of the International Committee of Medical Journal Editors.

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(Photos: Hannover Medical School)

DR. KAREN MEYER-WÜBBOLD,
Department of Conservative Dentistry, Periodontology and Preventive Dentistry, Hannover Medical School Carl-Neuberg-Str. 1, 30625 Hanover, Germany
Meyer-Wuebbold.Karen@mh-hannover.de

PROF. DR. HÜSAMETTIN GÜNAY
Department of Conservative Dentistry, Periodontology and Preventive Dentistry, Hannover Medical School Carl-Neuberg-Str. 1, 30625 Hanover, Germany
Guenay.H@mh-hannover.de

Steffen Müller, Stefan Rupf, Natalia Umanskaja, Matthias Hannig

Detection of Matrix Metalloproteinases (MMPs) in the root dentin of human teeth

Introduction: Matrix metalloproteinases (MMPs) play an important role in the metabolism of dental hard tissue. They are proteolytic enzymes whose function is the degradation of extracellular matrix proteins in the oral cavity. They influence dental substances and tissues and their uncontrolled activity is associated with disease processes such as the progression of carious lesions. Since the distribution of MMPs in the dentin has not previously been investigated, this was addressed by this study.

Material and Methods: Root dentin from 30 human teeth was used for this study, divided into 3 groups of 10 each as follows: Gp1(Group1): teeth with root canal fillings, Gp2: non-endodontically treated teeth and Gp3: unerupted teeth. 90 dentin disks were obtained by taking a coronal, medial and apical slice from each tooth. Enzyme activity was measured by a gelatinase/collagenase assay over a 2 hour period with every specimen having 4 readings recorded at time = 0 min, 30 min, 60 min and 120 min. The mean values of MMP activity over the 2 hours in µU/mg dentin were tested for normal distribution by using the Shapiro-Wilk test. The coronal, medial and apical values within the groups were compared with the paired T-test, while the differences in mean values between the study groups were checked with the unpaired T-test. The significance level was set at $p \leq 0.05$.

Results: All the dentin disks exhibited evidence of enzyme activity. Mean values calculated for the entire roots were 4.8×10^{-1} µU/mg for Gp1 (root canal filled), 4.7×10^{-1} µU/mg for Gp2 (non- endodontically treated) and lastly for Gp3 (unerupted), 4.8×10^{-1} µU/mg. These differences in enzyme activity between the 3 groups were not statistically significant. However, all 3 groups displayed statistically significant increases in enzyme activities in the apical direction i.e. moving from coronal to apical. For Gp1, 4.4×10^{-1} µU/mg coronally, 4.7×10^{-1} µU/mg medially and 5.4×10^{-1} µU/mg apically were measured. For Gp2, 4.2×10^{-1} µU/mg coronally, 4.7×10^{-1} µU/mg medially and 5.1×10^{-1} µU/mg apically were measured. Lastly Gp3, 3.8×10^{-1} µU/mg coronally, 4.5×10^{-1} µU/mg medially and 6.0×10^{-1} µU/mg apically were measured.

Conclusion: The results of this study demonstrated for the first time MMP activity within mineralized hard dentin. In all 3 groups, MMP activity increased from the coronal to apical regions of the roots. MMPs which were

Dental Practice, Brettener Straße 2, 75438 Knittlingen: Dr. Steffen Müller, M.A., M.Sc
Department of Operative Dentistry, Periodontology and Preventive Dentistry, Saarland University Medical Center, Homburg: Prof. Dr. Stefan Rupf, Dr. Natalia Umanskaya, Prof. Dr. Matthias Hannig
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originally synthesized and secreted during dentinogenesis and became incorporated into the mineralized dentin matrix, retain their reactive potential. The study demonstrated the extraordinary stability and longevity of these enzymes.

Keywords: matrix metalloproteinases; proteolytic activity; gelatinase/collagenase assay; root dentin; dentin disk/slice

Introduction

Matrix metalloproteinases (MMPs) are members of the large family of calcium-dependent, zinc-containing endopeptidases [1, 2, 17, 26, 33, 36]. Being proteolytic enzymes they play a role in the synthesis and degradation of proteins, predominantly in the extracellular matrix (ECM) [1, 11]. In the oral cavity these modifying and decomposing mechanisms have consequences with respect to dental hard tissues [5, 8].

MMPs play a key role in the normal physiology of connective tissues, during development, morphogenesis and wound healing [4]. However, their unregulated activity has general medical implications, such as in the pathogenesis of arthritis, tumor metastases, atherosclerosis [4], cardiovascular diseases, nephritis, neurological diseases, blood-brain barrier failure, skin and gastric ulcer formation, liver fibrosis, pulmonary emphysema, and, in periodontal disease [21]. For some time, the involvement of MMPs in the progression of carious lesions has also been discussed [25].

MMPs are secreted by a variety of connective tissue- and proinflammatory cells, including fibroblasts, osteoblasts and odontoblasts [5] as well as endothelial cells, macrophages and neutrophils [33]. They have diverse genetic origins, but as a structurally related group these enzymes are responsible for the degradation of many extracellular matrix proteins (ECM) together with the components of basement membranes [25]. Gross and Lapiere firstly reported MMPs in 1962 as a part of their studies about tadpole development [10].

Research issues for consideration

It is established that MMPs are proteolytic enzymes that are crucial in the synthesis and degradation of extracellular matrix proteins [22, 32–35]. The extracellular matrix comprises fibrillar collagens [7], and this in particular includes a major constituent of dentin, namely 90 % of Type I collagen [6]. If during dentin demineralization the exposure of collagen fibers occurs simultaneously with the activation of pro-MMPs then the discharged collagenases and gelatinases could decompose the exposed collagen fibers [20]. In dentistry, the literature highlights various areas where this could be of crucial importance, but it is especially relevant with respect to adhesive restorative dentistry. Some studies have demonstrated that collagenolytic and gelatinolytic activity in dentin can occur in the coronal root dentin. This becomes particularly crucial for endocrowns which have no post reinforcement but just rely on preparation within the pulp chamber and the adhesive microtags of the bonding resin. Therefore, the question of the stability of this hybrid layer is imperative.

Similarly, the literature does not assume that root fractures occur purely for mechanical reasons, but also, because of a weakening of the collagen matrix in the dentin, caused as a result of its decomposition by MMP activity. An in vitro study examined endodontically treated teeth that had post-retained restorations. These had been clinically monitored over a suitable time period and upon restoration failure electron microscopy techniques demonstrated that the structural integrity of the collagen matrix in the root dentin had been compromised [9]. The experimental protocol prevented the detection of any definitive causality for why this weakening had occurred. However, two central hypotheses were postulated. Either the decomposition of the collagen matrix was caused by bacteria and their virulence factors as collagenases, or else it was because the host‘s own MMPs had been activated.

On the basis of previous study results, consideration is currently given to the enzyme activity found in the root dentin of teeth both of those that have had endodontic treatment and of those that haven‘t. The following questions were formulated:

1. Is there any MMPs activity in the root dentin of teeth either with or without endodontic therapy, and can this be detected directly on the dentin surfaces?
2. Can any differences in the distribution of MMPs activity be demonstrated in the dentin, when moving along a root from coronally to apically?
3. Can any differences in the amount or distribution of MMPs activity be demonstrated between specific groups of teeth: a) endodontically treated b) no endodontic therapy c) unerupted?

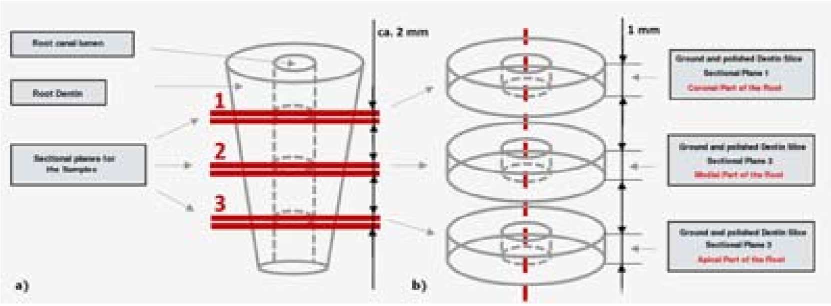


Figure 1 Schematic illustration of the cuts in the roots (**a**) and the corresponding prepared dentin slices (**b**).

Materials and method

Extracted teeth ($n = 30$) were assigned to 3 groups (Gp) with $n = 10$ each: Gp1 (root canal filled or endodontically treated teeth), Gp2 (non-endodontically treated teeth) and Gp3 (unerupted teeth). There was no root caries affecting any of these teeth. Teeth from Gp2 and Gp3 did not have any restorations. The teeth of Gp1 had by definition a completed root filling which was confirmed radiographically. They also had extensive coronal restorations, such as extensive amalgam/composite fillings or metal crowns. The age of the root fillings could not be determined because the teeth had been collected anonymously. All teeth had completely formed root apices. Gp1 and Gp2 comprised all tooth types (i.e. anteriors, premolars and molars), whilst Gp3 contained exclusively surgically removed unerupted third molars (upper and lower).

The teeth of Gp2 and Gp3 were subjected to root canal preparations following standard clinical protocols. The pulp chambers were opened, the pulp tissues removed and the root canal orifices identified. The canals were then mechanically prepared using files of ISO size 25/06 and 25/07 from the Mtwo rotary file System (VDW, Munich, Germany), and also Hedström files of ISO sizes 40 and 45. Gp1 teeth had their existing root fillings removed by means of Mtwo revision files of ISO size 15/05 and 25/05 followed by the same methodology as the other two groups. 1 % physiological saline was used for irrigating the root canals.

Next, the crowns of the teeth, the apical 2 mm of the roots and the complete root cementum were removed using diamond instruments. To prepare dentin slices for measuring enzyme activity the roots were first sectioned into thirds (coronal, medial, apical) and then segments of 2 mm in thickness were cut horizontally from each piece (Figure 1). Slices were prepared using a sawing machine with water cooling (Conrad Apparatebau GmbH, Clausthal-Zellerfeld, Germany, diamond-cut blade, Ø 100 × 0.6, Scott Diamant Tools GmbH, Stadtoldendorf, Germany). Finally, the 2 mm thick dentin slices were reduced to a thickness of 1 mm by polishing (Polisher Metkon GRiPO 2V, Buehler, Lake Bluff, USA, Silicon Carbide Grinding Paper P 1200, P 2500, Buehler). The completed specimens were then stored in a 0.1 % thymol solution.

Before testing for enzyme activity the slices were stored in water for 24 hours. Next, to expose the collagen and to activate the MMPs incorporated in the dentin, the slices were placed in a 0.5 M EDTA demineralizing solution for 10 min at room temperature, supported by constant agitation with a mechanical shaker set at 10 rpm. Following demineralization, the dentin slices were placed in the wells of a laboratory microplate (Greiner 96 Well Flat Bottomed Black/Polystyrene), covered with 80 µl buffering reagent solution together with 20 µl DQ gelatin solution from an EnzChek Gelatinase/Collagenase Assay Kit E12055 (Fisher Scientific GmbH, Schwerte, Germany).

The fluorescence-labeled cleavage products of DQ gelatinase have an absorption maximum of 495 nm and a fluorescence emission maximum of 515 nm. An electronic fluorescence detector (GENios, Tecan, Salzburg, Austria) was used (Firmware V4.62–07/01 GENios, software Tecan-i-control 1.10.4.0). Fluorescence measurements (10 x i.e. once for each sample tooth within every study group) were recorded during a 2 hour incubation period (at 37°C) after $t = 0$ min, $t = 30$ min, $t = 60$ min and $t = 120$ min.

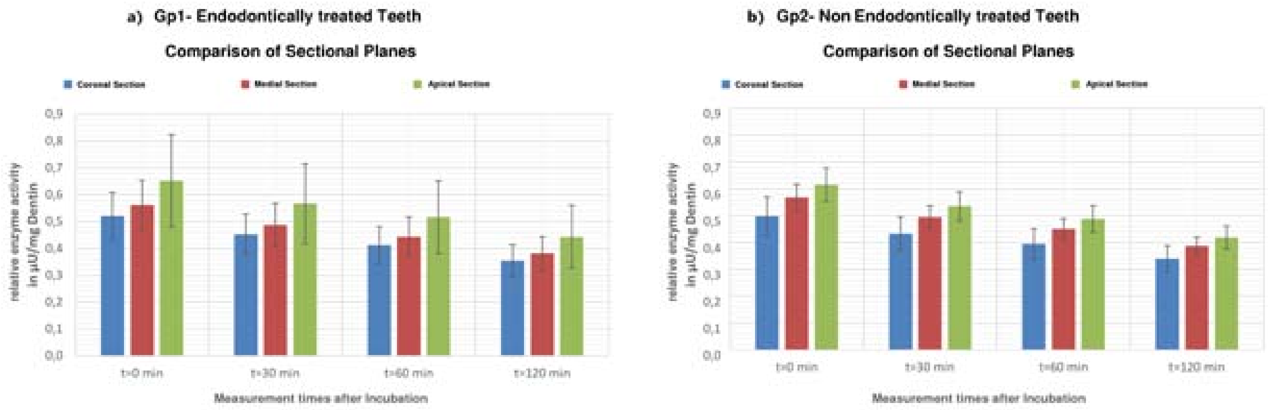
Levels of enzyme activity were then calculated by converting the measured fluorescence values by comparison with corresponding standard curves of standard collagenase type IV from Clostridium histolyticum. Preliminary tests had determined that the expected measuring range would be 0 to 25 µU per ml and therefore serial dilutions were necessary to adjust the original concentration of 1 U per ml. Based on the weight of the dentin slices and the calculated enzyme concentration, the mean enzyme activity per mg of dentin was calculated for every slice at each of the 4 measurement intervals. The enzyme activity values in µU/mg dentin were tested for conformation to a normal distribution using the Shapiro-Wilk test. To compare the enzyme activity values between the 3 root areas (coronal, medial and apical) a paired T-test was performed. The differences in the mean values of the respective study groups as related to the total dentin were checked with the unpaired T-test. The significance level was set at $p < 0.05$.

Results

For each of the three groups, Gp1 (endodontically treated teeth), Gp2 (non-endodontically treated teeth) and Gp3 (unerupted teeth), enzyme activity was detected at every measurement interval. It declined according to the enzyme kinetics, both continuously and in parallel in the 3 study groups.

Gp1 (endodontically treated teeth)

The dentin discs of Gp1 had an average weight of 35.2 mg (± 8.9 mg) per sample. Figure 2a shows enzyme activity values for the 3 root areas over the 2 hour measurement period at



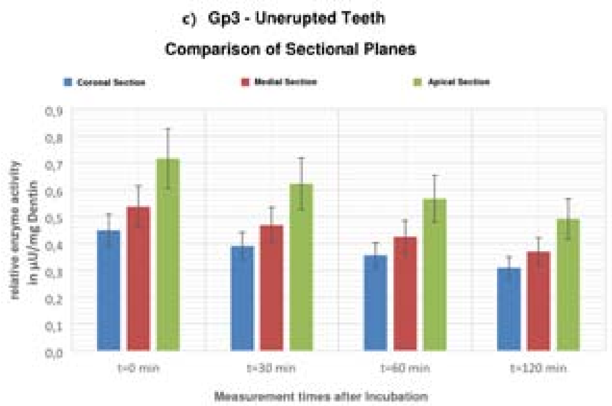


Figure 2 Presentation of the relative MMP activity of the coronal, medial and apical dentin slices in µU/mg dentin. Mean values (+ SD) for each group of 10 teeth (n = 10) during the 2hr incubation at t = 0 min, 30 min, 60 min and 120 min. (**a**) Gp1: endodontically treated teeth, (**b**) Gp2: non-endodontically treated teeth, (**c**) Gp3: unerupted teeth.

0 min, 30 min, 60 min and 120 min. A characteristic distribution pattern of the enzyme activity values over the 3 root areas is obvious. Mean enzyme activity values for the total measurement period were 4.4×10^{-1} µU/mg dentin coronally, 4.7×10^{-1} µU/mg dentin medially and 5.4×10^{-1} µU/mg dentin apically. The differences between all 3 areas were statistically significant (coronal vs. medial: $p = 0.008$, coronal vs. apical: $p = 0.006$, medial vs. apical $p = 0.04$; Fig. 3a).

Gp2 (non-endodontically treated teeth)

Gp2 had an average weight per sample of 35.2 mg (± 5.1 mg). Throughout the measurement period the distribution pattern of the enzyme activity values for the 3 root areas was comparable to Gp1 (Fig. 2b). In each case, the highest values were apically and the lowest were measured coronally. Mean enzyme activity values were 4.2×10^{-1} µU/mg dentin coronally, 4.7×10^{-1} µU/mg dentin medially and 5.1×10^{-1} µU/mg dentin apically. The differences between two of the areas were statistically significant (coronal vs. medial: $p = 0.002$ and coronal vs. apical: $p = 0.0002$). There was no significant difference for medial vs. apical with $p = 0.07$ (Fig. 3a).

Gp3 (unerupted teeth)

Gp3 had an average weight per sample of 34.4 mg (± 7.9 mg). Figure 2c again illustrates the characteristic pattern of the enzyme activity values increasing over the 3 root areas from coronally to apically that was also seen in both Gp1 and Gp2. Mean enzyme activity values were 3.8×10^{-1} µU/mg dentin coronally, 4.5×10^{-1} µU/mg dentin medially and 6.0×10^{-1} µU/mg dentin apically. The differences between all 3 areas at every time interval were statistically significant ($p = 0.001$ to $p = 0.0001$; Fig. 3a).

Comparison of enzyme activity values between Gp1, Gp2 and Gp3

The mean enzyme activity values over the 2 hours for the coronal and medial areas of Gp1 (4.8×10^{-1} µU/mg), Gp2 (4.7×10^{-1} µU/mg) and Gp3 (4.8×10^{-1} µU/mg) did not differ with statistical significance ($p = 0{,}05$ to 0.83). Only the comparison between the apical areas of Gp2 and Gp3 showed a statistically significant difference ($p < 0.02$, Fig. 3a).

There were no statistically significant differences in the total root dentin enzymatic activities between any of the groups ($p = 0.53$ to $p = 0.79$, Fig. 3b).

Discussion

In this study the activity of MMPs was detected for the first time on root dentin slices by using the gelatinase/collagenase assay method. In contrast, previous studies by Pashley et al. [24], von Hebling et al. [12] and Nishitani [23] used dentin milled into a fine powder by using a vibrating mill. By this method it was not possible to measure the distribution of the MMPs in the root dentin. Changes of the collagen structure and the MMPs could also not be completely ruled out.

In contrast, this study used solid dentin in the form of dentin slices to

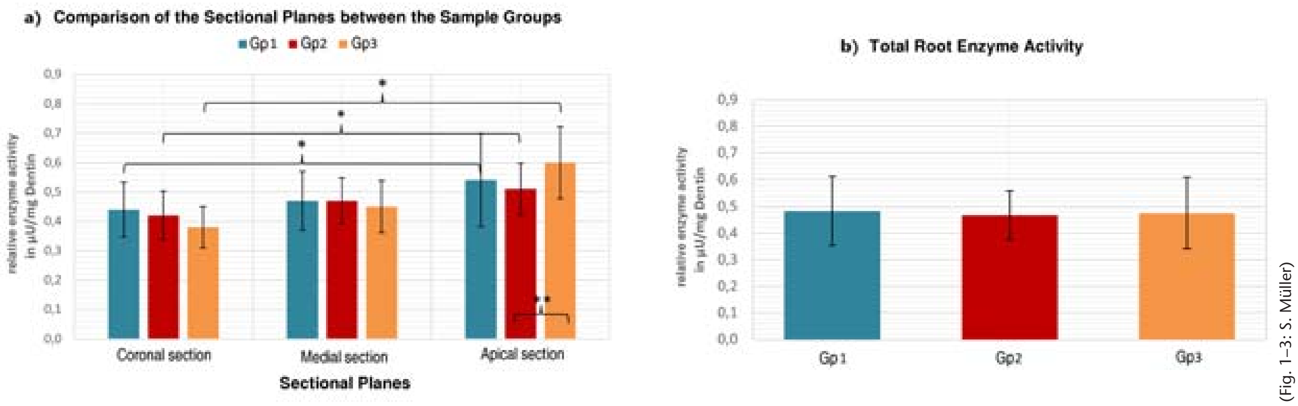


Figure 3 Comparison of MMP activity in µU/mg dentin of groups (Gp1: endodontically treated teeth, Gp2: non-endodontically treated teeth, Gp3: unerupted teeth). Mean values (+ SD) from the measurements at t = 0 min to t = 120 min. (**a**) coronal, medial and apical dentin slices of Gp1 to Gp3, statistical differences within the groups: *; between the groups: **; $p < 0.05$ (**b**) mean MMP activity of the entire roots of Gp1, Gp2 and Gp3. No statistically significant differences.

investigate MMP enzyme activity. In addition, the distribution of enzyme activity within the roots could be visualized over the 3 anatomical root areas, (coronal, medial and apical). The method used, however, is also an in vitro method for which the results cannot be fully transferred to the clinical situation. For the fluorescence measurement, a method established in the literature was used [12, 23, 24, 29] which, in contrast to the Western blot method [6, 7, 19, 27, 30] and zymography [13, 32], represents a rather time-consuming and costly procedure. According to Lynch and Matrisian [17] this method is appropriate for MMP-1, MMP-3, MMP-8, as well as for MMP-2 and MMP-9.

The results of this study proved that similar enzyme activity is present in endodontically treated teeth (Gp1), those without endodontic interference (Gp2), but also in unerupted teeth that had been surgically removed (Gp3). This study verified MMP activity from different regions of the roots and demonstrated that the enzyme activity increased from the coronal to the apical zones. This result could be expected, but interestingly, until now hasn't been presented in the literature. The basic condition for inclusion in the study was caries-free root dentin. The used endodontically treated teeth had extensive coronal restorations, the non endodontically treated teeth and the teeth not exposed in the oral cavity had intact crowns. Since the influence of the restoration of the teeth crown on the quality of the root dentin is not known, no statement can be made about the influence of coronal restorations on the activity of MMPs in the root dentin area. The teeth of groups Gp1 and Gp2 could have had their roots exposed to the oral cavity because of periodontal bone loss or other non-caries lesions. The effect that any such exposure to the oral environment may have had on MMP activity cannot be estimated [5, 30]. This can only be excluded for teeth of the Gp3. In our study, however, the possible influencing factors for a decrease in enzyme activity described above had no effect. MMP activity was detectable in all the teeth of Gp1 and Gp2.

The MMPs inside the dentin matrix structure remain inactivated as long as the dentin matrix structure is mineralized [23, 28]. The literature reports that limited demineralization of dentin, e.g. when using etch-and-rinse or self-etching bonding techniques, results in MMP activation (MMP-2 and MMP-9) in the dentin [20]. Adhesive dentistry procedures demineralize the dentin for 15 sec with 30 to 37 % phosphoric acid. Longer acid exposure times result in a deeper demineralization, which then results in deeper resin impregnation and consequently in a thicker hybrid layer. However, a thicker hybrid layer does not necessarily improve bond [31]. Here, a possible decrease in the stability of the composite dentin interaction is suspected due to a loss of the integrity of the dentin matrix [9]. After acid etching, incomplete wetting with primers may result in imperfect impregnation of the collagen matrix [37]. It has been shown that MMP's enclosed in the dentin matrix can attack and degrade exposed collagen fibers in the hybrid layer [20, 23, 24, 37].

While the exposure of the dentin collagen network by acid etching with etch-and-rinse adhesives and thus an activation of dentin-trapped MMP's is verified, the present study situation regarding an activation of the MMP's by self-etch adhesives is not conclusive [20, 23, 24]. It should be considered that thin adhesive layers can have the effect of a semipermeable membrane. Due to its water permeability it offers activated MMP's the possibility to perform their hydrolytic function against collagen fibrils and thus destroy the collagen network [37]. From a clinical perspective, it would therefore be advantageous to prevent the activation of MMPs [24]. Therefore, the use of MMP inhibitors such as EDTA [19, 24] and chlorhexidine [12, 37] seems to be recommendable at present. Under physiological conditions, the activities of MMP's are regulated at the level of transcription, at the level of activation of zymogenic precursors of so-called Pro-MMP's, but also at the level of inhibition by endogenous inhibitors such as tissue inhibitors, metalloproteinases themselves and TIMP's [14]. The MMP's also have a high variability to act in complex processes under pathophysiological conditions. The most interesting implications for dentistry are where MMPs are involved in the physiological and pathophysiological processes of the oral cavity and the teeth. For example, it is discussed that dentin-bound MMPs may play a role in regenerative processes and in the regulation of complex dentin-pulpa defense reactions in carious lesions by solving dentin-bound growth factors [30, 27].

MMPs which at the time of their synthesis and secretion, were incorporated into mineralized dentin during dentinogenesis, possess a remarkable capability to reactivate; but, these enzymes also have the impressive property of being able to remain inert and stable for long periods, even in endodontically treated teeth [15].

Summary and conclusion

In the present study, root dentin slices were tested for non-specific enzymatic activity of MMPs. In each of the 3 studied groups, Gp1 (endodontically treated teeth), Gp2 (non-endodontically treated teeth) and Gp3 (unerupted teeth), enzyme activity in the dentin could be detected. In addition, for the first time information on the distribution of MMPs from coronal to apical within the root dentin could be obtained.

Follow-up studies are necessary to classify the non-specific MMP activity to specific MMPs. Also, the distribution of MMP's within the root from the root canal to the dentin root cement junction has not yet been clarified.

Clinically, the detection MMP activity in endodontically treated teeth is relevant. The therapeutic use of MMP inhibitors, e.g. Chlorhexidine, is recommended during endodontic procedures. The use of an MMP inhibitor is also important for adhesive dentistry (etch-and-rinse techniques) to ensure durable bonding to dentin.

Conflicts of interest:

The authors declare that there is no conflict of interest within the meaning of the guidelines of the International Committee of Medical Journal Editors.

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(Photo: private)

DR. STEFFEN MÜLLER, M.A., M.SC.
Practice for dentistry
Brettener Str. 2
75438 Knittlingen
zahnarztpraxis.mueller@gmx.de

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Editors
Prof. Dr. Guido Heydecke
Editor in Chief | DZZ International
Chairman Department of Prosthetic Dentistry
University Medical Center Hamburg-Eppendorf
Martinistraße 52 | 20246 Hamburg
Phone +49 (0) 40 7410 – 53261
Fax +49 (0) 40 7410 – 54096

Prof. Dr. Werner Geurtsen
Editor | DZZ International
Chairman, Department of Conservative Dentistry, Periodontology and Preventive Dentistry
Hannover Medical School
Carl-Neuberg-Str. 1 | 30625 Hannover
Phone +49 (0) 511 – 5324816
Fax +49 (0) 511 – 5324811

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