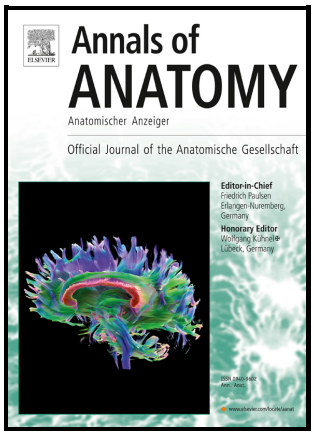

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PII: S0940-9602(22)00064-4

DOI: https://doi.org/10.1016/j.aanat.2022.151949

Reference: AANAT151949

To appear in: *Annals of Anatomy*

Received date: 5 January 2022
Revised date: 10 February 2022
Accepted date: 16 April 2022

Please cite this article as: Jens Weusmann, James Deschner, Jean-Claude Imber, Anna Damanaki, Paulo Sérgio Cerri, Natalia Da Ponte Leguizamón, Svenja Beisel-Memmert and Andressa Vilas Boas Nogueira, Impact of glycine and erythritol/chlorhexidine air-polishing powders on human gingival fibroblasts: an in vitro study, *Annals of Anatomy,* (2022) doi:https://doi.org/10.1016/j.aanat.2022.151949

Impact of glycine and erythritol/chlorhexidine air-polishing powders on human gingival fibroblasts: an in vitro study

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Abstract

Background

Supra- and subgingival air-polishing has been used in periodontitis and gingivitis therapy for years. Low-abrasive types of powders have facilitated the application in subgingival areas. In this study, the cellular effects of a glycine powder and an erythritol/chlorhexidine (CHX) powder on human gingival fibroblasts (HGF) were investigated.

Methods

HGF were obtained from sound gingiva of three healthy donors. After 12 hours and 24 hours of incubation time, cell viability testing and, after 24 hours and 48 hours, a cell proliferation assay was conducted. Additionally, the individual components erythritol and CHX were investigated for cell viability. In vitro wound healing was monitored for 48 hours and scanning electron microscopy (SEM) analysis was performed after 24 hours. Statistical analysis was accomplished by ANOVA and post hoc Dunnett's and Tukey's tests ($p < 0.05$) were performed.

Results

Erythritol/CHX powder and in a lower extent, glycine powder decreased cell viability and cell proliferation. The negative effect of erythritol/CHX was mainly based on the CHX component. In vitro wound healing was negatively influenced in both types of powders compared to control. Cell size was altered in both test groups, whereas cell morphology was affected only in the erythritol/CHX group.

Conclusions

The investigated powders for subgingival air-polishing can influence cell viability, morphology, and proliferation, as well as wound closure in vitro. These actions on fibroblasts are discernible, with the cytotoxic effect of erythritol/CHX powder being very clear and mainly due to the CHX component. Our results suggest that subgingivally applied powders can exert direct effects on gingival fibroblasts.

Keywords:

air polishing – periodontology – cell biology – glycine – erythritol – chlorhexidine

1. Introduction

Periodontitis is a highly prevalent chronic inflammatory disease that has a negative effect on socioeconomic, physical, and psychological factors of the affected individual (Papapanou et al., 2018). The disease is associated with dysbiosis of the oral biofilm and characterized by irreversible destruction of the periodontium structures (Hajishengallis et al., 2020; Joseph and Curtis, 2021). Furthermore, periodontitis may lead to tooth loss if untreated or ineffectively treated.

Periodontal therapy consists mainly of the disintegration of the biofilm on the tooth root surface that is often achieved by subgingival instrumentation with hand instruments or

mechanical devices, i.e. sonic or ultrasonic scalers (Cobb, 2002; Suvan et al., 2020; Walmsley et al., 2008). After subgingival instrumentation in the second step of periodontal therapy and, if necessary, periodontal surgical measures or re-instrumentation in the third step of periodontal therapy, the disease is controlled by regular professional mechanical plaque and calculus removal and guidance on individual oral hygiene measures at home in the so-called supportive periodontal care (SPC) (Sanz et al., 2020). Regular SPC is crucial for the prevention of periodontitis progression and recurrence (Axelsson and Lindhe, 1981; Axelsson et al., 2004). In these cases, repeated instrumentation with hand or sonic/ultrasonic instruments can lead to loss of tooth structure over many years (Flemmig et al., 1998a, b; Flemmig et al., 1997; Zappa et al., 1991).

Subgingival air polishing is able to reliably remove plaque in pockets up to 5 mm (Petersilka, 2011). Due to good patient acceptance and safety, subgingival air polishing is particularly important for use in SPC (Moene et al., 2010). Additionally, subgingival air-polishing is considered a promising method for periimplantitis therapy (Schwarz et al., 2015).

There are various requirements for powders that can be used subgingivally, such as protection of hard and soft tissues and non-cariogenicity as well as plaque removal efficacy. The first powders on the market were based on sodium bicarbonate. However, the relatively large particle size and the angular surface structure made these powders inappropriate for subgingival use, as they may cause tooth wear (Atkinson et al., 1984). Within the last decade, additional low-abrasive air-polishing powders based on glycine and erythritol have become available.

Glycine is a non-essential proteinogenic amino acid that is processed to air-polishing powders of smaller particle size (less than 45 µm) (Petersilka, 2011). In pockets with a depth of up to 5 mm, glycine-based powders showed a good cleaning effect and, compared to sodium bicarbonate, the abrasiveness on the root surface is reduced by about 80% (Petersilka et al., 2003a; Petersilka et al., 2003b; Petersilka et al., 2003c).

Erythritol is a non-cariogenic sugar alcohol that is moderately soluble in water. According to the manufacturer, the particle size of the tested erythritol-based powder is a maximum of 14 µm, which leads to the assumption of low abrasion on root surfaces. This commercially available erythritol-based powder has 0.3% chlorhexidine diacetate (CHX) added at the factory. Clinically, the erythritol/CHX mixture is approved for a wide range of indications and, according to the manufacturer, can be used without hesitation for supra- and subgingival therapy.

Interestingly, data regarding the effect of subgingivally applicable powders on periodontal cells are limited, although these powders come into direct contact with these cells during therapy. Topically applicable substances for the oral cavity are known to exert effects on the gingiva cells (López-García et al., 2021). Therefore, the effects of an erythritol/CHX and a glycine powder on human gingival fibroblasts were examined in this study.

2. Material and methods

2.1 Cell culture

Human gingival fibroblasts (HGF) were obtained from sound keratinized gingiva of individuals who were submitted to wisdom tooth extraction at the University Medical Center Mainz, Germany, after written informed consent was obtained. HGF were cultivated in a humid atmosphere containing 5% CO_2 at 37 °C in Dulbecco's Modified Eagle's Medium (DMEM, Invitrogen, Karlsruhe, Germany) supplemented with 10% fetal bovine serum (FBS, Invitrogen), 100 units of penicillin, and 100 µg/ml streptomycin (Invitrogen). Cells with passage number between 3 and 6 were cultured on different culture plates and grown to 80% confluence. Culture medium was changed every other day. The FBS concentration was decreased to 1% 24 hours before the experiment (Rath-Deschner et al., 2021).

2.2 Cell stimulation

Two different commercially available powders approved for subgingival use in air-polishing devices were used to stimulate HGF. A glycine-based powder (Perio-Mate Powder, NSK Europe, Eschborn, Germany) and an erythritol-based powder with the addition of 0.3% CHX (Air-Flow Plus, EMS Electro Medical Systems, Nyon, Switzerland) were investigated. Both powders were each dissolved in culture medium at a concentration of 1 g/20 ml. This concentration is based on previous research (Sygkounas et al., 2018). In order to be able to investigate the individual components of the powder based on erythritol/CHX, a solution of 30 mg/20 ml was prepared with chlorhexidine diacetate (Sigma Aldrich, Munich, Germany) according to the proportion. Correspondingly, a solution with pure erythritol (Sigma Aldrich) was also prepared at a ratio of 970 mg/20 ml. Unstimulated cells served as control.

2.3 Cell viability

Cell viability was determined by a commercially available assay kit (LIVE/DEAD Viability/Cytotoxicity Kit, Invitrogen) as per the manufacturer's protocol. This assay is based on two fluorescent dyes, calcein AM and ethidium homodimer-1 that measure intracellular esterase activity and plasma membrane integrity. For this assay, HGF were cultivated in 96-well culture plates and grown until 80% confluence as described above. One day before cell stimulation, FBS was reduced to 1%. Then, the cells were exposed to glycine, erythritol/CHX, erythritol, or CHX for 12 hours and 24 hours. Unstimulated cells served as control. Afterwards, the medium with the respective powders was removed and the monolayer cells were rinsed twice with phosphate-buffered saline (PBS, Invitrogen) and incubated with the staining solution of the cell viability kit. After 30 minutes of incubation, the fluorescence intensity was measured with a microplate reader (SynergyHT, Biotek, Winooski, VT, USA).

2.4 Cell proliferation

The Cell Proliferation Reagent WST-1 assay (Roche, Basel, Switzerland) was performed according to the manufacturer's instructions. HGF were seeded in 96-well plates (10,000 cells/well) and grown for 24 hours. Afterwards, the FBS concentration was reduced to 1% for 24 hours and then the cells were stimulated as described above. Unstimulated cells were used as control. After 24 hours and 48 hours, the WST-1 reagent solution was added to each well (10 µl) and incubated for 3 hours at 37 °C and 5% CO_2. Subsequently, the absorbance of the samples was determined at 450 nm with 640 nm as the reference wavelength using a microplate reader (SynergyHT).

2.5 In vitro wound healing and cell migration

In order to evaluate in vitro wound healing upon exposure to glycine and erythritol/CHX, a well-established in vitro wound healing model was used according to previous studies (Memmert et al., 2018; Nokhbehsaim et al., 2011; Weusmann et al., 2021). HGF were cultured to 100% confluence on 35 mm culture dishes. One day after reducing the FBS concentration to 1%, a 3-4 mm wide wound was performed using a sterile 100 µl pipette tip, leading to cell-free areas in the cell monolayers (Weusmann et al., 2021). Then, a number of washing steps with PBS and DMEM were performed to remove all non-adherent cells. After that, the cells were cultured in the presence of glycine or erythritol/CHX for 48 hours. In vitro wound closure was monitored over time using a JuLI™ Br and JuLI™ Br PC software (both NanoEnTek, Seoul, Korea). The open source image analysis system ImageJ 1.53 was used to analyze the images and the percentage of in vitro wound closure area was calculated (Schneider et al., 2012).

Furthermore, the migration of individual cells could be monitored using the JuLI™ Br and JuLI™ Br PC software. The cells that moved the farthest into the cell-free region were marked and traced using the ImageJ software. A total of six cells per group and donor were monitored.

2.6 SEM analysis

HGF were cultured on glass coverslips (Carl Roth, Karlsruhe, Germany) in 24-well plates and stimulated as described above. After 24 hours of stimulation DMEM with or without powder was removed, cells were washed with PBS (Sigma-Aldrich, Munich, Germany) and prepared for SEM analysis by fixation in 4% formaldehyde (Sigma-Aldrich, Munich, Germany) at pH 7.4 and room temperature for 1 h. This fixation step was followed by two washing steps one with PBS and one with distilled water. Afterwards, cells were treated with an ascending ethanol series. Cells were left twice in 20% ethanol for 30 minutes. This was repeated with 40% and 60% ethanol before cells were washed with 80% ethanol once and left in 80% ethanol overnight. Washing and incubation overnight were repeated with 90%, 96%, and 100% ethanol before ethanol was removed and coverslips were left to dry for 48 hours. Dried glass coverslips were mounted on sample stubs and one droplet of conductive carbon cement after Göcke (Plano GmbH, Wetzlar, Germany) was applied at the margin of the samples. Samples were vaporized with argon gas in a Scancoat six® sputter device (Edwards, Crawley, Great Britain) for 40 s. A scanning electron microscope (Philips XL 30, FEI Company, Eindhoven, Netherlands) was used to take detailed photographs of the gingival fibroblasts at magnifications of ×100, ×1,000 and ×2,000 magnifications

2.7 Statistics

The statistical analysis was performed using GraphPad Prism (8.0 software, San Diego, USA). Shapiro-Wilk test was applied for normality. For quantitative analysis, mean values and standard errors of the mean were determined. ANOVA followed by the post-hoc Tukey's test or Kruskal-Wallis followed by the post hoc Dunn's test were used for multiple comparisons. Comparisons between two groups were performed using Mann-Whitney U test. The significance level was 0.05.

3. Results

3.1 Effect of the different powders and their components on cell viability

As shown in Figure 1 (a, b), the rate of live cells was significantly ($p < 0.05$) decreased for cells exposed to glycine or erythritol/CHX compared to the control group after 12 hours and 24 hours. Moreover, the erythritol/CHX-based powder decreased the percentage of live cells after 12 hours and 24 hours significantly ($p < 0.05$) more than the glycine-based powder (Fig. 1a, b). The further comparison of erythritol with and without CHX to the control revealed that the inhibitory effect of erythritol/CHX on the cell viability was only found in the groups with a CHX component (Fig. 1c, d). After 12 hours and 24 hours, the groups erythritol/CHX and CHX groups presented a significant ($p < 0.05$) reduction in the percentage of live cells as compared to the control and erythritol groups after 12 hours and 24 hours (Fig. 1c, d).

3.2 Effect of glycine and erythritol/CHX on cell proliferation

Proliferation of HGF was evaluated in the presence and absence of glycine or erythritol/CHX for 24 hours and 48 hours. Both tested powders induced a significant reduction in cell proliferation after 24 hours and 48 hours (Fig. 2a, b). For HGF exposed to glycine, the percentage of cell proliferation compared to the control group was significantly reduced to 21.13% at 24 hours and 30.30% at 48 hours. Furthermore, the erythritol/CHX-based powder totally inhibited the HGF proliferation (Fig. 2a,b).

3.3 Effect of glycine and erythritol/CHX on in vitro wound healing and cell migration

Figure 3a illustrates the inhibition of in vitro wound closure by both erythritol/CHX- and glycine- based powders. Wounded HGF monolayers treated with glycine or erythritol/CHX ($p < 0.05$) decreased significantly the rate of in vitro wound closure compared to the control

group (Fig. 3b). In fact, in vitro wound healing occurred only in the control group, while in the two test groups no in vitro wound closure was found. (Fig. 3b). The results of glycine and erythritol/CHX over time look largely similar (Fig. 3b). Furthermore, the migration of HGF was evaluated by tracking cells that moved the farthest into the cell-free area during 24 h (Fig. 4a). Glycine- and erythritol/CHX-stimulated HGF showed only minor to no migration, respectively, as compared to the control group (Fig. 4b).

3.4 Effect of glycine and erythritol/CHX on cell morphology

Control group showed HGF distributed on glass coverslip with an elongated profile after 24 h (Fig. 5a). Cytoplasmic projections extended from these fibroblasts towards to neighboring fibroblasts were observed (Fig. 5b). Moreover, these fibroblasts exhibited round or elliptic nuclei occupying the central area of the large cytoplasm (Figs. 5b and 5c). After treatment with glycine, an apparent reduction in the HGF count was noticed (Fig. 5d). However, the fibroblasts had similar morphology to the control group (Figs. 5e and 5f). In contrast, the HGF subjected to erythritol/CHX contained irregular-shaped cells with long and slender cytoplasmic extensions (Fig. 5g). Often, these irregularly shaped cells exhibited flattened nuclei and several filopodia (Fig. 5h). Small structures, probably from a fragmented cell, with varied shapes were found dispersed in the HGF cultures. Sometimes, these structures contained round body with morphological features indistinguishable from that of the nucleus (Fig. 5i).

4. Discussion

The present study provides new evidence that subgingivally applicable powders for air-polishing devices can interfere with cell proliferation, cell viability, and wound closure in vitro. In addition, morphological changes were also observed (Fig. 5). Glycine and

erythritol/CHX powders led to a reduction in cell proliferation and percentage of living cells, with the inhibitory effect of erythritol/CHX powder being attributable to the CHX component. Moreover, both glycine and erythritol/CHX powders caused inhibitory effects on in vitro wound healing. Our in vitro study suggests that the tested powders may influence gingival healing and HGF viability.

According to the cell viability assay, the erythritol/CHX-based powder had the most negative effect on HGF compared to the other two groups at 12 hours and 24 hours. This result is consistent with the findings of a previous study, where erythritol/CHX showed an adverse effect on oral cells after six hours (Sygkounas et al., 2018). However, Sygkounas and co-workers have found that glycine-containing powders demonstrated an - although not significant - superior cell viability at six hours compared to control (Sygkounas et al., 2018). In contrast, our data showed a significant decrease in the cell viability for the glycine group. The different findings between the studies can be explained by the different stimulation periods of time chosen.

As erythritol/CHX powder had a negative effect on cell viability and since it contains CHX in its formula, we decided to test the substances alone or in combination. The strongest inhibitory effect on cell viability was observed for HGF incubated with erythritol/CHX and pure CHX powders, whereas pure erythritol had almost no effect on cell viability. Thus, it can be concluded that the negative influence of the erythritol/CHX powder on cell viability is caused by the addition of the CHX component. Erythritol in pure substance showed the least negative impact on cell viability compared to control.

No in vitro wound healing was observed with the use of glycine- and erythritol/CHX-based powders. This suggests that glycine and erythritol/CHX negatively influence the regenerative capacity of HGF. Our in vitro wound healing experiment also depends on cell migration. As described above, the two powders studied affect cell viability; therefore, the lack of in vitro wound closure in the monolayer model could be explained, at least in part, by powder-

induced cell death. In addition, the powders decreased cell proliferation and cell migration. Therefore, it can be assumed that the lack of closure of the monolayer wound was due to cell death, reduced proliferation, and slowed cell migration. From a clinical point of view, it is highly probable that powder residues remain in the periodontal sulcus and adjacent tissues when using air-polishing devices. This is even more likely to occur in the peri-implant sulcus, since on the one hand, the rinsing effect of the crevicular fluid is missing here, and on the other hand, there is space for powder retention due to the properties of the implant surfaces with their rough structure and the threads in the presence of periimplantitis. Additionally, the implant- abutment junction can be of interest in this regard, since there is a microgap ranging from 0 to 135 µm (Callan et al., 1998; Dellow et al., 1997; Taheri et al., 2020). However, if those powder residues have a negative effect on in vivo wound healing remains unclear.

Interestingly, the SEM analysis showed a change in the morphological aspects of the cells, especially for the erythritol/CHX group. Here, the cells developed an irregular shape with flattened nuclei and several filopodia, whereas cell size was decreased in the glycine group. CHX may alter cell shape and simultaneously lead to growth inhibition (Wyganowska-Swiatkowska et al., 2016). Osteoblasts treated with 0.01% CHX for 1 min displayed signs of necrotic and authophagic cell death as analyzed by transmission electron microscopy (Giannelli et al., 2008). Our SEM analysis showed irregular cell shape in the erythritol/CHX group due to CHX effects, which was similar to the effects reported on mouse fibroblasts, although in this case the concentrations of CHX used were lower than in our study (Faria et al., 2009). CHX-related necrosis and apoptosis in fibroblasts are caused by an overload of the endoplasmatic reticulum (Faria et al., 2009). Detachment and inhibition of HGF growth in vitro can occur on root surfaces after exposure to CHX, even at a minimal concentration of 0.2% (Cline and Layman, 1992).

The inhibition of cell proliferation and the effect on cell morphology by CHX in HGF has already been shown (Mariotti and Rumpf, 1999; Wyganowska-Swiatkowska et al., 2016).

This is in line with the anti-migration and pro-apoptotic effect of CHX on periodontal ligament fibroblasts (Tsourounakis et al., 2013). In addition, there is a proven genotoxic effect of CHX on oral epithelial cells that is related to the duration of use (Khan et al., 2016). Our study did not investigate this genotoxic effect; however, it may have contributed to the observed cytotoxic effect. However, it should not be forgotten that the subgingival area treated with the use of air-polishing powders is inflamed and the tooth surface is colonized by an oral biofilm. CHX inhibits most bacterial species including periodontal pathogenic microorganisms (Stanley et al., 1989).

Notably, glycine affected cell viability, morphology and proliferation clearly less than erythritol/CHX. This concurs with findings about the anti-inflammatory, immunomodulatory, and cytoprotective effects of glycine that have been linked to different intracellular signaling pathways (Zhong et al., 2003).

The powder concentration used in the present study was based on the only study we are aware of that investigated the cellular effects of subgingivally applicable air-polishing powders in vitro, apart from our research group (Sygkounas et al., 2018). This study by Sygkounas et al. examined the effect of five different air-polishing powders on human epithelial cells, human gingival fibroblasts, and periodontal ligament cells. The powders tested in the abovementioned study were one sodium bicarbonate-based powder, two glycine-based powders, one erythritol/CHX-based powder and one glycine/tricalcium phosphate-based powder. Moreover, in the present study, as in the previous publication of our group, an influence of air-polishing powders on cell proliferation and in vitro wound healing was shown (Weusmann et al., 2021).

Our study has some limitations. First, this is an in vitro experiment, and the results have to be interpreted with caution. It remains unclear if the negative impact of the tested air-polishing powders on cells have an impact on clinical results. Additionally, it remains unclear how long the tested agents remain in the gingival sulcus after treatments in vivo. Since the

concentration and stability of the dissolved air-polishing powders in the periodontal and periimplant sulcus is unclear, we had resorted to a concentration that had already been investigated (Sygkounas et al., 2018). It is also questionable how long the dissolved powders remain stable in the sulcus. Moreover, only one cell type was investigated in this study, whereas the powders are in contact with different cell types (e.g. fibroblasts of the periodontal ligament, epithelial cells, osteocytes) during clinical application. Therefore, future studies should also investigate the effect of air-polishing powders on cells and tissues in vivo.

5. Conclusions

Overall, this study showed that subgingivally applicable powders for air-polishing devices can affect cell viability, morphology, and proliferation, as well as wound closure in vitro. The extent to which the negative effects observed in vitro have clinical relevance needs to be investigated in future research. It can be concluded that the effects of subgingivally applied powders on fibroblasts are discernible, with the cytotoxic effect of erythritol/CHX powder being very clear and mainly to CHX. Our results suggest that subgingivally applied powders can exert direct effects on gingival fibroblasts.

Acknowledgements

The authors would like to thank Mrs. Irmel Schneiders, Mrs. Claudia Darmstadt and Mrs. Anna Weber for their valuable support.

Figures

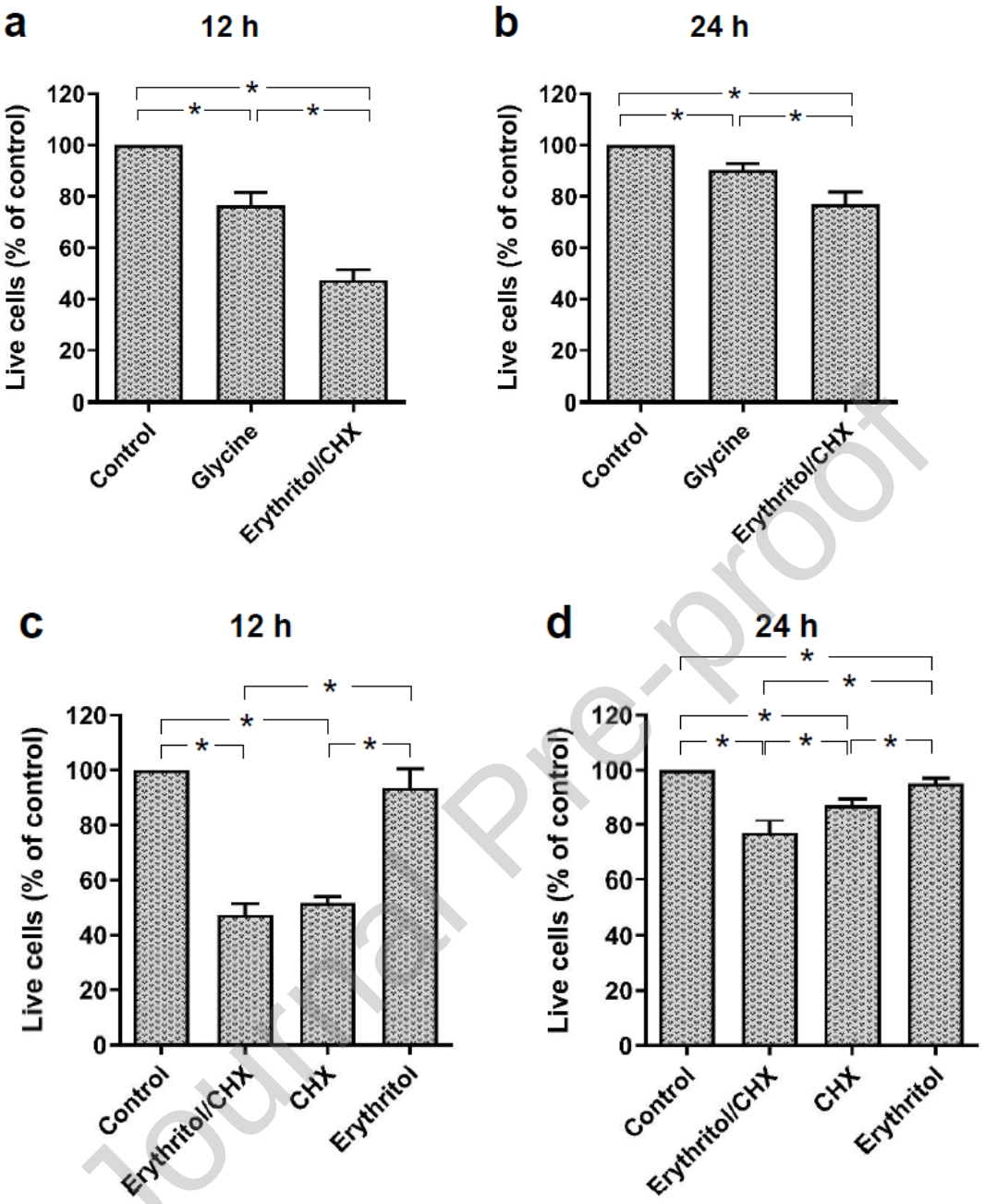


Fig. 1: Live HGF cells in the presence of glycine (1 g/20 ml) and erythritol/CHX (1 g/20 ml) at 12 h (a) and 24 h (b) and in the presence of erythritol/CHX (1 g/20 ml), CHX (30 mg/20 ml) or erythritol (970 mg/20ml) at 12 h (c) and 24 h (d). Unstimulated cells served as control. Mean ± SEM (n=9). *significant ($p<0.05$) difference between groups.

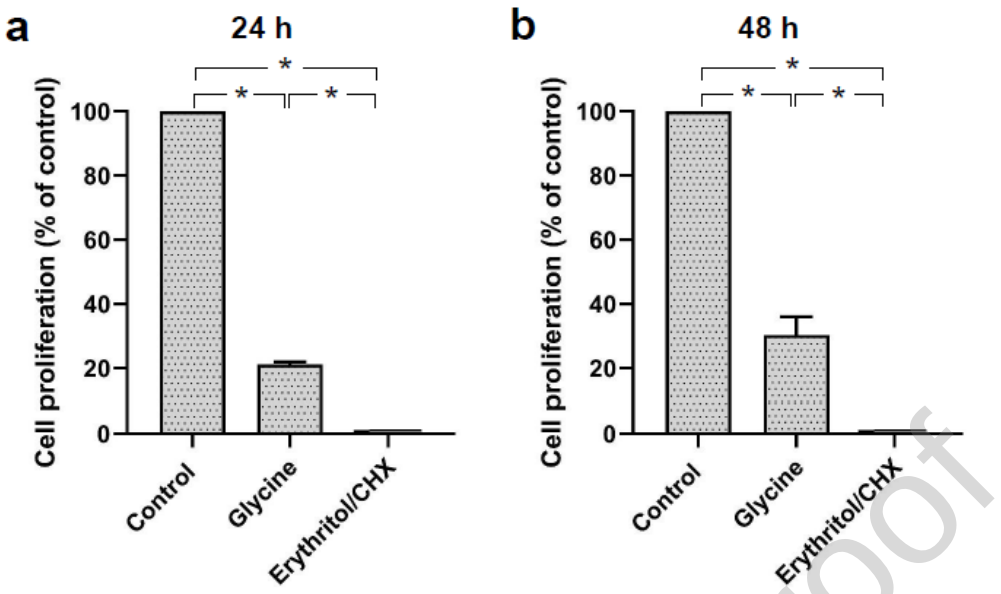
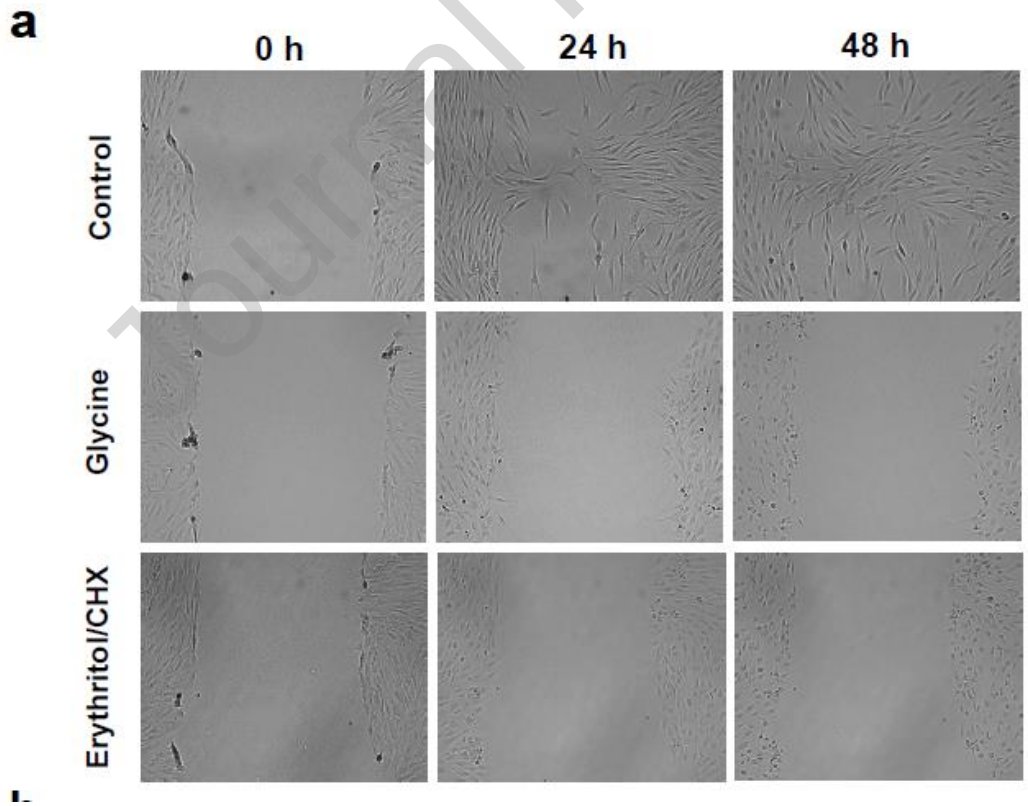


Fig. 2: HGF cell proliferation in the presence of glycine (1 g/20 ml) or erythritol/CHX (1 g/20 ml) at 12 h (a) and 24 h (b). Unstimulated cells served as control. Mean ± SEM (n=6). *significant ($p<0.05$) difference between groups.



b

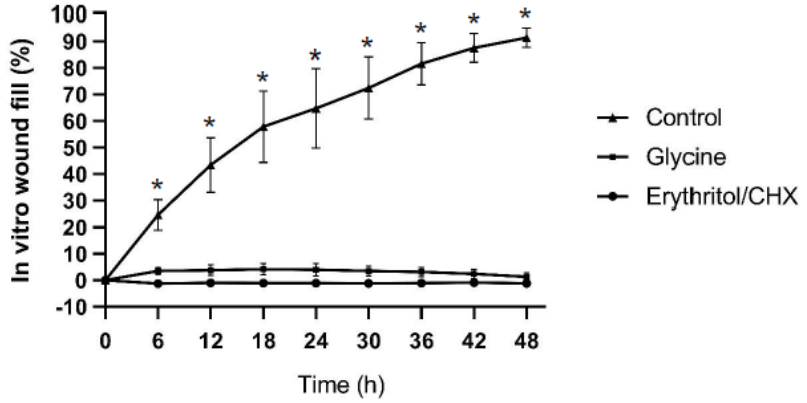
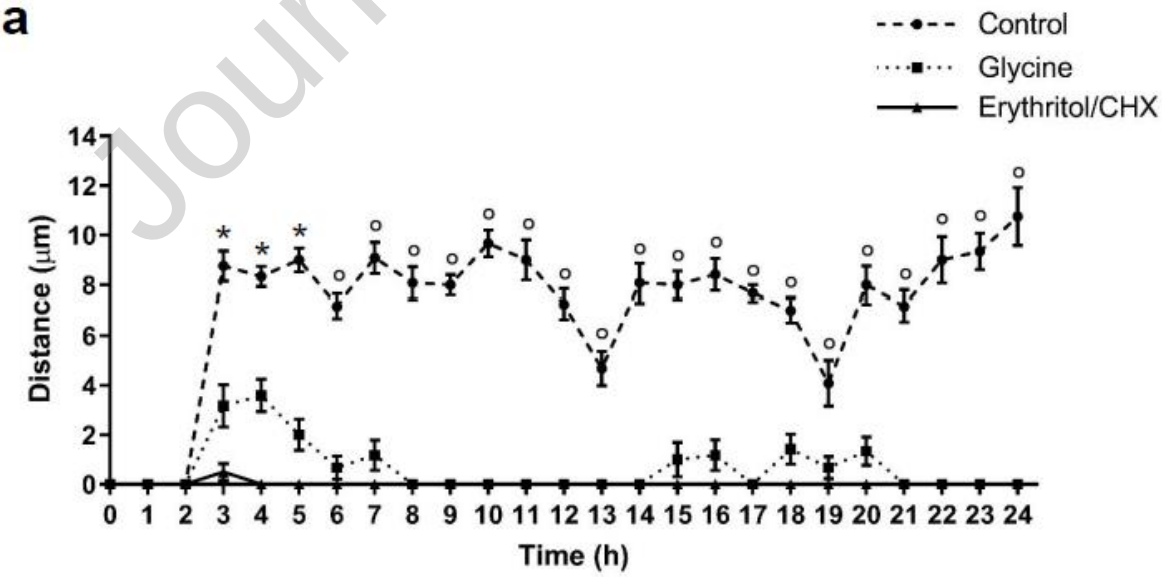


Fig. 3: a) In vitro wound closure of HGF cell monolayers in the presence or absence of glycine (1 g/20 ml) or erythrithol/CHX (1 g/20 ml) at 0 h, 24 h, and 48 h. Images from one representative donor are shown. b) In vitro wound closure of HGF in the presence of glycine (1 g/20 ml) and erythrithol/CHX (1 g/20 ml) over time. The in vitro wound closure, i.e., the percentage of cell coverage of the initially cell-free zones created by wounding, were analyzed by image analysis. Mean ± SEM. *significant ($p<0.05$) difference from all groups.

a



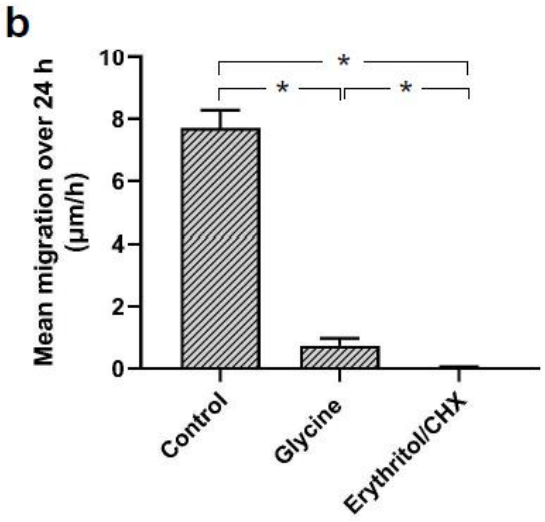


Fig. 4: a) Cell migration distance at different time points in HGF in the presence of glycine (1 g/20 ml) or erythritol/CHX (1 g/20 ml). *significant ($p<0.05$) difference between all groups. °significant ($p<0.05$) difference from glycine and eythritol/CHX b) Mean migration over 24 h in HGF in the presence of glycine (1 g/20 ml) and erythritol/CHX (1 g/20 ml). Mean ± SEM. *significant ($p<0.05$) difference from all groups.

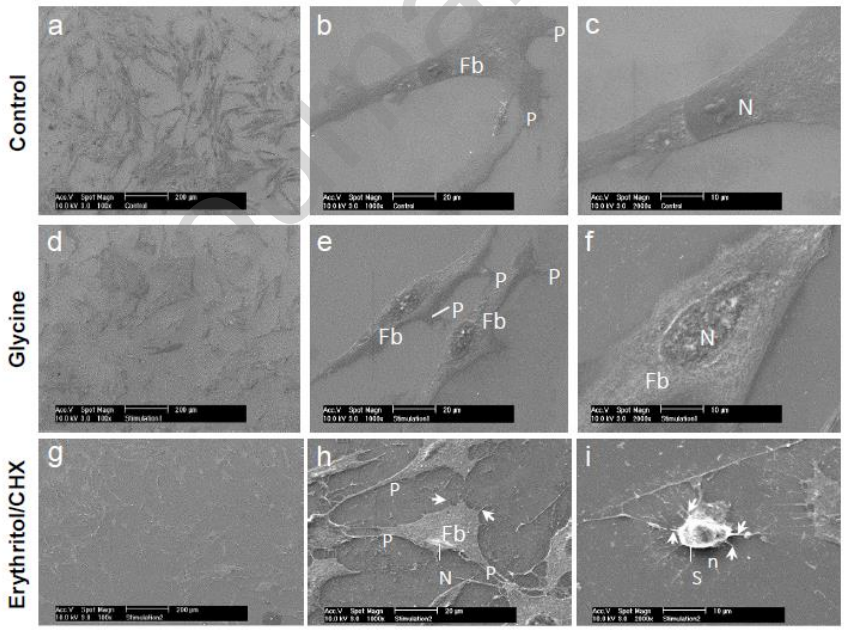


Fig. 5: a-c) – Electron micrographs of cultured human gingival fibroblast cells (HGFs) of control group. A – several fibroblasts arranged randomly cover the glass coverslips. b) An elongated fibroblast (Fb) contains short cytoplasmic projections (P). c) Higher magnification

of Fig. 5b. Rounded nucleus (N) is located in the central area of large cytoplasm with typical morphological features. d-f - Electron micrographs of cultured HGFs subjected to glycine (1 g/20 ml). d) A reduced number of HGFs in comparison with control group (A) is seen. e) Two elongated fibroblasts exhibit short cytoplasmic projections (P) towards each other. f) Higher magnification of a portion of a cell of cultured HGFs. An elliptic nucleus (N) with intact contour inside large cytoplasm of cell (Fb). g-i) Electron micrographs of HGFs subjected to erythritol/CHX (1 g/20 ml). g) Few irregular cells with slender cytoplasmic projections maintain the contact with each other. h) An irregular fibroblast (Fb) with flattened nucleus (N) emits slender cytoplasmic projections towards the neighboring cells; this cell (Fb) exhibits several filopodia (arrows). Note that several cytoplasmic projections (P) are observed in the neighboring cells. i) An irregular structure (S) containing a rounded body with similar aspect to that of a nuclear fragment (N) shows various filopodia (arrows).

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Ethical Statement

Hereby, I, Jens Weusmann, consciously assure that the manuscript "Impact of glycine and erythritol/chlorhexidine air-polishing powders on human gingival fibroblasts: an in vitro study"
has been carried out in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki) for experiments involving humans.

Declaration of interests

☐ The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

☒The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

Dr. Weusmann has given lectures for a powder supplier in the past but sees no competing interests.