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ORIGINAL ARTICLE

Ultrasonic activation of irrigants increases growth factor release from human dentine

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Abstract
Objectives Bioactive proteins are sequestered in human dentine and play a decisive role in dental pulp regeneration and repair. They can be released and exposed on the dentine surface by acids, but also chelators, such as ethylenediaminetetraacetic acid (EDTA). The objectives of this study were (i) to evaluate whether ultrasonic activation of irrigants in the root canal will promote growth factor release from dentine and (ii) to collect bioactive proteins in a physiological solution.
Materials and methods Human dentine disks underwent irrigation with and without ultrasonic activation. The protocols included treatment by either a single or two consecutive steps with 10 % EDTA and phosphate-buffered saline (PBS), where each sample was treated three times. To mimic clinical conditions, selected irrigation regimens were applied to root canals of extracted human teeth after preparation. Amounts of transforming growth factor β1 (TGF-β1) in solution were quantified using enzyme-linked immunosorbent assays. Nonparametric statistical analysis was performed to compare different groups as well as repetitions within a group (Mann-Whitney *U* test, $\alpha = 0.05$). Additionally, morphological changes of dentine surfaces were visualized by scanning electron microscopy (SEM).
Results TGF-β1 was not detectable after irrigation of dentine with PBS, neither with nor without ultrasonic activation. Irrigation with EDTA released TGF-β1, and ultrasonic activation of EDTA enhanced this effect. However, preceding EDTA conditioning enabled the release of bioactive proteins into PBS solution. Similar results were observed in dentine disks and root canals. Visualization of dentine surfaces after different treatment revealed superficial erosion after ultrasonic activation irrespective of the irrigant solution, but different degrees of exposure of organic substance.
Conclusions Ultrasonic activation enhances growth factor release from human dentine. Bioactive proteins can be isolated in physiological solvents and may act as autologous supplements for regenerative endodontic treatment or pulp tissue engineering.
Clinical relevance Autologous growth factors from human dentine can advance treatment strategies in dental pulp tissue engineering.

Keywords Ultrasonic activation · Dentine · Transforming growth factor beta1 · Ethylenediaminetetraacetic acid · Tissue engineering · Regenerative endodontic procedure

Introduction

During dentinogenesis, growth factors and other bioactive proteins are secreted by differentiated odontoblasts and embedded in the extracellular matrix of dentine [1, 2], where they preserve their ability for biochemical signaling. The entrapped molecules can be released later by lactic acid in carious lesions [3], by dental materials such as mineral trioxide aggregate [4], calcium hydroxide [5], or self-etching adhesives [6], and stimulate reactive or reparative dentine formation, repair, or regeneration. Furthermore, these growth factors can be liberated from dentine by demineralizing agents such as EDTA [1, 7], which is a hexadentate chelator and commonly used for smear layer removal from the root canal during endodontic therapy [8]. A variety of bioactive

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molecules have been detected in the dentine matrix, including transforming growth factor β1 (TGF-β1), fibroblast growth factor 2 (FGF-2), insulin-like growth factors 1 and 2 (IGF-1 and IGF-2), placenta growth factor (PlGF), epidermal growth factor (EGF), and bone morphogenetic protein 2 (BMP-2) [9–11], additionally angiogenic factors such as vascular endothelial growth factor (VEGF) and platelet-derived growth factor (PDGF) [12, 13]. They have been shown to exert chemotactic effects on dental pulp stem cells [14, 15], induce their proliferation and differentiation [16–18], promote mineralization [9, 19], and support angiogenesis [13, 20].

The release of endogenous growth factors from dentine might play a decisive role in the present and future therapies for dental pulp regeneration and tissue engineering, as they are based on exactly these cellular reactions. In a regenerative endodontic procedure, EDTA removes the smear layer on root canal walls and thus provides a physiological environment for incoming cells with exposed dentine tubules and collagen fibrils [21]. Growth factors on the dentine surface can act as chemoattractants and differentiation factors for mesenchymal stem cells from the apical papilla or periapical tissues [22, 23] and promote odontoblast-like cell differentiation at the dentine interface [7, 14, 24, 25].

For dental pulp tissue engineering, autologous growth factors could be collected in solution after irrigation of root canal dentine and re-inserted into the canal as bioactive components in combination with a suitable scaffold material. This approach exhibits considerable benefits compared to the use of recombinant growth factors, which is common for laboratory experiments, but poses questions regarding the choice of growth factors, their concentration, and whether recombinant proteins could evoke immunogenicity and undesirable effects as far as tumor formation in a clinical setting [26]. Beyond that, dentine matrix proteins might provide the required combination and concentrations of bioactive molecules to promote odontoblast differentiation versus ectopic tissue formation [27–29] and thus favor pulp regeneration instead of repair inside the root canal.

The cytotoxic effect of EDTA to suspend growth factors constitutes an obstacle [30–32], which might be circumvented by an improved two-step irrigation protocol that permits enrichment of growth factors in a physiological solution. In the context of root canal irrigation, ultrasonic-activated instrumentation has been proven to enhance the effects of irrigation solutions, such as better removal of smear layer and debris [33–37]. We hypothesized that ultrasonic activation of irrigants will increase the release of bioactive dentine matrix components, as shown for root dentine in periodontal research [38, 39]. Thus, the aim of this study was to analyze the impact of irrigation (one-step and two-step irrigation) with and without ultrasonic activation on the release of growth factors as well as on the surface morphology of human dentine.

Materials and methods

Quantification of TGF-β1 release from dentine disks

For analysis of growth factor release, dentine disks of 6 mm diameter and a thickness of 200 μm were prepared from the coronal part of human third molars from donors at age 15 to 25. After sectioning with an annular saw (Leitz 1600, Ernst Leitz Wetzlar, Wetzlar, Germany) under constant water flow at 600 rpm and a crosshead speed of 0.6 mm/s, the disks were stored in 0.5 % chloramine-T solution (Chloramine T trihydrate, Merck, Darmstadt, Germany), which was exchanged with distilled water 24 h before experimentation. Human tissue was obtained according to an informed consent protocol approved by an appropriate review board at the University of Regensburg.

Dentine disks in 96-well plates (Costar® 96-well plates, Corning Inc., Lowell, MA, USA) were subjected to one- and two-step irrigation protocols with or without ultrasonic activation as described in Table 1. Irrigants used for experimentation were 10 % EDTA at pH 7 (268 mM, EDTA Disodium Salt Dihydrate, Molecular Biology Grade, Merck) and 1× PBS (Instamed 9.55 g/l PBS Dulbecco w/o Ca^{2+} and Mg^{2+}, Biochrom AG, Berlin, Germany). Optional activation of 100 μl solution per disk was performed with an ultrasonic file (IRRI K 25/25, VDW GmbH, Munich, Germany) and the appendant unit (VDW.ULTRA®, VDW GmbH) in irrigation mode. During activation, the experimenter touched the dentine surface with the file continuously and without pressure in a 30° angulation and performed circular motions over the samples. Each individual dentine disk underwent the respective protocol three times. After treatment, irrigation solutions were removed individually from the samples, immediately frozen in liquid nitrogen, and stored at −80 °C (only final solution after two irrigation steps). All collected samples were thawed and subjected to quantification of TGF-β1, which was chosen as a representative growth factor, by use of a solid phase sandwich enzyme-linked immunosorbent assay (ELISA) test system (Human TGF-beta 1

Table 1 Treatment of dentine samples by at least one irrigant with or without ultrasonic activation

	First irrigant	Second irrigant
One-step	PBS for 10 min ± US	
	EDTA for 1 min ± US	
	EDTA for 3 min ± US	
	EDTA for 10 min − US	
Two-step	PBS for 10 min − US	PBS for 1 min + US
	EDTA for 1 min − US	PBS for 1 min + US
	EDTA for 10 min − US	PBS for 3 min ± US
	EDTA for 10 min − US	PBS for 5 min ± US

±US = irrigation with (+US) and without (−US) ultrasonic activation

Quantikine ELISA Kit, R&D Systems™, Wiesbaden, Germany). Spectrophotometrical analysis was performed on a microplate reader at λ = 450 nm (Infinite® 200, Tecan, Männedorf, Switzerland). Interference of the test solutions with antibody binding on the ELISA plates had been excluded in pilot experiments. Median values and 25–75 % percentiles for the individual cycles were obtained from nine independent samples (n = 9). Single measurements provided a basis for calculation of cumulative growth factor release over three cycles of irrigation from each dentine disk.

Root canal model

A root canal model was established to analyze growth factor release in a clinically relevant setup. The first and second molars from donors of all age groups without endodontic treatment were included, and roots without curvature but complete apical closure were chosen. Each tooth was decoronated to create a standardized canal length of 10 mm. Mechanical preparation of the root canals was performed with NiTi instruments (ProTaper NEXT®, DENTSPLY Tulsa Dental Specialities, Johnson City, TN, USA) at a working length of 9 mm and 0.9 % saline solution as irrigant (Sodium chloride solution, Sigma-Aldrich Chemie GmbH, Steinheim, Germany). Rotary files (X1 to X5) were used with an endodontic motor (X-Smart™Plus, DENSPLY Tulsa Dental Specialities) at the recommended speed of 300 rpm and a torque control of 2.0 N cm. Tooth roots were fixed in a pipette tip with impression material (Panasil® tray Soft Heavy, Kettenbach GmbH & Co. KG, Eschenburg, Germany) and underwent the selected irrigation protocols three times: PBS for 10 min with ultrasonic activation, EDTA for 10 min followed by 5 min PBS with ultrasonic activation, and EDTA for 3 min with ultrasonic activation. The ultrasonic file (IRRI K 25/25, VDW GmbH) was introduced until 1 mm from the anatomical apex with small amplitude filing movements in irrigation mode (VDW.ULTRA®, VDW GmbH). A volume of 100 μl was used in this model, and quantification of released TGF-β1 was conducted as described above. Data from 12 independent samples (n = 12) were analyzed to calculate median values and 25–75 % percentiles for individual cycles as well as the cumulative growth factor release per sample from three cycles.

Scanning electron microscopy

SEM analysis was performed for a selection of groups: 10 min PBS, 10 min EDTA, 5 min EDTA with ultrasound, 5 min PBS with ultrasound, as well as 10 min EDTA followed by 5 min PBS with ultrasound. Another group was established with a third step of 5.25 % sodium hypochlorite (NaOCl; Hypochlorite-Speiko®, Speiko®- Dr. Speier, Münster, Germany) for 5 min after EDTA for 10 min and ultrasonic-activated PBS for 5 min. Dentine cross-sections of 500 μm thickness and a diameter of 6 mm were prepared, and one third of the surface was covered with impression material (Impregum™ Penta™, 3M ESPE, Seefeld, Germany) to prevent effects from irrigants or the ultrasonic tip on this area (baseline). Samples were treated as described above, and the cover was removed before analysis. To display the sample profile, specimens were embedded in resin (Buehler EpoThin™ Resin, ITW Test & Measurement GmbH, Düsseldorf, Germany), cut vertically to the boundary between treated and untreated part, and polished. For surface view, dentine disks were mounted onto aluminum stubs using self-adhesive carbon disks (Leit-Tabs, PROVAC GmbH, Sprendlingen, Germany). After sample preparation, images were taken on a FEI Quanta 400 environmental scanning electron microscope with a field emitter (FEI Europe B.V., Eindhoven, The Netherlands) and operated at low-vacuum scanning electron microscopy (LVSEM) imaging mode. For surface analysis, the transition area from untreated to treated dentine was inspected. Single pictures were assembled to create a complete profile view of the cross-sections.

Statistical analysis

For enzyme-linked immunosorbent assays, the detection limit (DL) was defined as the lowest concentration of the delivered kit standard divided by 2. Medians and 25–75 % percentiles were computed and depicted graphically. Data were treated non-parametrically, and results were analyzed statistically using the Mann-Whitney U test on the α = 0.05 level of significance (SPSS, version 22.0, SPSS Inc., Chicago, IL, USA).

Results

Quantification of TGF-β1 release from dentine disks

The cumulative release of TGF-β1 from human dentine disks after three cycles of irrigation is depicted in Fig. 1. Treatment with PBS for 10 min did not induce growth factor release, neither with nor without ultrasonic activation (Fig. 1a). For one-step irrigation, a significantly (p = 0.000) higher amount of TGF-β1 was released by EDTA conditioning, which increased in a time-dependent manner (197 pg/ml after 1 min, 535 pg/ml after 3 min, and 908 pg/ml after 10 min). Ultrasonic-activated irrigation of dentine disks with EDTA further increased TGF-β1 release, which was statistically significant (p = 0.000) compared to non-activated irrigation for 1 min (197 pg/ml without and 313 pg/ml with ultrasonic activation).

Compared to PBS alone, prior treatment with EDTA for 1 min followed by 1 min of activated irrigation with PBS (two-step protocol) resulted in a significantly (p = 0.000) increased release of TGF-β1 (286 pg/ml). EDTA conditioning for 10 min allowed TGF-β1 release into PBS, which increased

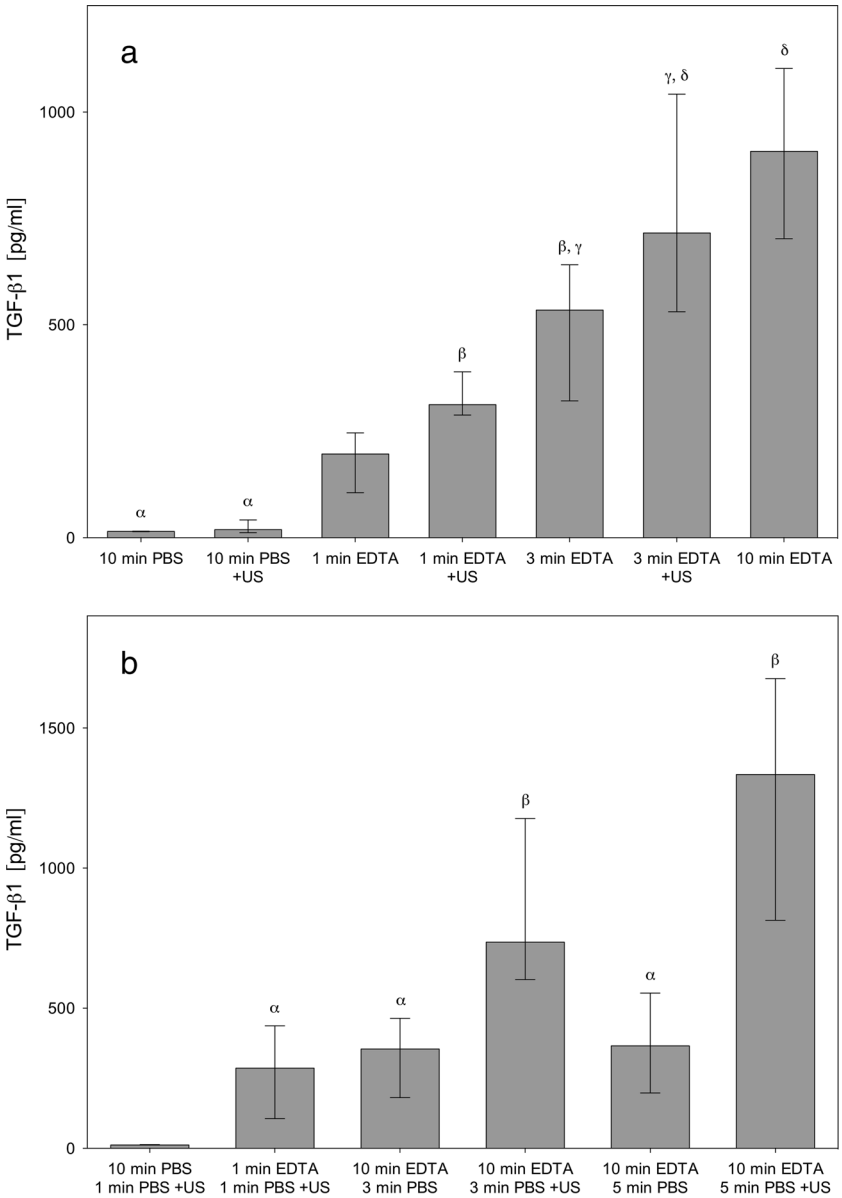

Fig. 1 Cumulative release of TGF-β1 from dentine disks after three cycles of treatment with EDTA or PBS in a one-step (**a**) or two-step protocol (**b**) with or without ultrasonic activation. Median values and 25–75 % percentiles computed from threefold treatment of nine independent samples ($n = 9$). Groups without statistical differences are indicated with *the same Greek letters* (α, β, γ, and δ)

significantly ($p \leq 0.004$) with ultrasonic activation (735 pg/ml after 3 min and 1334 pg/ml after 5 min).

Figure 2 illustrates TGF-β1 release after single cycles of the threefold application of irrigation to the same dentine disks. Whereas growth factor release by treatment with PBS was negligible, repeated irrigation with EDTA or EDTA followed by PBS with ultrasonic activation led to an increased dissolution of TGF-β1 in the second and third cycle. As indicated in Fig. 2, the increase between the first and the second treatment was significant ($p \leq 0.05$) in most groups, but not between the second and the third cycle.

Root canal model

The release of TGF-β1 from single root canals is depicted in Fig. 3. In contrast to the experiments with dentine disks, ultrasonic-activated irrigation with PBS in the root canal for 10 min released a considerable amount of growth factor (382 pg/ml) with no statistical difference among the three cycles (Fig. 3b). A cumulative concentration of 1023 pg/ml TGF-β1 was obtained with 5 min of activated irrigation with PBS after EDTA conditioning for 10 min (Fig. 3a); this amount increased slightly in the second and third treatment (Fig. 3b). The highest value was measured with ultrasonic activation of EDTA for 3 min (3445 pg/ml) without statistical differences between the cycles.

Scanning electron microscopy

Figure 4 shows composite SEM images to display cross-sections of whole dentine disks after different treatment protocols (Fig. 4a–d) as well as surface structures in top view

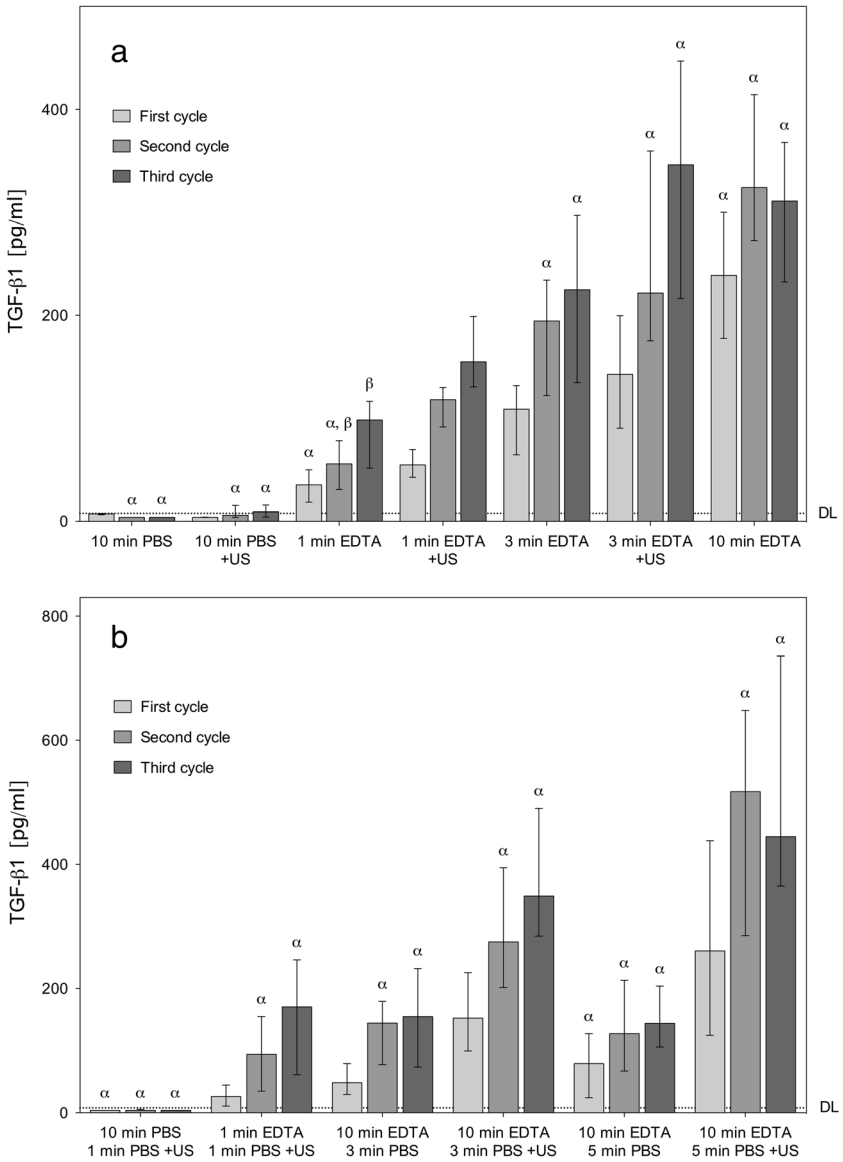

Fig. 2 TGF-β1 release from dentine disks after each of three cycles of irrigation with EDTA or PBS with and without ultrasonic activation. **a** One-step protocol and **b** two-step protocol with a combination of both irrigants. Depicted are median values and 25–75 % percentiles of three cycles, which were calculated from nine independent samples ($n = 9$). *The same Greek letters* (α and β) indicate nonsignificant differences among three consecutive cycles within a group

(Fig. 4e–j). Irrigation with EDTA for 10 min resulted in minimal erosions in the treated area and a smooth surface with open dentinal tubules as well as smear layer removal (Fig. 4a, h). After ultrasonic activation of EDTA for 5 min, superficial erosions were clearly visible (Fig. 4b). While intratubular debris was removed and tubules were visible, the surface was partly coated with organic substance (Fig. 4g). Ultrasonic activation of PBS also led to surface erosions, but elimination of intratubular debris remained incomplete (Fig. 4c, f). Ten minutes of EDTA conditioning prior to ultrasonic-activated irrigation with PBS removed intratubular debris and exposed organic matrix on the eroded surface (Fig. 4d, i). An additional irrigation step with NaOCl for 5 min after the described treatment (10 min EDTA/5 min activated irrigation with PBS) led to a complete removal of organic components and wide-open dentinal tubules. Partly, elimination of intratubular dentine was observed.

Discussion

In the present study, the effect of ultrasonic-activated irrigation on growth factor release from human dentine was investigated. TGF-β1 was quantified as a representative growth factor as it plays a key role in regenerative endodontics [40] and can be detected in far greater amounts compared with others, which was shown in previous work [41]. Additionally, morphological changes in the dentine surface by ultrasound treatment were analyzed.

EDTA is recommended for final irrigation during regenerative endodontic procedures as it creates an optimal biomimetic environment by release of growth factors and their exposition on the root canal walls [41, 42].

As plenty of bioactive proteins go into solution during the irrigation process [41] but cannot be utilized for tissue engineering when solved in cytotoxic EDTA [30–32], an irrigation

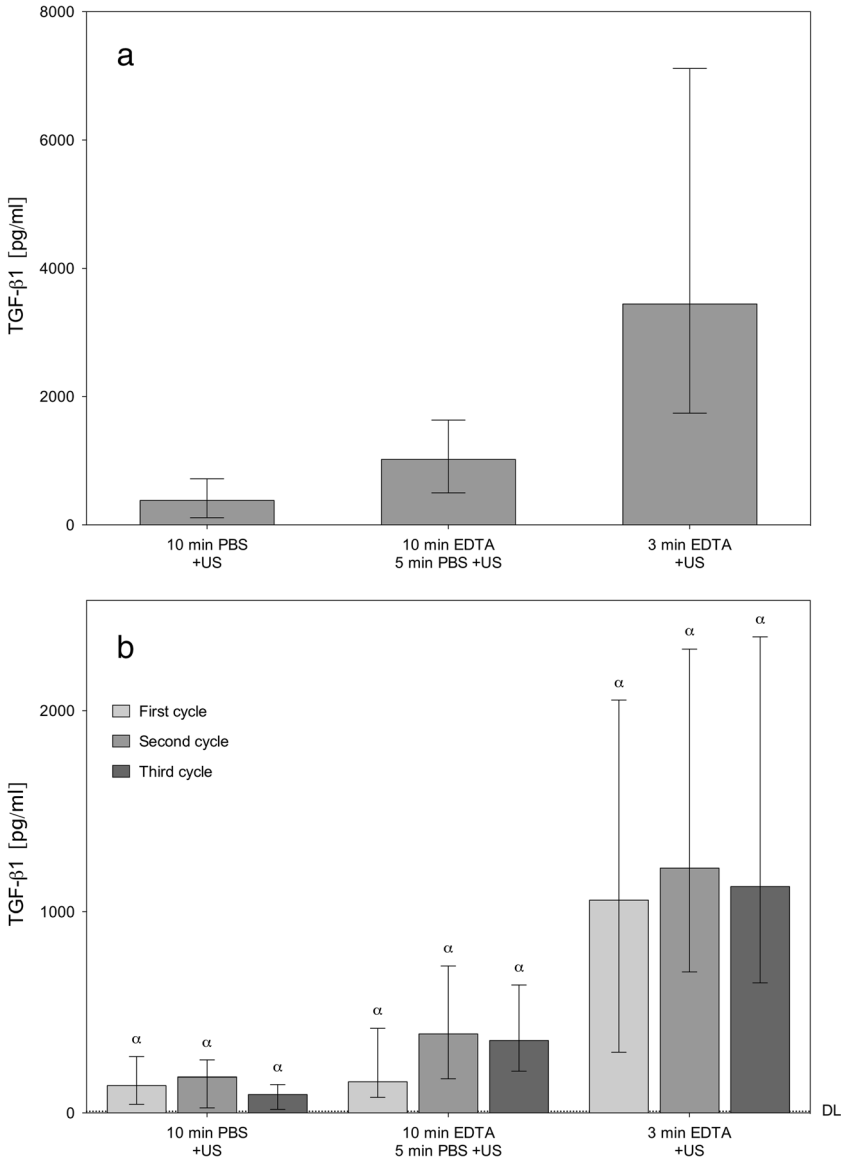

Fig. 3 Release of TGF-β1 from standardized root canals after ultrasonic activation of EDTA, PBS, or a combination of both. **a** Cumulative amount of TGF-β1 after three cycles of treatment. **b** Growth factor release from root canals per cycle. *Bars* represent median values and 25–75 % percentiles computed from 12 independent root canals treated three times each ($n = 12$). Differences among test groups (**a**) are significant, while differences between treatment cycles are non-significant (**b**), as indicated by α

sequence was established that allows the isolation of growth factors in a physiological solvent (PBS). The irrigation protocols were established according to clinical feasibility and practical requirements. It was intended to keep all irrigation steps short and limit ultrasonic activation to a maximum of 10 min to prevent heat generation and ensure the stability of growth factors in solution.

Further pretreatment of the dentine samples with disinfectants or medicaments was foregone consciously to gain distinct results with regard to the hypothesis. It was demonstrated in a recent study that a precedent dentine disinfection with sodium hypochlorite, which is necessarily applied in the first session of regenerative treatment, has only a slight negative effect on growth factor release in EDTA [41], and adverse effects of NaOCl on cell survival were largely reversed by consecutive EDTA irrigation [43]. Furthermore, isolation of growth factors would be conducted in the second session of regenerative treatment after an intracanal dressing with calcium hydroxide, which is described even to enhance growth factor release from dentine [41] and to increase proliferation of stem cells of the apical papilla on human dentine [43, 44].

Growth factor release from dentine disks

Dentine disks were chosen as a test system in the first part of the study to provide standardized samples with a flat and well-defined surface area. Despite a uniform shape, differences among individual samples regarding the amount of released TGF-β1 existed, however, with similar results over three cycles of treatment. The quantity of growth factors in dentine disks may depend on the donor age due to calcification processes taking place during life span [45]. Furthermore, number and size of dentine tubules increase from the enamel-dentine junction to the pulp-near dentine [46], which may be the

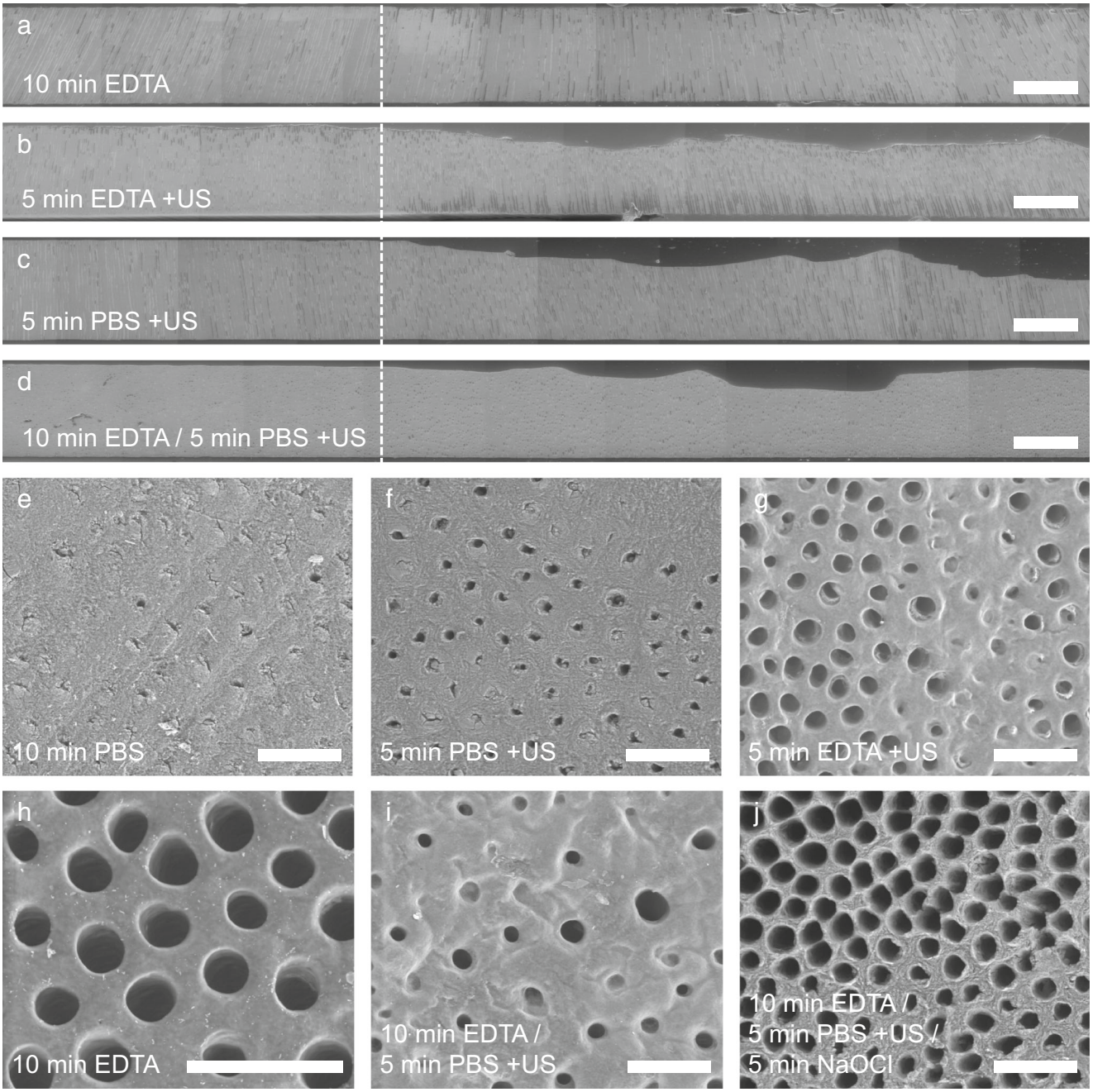

Fig. 4 SEM imaging of disk profiles (**a**–**d**), which were not treated in the left third (see *dotted line*), and surface view (**e**–**j**) from human dentine disks after different irrigation protocols: Irrigation with PBS (**e**) or EDTA (**a**, **h**) for 10 min, activated irrigation with PBS (**c**, **f**) or EDTA (**b**, **g**) for 5 min, activated irrigation with PBS after 10 min EDTA conditioning (**d**, **i**), and a consecutive step with NaOCl for 5 min (**j**). EDTA removed superficial and intratubular debris (**a**, **b**, **d**, **g**–**j**), whereas PBS insufficiently detached the smear layer (**e**) even with ultrasonic activation (**f**). The ultrasound tip created superficial erosions (**b**–**d**) and mobilized organic compounds, which deposited on the dentine surface (**g**, **i**) and could be dissolved with NaOCl (**j**). *Scale bars*: (**a**–**d**) 400 μm and (**e**–**j**) 10 μm

reason for comparatively large variations of the results, as the microscopic surfaces of the samples seem to diversify. Analogous to this, results from root canal experiments showed considerable interindividual differences in growth factor extractability among the specimens.

As results show, high amounts of growth factors were released from dentine disks into EDTA in a time-dependent manner, which was increased by ultrasonic activation. In contrast, barely any TGF-β1 was isolated with PBS alone, even after activated irrigation. SEM imaging as well as the observed turbidity of PBS after irrigation indicated superficial erosion of dentine during activated treatment. However, no growth factors were detected by ELISA, which indicates that TGF-β1 remained bound to the matrix of dissociated dentine particles

and therefore biologically inactive. With the two-step irrigation regimens, conditioning with EDTA was utilized to remove hydroxyapatite, expose the matrix proteins, and make them accessible to go into solution in PBS. Preceding incubation with the chelator for 1- and 10 min enabled TGF-β1 release into PBS, which was also increased significantly by ultrasonic activation and reached similar concentrations as EDTA alone. Without ultrasound, an extended incubation time for PBS after EDTA pretreatment from 3- to 5 min did not result in higher growth factor concentrations, which implies a restricted penetration depth of PBS. Ultrasonic activation of PBS led to a significantly increased TGF-β1 concentration, which might be associated with an improved ingression into dentine tubules.

Albeit comparable studies are missing, the presentation of the individual cycles provides further information about the trend of TGF-β1 liberation and possible microstructural changes in dentine. In almost every group, growth factor release increased significantly in the second cycle of irrigation and only slightly in the third treatment. The dissolution of the smear layer and removal of debris may play a role, as they primarily cover the dentine tubules. Penetrating EDTA seemed to unblock tubules' orifices resulting in an enlarged accessible dentine surface, which provided an increased contact area in the next cycle. Because similar effects have been observed with and without ultrasound, structural changes by the ultrasonic tip itself seemed to be negligible.

Root canal model

A closed apical root model was established to assess clinical transferability and potential of this approach. Three regimens of activated irrigation were selected to define the range of isolatable growth factors in vivo. Complete wetting of the dentinal walls with the irrigants was assured by sufficient canal enlargement [47] and prevention of vapor lock by manual or ultrasound instrumentation [48].

As seen for dentine disks, growth factors were released from root canals by ultrasonic-activated treatment with EDTA as well as by activated irrigation with PBS after EDTA conditioning. Interestingly, TGF-β1 was even detectable in PBS when activated for 10 min, and higher amounts of TGF-β1 were obtained in the root canal model than from dentine disks despite a smaller surface area. Whereas the prepared dentine disks have a surface of 60 mm^2, the dissected roots (prepared up to size X5/50.06) provide a contact area of only 10 mm^2. Considering the different surface areas in both models, higher concentrations were obtained in root canals. Taking into account that single roots without access cavities were treated, even higher concentrations can be expected in vivo.

Although tooth types may also play a role (no third molars were used for the root canal model), the reason for a higher release in this setup might be that the effect of ultrasonic activation was even more pronounced in the root canal model, as the file created more turbulence. Whereas the dentine disks were affected only by the oscillating tip, acoustic streaming occurred in root canals along the whole length of the instrument [34, 49, 50]. The trend of an increasing growth factor concentration over three cycles, which was found for dentine disks, was barely visible in the root canal model. This may be attributed to a lower number of dentine tubules in root canal dentine as multiple cycles of activated irrigation did not increase the microsurface and the amount of released growth factors, which was observed with disks from coronal dentine.

The continuity of TGF-β1 release over three cycles demonstrates that EDTA irrigation not only exposes growth factors on the dentine surface but also allows a repeatable and continuous dissolution from root canal dentine.

This seems to be an important aspect in face of a cell-free pulp tissue engineering approach [28, 42]. Clinical outcomes might be optimized by the use of autologous growth factors, which can be isolated during treatment and subsequently be supplemented to the scaffold. They may support migration of progenitor cells from the periapex into the canal lumen and finally to the dentinal walls where a gradient of bioactive molecules is created by both growth factor liberation into solution as well as exposure on the surface [1, 7, 40]. This condition may allow differentiation into odontoblast-like cells and finally lead to the formation of a pulp-like tissue. As there are a lot of open questions with regard to the effect of soluble matrix proteins in the context of pulp regeneration, future studies have to shed light on the effects of bioactive molecules on cellular behavior and odontoblast-like cell differentiation.

Effect of ultrasound on dentine surface

Scanning electron microscopic analysis of the profiles showed superficial erosions and grooves on the dentine disks after treatment with ultrasound irrespective of irrigant. Whereas an activated irrigation with PBS only insufficiently removed debris, non-activated treatment with EDTA eliminated intratubular particles on both sides of the disk. Ultrasonic activation of EDTA generated a clean surface with empty tubules, but also revealed organic matrix. A similar effect was observed with ultrasonic-activated treatment with PBS after EDTA conditioning. So far, similar exposure of organic substances by ultrasound was described mainly for root dentine in periodontal research, where ultrasonic instrumentation in combination with EDTA or citric acid laid bare collagen fibrils [38, 39].

A group with a final rinse of NaOCl was established to illustrate the dentine surface after removal of organic substance, which was exposed by ultrasonic treatment. Nevertheless, a final rinse with NaOCl is not reasonable due to cytotoxicity and growth factor destruction [41, 43]. The illustrated deproteinized dentine surface constrained the presence of organic substance after ultrasound application. Intratubular

dentine was dissolved, and orifices seemed irregular and rough, which was described similarly in former studies for EDTA followed by NaOCl [33, 51].

Conclusions

In these experiments, it was shown that (i) ultrasonic activation increases the release of growth factors into EDTA and (ii) leads to dissolution in PBS after EDTA conditioning. This effect can be utilized to collect bioactive proteins in physiological solution from root canals, which may be incorporated as endogenous growth factors into scaffold materials for dental pulp tissue engineering.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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Informed consent Human tissue was obtained according to an informed consent protocol approved by an appropriate review board at the University of Regensburg.

Human and animal rights This article does not contain any studies with human participants or animals performed by any of the authors.

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