THE EFFECT OF DISINFECTION ON THE DIMENSIONAL ACCURACY OF 3D PRINTED SURGICAL GUIDES (IN VITRO STUDY)

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ABSTRACT

INTRODUCTION: Disinfection of surgical guides is mandatory for intraoperative use. Virgin Coconut Oil may be a potent alternative disinfectant; however, its effect has not been fully discussed in dentistry.
OBJECTIVES: To evaluate the dimensional accuracy of surgical guides after immersion in three disinfectants: 100% Virgin Coconut Oil (VCO), 2% Glutaraldehyde (GA), and 70% Ethyl Alcohol (EA) and estimate the antimicrobial potential of these three disinfectants.
MATERIALS AND METHODS: Thirty identical surgical guides were printed and cut into two halves. The first half was scanned before and after disinfection-disinfection scanned files were compared to post-disinfection ones for the evaluation of the dimensional accuracy. The second half was immersed in sterile distilled water as the control groups for the estimation of the antimicrobial potential of the tested disinfectants. The comparisons between the three study groups were done using the One-Way ANOVA test.
RESULTS: At the morphological assessment of the dimensional accuracy, group VCO was the most accurate with the lowest mean deviation value of 0.12 ± 0.02mm and root mean square value of 0.12 mm, group GA and group EA were less accurate with mean deviation value of = 0.22 ± 0.05mm and = 0.19 ± 0.03mm and root mean square value of 0.22 and 0.20 respectively ($P < .001$). For the antimicrobial potential, the tested disinfectants showed 100% of reduction in the mean microbial counts.
CONCLUSIONS: Virgin Coconut Oil showed higher dimensional accuracy of the guides than Glutaraldehyde and Ethyl Alcohol. The antimicrobial potential was the same between the three disinfectants.
KEYWORDS: Disinfection; Dimensional accuracy;3D printing; Surgical guides
RUNNING TITLE: The Effect of Disinfection on Surgical Guides

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INTRODUCTION

One of the challenges facing modern dentistry is the method of successfully disinfecting three-dimensional (3D) printed objects which is an obligatory protocol prior to introducing in an operating room (1).The 3D printed surgical guide plays a crucial role in facilitating the precise placement and angulation of implants (2). As it will surely be exposed to blood, bone, and oral tissues during invasive implant procedures, it is categorized as a semi-critical product, which can be contaminated with various microorganisms during production, or in transition from/to, the dental laboratory (3). Owing to the nature of the resin material, from which stereolithographic surgical guides are usually made, they are known to be porous, water absorbable, and heat-sensitive, that being so, they may undergo deformation during steam sterilization (3-5). The application guide suggested the use of Ethyl Alcohol(EA), or non-chemical products for the disinfecting process (1,6). Consequently, high-level chemical disinfectants such as 2% Glutaraldehyde (GA) or 70% EA, are employed for 15-20 minutes, before surgery (3,7). As there are always drawbacks with such disinfectants that have a potential cause for concern to be in such proximity with oral tissues in such a procedure. EA has demonstrated more favorable effects in antimicrobial achievement in comparison with Chlorhexidine. Even though, EA is immensely flammable, must be stored in a cool place, irritates the oral tissues, and is not effective against bacterial spores and non-enveloped viruses. While GA is toxic, a skin irritant, harsh to mucous membranes, must be used in well-ventilated areas, and is not recommended as a spray or solution for decontamination of surfaces (8-10). Most recently, Virgin Coconut Oil (VCO) has gained popularity due to its unique natural antimicrobial, anti-inflammatory, antinociceptive, and antioxidant properties (11,12). As it was proven, VCO has an exceptional antimicrobial activity when compared to other edible oils due to its high saponification index leading to a reduction in microorganism accumulation and employs a powerful cleansing effect (13). Eventually, the antibacterial effect of the VCO is attributed not only to the lower acidic pH nature - between 2.52 and 4.38 which generally increases lauric acid activity - but, also to the medium-chain mono-glycerides as monolauric and monocarpic acid which destroy a wide variety of lipid-coated bacteria by disrupting their lipid membranes and inhibiting the enzymes involved in energy production and nutrient transfer (14,15). Furthermore, the high viscosity of the VCO reduces the aggregation and adhesion of microorganisms which has a great ability to inhibit the growth of Candida Albicans (13). VCO is remarkably very effective against many viruses due to its effective disintegration of the virus particles which disrupts its maturation; hence, preventing the binding of viral M proteins to the host cell membrane.(16) Additionally, the anti-inflammatory activity of VCO is outstanding by inhibiting the synthesis of inflammatory mediators responsible for the formation of pain and edema. Remarkably, the anti-nociceptive property of VCO inhibits the proliferative phase during the inflammatory process (14,17). VCO is extracted directly from fresh, mature coconut kernel without going through a refined process. This preserves the natural organic active compounds with antioxidant properties that accelerate the healing process of damaged tissues (13,15,18). The clinical necessity for chemical-free, effective, and tissue-friendly disinfectant, which inhibits the growth of microorganisms without affecting the dimensional accuracy or the surface details of the surgical guides, has been long overlooked.Thus, this study was carried out to evaluate the dimensional accuracy of a 3D printed surgical guide material before and after immersion in three disinfectants and estimate the antimicrobial potential of these disinfectants used to decontaminate the tested surgical guides. The null hypothesis was that no significant changes would be found in the dimensional accuracy of a 3D-printed surgical guide material after disinfection and the antimicrobial potential of VCO would be similar to GA and EA.

MATERIALS AND METHODS

This in vitro comparative study was held at the Department of Prosthodontics, Faculty of Dentistry, and the Department of Microbiology, Medical Research Institute, Alexandria University. A surgical guide and a model with 1mm diameter marker were fabricated by using 3D-printing technology (formlabs 2, Formlabs Inc) (19,20). Thirty identical surgical guides were designed and printed (Resin cartridge form2; Formlabs Inc) then cut into two halves (Fig. 1).Pre- and post-disinfection CBCT (Acteon, X-mind Trium) scans of the first halves placed onto 3D printed model were performed using low dose, high-resolution protocol at a proper field of view, and the DICOM (Digital Imaging and Communications in Medicine) data were exported as Standard Tessellation Language (STL)files. 3D analyzing CAD software (GOM Inspect) was used for the morphological assessment through the surface comparison analysis where a curve was drawn on the surface of the surgical guides before disinfection and seven points were indicated. The same points were also indicated on the corresponding positions after disinfection. In each study group, the pre-disinfection guides were registered as the control groups (actual data) and the post-disinfection guides were set as study groups (nominal CAD data). Then each post-disinfection guide was superimposed on the pre-disinfection one with the help of the 1mm diameter reference marker which was fixed and attached to the 3D printed model. At each point, the deviation in x, y, and z axes(dxyz) was measured to calculate the mean, the standard deviation (SD), and the root mean square (RMS) (21,22) (Fig. 2). A microbiological trial was carried out to assess the antimicrobial potential of the three tested disinfectants. All surgical guides were contaminated with a defined amount of human saliva collected from partially edentulous patients attending the Department of Prosthodontics, Faculty of Dentistry, Alexandria University, and then placed in sterile glass containers to be investigated. Each half of the three study groups was immersed in one of the three disinfectants for 20 minutes then left to dry for an additional 10 minutes then soaked in sterile glass containers containing 100 ml. of sterile distilled water for 10 minutes. An extra step was done, in which a sample was taken directly from the surface of the surgical guides disinfected with 100% VCO before its immersion in the sterile distilled water (13,14,23). Each half of the three control groups was immersed in sterile glass containers containing 100 ml. of sterile distilled water for 20 minutes. From each half, three samples were pipetted and cultured on microbiological media; Blood, MacConkey, and Sabouraud dextrose agar plates then the microbial count was expressed as colony-forming units per plate (CFU/plate). Percentage (%) of reduction was calculated by the following equation:[24]

% Of reduction = No of CFU/control plate - CFU/ study plate /No of CFU/control plate X100

Figure 1. Two halves of surgical guides placed on 3D printed model with 1mm diameter marker.

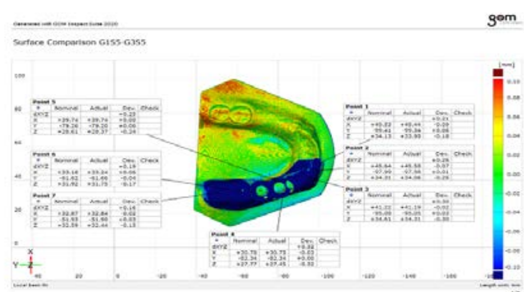

Figure 2. Morphological assessment of dimensional accuracy at seven points selected on the surface of the first half of the surgical guide pre-and post-disinfection.

Statistical analysis

Statistical analysis was performed using a statistical software program (IBM SPSS Statistics,v 23.0; IBM Corp). Normality was tested for all variables using descriptive statistics, plots, and normality tests. All variables showed normal distribution, so means, SD, and RMS were calculated, and parametric tests were used. Comparisons between the three study groups were done using the One-Way ANOVA test followed by multiple pairwise comparisons using Bonferroni post-hoc test to adjust the significance levels. Significance was inferred at (alpha=.05)

RESULTS

At the morphological assessment of the dimensional accuracy, statistically significant changes were found between the three study groups; group VCO with mean deviation = 0.12 ± 0.02mm and RMS=0.12, group GA with mean deviation = 0.22 ± 0.05mm and RMS=0.22 mm, and group EA with mean deviation = 0.19 ± 0.03mm and RMS= 0.20 (P < .001) (Table 1). Multiple pairwise comparisons were carried out when the One-Way ANOVA test was significant using Bonferroni adjusted significance levels. Group GA versus group EA comparison showed no statistically significant changes in accuracy with P=.51, while group VCO versus group GA and group EA comparisons showed statistically significant changes in accuracy with P<.001, P=.001 respectively (Table 2) For the antimicrobial potential of the three disinfectants, statistically significant differences were found between the three control and three study groups (P < .001) with the highest % of reduction in the mean number of oral microorganisms (about100%) between the three study groups. The culture results showed an uncountable bacterial growth on the control plates (more than 100 CFU/ml) representing the baseline of the oral microorganisms and the study plates showed no bacterial growth after the use of the three disinfectants (Table 3, Fig. 3,4,5). Regarding the direct sample that was collected from the surface of the surgical guides disinfected with 100% VCO, no bacterial growth was revealed (Fig. 6,7).

Table 1. Morphological assessment of dimensional accuracy between the three study groups.

		group VCO	group GA	group EA	P
Point 1	**Mean ± SD**	0.11 ± 0.04	0.20 ± 0.07	0.25 ± 0.06	**<0.001***
	RMS	0.12	0.21	0.26	
Point 2	**Mean ± SD**	0.11 ± 0.06	0.21 ± 0.09	0.23 ± 0.10	**0.008***
	RMS	0.12	0.23	0.24	
Point 3	**Mean ± SD**	0.14 ± 0.04	0.22 ± 0.05	0.20 ± 0.08	**0.02***
	RMS	0.14	0.23	0.22	
Point 4	**Mean ± SD**	0.12 ± 0.06	0.26 ± 0.06	0.25 ± 0.06	**<0.001***
	RMS	0.13	0.26	0.26	
Point 5	**Mean ± SD**	0.14 ± 0.04	0.22 ± 0.05	0.18 ± 0.04	**0.001***
	RMS	0.14	0.22	0.18	
Point 6	**Mean ± SD**	0.12 ± 0.02	0.21 ± 0.13	0.13 ± 0.05	**0.04***
	RMS	0.12	0.25	0.14	
Point 7	**Mean ± SD**	0.12 ± 0.03	0.18 ± 0.17	0.10 ± 0.05	0.25
	RMS	0.12	0.24	0.11	
Average of 7 points	**Mean ± SD**	0.12 ± 0.02	0.22 ± 0.05	0.19 ± 0.03	**<0.001***
	RMS	0.12	0.22	0.20	

SD: Standard deviation, RMS: Root mean square
*statistically significant at p < .05

Table 2. Pairwise comparisons using Bonferroni adjusted significance level.

	Groups	Compared to	*P*
Point 1	group VCO	group GA	**0.006***
		group EA	**<0.001***
	group GA	group EA	0.18
Point 2	group VCO	group GA	**0.04***
		group EA	**0.01***
	group GA	group EA	1.00
Point 3	group VCO	group GA	**0.02***
		group EA	0.09
	group GA	group EA	1.00
Point 4	group VCO	group GA	**<0.001***
		group EA	**<0.001***
	group GA	group EA	1.00
Point 5	group VCO	group GA	**0.001***
		group EA	0.15
	group GA	group EA	0.15
Point 6	group VCO	group GA	**0.049***
		group EA	**1.00**
	group GA	group EA	**0.12**
Point 7	group VCO	group GA	**0.66**
		group EA	**1.00**
	group GA	group EA	**0.34**
Average of 7 points	group VCO	group GA	**<0.001***
		group EA	**0.001***
	group GA	group EA	**0.51**

*Statistically significant at p < .05

Table 3 . Mean bacterial count between control and study groups after the addition of saliva to the two halves of the surgical guides.

Three types of culturing media	Control groups n=30	Study groups n=30	*P* (Between groups)
Blood agar	Uncountable (>100)	0	< .001*
MacConkey agar	Uncountable(>100)	0	< .001*
Sabouraud dextrose agar plates	Mean ± SD 10.4 ± 11.02	0	< .001*
	Median (IQR) 6.5 (3.5, 10)	0	
P (Within group)	< .001**	N/A**	

SD: standard deviation, IQR: Interquartile range,
*Statistically significant at p <.05 ,** Non applicable because mean bacterial count in all study groups = 0

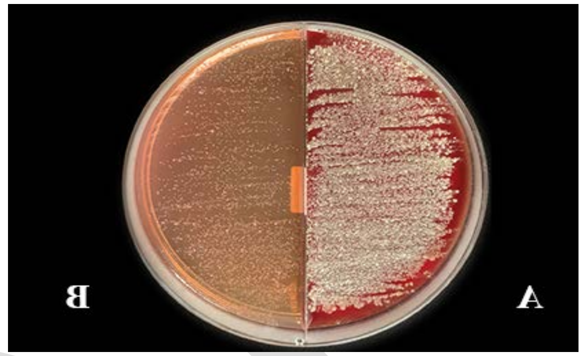

Figure 3. Culture results after the addition of saliva from the control group on Blood (A) and Mac-Conkey (B) agar plate

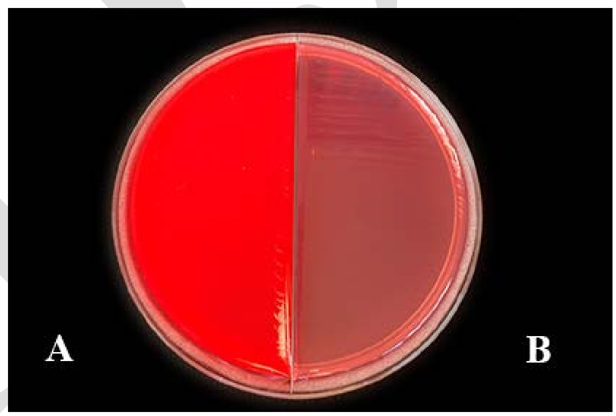

Figure 4. Culture results after the addition of saliva from the study group disinfected with2% GA on Blood (A) and MacConkey (B) agar plate

Figure 5. Culture results after the addition of saliva from the control (A) and the study group disinfected with 70% EA (B) on Sabouraud agar plate.

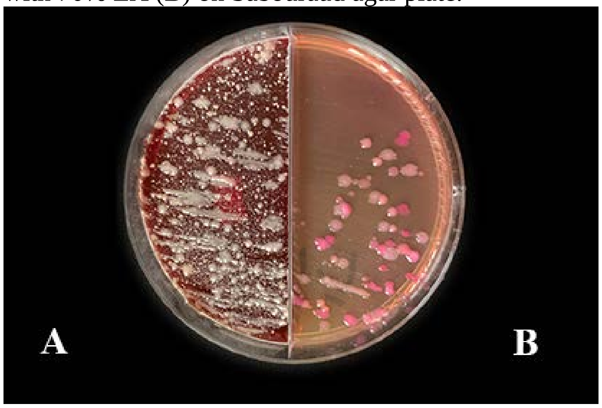

Figure 6. Culture results of the direct sample taken after addition of saliva from the control group on Blood (A) and MacConkey (B)agar plate.

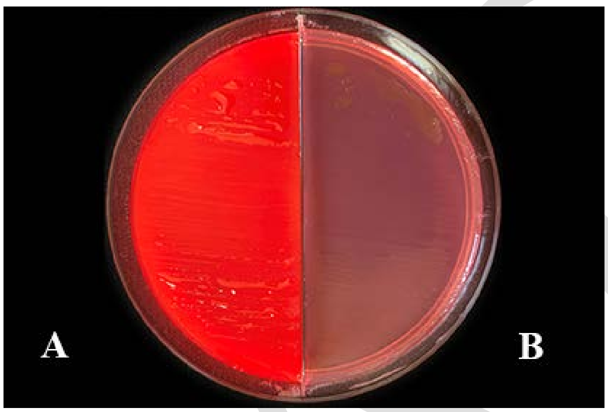

Figure 7. Culture results of the direct sample taken after addition of saliva from the study group disinfected with 100% VCO on Blood (A) and MacConkey (B) agar plate.

DISCUSSION

The effect of disinfection on the morphological dimensional accuracy of a 3D printed surgical guide material was evaluated and the antimicrobial potential of the three disinfectants used to decontaminate the tested surgical guides was estimated. The null hypothesis was rejected regarding the dimensional accuracy of the 3D printed surgical guide material as significant changes were found in the morphological assessment, nonetheless, the null hypothesis was accepted regarding the antimicrobial potential of the three disinfectants showing 100% reduction of the count of the oral microorganisms. It is worth noting that many articles were related to the application of 3D printed devices in dentistry; still, the technique and effect of disinfection on the surgical guides, in conjunction with the evaluation of the possible dimensional changes, were scarcely addressed. Thereby, the results of the present study seem to confirm that there can be statistically significant variances in the morphological assessment between the three study groups. As expected, group VCO was more accurate showing the least morphological changes in comparison to group GA and EA, which were also submillimeters. This proven correlation is directly attributed to the potential of water absorption and the porous nature of the 3D-printed resin material. This became a major concern, as learned, in the present study due to the prolonged immersion in each respective disinfectant for 20 minutes, leading to the high possibility of absorption into the surgical guide material (3-5). Subsequently, the high viscosity of VCO could explain why it displayed minimal morphological changes, in comparison to GA and EA trials, which absorbed more easily into the surface area of the guides.[13] Therefore, as referenced in Fleischer et al. (4) suggested reduction of porosity in the trial 3D-printed surface by employing appropriate printer settings which minimized the absorption of the disinfectant. This adjustment avoids the risk of its contact with oral tissues. Proving the dimensional accuracy of 0.2 mm appeared to be favorable, from the surgical point of view, and, further, did not influence the clinical use of the surgical guides (19,20). The current results were consistent with Dewi et al. (11) that reported a significant change in the dimensions of alginate dental impressions when using VCO as a disinfectant in comparison to Sodium Hypochlorite and GA solutions, within the defined range, stated by the American Dental Association. Similar results were reinforced with Ósk Thorgeirsdóttir et al. (25) studying the effect of monocarpic acid as a disinfectant for dentures with strong antimicrobial activity against Candida when applied topically. The findings of the present study were comparable to the results of Sennhenn-Kirchner et al. (7) which recommended the pre-surgical use of 70% EA for 15-20 minutes; yet, the influence on the dimensions of the surgical guides was not investigated. Török et al. (3) research was in opposition to the present efforts due to the implication of dissimilar 3D-printed material produced by Polyjet technology and a contrasting disinfectant (4% Gigasept), so the effect of the disinfection method was based only on the aspect of the tested material. Despite that, the microbiological results exhibited potential significant inhibitory properties, respective of the effectiveness of the tested disinfectants against the oral pathogens found in the saliva samples harvested from partially edentulous patients. As conducted, this method identified various, practical oral environments and simulated variants of microorganisms, normally found in the oral flora of such patients (24). Moreover, the beneficial topical effect of VCO showed promising results by eliminating100% of the microorganisms found on the surgical guides. These results were in line with many studies that supported local oral action of VCO when used during the oil pulling therapy – an alternate, substantial, cleansing method consisting of rinsing the oral cavity by ingesting a tablespoon of VCO for 20 minutes of contact time – to reduce the oral bacteria and improve oral health (13,14,23). Ripari, Francesca et al. (17) mentioned that VCO-based mouthwash was effective in treating plaque-induced gingivitis and reduced the aggregation of microorganisms without showing any harmful effects. Furthermore, Horas et al. (26) found that the topical effect of VCO on the palatal surgical wound during the palatoplasty procedure accelerated the wound healing, increased the number of fibroblast cells that appeared in the wound, and diminished the pain symptoms. Exemplified this, Dayrit et al. (16) established a scientific motivation for the utilization of VCO as a potential adjuvant therapy for COVID-19 patients and a general prophylactic agent against various microbial infections. In conclusion, VCO has an extensive history of application as an antimicrobial agent. This study should further cement, as the foundation, for in vivo studies that have the potential to assess the antimicrobial outcomes of VCO during preoperative surgical conditions.

CONCLUSIONS

Based on the findings of this in vitro study, the following conclusions were drawn:

1-Disinfection using 100% VCO for 20 minutes had a minimal morphological effect on the dimensional accuracy of the tested surgical guides that was clinically acceptable.

2-The antimicrobial potential of 100% VCO was comparable and equivalent to 2% GA and 70% EA with enormous potential benefits.

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Conflict of interest:

The authors declare that they have no conflict of interest.

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