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ORIGINAL ARTICLE

Effects of air polishing and an amino acid buffered hypochlorite solution to dentin surfaces and periodontal ligament cell survival, attachment, and spreading

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Abstract
Objectives The aim of this study is to examine morphological changes of dentin surfaces following air polishing or amino acid buffered hypochlorite solution application and to assess their influence on periodontal ligament (PDL) cell survival, attachment, and spreading to dentin discs in vitro.
Materials and methods Bovine dentin discs were treated with either (i) Classic, (ii) Plus, or (iii) Perio powder (EMS). Furthermore, Perisolv® a hypochlorite solution buffered with various amino acids was investigated. Untreated dentin discs served as controls. Morphological changes to dentin discs were assessed using scanning electron microscopy (SEM). Human PDL cells were seeded onto the respectively treated discs, and samples were then investigated for PDL cell survival, attachment, and spreading using a live/dead assay, adhesion assay, and SEM imaging, respectively.
Results Both control and Perisolv®-rinsed dentin discs demonstrated smooth surfaces at low and high magnifications. The Classic powders demonstrated the thickest coating followed by the Powder Plus. The Perio powder demonstrated marked alterations of dentin discs by revealing the potential to open dentinal tubules even before rinsing. Seeding of PDL cells demonstrated an almost 100 % survival rate on all samples demonstrating very high biocompatibility for all materials. Significantly higher PDL cell numbers were observed on samples treated with the Perio powder and the Perisolv® solution (approximately 40 % more cells; $p < 0.05$). SEM imaging revealed the potential for PDL cells to attach and spread on all surfaces.
Conclusion The results from the present study demonstrate that cell survival and spreading of PDL cells on root surfaces is possible following either air polishing or application with Perisolv®. Future in vitro and animal testing is necessary to further characterize the beneficial effects of either system in a clinical setting.
Clinical relevance The use of air polishing or application with Perisolv amino acid buffered hypochlorite solution was effective in treating root surfaces and allowed for near 100 % PDL cell survival, attachment, and spreading onto all root surfaces.

Keywords Periodontal regeneration · Powder spraying · Air-Flow · Dentin discs · Dentinal tubules

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Introduction

Periodontitis is a widespread inflammatory disease of the tooth-supporting soft and hard tissues, which is modulated by the host [1]. Biofilms are regarded as the primary etiologic factor for both disease initiation and progression [2]. Therefore, any cause-related periodontal therapy is based on the strict removal of the pathogenic microbial challenge and the successful prevention of their re-establishment [3].

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Clinically, this is achieved traditionally by mechanical debridement using scalers, curettes, and/or ultrasonic instruments along with proper oral hygiene instructions [4, 5].

The preservation and creation of a biocompatible tooth surface during this periodontal therapeutic approach is crucial for successful tissue integration [6]. This is, however, particularly difficult when the surfaces display distinct morphological features, which are difficult to reach and to clean [7]. As a consequence, the overall aimed therapeutic goals are difficult to achieve and it is well documented that the deeper the initial periodontal lesions are, the less effective mechanical debridement may be [8–10].

A number of instruments have been developed and recommended over the years to assist clinicians in removing bacteria and their deposits in severely affected sites. Most of the classical mechanical instruments including curettes and ultrasonic instruments—despite being effective in hard deposit removal—often cause more excessive removal of cementum and/or dentin than is necessary [11]. Because past studies have documented that biofilm, rather than calculus, is the main culprit in triggering periodontal inflammation [12], other strategies of investigation include methods that eliminate or inactivate the purported periodontal pathogens in the biofilm. As a consequence, systemically and locally applied antibacterial agents (i.e., chemical agents) were used, which notably always bear the risk of bacterial resistance, tolerance, or other side effects [13]. Therefore, alternatives have been introduced to the market to serve as adjuncts during instrumentation in removing or—at least—reducing or modifying bacterial biofilms. The use of lasers and antimicrobial photodynamic therapy (tPDT) has also been the subject of much study recently [14, 15]. While the results of these studies have been inconclusive, the background theory of mechanism remains interesting: selective, light-induced elimination/reduction of microorganisms, with minimal damage to host tissues. As an alternative, but based on mechanical principles, glycine powders using small and soft amino acid particles have been developed for air abrasion, which can be applied in specially designed power jet devices directly on the root surfaces. They have become a real alternative with good clinical and microbiological outcomes and were shown to exhibit less abrasive effects on teeth as compared to hand or ultrasonic scaling or powder jet devices employing classical bicarbonate powder [16–21].

Another chemical line of investigation has recently opened up, with a new gel that was designed to detoxify and clean periodontal pockets. The active ingredients of this gel contain sodium hypochlorite (0.95 %) and amino acids (glutamic acid, leucine, lysine). Based on studies using a similar formulation for the removal of carious dentin lesions [22, 23], this further development now aims to extend the use of this gel mixture for subgingival use by disrupting bacterial biofilms and dissolving degenerated tissues [24]. These effects are purportedly achieved through the chemical reaction of sodium hypochlorite with the amino acids to form N-monochloroamino acids, which while capable of dissolving degenerated tissue, also minimize the detrimental effects of the hypochlorite on sound dentin and healthy soft tissues [25, 26].

Since the spraying of periodontal pockets using a variety of prophylactic powders has recently been introduced as a means to condition tooth root surfaces, little is known regarding its effect on alterations of root surface morphology or the potential cell repopulation thereafter. This also holds true for the application of the buffered hypochlorite gel. Because the regeneration of periodontal tissues relies on a biocompatible dentin surface with minimal surface alterations, the aim of the present study was to examine morphological changes of dentin surfaces following Air-Flow powder or gel application and to assess the influence on PDL cell survival, attachment, and spreading to dentin discs in vitro.

Materials and methods

Dentin disc preparation, cell source, and reagents

Bovine roots of freshly extracted teeth were separated from their crown and the approximate area was first ground flat and polished using water-cooled silicon carbide paper (Stuers, Erkrat, Germany) up to P4000 grit and discs with a diameter of 6.0 mm and a thickness of 1.5–1.6 mm to fit directly into 96-well in vitro culture plates. Dentin discs were prepared using a diamond-coated trephine under constant water-cooling. The discs were then stored in the dark in tap water at a temperature of 4 °C until the experiment started.

Air-Flow® powders (1) Classic, (2) Plus, and (3) Perio were kindly provided by Electro Medical Systems (EMS, Nyon, Switzerland). Perisolv®—composed of hypochlorite (NaOCl) solution buffered with different amino acids—was provided by Regedent (Zurich, Switzerland).

For dentin disc preparations, discs were air sprayed with each powder for 10 s per disc followed by 10 s of rinsing. Perisolv® dentin discs were rinsed with Perisolv® for 10 s followed by rinsing.

Primary human PDL cells were obtained from the middle third portion of three teeth extracted from healthy patients with no signs of periodontal disease extracted for orthodontic reasons as previously described [27, 28]. For ethical approval, informed written consent was obtained from all patients. Primary human PDL cells were detached from the tissue culture plastic using trypsin solution. Cells used for experimental seeding were from passages 4–6. Cells were cultured in a humidified atmosphere at 37 °C

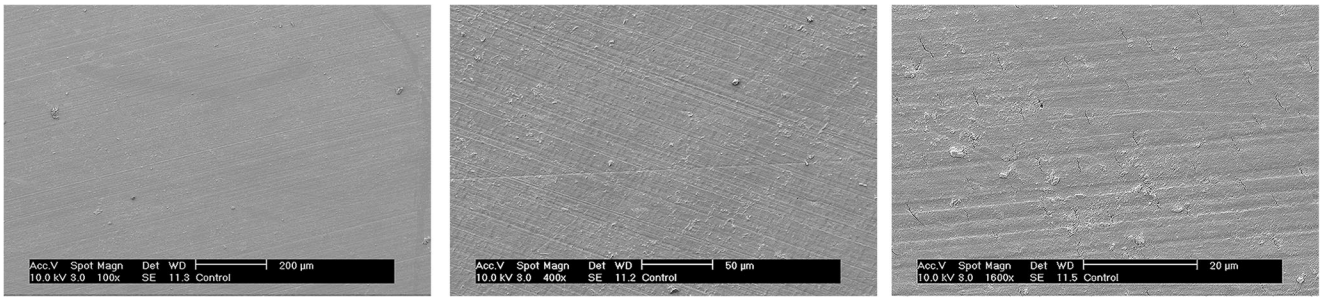

Fig. 1 SEM images of control dentin slices at low (×100), medium (×400), and high (×1600) magnification. Smooth surfaces were observed at low magnifications with slight variations observed at high magnification (×1600)

in growth medium consisting of DMEM (Gibco, Life technologies, Carlsbad, CA), 10 % fetal bovine serum (FBS; Gibco), and 1 % antibiotics (Gibco). For in vitro experiments, cells were seeded with HA in 96-well culture plates at a density of 5000 cells per well for all experiments including cell attachment, cell survival (live/dead assay), and morphological variation as qualitatively assessed via SEM.

Scanning electron microscopy

Dentin discs from samples including (1) control, (2) Powder Classic, (3) Powder Plus, (4) Powder Perio, and (5) Perisolv® rinsing were fixed in 1 % glutaraldehyde and 1 % formaldehyde for 2 days for scanning electron microscopy (SEM). Following serial dehydration with ethanol,

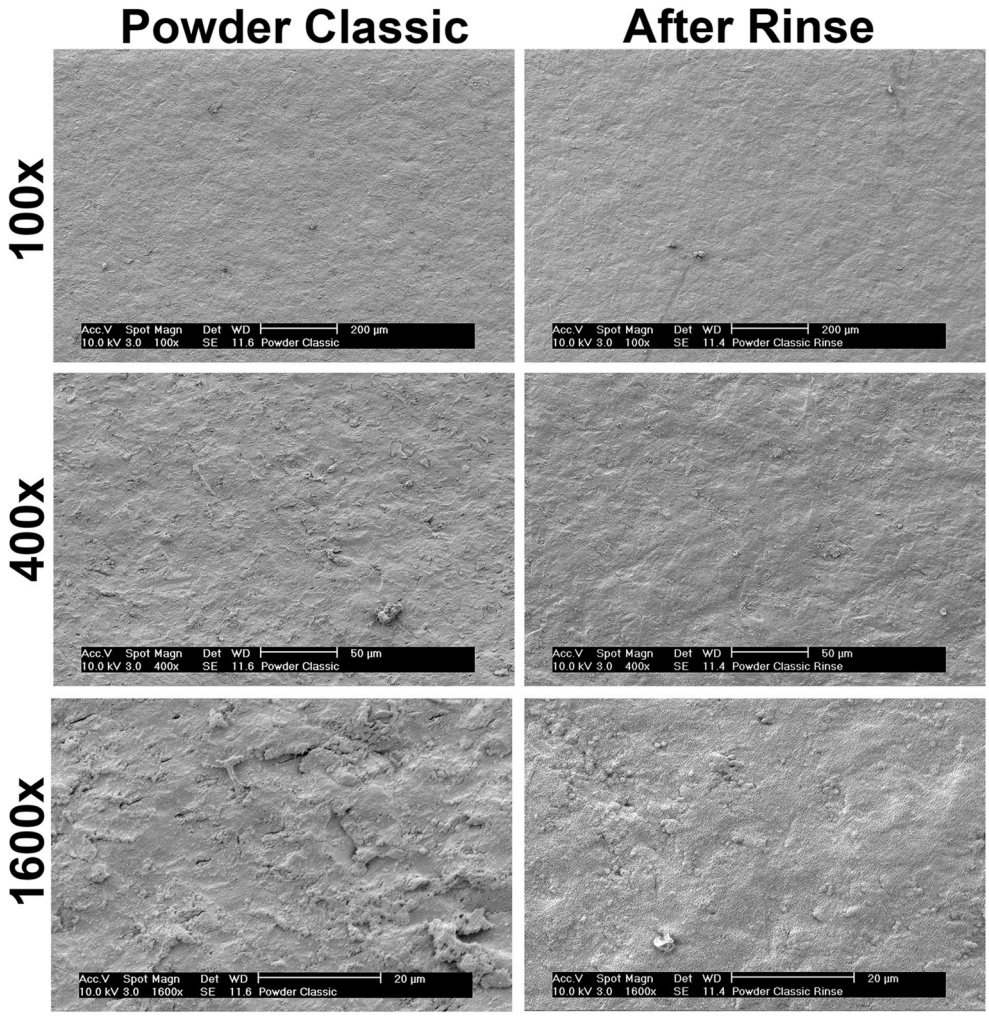

Fig. 2 SEM images of dentin discs Air-Flow sprayed for 10 s with Powder Classic before and after 10 s of rinsing with saline solution at various magnifications. A thin layer of collected powder was observed on dentin discs before and after rinsing

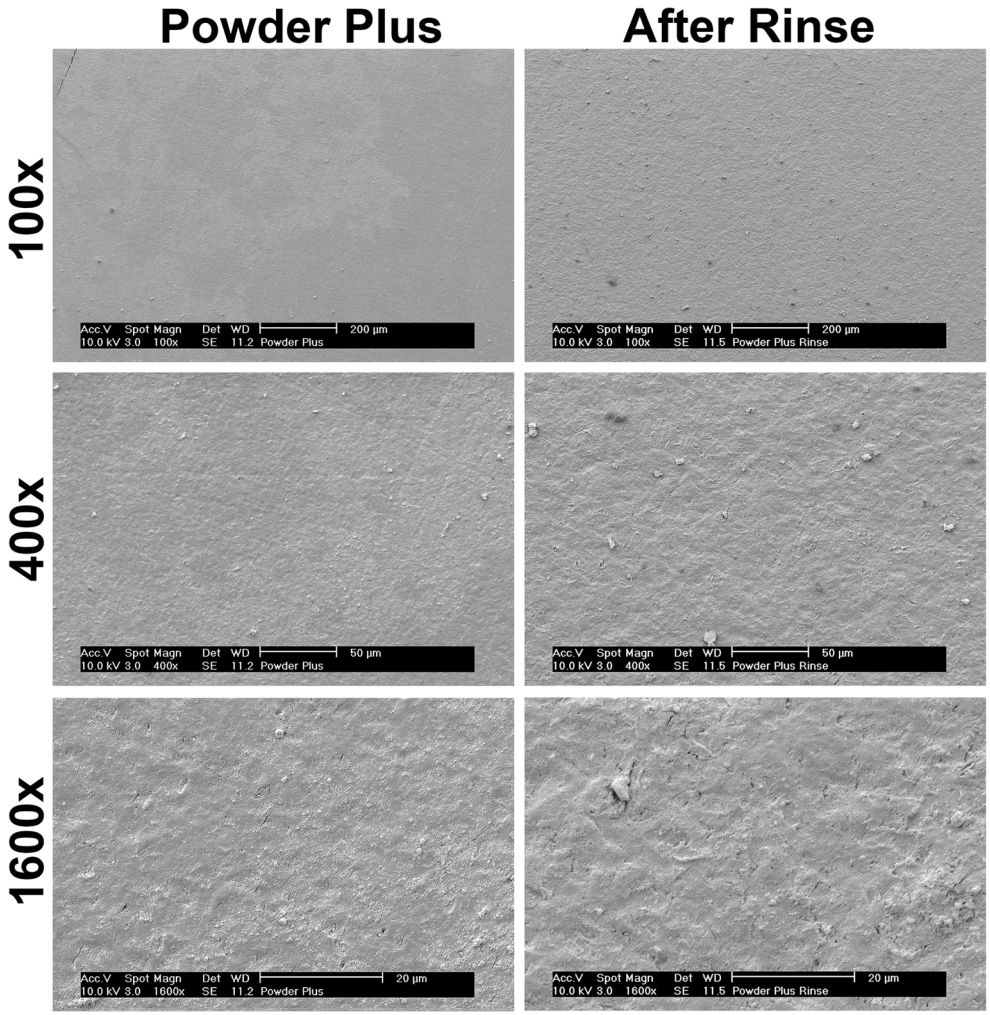

Fig. 3 SEM images of dentin discs Air-Flow sprayed for 10 s with Powder Plus before and after 10 s of rinsing with saline solution at various magnifications. Similarly to Powder Classic, a thin layer of powder was observed on dentin surfaces following spraying

samples were critical point dried (Type M.9202 Critical Point Dryer, Roth & Co. Hatfield, PA, USA) and allowed to dry overnight as previously described [29, 30]. The following day, samples were sputter-coated using a Balzers Union Sputtering Device (DCM-010, Balzers, Liechtenstein) with 10 nm of gold and analyzed microscopically using a Philips XL30 FEG scanning electron microscope to determine surface variations between samples. Furthermore, primary human PDL cells seeded onto dentin discs with each treatment modality were also investigated for PDL cell surface spreading in response to the various Air-Flow powders and Perisolv® rinsing.

Cell viability

Primary human PDL cells were seeded in 96-well plates at a density of 5000 cells per well onto dentin discs including (1) control, (2) Powder Classic, (3) Powder Plus, (4) Powder Perio, and (5) Perisolv®. PDL cells were evaluated using a live-dead staining assay according to the manufacturer's protocol (Enzo Life Sciences AG; Lausen, Switzerland) as previously described [31]. Experiments were performed in triplicate with three fluorescent images taken per experimental condition with a fluorescent microscope (OLYMPUS BX51, Tokyo, Japan).

Adhesion assay

Primary human PDL cells were seeded in 96-well plates at a density of 5000 cells per well onto dentin slices either (1) control, (2) Powder Classic, (3) Powder Plus, (4) Powder Perio, and (5) Perisolv®. PDL cells were quantified using fluorescent imaging (from live/dead assay) at 8 h for cell numbers as previously described [32]. At desired time point of 8 h, cells were washed with phosphate-buffered solution (PBS), fixed with 4 % formaldehyde solution

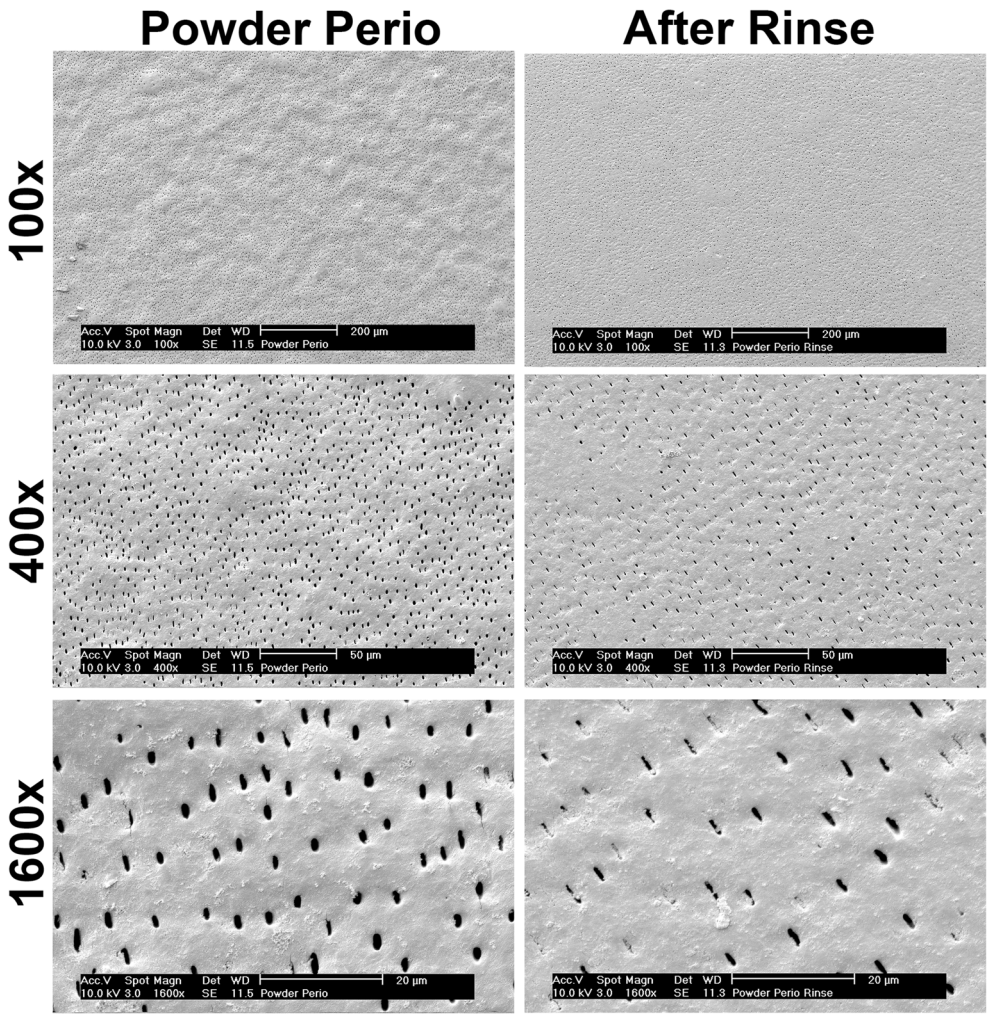

Fig. 4 SEM images of dentin discs Air-Flow sprayed for 10 s with Powder Perio before and after 10 s of rinsing with saline solution at various magnifications. Interestingly, Air-Flow spray with Powder Perio revealed the opening of dentinal tubules both before and after rinsing

(Grogg-Chemie AG, Stettlen, Switzerland) for 5 min, and mounted with VECTASHILD containing DAPI (Vector, Burlingame, CA). Fluorescent images were quantified with a fluorescent microscope. Experiments were performed in triplicate with five images captured per group. Data were analyzed for statistical significance using one-way analysis of variance with Tukey's test (*, p values <0.05 was considered significant).

Results

Surfaces characteristics of dentin slices with or without air polishing or Perisolv® rinsing

Morphological changes to dentin slices were first visualized using SEM imaging (Figs. 1, 2, 3, 4, and 5). First, uncoated control dentin slices demonstrated smooth surfaces at low magnification and demonstrated only slight irregularities at high magnification (Fig. 1). Thereafter, dentin discs were Air-Flow sprayed for 10 s with various powders and visualized before and after rinsing with saline (Figs. 2, 3, and 4). The Classic powder demonstrated the additional layer of powder following Air-Flow, and even after rinsing with saline, fine particles were still observed at high magnification (Fig. 2). A similar observation was observed for Powder Plus however to a lesser extent (Fig. 3). Following rinsing, the dentin surfaces revealed surfaces with many additional micro-rough patterns as a result from the Air-Flow spraying (Fig. 3). Interestingly, dentin discs that were sprayed with Powder Perio demonstrated very profound changes to dentin discs (Fig. 4). It was found that spraying surfaces with Powder Perio revealed the open of dentinal tubules both before and after rinsing (Fig. 4). Lastly, the use of Perisolv® rinsing did not affect surface morphology of dentin discs (Fig. 5).

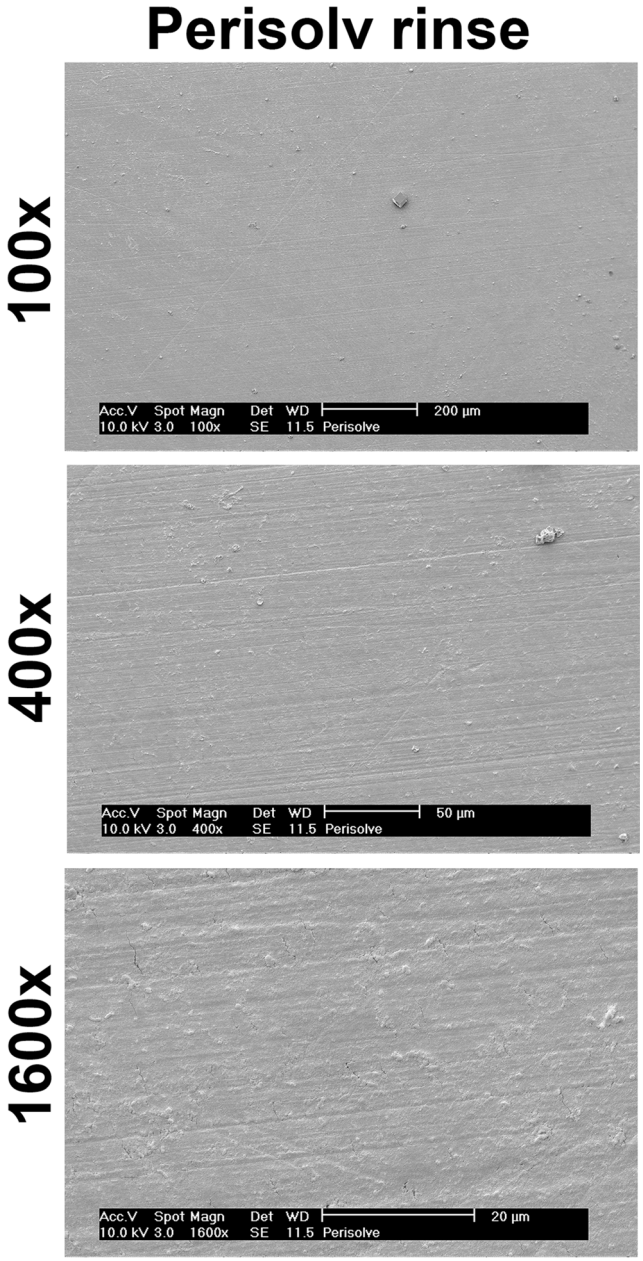

Fig. 5 SEM images of dentin discs that were rinsed with Perisolv® for 10 s at various magnifications. No change in surface morphology was observed when compared to control dentin discs

PDL cell survival, attachment, and spreading

Each of the modifications to dentin discs was then investigated for their effect on PDL cell survival, attachment, and spreading of PDL cells (Figs. 6, 7, and 8). It was first observed that cell survival was near 100 % for all samples (Fig. 6, green cells label live cells versus red cells label dead cells). Thereafter, cell numbers were quantified using DAPI staining at 8 h to investigate the total number of attached cells following each of the treatment groups (Fig. 7). It was found that significantly more cells attached to dentin discs having been Air-Flow sprayed with Perio Powder or rinsed with Perisolv® (Fig. 7). Investigation of cell spreading and cell attachment via SEM imaging did not reveal any discernable differences between treatment groups at 8 h (Fig. 8).

Discussion

Successful periodontal regeneration requires adequate infection control and implies afterwards migration, adhesion, and proliferation of periodontal progenitor and mesenchymal stem cells located in the periodontal ligament [33, 34]. In this context, we focused on biological effects after modern non-destructive root surface cleaning procedures like air polishing or amino acid buffered hypochlorite solution application and determined their influence on PDL cell survival, attachment, and spreading to dentin discs in vitro. This study showed that the Classic and Plus powders demonstrated some coating effects, whereas the Perio powder opened the dentinal tubules even before rinsing. Seeding of PDL cells, however, showed an almost 100 % survival rate on all samples demonstrating very high biocompatibility for all materials despite the smear remnants. Nevertheless, significantly higher cell numbers were observed on samples treated with the Perio powder and the Perisolv® solution, which was corroborated by SEM.

A shortcoming of the present study was that treatments were performed on clean dentin surfaces and that the samples were polished. This comparative screening study, however, primarily focused on material-induced surface changes and the potential influence of the applied materials and their remnants. Therefore, we did not try to imitate the clinical situation in the first instance. Hägi and co-workers assessed air polishing with erythritol with and without chlorhexidine (Plus powder in this study) using a specially designed nozzle for subgingival application and showed that this treatment caused no substance loss and resulted in a smooth surface with nearly no residual biofilm, which also promoted the reattachment of PDL fibroblasts [35]. However, it must be noted that a onefold treatment of the specimens was not sufficient, and that the bacteria had to be additionally killed by UV. And still, the remaining bacterial compounds (e.g., lipopolysaccharides (LPS)) have interfered with PDL fibroblast orientation. In that study, only a fivefold treatment was, however, sufficient to enable a so-called contaminant free and biocompatible surface.

Schwarz and co-workers studied the influence of different air-abrasive powders, glycine, and sodium bicarbonate particles, on cell viability as well [36]. In contrast to the present study, contaminated titanium discs were studied and

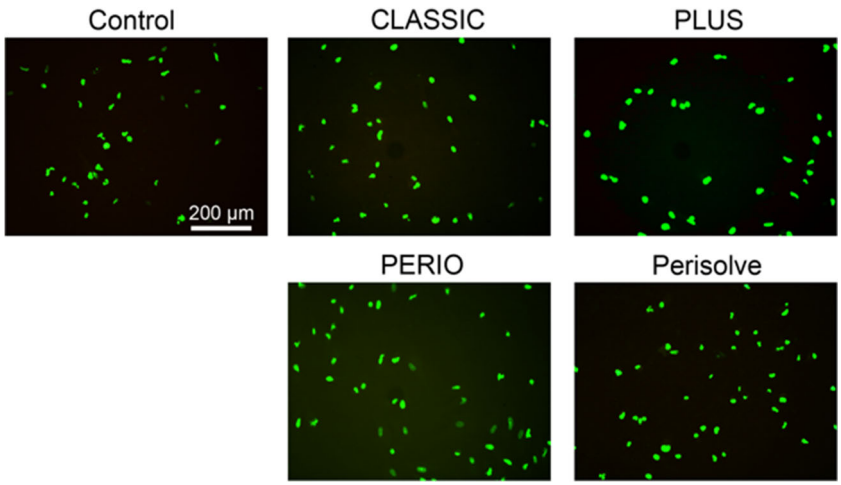

Fig. 6 Live/dead staining of primary human primary PDL cells on control, Powder Classic, Powder Plus, Powder Perio, and Perisolv® dentin discs. For cell viability, live-dead staining was done with viable cell appearing in *green* and dead cells in *red*. The results from these experiments demonstrated that all treatment modalities are highly biocompatible with little to no cell death observed. 13

osteoblastic osteosarcoma cell attachment was measured using a mitochondrial activity assay. Whereas both powders removed almost completely the biofilm, the luminescent cell viability test revealed better cell growth on samples treated with the Classic powder when compared to the Perio powder, which is in contrast to the present study and may be explained at least in part by the different substrates and methods used. The rough titanium surface may have been more efficiently cleaned by the sodium bicarbonate powder, which is characterized by harder particles of a bigger size, which may display an advantage when cleaning this kind of more complex surface structures [36]. On smooth dentin surfaces, in contrast, biofilms are to be removed, whereas the tooth surface preferably remains intact. With bicarbonate powder, considerable substance defects are associated even after short application times of 5 s, whereas glycine powder shows significantly less defect formation. The latter shows no detectable substance loss within the first 5 s [21] and only moderate superficial defects after 20 s of application time. Again, the laboratory condition may differ from the clinical situation in terms that cementum may cover the roots. Both tooth substances differ slightly in their chemical and histologic composition, i.e., that dentin is more mineralized, whereas cementum contains a bigger organic component and more water. This fact should also be taken into consideration when interpreting the current results. Cell attachment may vary as well on cementum. But to obtain samples with intact cementum is (i) difficult, and (ii) we used machined surfaces because this more reflects the clinical reality. However, flat surfaces had to be used under the current laboratory conditions to perform our experiments as planned. In addition, periodontally affected roots were pretreated in most cases. This inflicts partial removal of intact cementum and flattening in due course of the debridement procedures and the root material is abraded in order to ensure a clean and smooth surface. This is necessary—as mentioned above—to obtain a biocompatible surface. But atraumatic surface treatments are still warranted.

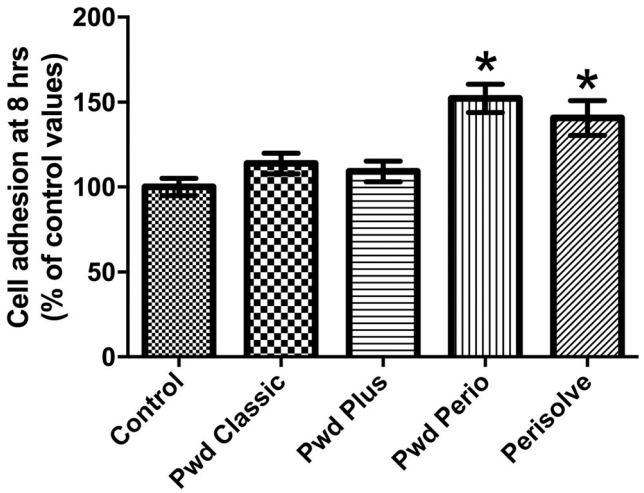

Fig. 7 Cell number of primary human PDL cells seeded on control, Powder Classic, Powder Plus, Powder Perio, and Perisolv® dentin discs. A significant increase in cell numbers was observed on Powder Perio and Perisolv® dentin discs when compared to control samples (*asterisk* denotes significant difference when compared to control samples, $p < 0.05$)

Based on studies using a similar formulation for the removal of carious dentin lesions, this further development of the gel mixture for use subgingivally has been reported in a case study treating 15 patients and a total of 158 residual pockets (non-responding sites persisting beyond the normal healing time of 6–12 months) [22, 23]. The manufacturer's claim is that the gel aids in hard deposit removal (reduced friction during instrumentation, softening of calculus), disruption of biofilm, and dissolving the generated tissue and therefore facilitating its removal from the periodontal pocket by scaling and root planning and aids in the healing process through its antibacterial properties [24].

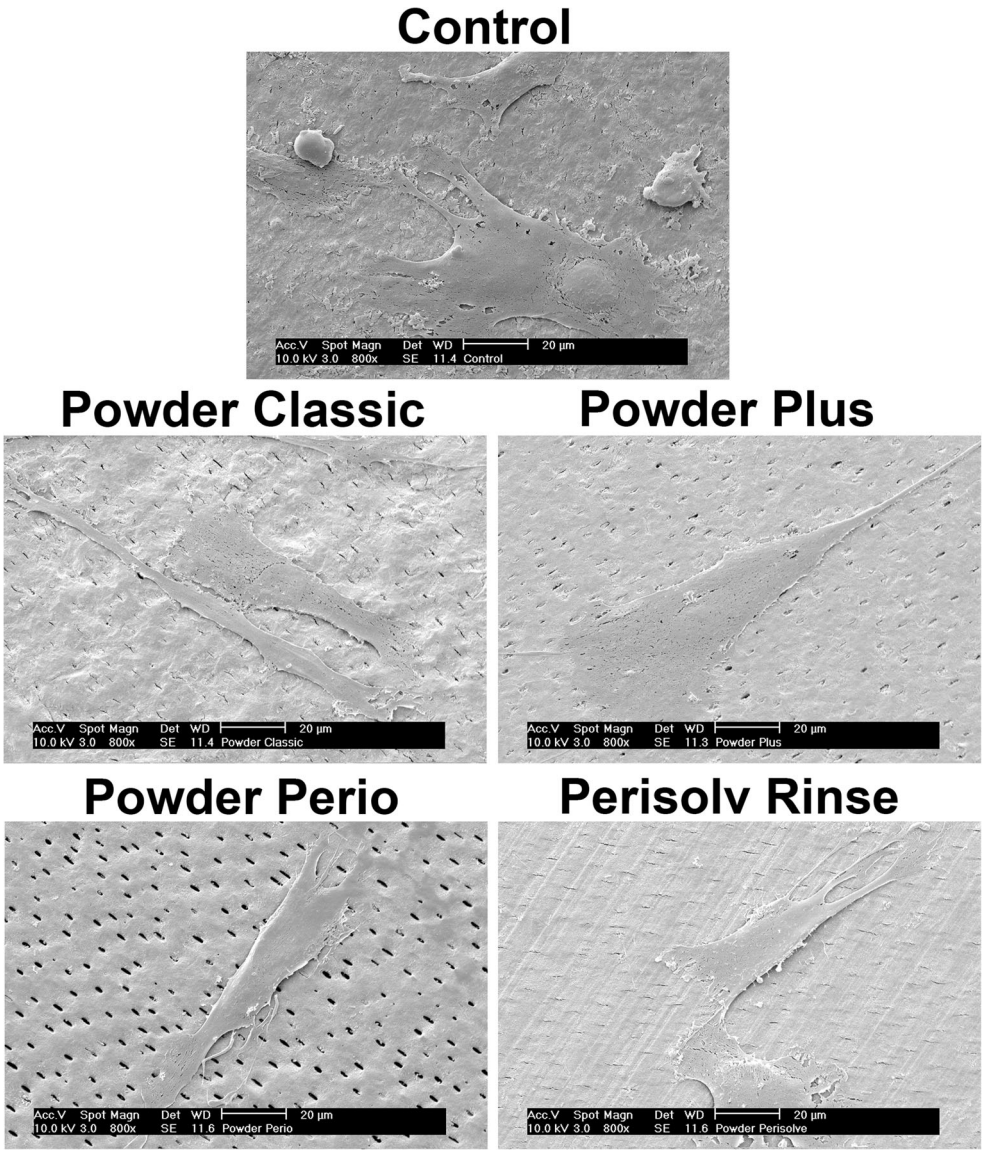

Fig. 8 SEM images of primary human PDL cells seeded on control, Powder Classic, Powder Plus, Powder Perio, and Perisolv® dentin discs. No discernable differences could be observed with respect to cell shape or spreading following surface modifications between groups

Therefore, no harmful side effects have been reported in over its 15-year use for caries removal, and none are to be expected with its use in the treatment of periodontal pockets. However, laboratory or clinical data regarding the latter indication are still scarce. Therefore, this study was justified and the results corroborated some assumptions within the limitations of the present investigation. One other reported limitation of the present study was the time course investigation culturing primary human PDL cells onto dentin surfaces. While we report that all treatment modalities were able to re-establish periodontal cell repopulation, future investigation with longer time points is of interest to further determine the ability for each treatment modality to influence PDL cell proliferation and mineralization. Furthermore, numerous cell types are in contact with dentin/cementum surfaces including gingival fibroblasts and epithelial cells. Future research investigating the various cell types found in contact with dentin and cementum surfaces are needed to evaluate the potential of each air polishing or amino acid buffered hypochlorite solution technique on cell behavior of gingival fibroblasts and epithelial cells.

In summary, the present study demonstrated that cell survival and repopulation of root surfaces is possible following either air polishing or application with Perisolv®. Additional in vitro and animal testing is necessary to further characterize the beneficial effects of either system in clinical setting. Potential side effects when applying these techniques and materials should also be taken into consideration, when it comes to the opening of dentinal tubules and related consequences, especially when treating sensitive areas and patients.

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Compliance with ethical standards

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

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Ethical approval This article does not contain any studies with identifiable human participants or animals. An IRB was therefore not required.

Informed consent For this type of study, signed informed consent was obtained for PDL cell isolation.

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