5 | 2019
1. VOLUME

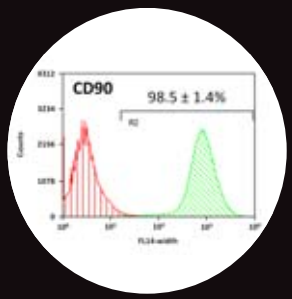
DZZ
INTERNATIONAL

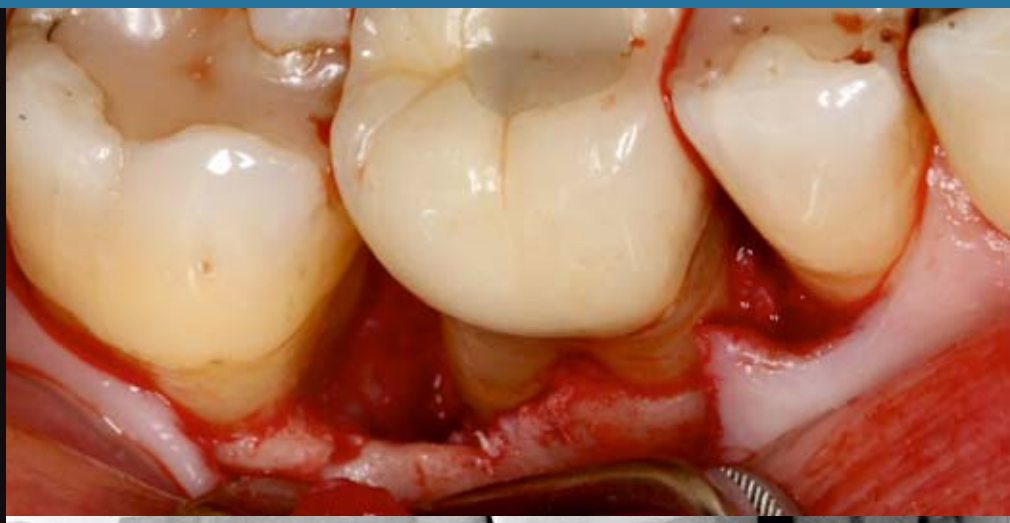
German Dental Journal International – **www.online-dzz.com**
International Journal of the German Society of Dentistry and Oral Medicine

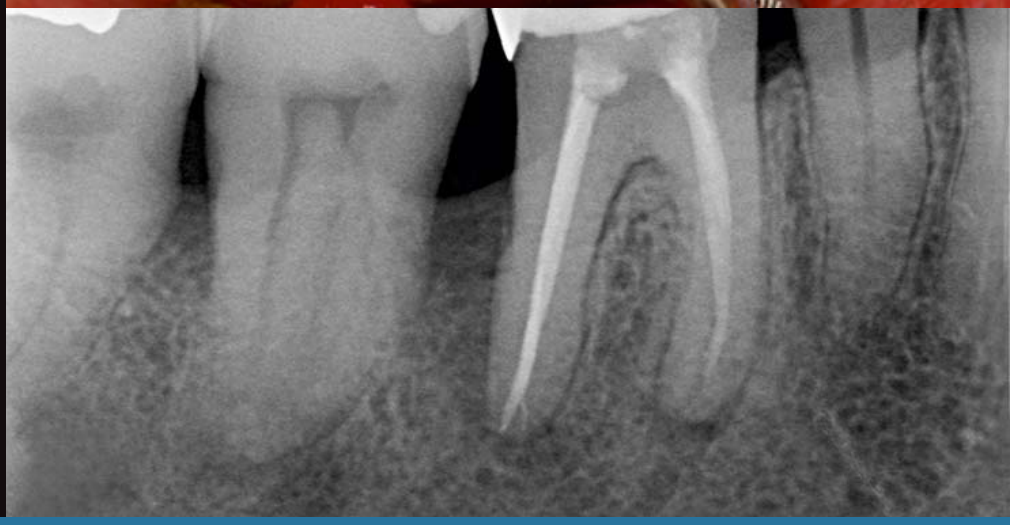
The impact of the stomatognathic system on the development of human beings

The effectiveness of an electric "wash toothbrush" on oral plaque control

Characterization of cells derived from inflamed intrabony periodontal defects







This journal is regularly listed in CCMED / LIVIVO.

Deutscher Ärzteverlag

PRACTICE

RESEARCH

Title picture hint: From the original article of Knut Adam et al., here (upper picture) figure 1C: The mucoperiosteal flap including the intra-lesional granulation tissue was thoroughly detached from the intra-bony periodontal defect and mobilized as far as it was necessary for an effective scaling and root planning and (picture below) figure 1E: The radiograph 2 years after surgery and endodontic treatment revealed a significant bone fill in the former defect and (round figure left) figure 2: Representative data of the immunophenotypic characterization with flow cytometry: The single-parameter histogram shows the expression of the mesenchymal stem cell marker CD90, p. 182–194

Online-Version of DZZ International: www.online-dzz.com

The impact of the stomatognathic system on the development of human beings

Question

Which of the versatile complex functions of the stomatognathic system play a key role in human development?

Background

The stomatognathic system and its comprehensiveness and meaning for the entire organism is underestimated even by dentists. We usually only speak of the chewing organ and this concept alone seems to reduce our operating field to restoring the function of "chewing". However, the stomatognathic system has many other functions and plays a key role in the evolution from hominoids to homo sapiens as opposed to other organ systems. It consists of numerous structures that form a complex cypernetic regulatory circuit (Fig. 1a, Fig. 1b), which themselves show osseous, chondral, ligamentary, muscular, fascial, organic and neuronal connection with other systems.

The primary functions of the stomatognathic system were food intake, defense and the presentation of threatening gestures in order to establish a social ranking order. Presently, we find these simple functions in various phylogenetic inferior animal species. From the ectoderm, fangs evolved from what used to originally be skin scales to capture and fixate food [8]. A simple hinge joint with a one-dimensional flap motion was sufficient for this function. Food was devoured without chewing. A large part of the energy contained in food was therefore needed in processing the food in the intestinal tract. Multidimensional chewing, grinding of the food and predigesting through the addition of saliva's enzymes occurred more and more in the course of evolution. Chewing movements became increasingly complex and eventually led to the development of a new mandibular joint, which is still found in mammals (mammalia) today. The original hinge joint evolved to become the ossicles of the middle ear (ossicular chain). The osseous mandibular corpus is a mesoderm derivative that has a growth center specifically in the area of the ascending jawbone that links the condyle and caput mandibulae to the structures of the neurocranium. It is characteristic for our mandibular jaw that osseous and cartilaginous structures such as condyle, condylar cartilage, articular disk and articular capsule evolve in parallel and cluster together. This evolutionary process required perfect coordination of all growth processes considering the spatial limitation of the fast evolving structures of the neurocranium and viscerocranium as well as the neck. It may explain the difficulty in diagnosis of dysfunction of the stomatognathic system. Due to its many individual functions, the stomatognathic system in the human organism is linked to the brain, or CNS, in the most complex and versatile way. The brain stem is largely in charge of neuronal control of "simple" functions such as chewing, defending or threatening others. Among others, a central area referred to as a masticatory center is located here. It controls the chewing process after initiation by associated centers in the cerebral cortex mostly autonomously, but always fed back by sensible, sensory centers (that process peripheral information).

The basic functions of the stomatognathic system mentioned above are complemented by the formation of sounds in mammals (mammalia) and birds (aves) [2]. The main focus of the development of a sound is undoubtedly in the larynx, where its specific structure makes a modulation of sounds possible. But also the shape of the oral cavity plays a key role in sound formation and functions as a resonance space. The design and modification of the oral cavity with tongue, teeth, cheek and lip muscles, muscles of the soft palate and the mucosa all contribute significantly to the specific formation of sounds. Only in humans, the formation of sounds has evolved to the level of language. According to Popper [7] we differentiate the following stages of speech/articulation:

– Stage 1: Expressive or symptomatic function: The living being expresses inner emotional states

Translation: Yasmin Schmidt-Park
Citation: Behr M, Fanghänel J: The impact of the stomatognathic system on the development of human beings. Dtsch Zahnärztl Z Int 2019; 1: 171–174
DOI.org/10.3238/dzz-int.2019.0171–0174

such as fear or well-being, for example the purr of a cat
- Stage 2: Signal or signal-triggered function: For example, warning cries of birds that alert their fellow species to warn of danger and to trigger flight behavior.
- Stage 3: Descriptive function: items or conditions, such as the current or future weather, can be described to other living beings using articulation and can therefore be communicated
- Stage 4: Argumentative function: Exchange of abstract processes that can occur in different time levels (past, present, future) and spaces. Critical evaluations, plans and decision making on occurrences in the environment can be articulated.

Stage 3 and particularly stage 4 are only present in humans. Even chimpanzees (pan), our closest relatives are only capable of creating stage 1 and 2 sounds.

The attitudes differ on how far the primate morphology of the larynx does not allow speech. Unlike Liebermann [6], Tobias [10] shares the view that fundamentally, the higher primates' morphological differences in the upper airways are not an explanation for their inadequate speech functions. In a study in the USA a chimpanzee baby and a human baby were raised in the same family [4]. The surroundings and support were practically identical for both infants. While the human baby practiced its speech function through continuous babble and sound formation, the chimpanzee baby was mostly mute. It never learned our stage-3-speech function, to name objects in the room, and especially not stage-4-speech function, which the human baby learned in the course of its development.

Eccles sees an explanation of this situation in brain development as well. Brain size does not solely play a key role. In humans – approximately 30.000 years ago – the development of both brain halves took a new direction into cerebral hemispheres. The brain halves, which would have practically fulfilled inversely identical functions, specialized on the contrary to other mammals. In homo sapiens,

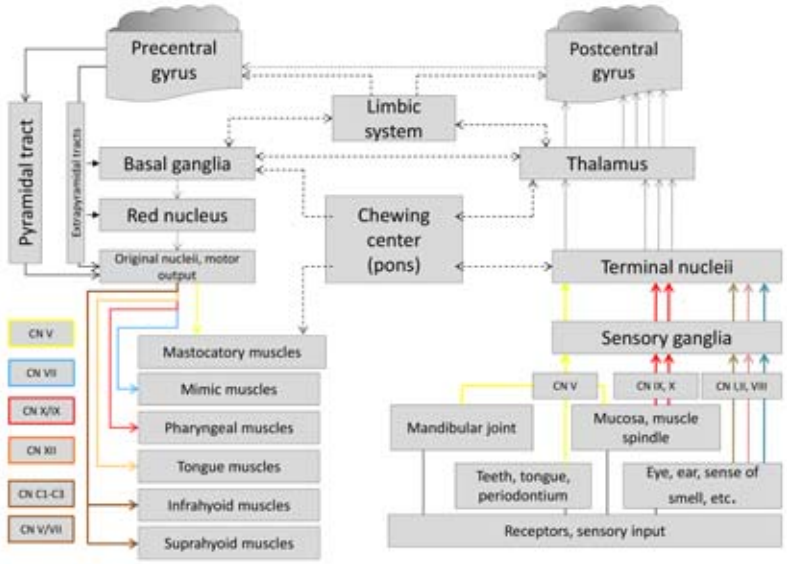


Figure 1a Cybernetic regulatory circuit and neuromuscular control of masticatory function.

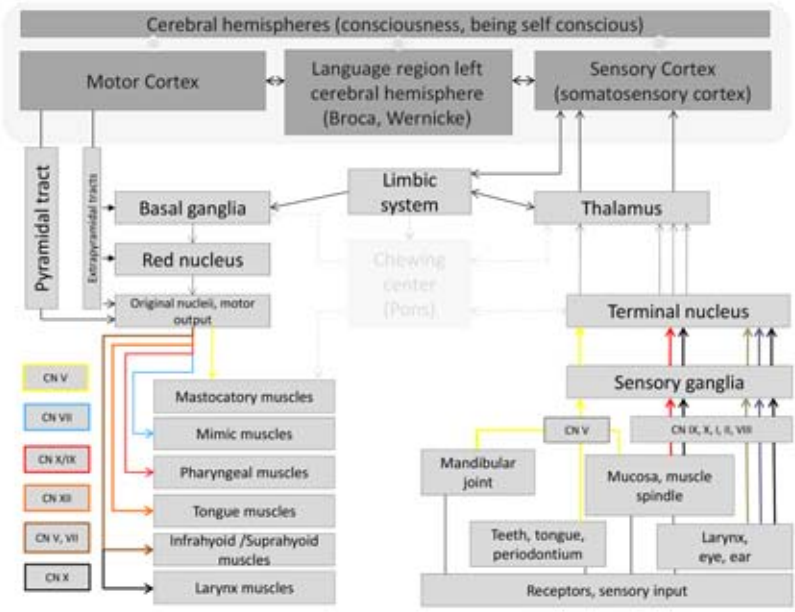


Figure 1b Cybernetic regulatory circuit and neuromuscular control of speech function.

we differentiate a dominant and a non-dominant brain half [2, 5]. The dominant left cerebral hemisphere has a connection to our self-consciousness as an independent person. It analyzes verbal, linguistic descriptions, conceptual similarities, analyzes time and is capable of arithmetic and computerized functions. (Fig. 2). The dominant left cerebral hemisphere has a connection to our self-consciousness as an independent person. It analyzes verbal, linguistic descriptions, conceptual similarities, analyzes time and is capable of arithmetic and computerized functions. The right cerebral hemisphere is linked to consciousness (however, not self-consciousness). It processes non-verbal information, tactile geometric information e.g. of the room and analyzes image and space patterns, visual similarities and can carry out syntheses on this time period.

We owe the ability of speech of stage 3 and 4 cross-modal links of different sensory centers of both hemispheres as well as specially developed areas of the left hemisphere. These are the anterior speech cortex (Broca's

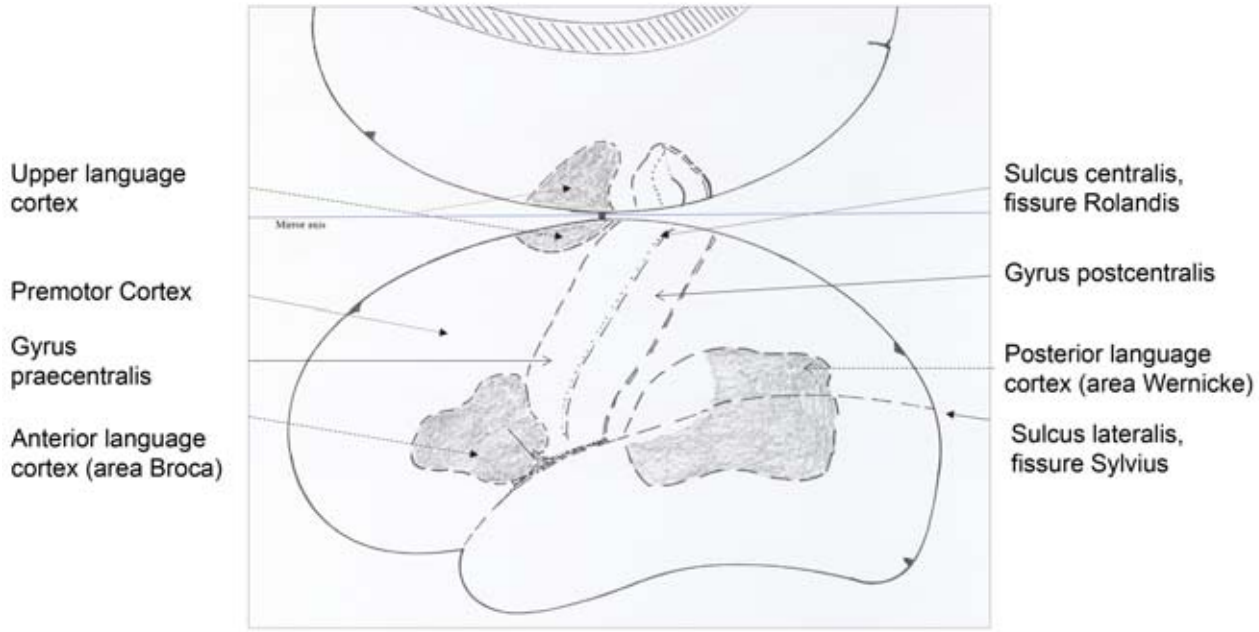
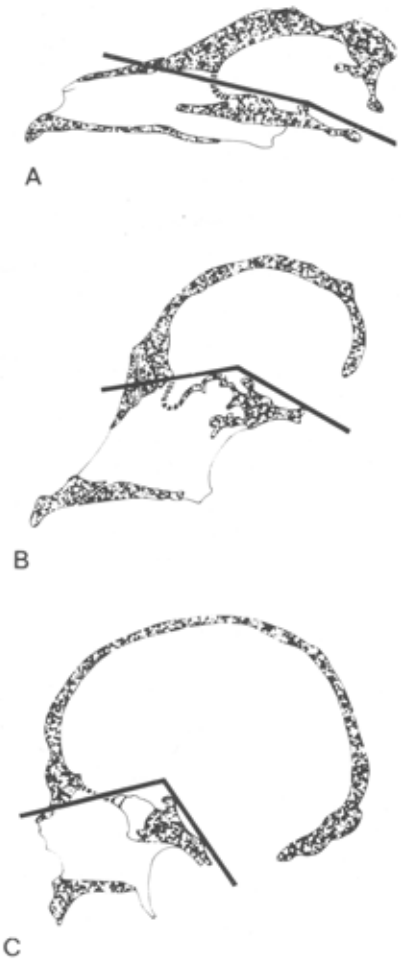


Figure 2 Cortical language fields of the left dominant cerebral hemisphere. The left cerebral hemisphere is depicted laterally (from below) and medially (from above). Re-drawing after [2].



(Fig. 3: By courtesy of Leopoldina)

Figure 3A–C The changing of sphenoid-clivus angles (basicranial angulation) of the skull of a dog (canis familiaris) (**A**), an ape (pan) (**B**) and a human (homo sapiens) (**C**) in the course of evolution [3].

speech area), the upper speech cortex and especially the posterior speech cortex (Wernicke's speech area) (Fig. 2). In the animal kingdom tactile, visual or auditory sensory stimuli are always connected with a "limbic stimulus" [2], and therefore determine the living beings action. Human beings can also associate not-limbic sensory stimuli, become conscious of them and can orient their actions accordingly. Using language, the division between different senses can be overcome. It helps us to merge different sensory modalities into one unit, recognition and experience. Teuber [9] expressed this fact in the following way: "Language frees us to a great extent of the tyranny of the senses." Through language, human beings have succeeded to formulate, evaluate, and share sensory experiences with others, or rather, to profit from the wealth of experience of other people. Through language, the benefits of the upright walk (verticalisation) and an subsequent modification of the skull base could fully unfold (Fig. 3) [3], which leaves the hands of manual tasks free to develop their full potential. The results of manual skills were "discussed" and more importantly passed on, so that a further growing wealth of experience could be formed. Language development encouraged it and made it possible to develop advantages of altruistic action and the value of cultural effort (Fig. 4). The potential of human evolution exponentiated explosively. It is no longer dependent on random "improvements" through mutations in genetic material, and is no longer reliant on other living beings [2].

The development of these unique, new cross-modal links are readable in the human brain's topography (Fig. 2). We find the anterior language center directly before zones that are responsible for controlling relevant muscles. In the case of motoric aphasia the cause of the malfunction is in the use of the muscles used for articulation, rather than their paralysis. The posterior language center of the left cerebral hemisphere is crucial for the initiation, execution and understanding of language. If this structure is disrupted, neither written nor spoken language can be understood. When looked at in a side by side comparison, a hypertrophy of the structure referred to as planum temporale of the posterior language center can be seen in the area of the superior temporal gyrus in the left hemisphere.

Topographic and functional relationships can be demonstrated in the

peripheral nervous system. The cranial nerves V (trigeminal nerve), VII (facial nerve), IX (glossopharyngeal nerve), X (vagal nerve) and XII (hypoglossal nerve) supply all structures of the masticatory apparatus. Cranial nerves IX (glossopharyngeal nerve) and X (vagal nerve) supply the speech apparatus structures within and outside of the larynx. Thus, cranial nerves as well as masticatory and speech apparatus are demanded simultaneously in various diseases (common cold, childhood illnesses).

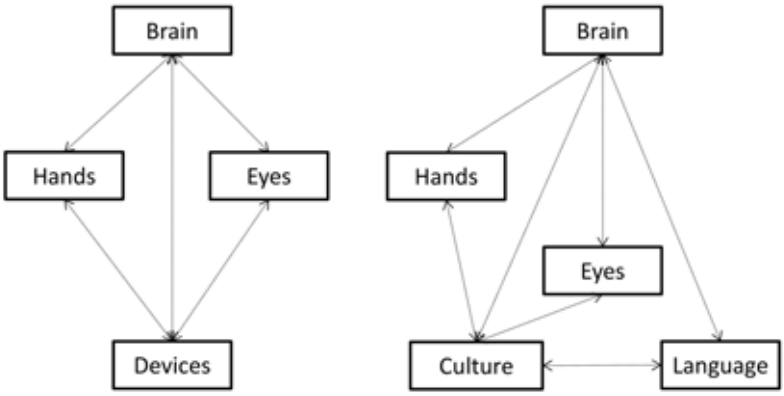
Lastly, the limbic system of the cerebrum is mentioned. This is a non-homogenous structure that consists of the cingulate gyrus, hippocampus formation, as well as the amygdalaoid corpus. This system is responsible for emotions and memory. It is functionally linked to the chewing and speech apparatus: For example, memorable emotions are created while eating meals that taste delicious or terrible. We then speak emotionally about our sensation. The stomatognathic system and speech apparatus are equally involved.

Statement

For the stomatognathic system, the widespread neural crosslinking imply that its muscles serve multiple functions [1]. While the control pulses for chewing are derived mainly from the older/more ancient parts of the hindbrain (pons, cerebellum), midbrain and basal ganglia, the neural impulses for speech formation are derived mainly from the more juvenile speech areas of the left cerebral hemisphere.

In addition to the afore mentioned spatial complexity in the set up of the stomatognathic system during the growth phase into skull structures, another complexity in neurological „wiring“ of the stomatognathic system exists (Fig. 1a, Fig. 1b), which contains impulses of different neurological centers with various tasks. Furthermore, this complexity explains the difficulty to diagnose and understand problems in the stomatognathic system's function.

As dentists, we should be aware of this truth. For example, someone who changes the position of occlusion, moves teeth in the jaw or places implants in the jaw bone interacts with an extremely sensitive and highly complex biocybernetic regulatory circuit (Fig. 1a, Fig. 1b). We are not just practicing in a chewing organ, it is an essential part of our body that makes us who we are: humans.



(Fig. 1, 2 and 4: M. Behr, J. Fanghänel)

Figure 4 Left: System of positive reinforcement between 3 biological and a cultural element. Right: Modified feedback system across generations considering language, a biological determined skill to pass on cultural views and practices of survival value to further generations. Language possibly plays a key role in the autocatalytic system. Re-drawing after [2].

References

1. Behr M, Fanghänel J: Kraniomandibuläre Dysfunktionen. Antworten auf Fragen aus der Praxis. Thieme, Stuttgart, New York 2019

2. Eccles JC: Die Evolution des Gehirns – Die Erschaffung des Selbst. Piper, München, Zürich 1994

3. Fanghänel J, Schumacher GH: Schädelwachstum und Statik. Nova acta Leopoldina NF 1986; 262: 585–595

4. Gardner RA, Gardner BT: Comparative psychology and language acquisition. In: Sebeok TA, Umiker-Seboek DJ (eds.): Speaking of apes. Plenum Press, New York 1980, 287–330

5. Levy-Agresti J, Sperry RW: Differential perceptual capacities in major and minor hemispheres. Proc Natl Acad Sci 1968; 61: 1151

6. Liebermann P: On the origin of speech. Macmillian, New York 1985

7. Popper KR, Eccles JC: Das Ich und sein Gehirn. Piper, München, Zürich 1982

8. Schumacher GH, Schmidt H, Richter W: Anatomie und Biochemie der Zähne. Volk und Gesundheit, Berlin 1982, 33–51

9. Teuber HL: Lacunae and research approaches to them. In: Millikan CH, Darley FL (eds.): Brain mechanisms underlying speech and language. Grune & Stratton, New York, London 1976, 204–216

10. Tobias PV: Recent advances in the evolution of hominids with special reference to brain and speech. In: Chagas C: Recent advances in the evolution of primates. Pontificiae academiae scientarum. Scripta varia, Vatican City 1983, 85–140

(Photo: UKR)

PROF. DR. MICHAEL BEHR
University of Regensburg
Faculty of Medicine
Franz-Josef-Strauss-Allee 11
93053 Regensburg
michael.behr@klinik.uni-regensburg.de

(Photo: UKR)

PROF. DR. JOCHEN FANGHÄNEL
University of Regensburg
Faculty of Medicine
Franz-Josef-Strauss-Allee 11
93053 Regensburg
jochen.fanghaenel@ukr.de

Karen Meyer-Wübbold, Hüsamettin Günay, Kira Ebert

The effectiveness of an electric "wash toothbrush" on oral plaque control – A pilot study

Introduction: Mechanical plaque control by means of self-responsible, home-based oral hygiene is essential for the prevention of caries and periodontal diseases. In this respect, many elderly patients are at increased risk. It has already been shown that an electric toothbrush with oscillating-rotating movement and a continuous water supply has a positive effect on dental plaque control when compared to a manual toothbrush. The aim of the present pilot study was to evaluate if sonic toothbrushes likewise benefit from a continuous water supply during the brushing process and if they have a positive effect on dental plaque control in younger seniors.

Methods: The study included 12 subjects (mean age 72.08 ± 3.88 years, 6 females, 6 males). Following a plaque accumulation phase of 48 hours, an electric toothbrush with oscillating-rotating movement and a sonic toothbrush with (wash toothbrush) and without continuous water supply were tested in a single application. The Quigley-Hein-Index (QHI) and the Approximal-Plaque-Index (API) were each determined before and after brushing to assess plaque reduction.

Results: The electric toothbrush with an oscillating-rotating movement pattern with continuous water supply (WORT) showed a higher reduction of the plaque index readings compared to the electric toothbrush with oscillating-rotating movement pattern without water supply (ORT) in the area of the smooth surfaces (WORT: Δ QHI 1.68 ± 0.28; ORT: Δ QHI 1.41 ± 0.34) and approximal surfaces (Δ API WORT: 20.43 ± 18.7 %; Δ API ORT: 19.85 ± 18.03 %). These results, however, were not statistically significant. The sonic toothbrush with continuous water supply (WST) showed a significantly higher reduction of plaque index compared to the sonic toothbrush without water supply (ST) on the smooth surfaces (WST: Δ QHI 1.88 ± 0.33, ST: Δ QHI 1.27 ± 0.25, $p < 0.001$) and approximal surfaces (Δ API WST: 30.14 ± 14.85 %, Δ API ST: 14.12 ± 10.6 %, $p = 0.006$). A higher reduction of the plaque index value was determined on both the smooth and approximal surfaces using the WST as compared to the WORT, although the results were not statistically significant.

Conclusion: An electric toothbrush with a continuous water supply has a positive effect on dental plaque control in elderly subjects. Sonic toothbrushes benefit from a continuous water supply to a greater extent than electric toothbrushes with an oscillating-rotating movement pattern. Further investigations should evaluate if the use of an electric toothbrush increases the "hydrodynamic effect", thereby facilitating that difficult-to-clean niches such as exposed root surfaces or crown margins are reached.

Keywords: oscillating-electric toothbrush; sonic toothbrush; continuous water supply; plaque control

Department of Conservative Dentistry, Periodontology and Preventive Dentistry, Hannover: Dr. Karen Meyer-Wübbold, Dr. Kira Ebert, Prof. Dr. Hüsamettin Günay
Translation: Christian Miron
Corresponding authors: Karen Meyer-Wübbold, Hüsamettin Günay; these authors are equal first authors: Karen Meyer-Wübbold, Hüsamettin Günay
Citation: Meyer-Wübbold K, Günay H, Ebert K: The effectiveness of an electric "wash toothbrush" on oral plaque control – A pilot study. Dtsch Zahnärztl Z Int 2019; 1: 175–181
Peer-reviewed article: submitted: 25.01.2019, revised version accepted: 23.04.2019
DOI.org/10.3238/dzz-int.2019.0175–0181

1. Introduction

Mechanical plaque control and biofilm removal play a critical role in the prevention of caries, gingivitis and periodontitis [2]. The removal of the biofilm is not just the responsibility of the dentist, but rather, primarily that of the patient who should be self-responsible for undertaking home-based oral hygiene measures on a regular basis [5]. Due to the fact that caries and inflammatory periodontal diseases continue to be "common diseases", it appears that the quality of home-based plaque removal in large parts of the population is inadequate. Especially older patients display a higher plaque affliction compared to younger ones [16]. In the Fifth German Oral Health Study (DMS V), 28 % of the examined senior citizens had at least one root surface caries or root surface filling. With respect to the dentulous study participants, this was as high as 32 % [11]. For this reason, they should be counted as patients at risk for root surface and crown marginal caries [11]. The cause for increased root and crown margin caries susceptibility in older patients is multifactorial. As an example, exposed root surfaces or exposed restoration margins due to periodontal problems foster plaque retention and caries predilection sites [3].

As part of gingivitis and caries prophylaxis, it is not only necessary that smooth surfaces are cleaned, but also interdental spaces, especially given that tooth surfaces below the approximal contacts represent predilection sites for caries and gingivitis [19]. In spite of this, these areas are often not sufficiently reached when simply using a hand and/or an electric toothbrush [24]. If biofilm or food particles cannot be removed with a toothbrush alone, additional hygiene tools such as dental floss or interdental brushes are recommended [7, 23]. However, user acceptance of these additional hygiene tools is to date still considered to be low [11, 31].

Studies in behavioral science have shown that it is difficult to achieve health-related behavioral changes in adults [1]. Thus, patients often overlook dentists' recommendations related to making changes in their oral hygiene habits; such changes may include brushing technique and brushing system, or the additional use of hygiene tools for approximal space cleaning. Moreover, in older patients, a decrease of acuity and motor dexterity (limitations in gross and fine motor skills, decreased vision, decreased cognitive performance) [21] occurs with age; many of them are unable to use conventional toothbrushes to brush their teeth or employ hygiene tools to clean approximal spaces.

In order to assertively improve cleaning performance independent of individual factors such as dexterity, motivation and brushing time, new and more effective toothbrushes are continuously being developed and worked upon. Especially due to the low acceptance of hygiene tools for interdental cleaning, toothbrushes with increased efficiency in this area are desirable. In a survey using a representative sample of the population in the Federal Republic of Germany, it was found that 53 % of respondents used a manual toothbrush and 38 % used an electric toothbrush in the context of home-based oral hygiene [31]. The most common electric toothbrushes have an oscillating-rotating movement pattern or are activated by sound or ultrasound based on the manufacturer's specifications. In literature, oscillating-rotating brushes receive an advantage as a high level of evidence exists with regards to the effectiveness of these brushes [29, 30]. The bristles of sound-activated toothbrushes work mainly using "side-to-side movements" [12]. Cleaning occurs mechanically through the moving filaments themselves, on the one hand, while on the other, vibrations of the toothpaste-saliva mixture in the mouth generate turbulence (hydrodynamic effect). Due to the generated turbulence, the mixture should reach areas which are inaccessible to the toothbrush. In the case of ultrasonic toothbrushes, an additional cavitation effect occurs, thus leading to the removal of the biofilm and the attached plaque [12].

So-called "wash brushes" have been used for many years in households and in industry. These brushes are connected to a high-pressure cleaner or a normal water duct and are thus equipped with a continuous water supply. They are recommended by various manufacturers for their effective as well as gentle cleaning of smooth and sensitive surfaces.

In a pilot study in which the effectiveness of a manual and electric toothbrush was tested with ("wash toothbrush") and without continuous water supply, it could already be shown that an electric toothbrush, equipped with a continuous water supply and having an oscillating-rotating movement pattern, has a positive effect on dental plaque control in both younger and older subjects compared to a manual toothbrush [9]. The aim of this pilot study was to evaluate whether sonic toothbrushes benefit from a continuous water supply during the brushing process and if this has a positive effect on dental plaque control in younger elderly people.

2. Methods

2.1 Study design

The present study is a prospective, single-blind pilot study with crossover design. The study has received a positive vote from the Ethics Committee of the Hannover Medical School (Vote No. 1615–2012).

2.2 Subjects

In the current pilot study, a total of 12 subjects participated voluntarily with their prior written consent, which could thereafter be revoked at any time without giving reasons. The participants were between the ages of 66 and 79 years (72.08 ± 3.88 years); 6 subjects were male and 6 were female. Moreover, the participants were patients receiving a systematic periodontal therapy and part of the recall system at the Clinic for Conservative Dentistry, Periodontology and Preventive Dentistry of the Hannover Medical School. All subjects presented a history of periodontitis, but were periodontally healthy/rehabilitated.

Exclusion criteria included removable dentures, fewer than 20 teeth, PSI code > 2, taking anti-

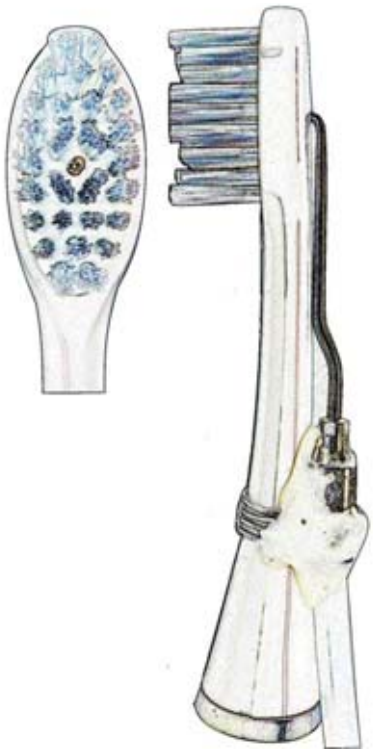


Figure 1 Brush head of the sonic toothbrush with continuous water supply – "washsonic-toothbrush" (WST)

inflammatory or anti-bacterial drugs, systemic disorders that influence oral findings, an age less than 65 years, and motor or sensory limitations.

2.3 Toothbrushes used

Each participant received a conventional electric toothbrush (Oral-B Professional Care Triumph 5000 with an attached Oral-B Precision Clean brush, Procter & Gamble), which had an oscillating-rotating movement pattern (ORT) as well as a sonic toothbrush (ST) (Hydrosonic CHS 100 with the brush head Hydrosonic sensitive (CHS 200), set at 32,000 movements per minute at "intensive" level, Curaprox). Additionally, each subject received a modified electric toothbrush with oscillating movement pattern (WORT) and a modified sonic toothbrush (WST). For this purpose, the conventional toothbrushes described above were modified and equipped with a continuous water supply (Fig. 1 and 2). The water supply was centrally located on the bristle field and was generated by a conventional irrigator (MD 5613 AEG) with a water flow rate of 65 ml per minute. In contrast to a conventional oral irrigator, the supplied water does not exactly strike the tooth surface, nor any existing pockets, but rather is distributed across the bristle field. In this manner, the supplied water is not used for mechanical biofilm disruption, but rather to support the cleaning action of the bristles. Hereafter, the term "wash toothbrush" is used to generally denote a modified toothbrush with continuous water supply.

2.4 Collected parameters

As part of an initial examination (baseline), a general medical history as well as the following parameters were collected:

- General dental examination (01 and resulting DMF-T/-S)
- Periodontal Screening Index (PSI) [15]
- Papilla Bleeding Index (PBI) [20]
- Approximal-Plaque-Index (API) [13]
- Modified Quigley-Hein-Plaque-Index (QHI) [27]

In order to create uniform starting conditions, all subjects received a professional tooth cleaning following the baseline examination. Both groups tested the 4 different toothbrushes in a single application. As part of the baseline investigation, all participants received thorough clarification and instructions by means of models and videos with regard to how to employ the various toothbrushes. The use of each toothbrush was preceded by a 2-day plaque accumulation phase (no home-based oral hygiene, no use of oral hygiene products or dental care products such as candies or chewing gum containing menthol). After testing each toothbrush, a 2-day "wash-out phase" was followed, during which the subjects performed home-based oral hygiene with their usual oral hygiene tools. After this phase, the next 2-day plaque accumulation phase began before testing the next toothbrush.

After the 2-day accumulation phase, the QHI and API indices were used to quantify plaque following staining with a plaque disclosing solution (Mira-2 tone, Hager & Werken). Then, the subjects received different toothbrushes in each of the 4 phases in the following order: an electric toothbrush without and with continuous water supply (ORT and WORT) and a sonic toothbrush without and with continuous water supply (ST and WST). After brushing with each respective toothbrush using medium abrasiveness toothpaste (Elmex Sensitive Professional Repair & Prevent, CP-GABA GmbH), residual plaque was visualized again using plaque disclosing solution and quantified using the QHI and API indices.

All parameters were collected by the same examiner after initial calibration took place together with the project manager. The examiner was unaware that a pre-determined sequence existed, and thus, did not know which toothbrush was being used.

In order to evaluate cleaning effectiveness, the differences of the QHI and API before and after brushing were calculated (below Δ QHI and Δ API). The collection of the plaque indices took place with the help of magnifying loupes (2-fold magnification). A questionnaire was used as part of the initial examination in order to record the oral hygiene habits of the subjects. After the last appointment, the subjects completed a further questionnaire with the purpose of documenting their subjective impression of the tested toothbrushes.

2.5 Statistical analysis

The statistical software program SPSS Statistics 21 for Windows was used to analyze data. First, mean values, standard deviations and frequencies were calculated as part of the descriptive statistics. Subsequently, the calculated mean values were tested for normal distribution using the Kolmogorow-Smirnow test (KS test). Since the tested variables (QHI, API values) were > 0.05, a normal distribution could be assumed. Therefore, a parametric paired t-test was employed to analyze variance for repeated measures within a group (electric toothbrush with oscillating-rotating movement pattern without and with continuous water supply, sonic

	QHI before	QHI after	QHI Difference	API before (%)	API after (%)	API Difference (%)
ST	2.31 ± 0.47	1.04 ± 0.38	1.27 ± 0.25	99.04 ± 3.33	84.92 ± 12.59	14.12 ± 10.6
WST	2.54 ± 0.4	0.66 ± 0.27	1.88 ± 0.33	98.72 ± 4.44	68.58 ± 14.85	30.14 ± 14.85
ORT	2.29 ± 0.20	0.88 ± 0.37	1.41 ± 0.34	97.88 ± 3.93	78.03 ± 18.5	19.85 ± 18.03
WORT	2.44 ± 0.22	0.76 ± 0.33	1.68 ± 0.28	99.04 ± 2.78	78.61 ± 19.43	20.43 ± 18.7

Table 1 QHI and API of the participants before and after toothbrushing with ST, WST, ORT and WORT, as well as QHI and API differences (* statistically significant)

toothbrush without and with continuous water supply). The means between the tested toothbrushes were compared with the unpaired t-test. The statistical significance level was set at p = 0.05.

3. Results

3.1 Results from baseline examination

No participants in the project had a need for periodontal treatment and all were caries free. The participants had a mean PBI of 0.7 ± 0.3 and a mean DMF-T of 17.8 ± 4.7 (DMF-S: 61.3 ± 23.4). The plaque index value in the area of smooth surfaces (QHI) was on average 1.4 ± 0.3 and 91.5 ± 8.7% in the approximal area (API).

3.2 Comparison of plaque index value reduction between ORT and WORT

With the WORT, the plaque index value on the smooth surfaces (Δ QHI) was reduced on average by 1.68 ± 0.28 and by 20.43 ± 18.7 % in the approximal region (Δ API). With the ORT, on average, a reduction of the plaque index value (Δ QHI) by 1.41 ± 0.34 was achieved on the smooth surfaces and 19.85 ± 18.03 % in the approximal areas. Comparison of the means between WORT and ORT showed a tendency towards a higher reduction of the plaque index value in the area of the smooth surfaces for the WORT, but this was not statistically significant (p = 0.062). There were only slight differences between the two toothbrushes in the area of the approximal surfaces (Table 1).

3.3 Comparison of plaque index value reduction between ST and WST

The WST showed a significantly higher reduction of the plaque index value on the smooth surfaces (p < 0,001) and in approximal areas (p = 0,006) compared to the ST. With the ST, the plaque index value (Δ QHI) was reduced by 1.27 ± 0.25 on the smooth surfaces and by 14.12 ± 10.6 % in approximal areas. With the WST, on average, a reduction of the plaque index value (Δ QHI) by 1.88 ± 0.33 was achieved on the smooth surfaces and 30.14 ± 14.85 % in approximal areas (Table 1).

3.4 Comparison of the reduction in the plaque index values between ST and ORT

With the ORT, the subjects tended to achieve a higher reduction of the plaque index value on the smooth surfaces as well as in the approximal areas than with the ST, but this was not statistically significant (Table 1).

3.5 Comparison of the reduction in plaque index values between WST and WORT

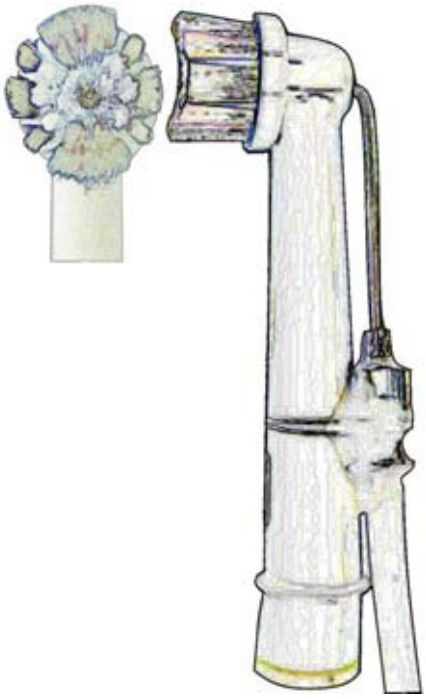
The subjects tended to achieve a higher reduction of the plaque index value with the WST than with the WORT, both on the smooth surfaces and in the approximal areas, but this was not statistically significant (Table 1).

3.6 Evaluation of the questionnaire

75 % of the participants already used an electric toothbrush as part of their home-based oral hygiene. All project participants stated that they had a better "feeling in the mouth" after using the "wash toothbrushes" (WORT, WST) compared with the conventional electric toothbrushes (ORT, ST). Moreover, 83.3 % and 16.7 % preferred the WST and WORT, respectively.

4. Discussion

In the present study, it was observed that there was a tendency towards a higher reduction of the plaque index value on both smooth and approximal surfaces for the electric toothbrush with an oscillating-rotating movement pattern (ORT) compared to the sonic toothbrush (ST). In literature, the efficiency between sonic toothbrushes and electric toothbrushes with oscillating-rotating movement pattern is controversially debated. Some studies affirm that electric toothbrushes with oscillational rotating patterns of movement have a higher plaque and gingivitis reduction as compared to sonic toothbrushes [4, 8]. Other studies, however, observe the opposite [17, 25]. Clinical studies should take into account that home-based oral hygiene may also be incorrectly or inadequately exercised. Ganss et al. (2018) used videos to observe subjects during brushing with an electric as well as a manual toothbrush [6]. For both toothbrushes, identical movement patterns (horizontal and circular brushing movements) were registered. Only 50.5 % of subjects allowed for "passive movements" using the electric toothbrush (positioning the brush head on the tooth with less than 2 movements). This



(Fig. 1 and 2, Table 1: K. Meyer-Wübbold, H. Günay)

Figure 2 Brush head of the electric toothbrush with continuous water supply – "washelectric-toothbrush" (WORT)

"passive brushing" took up less than 10 % of the total brushing time [6]. In spite of this, in order to achieve an optimal brushing performance with electric toothbrushes, "passive movement" makes sense. In order to achieve an optimal brushing result, the brush head should be guided both along the gingival margin as well as along the contour of the tooth and into the interdental space using a small pivoting movement. Also with the sonic toothbrush employed in the present study, the manufacturer recommends an angulation of 45° to the tooth surface for optimal cleaning in the area of the gingival margin. In doing this, the bristles should be placed only lightly without pressure on the tooth surface. For each tooth, the user should stay for 2 to 3 seconds and then slowly perform tilting movements without pressure [Source: instruction manual and instruction video Hydrosonic, Curaprox]. The patients in the present study were indeed intensively instructed at the beginning with regard to the use of the respective toothbrush with the aid of models and videos. However, it cannot be ruled out whether or not the technique specified by the manufacturer was or was not fully implemented. The technique specified by the manufacturer is very similar to the "bass technique" and was therefore difficult for the project participants to implement. In addition, it could be observed that the manufacturer's recommendation of a short, motionless pause of the brush head on the tooth was also difficult for the participants to implement. The subjects quickly became impatient, which was possibly related to the feeling of "being under surveillance". During the use of the ST, the participants repeatedly came back to the movement pattern of a manual toothbrush. Moreover, the subjects were probably not used to perform brushing with minimal pressure. Also, since the sonic toothbrush used in the present study did not have a pressure control, it cannot be ruled out whether or not too much pressure was exerted by the patients; this could possibly have reduced the cleaning performance of the sonic toothbrush.

In the present study, for the modified sonic toothbrush with continuous water supply (WST), a higher reduction in the plaque index was observed compared to all other toothbrushes tested, both on smooth and in approximal surfaces. For sonic toothbrushes, the bristles of the brush head are moved with rapid oscillations, thereby achieving a "hydrodynamic effect". This means that the bristles do not only mechanically clean, but also generate turbulence through vibrations, thus allowing the toothpaste-saliva mixture to remove plaque and bacteria in poorly accessible areas. This effect has been demonstrated in some studies [22]. In the present study, however, the lowest reduction of the plaque index value was observed on smooth and in approximal surfaces for the sonic brush without continuous water supply (ST). Apparently, a normal "toothpaste saliva mixture" does not seem to be sufficient to achieve an efficient "hydrodynamic effect". If, however, the sonic toothbrush is combined with a continuous water supply (WST), significantly higher reductions in the plaque index value are achieved both in the areas of smooth and approximal surfaces. The WORT also showed higher reductions in the plaque index value compared to the ORT, both on the smooth and approximal surfaces, but this was not statistically significant. Movement of liquid around the bristles is not only observed for sonic toothbrushes, but also for other electric toothbrushes, which may have an additive effect on the purely mechanical action of the toothbrush [18]. Sahota et al. (1998) concluded that plaque removal depends both on direct bristle contact and on the presence of fluid [18]. They could observe that although plaque removal is mainly due to the mechanical action of the bristles, additional plaque removal occurs through turbulence; this arises when the bristles work in an aqueous environment. The results of the present study confirm this hypothesis.

A dry mouth has been observed in many elderly patients [14]. The causes of decreased salivation at old age are manifold; among them are a decrease in chewing activity, changing dietary habits, lower fluid intake or a systemic medication that reduces salivation [14]. Sufficient toothpaste-saliva mixture during the brushing process may not be formed for these patients. Therefore, these patients could benefit from a toothbrush with continuous water supply. With regard to toothbrushes with continuous water supply, it is difficult to make comparisons of literature due to the low number of studies. Sumi et al. (2003) examined the efficiency of an electric toothbrush with an oscillating motion pattern and continuous water supply as compared to a conventional electric toothbrush with oscillating motion pattern in elderly patients in terms of plaque removal in the area of smooth surfaces. They concluded that the modified toothbrush removed significantly more plaque in the area of smooth surfaces [26]. This result is comparable to those of this pilot study. In this study, higher reductions in plaque index value were observed for the modified toothbrush with oscillating motion pattern as compared to the conventional toothbrush as well.

In the approximal area, the ORT barely showed any differences in the reduction of the plaque index value compared to the WORT. Even with the WST, only a 30.14 % reduction of the plaque index value was achieved in the approximal area. These results suggest that sufficient cleaning in approximal spaces is difficult without additional hygiene tools. However, it should be noted that the index used in the present study to assess plaque in approximal areas is an index that only makes a yes/no decision on the presence of plaque in the approximal areas. The extent of plaque is therefore not taken into account. In order to assess cleaning efficiency as well as motivate the patient, a statement regarding the extent of plaque reduction would be more meaningful than just a statement regarding complete plaque removal [10]. Furthermore, in assessing the approximal cleaning performance of the toothbrushes, the possible "user errors" already mentioned above should also be taken into consideration.

In addition to the cleaning efficiency of the different toothbrushes, the subjective impression of the participants was also evaluated by means of a questionnaire. All subjects stated that they had a better "feeling in the mouth" after using the "wash toothbrushes" compared with the conventional electric toothbrushes. In choosing between the two "wash toothbrushes", over 80 % of the subjects preferred the WST over the WORT. This subjective impression is reflected in the clinical values as well.

The toothbrushes were only tested once by the participants. Many participants claimed that they needed to get used to the continuous water supply of the modified toothbrushes and that it was difficult to concentrate on the brushing process when the water supply was switched on. Moreover, in interpreting these results, it should be taken into account that not all participants were already familiar with electric toothbrushes, as 25 % of them used a manual toothbrush for their home-based oral hygiene. An influence of these aspects on the results of the reduction of the plaque index values cannot be ruled out. Hence, in future studies, an "adjustment period" should take place in advance so that the participants have time to familiarize themselves with the use of different toothbrushes. It would also be desirable to evaluate the corresponding toothbrushes over a longer time period in the context of home-based home oral hygiene.

In order to collect the indices, the plaque was visualized using a plaque disclosing solution. However, neither a demonstration nor an explanation of the plaque afflicted sites ensued for the participants. The brushing process was performed by the retirement-aged participants in an oral hygiene room, which had a sink and an unlit mirror. Moreover, the subjects were at least 50 cm away from the mirror. In this manner, the participants had no possibility to recognize the plaque-prone spots in detail on site. Therefore, the visualization of plaque could not have influenced the brushing results.

In the present pilot study, mechanical plaque removal with the various toothbrushes was combined with the use of toothpaste since the majority of the population also uses toothpaste for home-based oral hygiene. All patients used the same toothpaste with medium abrasiveness at all times. In a systematic review, it was shown that the use of toothpaste plays a rather disorderly role in supporting mechanical plaque removal. Valkenburg et al. (2016) determined that, as part of mechanical plaque removal, 49.2 % of the plaque was removed in combination with toothpaste and 50.3 % without toothpaste [28]. In this regard, an additive effect of toothpaste on plaque removal can also be neglected in the present pilot study.

5. Conclusion

Taking the limitations of this pilot study into account, it appears that an electric toothbrush equipped with a continuous water supply has a positive effect on dental plaque control in elderly subjects. Moreover, it appears that a sonic toothbrush profits from a continuous water supply to a greater extent than an electric toothbrush with an oscillating-rotating movement pattern. Further investigations should evaluate whether the application of an electric wash toothbrush can increase the "hydrodynamic effect" and whether it can thereby also reach hard-to-access niches such as exposed root surfaces or crown margins.

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.

References

1. Ashenden R, Silagy C, Weller D: A systematic review of the effectiveness of promoting lifestyle change in general practice. Family Practice 1997; 14: 160–176

2. Axelsson P, Nyström B, Lindhe J: The long-term effect of a plaque control program on tooth mortality, caries and periodontal disease in adults – results after 30 years of maintenance. J Clin Periodontol 2004; 31: 749–757

3. Bizhang M, Zimmer S: Oralprophylaxe für ältere Menschen. Wissen kompakt 2012; 6: 39–52

4. Ccahuana-Vasquez RA, Conde EL, Cunningham P, Grender JM, Goyal CR, Qaqish J: An 8-week clinical comparison of an oscillating-rotating electric rechargeable toothbrush and a sonic toothbrush in the reduction of gingivitis and plaque. J Clin Dent 2018; 29: 27–32

5. Dörfer CE, Staehle HJ: Strategien der häuslichen Plaquekontrolle: Zahnmed up2date 2010; 3: 231–256

6. Ganß C, Duran R, Winterfeld T et al.: Tooth brushing motion patterns with manual and powered toothbrushes – a randomized video observation study. Clin Oral Investig 2018; 22: 715–720

7. Geurtsen W, Hellwig E, Klimek J: Grundlegende Empfehlungen zur Kariesprophylaxe im bleibenden Gebiss. Dtsch Zahnärztl Z 2013; 68: 639–646

8. Grender J, Williams K, Walters P, Klukowska M, Reick H: Plaque removal efficacy of oscillatin-rotating power toothbrushes: review of six comperative clinical trials. Am J Dent 2013; 26: 68–74

9. Günay H, Niehus K, Staufenbiel I, Geurtsen W, Meyer K: Effektivität einer modifizierten Zahnbürste mit kontinuierlicher Wasserzufuhr auf die Plaquekontrolle. Dtsch Zahnärztl Z 2017; 71: 46–53

10. Günay H, Meyer-Wübbold K: Effekt des zweimaligen Zähneputzens auf die dentale Plaqueentfernung bei jungen Senioren. Dtsch Zahnärztl Z 2018; 73: 153–163

11. IDZ, Institut der Deutschen Zahnärzte (Hrsg): Fünfte Deutsche Mundgesundheitsstudie (DMS V). Deutscher Zahnärzte Verlag DÄV, Köln 2016

12. Klocke A, Sonntag D, Beikler T: Elektrische Zahnbürsten – gibt es was Neues? Quintessenz 2015; 66: 7–19

13. Lange DE, Plagmann HC, Eenboom A, Promesberger A: Klinische Bewertungsverfahren zur Objektivierung der Mundhygiene. Dtsch Zahnärztl Z 1977; 32: 44–47

14. Laurisch L: Mundtrockenheit – Hintergründe und Therapie eines zunehmenden Problems. Dtsch Zahnärztl Z 2012; 67: 430–437

15. Meyle J, Jepsen S: Der parodontale Screening-Index (PSI). Parodontologie 2000; 11: 17–21

16. Micheelis W, Schiffner U, Hoffmann T: Vierte Deutsche Mundgesundheitsstudie (DMS IV): Neue Ergebnisse zu oralen Erkrankungsprävalenzen, Risikogruppen und zum zahnärztlichen Versorgungsgrad in Deutschland 2005. Deutscher Zahnärzte Verlag DÄV, Köln 2006

17. Robinson PJ, Maddalozzo D, Breslin S: A six-month clinical comparison of the efficacy of the Sonicare and the Braun Oral-B electric toothbrushes on improving periodontal health in adult periodontitis patients. J Clin Dent 1997; 8: 4–9

18. Sahota H, Landini G, Walmsley AD: A testing system for electric toothbrushes. Am J Dent 1998; 11: 271–275

19. Sälzer S, Slot DE, Van der Weijden FA, Dörfer CE: Efficacy of interdental mechanical plaque control in managing gingivitis – a meta-review. J Clin Periodontol 2015; 42: 92–105

20. Saxer UP, Mühlemann HR: Motivation and education. SSO Schweiz Monatsschr Zahnheilkd 1975; 85: 905–919

21. Schlüter IM: Zahnproblemen im Alter vorbeugen – Tipps zur Mundhygiene bei Senioren. Geriatrie-Report 2018; 13: 26–29

22. Schmidt JC, Zaugg C, Weiger R, Walter C: Brushing without brushing? – a review of the efficacy of powered toothbrushes in noncontact biofilm removal. Clin Oral Invest 2013; 17: 687–709

23. S2k-Leitlinie (Langversion): Kariesprophylaxe bei bleibenden Zähnen – grundlegende Empfehlungen; AWMF-Registernummer: 083–021; 2016. www.awmf.org/leitlinien/detail/ll/083–021.html

24. Slot DE, Dörfer CE, Van der Weijden GA: The efficacy of interdental brushes on plaque and parameters of periodontal inflammation: a systematic review. Int J Dent Hyg 2008; 8: 253–264

25. Starke M, Delaurenti M, Ward M, Souza S, Milleman KR, Melleman JL: A comparison of the effect of two power toothbrushes on the gingival health and plaque status of subjects with moderate gingivitis. J Clin Dent 2017; 28: A29–35

26. Sumi Y, Nakajima K, Tamura T, Nagaya M, Michiwaki Y: Developing an instrument to support oral care in the elderly. Gerodontology 2003; 20: 3–8

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

28. Valkenburg C, Slot DE, Bakker EWP, Van der Weijden FA: Does dentifrice use help to remove plaque? A systematic review. J Clin Periodontol 2016; 43: 1050–1058

29. Van der Weijden FA, Slot DE: Efficacy of homecare regimens for mechanical plaque removal in managing gingivitis a meta review. J Clin Periodontol 2015; 42: 77–91

30. Yaacob M, Worthington HV, Deacon SA et al.: Powered versus manual toothbrushing for oral health. Cochrane Database Syst Rev 2014; 17: CD002281. doi: 10.1002/14651858.CD002281.pub3

31. Zimmer S, Lieding L: Gewohnheiten und Kenntnisse zur Mundhygiene in Deutschland – Ergebnisse einer bevölkerungsrepräsentativen Befragung. Dtsch Zahnärztl Z 2014; 69: 584–593

(Photos: Hannover Medical School)

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

Knut Adam, Evangelia Gousopoulou, Athina Bakopoulou, Gabriele Leyhausen, Joachim Volk, Ingmar Staufenbiel, Hüsamettin Günay, Peter Paul Josef Schertl, Werner Geurtsen

Characterization of cells derived from inflamed intra-bony periodontal defects

Introduction: Regeneration of intra-bony periodontal defects should be supported by formation of new blood vessels and nerve fibres to ensure nutrition and innervation of the newly formed tissues. Aim of the present study was to evaluate the neurovascular properties of human stem cells derived from inflamed periodontal ligaments (ihPDLSCs).

Methods: Cultures of ihPDLSCs were established from granulation tissue of intra-bony periodontal defects (n = 4). Expression of epitopes characteristic for mesenchymal (CD73, CD90, CD105, CD146, STRO-1), embryonic (SSEA-4) and hematopoietic (CD34, CD45) stem cells were analysed by flow cytometry. Neuronal, endothelial and osteoblastic differentiation was induced by respective media. Changes in cell morphology were observed microscopically. Matrix mineralization was visualized and quantified using Alizarin Red S staining. Gene expression of neurogenic (NEFL, NCAM1, ENO2, TUBB3), angiogenic (VEGFR1, VEGFR2, PECAM1, ANGPT2) and osteogenic (RUNX2, SP7, APL, BMP2, BGLAP, SPP1, IBSP) markers was assessed by qRT-PCR.

Results: Cultures of ihPDLSCs showed high expression of CD73 and CD90, medium to high expression of CD105 and CD146, low to medium expression of SSEA-4 and low expression of STRO-1, CD34 and CD45. Trilineage differentiation potential was documented by histomorphologic changes, pronounced matrix mineralization and significant upregulation of stage-specific markers characteristic for neuronal (NEFL, NCAM1, ENO2), endothelial (VEGFR1, VEGFR2, PECAM1) and osteoblastic (ALP, BMP2) differentiation.

Conclusions: Our data provide evidence that cells isolated from granulation tissue of intra-bony periodontal defects have properties characteristic for mesenchymal stem cells. As these cells have the potential to undergo neuronal, endothelial and osteoblastic differentiation, the preservation of granulation tissue during regenerative periodontal surgery may be valuable to promote the healing of intra-bony periodontal defects.

Keywords: mesenchymal stem cells; regenerative periodontal surgery; inflamed intra-bony defects; granulation tissue; trilineage differentiation potential; in vitro

Department of Conservative Dentistry, Periodontology and Preventive Dentistry, Hannover Medical School, Germany: Dr. Knut Adam, Evangelia Gousopoulou, Assist. Prof. Dr. Athina Bakopoulou, Dr. Gabriele Leyhausen, Dr. Joachim Volk, PD Dr. Ingmar Staufenbiel, Prof. Dr. Hüsamettin Günay, Dr. Peter Paul Josef Schertl, Prof. Dr. Werner Geurtsen
Department of Preventive Dentistry, Periodontology and Implant Biology, School of Dentistry, Faculty of Health Sciences, Aristotle University of Thessaloniki, Greece: Evangelia Gousopoulou
Department of Prosthodontics, School of Dentistry, Faculty of Health Sciences, Aristotle University of Thessaloniki, Greece: Assist. Prof. Dr. Athina Bakopoulou
Citation: Adam K, Gousopoulou E, Bakopoulou A et al.: Characterization of cells derived from inflamed intra-bony periodontal defects. Dtsch Zahnärztl Z Int 2019; 1: 182–194
Peer-reviewed article: submitted: 09.05.2019, revised version accepted: 25.06.2019
DOI.org/10.3238/dzz-int.2019.0182–0194

Background

Intra-bony periodontal defects caused by periodontitis often show a rapid progression and represent a serious risk for tooth loss. During the destructive process, an inflammatory soft tissue – the granulation tissue – progressively replaces the healthy periodontium. The granulation tissue, considered as tissue of minor value, is generally resected during regenerative periodontal surgery [12, 13, 51, 62, 63]. The resulting tissue deficiency is usually associated with the development of gingival recessions, in particular in patients with advanced intra-bony periodontal defects [57]. Bone substitutes and occlusive membranes acting as defect fillers and barriers are routinely used to avoid a soft tissue collapse into the defect [17]. However, these exogenous materials can lead to unwanted side effects, like incomplete resorption of the bone substitutes or membrane exposure, and adversely affect the regenerative healing processes [43, 64].

Therefore, our group has introduced the granulation tissue preservation technique (GTPT, Fig. 1), which attempts to maintain as much granulation tissue as possible during regenerative periodontal surgery. We have shown in a case series that the preservation of granulation tissue as 'autologous' material has a positive influence on the clinical and radiographic treatment outcome [28]. Thus, we observed only a negligible development of gingival recessions and a significant bone fill in most cases. The maintenance of the vascular network, the increased wound stability and the preservation of mesenchymal stem cells (MSCs) are possible explanations for that. It has been suggested that the recruitment of progenitor cells, which are able to differentiate into specialized cells, plays a critical role within periodontal wound healing processes [8]. As all periodontal tissues originate from the dental mesenchyme, which is derived from the cranial neural crest [35], MSCs are considered as the main progenitor cells for periodontal regeneration.

Various sources of MSCs have been identified in the oral cavity. MSCs have been shown to reside in the pulp (dental pulp stem cells – DPSCs) [27] and the apical papilla (stem cells from the apical papilla – SCAP) [67] of permanent teeth, in the pulp of deciduous teeth (stem cells from human exfoliated deciduous teeth – SHED) [48], in the alveolar bone (bone marrow stem cells – BMSCs) [66], in the gingiva (gingival mesenchymal stem cells – GMSCs) [78] and in the periodontal ligament (healthy human periodontal ligament stem cells – hhPDLSCs) [65]. It has been also reported that MSCs may survive under inflammatory conditions. Therefore, stem cell properties have been found in cells isolated from the inflamed dental pulp [56], the inflamed gingiva [25] and the inflamed periodontal granulation tissue (inflamed human periodontal ligament stem cells – ihPDLSCs) [54].

Regenerative periodontal surgery aims at renewing all tooth supporting tissues including the periodontal ligament (PDL), the root cementum and the alveolar bone [36]. Angiogenesis and neurogenesis play a decisive role in periodontal regeneration, because formation of blood vessels and nerve fibres is required to ensure nutrition and innervation of the newly formed tissues [3, 42, 71]. Objective of the present study was to examine, if ihPDLSCs have the potential to promote the healing processes following regenerative periodontal surgery. The hypothesis of the present study was that ihPDLSC cultures derived from intra-bony periodontal defects contain a MSC population that is able to

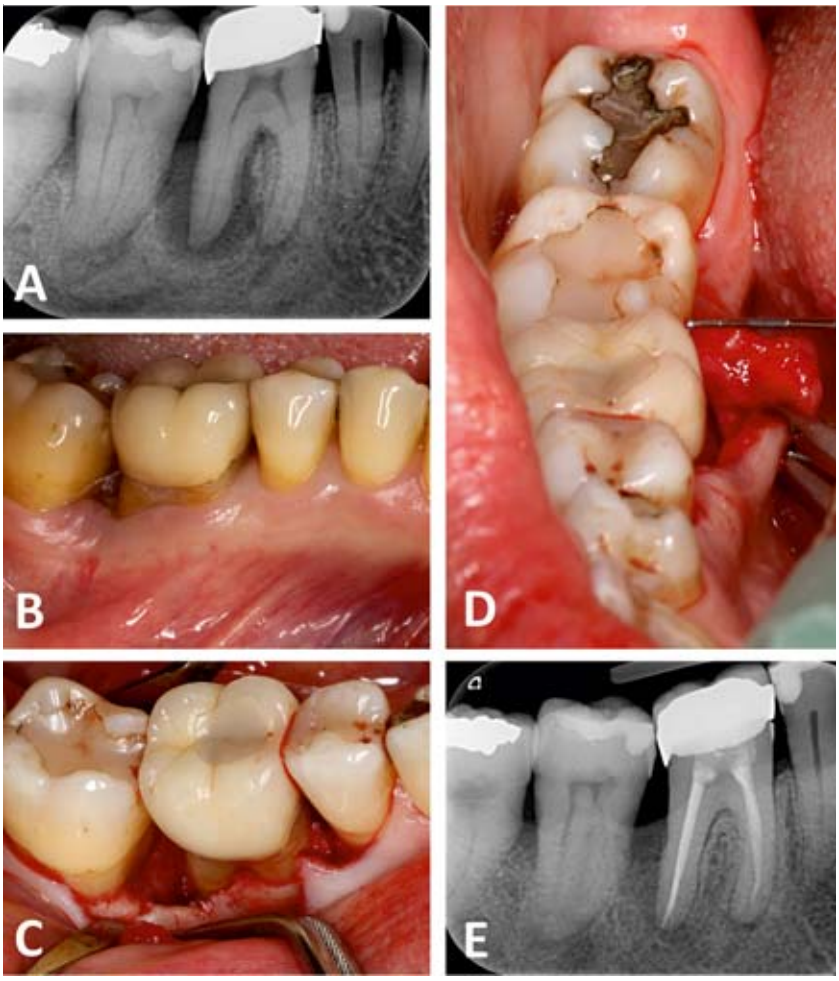


Figure 1A–E Representative treatment of an intra-bony periodontal defect (IPD) using the granulation tissue preservation technique (GTPT): The preoperative radiograph showed a pronounced bone loss at the distal root of the lower right first molar (**A**). The mucoperiosteal flap including the intra-lesional granulation tissue (GT) was thoroughly detached from the IPD and mobilized as far as it was necessary for an effective scaling and root planning (**B–D**). The radiograph 2 years after surgery and endodontic treatment revealed a significant bone fill in the former defect (**E**).

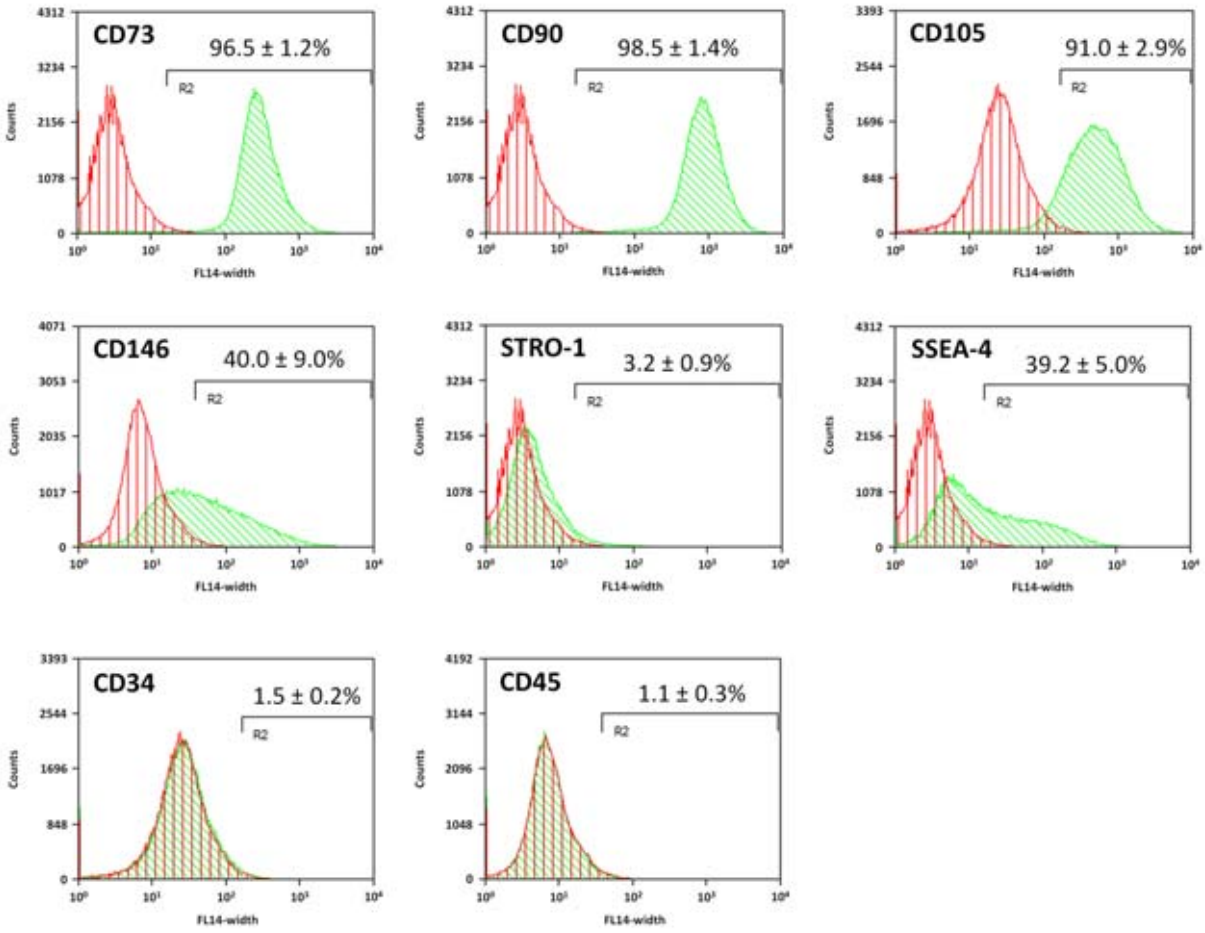


Figure 2 Representative data of the immunophenotypic characterization with flow cytometry: Single-parameter histograms show expression of mesenchymal (CD73, CD90, CD105, CD146, STRO-1), hematopoietic (CD34, CD45) and embryonic (SSEA-4) stem cell markers (red/vertical lines: unstained control; green/diagonal lines: cells expressing marker of interest).

undergo neurogenic, angiogenic and osteogenic differentiation.

Methods

Isolation and culture of ihPDLSCs

Four systemically healthy patients (aged 46.75 ± 2.63 years, 2 women, 2 men) diagnosed for severe chronic periodontitis were selected as donors for the biopsies. All patients received a non-surgical periodontal therapy to reduce local signs of inflammation. The surgical intervention was performed at teeth with a residual periodontal defect exhibiting a pocket probing depth > 6 mm, bleeding on probing and a radiographically evident intra-bony component of ≥ 3 mm. Since all surgical interventions were conducted for the purpose of periodontal regeneration, the GTPT was applied [28]. To get access to the bacterially contaminated root surface(s), a circumferential marginal incision was conducted at the defect-related teeth and the buccal and oral gums including the intra-lesional granulation tissue were sharply dissected from the underlying bone using microsurgical instruments. The mucoperiosteal flaps with the adherent granulation tissue were elevated as far as needed for an effective mechanical debridement (Fig. 1). The residual inflammatory granulation tissue was collected from the bottom of the intra-bony periodontal defect using curettes and scalers and used for the cell culture establishment of the present study. The defect-related root surface(s) were thoroughly debrided with hand instruments and a sonic device. Afterwards, the regenerative procedure was conducted. This included the application of a 24 % ethylene-diamine-tetra-acetic acid (EDTA) gel (PrefGel, Straumann, Freiburg, Germany), irrigation with sterile physiologic saline solution and application of enamel matrix proteins (Emdogain, Straumann). At the end of the surgical procedure, the intra-lesional granulation tissue and mucoperiosteal flaps were repositioned and fixed with interrupted sutures.

Immediately after collection, the inflammatory granulation tissue was minced into the smallest pieces possible and digested in α-minimal essential medium (α-MEM; Gibco, Grand Island, NY, USA) containing 3 mg/ml collagenase type I (Gibco) and 4 mg/ml dispase II (Sigma-Aldrich, Steinheim, Germany) for 1 h at 37°C. Cell-containing medium was passed through a strainer with a pore size of 70 µm (EASYstrainer, Greiner bio-one, Frickenhausen, Germany). The resulting ihPDLSCs were seeded into

	QuantiTect Primer Assay	Protein/enzyme (abbreviation)	Catalogue number	Detected transcript(s)
Neurogenic	Hs_ENO2_1_SG	enolase 2 (ENO2)	QT00084889	NM_001975 (2423 bp)
	Hs_NCAM1_1_SG	neural cell adhesion molecule (NCAM1)	QT00071211	NM_000615 (5977 bp) NM_001076682 (4944 bp) NM_001242608 (4831 bp)
	Hs_NEFL_1_SG	neurofilament, light polypeptide (NEFL)	QT00096369	NM_006158 (3854 bp)
	Hs_TUBB3_1_SG	tubulin, beta 3 class III (TUBB3)	QT00083713	NM_006086 (1794 bp)
Angiogenic	Hs_ANGPT2_1_SG	angiopoietin 2 (ANGPT2)	QT00100947	NM_001118887 (5267 bp) NM_001118888 (5114 bp) NM_001147 (5270 bp)
	Hs_PECAM1_1_SG	platelet and endothelial cell adhesion molecule 1 (PECAM1)	QT00081172	NM_000442 (6831 bp) XM_005276880 (4006 bp) XM_005276881 (3972 bp) XM_005276882 (3966 bp) XM_005276883 (3943 bp) XM_006721944 (2438 bp) XM_006721945 (2452 bp)
	Hs_FLT1_1_SG	fms-related tyrosine kinase 1 (FLT1) or vascular endothelial growth factor receptor 1 (VEGFR1)	QT00073640	NM_002019 (7123 bp)
	Hs_KDR_1_SG	kinase insert domain receptor (KDR) or vascular endothelial growth factor receptor 2 (VEGFR2)	QT00069818	NM_002253 (6055 bp)
Osteogenic	Hs_ALPL_1_SG	alkaline phosphatase (ALPL)	QT00012957	NM_000478 (2606 bp) NM_001127501 (2441 bp) NM_001177520 (2325 bp) XM_005245818 (2573 bp) XM_005245820 (1379 bp) XM_006710546 (2558 bp)
	Hs_BGLAP_1_SG	bone gamma carboxyglutamic acid-containing protein (BGLAP)	QT00232771	NM_199173 (562 bp)
	Hs_BMP2_1_SG	bone morphogenic protein 2 (BMP2)	QT00012544	NM_001200 (3150 bp)
	Hs_IBSP_1_SG	integrin-binding sialoprotein (IBSP)	QT00093709	NM_004967 (1595 bp)
	Hs_RUNX2_1_SG	runt-related transcription factor 2 (RUNX2)	QT00020517	NM_001015051 (5487 bp) NM_001024630 (5553 bp) NM_004348 (5720 bp) NM_001278478 (5235 bp) XM_006715231 (5304 bp) XM_006715233 (2944 bp) XM_006715234 (875 bp)
	Hs_SP7_1_SG	sp7 transcription factor (SP7)	QT00213514	NM_001173467 (3173 bp) NM_152860 (2995 bp) NM_001300837 (3211 bp)
	Hs_SPP1_1_SG	secreted phosphoprotein 1 (SPP1)	QT01008798	NM_000582 (1616 bp)

Table 1 QuantiTect Primer Assays (Qiagen) used for the qRT-PCR analyses of genes related to neuronal, endothelial and osteoblastic differentiation

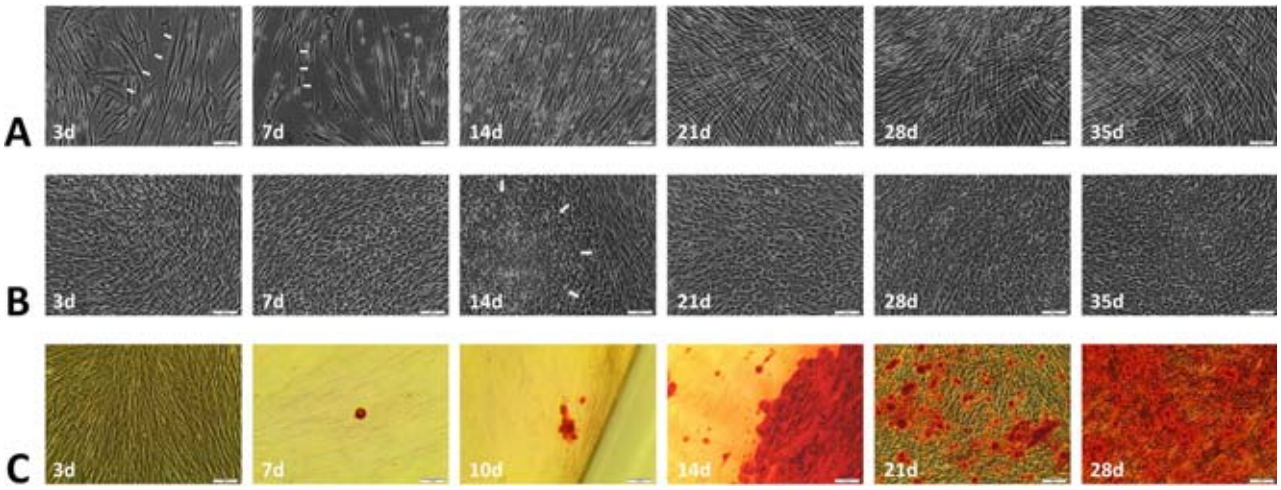


Figure 3A–C Microscopic images documenting the histomorphologic changes occurring during neuronal (**A**), endothelial (**B**) and osteoblastic (**C**) differentiation: Already 3 days after exposure to neurogenic medium, the formation of a neuron-like phenotype with a 'drawn-out' cell body (open arrow) and dendrite-like processes (white arrows) was observed (**A**). During angiogenic differentiation, the cell morphology changed from spindle-shaped (characteristic for fibroblasts) to polygonal (characteristic for endothelial cells). At the beginning of the endothelial differentiation, both morphologies were simultaneously present, as illustrated by the microscopic image at day 14 (white arrows indicating endothelial-like cells). Afterwards, a continuously increasing amount of the polygonal phenotype was observed (**B**). During osteoblastic differentiation, an increasing amount of AR-S positive mineralized deposits was detectable. At the end of the osteogenic differentiation experiment, the entire surface of the well was covered by AR-S positive mineralized tissue (**C**).

cell culture flasks containing complete culture medium (CCM) consisting of α-MEM supplemented with 15 % foetal bovine serum (FBS, Biochrom, Berlin, Germany), 100 U/ml penicillin, 100 µg/ml streptomycin (both from Biochrom), 2.5 µg/ml Amphotericin B (Capricorn Scientific, Ebsdorfergrund, Germany) and 100 µM L-ascorbic acid phosphate (Sigma-Aldrich). The cultures were incubated in a humidified atmosphere at 37 °C and 5 % CO_2. Cells from passages 2 to 4 were used for the experiments.

Characterization of ihPDLSC cultures with flow cytometry

The antigen profiles of ihPDLSC cultures at passages 2 and 3 were analysed by flow cytometry, as previously described [2]. Cells were seeded in 75 cm^2 culture flasks and expanded in CCM until confluency. Cells were trypsinized, washed with phosphate-buffered saline (PBS) and re-suspended in FACS buffer consisting of PBS supplemented with 1 % bovine serum albumin (BSA) and 0.1 % sodium azide (NaN_3). For each sample, 1x 10^6 cells/100 µl FACS buffer were Fc-blocked with 1 µg of human IgG (Sigma-Aldrich) for 15 min on ice. Afterwards, cells were stained by incubation with the following fluorochrome-conjugated mouse anti-human antibodies for 25 min in the dark on ice: CD73-FITC (fluorescein isothiocyanate), CD90-FITC, CD105-APC (allophycocyanin), CD146-PE (phycoerythrin), STRO-1-FITC, CD34-APC, CD45-PE and SSEA-4-FITC (all from BioLegend, Fell, Germany). For flow cytometry analysis, a BD LSR II Flow Cytometer (BD Biosciences, Heidelberg, Germany) was used. A total of 100,000 events were acquired for each sample. Data were analysed using the Summit 5.1 software (Beckman Coulter, Fullerton, USA). Flow cytometry experiments were repeated 4 times for each donor.

Induction of neurogenic differentiation

For neurogenic differentiation, ihPDLSCs were seeded into six-well plates coated with 0.1 % gelatine (Sigma-Aldrich) at 1x 10^5 cells/well and expanded in neurobasal A medium (Gibco) supplemented with B27 supplement (Gibco), 2 mM L-glutamine (Gibco), 20 ng/ml epidermal growth factor (EGF, Biochrom), 40 ng/ml recombinant human basic fibroblast growth factor (rh-bFGF, Biochrom), 100 U/ml penicillin, 100 µg/ml streptomycin and 2.5 µg/ml amphotericin B. Cells were cultured for 5 weeks and the neurogenic medium was changed every 2 to 3 d. Neurogenic differentiation was assessed with an inverted microscope for the detection of morphological changes towards a neuron-like phenotype and with quantitative reverse transcriptase polymerase chain reaction (qRT-PCR) for the expression of the neuronal markers neurofilament light polypeptide (NEFL), neural cell adhesion molecule 1 (NCAM1), tubulin beta 3 class III (TUBB3), and enolase 2 (ENO2).

Induction of angiogenic differentiation

For induction of angiogenic differentiation, cells were seeded into six-well plates coated with collagen I (Santa Cruz Biotechnology, Heidelberg, Germany) at 1x 10^5 cells/well. Cultures were expanded in CCM until they reached confluency. Afterwards, cells were exposed to angiogenic medium consisting of M199 medium (Gibco) supplemented with 5 % FBS, 100 U/ml penicillin, 100 µg/ml streptomycin, 2.5 µg/ml amphotericin B, 50 µg/ml heparin (Sigma-Aldrich), 1 µg/ml hydrocortisone (Sigma-Aldrich), 60 µg/ml en-

dothelial cell growth supplement (ECGS, PromoCell, Heidelberg, Germany), 10 ng/ml EGF (Biochrom), 25 ng/ml rh-bFGF (Biochrom) and 50 ng/ml vascular endothelial growth factor (VEGF, Gibco). Cells were cultured for 5 weeks; the angiogenic medium was changed every 2 to 3 d. Angiogenic differentiation was assessed by evaluation of phenotypic changes and qRT-PCR for the expression of the angiogenic markers angiopoietin 2 (ANGPT2), vascular endothelial growth factor receptor 1 (VEGFR1), vascular endothelial growth factor receptor 2 (VEGFR2) and platelet endothelial cell adhesion molecule 1 (PECAM1).

Induction of osteogenic differentiation

For osteogenic differentiation, cells were seeded into six-well plates at 1x 10^5 cells/well and expanded in CCM until they reached confluency. Subsequently, cells were exposed to osteogenic medium consisting of CCM supplemented with 5 mM β-glycerophosphate (Sigma-Aldrich), 1.8 mM monopotassium phosphate (KH_2PO_4, Sigma-Aldrich) and 10 nM dexamethasone (Sigma-Aldrich). Cells were cultured for 4 weeks; the osteogenic medium was changed every 2 to 3 d. Osteogenic differentiation was analysed by the Alizarin Red S (AR-S) mineralization assay and by qRT-PCR for the expression of the osteogenic markers bone morphogenic protein 2 (BMP2), secreted phosphoprotein 1 (SPP1), runt related transcription factor 2 (RUNX2), Sp7 transcription factor (SP7), bone gamma-carboxyglutamate protein (BGLAP), integrin binding sialoprotein (IBSP) and alkaline phosphatase (ALP). For the in vitro mineralization assay, cultures were washed twice with PBS without Ca^{2+} and Mg^{2+} (Biochrom) and fixed with 10 % neutral buffered formalin solution (Sigma-Aldrich) for 1 h at room temperature. Afterwards, cells were washed twice with distilled water and stained with 1 % AR-S (pH 4.0, Sigma-Aldrich) for 20 min at room temperature. To reduce non-specific staining, cells were washed 4 times with 2 ml distilled water and mineralized deposits were visualized and photographed with an inverted microscope (Olympus Optical Co., Ltd., Japan). For quantification of calcified tissues, AR-S was extracted by adding 1.5 ml cetylpyridinium chloride (CPC) buffer (10 %, w/v) in 10 mM disodium monohydrogen phosphate (Na_2HPO_4, pH 7.0) for 2 h at 37°C. Aliquots of 200 µl were transferred to a 96-well plate and the optical density of the solution was measured at 550 nm using a microplate spectrophotometer (Spectra Max 250, MWG Biotech, Sunnyvale, CA, USA).

Quantitative real-time reverse transcription polymerase chain reaction (qRT-PCR)

To analyse changes in gene expression during neurogenic, angiogenic and osteogenic differentiation, a two-step qRT-PCR was applied. Total mRNA was isolated using the RNeasy Plant Mini Kit (Qiagen, Hilden, Germany). An additional on-column DNA digestion was conducted to eliminate genomic DNA (RNase-free DNase Set, Qiagen). Afterwards, the RNA concentration was measured using a microplate reader (Synergy H1, BioTek, Bad Friedrichshall, Germany). The cDNA was synthetized using 1 µg of the isolated RNA and the QuantiTect Reverse Transcription Kit (Qiagen). For amplification and real-time quantification of cDNA targets, the QuantiTect SYBR Green PCR Kit, QuantiTect Primer Assays (Table 1) and the Rotor-Gene Q cycler (all from Qiagen) were used. All PCR reactions consisted of an initial incubation step of 5 min at 95°C to activate the HotStarTaq DNA polymerase and 40 cycles of denaturation (at 95°C for 5 sec), annealing and extension (at 60°C for 10 sec). A standard melting curve was used to validate the specificity of the reaction products. PCR raw data were processed using LinRegPCR to perform baseline correction, to determine the window-of-linearity and to determine the PCR efficiency per sample and per amplicon group [59]. Actin beta (ACTB), beta-2-microglobulin (B2M), glyceraldehyde-3-phosphate dehydrogenase (GAPDH), 18S ribosomal RNA (RRN18S), succinate dehydrogenase flavoprotein subunit (SDHA2) and tyrosine 3-monooxygenase/tryptophan 5-monooxygenase activation protein zeta (YWHAZ) were used as housekeeping genes. The 2 most stable housekeeping genes selected by geNorm were used to normalise the adjusted PCR data [73].

Statistics

The gene expression of independent biological replicates was standardized by logarithmic transformation, mean centring and auto-scaling, as described by Willems et al. [76]. Statistical analysis was performed by one-way ANOVA with Tukey's multiple comparison test using GraphPad Prism 6.0 (GraphPad Software Inc., La Jolla, CA 92037, USA). A value of $p \leq 0.05$ was considered statistically significant.

Results

Immunophenotypic characterization

Specific cell surface markers were selected to show that the established and in vitro expanded ihPDLSC cultures contain cells with MSC-like properties. Despite certain intra- and inter-individual differences, all ihPDLSC cultures exhibited a similar expression pattern of cell surface molecules (Table 2, Fig. 2). A high expression was detected for the MSC markers CD73 and CD90 (> 95 % of the population), a medium to high expression was observed for CD146 (76.2 ± 24.3) and CD105 (82.6 ± 14.6) and a low expression was documented for STRO-1 (5.2 ± 3.8). The embryonic marker SSEA-4 exhibited low to medium expression levels (30.3 ± 12.5). Furthermore, the hematopoietic progenitor cell antigen CD34 and the leukocyte common antigen CD45 showed a negligible expression level (< 2 %) in almost all cases. Considerable intra- and inter-individual differences were found for the markers CD105, CD146, SSEA-4 and CD34.

Multilineage differentiation potential

Multipotency belongs to the key properties of MSCs. Therefore, the neurogenic, angiogenic and osteo-

CD73	CD90	CD105	CD146	STRO-1	SSEA-4	CD34	CD45
97.6 ± 1.8	98.9 ± 0.9	82.6 ± 14.6	76.2 ± 24.3	5.2 ± 3.8	30.3 ± 12.5	3.6 ± 4.0	1.3 ± 0.5

Table 2 Immunophenotypic characterization with flow cytometry (expression of mesenchymal [CD73, CD90, CD105, CD146, STRO-1], embryonic [SSEA-4] and hematopoietic [CD34, CD45] stem cell markers of all donors [n = 4] expressed as mean value [%] ± standard deviation)

genic differentiation potential was evaluated. Initial morphological changes characteristic for neurogenic differentiation were microscopically detected 3 d after induction. During the differentiation process, the fibroblast specific, spindle-shaped morphology changed into a neuron-like phenotype with a 'drawn-out' cell body and dendrite-like extensions. In addition, the longer the differentiation process lasted, the more the orientation of cells changed from a random to a parallel pattern (Fig. 3A). To analyse the neurogenic differentiation potential of ihPDLSCs at mRNA level, qRT-PCR was conducted for neurogenic markers. A continuously increasing expression of NEFL, NCAM1 and ENO2 could be observed (Fig. 4A). Expression of NEFL was significantly increased at day 14, 21, 28 and 35 ($p < 0.001$), expression of NCAM1 was significantly increased at day 21 ($p < 0.05$), 28 ($p < 0.01$) and 35 ($p < 0.001$), and expression of ENO2 was significantly increased at day 3, 7, 14, 21, 28 and 35 ($p < 0.001$) when compared to day 0 (reference). Expression of TUBB3 did not significantly change during the entire observation period.

The spindle-shaped morphology of fibroblasts changed toward a polygonal endothelial cell-like phenotype during cultivation in angiogenic medium. In addition, the shoal-like arrangement of fibroblasts converted into a cobblestone-like pattern (Fig. 3B). At mRNA level, a continuously increasing expression of VEGFR2, VEGFR1 and PECAM1 was detected (Fig. 4B). Expression of VEGFR2 and PECAM1 was significantly increased at day 3, 7, 14, 21, 28 and 35 ($p < 0.001$) and expression of VEGFR1 was significantly increased at day 7, 14, 21, 28 and 35 ($p < 0.001$) when compared to day 0. As ANGPT2 was not detectable in 3 out of our 4 donors, statistics were not performed for this marker.

During the 4 weeks of induction, ihPDLSCs exhibited osteogenic potential as determined by the presence of AR-S positive mineralized deposits and by increased expression of osteogenic markers. First AR-S positive nodules were observed 3 to 7 d after induction. These were primarily located in the periphery of the well. Afterwards, mineralization rapidly increased and after 14 to 21 d of induction 70–80 % of the adherent monolayer was covered by AR-S positive calcium accumulations (Fig. 3C). These microscopic observations were confirmed by spectrophotometric AR-S quantification using the CPC extraction method (Fig. 5). AR-S concentration per well was significantly increased at day 14, 21 and 28 ($p < 0.001$) when compared to day 3, 7 and 10. Significant changes in gene expression were observed for ALP and BMP2 (Fig. 4C). Expression of ALP significantly increased at day 3, 7, 10 and 14 ($p < 0.001$) and subsequently decreased to non-significant values. Expression of BMP2 was significantly increased at day 3, 7, 10, 14, 21 and 28 ($p < 0.001$) when compared to day 0 and showed the highest values at day 21. Expression of RUNX2 and BGLAP did not significantly change throughout the entire observation period. As IBSP, SP7 and SPP1 were not reliably detectable, data were not further analysed for these markers.

Discussion

Re-establishment of a biocompatible root surface, exclusion of the gingival epithelium, sufficient wound stability and presence of progenitor cells are prerequisites to regenerate destroyed periodontal structures. MSCs as the ideal progenitor cells for periodontal regeneration have been shown to reside in the intact periodontal ligament, in the adjacent alveolar bone and in the blood stream [33]. During wound healing, MSCs are assumed to migrate into the periodontal defect and subsequently differentiate into the cell types required for periodontal regeneration [34]. Due to the bacterial contamination and the inflammatory properties, granulation tissue of intra-bony periodontal defects has not been considered as appropriate source for MSCs that could be useful in regenerating destroyed tissues. Therefore, it is routinely removed during regenerative periodontal surgery [12, 13, 51, 62, 63]. However, the clinical and radiographic outcomes of the GTPT, which has been developed and documented by our group, suggest that the preservation of granulation tissue may positively influence the healing processes following regenerative periodontal surgery.

Hypothesis of the present study was that ihPDLSCs show characteristics of MSCs and maintain their multilineage differentiation potential, which is needed for periodontal regeneration. Microscopic observations revealed that ihPDLSCs comprise a heterogeneous mixture of cells that predominantly show a spindle-shaped, fibroblast-specific morphology. Flow cytometry analysis was used for immune-phenotypic characterization. Although several articles have been published regarding MSC surface antigens, there is no general consensus, which combination of CD markers is appropriate to identify MSCs with sufficient accuracy. Dominici et al. [15] have published minimal criteria for defining MSCs. These criteria include that more than 95 % of the MSC population must express CD73, CD90 and CD105. Since expression of CD73, CD90, CD105 and CD44 is not only observed in MSCs but also

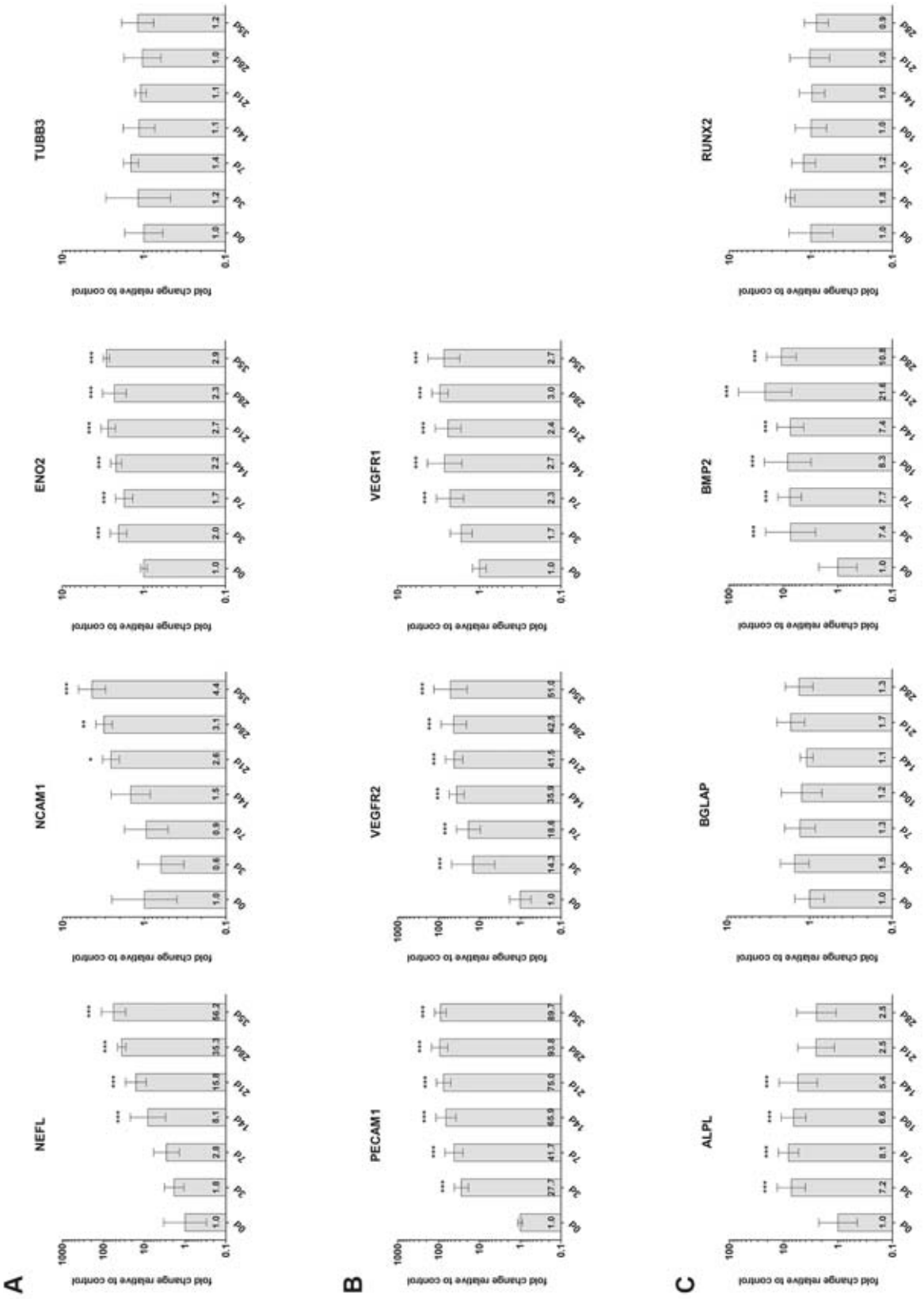
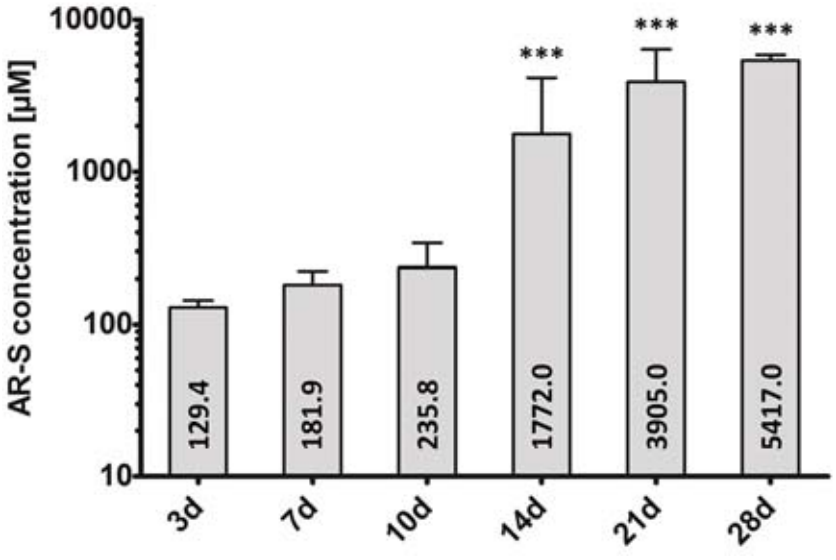


Figure 4A–C Gene expression of markers characteristic for neuronal (**A**), endothelial (**B**) and osteoblastic (**C**) differentiation: The qRT-PCR data of all donors (n = 4) were standardized using logarithmic transformation, mean centring and auto-scaling, as described by Willems et al. [76]. Data are expressed as recalculated average with upper and lower confidence interval. One-way ANOVA with Tukey's multiple comparison test was used to detect significant differences to day 0 (*P < 0.05; **P < 0.01; ***P < 0.001). The neuronal markers included neurofilament light polypeptide (NEFL), neural cell adhesion molecule 1 (NCAM1), tubulin beta 3 class III (TUBB3) and enolase 2 (ENO2). The endothelial markers included vascular endothelial growth factor receptor 1 (VEGFR1), vascular endothelial growth factor receptor 2 (VEGFR2) and platelet and endothelial cell adhesion molecule 1 (PECAM1). The osteoblastic markers included alkaline phosphatase (ALP), bone gamma-carboxyglutamate protein (BGLAP), bone morphogenic protein 2 (BMP2) and runt related transcription factor 2 (RUNX2).



(Fig. 1–5, Tab. 1 and 2: Knut Adam)

Figure 5 Quantification of matrix mineralization during the osteoblastic differentiation experiments using the AR-S extraction method. Data from all donors (n = 4) are expressed as mean AR-S concentration [µM] ± standard deviation. AR-S concentrations were significantly increased at day 14, 21 and 28 when compared to day 3, 7 and 10 (***P < 0.001; one-way ANOVA with Tukey's multiple comparison test).

in fibroblasts and stromal cells [49], further markers are required. Thus, expression of CD146, Stro-1 and SSEA-4 was additionally investigated in the present study. Our data revealed that ihPDLSCs exhibit a high expression of CD73, CD90, CD105 and CD146. Considerable inter-individual differences in the expression of CD105 and CD146 were detected. This heterogeneity is reflected by the results of other studies about ihPDLSCs, where expression of CD105 ranged from 51.9 to 96.0 % and expression of CD146 from 9.2 to 81.1 % [32, 41, 44, 54, 72]. Expression of Stro-1 was quite low and obviously lower than in other studies about ihPDLSCs [32, 41, 44, 54]. In this context, Lv et al. [45] reported that Stro-1 is not universally expressed in all types of MSCs and that its expression gradually may become lost in culture. A similar phenomenon can be observed for CD34, a marker for hematopoietic stem cells (HSCs) and endothelial progenitors, which is commonly used to distinguish MSCs from HSCs. Mitchell et al. [47] reported that CD34 expression dramatically decreases with each passage in culture. This could explain why CD34 expression showed significant inter-individual differences and was higher than 2 % in 2 out of our 4 donors. Stage-specific embryonic antigen 4 (SSEA-4) is known as an embryonic stem cell-associated marker. However, SSEA-4 is not only expressed in human embryonic stem cells but also in adult MSC populations [24]. Liu et al. [44] reported that ihPDLSCs express SSEA-4 by 13.56 ± 4.21 %, which is remarkably lower than in our study (30.27 ± 12.50). Although the exact percentage remains unclear, the flow cytometry data of our study indicate that a relevant fraction of the heterogeneous ihPDLSC population expresses surface antigens, which allow to identify them as MSCs.

The ability to differentiate into 3 different lineages under inductive culture conditions represents another characteristic of MSCs. Generally, the potential to generate adipocytes, osteoblasts and chondrocytes is regarded sufficient to prove multipotency. However, differentiation potential of MSCs is not only restricted to tissues of mesodermal origin. Thus, trans-differentiation into ectodermal (e.g. neurons) and endodermal (e.g. hepatocytes) lineages was successfully conducted in vitro [18, 39]. As neurogenesis, angiogenesis and osteogenesis belong to the biological key processes in periodontal regeneration, we attempted to generate neurons of ectodermal origin as well as endothelial cells and osteoblasts of mesodermal origin in vitro. Our data provide a detailed view on the expression changes chronologically occurring during neural, endothelial and osteoblastic differentiation.

As all periodontal tissues originate from the neural crest, it is not surprising that hhPDLSCs spontaneously express neuronal markers, as shown by Heng et al. [30]. However, we could show that a baseline expression of neuronal markers is also present in ihPDLSCs. After induction of neurogenic differentiation, ihPDLSCs showed a continuously increasing expression of the neuronal markers NEFL, NCAM1 and ENO2. Neurofilaments, like NEFL, are neuronal intermediate filaments representing the most abundant structural cytoskeletal proteins of myelinated axons [70]. During development of the nervous system, the expression of neurofilaments follows a stereotypic program, which is phylogenetically conserved and repeated during axonal regeneration [70]. NCAM1 belongs to the immunoglobulin superfamily and regulates neurite outgrowth in the developing nervous system [58]. In addition, regeneration of central and peripheral neuronal fibres was reported to be associated with an upregulation of NCAM1 expression [53]. ENO2 is a glycolytic enzyme predominantly localized in the cytoplasm of neurons. Expression of ENO2 is upregulated when axons are damaged suggesting that ENO2 is involved in neuronal regeneration [10]. The significant increase of these neuronal markers strongly indicates that ihPDLSCs have the potential to generate neuron-like cells and to play an important role in the re-innervation of tissues regenerated following periodontal surgery.

However, expression of TUBB3 did not significantly change. TUBB3 is a component of neuronal microtubules and involved in many cellular functions such as intracellular organization, coordinated vesicle transport and cell division [37]. TUBB3 is one of the earliest neuronal cytoskeletal proteins in the development of

the central nervous system and seems to participate in early neuritogenesis [38]. Foudah et al. [23] reported that a very high percentage of undifferentiated hhPDLSCs and other types of MSCs are positive for TUBB3 and that this expression does not change up to passage 16. This phenomenon implies that TUBB3 is constitutively expressed in MSCs, not influenced by spontaneously occurring differentiation or senescence processes and, therefore, not solely neuron-specific. Widera et al. [75] investigated the neurogenic differentiation potential of ihPDLSCs. They were able to detect high levels of neuron-specific markers, such as MAP2, NEFL, NEFM and NEFH after neural induction with retinoic acid. Their conclusion that ihPDLSCs possess the ability to generate neural precursors was confirmed by the results of our study.

Angiogenesis, defined as new blood vessel sprouting from pre-existing ones, is an essential process involved in development, wound healing and regeneration [16, 19, 22]. After induction of angiogenic differentiation, a significant increase in the expression of the endothelial marker proteins VEGFR2, VEGFR1 and PECAM1 was observed. VEGF is regarded as the most important factor stimulating angiogenesis in healthy and diseased tissues [20, 50]. Most angiogenic functions of VEGF, including proliferation, survival, migration and permeability of endothelial cells, are triggered by VEGFR2 [14]. Although its precise function remains unclear, VEGFR1 seems to be a decoy receptor for VEGF [6, 19]. Thus, VEGFR1 moderates the angiogenic effects of VEGF by preventing VEGF binding to VEGFR2. PECAM1 is an efficient signalling molecule, expressed on all cells within the vascular compartment and involved in angiogenesis [55, 77]. The expression profiles of VEGFR2, VEGFR1 and PECAM1 in the present study strongly suggest that endothelial trans-differentiation occurred. However, ANGPT2 was generally not expressed during the entire angiogenic differentiation experiments. Endothelial cells in adults persist in a quiescent state and proliferate only following activation. ANGPT1-mediated Tie2 activation is required to maintain this vascular quiescence [21]. ANGPT2, as functional antagonist of ANGPT1, destabilizes quiescent endothelia and, thus, promotes angiogenesis in particular in combination with VEGF [21, 61]. The lack of ANGPT2 expression in our experiments can be explained by the 'artificial' in vitro conditions, which are not unconfined comparable to the in vivo situation of a quiescent-resting endothelium. In this context, Korff et al. [40] investigated the synergistic effects of VEGF and ANGPT2 in a 3-dimensional spheroidal co-culture model. They reported that endothelial cells exhibit different responsiveness to VEGF stimulation depending on the culture conditions. Thus, endothelial cells grown in monoculture were able to form capillary-like sprouts after stimulation with VEGF, while endothelial cells grown in co-culture with smooth muscle cells were only able to generate sprouts, when VEGF was combined with ANGPT2. This suggests that ANGPT2 is obligatory for endothelial differentiation when cells are grown in co-culture, but dispensable when cells are grown in monoculture like in our study. Amin et al. [1] investigated the angiogenic and vasculogenic differentiation potential of hhPDLSCs. They detected an upregulation of the endothelial markers VEGFR2, Tie1, Tie2, VE-cadherin and vWF. Further studies consistently revealed that MSCs derived from healthy oral tissues are able to differentiate into endothelial-like cells [4, 60]. Our study discovered for the first time, that ihPDLSCs as a MSC population derived from inflamed tissue retain their angiogenic differentiation potential required for neovascularization.

As regeneration of intra-bony periodontal defects involves formation of new alveolar bone, differentiation of MSCs to osteoblasts is a prerequisite. Osteoblast differentiation is dynamically controlled by stage-specific signal transduction and transcription factors, such as RUNX2 and SP7 [52]. In vivo, RUNX2 is strongly expressed in pre-osteoblasts and immature osteoblasts, but finally down-regulated in mature osteoblasts [46]. This indicates that RUNX2 is crucial for the initial steps of osteoblast differentiation. In the present study, RUNX2 was strongly expressed in ihPDLSCs throughout the entire differentiation experiment. A definite increase in the expression of RUNX2 was detectable 3 days after induction. Wang et al. [74] investigated the osteogenic differentiation potential of SCAP using osteogenic differentiation media containing dexamethasone, β-glycerophosphate and KH_2PO_4, as used in our study. Similar to our results, they reported that expression of RUNX2 was upregulated 3 days after induction and reduced afterwards. SP7 represents another transcription factor essential for bone development and osteoblastogenesis [52]. Despite some obvious inter-individual differences, a reproducible expression pattern was detected. Thus, expression of SP7 was initially upregulated (peaking at day 3, 7, or 10), afterwards considerably downregulated and frequently not detectable (data not shown). Therefore, expression of RUNX2 and SP7 seems to be associated with the early stages of osteoblast differentiation, but not with matrix mineralization in vitro. Bone morphogenetic proteins (BMPs) are multi-functional growth factors that belong to the transforming growth factor β (TGF-β) superfamily [7]. It has been reported that BMP2 strongly promotes the differentiation of MSCs into osteoblasts [29, 52] and also enhances bone matrix production by osteoblastic cells [69]. Expression of BMP2 was significantly upregulated during the entire osteogenic differentiation experiment. It showed highest values at day 21 when matrix mineralization had largely covered the well surface. Conversely, ALP expression was significantly upregulated from day 3 to 14 and downregulated at day 21. These results are in agreement with data reported by Hoemann et al. [31] who investigated in vitro osteogenesis assays in BMSCs. They documented that confluent osteogenic cultures follow a two-stage developmental process consisting of a 1– to 2-week initiation phase with increased ALP activity and a subsequent maturation phase, in which matrix

mineralization occurs. During increased ALP activity, the extracellular matrix undergoes modifications in composition and organization, which prepares it effectively for the following mineralization process [68]. While the upregulation of ALP expression is stimulated by dexamethasone, the matrix mineralization is induced by β-glycerophosphate, which is rapidly converted to glycerol and inorganic phosphate in vitro [31]. BGLAP, SPP1 and IBSP belong to the non-collagenous proteins in the bone matrix. They are produced at the late stages of osteoblastic maturation and participate in matrix mineralization in vivo [68]. BGLAP was strongly expressed and did not significantly change in our study. However, expression of SPP1 and IBSP was significantly decreased and frequently suppressed to undetectable levels (data not shown), although an increased expression of both proteins is considered essential for matrix mineralization in vivo [68]. Similar to the results of our study, Cheng et al. [11] reported that exposure of BMSCs to dexamethasone resulted not only in substantial matrix mineralization, but also in drastically suppressed levels of SPP1 and IBSP in vitro. Since high concentrations of SPP1 and IBSP inhibit hydroxyapatite formation in vitro [5, 26], it seems likely, that already minor concentrations of SPP1 and IBSP are sufficient to initiate and perpetuate the mineralization process.

Other investigations have already shown the osteogenic differentiation potential of ihPDLSCs [9, 32, 54]. However, the data of the present study provide for the first time a detailed view on both, the process of matrix mineralization and the expression of osteogenic markers, chronologically occurring after induction with dexamethasone. These data strongly indicate that ihPDLSCs are able to generate osteoblast-like cells and that ihPDLSCs have many features in common with BMSCs during osteoblastic differentiation.

The cell cultures used in the present study were unexceptionally isolated from granulation tissues harvested from the bottom of intra-bony periodontal defects. Attachment loss is continuously proceeding in inflammatory periodontal diseases. Thus, granulation tissues derived from the bottom of intra-bony periodontal defects have been exposed to the inflammatory conditions for a shorter period of time than granulation tissues harvested from a more coronal part. It is conceivable that the regenerative capacity of the granulation tissue may increase gradually to the bottom of the periodontal defect and that the cell populations differ in the different parts of the defect. Further investigations are required to clarify this uncertainty.

Conclusions

Granulation tissue derived from intra-bony periodontal defects represents an inflamed tissue containing cell populations with properties characteristic for mesenchymal stem cells. The present in-vitro-study highlights the expression changes chronologically occurring during neurogenic, angiogenic and osteogenic differentiation of inflamed human periodontal ligament stem cells. Our data strongly suggest that these cells are able to undergo neuronal, endothelial and osteoblastic differentiation. As formation of nerves, blood vessels and alveolar bone are key processes in the regeneration of periodontal tissues, we strongly believe that the preservation of granulation tissue, to date considered as tissue of minor value and routinely removed, could promote the healing processes in intra-bony periodontal defects. This knowledge may lead to a paradigm shift in regenerative periodontal surgery, where the preservation of granulation tissue as 'autologous' tissue could replace exogenous materials like bone substitutes or occlusive membranes. In addition, the granulation tissue of intra-bony periodontal defects may be considered as an easily accessible source for MSC in regenerative medicine.

Declarations

Ethics approval and consent to participate

The present study was reviewed and approved by the Ethical Committee of Hannover Medical School (No. 1096). A written informed consent was obtained from all subjects included in the study.

Consent for publication

The patient who contributed to the realization of the manuscript through clinical and radiographic pictures gave her written consent for publication.

Availability of data and materials

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Funding

The present study was supported by the research grant of the German Society of Dental, Oral and Craniomandibular Sciences (Deutsche Gesellschaft für Zahn-, Mund- und Kieferheilkunde).

Acknowledgements

The authors would like to thank Dr. Matthias Ballmaier for his expertise during the flow cytometry experiments and analyses.

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.

References

1. Amin HD, Olsen I, Knowles J, Dard M, Donos N: A tyrosine-rich amelogenin peptide promotes neovasculogenesis in vitro and ex vivo. Acta Biomater 2014; 10: 1930–1939

2. Bakopoulou A, Kritis A, Andreadis D et al: Angiogenic potential and secretome of human apical papilla mesenchymal stem cells in various stress microenvironments. Stem Cells Dev 2015; 24: 2496–2512

3. Bartold PM, McCulloch CA, Narayanan AS, Pitaru S: Tissue engineering: a new paradigm for periodontal regeneration based on molecular and cell biology. Periodontol 2000: 24: 253–269

4. Bento LW, Zhang Z, Imai A et al.: Endothelial differentiation of SHED requires

MEK1/ERK signaling. J Dent Res 2013; 92: 51–57

5. Boskey AL: Mineral-matrix interactions in bone and cartilage. Clin Orthop Relat Res 1992; 281: 244–274

6. Carmeliet P, Jain RK: Molecular mechanisms and clinical applications of angiogenesis. Nature 2011; 473: 298–307

7. Chen D, Zhao M, Mundy GR: Bone morphogenetic proteins. Growth Factors 2004; 22: 233–241

8. Chen FM, Jin Y: Periodontal tissue engineering and regeneration: current approaches and expanding opportunities. Tissue Eng Part B Rev 2010; 16: 219–255

9. Chen X, Hu C, Wang G et al.: Nuclear factor-κB modulates osteogenesis of periodontal ligament stem cells through competition with β-catenin signaling in inflammatory microenvironments. Cell Death 2013; Dis 4: e510

10. Cheng F, Yuan Q, Yang J, Wang W, Liu H: The prognostic value of serum neuron-specific enolase in traumatic brain injury: systematic review and meta-analysis. PLoS One 2014; 9: e106680

11. Cheng SL, Zhang SF, Avioli LV: Expression of bone matrix proteins during dexamethasone-induced mineralization of human bone marrow stromal cells. J Cell Biochem 1996; 61: 182–193

12. Cortellini P, Tonetti MS: Clinical and radiographic outcomes of the modified minimally invasive surgical technique with and without regenerative materials: a randomized-controlled trial in intra-bony defects. J Clin Periodontol 2011; 38: 365–373

13. Cortellini P, Tonetti MS: Clinical concepts for regenerative therapy in intra-bony defects. Periodontol 2000 2015; 68: 282–307

14. Cross MJ, Dixelius J, Matsumoto T, Claesson-Welsh L: VEGF-receptor signal transduction. Trends Biochem Sci 2003; 28: 488–494

15. Dominici M, Le Blanc K, Mueller I et al.: Minimal criteria for defining multipotent mesenchymal stromal cells. The International Society for Cellular Therapy position statement. Cytotherapy 2006; 8: 315–317

16. Dvorak HF: Angiogenesis: update 2005. J Thromb Haemost 2005; 3: 1835–1842

17. Esposito M, Grusovin MG, Papanikolaou N, Coulthard P, Worthington HV: Enamel matrix derivative (Emdogain®) for periodontal tissue regeneration in intrabony defects. Cochrane Database Syst Rev 2009; 7: CD003875

18. Fairbairn NG, Meppelink AM, Ng-Glazier J, Randolph MA, Winograd JM: Augmenting peripheral nerve regeneration using stem cells: A review of current opinion. World J Stem Cells 2015; 7: 11–26

19. Ferrara N, Gerber HP, LeCouter J: The biology of VEGF and its receptors. Nat Med 2003; 9: 669–676

20. Ferrara N: VEGF-A: a critical regulator of blood vessel growth. Eur Cytokine Netw 2009; 20: 158–163

21. Fiedler U, Augustin HG: Angiopoietins: a link between angiogenesis and inflammation. Trends Immunol 2006; 27: 552–558

22. Folkman J: Role of angiogenesis in tumor growth and metastasis. Semin Oncol 2002; 29: 15–18

23. Foudah D, Monfrini M, Donzelli E et al.: Expression of neural markers by undifferentiated mesenchymal-like stem cells from different sources. J Immunol Res 2014, 987678

24. Gang EJ, Bosnakovski D, Figueiredo CA, Visser JW, Perlingeiro RC: SSEA-4 identifies mesenchymal stem cells from bone marrow. Blood 2007; 109: 1743–1751

25. Ge S, Mrozik KM, Menicanin D, Gronthos S, Bartold PM: Isolation and characterization of mesenchymal stem cell-like cells from healthy and inflamed gingival tissue: potential use for clinical therapy. Regen Med 2012; 7: 819–832

26. Gorski JP: Acidic phosphoproteins from bone matrix: a structural rationalization of their role in biomineralization. Calcif Tissue Int 1992; 50: 391–396

27. Gronthos S, Mankani M, Brahim J, Robey PG, Shi S: Postnatal human dental pulp stem cells (DPSCs) in vitro and in vivo. Proc Natl Acad Sci USA 2000; 97: 13625–13630

28. Günay H, Weinspach K, Geurtsen W, Staufenbiel I: Relevance of the intralesional granulation tissue in regenerative periodontal surgery – case reports. Dtsch Zahnärztl Z 2013; 68: 526–537

29. Hayrapetyan A, Jansen JA, van den Beucken JJ: Signaling pathways involved in osteogenesis and their application for bone regenerative medicine. Tissue Eng Part B Rev 2015; 21: 75–87

30. Heng BC, Lim LW, Wu W, Zhang C: An overview of protocols for the neural induction of dental and oral stem cells in vitro. Tissue Eng Part B Rev 2016; 22: 220–250

31. Hoemann CD, El-Gabalawy H, McKee MD: In vitro osteogenesis assays: influence of the primary cell source on alkaline phosphatase activity and mineralization. Pathol Biol (Paris) 2009; 57: 318–323

32. Hung TY, Lin HC, Chan YJ, Yuan K: Isolating stromal stem cells from periodontal granulation tissues. Clin Oral Investig 2012; 16: 1171–1180

33. Hynes K, Menicanin D, Gronthos S, Bartold PM: Clinical utility of stem cells for periodontal regeneration. Periodontol 2000 2012; 59: 203–227

34. Ivanovski S: Periodontal regeneration. Aust Dent J 2009; 54: 118–128

35. Jussila M, Thesleff I: Signaling networks regulating tooth organogenesis and regeneration, and the specification of dental mesenchymal and epithelial cell lineages. Cold Spring Harb Perspect Biol 2012; 4: a008425

36. Kao RT, Nares S, Reynolds MA: Periodontal regeneration – intrabony defects: a systematic review from the AAP Regeneration Workshop. J Periodontol 2015; 86: 77–104

37. Katsetos CD, Dráberová E, Legido A, Dumontet C, Dráber P: Tubulin targets in the pathobiology and therapy of glioblastoma multiforme. I. Class III beta-tubulin. J Cell Physiol 2009; 221: 505–513

38. Katsetos CD, Herman MM, Mörk SJ: Class III beta-tubulin in human development and cancer. Cell Motil Cytoskeleton 2003; 55: 77–96

39. Kawanabe N, Murata S, Murakami K et al.: Isolation of multipotent stem cells in human periodontal ligament using stage-specific embryonic antigen-4. Differentiation 2010; 79: 74–83

40. Korff T, Kimmina S, Martiny-Baron G, Augustin HG: Blood vessel maturation in a 3-dimensional spheroidal coculture model: direct contact with smooth muscle cells regulates endothelial cell quiescence and abrogates VEGF responsiveness. FASEB J 2001; 15: 447–457

41. Li C, Wang X, Tan J, Wang T, Wang Q: The immunomodulatory properties of periodontal ligament stem cells isolated from inflamed periodontal granulation. Cells Tissues Organs 2014; 199: 256–265

42. Lin Z, Rios HF, Cochran DL: Emerging regenerative approaches for periodontal reconstruction: a systematic review from the AAP Regeneration Workshop. J Periodontol 2015; 86: 134–152

43. Ling LJ, Hung SL, Lee CF, Chen YT, Wu KM: The influence of membrane exposure on the outcomes of guided tissue regeneration: clinical and microbiological aspects. J Periodontal Res 2003; 38, 57–63

44. Liu D, Xu J, Liu O: Mesenchymal stem cells derived from inflamed periodontal ligaments exhibit impaired immunomodulation. J Clin Periodontol 2012; 39: 1174–1182

45. Lv FJ, Tuan RS, Cheung KM, Leung VY: Concise review: the surface markers and identity of human mesenchymal stem cells. Stem Cells 2014; 32: 1408–1419

46. Maruyama Z, Yoshida CA, Furuichi T et al.: Runx2 determines bone maturity and turnover rate in postnatal bone development and is involved in bone loss in estrogen deficiency. Dev Dyn 2007; 236: 1876–1890

47. Mitchell JB, McIntosh K, Zvonic S et al.: Immunophenotype of human adipose-derived cells: temporal changes in stromal-associated and stem cell-associated markers. Stem Cells 2006; 24: 376–385

48. Miura M, Gronthos S, Zhao M et al.: SHED: stem cells from human exfoliated deciduous teeth. Proc Natl Acad Sci USA 2003; 100: 5807–5812

49. Murphy MB, Moncivais K, Caplan AI: Mesenchymal stem cells: environmentally responsive therapeutics for regenerative medicine. Exp Mol Med 2013; 45: e54

50. Nagy JA, Dvorak AM, Dvorak HF: VEGF-A and the induction of pathological angiogenesis. Annu Rev Pathol 2007; 2: 251–275

51. Nickles K, Ratka-Krüger P, Neukranz E, Raetzke P, Eickholz P: Open flap debridement and guided tissue regeneration after 10 years in infrabony defects. J Clin Periodontol 2009; 36: 976–983

52. Nishimura R, Hata K, Ikeda Fet al.: Signal transduction and transcriptional regulation during mesenchymal cell differentiation. J Bone Miner Metab 2008; 26: 203–212

53. Olsen M, Zuber C, Roth J, Linnemann D, Bock E: The ability to re-express polysialylated NCAM in soleus muscle after denervation is reduced in aged rats compared to young adult rats. Int J Dev Neurosci 1995; 13: 97–104

54. Park JC, Kim JM, Jung IH et al.: Isolation and characterization of human periodontal ligament (PDL) stem cells (PDLSCs) from the inflamed PDL tissue: in vitro and in vivo evaluations. J Clin Periodontol 2011; 38: 721–731

55. Park S, Sorenson CM, Sheibani N: PECAM-1 isoforms, eNOS and endoglin axis in regulation of angiogenesis. Clin Sci (Lond) 2015; 129: 217–234

56. Pereira LO, Rubini MR, Silva JR et al.: Comparison of stem cell properties of cells isolated from normal and inflamed dental pulps. Int Endod J 2012; 45: 1080–1090

57. Pietruska M, Skurska A, Pietruski J et al.: Clinical and radiographic evaluation of intrabony periodontal defect treatment by open flap debridement alone or in combination with nanocrystalline hydroxyapatite bone substitute. Ann Anat 2012; 194: 533–537

58. Rønn LC, Berezin V, Bock E: The neural cell adhesion molecule in synaptic plasticity and ageing. Int J Dev Neurosci 2000; 18: 193–199

59. Ruijter JM, Ramakers C, Hoogaars W et al.: Amplification efficiency: linking baseline and bias in the analysis of quantitative PCR data. Nucleic Acids Res 2009; 37: e45

60. Sakai VT, Zhang Z, Dong Z et al.: SHED differentiate into functional odontoblasts and endothelium. J Dent Res 2010; 89: 791–796

61. Scharpfenecker M, Fiedler U, Reiss Y, Augustin HG: The Tie-2 ligand angiopoietin-2 destabilizes quiescent endothelium through an internal autocrine loop mechanism. J Cell Sci 2005; 118: 771–780

62. Sculean A, Donos N, Miliauskaite A, Arweiler N, Brecx M: Treatment of intrabony defects with enamel matrix proteins or bioabsorbable membranes. A 4-year follow-up split-mouth study. J Periodontol 2001; 72: 1695–1701

63. Sculean A, Kiss A, Miliauskaite A, Schwarz F, Arweiler NB, Hannig M: Ten-year results following treatment of intrabony defects with enamel matrix proteins and guided tissue regeneration. J Clin Periodontol 2008; 35: 817–824

64. Sculean A, Nikolidakis D, Nikou G, Ivanovic A, Chapple IL, Stavropoulos A: Biomaterials for promoting periodontal regeneration in human intrabony defects: a systematic review. Periodontol 2000 2015; 68: 182–216

65. Seo BM, Miura M, Gronthos S et al.: Investigation of multipotent postnatal stem cells from human periodontal ligament. Lancet 2004; 364: 149–155

66. Shi S, Gronthos S: Perivascular niche of postnatal mesenchymal stem cells in human bone marrow and dental pulp. J Bone Miner Res 2003; 18: 696–704

67. Sonoyama W, Liu Y, Yamaza T et al.: Characterization of the apical papilla and its residing stem cells from human immature permanent teeth: a pilot study. J Endod 2008; 34: 166–171

68. Stein GS, Lian JB, Owen TA: Relationship of cell growth to the regulation of tissue-specific gene expression during osteoblast differentiation. FASEB J 1990; 4: 3111–3123

69. Susperregui AR, Viñals F, Ho PW, Gillespie MT, Martin TJ, Ventura F: BMP-2 regulation of PTHrP and osteoclastogenic factors during osteoblast differentiation of C2C12 cells. J Cell Physiol 2008; 216: 144–152

70. Szaro BG, Strong MJ: Post-transcriptional control of neurofilaments: New roles in development, regeneration and neurodegenerative disease. Trends Neurosci 2010; 33: 27–37

71. Taba M Jr, Jin Q, Sugai JV, Giannobile WV: Current concepts in periodontal bioengineering. Orthod Craniofac Res 2005; 8: 292–302

72. Tang HN, Xia Y, Yu Y, Wu RX, Gao LN, Chen FM: Stem cells derived from "inflamed" and healthy periodontal ligament tissues and their sheet functionalities: a patient-matched comparison. J Clin Periodontol 2016; 43: 72–84

73. Vandesompele J, De Preter K, Pattyn F et al.: Accurate normalization of real-time quantitative RT-PCR data by geometric averaging of multiple internal control genes. Genome Biol 2002; 3: RESEARCH0034

74. Wang L, Yan M, Wang Y et al.: Proliferation and osteo/odontoblastic differentiation of stem cells from dental apical papilla in mineralization-inducing medium containing additional KH(2)PO(4). Cell Prolif 2013; 46: 214–222

75. Widera D, Grimm WD, Moebius JM et al.: Highly efficient neural differentiation of human somatic stem cells, isolated by minimally invasive periodontal surgery. Stem Cells Dev 2007; 16, 447–460

76. Willems E, Leyns L, Vandesompele J: Standardization of real-time PCR gene expression data from independent biological replicates. Anal Biochem 2008; 379: 127–129

77. Woodfin A, Voisin MB, Nourshargh S: PECAM-1: a multi-functional molecule in inflammation and vascular biology. Arterioscler Thromb Vasc Biol 2007; 27: 2514–2523

78. Zhang Q, Shi S, Liu Y et al.: Mesenchymal stem cells derived from human gingiva are capable of immunomodulatory functions and ameliorate inflammation-related tissue destruction in experimental colitis. J Immunol 2009; 183: 7787–7798

(Photo: Hannover Medical School)

DR. KNUT ADAM
Department of Conservative Dentistry, Periodontology and Preventive Dentistry
Hannover Medical School
Carl-Neuberg-Str. 1, 30625 Hannover, Germany
Phone: 0049 511 532 4712
Fax: 0049 511 532 4811
adam.knut@mh-hannover.de

Philipp Kanzow, Mona Shaghayegh Maes, Annette Wiegand, Valentina Hraský

Radiographic alveolar bone loss in German patients with disabilities and treatment in general anesthesia

Introduction: The cross-sectional study aimed at assessing the periodontal status of German adult patients with disabilities (intellectual, physical, and/or dementia) requiring dental treatment in general anesthesia.

Material and Methods: Between 2011 and 2017, 206 patients received dental treatment(s) in general anesthesia. Periodontal status was retrospectively assessed based on the radiographically visible alveolar bone loss (%). Staging and grading of periodontal disease according to the 2017 classification for periodontal disease was performed. Various general and periodontal parameters, medications, and diagnoses of systemic diseases in association with periodontal diseases were analyzed as potential risk factors for bone loss. Statistical analysis was performed using Pearson correlations, Wilcoxon rank-sum tests, Kruskal-Wallis tests, and multiple linear regressions ($p < 0.05$).

Results: Periapical radiographs were available from 199 patients (86 females; age: 41.1 ± 15.0 years). Based on a distance from the cemento-enamel junction to the marginal bone level exceeding 2 mm, 174 (87.4 %) patients were diagnosed with periodontitis (22.4 ± 20.6 % bone loss). Most periodontitis patients were classified as stage I (39.7 %), followed by stage II (29.1 %), stage III (14.1 %), and stage IV (4.5 %). Generalized periodontitis was most frequently observed in stage I patients ($p \leq 0.047$). Significant predictors of % bone loss were age ($\beta = 0.65$; 95%-CI: 0.40–0.89; $p < 0.001$), intellectual disability ($\beta = 11.87$; 95%-CI: 1.21–22.52; $p = 0.029$), and smoking/nicotine dependence ($\beta = 17.29$; 95%-CI: 3.42–31.16; $p = 0.015$).

Conclusion: Periodontal disease is common in German patients with disabilities. Bone loss is associated with older age, intellectual disability, and smoking/nicotine dependence.

Keywords: alveolar bone loss; patients with disabilities; general anesthesia; radiographic bone loss

Department of Preventive Dentistry, Periodontology and Cariology, University Medical Center Göttingen: Dr. Dr. Philipp Kanzow, Mona Shaghaeygh Maes, Prof. Dr. Annette Wiegand, Dr. Valentina Hraský, MSc

Citation: Kanzow P, Maes MS, Wiegand A, Hraský V: Radiographic alveolar bone loss in German patients with disabilities and treatment in general anesthesia. Dtsch Zahnärztl Z Int 2019; 1: 195–203

Peer-reviewed article: submitted: 12.02.2019, revised version accepted: 13.05.2019

DOI.org/10.3238/dzz-int.2019.0195–0203

General parameters	– Age – Gender – Type of disability (intellectual, physical, dementia) – Legal guardian (yes, no) – Living situation (care facility, alone, with family) – Nutrition (without restriction, pureed/liquid food, feeding tube) – Oral hygiene (alone, with support, impossible) – Reasons for initial consultation (pain, swelling, caries, prophylaxis, other) – Medications (antihypertensives, anticoagulants, anticonvulsants, sedative drugs, antidepressants, muscle relaxants) – Systemic disorders (diabetes mellitus, obesity, smoking/nicotine dependence) – Immunologic disorders (Down syndrome, HIV infection)
Periodontal parameters	– Tooth loss (excluding wisdom teeth as assessed on radiographs) – Bone loss as a function of age – Presence of subgingival calculus – Radiographic furcation involvement

Table 1 Extracted general and periodontal parameters

1. Introduction

In 2017, about 7.8 million people with severe disabilities lived in Germany. This number is equivalent to 9.4 % of the total population [20]. According to definition of Book IX of the German Social Law Code (§2, SGB IX), the physical function, mental ability, and/or mental health of people with severe disabilities deviate from the age-typical condition for more than 6 months, so that participation in society is permanently impaired. The most common causes included physical disabilities (59.2 %) as well as cerebral disorders, intellectual, and/or mental disabilities (21.4 %) [21].

As patients with disabilities often show a reduced ability to cooperate, dental treatments have frequently to be performed in general anesthesia [10]. A systematic review revealed that patients with disabilities have a poorer oral hygiene leading to a stronger accumulation of plaque. As a consequence, they show a higher prevalence and greater severity of periodontal disease [1].

Regarding the periodontal status of German adult patients with disabilities, only 3 studies with conflicting results have been published. A recent study found a high prevalence of periodontitis as assessed by Periodontal Screening and Recording (PSR) index among adult patients with intellectual disability undergoing dental treatment in general anesthesia. Within the study population, a PSR code 3 or 4, both indicating periodontitis, was present in 92.3 % [9]. A previous study found that 34 % of adults with disabilities presented deep pockets (6 mm or more). According to the Community Periodontal Index of Treatment Needs (CPITN), 83 % of patients required scaling or complex treatment (categories II and III, equivalent to PSR codes 2 to 4) [18]. Besides these, only one further study evaluated the oral health of adults with disabilities attending Special Olympics Germany by visually assessing gingivitis. Gingivitis prevalence amounted to 58.5 % [19]. Based on this data, the current level of evidence regarding the periodontal status among German patients with disabilities is insufficient.

This cross-sectional study therefore aimed at determining the periodontal status of German patients with disabilities requiring dental treatment in general anesthesia by assessing the radiographically visible alveolar bone loss. Furthermore, potential risk factors (e.g. medications and systemic diseases) for bone loss

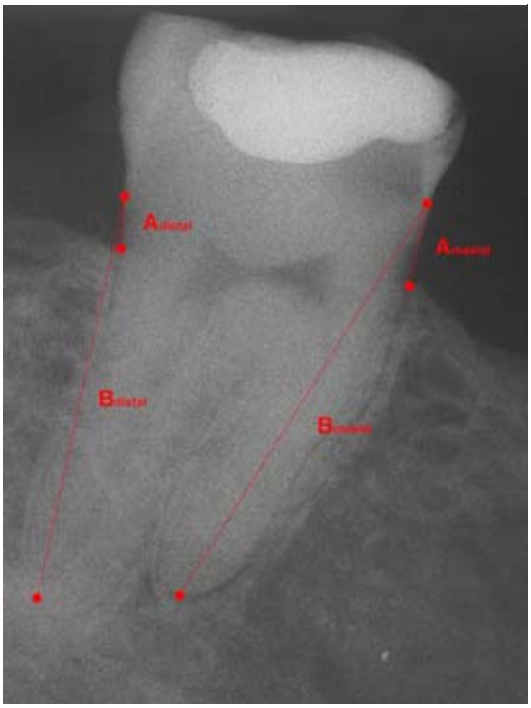


Figure 1 Distance from the cemento-enamel junction to the marginal bone level (*A*). Measurements were taken on the mesial (A_{mesial}) and distal (A_{distal}) sides and averaged arithmetically. Total root length (*B*) as the distance from the cemento-enamel junction to the apex (also measured mesially and distally and averaged arithmetically).

	n	%	p
N	199	100.0	
Age (average years ± SD)	41.1 (15.0)		< **0.001**
BMI (average ± SD, n = 178)	26.0 (7.1)		0.782
Gender			< **0.001**
male	113	56.8	
female	86	43.2	
Type of disability* (n = 194)			
intellectual	170	85.4	< **0.001**
physical	126	63.3	< **0.001**
dementia	14	7.0	< **0.001**
Legal guardian	183	92.0	< **0.001**
Living situation (n = 194)			< **0.001**
care facility	109	56.5	
alone	74	38.3	
with family	10	5.2	
Nutrition (n = 194)			0.189
without restrictions	144	74.2	
pureed/liquid food	31	16.0	
feeding tube	19	9.8	
Oral hygiene (n = 194)			0.248
alone	75	38.7	
with support	93	47.9	
impossible	26	13.4	
Reasons for initial consultation*			
caries (n = 198)	127	63.8	< **0.001**
prophylaxis	73	36.7	< **0.001**
pain	47	23.6	< **0.001**
other	31	15.6	< **0.001**
swelling	16	8.0	< **0.001**

Medication*			
anticonvulsants	88	44.2	< **0.001**
sedative drugs	57	28.6	< **0.001**
antihypertensives	51	25.6	< **0.001**
muscle relaxants	43	21.6	< **0.001**
antidepressants (n = 198)	42	21.2	< **0.001**
anticoagulants	25	12.6	< **0.001**
Systemic/immunologic disorders			
Obesity** (n = 178)	41	23.0	< **0.001**
HIV	0	0.0	-
Diabetes***	13	6.5	< **0.001**
Down syndrome	7	3.5	< **0.001**
Smoking/nicotine dependence**** (n = 198)	10	5.1	< **0.001**

Table 2 Demographic data of all patients and information regarding BMI, type of disability, presence of a legal guardian, living situation, nutrition, oral hygiene, reasons for initial consultation, medication, and systemic/immunologic disorders. p-values indicate univariate effect on % bone loss. In case of missing values, number of included patients are indicated in brackets. Due to the effect of rounding, some numbers do not sum up to 100 %. *Multiple selections were possible. **Defined as body mass index (BMI) ≥ 30 [15]. ***Based on intake of anti-diabetic medication. ****Active smokers or those with less than 5 years since cessation.

were evaluated. Finally, staging and grading of periodontal disease was performed based on the 2017 classification for periodontal disease [16].

2. Material and methods

2.1 Patients

All adult patients with intellectual/physical disability and/or dementia (age ≥ 18 years) who received dental treatment in general anesthesia in the Department of Preventive Dentistry, Periodontology and Cariology between January 2011 and December 2017 were screened (n = 206). Only those patients with full-mouth periapical radiographs were included in the present study (n = 199). The retrospective evaluation study was approved by the local ethics committee of the University Medical Center Göttingen (application number: 15/1/18).

To identify potential risk factors for bone loss, various general and periodontal parameters, medications, and diagnoses of systemic diseases were extracted or calculated from the patient records (see Tab. 1).

Patients' weight and height were extracted from the patient records. Based on these data the body mass index (BMI) was calculated using the following formula:

$$Body\ Mass\ Index = \frac{weight\ [kg]}{height\ [m]^2}$$

A BMI ≥ 30 was defined as obesity [15].

For these parameters and diseases, an association with periodontal diseases has been shown [11].

2.2 Radiographic assessment

The extent of alveolar bone loss was assessed on analogous full-mouth periapical radiographs (Kodak Insight Films IP-21 Size 2; Carestream Health, Rochester, NY, USA). All radiographs were taken by trained dental nurses in parallel technique with the beam angled perpendicular to film. If radiographs were available from multiple time points, the evaluation was based on the latest images. The measurements were performed using a digital caliper (16 ER; Mahr, Göttingen, Germany) to 0.01 mm under 2.5x magnification and standardized conditions. An X-ray image viewer (DSK 15 x 30 ST; Maier, Garmisch-Partenkirchen, Germany) in a darkened room without direct influence of daylight was used.

Except for wisdom teeth and non-restorable retained roots, the distance (*A*) from the cemento-enamel junction or from the restoration margin (if present and exceeding the cemento-enamel junction) to the marginal bone level (most coronal level where the periodontal space still retained its normal width) was measured for each tooth [6]. If the respective tooth was visible on multiple radiographs, the one with best quality was used. Ex-

	Patients without periodontitis		Patients with periodontitis	
	n	%	n	%
N	25	12.6	174	87.4
Tooth loss (excluding wisdom teeth)				
≤ 4 lost teeth	18	72.0	98	56.3
5–8 lost teeth	3	12.0	45	25.9
≥ 9 lost teeth	4	16.0	31	17.8
Radiographic bone loss as a function of age				
< 0.25	25	100.0	52	29.9
0.25–0.5	0	0.0	64	36.8
0.51–1.0	0	0.0	35	20.1
≥ 1.0	0	0.0	23	13.2
Presence of subgingival calculus	5	20.0	120	69.0
Radiographic furcation involvement	0	0.0	52	29.9

Table 3 Measured periodontal parameters among patient with and without periodontitis. Due to the effect of rounding, some numbers do not sum up to 100 %.

tremely distorted images were excluded from the analysis. If possible, all teeth were measured at their mesial and distal sides and the final bone level calculated as the arithmetical average [3, 6, 12, 17]. In case of overlaps with adjacent structures resulting in unassessable measurement sites, only one site per tooth was assessed.

$$A_{tooth} = \frac{A_{mesial} + A_{distal}}{2}$$

At tooth level (A_{tooth}) a value of up to 2 mm was considered physiological (no bone loss), while a value above 2 mm was defined as periodontitis [14]. At patient level, the highest value A_{tooth} was decisive for the classification of periodontal disease.

Furthermore, the total root length (B) was calculated as the distance from the cemento-enamel junction or from the restoration margin (if present and exceeding the cemento-enamel junction) to the apex or apices of the mesial and distal roots. These measurements were performed mesially and distally and averaged (Fig. 1).

$$B_{tooth} = \frac{B_{mesial} + B_{distal}}{2}$$

If A_{tooth} exceeded 2 mm, the % bone loss was calculated for each tooth from the ratio of A_{tooth} and B_{tooth}.

$$Radiographic\ bone\ loss = \frac{A_{tooth} - 2\ mm}{B_{tooth} - 2\ mm}$$

In patients with periodontal disease, classification of periodontitis severity was based on the % radiographic bone loss and divided into different stages: stage I (< 15 %), stage II (15–33 %), and stage III (> 33 %). The presence of further complexity factors (vertical bone loss ≥ 3 mm and/or radiographically visible furcation involvement) also led to the classification in stage III. Cases of stage III were classified as stage IV if only fewer than 20 remaining teeth were present. In addition, information regarding the extent was added to the stage as a descriptor: periodontitis was either present as localized (< 30 % of teeth affected) or generalized manifestation [16].

Periodontitis grading was assessed by indirect evidence of progression based on the % radiographic bone loss as a function of age at the most affected tooth. Further risk factors (i.e. smoking status and diabetes) were extracted from the patient files and served as grade modifiers. Grading was divided into 3 grades: grade A (bone loss/age < 0.25, nonsmoker, and no diabetes), grade B (bone loss/age 0.25–1 or smoker < 10 cigarettes per day or diabetes), and grade C (bone loss/age > 1 or smoker with ≥ 10 cigarettes per day) [16]. As no information regarding the level of hyperglycemia (e.g. HbA1c

values) were available, no differentiation between grade B and C based on the diabetic status were made.

Furthermore, the presence of radiographically visible sub-gingival calculus was recorded.

All radiographs were assessed by one calibrated dentist (PK). A random sample (n = 20) was evaluated by another dentist (VH). The main examiner re-assessed the same random sample after several weeks. Both the inter-rater and intra-rater reliability were calculated for the assessment of periodontitis stage, periodontitis grade, presence of subgingival calculus, and radiographic furcation involvement.

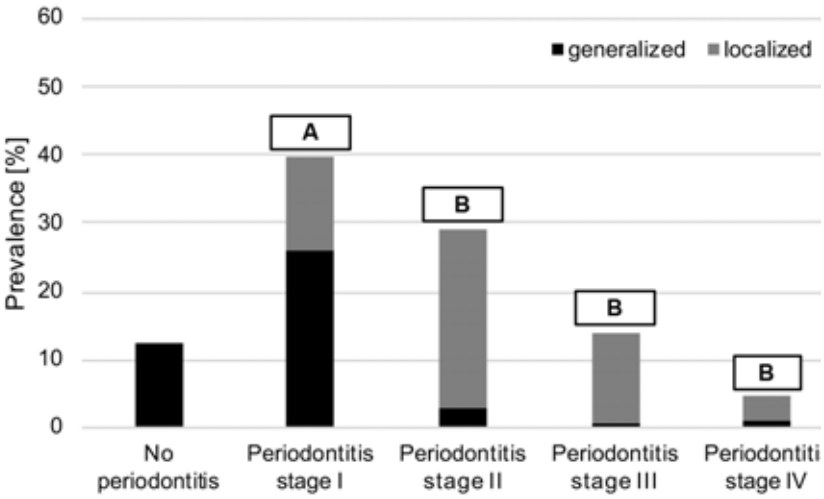


Figure 2 Prevalence and staging of periodontal disease. Different bold letters mark significant different distribution of extent (generalized vs. localized) between stages.

2.3 Statistical analysis

The statistical analysis was performed using the software R (version 3.5.2, www.r-project.org) with the package "irr" (version 0.84).

The extent of periodontal disease was compared between different stages using pairwise Wilcoxon rank-sum tests and adjusted according to Bonferroni-Holm.

As part of the univariate analysis, the correlation of patients' age and BMI (continuous variables) with % bone loss was analyzed by Pearson correlations. The influence of dichotomous variables, such as gender (male/female), legal guardianship (yes/no), intellectual disability (yes/no), physical disability (yes/no), dementia (yes/no), initial consultation due to pain (yes/no), initial consultation due to swelling (yes/no), initial consultation due to caries (yes/no), initial consultation for prophylaxis (yes/no), other reason for initial consultation (yes/no), intake of medications (i.e. antihypertensives, anticoagulants, anticonvulsants, sedative drugs, antidepressants, and muscle relaxants) and presence of systemic/immunologic disorders (obesity, diabetes, Down syndrome, smoking/nicotine dependence) on % bone loss was assessed using Wilcoxon rank-sum tests. The effect of multi-categorical variables, such as living situation (care facility, alone or with family), nutrition (without restrictions, pureed/liquid food or feeding tube), and oral hygiene status (alone, with support or impossible) on % bone loss was assessed using Kruskal-Wallis tests.

Subsequently, variables being significantly associated ($p < 0.05$) with % bone loss were used in a multiple linear regression model for the prediction of % bone loss.

Inter-rater and intra-rater reliability of the radiographic assessment were evaluated by Cohen's κ (dichotomous variables: presence of subgingival calculus and furcation involvement) and Kendall's W corrected for ties (ordinal variables: periodontitis stage and grade).

For all analyses, the level of significance was set to $p < 0.05$.

3. Results

199 patients were included in this study. Demographic data and information on BMI, type of disability, presence of a legal guardian, living situation, nutrition, oral hygiene, reasons for initial consultation, medication, and systemic diseases of all patients are shown in Table 2.

Periodontitis ($A_{tooth} > 2$ mm) was present in 174 patients (87.4 %). Among these patients, bone loss amounted to 22.4 ± 20.6 %. Further periodontal parameters such as tooth loss, % bone loss as a function of age, and radiographic presence of subgingival calculus and furcation involvement are shown in Table 3 for patients with and without periodontitis.

Among patients with periodontitis, distribution of stages and extent is shown in Figure 2. The extent (generalized vs. localized) of periodontal disease differed significantly between different stages. Generalized periodontitis was more frequent in less severe cases (stage I), while localized periodontitis was predominant in more severe stages ($p \leq 0.047$). Progression of periodontitis was rated as grade B in most patients (n = 123, 70.7 %), followed by grade A (n = 48, 27.6 %), and grade C (n = 3, 1.7 %).

3.1 Univariate analyses

Bone loss was significantly influenced by age ($p < 0.001$) and increased in older patients ($r = 0.38$). Smoking (+12.5 %), Down syndrome (+11.5 %), anticoagulants (+10.2 %), the existence of a legal guardian (+8.3 %), antihypertensives (+6.9 %), intellectual disability (+5.8 %), psychological disability (+5.4 %), living in a care facility (+4.0 %), consultation due to prophylaxis (+4.4 %), physical disability (+1.9 %), anticonvulsants (+1.8 %), antidepressants (+1.8 %), sedative drugs (+0.9 %), female gender (+0.8 %), consultation due to pain (+0.7 %), obesity (+0.4 %), and consultation due to other reasons than caries, pain, prophylaxis, or swelling (+0.02 %) were significantly related to increased % bone loss ($p < 0.001$). While consultation due to swelling (-8.7 %), living alone (-7.2 %) or with family (-4.1 %), muscle relaxants (-3.2 %), diabetes (-1.1 %), and consultation

due to caries (-0.1 %) were significantly related to decreased % bone loss ($p < 0.001$).

BMI, nutrition, and oral hygiene status had no significant effect on bone loss.

3.2 Multiple linear regression model

Significant variables from the previous univariate analysis were included in a multiple linear regression model for the prediction of % bone loss (Tab. 4). The model was significant at $p < 0.001$ with an adjusted R^2 of 0.19 and a Cohen's f^2 of 0.48 which can be regarded as large effect size [7]. When adjusted for the other variables in the model (gender, kind of disability, presence of a legal guardian, living situation, reasons for initial consultation, medications, obesity, diabetes, and Down syndrome), age ($\beta = 0.65$; 95%-CI: 0.40–0.89; $p < 0.001$), intellectual disability ($\beta = 11.87$; 95%-CI: 1.21–22.52; $p = 0.029$), and smoking/nicotine dependence ($\beta = 17.29$; 95%-CI: 3.42–31.16; $p = 0.015$) remained as significant independent predictors of % bone loss in patients with disabilities.

Intra-rater reliability was almost perfect [13] (Cohen's κ: 0.90 presence of sub-gingival calculus, 1.0 furcation involvement; Kendall's W: 0.91 periodontitis stage, 0.93 periodontitis grade). Inter-rater reliability of the radiographic assessment was mostly substantial [13] (Cohen's κ: 0.69 presence of sub-gingival calculus, 0.60 furcation involvement; Kendall's W: 0.80 periodontitis stage, 0.76 periodontitis grade).

4. Discussion

There is strong evidence that patients with intellectual disease show a greater prevalence and severity of periodontal disease than the general population [1]. These studies focused on patients with mental retardation (e.g. Down syndrome) and developmental disability (e.g. autism). While many studies are available from all over the world, information about the situation in Germany is rare.

In the present study among German patients with disabilities, periodontal disease was present in the majority of patients (n = 174, 87.4 %). Among these, bone loss amounted to 22.4 ± 20.6 %. Patients' age, intellectual disability, and smoking/nicotine dependence were significant independent predictors of increased % bone loss in the present population of patients with disabilities. Further factors, such as patients' gender, presence of a legal guardian, living situation, reasons for initial consultation, medications, obesity, diabetes, and Down syndrome were not significantly related to bone loss in the multiple linear regression model.

As limitation of the present study, the very heterogeneous group of patients (different kinds of/reasons for and extent of disabilities) must be noted. Patients with intellectual disabilities, physical disability, and/or dementia were included. This heterogeneity might lead to differences in patients' lifestyles and a large variation regarding the degree of autonomy. Furthermore, the course of life of patients with later-onset dementia is likely to be very different from those patients with inborn intellectual disabilities affecting their ability to perform oral hygiene measures. As a consequence, disabilities' impact on the oral hygiene status is likely to vary among the included patients. For example, patients with intellectual disabilities have difficulties to perform an acceptable oral hygiene from childhood on while patients with dementia usually have had a long phase in their life where they could perform oral hygiene in an acceptable way.

Due to missing periodontal measurements (e.g. clinical attachment loss, Periodontal Screening and Recording [PSR] index, and inflammatory activity), periodontal status was assessed on radiographs only. As most of the assessed radiographs were taken during general anesthesia where perfect parallel technique with the beam angled perpendicular to film was not always possible, minor inaccuracies are likely to have impacted on the meassurements. In addition, overlaps with adjacent structures (e.g. *Proc. zygomaticus*, wisdom teeth) or endotracheal tube resulted in sometimes unassessable measurement sites which were omitted. To address these issues, measurements were performed twice and averaged (mesially and distally), and bone loss was expressed as percentage of root length rather than absolute values.

Both age and smoking/nicotine dependence have been shown to be related to bone loss in the literature [11, 16]. Regarding further patient-related factors, results of the present study are partly in contrast to the existing knowledge as some of them have previously been shown to be significantly associated with periodontal disease [11]. This difference might be explained by the population of the present study: the number of patients bearing certain risk factors (e.g. Down syndrome or diabetes) was relatively small. As the assessment of diabetes was only based on the intake of anti-diabetic medications, undetected diabetes within the non-diabetic group cannot be ruled out. Within the group of already treated diabetes patients, the diabetic status (i.e. level of hyperglycemia) was not available. Therefore, results regarding the effect of diabetes have to be interpreted with caution [11]. Except for the radiographic bone and tooth loss, data on all variables were only self-reported by the patients, caregivers, and/or their legal guardians. This might have added further inaccuracy.

A direct comparison regarding the prevalence of periodontitis in patients with disabilities with the prevalence among patients without disabilities based on data derived from other studies is difficult due to inconsistent definitions of periodontitis. Even among studies which assess periodontal status radiographically, different treshholds are used. In the present study, a value of up to 2 mm was considered physiological (no bone loss), while a value above 2 mm was defined as periodontitis. This threshold is derived from the 2017 definition of periodontal health [14]. Even when adjusting this treshold to < 3 mm as used in previous studies [2, 4, 5], periodontitis was present in n = 132 patients (66.3 %). Based on this treshold, periodontitis based on radiographic assessment was more often present in patients with disabil-

	β	95% confidence interval for β		Std. error	t	Sig.
		Lower bound	Upper bound			
(Intercept)	-18.61	-41.32	4.10	11.49	-1.62	0.108
Age	0.65	0.40	0.89	0.13	5.17	**< 0.001**
Gender	-0.91	-6.87	5.06	3.02	-0.30	0.756
Intellectual disability	11.87	1.21	22.52	5.39	2.20	**0.029**
Physical disability	-1.98	-8.75	4.80	3.43	-0.58	0.566
Dementia	-9.77	-22.52	2.99	6.46	-1.51	0.133
Legal guardian	5.07	-7.86	18.00	6.54	0.77	0.440
Living situation	-2.00	-7.38	3.39	2.72	-0.73	0.465
Initial consultation due to caries	-0.85	-7.34	5.65	3.29	-0.26	0.797
Initial consultation due to prophylaxis	4.30	-1.97	10.57	3.18	1.36	0.177
Initial consultation due to pain	1.25	-5.77	8.28	3.56	0.35	0.725
Initial consultation due to swelling	-2.13	-12.46	8.21	5.23	-0.41	0.685
Initial consultation due to other reasons	2.70	-5.70	11.09	4.25	0.63	0.527
Antihypertensives	4.26	-3.42	11.94	3.89	1.10	0.275
Anticoagulants	5.64	-3.05	14.32	4.39	1.28	0.202
Anticonvulsants	2.23	-3.97	8.43	3.14	0.71	0.478
Sedative drugs	-6.45	-13.45	0.56	3.55	-1.82	0.071
Antidepressants	-0.93	-8.63	6.77	3.90	-0.24	0.811
Muscle relaxants	-4.74	-12.32	2.84	3.84	-1.24	0.219
Obesity*	-0.43	-7.66	6.80	3.66	-0.12	0.907
Diabetes**	-6.09	-17.91	5.73	5.98	-1.02	0.310
Down syndrome	6.23	-10.00	22.46	8.21	0.76	0.450
Smoking/nicotine dependence***	17.29	3.42	31.16	7.02	2.46	**0.015**

Table 4 Results of multiple linear regression analysis for prediction of % radiographic alveolar bone loss. *Defined as body mass index (BMI) ≥ 30 [15]. **Based on intake of anti-diabetic medication. ***Active smokers or those with less than 5 years since cessation.

ities, than in adults without disabilities with a reported prevalence between 17.9 % [4] and 26.0 % [5].

Regarding the prevalence of periodontitis in German patients with disabilities, only 2 studies of patients with disabilities have been published [9, 18]. In a small population (n = 52), periodontitis (PSR code 3 or 4) was present in 92.3 % [9]. However, the high prevalence of periodontitis might be related to the assessment tool (PSR index). Even initial periodontitis or gingiva hyperplasia caused by other reasons than peri-

odontitis (e.g. drug intake) might have been reported as periodontal disease [8]. As opposed to the PSR index, radiographs only allow for the evaluation of true bone loss as a consequence of periodontitis.

Another study among German adults with disabilities found a prevalence of 83 % based on CPITN (categories II and III) [18]. However, the same limitations as with the PSR index apply. Furthermore, a direct comparison between results assessed by PSR index and treatment need according to CPITN is not possible. As CPITN category II is equivalent to both PSR code 2 and 3, studies based on CPITN (categories II and III) are likely to show a higher prevalence of periodontitis than those based on PSR (code 3 + 4).

A third study reports on the periodontal status among German athletes with disabilities [19]. However, the authors visually assessed gingivitis rather than periodontitis; gingivitis prevalence amounted to 58.5 %. As gingivitis usually precedes periodontitis, prevalence of periodontitis can be expected to be less or maximal up to this value. Because mean age of athletes (30.8 years) was lower than the average age of patients included in the present study, a lower prevalence of periodontitis might be explained by age-related differences. Furthermore, athletes might favor a different lifestyle, be better cared for, and live more autonomously than average patients with disabilities in Germany.

Dental professionals, patients' caregivers, and legal guardians should be aware of periodontal disease among patients with disabilities. For the prevention of periodontal disease, dental hygiene instructions tailored for caregivers are necessary in order to improve dental hygiene performance among patients' caregivers. Since 2018, both patients and their caregivers are entitled to these measures according to Book V of the German Social Law Code (§22a, SGB V). German dentists should treat patients with disabilities and their caregivers according to these requirements.

5. Conclusion

Periodontal disease is common in German patients with disabilities. Older age, intellectual disability, and smoking/nicotine dependence are associated with increased bone loss.

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.

Literature

1. Anders PL, Davis EL: Oral health of patients with intellectual disabilities: a systematic review. Spec Care Dentist 2010; 30: 110–117

2. Bahrami G, Isidor F, Kirkevang LL, Vaeth M, Wenzel A: Marginal bone level in an adult Danish population. Oral Health Prev Dent 2006; 4: 119–127

3. Bahrami G, Vaeth M, Isidor F, Wenzel A: Marginal bone loss over 5 years in an adult Danish population. Oral Health Prev Dent 2007; 5: 113–118

4. Bahrami G, Vaeth M, Kirkevang LL, Wenzel A, Isidor F: The impact of smoking on marginal bone loss in a 10-year prospective longitudinal study. Community Dent Oral Epidemiol 2017; 45: 59–65

5. Bahrami G, Vaeth M, Wenzel A, Isidor F: Marginal bone level in two Danish cross-sectional population samples in 1997–1998 and 2007–2008. Acta Odontol Scand 2018; 76: 357–363

6. Björn H, Halling A, Thyberg H: Radiographic assessment of marginal bone loss. Odontol Revy 1969; 20: 165–179

7. Cohen J: A power primer. Psychol Bull 1992; 112: 155–159

8. Eickholz P: Parodontologische Diagnostik – Teil 5: PSI und Sondierungsparameter. Parodontologie 2010; 21: 177–187

9. Hillebrecht AL, Hrasky V, Anten C, Wiegand A: Changes in the oral health-related quality of life in adult patients with intellectual disabilities after dental treatment under general anesthesia. Clin Oral Investig, (2019)

10. Jaschinski S, Cichon P: Therapie parodontaler Erkrankungen bei Patienten mit Behinderungen. Teil 2: Behandlungskonzept für die Therapie entzündlicher Parodontalerkrankungen bei Patienten mit Behinderungen. Parodontologie 2009; 20: 117–137

11. Jepsen S, Caton JG, Albandar JM, Bissada NF et al.: Periodontal manifestations of systemic diseases and developmental and acquired conditions: Consensus report of workgroup 3 of the 2017 World Workshop on the Classification of Periodontal and Peri-Implant Diseases and Conditions. J Clin Periodontol 2018; 45(Suppl 20): S219–S229

12. Khocht A, Zohn H, Deasy M, Chang KM: Screening for periodontal disease: radiographs vs. PSR. J Am Dent Assoc 1996; 127: 749–756

13. Landis JR, Koch GG: The measurement of observer agreement for categorical data. Biometrics 1977; 33: 159–174

14. Lang NP, Bartold PM: Periodontal health. J Clin Periodontol 2018; 45: S9–S16

15. National Health Service. Obesity, London 2016

16. Papapanou PN, Sanz M, Buduneli N et al.: Periodontitis: Consensus report of workgroup 2 of the 2017 World Workshop on the Classification of Periodontal and Peri-Implant Diseases and Conditions. J Clin Periodontol 2018; 45(Suppl 20): S162–S170

17. Pepelassi EA, Tsiklakis K, Diamanti-Kipioti A: Radiographic detection and assessment of the periodontal endosseous defects. J Clin Periodontol 2000; 27: 224–230

18. Pieper K, Dirks B, Kessler P: Caries, oral hygiene and periodontal disease in handicapped adults. Community Dent Oral Epidemiol 1986; 14: 28–30

19. Schulte AG, Kaschke I, Bissar A: [Oral health in adult athletes with intellectual disabilities in Germany]. Gesundheitswesen 2011; 73: e78–83

20. Statistisches Bundesamt. Schwerbehinderte Menschen 2017 (Fachserie 13 Reihe 5.1). Wiesbaden, 2019

21. Statistisches Bundesamt. Schwerbehinderte Menschen am Jahresende. Wiesbaden, 2019

(Photo: UMG)

DR. MED. DENT. DR. RER. MEDIC. PHILIPP KANZOW
Department of Preventive Dentistry, Periodontology and Cariology
University Medical Center Göttingen
Robert-Koch-Str. 40; 37075 Göttingen
philipp.kanzow@med.uni-goettingen.de

DZZ International
German Dental Journal International

Publishing Institution
International Journal of the German Society of Dentistry and Oral Medicine/Deutsche Gesellschaft für Zahn-, Mund- und Kieferheilkunde e. V. (Zentralverein, gegr. 1859), Liesegangstr. 17a, 40211 Düsseldorf, Phone: +49 2 11 / 61 01 98 – 0, Fax: +49 2 11 / 61 01 98 – 11

Affiliations
German Society of Periodontolgy (DG PARO)
German Society for Prosthetic Dentistry and Biomaterials
German Association for Conservative Dentistry
German Society of Craniomandibular Function and Disorders in the DGZMK
German Society of Paediatric Dentistry
German Academy of Oral and Maxillofacial Surgery
German Association of Dento-Maxillo-Facial Radiology (GSDOM)
German Academy of Dental Ergonomics
Group of Basic Science in Dentistry

Publisher
Deutscher Ärzteverlag GmbH
Dieselstr. 2, 50859 Köln;
Postfach 40 02 65, 50832 Köln
Phone: +49 2234 7011-0; Fax: +49 2234 7011-6508
www.aerzteverlag.de

Executive Board
Jürgen Führer

Director Business Division Medicine and Dentistry
Katrin Groos

Product Management
Carmen Ohlendorf, Phone: +49 02234 7011-357; Fax: +49 2234 7011-6357; ohlendorf@aerzteverlag.de

Editorial Office
Irmingard Dey, Phone: +49 2234 7011-242; Fax: +49 2234 7011-6242; dey@aerzteverlag.de

Frequency
6 times a year

Layout
Linda Gehlen

Account
Deutsche Apotheker- und Ärztebank, Köln,
Kto. 010 1107410 (BLZ 370 606 15),
IBAN: DE 2830 0606 0101 0110 7410,
BIC: DAAEDEDD,
Postbank Köln 192 50–506 (BLZ 370 100 50),
IBAN: DE 8337 0100 5000 1925 0506,
BIC: PBNKDEFF

1. Volume
ISSN 2627-3489