

TITLE: INHIBITION OF BIOFILM FORMATION: AN IN VITRO STUDY ON DISINFECTION OF TITANIUM SURFACES

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ABSTRACT:

Modifications on surfaces of dental implants are performed in order to optimize osseointegration. However, this fact may favor bacterial colonization considered an important factor for the development of peri-implantitis. Alternative therapies with natural extracts such as Terpinen-4-ol that have antimicrobial properties open new perspectives to control and reduce microbial load. This study evaluated the efficacy of Terpinen-4-ol, for the decontamination of sandblasted titanium surfaces previously infected with *Porphyromonas gingivalis* (*Pg*) and *Fusobacterium nucleatum* (*Fn*) biofilms. The broth microdilution method was used to determine the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (CMB) of Terpinen-4-ol. Biofilm developed in microtiter plate was evaluated by the Tetrazolium Salt (XTT) assay. *P. gingivalis* and *F. nucleatum* biofilms were grown on titanium discs and then descontaminated for 5 minutes with Terpinen-4-ol (0.94% and 0.24%) and Chlorhexidine (CHX 0.2%). The decontamination effect was quantified in colony forming units (CFU mL⁻¹) and evaluated by Confocal Scanning Laser Microscopy (CLSM). The fibroblast cells were cultured and exposed to Terpinen-4-ol (0.94% and 0.24%) for 5 minutes, 1 and 24 hours. The colorimetric MTT (methyltetrazolium) assay was applied to assess their effects on the viability cells. The results were compared using the Kruskal Wallis and Mann Whitney test and ANOVA and Tukey HSD tests, with a significance level of 5%. The lowest concentration capable of inhibiting bacterial growth (MIC) were Terpinen-4-ol 0.06% for *Pg* and *Fn*. CMB values were similar to MIC. The XTT assay showed antimicrobial activity at Terpinen-4-ol 0.06% for *Pg* and 0.24% for *Fn*. It was observed through CFU mL⁻¹ and CLSM monospecies and multispecies antibiofilm activity on titanium disks using Terpinen-4-ol 0.94% and 0.24% respectively. The cytotoxic evaluation (MTT) of Terpinen-4-ol 0.94% on fibroblast cell culture showed results statistically similar to CHX 0.2%. However, Terpinen-4-ol 0.24% after 5 minutes showed higher cell viability when compared with CHX 0.2%. Terpinen-4-ol showed to be an efficient alternative therapy in reducing the biofilms formed on sandblasted titanium surfaces with *P. gingivalis* and *F. nucleatum* and does not present cytotoxicity. This natural compound represent an alternative agent to disinfection of titanium surfaces.

Keywords: antimicrobial activity; terpinen-4-ol; biofilm; periodontogenic bacteria

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