eDNA release from anaerobic-grown, oral polymicrobial biofilms made by *Aggregatibacter actinomycetemcomitans, Porphyromonas gingivalis* and *Candida albicans*

Santiago M. Lattar[1*], Rene Peter Schneider[2], Gabriel Padilla Maldonado[3] and Vidal Jorge Eugenio[4]

[1] Laboratory of Cellular Biology of Microorganisms , Department of Microbiology, Institute of Biological Science, Federal University of Minas Gerais, Belo Horizonte, BH.
[2] Polytechnic School, University of São Paulo,São Paulo, SP, Brazil.
[3]Bioproducts Laboratory, Department of Microbiology, Institute of Biomedical Science, University of São Paulo, São Paulo, SP
[4] Department of Microbiology and Immunology, University of Mississippi Medical Center, Jackson MS, USA.

Background: Extracellular DNA (eDNA) is a major component of the biofilm matrix. *A. actinomycetemcomitans* requires eDNA for the formation of a cohesive biofilm while *Porphyromonas gingivalis* biofilms contain large amounts of eDNA. *Candida albicans* eDNA also plays a crucial role in the formation of biofilms. In this study we analyzed the dynamics of eDNA release by monospecies and polymicrobial biofilms grown under anaerobic conditions, and studied the synergistic effect of DNase I and ciprofloxacin to disaggregate oral biofilms made by these three species.
Materials and Methods: *A. actinomycetemcomitans* JP2, *Porphyromonas gingivalis* ATCC 33277 and *C. albicans* ATCC 10231 were utilized. We first created a life-like substrate by incubating sterile saliva for 3 h at 37°C in 6-well plates. Saliva was then discarded and plates added with BHI containing hemin and menadione. Microorganism were inoculated at $2x10^6$ cfu/ml and treated, or not, with DNase I (2 U/µl) and/or Ciprofloxacin (100 ng/ml) as indicated. Experiments were incubate for 72 h at 37°C, under anaerobic conditions. Biofilm counts (cfu/ml) were obtained and eDNA was quantified by qPCR. The ultrastructure was analyzed by confocal microscopy.
Results: *P. gingivalis* significantly released more eDNA than *A. actinomycetemcomitans* and *C. albicans* in polymicrobial and monospecies biofilms. *A. actinomycetemcomitans* produced significantly more eDNA than *C. albicans*. A combined treatment with DNaseI and ciprofloxacin significantly reduced the eDNA and cell density from polymicrobial and monospecies biofilms compared to an individual treatment. Reduction of eDNA in polymicrobial biofilms of *P. gingivalis, A. actinomycetemcomitans* and *C. albicans* correlated with a decreased in the density of *P. gingivalis* and *A. actinomycetemcomitans* but not that of *C. albicans*. Accordingly, a decrease of eDNA in monospecies biofilms of *P. gingivalis, A. actinomycetemcomitans* and *C. albicans* correlated with a decreased density of *P. gingivalis* and *A. actinomycetemcomitans* but not that of *C. albicans*.
Conclusions: In monospecies and polymicrobial biofilms produced under anaerobic conditions *P. gingivalis* and *A. actinomycetemcomitans* (bacteria) release more eDNA than *C. albicans* (yeast). Degradation of the eDNA matrix with DNase I may provide a novel therapeutic strategy to destabilize biofilm growth and improves ciprofloxacin sensitivity causing the reduction of the density of biofilms.