Differentiating fluoride reservoirs in dental biofilm: methodology development
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Abstract
Fluoride is found in dental biofilm bound to bacteria cells or as precipitated minerals. The aim of this study was to test the dissolution rate of CaF₂ in Fluoride or Calcium rich solutions. The results show that in 10 washes, CaF₂ solubilizes partially in Ca or F-rich solutions.

Key words: Dental biofilm, fluoride, calcium.

Introduction
Fluoride (F) is found in dental biofilm bound to bacteria cells or as precipitated minerals (e.g. calcium fluoride, CaF₂), and determining the amount of F in each pool is relevant and challenging. Aiming to develop a method to differentiate these reservoirs in F-rich biofilm samples, in this preliminary study we tested the dissolution rate of CaF₂ in calcium (Ca) or F-rich solutions.

Results and Discussion
CaF₂ was precipitated in microcentrifuge tubes (n=18) from supersaturated 0.05 M PIPES buffer, pH 7.0, containing 10 mM F and 10 mM Ca. After centrifugation, supernatants were collected to determine Ca and F concentration. The CaF₂ precipitated (ppt) in the tubes (n=6/group) was: I – saved as control (no further extraction), II – extracted with PIPES buffer containing 5.0 mM Ca (mean concentration found in the supernatants) or III – extracted with PIPES buffer containing 0.88 mM F (mean concentration found in the supernatants). Extractions consisted of five 30-s washes under vortex with 2 mL of the treatment solutions at 37ºC, followed by five 1-min washes. Between each wash, tubes were centrifuged and the supernatant collected to check F (in group II) or Ca (in group III) release, which were, respectively, 0.29±0.02 and 0.14±0.01 mM, indicating CaF₂ dissolution. After the 10 washes, CaF₂ remaining in the tubes were dissolved with 0.5 M HCl for 3 h at room temperature to determine the amount (mg) of CaF₂ not extracted by the washes made. The amount (mg) of CaF₂ not extracted in groups II (0.18±0.04) and III (0.13±0.03) differed (p<0.01, ANOVA and Tukey test) from that found in group I (0.38±0.01).

Conclusions
The results show that in 10 washes, CaF₂ dissolves partially, 52 and 66% in solutions containing saturating concentrations of either Ca or F, respectively.

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