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CBD-Supplemented Polishing Powder Enhances Tooth Polishing by Inhibiting Dental Plaque Bacteria

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Abstract

Introduction

Dental health problems including dental plaque are common health problems affecting people of different age groups globally. Air-polishing is a safe tooth polishing technique used by dental professionals for stain and plaque removal and as preventive procedure for dental health. Here we report the technical improvisation of existing air-polishing technique by supplementing cannabinoid powder into the classic polishing powder for effective removal of supragingival and subgingival plaque and inhibition of plaque forming bacteria.

Methods

The cannabidiol (CBD) powder was added to the tooth polishing powder (AIR-N-GO, classic) at 1% (wt/wt) ratio. The study was conducted on 12 patients, of which 6 received regular polishing treatment and 6 received CBD-supplemented polishing treatment. The dental plaque samples were collected before and after each treatment and subjected to *in vitro* microbiological analysis and the colony forming units (CFU) were analyzed using automated colony counter.

Results

Based on *in vitro* microbiological analysis, the average CFU of interdental space samples collected from post-CBD-supplemented polishing treatment was significantly reduced (linear fold change between 3.9-18.4) compared to that of post-regular polishing (linear fold change between 1.0-2.6) treatment.

Conclusions

CBD-supplemented polishing powder can help in effective removal and killing of dental plaque bacteria during the polishing treatment. CBD powder can be added as enhancing supplement to the existing polishing powders.

Keywords: cannabinoid, CBD, dental care, dental plaque, tooth polish, polishing powder

Introduction

Dental health problems including dental plaque and staining are common oral health problems affecting people of various age groups globally. Dental plaque is a complex biofilm made of multiple species of bacteria and an etiologic agent of dental caries and periodontal diseases (Rosan and Lamont 2000). Dental health is influenced by various factors including diet and lifestyle (Scardina and Messina 2012; Hasselkvist et al. 2014). Alcohols such as red wine and

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soft drinks such as tea, cola and coffee can cause tooth staining and erosion (Karadas and Seven 2014; Grocock 2018; Manno et al. 2018). Apart from food related tooth staining, smoking causes severe tooth staining (Alkhatib et al. 2005). Tooth polishing is a dental procedure performed to remove the accumulated dental plaque, stains and to smooth the surface of the teeth and as well as to prevent dental problems. Tooth polishing involves removal of dental plaque, accumulated particles and stains (Sawai et al. 2015) and the procedure reduces onset or development of dental problems due to untreated accumulation of dental plaque and also helps in aesthetics.

Air-polishing technique involves a polishing powder, water and air applied under continuous, accurate and controlled spray. Air-polishing method is reported to be a safer and effective method with several advantages over the other tooth polishing methods, however is not suitable for patients with health complications including sodium restricted diet and infectious diseases (Graumann et al. 2013). Air-polishing method is reported to be effective in stain removal in smokers as well as non-smokers (Hongsathavij et al. 2017). A scanning electron microscopy study revealed that air-polishing method was able to more deeply clean without creating any damage to the enamel in contrary to polishing pastes (rubber cup method) which abrade the enamel surface (Camboni and Donnet 2016). However a comparative *in vitro* study on air polishing involving glycine and sodium bicarbonate powders have reported increased adhesion of *S. mutans* to the air polished dentin using sodium bicarbonate (Tada et al. 2014). Sodium bicarbonate, the commonly used powder for air-polisher and is abrasive and can increase the surface roughness of tooth thereby increasing the chances of bacterial adhesion and biofilm formation (Janiszewska-Olszowska et al. 2020). An improvisation of air-polishing technique is therefore required to reduce such risk of bacterial re-adhesion to the polished tooth surface. We recently reported the efficiency of cannabinoids in inhibiting dental plaque bacteria (Stahl and Vasudevan 2020; Vasudevan and Stahl 2020). In this study we tested the effect of cannabidiol (CBD) on tooth polishing by supplementing the crystalline CBD powder to the regular air-polisher powder and here we report the results of our finding based on *in vitro* microbiological analysis.

Methods

Study population

The study protocol was reviewed and cleared by the Ethics Committee of the Institutional Review Board (AZ Groeninge Kortrijk, Belgium). The study protocol and the purpose were explained orally to each participant. Oral and signed consent from each participant was obtained before the start of the study. A total of 12 adults (6 women and 6 men), aged between 24 and 83 were recruited for the study from Euro-Dent clinic, Mortsel 2640, Belgium. For convenience, the study candidates were chosen among the clinic patients who are eligible (criteria described below) and agreed to participate in the study with consent. The chosen 12 adults satisfied the following selection criteria for the study: (a) presence of a minimum number of teeth (seven), including one molar, (b) absence of dentures, (c) no recent history of antimicrobial therapy or other drug therapy, including immunosuppressive, (d) no history of diabetes, (e) presence of dental plaque and (f) were not taking any special treatments for dental plaque in the recent past.

Equipment and chemicals

The disposable sampling microbrush applicators were purchased from Microbrush International (Ireland). The plastic consumables like microtubes, petriplates, ready-to-use

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media of LB agar plates (90 mm) were purchased from VWR International (Belgium). Pure isolate of CBD crystalline powder was purchased from PharmaHemp (Slovenia). The Synbiosis-aCOLyte 3 HD colony counter and Memmert incubator were purchased from Wilten Instrumenten (Netherlands). AIR-N-GO Easy polisher equipment and the AIR-N-GO classic powder (raspberry flavor) were purchased from Acteongroup (USA).

CBD-supplemented polishing

The CBD crystalline powder was added to the AIR-N-GO classic powder at 1% (wt/wt) and mixed well. The CBD-supplemented powder was loaded to the pre-cleaned AIR-N-GO Easy polisher equipment and the polishing procedure was performed by standard method. For the control, the AIR-N-GO classic powered was directly used on a separate AIR-N-GO Easy polisher equipment to avoid any chances of CBD contamination.

Dental plaque sampling

Prior to plaque sampling, saliva on the tooth surface was removed by water spray, and the sampling target area was dried with cotton. Plaque samples were collected from interdental spaces using disposable microbrush applicator and immediately dispensed into a 2 ml microtube containing 1 ml of phosphate buffer saline (PBS). For samples after polishing treatment (dental plaque removed), the sampling was performed at the same interdental space spot using disposable microbrush. All the samples were processed for *in vitro* assay within 2h from sampling.

***In vitro* assay**

The dental plaque sample was mixed well using vortex and an aliquot of 100 µl of dental plaque sample was spread on the surface of LB agar plate using sterile spreader. *In vitro* assay of each sample was performed in triplicates. The petri dishes were sealed with thin layer parafilm and incubated at 37°C for 36 h. After incubation, the plates were analyzed using automated colony counter to compare the bacterial colonies. The colonies observed on *in vitro* culture plates represent total-culturable bacterial species (predominantly aerobic) present in the dental plaque samples. The pictures of plates were taken using automated colony counter. The colony forming unit (CFU) was calculated by the formula, CFU/mL = number of colonies per plate x 10.

Statistical analysis

The *in vitro* experiments were conducted in triplicates. The average values of total colony count were calculated (Supplementary Table 1). The average values were used to calculate the linear fold change and to represent in figures. Student's unpaired t-test was performed to compare the results before and after polishing treatment for test product (CBD-supplemented powder) and control product (AIR-N-GO classic powder) respectively, as well as to compare the results between test and control treatment (Supplementary Table 2).

Results and discussion

Tooth polishing by air-polishing method using polishing powder supplemented with CBD (1%) resulted in a significant reduction of average CFU than that of regular powder, based on *in vitro* microbiological analysis of samples from interdental space. There was marginal difference in CFU between samples before and after air-polishing with regular powder (Figure 1, Figure 2) with a linear fold change in the range of 1.0 – 2.6 (Supplementary Table 1). However, a significantly higher reduction in CFU was observed between samples before

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and after air-polishing with CBD-supplemented powder (Figure 1, Figure2) with a linear fold change in the range of 3.9 – 18.4 (Supplementary Table 1). In addition, we observed that some species of bacteria were completely absent in samples after CBD-supplemented polish treatment, as evident from the presence (before polish) and absence (after polish) of bacterial colonies with distinct morphology (Supplementary Figure 1, Supplementary Figure 2). For example, in sample 4 some of the bacterial colonies with distinct morphology (large, bright-white colonies) were observed before polish, but they were completely absent after CBD-supplemented polish treatment and such prominent differences were not observed in control treatments (Supplementary Figure 1, Supplementary Figure 2).

Based on statistical analysis, the difference in CFU of samples before and after regular polishing treatment was not significant (p-value >0.5). To the contrary, the difference in CFU of samples was significantly higher (p-value <0.001) between before and after CBD-supplemented polishing treatment. The difference in CFU was also significant (p-value <0.05) for samples post-CBD-supplemented polishing in comparison to post-regular polishing treatment (Supplementary Table 2).

Our results suggest that regular powder although effective in removing the dental plaque, was not effective in inhibiting the plaque forming bacteria. Although the chalk hard plaque was removed, there were still alive and active bacteria present in the treated tooth surface or interdental space as inferred by the *in vitro* bacterial culture results (Supplementary Figure 1, Supplementary Figure 2). However, addition of CBD powder appears to enhance the tooth polishing process by inhibiting the plaque forming bacteria as inferred by the significant reduction of CFU count on *in vitro* culture plates (Supplementary Figure 1, Supplementary Figure 2). The results suggest that addition of CBD to polishing powders might improve the tooth polishing treatments and might help to inhibit and reduce the rebinding ability of plaque forming bacteria and formation of new plaque.

Air-polishing technique is a safe and comfortable (for patients) treatment for tooth polishing without adverse effects on the enamel (Graumann et al. 2013; Camboni and Donnet 2016). However the technique has a drawback of creating extensive aerosols that contain bacteria, blood and saliva together with plaque and polishing powder particles from the patient (Harrel and Molinari 2004; Sawai et al. 2015). A recent study on microbial composition and spatial distribution of dental aerosols has reported the bacterial contamination of human origin and water origin and emphasized the importance of infection control measures in dental clinic including the dental water line (Zemouri et al. 2020). Aerosols in dental clinic pose potential threat to the dentist and dental assistants of various infections including respiratory track infection such as pneumonic plague, TB, SARS, MERS and COVID-19 (Harrel and Molinari 2004; Bizzoca et al. 2020; Ge et al. 2020; Peng et al. 2020). Especially, with the present pandemic situation of COVID-19, dentists and dental assistants are at tremendous risk as the virus spread through droplets in air by oral, nasal and eye mucus membranes (Ge et al. 2020; Peng et al. 2020). Several viral and bacterial infections can spread from patients to dentists and team and eventually to other patients through cross-contamination from aerosols (Bizzoca et al. 2020). It is essential therefore to upgrade the tooth polishing procedure to minimize the aerosol-mediated infections and it is feasible to achieve this by simple modification of polishing powders. Based on our previous reports (Stahl and Vasudevan 2020; Vasudevan and Stahl 2020) and this study results, CBD-supplemented powder has the potential to reduce the risk of aerosol-mediated infection by inhibiting the bacteria. Although the present study is limited to *in vitro* analysis, the results are significant and suggest a strong potential of cannabinoids in reducing the dental aerosol-mediated infections. However, this need to be tested by analysis of the aerosol samples collected from the close proximity of patient receiving CBD-supplemented polishing.

 doi:10.20944/preprints202007.0734.v1

Air-polishing was initially used for supragingival plaque removal, however by changing the powder with low-abrasive powder such as glycine powder, the same technique is also applied for subgingival plaque removal without causing damage to the soft tissues (Graumann et al. 2013; Sawai et al. 2015). Similarly, in addition to CBD-supplementation in the powder phase, a new liquid formulation with CBD-infusion to replace water for the liquid phase in the air-polisher can also be developed in future to further increase the inhibition of plaque forming bacteria. Dental water line may have bacterial contamination (Zemouri et al. 2020) and such liquid formulation with CBD might reduce the risk of such contaminants and further improve the subgingival and supragingival plaque removal and enhance the overall tooth polishing by inhibiting the bacteria as well as to further reduce the risk of infections from the dental aerosols. The present study demonstrates a simple improvisation of existing air-polishing technique and is easy to implement without any need for modification of existing protocol. With the help of its anti-quorum sensing properties (Soni et al. 2015), and as demonstrated in this study, cannabinoids have the potential to enhance the overall performance of tooth polishing treatment.

Declarations

Ethics approval and consent to participate

AZ Groeninge Kortrijk; Belgium issued approval B39S201836215; AZGS2018028. The study protocol was reviewed and cleared by the Ethics Committee of the Institutional Review Board (AZ Groeninge Kortrijk; Belgium). The study protocol and the purpose were explained orally to the participants. Oral and signed consent from each participant was obtained before the start of the study.

Consent for publication

The contribution of Euro-Dent BV in this study was only limited to dental plaque sampling which also involves the procedure of ethics approval and patients' consent to participate in the study. CannIBite bvba is the sole contributor for development of cannabinoid supplemented tooth polishing, for conduct of experiments, for data analysis and manuscript preparation. Consent to publication was agreed by all authors. Consent to publication was agreed by CannIBite bvba and Euro-Dent BV.

Availability of data and materials

Additional data are available as supplementary items. Any further details on results, data and methods can be requested directly to corresponding author.

Competing interests

Veronica Stahl is the founder of CannIBite bvba, which develops cannabinoids infused dental care products. CannIBite bvba agreed to publish this study report.

Intellectual property info: CannIBite bvba / Veronica Stahl has pending patents (in process) for application of cannabinoids in dental care, personalization and treatments.

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Authors' contributions

VS contributed to the concept, performed the dental polishing and sampling. KV contributed to experiment design, data acquisition and interpretation, performed the *in vitro* experiments, drafted and critically revised the manuscript. All the authors read and approved the final manuscript.

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Figures

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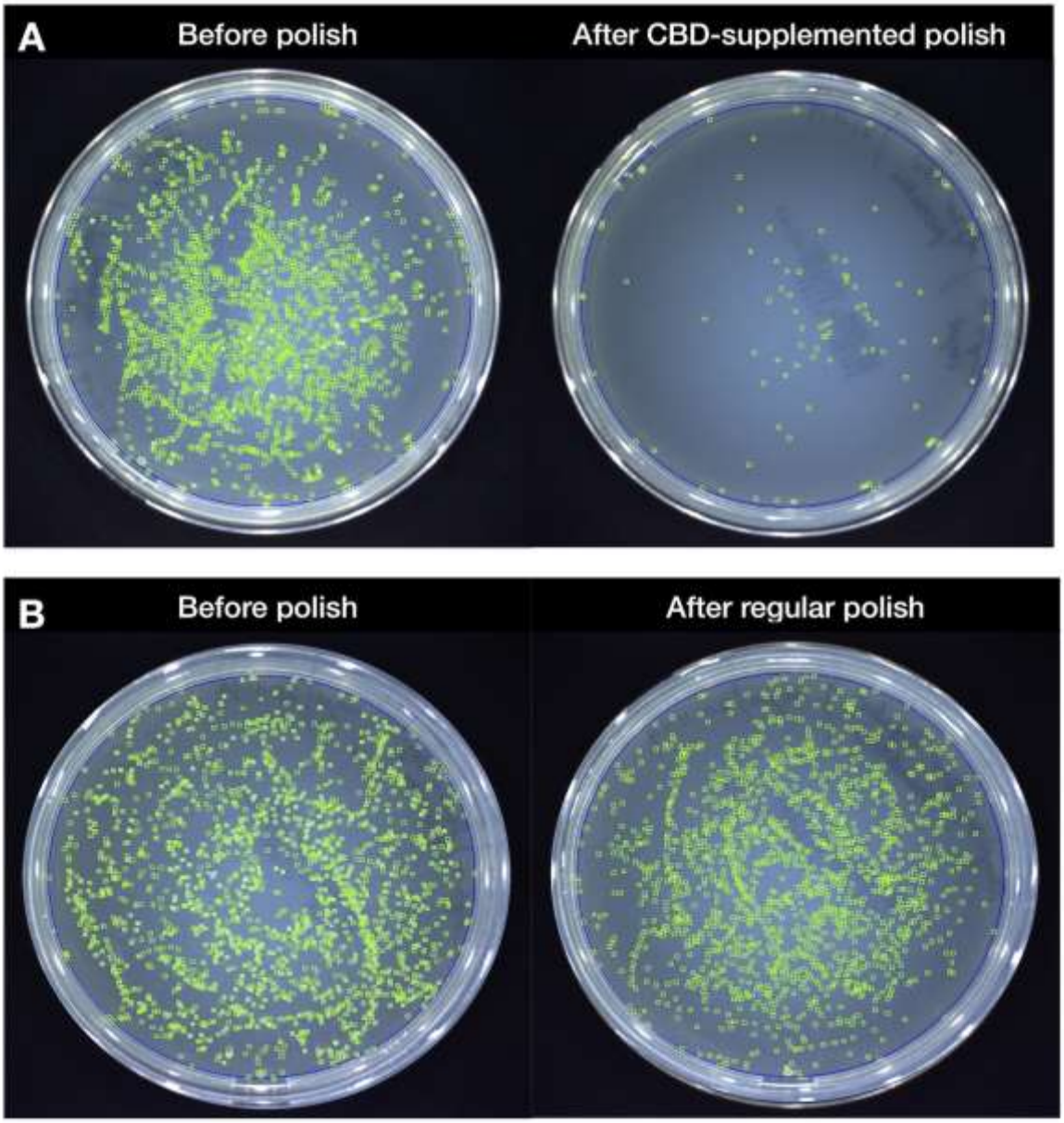

Figure 1. Sample pictures of bacterial culture plates representing bacterial colonies (A) before vs after CBD-supplemented air-polishing treatments, (B) before vs after regular air-polishing treatment. The green color highlights are generated using automated colony counter aCOLyte3 and each green mark represent single bacterial colony detected by aCOLyte3.

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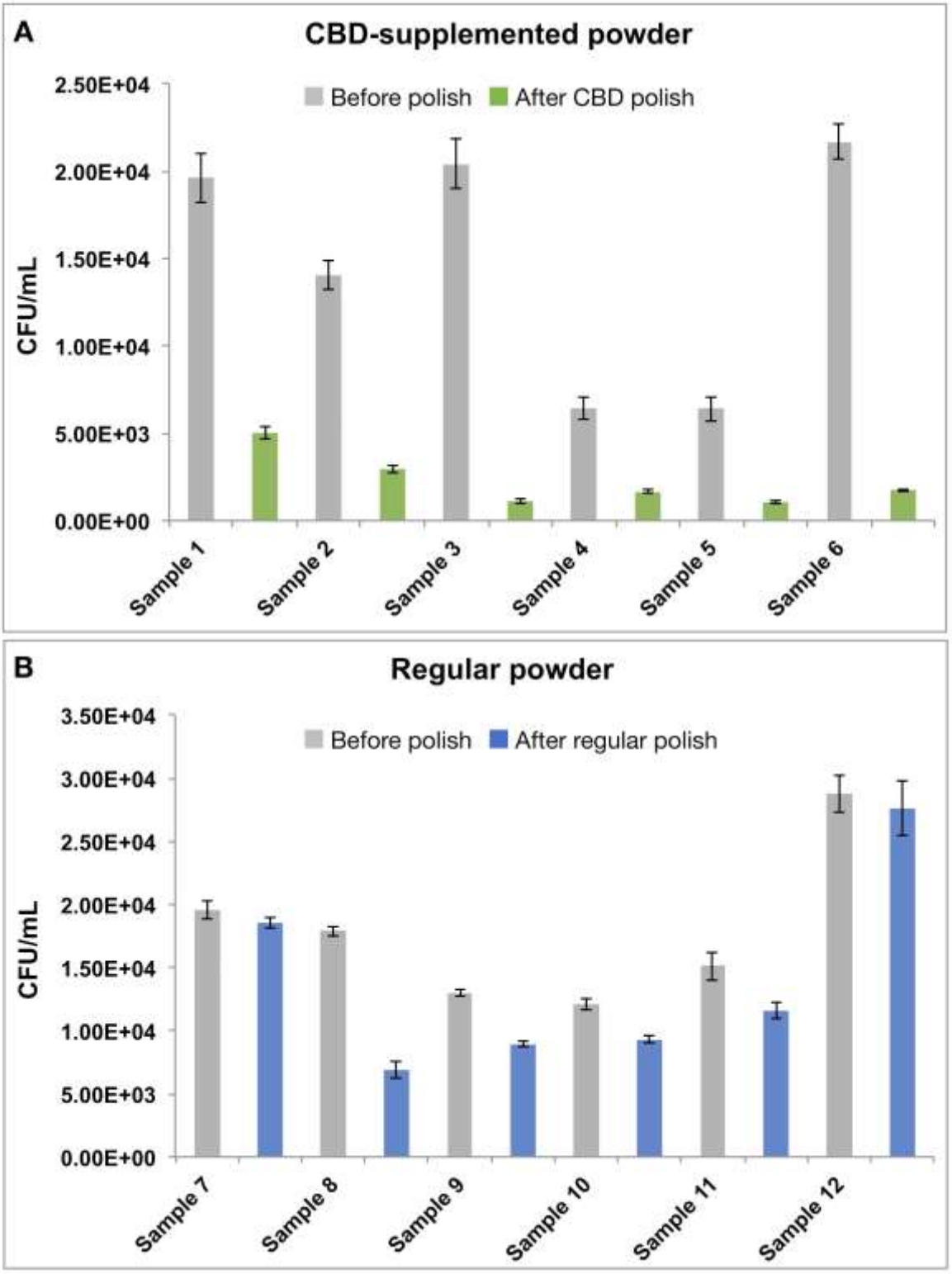

Figure 2. Graphical representation of CFU/mL data measured using automated colony counter. A, CBD-supplemented air-polishing; B, regular air-polishing.