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The evaluation of enamel matrix derivative on subgingival microbial environment in non-surgical periodontal therapy

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Abstract

Introduction and objective: Recent *in vitro* studies indicate that enamel matrix derivative (EMD) could modulate the growth of periodontopathogens. The aim of the presented study was an analysis of the influence of EMD on the presence of *Porphyromonas* and *Prevotella* bacteria in the periodontal pockets of patients with chronic periodontitis in non-surgical periodontal therapy.

Materials and Methods: The studies were conducted on 20 patients. The condition of the periodontium was evaluated by clinical indexes: API. SBI. PD. CAL before and 3 months after the therapy in two selected quadrants. The material was collected for investigation. The periodontopathogens were cultured and identified. Two days after EMD-scaling root planing (SRP) was applied into the pockets.

Results: In the group of patients under investigation before the EMD application the presence of *P. gingivalis* was found in 6 patients and *P. intermedia* in 8 patients. After root planing and EMD application no periodontopathogens were identified in those patients either in the periodontal pockets treated with EMD or in the periodontal pockets free from EMD (control). In the statistical analysis of changes in clinical indexes, the application of SRP and SRP combined with EMD was proved to significantly influence the improvement of the clinical state. However, no significant differences between the individual parameters were found in either group.

Conclusions: The SRP is an effective method of limiting the development of periopathogens in periodontal pockets. The non-surgical therapy with EMD does not change the clinical parameters significantly, compared with the SRP. Simultaneously, the application of EMD inhibits the development of periopathogens, such as *Porphyromonas gingivalis* and *Prevotella*.

Key words

enamel matrix derivative, EMD, periopathogens, *Porphyromonas*, *Prevotella*, scaling root planing

INTRODUCTION

The oral cavity is a specific ecosystem, characterised by the presence of micro-environments with different pH, oxygen partial pressure and reduction-oxidation potential. The microorganisms settling this ecosystem make a complex structure of bacterial colonies attached to the background, which is called biofilm. Due to the polysaccharide matrix, molecular diffusion between bacterial colonies in the biofilm is possible. This ensures its complexity, metabolic cooperation, exchange of genetic information, immunity to phagocytes and neutrophil granulocytes of the host's immune system and to antibiotics and, in consequence, increased pathogenicity and more complicated therapy [1]. The biofilm consists of supragingival and subgingival plaque. After establishing mutual relations they remain environments with completely different profiles. About 700 species of bacteria have been isolated in the oral cavity, whereas about 400 species can be found in the periodontal pocket environment [2]. This is a highly diversified environment [3] in which it is assumed that it is composed of four ecological niches: the dental surface, gingival fluid, the surface of epithelial cells and superficial portion of the pocket epithelium [1, 4]. There is a planktonic biofilm zone between the environment of the biofilm adjacent to the tooth, which is largely a continuation of the supragingival plaque environment, and the biofilm of the inner wall of the pocket which originally contains spirochetes and Gram-negative bacteria: *Porphyromonas gingivalis*, *Treponema denticola*, *Tannerella forsythia* [4, 5]. This creates the likelihood of different responses of individual biofilms to the applied therapeutic methods. To date, studies have proved the fact that the treatment of plaque and tartar removal combined with the root surface preparation brings the best effects in the biofilm elimination [6]. Thanks to the treatment, it is possible to eliminate up to 99% of bacteria immediately afterwards. Simultaneously. the number of periodontally inert microorganisms increases: *Streptococcus, Actinomyces*; the number of spirochetes is reduced; there is considerable reduction in the number of *T. forsythia, P. gingivalis, T. denticola* bacteria, and the pockets become shallow. However. numerous researches point to the fact that none of the applied methods is fully effective in the elimination of all bacteria and subgingival tartar. As the depth of the pockets increases, the percentage of root surfaces free from subgingival plaque and tartar decreases [7]. For the

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pockets not deeper than 3 mm the percentage of surfaces which continue to be covered with residue remains ranges from 4% – 43%, for pockets with a depth of 4–6 mm the percentage ranges from 15% – 38%, whereas for pockets deeper than 6 mm it ranges from 19% – 66%. Cleaning the furcation area without the application of surgical methods does not seem to be effective [8, 9]. The optimal result was observed after obtaining the biocompatible root surface as a result of non-surgical elimination of plaque from the pockets not deeper than 5 mm, and combining it with chemical plaque prophylaxis [7, 10, 11].

The search for an effective chemical maintaining activity in the subgingival pocket environment has been ongoing for many years. Enamel matrix proteins suspended in polyglycolic acid and exhibiting high efficacy against *P. gingivalis* [12] seem to be promising. After the application of EMD gel there is precipitation and aggregation of amelogenin molecules with low molecular weight and strong hydrophobic properties. The aggregates remain stable on the root surface, even after contact with saline [13]. In this way, the EMD layer on the root surface may work as an inhibitor for the pathogens recolonising the pocket, both as a protective protein layer limiting bacterial adhesion and growth, and as a mechanical membrane which is not dissolved in physiological conditions [13, 14]. Furthermore, research on EMD kinetics *in vivo* has shown that the EMD preparation becomes eliminated in polyglycolic acid according to the biphasic pattern. After application, at first the excess of the preparation is quickly eliminated into the oral cavity environment. However, the part remaining on the root surface becomes eliminated during the following two weeks [13]. This barrier to the pathogens recolonising the pocket seems to have positive effect on the healing process.

OBJECTIVE

The aim of the study was an analysis of the influence of enamel matrix derivative (EMD gel) on the presence of *Porphyromonas* and *Prevotella* bacteria in the periodontal pockets of patients with chronic periodontitis in non-surgical periodontal therapy.

MATERIALS AND METHOD

Twenty patients (aged 42.3 years on average) with clinically diagnosed medium or severe chronic periodontitis (PPD ≥ 5 mm and CAL ≥ 3 mm) were qualified for the research. The qualification criteria included the following: no periodontal disease treatment in the last six months, no concomitant general diseases, no antibiotic therapy in the last 3 months, and no smoking. The research was carried out in two upper quadrants with comparable clinical states (at least two pockets with PPD ≥ 6 mm) by means of the WHO probe. API (Approximal Plaque Index), SBI (Sulcus Bleeding Index), PPD (Probing Periodontal Depth) and CAL (Clinical Attachment Level) indexes (Tab. 1) were specified. The examination was performed manually with the WHO 621 Hu-Friedy periodontal probe (scale of up to 11.5 mm). Before the therapy, standardised sterile paper points (size 35 and 40) were used to collect biofilm samples from both quadrants (from each pocket in the quadrant), and scaling and root planing was carried out immediately in the whole oral cavity by means of manual and ultrasound tools, avoiding the use of antiseptics both during the time preceding the Emdogain application and afterwards (FMSRP).

Table 1. Baseline parameters for sites treated with SRP alone (control group) and with SRP and EMD gel.

	Control group	Group with EMD gel
API [%]	63.75±27.86	62.50±26.53
BOP [%]	50.75±31.80	49.25±30.71
PD [mm]	6.95±1.88	7.00±1.81
CAL [mm]	5.30±1.26	5.20±1.40

Two days after the therapy, Emdogain preparation (Straumann, Basel, Switzerland) from Schlamberger (Warsaw. Poland) was applied into the pockets in one quadrant. The application of the drug was preceded by inserting PrefGel preparation into the pockets for two minutes. The preparation was thoroughly washed away. applying subgingival irrigation (Perio Pic) with the use of saline. The patients were instructed about the need to thoroughly remove supragingival plaque with the application of the rules of standard home hygiene, but without the application of any antiseptics.

After three months, there was a follow-up examination comprising evaluation of the clinical parameters and microbiological environment. Sterile paper strips were used to collect specimens from the patients' gingival pockets for microbiological examination. The samples were cultured towards anaerobic bacteria on thioglycollate medium (Oxoid) and on Columbia agar (bioMerieux) enriched with 5% defibrinated sheep blood. The inoculations were incubated at a temperature of 37 °C for seven days before they were discarding as negative, in anaerobic condition with the use of GENbag anaer or GENbox microaer (bioMerieux). The cultured strains were identified by means of API 20A tests in the automatic system ATB Expression (bioMerieux). Clinical parameters were analysed by means of the *Mann–Whitney* and Wilcoxon tests.

RESULTS

In the group of examined patients before the EMD application, the presence of periodontopathogens was found in 14 patients. including *Porphyromonas gingivalis* in six patients and *Prevotella intermedia* in eight patients. After FMSRP and EMD gel application, no periodontopathogens were identified in those patients, neither in the periodontal pockets treated with EMD nor in the periodontal pockets free from EMD (control). Statistical characteristics of the obtained values of API, BOP, PD and CAL in both groups after three months re-evaluation is shown in Table 2.

Table 2. Clinical parameters for sites treated with SRP alone (control group) and with SRP and EMD gel at 3 month re-evaluation.

	Control group	Group with EMD gel
API [%]	18.00±10.18	16.25±10.24
BOP [%]	8.25±7.12	4.50±5.10
PD [mm]	4.30±1.08	4.10±0.97
CAL [mm]	4.00±0.86	3.85±0.93

The comparison of indexes before and after the therapy showed a significant difference both in the control group and the group with EMD (Wilcoxon test) (Tab. 3). The mean PD reduction was 2.90 ± 1.37 in the group with EMD and 2.65 ± 1.42 in the control group. The mean CAL change was 1.35 ± 0.93 and 1.30 ± 0.86 ($p<0.001$), respectively. The reduction of bleeding indexes was 44.75% and 42.5%, respectively, whereas the reduction of the plaque index was 46.25% and 45.75%, respectively.

Table 3. Clinical parameters for sites treated with SRP alone (control group) and with SRP and EMD gel changes.

N=20	Control group [mean ± SD]	Group with EMD gel [mean ± SD]	p
API [%]	63.75 ± 27.86	18.00 ± 10.18	p < 0.001
APIE [%]	62.50 ± 26.53	16.25 ± 10.24	p < 0.001
BOP [%]	50.75 ± 31.8	8.25 ± 7.12	p < 0.001
BOPE [%]	49.25 ± 30.71	4.50 ± 5.1	p < 0.001
CAL [mm]	5.30 ± 1.26	4.00 ± 0.86	p < 0.001
CALE [mm]	5.20 ± 1.4	3.85 ± 0.93	p < 0.001
PD [mm]	6.95 ± 1.88	4.30 ± 1.08	p < 0.001
PDE [mm]	7.00 ± 1.81	4.10 ± 0.97	p < 0.001

The statistical analysis with the *Mann–Whitney* U test proved that there was no significant difference between individual parameters specifying the results of treatment between the control quadrant and the one treated with the application of EMD gel.

DISCUSSION

The research conducted during three months proved that non-surgical therapy with EMD gel does not change the clinical parameters significantly, compared with the SRP. FMSRP was chosen as the method of mechanical plaque elimination in the research [15]. Analysis of the efficacy of non-surgical treatment of periodontal diseases: full mouth disinfection (FMD), scaling and root planing of all teeth conducted during one visit, and not combined with the use of antiseptics (FMSRP) and scaling and root planing conducted during consecutive visits in combination with the use of mild antiseptics (CSD), pointed to the comparable efficacy of FMD and FMSRP in PPD, CAL and BOP (Bleeding On Probing) change, which was higher than CSD. However, only the FMSRP method which assumed no application of antiseptics, showed the influence on the extended time of bacterial flora elimination [15]. Analysis of the clinical parameters in the research confirmed this tendency and the obtained effects were comparable with those presented in the literature [16]. Simultaneously, analysis revealed no statistically significant differences in the improvement of the state of periodontium in combination with the application of Emdogain preparation into periodontal pockets. Earlier, Gutierrez et al. [17] obtained similar results, but they applied a different method. They analysed clinical parameters in two single-rooted teeth with PPD ≥ 5 mm and radiological bone loss ≥ 3 mm in each patient, applying the preparation immediately after treatment, thus excluding the possibility of achieving no contact with blood. In the presented research, the preparation was applied within the quadrant where at least two pockets with PPD ≥ 6 mm were noted two days after the SRP treatment, which gave the chance for no bleeding in the pockets. Simultaneously, it protected the pocket environments from recolonisation with periopathogens from the other ecological niches of the oral cavity, according to the research by Ramberg et al. [18], which proved that after the applied SRP therapy the number of bacteria falls dramatically, but it also immediately returned. However. not all species returned at the same time and in the same number [19].

Microbiological analysis with the culture method also proved SRP to be an effective method of limiting the development of periopathogens in pockets ≥ 5 mm after three months of observation. The application of EMD gel as a local antiseptic did not change the results. Thus far, the research on EMD efficacy in inhibition of the development of periopathogens has been conducted *in vitro*. Sculean et al. [20] researched the influence of EMD on supragingival bacterial plaque. The samples of supragingival plaque collected from 24 patients with chronic periodontitis were connected with NaCl, EMD, Emdogain, propylene glycol alginate (PGA) and 0.2% chlorhexidine (CHX). Bacterial viability was examined by means of a fluorescent microscope. With EMD, 54% of bacteria retained their viability, with Emdogain – 21.4% and with PGA – 19.6%. NaCl and CHX were applied as negative and positive controls, respectively, and produced the results of 76.8% and 32.3% of living bacteria, respectively.

The aim of the research conducted by Spahr et al. [21] was to prove the influence of EMD *in vitro* on the growth of Gram-negative periopathogens, especially *P. gingivalis*. *Aggregatibacter actinomycetemcomitans, P. intermedia* and streptococci. The research conducted on agar medium resulted in a similar, positive influence on the inhibition of the growth of Gram-negative bacteria, both during the application of PGA on its own and in combination with EMD, and no or very little influence on Gram-positive bacteria. Arweiler et al. [22] researched the influence of EMD on supragingival plaque collected from healthy students. Similar to the research by Sculean [20], NaCl, EMD+PGA, PGA and CHX were applied. The antibacterial efficacy of all the substances to NaCl and a similar reaction of EMD+PGA to PGA on its own was proved. Walter et al. [23] and Newmann et al. [24] in their research obtained similar results, suggesting the activity of polyglycolic acid. Simultaneously, they proved the absence of reaction of amelogenin fractions to *P. gingivalis* and even its effect stimulating *P. gingivalis* growth [24].

The presented *in vivo* research proved that in comparison with the SRP the application of Emdogain as an antiseptic aiding non-surgical treatment of periodontal diseases did not have significant influence on the subgingival plaque environment. It is likely that Emdogain activity is also inhibited by the components of the gingival fluid [25] and by microorganisms themselves, especially *Porphyromonas gingivalis* [26]. Studies on coaggregation helped distinguish characteristic groups of bacteria, i.e. complexes [27]. The number of bacteria in those complexes clearly increases along with the increase in the depth of the pocket [3]. In the presented research conducted with the culture method, *Porphyromonas* bacteria were isolated in the patients with PPD pockets ≥ 5 mm, whereas *Prevotella* was observed in

the patients whose PPD pockets were ≥ 7 mm in depth. The dominance of *P. gingivalis* and *P. intermedia* correlates with higher depth of periodontal pockets and the bleeding, and also with higher likelihood of loss of attachment [28, 29]. As a result of scaling and root planning, the total number of microorganisms in periodontal pockets is reduced and bacterial dominance changes from Gram-negative to Gram-positive bacteria [9]. The number of red complex bacteria (*P. gingivalis*, *T. denticola*, *T. forsythia*) is significantly reduced. However, these treatments do not lead to complete eradication of periodontopathogens from the pockets, all the more so because some of them are capable of penetrating both cement and soft tissue [26, 30]. Simultaneously, the research by Valm et al. [31] on 15 different bacterial strains proved their interesting correlations in the process of biofilm formation. Cells of the genera *Prevotella* and *Actinomyces* showed the most interspecies associations, suggesting the central role of these genera in establishing and maintaining biofilm complexity. Besides, *Prevotella intermedia* includes a wide range of virulence factors, such as haemolytic activity. On the other hand, in studies *Porphyromonas gingivalis* exhibited the presence of virulence factors (mainly protease).

At present, it is also known that clinical isobaths both from the oral cavity and from other focuses are resistant to antibiotics [32], which considerably limits their application. It has also been proved that the activation of acquired resistance by *Porphyromonas gingivalis* reduces bone changes and resorption [33]. Numerous studies on the virulence of periopathogens confirm the fact that the periodontal disease is not caused by one species of bacteria, but is usually caused by complexes of bacterial genera.

The results of microbiological research with the application of the PCR technique point to the possibility of invasion of periodontopathogens into the cells of the oral cavity epithelium, which makes them inaccessible to eradication as a result of mechanical action [34]. The studies by Sbordonte et al. [35] indicate that if there is no control of supragingival plaque, recolonisation of the pocket with periodontopathogens (*P. gingivalis*. *P. intermedia* and *F. nucleatum*) takes place within two months after the end of non-surgical therapy. The presented study also confirms the fact that the methods of conservative treatment of chronic periodontitis may result in favourable microbiological changes and significant and permanent improvement of clinical parameters on condition that supragingival plaque is under constant control.

CONCLUSION

The SRP is an effective method of limiting the development of periopathogens in pockets ≥ 5 mm. Non-surgical therapy with EMD does not change the clinical parameters significantly, compared with the SRP. Simultaneously, application of EMD inhibits the development of periopathogens, such as *Porphyromonas gingivalis* and *Prevotella*.

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