

TITLE: THE OZONE EFFECT AGAINST MIXED BIOFILMS OF *Candida* species AND *Streptococcus mutans*

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ABSTRACT:

Purpose: This study explored the bacteria-fungal interactions in the mixed biofilms formation as well as the effect of ozonized oil on the same biofilms. **Methods:** The microorganisms *Candida albicans* ATCC 90028, *C. glabrata* ATCC 2001, *C. krusei* ATCC 6258, *C. orthopsilosis* ATCC 96141, *C. parapsilosis* ATCC 22019, *C. tropicalis* ATCC 13803 and *Streptococcus mutans* ATCC 25175 were used. Biofilms were developed from cell suspensions (10^6 cells/mL and 10^8 cells/mL, for yeast and bacteria, respectively) in a 96-well microtiter plate for 24 h at 37°C and treated with ozonized oil for 5 to 120 minutes. Cell viability was determined by the agar plating methodology. **Results:** *Candida* species benefited from the association with *S. mutans* since the count of colony forming units per milliliter (CFU/mL) was significantly higher ($p < 0.0001$) compared to monospecies biofilms. *S. mutans* benefited only in association with *C. krusei* and *C. orthopsilosis* ($p < 0.001$), where there was an increase in CFU/mL counts. Mixed biofilms exposure to ozonized oil during 120 minutes resulted in a reduction of approximately 11 Log CFU/mL and 13.43 Log CFU/mL of *Candida* spp. and *S. mutans*, respectively, showing remarkable fungicidal and bactericidal activity of the ozonized oil. **Conclusion:** The ability to form biofilms hinders the access of drugs and immune defenses to infection sites. However, ozone therapy, inserted in the Unified Health System through the National Policy on Integrative and Complementary Practices, can be explored as a strategy to control diseases associated with biofilms formed by *Candida* spp. and *S. mutans*.

Keywords: *Candida* spp., *Streptococcus mutans*, biofilm, ozone, complementary medicine

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