ABSTRACT

Dental caries is recognized as an oral disease caused by four main factors, namely the host, substrate, bacteria, and time. *Streptococcus mutans* and *Candida albicans* are identified as two microorganisms involved in the development of caries. Saliva is also considered to play an important role in the caries process. Hyposalivation, defined as a reduction in saliva production, is known to increase the susceptibility to caries. One approach used to manage hyposalivation is the administration of artificial saliva, in which the calcium content functions in the remineralization process to help prevent dental caries. This study was conducted to determine the effect of calcium ion supplementation in artificial saliva on the biofilm density of *Streptococcus mutans* and *Candida albicans* co-cultures.

The research was carried out using an in vitro laboratory experimental method. Four treatment groups with a total of 24 samples were assigned, consisting of a negative control group and groups supplemented with 0.3 mmol/L, 0.6 mmol/L, and 1.2 mmol/L calcium ions. Each group was provided with artificial saliva containing calcium concentrations corresponding to the assigned treatment. The co-culture biofilms were incubated for 48 hours at 37°C. After incubation, the biofilms were stained with 0.1% crystal violet for 15 minutes, rinsed with PBS, and washed with 95% ethanol. Biofilm density was then measured using a microplate reader at 540 nm. Data were analyzed using one-way ANOVA followed by an LSD post-hoc test.

The results indicated that significant differences ($p<0,05$) were observed among the negative control group and all calcium-supplemented groups. It was concluded that the addition of calcium ions increased the biofilm density of *Streptococcus mutans* and *Candida albicans* co-cultures.

Keywords: Calcium ions, Biofilm, Artificial saliva, *Streptococcus mutans*, *Candida albicans*, Co-culture.