Original Article

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

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ABSTRACT

Objective: Air polishing is a safe tooth polishing technique used by dental professionals for stain and plaque removal and as a preventive procedure for dental health. We previously reported the antibacterial properties of cannabinoids against dental plaque bacteria. The objective of this study was to analyze the possibilities to improvise the existing air-polishing technique by supplementing cannabinoid powder into the classic polishing powder for effective removal of supragingival and subgingival plaque and for inhibition of plaque-forming bacteria. **Materials and Methods:** The cannabidiol (CBD) powder was added to the tooth polishing powder (AIR-N-GO, classic) at a 1% (wt/wt) ratio. The study was conducted on 12 patients, of whom six received regular polishing treatment and six 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 by using an 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 and 18.4) compared with that of postregular polishing (linear fold change between 1.0 and 2.6) treatment. **Conclusions:** The CBD-supplemented polishing powder can help in effective removal and killing of dental plaque bacteria during the polishing treatment; it can also be added as an enhancing supplement to the existing polishing powders.

KEYWORDS: *Cannabinoid, CBD, dental care, dental plaque, polishing powder, tooth polish*

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INTRODUCTION

Dental health problems, including dental plaque and staining, are common oral health problems affecting people of various age groups globally. Dental plaque is made of a complex biofilm of multiple species of microbes, and is an etiologic agent of dental caries and periodontal diseases.[1] Dental health is influenced by various factors, including diet and lifestyle.[2,3] Alcohols such as red wine and soft drinks such as tea, cola, and coffee can cause tooth staining and erosion.[4-6] Apart from food-related tooth staining, smoking causes severe tooth staining.[7] Tooth polishing is a dental procedure that is performed to remove the accumulated dental plaque and stains, to smooth the surface of the teeth, and to prevent dental problems. Tooth polishing involves removal of dental plaque, accumulated particles, and stains[8] and the procedure reduces onset or development of dental problems due to untreated accumulation of dental plaque and also helps in aesthetics.

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The air-polishing technique involves a polishing powder, water, and air applied under continuous, accurate, and controlled spray. This method is reported to be a safer and effective method, with several advantages over the other tooth polishing methods; however, it is not suitable for patients with health complications, including a sodium restricted diet and infectious diseases.[9] The air-polishing method is reported to be effective in stain removal in smokers as well as nonsmokers.[10] A scanning electron microscopy study revealed that the air-polishing method was able to more deeply clean without creating any damage to the enamel, contrary to polishing pastes (rubber cup method) that abrade the enamel surface.[11] However, a comparative *in vitro* study on air polishing involving glycine and sodium bicarbonate powders has reported increased adhesion of *S. mutans* to the air-polished dentin by using sodium bicarbonate.[12] Sodium bicarbonate, the commonly used powder for air polishing, is abrasive and can increase the surface roughness of teeth, thereby increasing the chances of bacterial adhesion and biofilm formation.[13] An improvisation of the air-polishing technique is, therefore, required to reduce such risk of bacterial re-adhesion to the polished tooth surface. We recently reported the potential of cannabinoids in inhibiting the dental plaque associated with bacteria.[14,15] In this study, we tested the effect of cannabidiol (CBD) on tooth polishing by supplementing the crystalline CBD powder with the regular air-polisher powder; here, we report the results of our finding based on *in vitro* microbiological analysis.

Materials and Methods

Study population

The study protocol was reviewed and approved by the Ethics Committee of the Institutional Review Board (AZ Groeninge Kortrijk, Belgium). The purpose of the study and procedure were explained orally to each participant. Oral and signed consent was obtained from each participant before sampling. A total of 12 adults (six women and six men), aged between 24 and 83, were recruited for this study. For convenience, the candidates were chosen from Euro-Dent clinic, Mortsel 2640, Belgium from among the clinic patients who were eligible (criteria described later) and agreed to participate in the study. 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 being immunosuppressive; (d) no history of diabetes; (e) presence of dental plaque; and (f) not taking any special treatments for dental plaque in the recent past.

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 precleaned AIR-N-GO Easy polisher equipment, and the polishing procedure was performed by the standard method. For the control, the AIR-N-GO classic powder was directly used on a separate AIR-N-GO Easy polisher equipment to avoid any chances of CBD contamination.

Dental plaque sampling

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

In vitro assay

The dental plaque sample was mixed on vortex, and the sample aliquot of 100 µl was spread on the surface of an LB agar plate by using a sterile spreader. An *in vitro* assay of each sample was performed in three replicates. The petri dishes were sealed with parafilm and incubated for 36 h at 37°C in a temperature-controlled incubator. After incubation, the plates were analyzed by using an 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 automated colony counter was also used to highlight the colonies and to take pictures of plates. The colony forming unit (CFU) was calculated by the formula, CFU/mL = number of colonies per plate x 10.

Statistical analysis

The *in vitro* analysis of each sample was conducted in three replicates. The average values of total colony count were calculated [Table S1]. The average values were used to calculate the linear fold change and to represent them 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 [Table S2].

Results

Tooth polishing by the 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 the *in vitro* microbiological analysis of samples from interdental space. There was a marginal difference in CFU between samples before and after air polishing with regular powder [Figures 1, 2], with a linear fold change in the range of 1.0–2.6 [Table S1]. However, a significantly higher reduction in CFU was observed between samples before and after air polishing with CBD-supplemented powder [Figures 1, 2], with a linear fold change in the range of 3.9–18.4 [Table S1]. 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 a distinct morphology [Figures S1, S2]. For example, in sample 4, some of the bacterial colonies with a distinct morphology (large, bright-white colonies) were observed before polish treatment, but they were completely absent after CBD-supplemented polish treatment and such prominent differences were not observed in control treatments [Figures S1, S2].

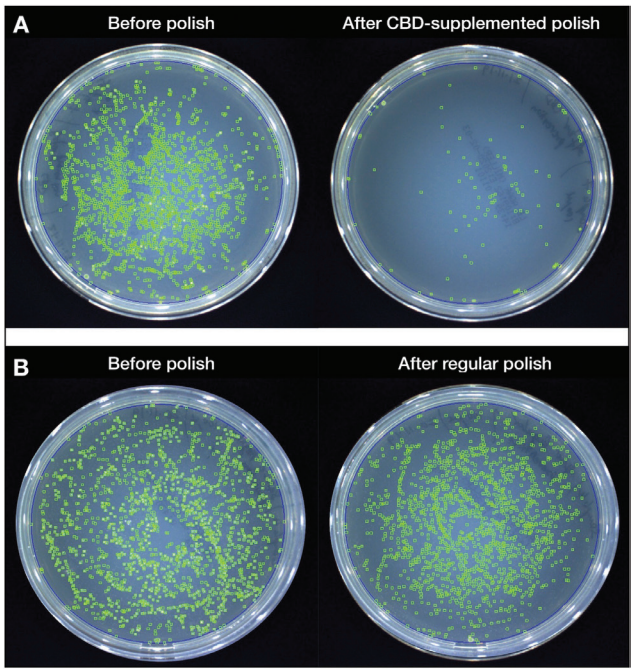

Figure 1: Sample pictures of bacterial culture plates representing bacterial colonies (A) before vs after CBD-supplemented air polishing, (B) before vs after regular air polishing. The green color highlights are generated by using an automated colony counter, and each green mark represents a single bacterial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

Based on statistical analysis, the difference in CFU of samples before and after regular polishing treatment was not significant (p-value >0.5). On 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 postregular polishing treatment [Table S2].

Discussion

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 [Figures S1, S2]. However, the 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 [Figures S1, S2]. The results suggest that the addition of CBD to polishing powders might improve the tooth polishing treatments and

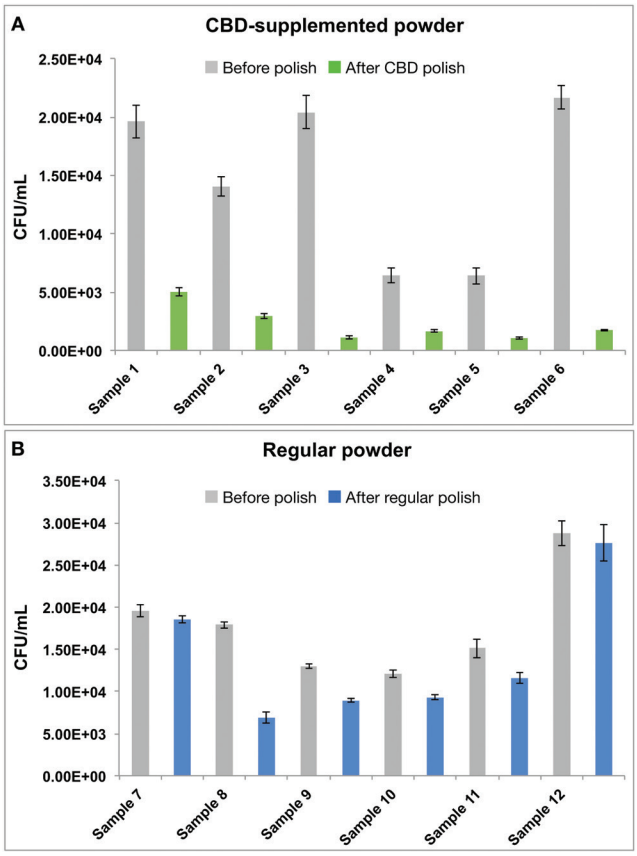

Figure 2: Graphical representation of CFU/mL data measured by using an automated colony counter. A, CBD-supplemented air polishing; B, regular air polishing

might help to inhibit and reduce the rebinding ability of plaque-forming bacteria and the formation of new plaque.

The air-polishing technique is a safe and comfortable (for patients) treatment for tooth polishing without adverse effects on the enamel.[9,11] 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.[8,16] 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 clinics, including the dental water line.[17] Aerosols in dental clinic pose a 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.[16,18-20] 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.[18,20] Several viral and bacterial infections can spread from patients to dentists and their team and eventually to other patients through cross-contamination from aerosols.[19] It is essential, therefore, to upgrade the tooth polishing procedure to minimize aerosol-mediated infections, and it is feasible to achieve this by simple modification of polishing powders. Based on our previous reports[14,15] and this study results, CBD-supplemented powder has the potential to reduce the risk of aerosol-mediated infection by inhibiting the bacteria. Although the current 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 needs to be tested by an analysis of the aerosol samples collected from the close proximity of patients receiving CBD-supplemented polishing.

Air polishing was initially used for supragingival plaque removal; however, by substituting the powder with a low-abrasive powder such as glycine powder, the same technique is also applied for subgingival plaque removal without causing damage to the soft tissues.[8,9] 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. The dental water line may have bacterial contamination[17]; such liquid formulation with CBD might reduce the risk of such contaminants, further improve the subgingival and supragingival plaque removal, enhance the overall tooth polishing by inhibiting the bacteria, and finally, further reduce the risk of infections from the dental aerosols. The current study demonstrates a simple improvisation of the existing air-polishing technique and is easy to implement without any need for modification of the existing protocol. With the help of its anti-quorum sensing properties,[21] and as demonstrated in this study, cannabinoids have the potential to enhance the overall performance of tooth polishing treatment.

Patents

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

Acknowledgement

Not applicable.

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Conflicts of interest

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

Authors' contributions

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

Ethics approval and consent to participate

Ethical approval date of issue 17.04.2018; Institute name, AZ Groeninge Kortrijk, Belgium; issued approval B39S201836215, AZGS2018028; letter reference Nr. 18030. 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 the development of cannabinoid-supplemented tooth polishing, for the conduct of experiments, data analysis, and article preparation. Consent to publication was agreed by all authors.

Consent to publication was agreed by CannIBite bvba and Euro-Dent BV.

Data availability statement

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

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Supplementary Materials

The following are available online as

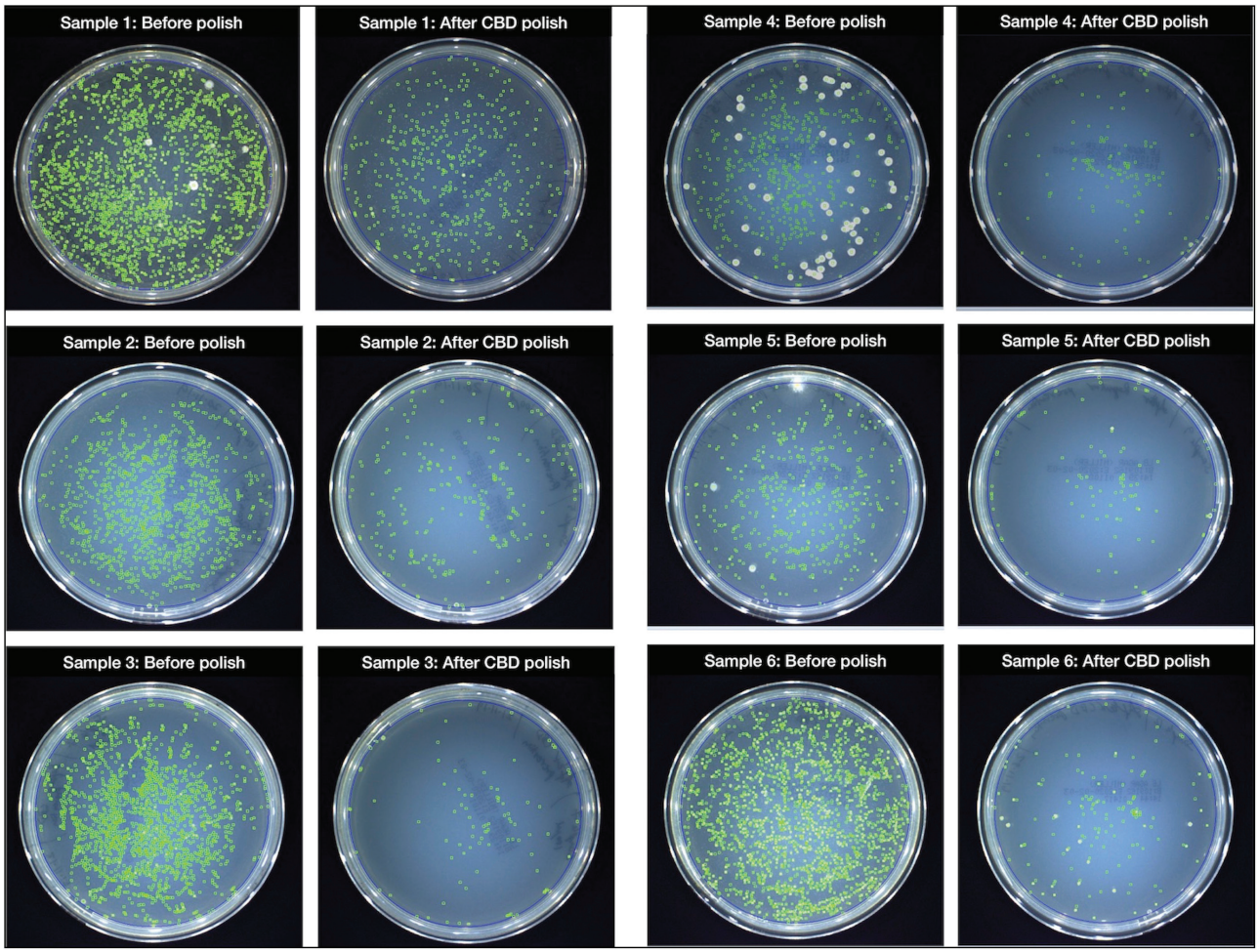

Figure S1: Bacterial culture plates of samples (1 to 6) before vs after CBD-supplemented air-polishing treatments. The green color highlights are generated by using automated colony counter aCOLyte3, and each green mark represents a single bacterial colony detected by aCOLyte3.

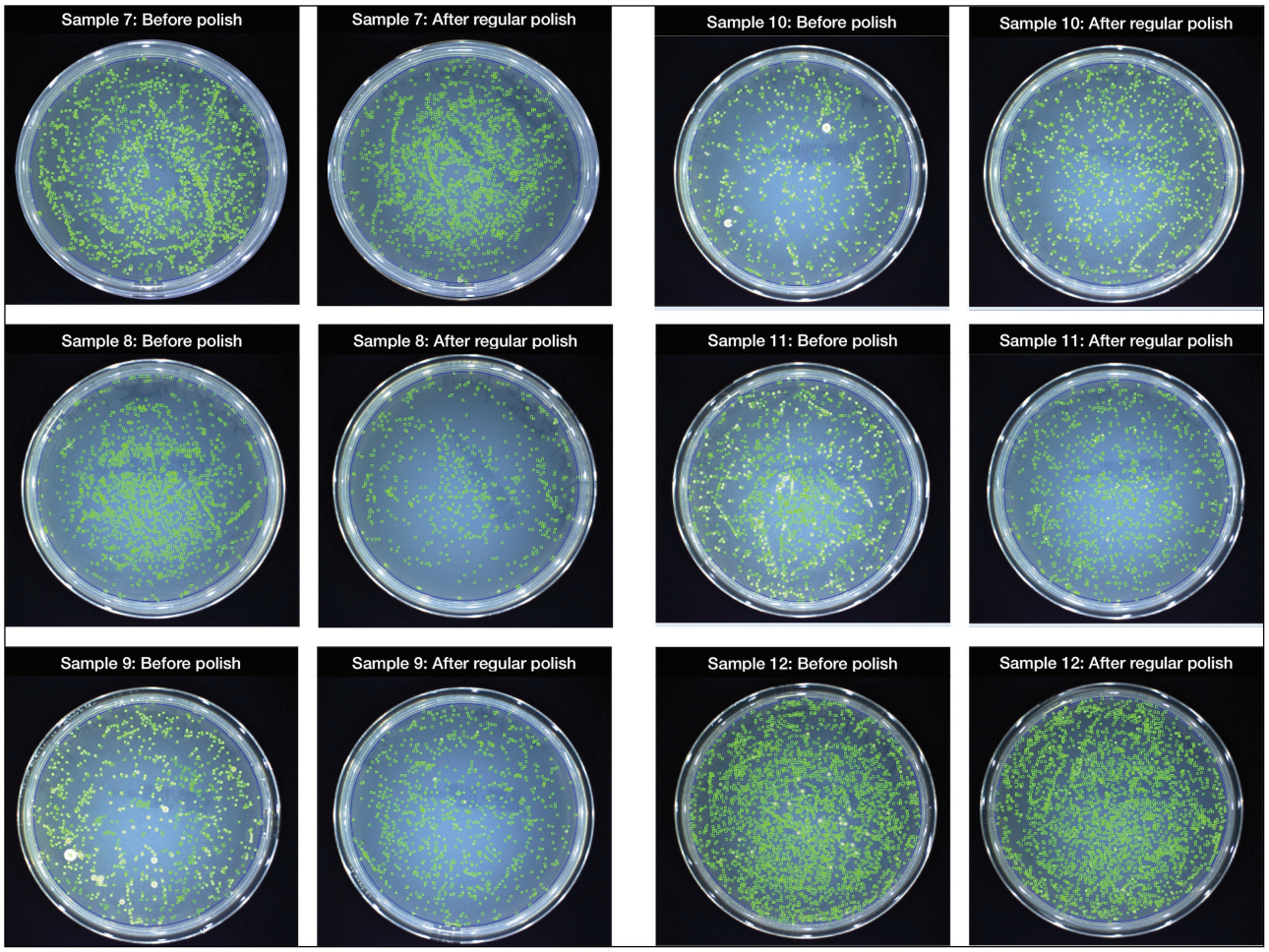

Figure S2: Bacterial culture plates of samples (7 to 12) before vs after regular air-polishing treatments. The green color highlights are generated by using automated colony counter aCOLyte3, and each green mark represents a single bacterial colony detected by aCOLyte3.

Table S1: Colony count measurement data and comparative statistical analysis data

Colony counter (aCOLyte3) settings

aCOLyte3 version	1.3.1.0
Sample volume	100 µL
Exposure time	0.07–0.09
Sensitivity	95–97
Maximum colony size	Off
Split colonies	Yes
Media	LB agar (90mm plate)

S. Nr.	Gender	Age	Treatment	Count / Plate			CFU / mL			Average CFU/ mL	SD	Linear fold change
				R1	R2	R3	R1	R2	R3			
1	M	30	Before polisher	1955	1823	2101	19550	18230	21010	1.96E+04	1.39E+03	
			After CBD polisher	467	502	536	4670	5020	5360	5.02E+03	3.45E+02	3.906312292
2	F	52	Before polisher	1407	1322	1487	14070	13220	14870	1.41E+04	8.25E+02	
			After CBD polisher	272	317	296	2720	3170	2960	2.95E+03	2.25E+02	4.763841808
3	F	52	Before polisher	2042	1901	2182	20420	19010	21820	2.04E+04	1.41E+03	
			After CBD polisher	110	123	99	1100	1230	990	1.11E+03	1.20E+02	18.44879518
4	M	83	Before polisher	712	611	598	7120	6110	5980	6.40E+03	6.24E+02	
			After CBD polisher	152	173	164	1520	1730	1640	1.63E+03	1.05E+02	3.928425358
5	M	47	Before polisher	600	721	593	6000	7210	5930	6.38E+03	7.20E+02	
			After CBD polisher	97	114	106	970	1140	1060	1.06E+03	8.50E+01	6.03785489
6	M	66	Before polisher	2263	2175	2063	22630	21750	20630	2.17E+04	1.00E+03	
			After CBD polisher	176	183	166	1760	1830	1660	1.75E+03	8.54E+01	12.38285714
7	M	27	Before polisher	1887	1958	2036	18870	19580	20360	1.96E+04	7.45E+02	
			After regular polisher	1859	1892	1811	18590	18920	18110	1.85E+04	4.07E+02	1.05735347
8	F	49	Before polisher	1787	1746	1829	17870	17460	18290	1.79E+04	4.15E+02	
			After regular polisher	753	687	625	7530	6870	6250	6.88E+03	6.40E+02	2.596610169
9	F	24	Before polisher	1300	1276	1334	13000	12760	13340	1.30E+04	2.91E+02	
			After regular polisher	913	868	890	9130	8680	8900	8.90E+03	2.25E+02	1.463871209
10	F	42	Before polisher	1207	1166	1253	12070	11660	12530	1.21E+04	4.35E+02	
			After regular polisher	899	928	953	8990	9280	9530	9.27E+03	2.70E+02	1.304316547
11	M	48	Before polisher	1509	1398	1627	15090	13980	16270	1.51E+04	1.15E+03	
			After regular polisher	1218	1096	1159	12180	10960	11590	1.16E+04	6.10E+02	1.305499568
12	F	38	Before polisher	2756	2822	3036	27560	28220	30360	2.87E+04	1.46E+03	
			After regular polisher	3006	2623	2652	30060	26230	26520	2.76E+04	2.13E+03	1.040212535

Table S2: Comparative statistical analysis

t-Test: Two-Sample Assuming Unequal Variances	Before polish	After CBD polish
Mean	16245.27778	2251.666667
Variance	41393049.41	2302016.667
Observations	12	6
Hypothesized mean difference	0	
df	13	
t Stat	7.147518355	
P(T<=t) one-tail	3.75263E-06	
t Critical one-tail	1.770933396	
P(T<=t) two-tail	7.50526E-06	
t Critical two-tail	2.160368656	

t-Test: Two-Sample Assuming Unequal Variances	Before polish	After regular polish
Mean	16245.27778	13795.55556
Variance	41393049.41	62062287.41
Observations	12	6
Hypothesized mean difference	0	
df	8	
t Stat	0.659607132	
P(T<=t) one-tail	0.264016925	
t Critical one-tail	1.859548038	
P(T<=t) two-tail	0.52803385	
t Critical two-tail	2.306004135	

t-Test: Two-Sample Assuming Unequal Variances	After CBD polish	After regular polish
Mean	2251.666667	13795.55556
Variance	2302016.667	62062287.41
Observations	6	6
Hypothesized mean difference	0	
df	5	
t Stat	-3.524562565	
P(T<=t) one-tail	0.008418509	
t Critical one-tail	2.015048373	
P(T<=t) two-tail	0.016837018	
t Critical two-tail	2.570581836	