9. ABSTRACT

Optimization of Er:YAG Laser Parameters for Microbiological Control of Erosive and Ulcerative Lesions *In Vitro*

Background. Erosive and ulcerative oral mucosal lesions, particularly in the course of oral lichen planus (OLP), are characterised by a chronic course and susceptibility to secondary microbial colonisation. Polymicrobial biofilm, including *Candida* spp., perpetuates local inflammation and delays epithelial healing. Long-term topical corticosteroid therapy, the mainstay of OLP management, further promotes fungal overgrowth. The erbium:yttrium-aluminium-garnet laser (Er:YAG, λ = 2940 nm) demonstrates high water absorption and superficial tissue interaction, offering potential for selective microbial decontamination. Optimal exposure parameters balancing antimicrobial efficacy with biological safety have not been systematically defined.

Aim. To evaluate the biological and antimicrobial effects of Er:YAG laser irradiation *in vitro* in the context of microbiological control of erosive and ulcerative oral mucosal lesions.

Material and Methods. The dissertation comprises two PRISMA 2020-compliant systematic reviews registered in PROSPERO and two original *in vitro* experimental studies. Study 1 assessed dose-response relationships across 30–400 mJ against six ATCC reference strains (*Escherichia coli*, *Staphylococcus aureus* MSSA and MRSA, *Enterococcus faecalis*, *Pseudomonas aeruginosa*, *Candida albicans*) in planktonic and mature agar biofilm model, including a comparison of two laser tip types (PS600T vs. C600F). Study 2 evaluated the cytotoxicity of 80, 130, and 180 mJ on human foreskin fibroblasts (HFF-1) using time-lapse proliferation imaging, Annexin V/Ethidium Homodimer III assay, and immunofluorescent detection of phosphorylated histone H2AX (γH2AX). Statistical analysis employed two-way ANOVA with Dunnett's and Tukey's post hoc tests ($p < 0.05$).

Results. Both systematic reviews confirmed the antimicrobial activity of Er:YAG laser and its clinical utility in OLP, while indicating substantial parameter heterogeneity and low-to-moderate certainty of evidence. Study 1 demonstrated an energy-dependent increase in growth inhibition zones ($p < 0.001$), with optimal effect at 180–230 mJ, and

reduction of viable biofilm coverage to 10–15% for *E. coli* and *C. albicans* at 230 mJ; *E. faecalis* and *MRSA* showed relative resistance (25–30% residual coverage). Both laser tips produced similar dose-effect profiles, with PS600T yielding marginally wider inhibition zones. Study 2 showed that 80–130 mJ did not significantly affect fibroblast proliferation, apoptosis, necrosis, or γH2AX expression, whereas 180 mJ caused significant proliferation inhibition, elevated apoptotic and necrotic cell fractions, and an approximately twofold increase in γH2AX-positive cells ($p < 0.001$).

Conclusions. The energy range of 80–130 mJ constitutes an optimal therapeutic window providing effective microbial decontamination while preserving host tissue viability. An energy of 180 mJ exceeds the biological safety threshold. These findings provide a scientific rationale for future clinical trials evaluating Er:YAG laser as an adjunctive modality in erosive oral mucosal disease, pending validation in *in vivo* models.

Key words: Lasers; Biofilms; Candidiasis, Oral; Lichen Planus, Oral; Mouth Mucosa; Fibroblasts