Prevention and treatment of peri-implant diseases

Cleaning of titanium dental implant surfaces

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Chapter 5

Influence of various air-abrasive powders on the viability and density of periodontal cells: an *in vitro* study

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Introduction

Air-abrasive treatment uses an abrasive powder brought into a stream of compressed air to clean and polish all kinds of surfaces by removing deposits or smoothing its texture (Moëne et al. 2010). The air-abrasive devices are commonly used during nonsurgical treatment for supra- and subgingival biofilm removal from teeth and implants (Petersilka et al. 2003). These devices have also been used with promising results during periodontal flap surgery as well as during the surgical treatment of peri-implantitis (Horning et al. 1987; Toma et al. 2014). The air-abrasive devices can be used with different powders. Since the 1980’s, sodium bicarbonate has been used and has been proven to be safe and efficient for removing supragingival plaque and stains from intact enamel surfaces (Petersilka 2011). However, sodium bicarbonate can be extremely abrasive to root cementum and dentin and may induce changes on implant surfaces (Petersilka et al. a. 2003; Louropoulou et al. 2012).

To facilitate the removal of biofilm from dental root and implant surfaces whilst minimizing trauma to hard and soft tissues, a less abrasive amino acid glycine powder was introduced (Petersilka et al. 2003). This powder has been shown to induce minimum tooth and implant surface alterations while still removing biofilm efficiently in vitro and in vivo (Louropoulou et al. 2012, 2014). Since the introduction of glycine powders other types of presumably low-abrasive powders began to appear in the market, like powders based on aluminum trioxide or calcium carbonate (Petersilka, 2011). More recently an erythritol-containing powder with chlorhexidine gluconate as preservative (CHX) (0.3%) has also been introduced for use with air-polishing devices (Hägi et al. 2013).

Scarce and small powder remnants have been detected on surfaces after powder treatment in vitro (Schwarz et al. 2009; Tastepe et al. 2013; John et al. 2016). Also, in clinical situations remnants of the powder are expected in peri-implant and periodontal pockets or in the tissues surrounding teeth and implants during surgery. It has been speculated that these fragments have an effect on the biocompatibility of the treated surfaces and may affect biologic responses during healing (Schwarz et al. 2009; Tastepe et al. 2013; John et al. 2016). The aim of the present in vitro study was to investigate the possible effect of five commercially available air-abrasive powders, on the viability and density of three types of cells: epithelial cells (EC), gingival fibroblasts (GF) and periodontal ligament fibroblasts (PDLF).
Materials and Methods

Powders and solutions
In the present study, five commercially available powders, developed for use with a dental air-abrasive system, were evaluated. Table 1 provides an overview of the study products and details regarding main ingredients and particle size. A sodium bicarbonate powder (SBP), two amino acid glycine powders with the same particle size from two different manufacturers (AGP-1 and AGP-2), an amino acid glycine and tricalcium phosphate powder (TCP) and an erythritol powder, in which chlorhexidine gluconate was added as preservative, (ECP) were used.

Suspensions of these powders in three different concentrations were prepared in culture medium: the maximum soluble concentration, the maximum diluted 10-times (1:10) and 100-times (1:100). Details regarding the maximum soluble concentration and pH of this suspension for the different powders can be found in Table 1. The criterion used to define the maximum soluble concentration was the highest degree of powder solubility, beginning from the 3gr/60ml, which is the ratio of powder/water emitted from the nozzle of the air-powder device, as given by the manufacturer.

Cell types
Three cell types were used: epithelial cells from a human buccal epithelial cell line (epithelial cell line -Tr146), human gingival fibroblasts (primary gingival cells- Gin) and human periodontal ligament fibroblasts (primary periodontal ligament cells-PDL).

The two types of fibroblasts were derived from one donor and harvested from an extracted third molar. Informed consent was obtained from the donor. The cells were taken from a site without signs of inflammation and periodontal attachment loss (probing pocket depth ≤3 mm, no bleeding on probing and no loss of attachment). The cell propagation was performed as described by de Vries et al. (2006).

Time point
A pilot study was conducted to evaluate the effect of SBP and AGP-1 powders on the viability and cell density of epithelial cells and gingival fibroblasts, when the cells were cultured in the presence of the powders’ suspensions. Three different time points were tested: two hours, six hours and three days. No effect was observed for any of the powders after two hours, whereas some effect on both cell viability and cell density, as compared to the control,
was observed after six hours and three days of incubation. Based on the results of the pilot study, in the present study the effect of the different powders after six hours of incubation was investigated.

**Culturing**

Cells were cultured in culture medium in 96 well plates with 15,000 cells/well. The culture medium used was DMEM (Thermo Fisher Scientific, Waltham, MA) supplemented with 10% fetal clone serum (HyClone I, Thermo Fisher Scientific) and 1% penicillin-streptomycin (Sigma-Aldrich, St. Louis, MO). After overnight culturing the medium was replaced with the media with or without the different powders and incubated for six hours. The medium without powder served as a control. Four replicates were plated per condition.

**Cell Viability**

The viability was assessed by measuring the mitochondrial activity using an Alamar blue assay (Life Technologies, Carlsbad, CA), according to the manufacture’s protocol.

**Cell density**

After measuring the viability the medium was removed, cells were washed once with PBS and subsequently lysed by adding 100 ul of Cyquant Lysis buffer per well. The amount of DNA, as a measure for cell density was measured using the Cyquant cell proliferation kit (Life Technologies, Carlsbad, CA), according to the manufacture’s protocol. More specifically, the above technique is based on a sensitive nucleic acid stain-based assay for determining numbers of cells in culture, since the cellular nucleic acid content is considered a reasonable indicator of cell number (Jones et al. 2001).

**Statistical analysis**

A software package (SPSS for Windows, 21.0, SPSS Inc., Chicago, MA, USA) was used for the statistical analysis. The experimental groups were considered to be independent. Mean values and standard deviations were calculated for each group. One Way Analysis of Variance (1-Way ANOVA) was applied with Bonferroni’s correction for detecting the significance among the multiple comparisons within and between groups. Results were considered statistically significant at p < 0.05.
Results

In the present study, the effect of five commercially available air-abrasive powders on cell density and viability of epithelial cells (EC), periodontal ligament fibroblasts (PDLF) and gingival fibroblasts (GF) was assessed. Three different suspensions of the powders were prepared. The results for the maximum soluble concentration of the powders are presented in Figures 1-2. Data for the two other dilutions are provided in Figures 3-6.

Sodium bicarbonate powder (SB)
In the maximum concentration, sodium bicarbonate powder resulted in a significant decrease in both cell density and cell viability of all types of cells (Figure 1,2). There was at least a 5-time reduction in the number of cells compared with the control (Figure 1). The viability remained reduced in the other two dilutions (Figure 4, 6). Only in the case of gingival fibroblasts and in the highest dilution of the powder (100-time), differences with the control could no longer be observed (Figure 6). Regarding cell density, the reduction in numbers was less pronounced with the powder 10-time diluted, while no difference compared with control was observed, when the powder was diluted 100-times.

Amino acid glycine powders (AGP-1, AGP-2)
The amino acid glycine powders had different effects on the cells. The AGP-1 powder in the maximum soluble concentration resulted in a statistically significant reduction in the number of all cells (Figure 1) When diluted 10-times, reduced numbers of epithelial cells and PDL fibroblasts were noted. When 100-time diluted, the cell density for all cells was comparable with the control (Figure 3, 5).

The AGP-2 powder at the maximum soluble concentration caused a significant reduction only in the number of PDLF fibroblasts (Figure 1). Further, no effect on the cell density was observed (Figure 1, 3, 5).

Regarding viability, epithelial cells and fibroblasts exhibited different responses. More specifically, both glycine-based powders resulted in a significant reduction in the viability of epithelial cells, irrespective of the concentration of the powder. A reduction in the viability of PDL fibroblasts was noted with the AGP-1 powder, when diluted. Both glycine-based powders had no effect on the viability of gingival fibroblasts, regardless the concentration of the powder (Figure 2, 4, 6).
Amino acid glycine with tricalcium phosphate powder (TCP)
The density of gingival and PDL fibroblasts was not affected, when the amino acid glycine powder with tricalcium phosphate was used. Interestingly enough, and for all concentrations tested, increased numbers of epithelial cells compared with the control were observed (Figure 1, 3, 5). However, the viability of the epithelial cells was significantly reduced, in the maximum soluble concentration and 10-time dilution. No significant effect on the viability of both types of fibroblasts could be observed (Figure 2, 4, 6).

Erythritol powder (ECP)
In the maximum soluble concentration, a significant reduction in both cell number and viability was observed, for all cell types. The viability of epithelial cells and PDL fibroblasts was reduced also when the powder was diluted (Figure 2, 4).

Effect on epithelial cells
All powders and in all concentrations reduced the viability of epithelial cells (Figure 2, 4, 6). The only exception was the TCP powder 100-time diluted. Interestingly enough, increased numbers of epithelial cells were observed. AGP-2 powder had no significant effect in the cell density. Compared to the other two glycine-containing powders (AGP-2, TCP), AGP-1 had a more pronounced effect on the counts of epithelial cells. SB, AGP-1 and EC reduced the numbers of epithelial cells, especially in the highest concentration (Figure 1, 3, 5).

Effect on gingival fibroblasts
The glycine-based powders (AGP-1, AGP-2, TCP) did not have any effect on the viability of gingival fibroblasts, irrespective of the concentration of the powder. The AGP-2 and TCP powders also had no significant effect on the cell density. A decrease in the number of cells was noted with the maximum concentration of the AGP-1 powder. The other two powders (sodium bicarbonate and erythritol) caused a decrease in the numbers and viability of gingival fibroblasts, when used in the highest concentration. This effect could no longer be observed in the other, lower concentrations.
Effect on PDL fibroblasts
Reduction in the viability was observed when the sodium bicarbonate and erythritol powders were used, independent of the concentration. When the glycine-based powders were used, no effect on the viability was observed with the TCP and AGP-2 powders. Reduced viability was noted with the AGP-1 powder diluted. The TCP powder had no effect on the number of fibroblasts. The other powders in the maximum concentration caused a reduction in the number of these cells.

Discussion
The use of air-abrasive devices can lead to residual powder fragments on the treated surfaces and in the surrounding tissues. It has been speculated, especially in the case of implants, that these powder remnants may account, at some level, for changes in the biocompatibility of the implant surfaces and may, therefore, affect the biologic responses. In the present study we investigated the possible influence of five commercially available air-abrasive powders on periodontal tissue cells. Due to their important role in wound healing both epithelial cells and fibroblasts were included. What the concentration is of the powder remaining in the tissues or on the treated surfaces is not known. That is why we used three different suspensions of the powders. The results of the present study indicate that the effect of the different kinds of powders on the various cell types may differ considerably depending on the cell type and the type and concentration of the powder used.

The present study indicates that sodium bicarbonate powder decreases the viability and the number of human gingival fibroblasts. These findings are in accordance with the findings of Shibly and colleagues (2003). In their study it was shown that fibroblasts' counts were reduced after treatment of machined titanium surfaces with a sodium bicarbonate powder. In the present study a significant reduction in the number of gingival fibroblasts was also observed when one of the two tested amino acid glycine based powders (AGP-1) was used. No effect was observed when the AGP-2 powder was used. The AGP-1 and the AGP-2 are both amino acid glycine based powders with a slight difference in their composition (3-4% approximately, according to the information provided by the manufacturers). This small difference in composition, for which the manufacturers provided no details, could be an explanation for the difference observed on the gingival fibroblasts.

It has been shown that cells residing within the periodontal ligament have phenotypic
characteristic of osteoblast-like cells, exhibiting potential osteoblastic activity (Basdra et al. 1997). We observed that sodium bicarbonate powder causes a significant reduction in both cell density and viability of these cells. Also, one of the amino acid glycine powders (AGP-1) reduced the viability of these cells. This is in accordance with the findings of Schwarz and colleagues (2009). These authors assessed the effect of different air-abrasive powders on the viability of osteoblast-like cells (SAOS2) at biologically contaminated titanium implant surfaces. The powders used were a sodium bicarbonate powder and amino acid glycine powders with different particle sizes. One of the glycine powders that they tested was the AGP-1 powder that we used in our study. They observed a reduction in the viability on the SAOS2 cells, which was more pronounced in the case of the amino acid glycine powders. However, another study that assessed the viability of SAOS2 cells after treatment of titanium discs with the same (AGP-1) glycine powder reported similar or increased cell viability compared with the controls after three and six days of incubation respectively (Toma et al. 2016).

To the best of our knowledge, this is the only in vitro study that investigated the possible effect of different air-abrasive powders on epithelial cells. These cells are an important component of the soft tissue seal and are the first cells that come in contact with the powders during non-surgical treatment. According to the results of this study all powders reduce the viability of epithelial cells. The most pronounced reduction was observed with the sodium bicarbonate and erythritol powders especially when respectable amounts of the powder come in contact with the epithelial cells.

Sodium bicarbonate and amino acid glycine powders are commonly used. However, new powders are being developed based on different ingredients such as erythritol or tricalcium phosphate, which are considered to be less abrasive. The erythritol-containing powder in its commercially available form is combined with chlorhexidine gluconate as preservative (CHX) (0.3%). This was the powder used in our study (ECP). An in vitro study evaluating the above combination of erythritol and CHX showed that this combination seems to be a viable alternative to glycine treatment for biofilm removal since it constitutes a combination of an antimicrobial substance (CHX) with an antibiofilm substance (erythritol) (Drago et al. 2014). In the present study we investigated the effect of this powder on three different types of cells. In the maximum soluble concentration a reduced density and viability was observed for all types of cells. To which of the compounds of the powder these results could be attributed is not clear. Erythritol is a four-carbon sugar alcohol and can be found naturally in many organisms, which indicates that it is a byproduct of metabolism of sugar. However, a possible
contribution to the abovementioned negative effect cannot be excluded. Chlorhexidine gluconate is a cationic polybiguanide (bisbiguanide) and it is primarily used as its salts (e.g., the dihydrochloride, diacetate and digluconate) with antiseptic and bacteriostatic properties. There are a number of studies that examined the possible effect of chlorhexidine gluconate (CHX) on various types of cells. Different studies have shown that direct exposure of cells to CHX resulted in inhibition of growth even when CHX was used at very low concentrations (0.0025 to 0.01%) (Helgeland et al. 1971; Cline et al. 1992; Lessa et al. 2010).

Another novel powder that was tested in the present study was TCP, a combination of amino acid glycine and tricalcium phosphate. A rational for using this type of powder is the less abrasive nature of the powder and its possible osteoconductive properties. More specifically, tricalcium phosphate is considered to have excellent biological properties (osteocostruction, osteoinduction), adequate setting time, excellent moldability for surgical applications and the capability to deliver different bone-enhancing proteins (Ambard & Mueninghoff 2006). A recently published study concluded that decontamination with glycine and tricalcium phosphate powder seems to be more efficient than treatment with glycine or sodium bicarbonate alone (John et al. 2016). At the cellular level it has been shown that tricalcium phosphate enhances the cellular performance of osteoblast-like cells, leading to the reconstruction of hard tissues (Oh et al. 2010; Wu et al. 2014). We observed that this powder did not have any effect on the cell density. Interestingly enough, increased numbers of epithelial cells and to a certain extent of PDL fibroblasts were noted. Also no adverse effect in the viability of both gingival and PDL fibroblasts were noted. It has been suggested that if remnants of this powder remain on the surface or in the tissues after treatment this may have a beneficial effect on tissue responses (Tastepe et al. 2013; John et al. 2016). The results of the present study are in support of this supposition.

An important limitation of this study is that only fibroblasts from one donor have been used. Therefore, the results regarding the fibroblasts should be interpreted with caution. This is not the case for epithelial cells, as for these cells an epithelial cell line was used.

In conclusion, different effects were observed on different types of cells. All powders caused a reduction in the viability of the epithelial cells. The most pronounced effect was observed with the sodium bicarbonate and erythritol-containing powders and for the highest concentration. When the glycine powder with tricalcium phosphate was tested with fibroblasts, no adverse effect on both the viability and cell density was observed. Within the limitation of this study, it seems that while some of the powders may adversely affect the
counts and viability of periodontal cells some other powders may have a beneficial effect on the cells. It can thus be speculated that in clinical situations a careful selection of the powder should be done by the clinician, depending on the area that the powder is going to be used, i.e. supragingivally, subgingivally or during flap procedures. The clinical significance of this finding in terms of tissue healing should be the subject of further investigation.

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Declaration of interest
The authors declare that they have no conflict of interest.
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Authors’ contributions:
E. Sygkounas contributed to the design, acquisition, analysis, interpretation of data, drafted the manuscript.
A. Louropoulou contributed to the conception, design, acquisition, analysis, interpretation of data, drafted the manuscript.
T. Schoenmaker contributed to the design, analysis, interpretation of data, critically revised the manuscript for important intellectual content.
T.J. de Vries contributed to the design, analysis, interpretation of data, critically revised the manuscript for important intellectual content.
G.A. van der Weijden contributed to the conception, design, analysis, interpretation of data, critically revised the manuscript for important intellectual content.

All authors gave final approval and agree to be accountable for all aspects of the work in ensuring that questions relating to the accuracy or integrity of any part of the work are appropriately investigated and resolved.
References


Chapter 5
…and density of periodontal cells: an in vitro study


Table 1. Powder characteristics and properties of the suspension with the maximum soluble powder concentration

<table>
<thead>
<tr>
<th>Powder</th>
<th>Abbreviation</th>
<th>Main ingredient(s)</th>
<th>Mean particle size (μm)</th>
<th>Manufacturer</th>
<th>Concentration (mg/ml) ¶</th>
<th>pH*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Air Flow® Classic</td>
<td>SBP</td>
<td>Sodium bicarbonate</td>
<td>65 μm</td>
<td>EMS, Nyon, Switzerland</td>
<td>17</td>
<td>8.3</td>
</tr>
<tr>
<td>Air Flow® Perio</td>
<td>AGP-1</td>
<td>Amino acid glycine</td>
<td>25 μm</td>
<td>EMS, Nyon, Switzerland</td>
<td>50</td>
<td>7.8</td>
</tr>
<tr>
<td>AIR-N-GO® Perio</td>
<td>AGP-2</td>
<td>Amino acid glycine</td>
<td>25 μm</td>
<td>SATELEC SAS, ACTEON group, Bordeaux, France</td>
<td>50</td>
<td>7.7</td>
</tr>
<tr>
<td>Air Flow® Plus</td>
<td>ECP</td>
<td>Erythritol Chlorhexidine gluconate</td>
<td>14 μm</td>
<td>EMS, Nyon, Switzerland</td>
<td>50</td>
<td>8.5</td>
</tr>
<tr>
<td>Clinpro® Prophy Powder</td>
<td>TCP</td>
<td>Amino acid glycine Tricalcium phosphate</td>
<td>25 μm 45 μm</td>
<td>3M ESPE, Bracknell, Berkshire, United Kingdom</td>
<td>5</td>
<td>7.8</td>
</tr>
</tbody>
</table>

¶ maximum soluble powder concentration

* pH of the suspension with the maximum soluble powder concentration
Figure 1. Effect of air-abrasive powders on cell density (maximum soluble concentration)

DNA (ng/ml) was measured after six hours of incubation with the maximum soluble concentration of the air-abrasive powders. Averages +/- SE are shown. The * indicates statistical significance when compared to control (p < 0.05). The † indicates statistical significance when the three glycine-containing powders were compared to each other (p < 0.05).
Figure 2. Effect of air-abrasive powders on cell viability (maximum soluble concentration)

Viability (in arbitrary units) was measured after six hours of incubation with the maximum soluble concentration of the air-abrasive powders. Means +/- SE are shown. The * indicates statistical significance when compared to control (p < 0.05). The ** indicates statistical significance when the three glycine-containing powders were compared to each other (p < 0.05)
Figure 3. Effect of air-abrasive powders on cell density (10-times dilution)

DNA (ng/ml) was measured after six hours of incubation with the maximum soluble concentration of the air-abrasive powders diluted 10 times (1:10). Averages +/- SE are shown. The * indicates statistical significance when compared to control (p< 0.05). The † indicates statistical significance when the three glycine-containing powders were compared to each other (p< 0.05).
Figure 4. Effect of air-abrasive powders on cell viability (10-times dilution)

Viability (in arbitrary units) was measured after six hours of incubation with the maximum soluble concentration of the air-abrasive powders diluted 10 times (1:10). Means +/- SE are shown. The * indicates statistical significance when compared to control (p< 0.05). The † indicates statistical significance when the three glycine-containing powders were compared to each other (p< 0.05).
**Figure 5.** Effect of air-abrasive powders on cell density (100-times dilution)

DNA (ng/ml) was measured after six hours of incubation with the maximum soluble concentration of the air-abrasive powders diluted 100 times (1:100). Averages +/- SE are shown. The * indicates statistical significance when compared to control (p < 0.05). The ** indicates statistical significance when the three glycine-containing powders were compared to each other (p < 0.05).
Figure 6. Effect of air-abrasive powders on cell viability (100-times dilution)

Viability (in arbitrary units) was measured after six hours of incubation with the maximum soluble concentration of the air-abrasive powders diluted 100 times (1:100). Means +/- SE are shown. The * indicates statistical significance when compared to control (p < 0.05). The ▼ indicates statistical significance when the three glycine-containing powders were compared to each other (p < 0.05).