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# **PHD THESIS**

**„LOW-COST” ANTIMICROBIALS USED AS ADJUNCTIVES  
IN STEP 2 OF PERIODONTAL THERAPY FOR PATIENTS WITH  
STAGE III-IV PERIODONTITIS: SAFETY  
EVALUATION, TREATMENT PROTOCOLS AND BIOFILM  
REMOVAL EFFICIENCY.**

## **A B S T R A C T**

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## INTRODUCTION

Periodontitis is a widespread and multifactorial inflammatory disease characterized by the progressive destruction of tooth-supporting structures, often leading to tooth loss if left untreated. It involves complex interactions among active herpesviruses, specific bacterial pathogens, and destructive immune responses. Traditional periodontal therapy, which primarily involves mechanical debridement, often proves inadequate in managing advanced stages of the disease. Periodontitis remains a significant global health issue, particularly in low-income populations where the disease is prevalent and often untreated. This underscores the urgent need for effective adjunctive antimicrobial treatments that are both affordable and accessible in these resource-limited settings. The European Federation of Periodontology (EFP) guidelines emphasize that managing stages I-III periodontitis involves effective biofilm control and the judicious use of adjunctive antimicrobials, but many of recommended adjunctive means come with additional costs. Slots & Jorgensen's research team have addressed these concerns over the years 2002-2020, providing evidence supporting low-cost solutions that maintain high standards of periodontal care.

The research direction of the Timisoara University Clinic of Periodontology is marked by a strong commitment to advancing treatment protocols and diagnostic methods for periodontal and peri-implant diseases. This clinic has been continuously exploring innovative and cost-effective therapies, as evidenced by a series of rigorous studies aimed at enhancing traditional treatment regimens for severe periodontitis and peri-implant mucositis.

The research thesis aims to contribute with results of fundamental and clinical research in support of affordable and effective adjunctive antimicrobials for steps 2 of periodontal therapy, to further optimize existing, but so far never applied in clinical trials. The general motivation for our research arises from the critical need to develop affordable, effective, and accessible periodontal treatments, particularly for low-income populations where periodontal disease is prevalent and often improperly treated. Previous studies have highlighted the use of sodium hypochlorite (NaOCl), well-known for its potent antimicrobial properties, broad-spectrum activity, and cost-effectiveness, making it a viable candidate for adjunctive in periodontal treatment. As patient self-care remains a critical part of maintaining periodontal health, oral rinsing with 0.10-0.25% NaOCl has been recommended as a valuable adjunct to conventional anti-plaque, anti-gingivitis treatments and home self-periodontal care, therefore *in vitro* safety evaluation of sodium hypochlorite was of interest in the context of our research. A

second research included in the special part is a single-blind randomized clinical trial comparing the clinical and microbiological efficacy of a low-cost protocol integrating antivirals, low-cost potent local antimicrobials povidone-iodine (PVP-I) and NaOCl and systemic antibiotics, to enhance the clinical outcomes of subgingival instrumentation in step 2 of periodontal therapy. Although recommended by Slots et al. in various versions over the years, this complex and highly effective low-cost protocol has not been tested so far in a controlled clinical study. The actual mechanism of action and the effectiveness of biofilm-disrupting and removing substances, currently widely used in periodontal therapy under various commercial formulations, is well known. Despite its long-standing use in endodontic therapy, NaOCl displays still insufficient research chairside data on its effectiveness. To address the existing gap, a pilot longitudinal study has been designed, aiming to assess the biofilm disruptive properties of a 0.2% NaOCl solution by using 3D scanning and disclosure agents in patients undergoing Guided Biofilm Therapy® (GBT) protocol. The doctoral research scientific objectives were to evaluate the *in vitro* safety of NaOCl home-care rinsing solutions at concentrations  $\geq 0.5\%$  (below the limits of patient's tolerance), compare the clinical and microbiological efficacy of a low-cost protocol using povidone-iodine, sodium hypochlorite, antivirals, and antibiotics with chlorhexidine in step 2 of periodontal therapy in stage IV periodontitis patients, and investigate the reductive effect of the 0.2% NaOCl rinsing solution on plaque covered areas in a pilot longitudinal study.

The thesis begins with an introduction that outlines the scope and significance of the research on periodontal disease and its cost-related challenges. In the first chapter of the general part, the focus is on understanding periodontal disease and the financial burdens associated with its treatment. This section outlines the prevalence of periodontal disease and the economic challenges it poses to both patients and healthcare systems. The second chapter describes the critical role of specific bacteria and viruses in the development and progression of periodontal disease and includes following sections: the identification and significance of keystone bacteria that contribute to periodontal disease, exploring their interactions and impact on oral health; keystone bacteria influence on the immune response in periodontal disease and the mechanisms by which they alter host immunity and contribute to disease pathology; the role of viruses in periodontal disease, including how they interact with bacteria and the host to exacerbate the condition. The chapter concludes with a summary of the findings on keystone bacteria and viruses, providing clinical insights and potential implications for treatment strategies. The third chapter outlines the recommended treatment

sequence for stages I-IV of periodontitis, based on the European Federation of Periodontology (EFP) S3 guidelines; explores advanced methodologies in antimicrobial therapy for periodontal disease, highlighting the complexities and innovations in treatment approaches, reviews the evolution of cost-effective antimicrobial protocols. The final section focuses on self-management strategies for patients, emphasizing the importance of cost-effective approaches in the ongoing care and management of periodontal disease.

The special part begins with an *in vitro* safety evaluation of NaOCl at concentrations deemed safe and acceptable for patients with periodontitis, using both 2D and 3D models to assess the cytotoxicity of NaOCl on human skin keratinocytes (HaCaT) and human gingival fibroblasts (HGF). The second part of the research is a single-blind randomized clinical trial comparing the clinical and microbiological efficacy of a low-cost protocol using NaOCl, povidone-iodine, antibiotics, and antivirals as adjuncts to non-surgical therapy for patients with stage IV periodontitis, opposed to the standard use of chlorhexidine. The third part of the research is a pilot longitudinal study designed to assess the full-mouth dental plaque-covered area reduction using a 3D intraoral scanner after rinsing with 0.2% sodium hypochlorite. The work concludes with a summary of findings, personal contributions, and a bibliography.

Main results and conclusions of the doctoral research are:

- a. For the fundamental study, an *in silico* evaluation predicted that NaOCl is free from mutagenic, tumorigenic, irritant, and reproductive toxicity and has acceptable drug-like properties. The *in vitro* experiment used 2D and 3D models to assess the cytotoxicity of NaOCl on human skin keratinocytes (HaCaT) and human gingival fibroblasts (HGF). HaCaT and HGF cells were exposed to NaOCl at concentrations ranging from 0.05% to 0.5% for 10, 30, and 60 seconds. The most significant cytotoxic effect was observed in HaCaT cells after 60 seconds at 0.5%. However, in a 3D reconstructed human epidermis model (EpiDerm), NaOCl at 0.05% and 0.25% showed no irritative potential.
- b. In the clinical trial, 45 patients with stage IV periodontitis were randomly assigned to either a control group (subgingival instrumentation with chlorhexidine) or a test group (subgingival instrumentation with povidone-iodine, NaOCl rinsing solution, antibiotics, and antivirals). Clinical and microbiological assessments were conducted at baseline and after 3 months. The test group showed significant reductions in the detection of key periodontal pathogens, including *Porphyromonas gingivalis* ( $p =$

0.021), *Tannerella forsythia*, and *Treponema denticola* ( $p < 0.0001$ ). Additionally, the test group experienced significant improvements in pocket probing depth ( $p = 0.0005$ ) and bleeding on probing ( $p < 0.0001$ ), though changes in clinical attachment loss and full-mouth plaque score were not statistically significant. The proposed protocol demonstrated substantial clinical and microbiological benefits compared to current antimicrobial recommendations.

- c. For the pilot longitudinal study, 8 subjects, aged 26-70 were involved. Clinical procedures included the Guided Biofilm Therapy® (GBT) protocol implementation, supplemented with 0.2% NaOCl solutions rinsing for 30 seconds, followed by 3D scans of dental arches using the Medit i700 intraoral scanner. The study found significant reductions in plaque-covered areas after rinsing with 0.2% NaOCl. The mean stained area for the upper arch decreased by 39.651%, and for the lower arch by 38.26%. Overall, the full mouth mean stained area decreased by 38.955%. Statistical analysis showed that the mean percent reductions for both upper and lower jaws were significantly higher than the clinically significant threshold of 15% ( $p=0.003$  for upper jaw,  $p=0.027$  for lower jaw). These findings suggest that 0.2% NaOCl is an effective biofilm disruptor when integrated into periodontal therapy protocols.

## PERSONAL CONTRIBUTIONS AND ORIGINALITY OF THE DOCTORAL RESEARCH

This doctoral research has made several original contributions to the field of periodontal therapy. Firstly, it provided valuable data on the cytotoxicity and safety of sodium hypochlorite (NaOCl), confirming its safe application at lower concentrations (0.2%) as an adjunctive treatment in periodontal therapy. These findings represent the first in-vitro results addressing the potential irritation effects of NaOCl in 3D-reconstructed epidermis. Additionally, the research tested a complex, low-cost clinical protocol for periodontal therapy. This protocol involved the use of potent, low-cost antimicrobials for irrigation and home-care rinsing, combined with systemic antibiotics and antivirals as an adjunct to subgingival instrumentation in the second step of periodontal therapy and provided patient-centered data regarding the tolerance and acceptability of 0.2% NaOCl rinsing solutions. Moreover, this research was the first to utilize 3D planimetric analysis to measure full-mouth plaque reduction following the use of a 0.2% NaOCl rinsing solution as part of a modified Guided Biofilm Therapy (GBT) protocol. Unlike previous studies that focus on *in vitro* experiments, this study applied its findings in a clinical setting on human subjects, providing robust evidence of the efficacy of NaOCl in

reducing biofilm areas on complete dental arches. In conclusion, the doctoral research fully achieved its scientific objectives, providing significant original contributions to the field of periodontal therapy.