MECHANICAL THERAPY EFFECT IN CHRONIC PERIODONTITIS

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Summary
Chronic periodontal diseases are bacterial infections affecting the periodontium resulting in the loss of tooth support and are associated with bacteremia, inflammation, strong immune response and important tissue destroy. The aim of this study was to determine the effect of scaling and root planing (SRP) on gingival mucosa from generalized chronic periodontitis (GCP) patients. The evaluation was performed by light microscopy (LM) and transmission electron microscopy (TEM). The results were compared to those from untreated GCP controls. GCP subjects important epithelium and subepitelial lesions, PMN infiltrates and bacteria were visualized. After SRP gingival epithelium regenerated, leukocyte infiltrate decreased, and no bacteria was in the biopsy tissue. Conclusion: These findings conclusively reveal the possible use of SRP as a treatment tool for generalized chronic periodontitis patients.

Keywords: periodontitis, light microscopy, TEM, SRP

Introduction
The pathogenesis of periodontitis involves the interplay of microbiota present in the subgingival plaque and the host responses. Although periodontal disease is initiated and maintained by a pathogenic oral flora, an excessive host response is thought to contribute significantly to periodontal tissue destruction (Signat, et al., 2002; Sakka and Coulthard, 2010).

Inflammation and destruction of periodontal tissues are considered to result from the response of a susceptible host to a microbial biofilm containing gram-negative pathogens (Signat, et al., 2002; Han, et al., 2000).

There are two major types of destructive periodontal disease currently recognized. The first is chronic periodontitis and the second is aggressive periodontitis (Arabac, 2010). Since treatment approach varies for these both forms, differential diagnosis is mandatory, and must be based on specific information about the patient’s medical history, comprehensive clinical examination as well as, if necessary, on the analysis of various tests. To make an accurate periodontal diagnosis, gingival inflammation and the extent of periodontal tissue destruction should be determined (Bloom, 1975; Stawinska, 2009).

According to the latest treatment guidelines, periodontal health should be achieved in the least invasive and most cost-effective manner. Often this is accomplished with scaling and root planing (SRP) and adjunctive therapy such as local delivery antimicrobials and host modulation. The treatment of cavities, inadequate fillings, broken fillings, crowding of teeth, rotated or tilting teeth are also a component of non-surgical therapy. These patients are then maintained with proper home care and periodic professional maintenance cleanings (Stawinska, 2009; Park, 2010).

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When disease does recur, despite frequent recall, it can usually be attributed to lack of sufficient plaque control or to other risk factors that influence host response. However, to date no treatment available can completely control or eliminate the pathogenic plaques associated with periodontal diseases for extended periods of time. Daily home care and frequent re-evaluation and careful monitoring are still paramount for long-term success (Drisko, 2001).

Nonsurgical therapy remains the cornerstone of periodontal treatment, but it does have its limitations. When it does not achieve periodontal health, surgery may be indicated to restore periodontal anatomy and facilitate oral hygiene practices (Keremy, 2009).

The purpose of this study was to evaluate by structural and ultrastructural examinations the utility of SRP at generalized chronic periodontitis (GCP) diagnosed patients.

### Material and methods

#### Patients and study design

At the initial examination, the test group members were selected after a clinical and radiographic examination. The test group included 12 patients (6 male, 6 female) diagnosed with GCP in the Department of Periodontology, University of Medicine and Pharmacy Iuliu Hatieganu, Cluj Napoca, Romania from January to December 2010. A diagnosis of GCP was performed according to the criteria established in 1999 (Armitage, 1999). The patients diagnosed with GCP had more than two areas with a 5 mm or more probing depth (PD) in the quadrant and bone loss > 30% of the root length on the radiographs. The test groups had more than 20 functional teeth.

The periodontal status of potential study participants was examined by a single periodontist at recruitment (Table 1). A physical examination and structured interview was performed by a certified physician to assess eligibility (table 2).

#### Table 1: Clinical data (mean±SD)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>GCP group</th>
<th>GCP after SRP</th>
</tr>
</thead>
<tbody>
<tr>
<td>n (males/females)</td>
<td>12 (6/6)</td>
<td>12 (6/6)</td>
</tr>
<tr>
<td>Age</td>
<td>56.2±5</td>
<td>45.3±2.2</td>
</tr>
<tr>
<td>Probing depth - PD (mm)</td>
<td>3.5±1.02</td>
<td>3±0.8</td>
</tr>
<tr>
<td>Clinical attachment level – CAL (mm)</td>
<td>4.4±1.2</td>
<td>3.2±1.5</td>
</tr>
<tr>
<td>Plaque index – PI</td>
<td>40.2±6.8</td>
<td>28±1.9</td>
</tr>
<tr>
<td>Bleeding index - BI</td>
<td>56.3±14.7</td>
<td>19.8±8.4</td>
</tr>
</tbody>
</table>

#### Table 2: Inclusion and exclusion criteria

**Inclusion criteria**
- Male or female
- 25 years of age or older
- Three or more periodontal pockets with a probing depth (PD) > 5 mm
- Have at least 16 natural teeth excluding third molars
- Provide informed consent and willingness to cooperate with the study protocol

**Exclusion Criteria**
- History of antibiotic use in the previous three months
- Pregnant or lactating females
- Treatment with antihypertensive, antilipemic, antiarrhythmic, and other cardiovascular drugs
- Systemic diseases such as diabetes, HIV/AIDS, liver disease, chronic renal failure, tuberculosis, and autoimmune diseases
- Previous history of cardiovascular disease: Acute myocardial infarct, stable angina, unstable angina, heart failure, atrial fibrillation, AV blockade, peripheral vascular disease, and cerebrovascular accident
- Patients who received periodontal treatment within the last 6 months
- Patients who require antibiotic prophylaxis before examination or treatment
- Patients with some mental disability
Eligible subjects for the present study were invited to the second visit for gingival mucosa samples collection. One month later, scaling and root planing (SRP) with ultrasonic devices and curettes, four quadrants in one session, was performed. One month after mechanical periodontal treatment subjects were invited to the following visit for gingival mucosa samples collection.

The protocol was approved by the Ethics Committee of University of Medicine and Pharmacy, Iuliu Hatieganu Cluj-Napoca. All patients gave informed consent to participate.

Collection of gingival tissues

Biopsy specimens of gingival mucosa were harvested from healthy subjects with normal mucosa (controls) and GCP patients. The biopsies were taken under local anesthesia (1% Lidocain).

Light and electron microscopy

Immediately after excision, all specimens were transferred to a 2.7% glutaraldehyde solution in phosphate buffered saline (PBS)0.1M, pH 7.2 for 90min at 4°C for pre-fixation. Than they were washed in four successive baths with PBS 0.15M pH 7.2 for 4h at 4°C. Biopsies were post-fixed with 2 % osmic acid in PBS 0.15M pH 7.2 for 75 min at 4°C. Dehydration was performed in a graded acetone series (50%, 70%, 80%, 90%, absolute) at room temperature, 30min in each bath. The samples were infiltrated and embedded with Epon 812. Sections were cut on a Leica UC 6 ultramicrotome (DDK diamond wheel (Craciun and Horobin, 1989; Hayat, 2000; Toader,1996;).

Semi-thin sections of 200-400 nm thick were stained with Epoxy tissue stain for light microscopy (LM) and orientation purposes. For light microscopy were used an Olympus BX 51 microscope, a CCD Media Cibernetics camera, and Image Pro Plus software (Kuo, 2007; Kay, 1967).

Ultra-thin sections of 20-40 nm were cut and stained with uranyl acetate and lead citrate and examined on a JEOL JEM 1010 transmission electron microscope (Japan Electron Optical, Ltd., Tokyo, Japan). For TEM were used a Megaview III camera and Soft Imaging Analysis Analysis software (Pavelka and Roth, 2005; Ploaie and Petre, 1979).

Results and discussions

Gingival epithelium is a stratified squamous epithelium surrounding the tooth and forming an attachment to the tooth surface. It functions as a protective barrier against pathogenic microorganisms in dental plaque. Epithelial cells are seen as providing not only a physical barrier to infection but playing an active role in innate host defense, because they are in constant contact with bacteria or bacterial products from supra- and subgingival biofilms on the tooth surface. They respond to bacteria in an interactive way, by producing antimicrobial peptides, as chemokines that attract monocytes and neutrophils, cytokines that activate the adaptive immune system (Signat, 2002; Arabac, et al., 2010).

The gingival mucosa must resist to mechanical stimuli during mastication as well as having large accumulations of plaque bacteria in direct contact with the gingival sulcus. Gingival tissue must form a seal in the sulcus that protects the underlying tissue from subgingival plaque.

LM examination of the gingival biopsy from GCP subjects showed important lesions that involve all layers, as previously described (Matthews, 2010; Usineviciu, et all., 1981).

TEM of gingival mucosa from chronic periodontal patients showed important degenerative changes. Stratum corneum was thinner, and in some regions detached, desmosomes partially destroyed. In the granular and spinous layers the distances between the cells increased due to the injured desmosomes. The basal lamina had lost its continuity, being partially destroyed. Connective tissue had fragmentized collagen fibers, epithelial cells migrated and leukocytes were infiltrated into it.
Bacterial colonization and subsequent bacterial invasion into the gingival sulcus are the primary etiologic factors for periodontal diseases. The interactions between microorganisms and host response play a key role in the disease’s pathogenesis. The immune and inflammatory responses to the chronic presence of microorganisms result in the destruction of structural components in the periodontium. Both, the host and the bacteria in the periodontal biofilm release proteolytic enzymes that cause tissue damage. Consequently, examining the gingival tissues after SRP treatment it may be appreciated if this method is efficient for GCP (Drisco, 2001; Arabac, 2010).

Before any treatment, GCP subjects bacteria were observed between the degenerated gingival structures. At the SRP patients stratum corneum is present but patchy. It is thicker than before the treatment but it is thinner than in healthy controls (fig.1) Because it is partially regenerated, desmosomes are rare, predisposing to cell detachment (fig.2).

The spinous and granulated layer almost all cells have normal morphology regarding nucleus, cytoplasm, and tomofibrils. Desmosomes are nor fully regenerated and intercellular spaces are still larger (fig.3).

At GCP patients the cells from the basal layer were partially injured, hemidesmosomes destroyed, allowing the epithelial cells to migrate into the connective tissue. In the basal layer of the GCP patients treated with SRP, cells are fully recovered. This is a proof that the regenerative process is ongoing (fig.4) (Matthews, 2010).

All the changes from the gingival epithelium show that it started to regenerate at 30 days after SRP treatment, as previously mentioned (Matthews, 2010).

The connective tissue leukocyte infiltrate was significantly reduced or disappeared (fig.7).

Comparing to the connective tissue of the GCP patient before SRP treatment, it is clear that collagen fibers are not as fragmentized anymore, and the leukocytes infiltrate decreased significantly (fig.8) (Jung, 2010; Kim, et al, 2010).

At the GCP patients without treatment bacteria could be observed in the interstitial tissue of the epithelium and in the connective tissue. After SRP treatment bacteria were not found anymore (Jung, 2010; Drisko, 2001)

Conclusions
These findings conclusively reveal the possible use of SRP as a treatment tool for generalized chronic periodontitis patients. Mechanical non-surgical therapy in GCP patients is suggested to be an effective approach to enhance the periodontal health.

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References


**Figure 1:** Stratum corneum at SRP patients is present but patchy.

**Figure 2:** Stratum corneum at SRP patients is partially regenerated, desmosomes are rare, predisposing to cell detachment.
Figure 3: Spinous and granulated layer almost all cells have normal morphology at SRP patients.

Figure 4: In the basal layer of the GCP patients treated with SRP, cells are fully recovered.

Figure 5: At SRP subjects connective tissue has a mosaic structure, with normal and altered areas.

Figure 6: At SRP subjects, degranulated or destroyed mast cells can be seen.

Figure 7: After SRP treatment collagen fibers of chorion are not so fragmentized anymore.

Figure 8: At SRP subjects, leukocyte infiltrate decreased.