

Methods

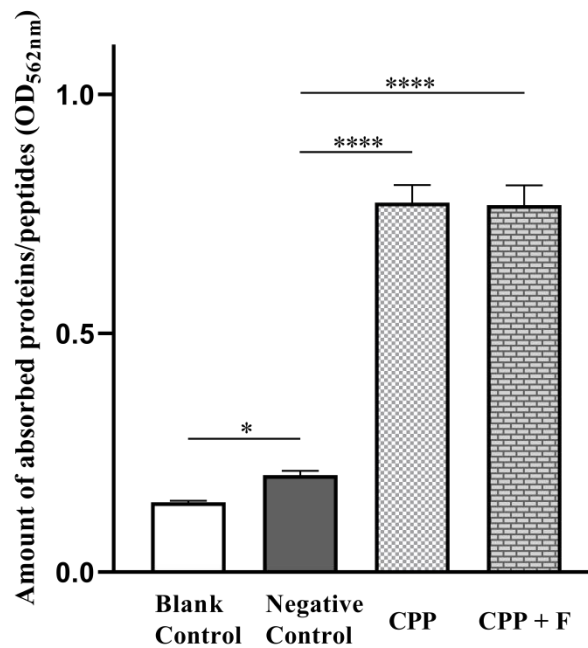
The amount of proteins/peptides adsorbed onto the HA disc before and after pellicle formation and modification was measured according to a previously described method [1]. Twelve HA discs were individually placed into the wells of 24-well plates and randomly divided into four groups (n = 3 per group): blank control, negative control, CPP, and CPP + F groups. The native HA disc (n = 3) incubated with a volume of 1 mL of PBS was used as the blank control group. In the negative control, CPP, and CPP + F groups, s-HA discs were performed as described in the main manuscript. After rinsing twice with sterile PBS, the s-HA discs were incubated with 1 mL of PBS (negative control), 2.5% CPP (CPP) or 2.5% CPP supplemented with 900 ppm fluoride (CPP + F) for 2 h at 37 °C. After rinsing twice with PBS, all the HA discs were transferred to a 48-well plate individually, and treated with 200 µL of micro-bicinchoninic acid (Micro BCA™ Protein Assay Kit, Thermo Scientific, USA) to react with the adsorbed proteins/peptides at 37 °C for 30 min. The optical density of the supernatant at 562 nm (OD_{562nm}) was measured using a micro-plate reader (Epoch; BioTek Instruments, VT, USA). Experiments were performed three times in triplicate.

Results

Pellicle formation and modification by CPP and fluoride-doped CPP

As shown in the Supplementary Figure below, the amount of adsorbed proteins/peptides was significantly higher in the negative control (0.20 ± 0.01 , $p < 0.05$), CPP (0.77 ± 0.04 , $p < 0.0001$) and CPP + F (0.77 ± 0.04 , $p < 0.0001$) groups

than in the blank control group (0.15 ± 0.00). Both CPP and CPP + F groups showed significantly higher proteins/peptides amounts than the negative control group ($p < 0.0001$), whereas there was no significant difference between the CPP and CPP + F groups.



Supplementary Figure

* $p < 0.05$, **** $p < 0.0001$.

References:

1. Kwon JS, Lee MJ, Kim JY, Kim D, Ryu JH, Jang S, Kim KM, Hwang CJ, Choi SH. Novel anti-biofouling light-curable fluoride varnish containing 2-methacryloyloxyethyl phosphorylcholine to prevent enamel demineralization. *Sci Rep.* 2019;9:1432.