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Can Scanning Electron Microscopy be used to quantitatively measure dentine roughness?

A microscopia eletrônica de varredura pode ser usada para medir quantitativamente a rugosidade da dentina?

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ABSTRACT

Objective: The aim of this study was to determine if SEM used with imaging software could be used to quantitatively determine the extent of dentine roughness due to tooth-brushing. **Material and methods:** Flat, polished dentine surfaces were subjected to 25,000 cycles of simulated tooth-brushing at 2 strokes per second with a load of 200 g. At the end of the brushing cycle, dentine surfaces were first assessed using profilometry and then subjected to SEM analysis. Ra (average roughness) readings were obtained for profilometric assessment. Using imaging software, the horizontal distance between adjacent characteristic grooves noted on micrographs was measured, ensuring that the middle of each crest of the associated groove was used as the reference point, the Crest-to-Crest distance (C-C distance). These two parameters were examined statistically for correlation. **Results:** When Spearman Rank tests were utilized the correlation between average Ra and crest to crest measurements was 0.709 ($p < 0.01$). The Bland Altman plot, however, showed poor agreement between the two test parameters. **Conclusion:** Further work is needed to validate the use of this methodology in the quantitative assessment of tooth surface loss due to abrasion.

KEYWORDS

Abrasion; Bland-Altman; Dentine roughness; Profilometry; Scanning electron microscopy.

RESUMO

Objetivo: O objetivo deste estudo foi determinar se o MEV usado com software de imagem poderia ser usado para determinar quantitativamente a extensão da rugosidade da dentina devido à escovação dentária. **Material e Métodos:** Superfícies dentinárias planas e polidas foram submetidas a 25.000 ciclos de escovação dentária simulada a 2 movimentos por segundo com uma carga de 200g. No final do ciclo de escovação, as superfícies dentinárias foram primeiro avaliadas por perfilometria e depois submetidas à análise MEV. Leituras de Ra (rugosidade média) foram obtidas para avaliação perfilométrica. Utilizando um software de imagem, foi medida a distância horizontal entre sulcos característicos adjacentes observados nas micrografias, garantindo que o meio de cada crista do sulco associado fosse usado como ponto de referência, a distância crista a crista (distância CC). Esses dois parâmetros foram examinados estatisticamente para correlação. **Resultados:** Quando foram utilizados os testes de Spearman Rank, a correlação entre a média de Ra e as medidas de crista a crista foi de 0,709 ($p<0,01$). O gráfico de Bland Altman, no entanto, mostrou fraca concordância entre os dois parâmetros de teste. **Conclusão:** Mais trabalhos são necessários para validar o uso desta metodologia na avaliação quantitativa da perda da superfície dentária devido à abrasão.

PALAVRAS-CHAVE

Abrasão dentária; Escovação dentária; Dentina; Propriedades de superfície; Microscopia eletrônica de varredura.

INTRODUCTION

Methodologies that assess tooth brushing abrasion are well described in the literature. Such in vitro tests of dental hard tissue abrasion are based on an assessment of surface roughness and the differences between surface roughness measurements before and after an abrasive challenge [1]. Using profilometric methods, differences in roughness between worn and unworn surfaces can be calculated to understand the amount of tooth structure lost [1]. In vitro methods of determining tooth surface loss (abrasion and/or erosion) have included measurements of changes in either linear or volumetric surface roughness [2,3]. Increases in surface roughness indicate increasing tooth surface loss [4,5].

While contact profilometry can be used to assess tooth wear due to abrasion, the use of contact profilometry to assess tooth wear due to a combination of both abrasion and erosion is limited, since contact with an acid-softened enamel or dentine surface may produce erroneous roughness measurements [6]. With mixed abrasive/erosive lesions, surface roughness should be assessed using non-contact profilometry in assessments of roughness to mitigate misleading results on already softened enamel or dentine surfaces caused by acidic challenges [6]. However, the use of contact profilometry to assess the effects of tooth-brushing abrasion is still relevant due to the nature of bulk tissue removal caused by prolonged interaction and motion of toothbrush bristles with tooth structure, mediated by toothpaste [4].

Visual analysis of micrographs obtained from scanning electron microscopy (SEM) has also been used to assess erosive, abrasive, or mixed challenges on tooth structure [7]. The use of SEM has been mainly qualitative with descriptions of the alterations of either dentin or enamel [7,8]. A review of the literature reveals few descriptions of quantitative methods assessing dentin abrasion using micrographs obtained from SEM.

As part of a larger study, the authors demonstrated a distinct and characteristic pattern of grooves on dentin surfaces due to simulated toothbrushing with a soft-bristled toothbrush and toothpastes with known abrasives. Such characteristic grooves: with distinct and identifiable peaks and depressions, were previously described, but not visualized, in a profilometric study of dentine abrasion [9]. Initial comparisons of micrographs revealed variations in created groove patterns in terms of relative width when brushed with different toothpastes. This study aims to validate a methodology for quantifying roughness changes in dentin as a result of long-term tooth-brushing abrasion using micrographs obtained from SEM. The null hypothesis stated there would be no significant linear relationship different from zero between average roughness Ra and C-C measurements when used to measure the surface roughness.

MATERIALS AND METHODS

An exemption to ethical approval was given by the university's ethics committee prior to the start of the larger study (CREC-SA.0181/02/2020). The power analysis (G Power, Universitat Kiel, Germany) was performed as part of a larger study, on toothbrushing abrasion with various types of toothpastes, that calculated the amount of samples in each experimental group at 6 given an effect size of 0.25, and a power of 0.8.

The occlusal thirds of human molars were removed using a diamond water-cooled saw to reveal dentine with a periphery of enamel. Exposed dentin was polished using decreasing grits of silicon carbide paper, 600, 800, 1000, 1200 grit, and culminating in 1500 grit. Baseline average Ra measurements were taken using a profilometric technique using Mitutoyo Surftest 401 surface roughness analyzer (Mitutoyo America Corporation, Aurora, IL, USA) with a cutoff of 0.25 mm, a transverse length of 1.6 mm, a sample length of 1.25 mm, and a vertical bandwidth of 50μm. For each evaluated surface, 3 random readings were taken to give a mean Ra for each specimen. Only samples with mean roughness $\leq 0.4\mu$m were included. Samples were either polished until the required value for inclusion was obtained or the specimen was replaced by another altogether. The specimens were allocated to one of 5 groups; one negative control (distilled water) and four experimental groups of charcoal containing toothpastes with known abrasive particles (Table I). Auto-polymerizing polymethylmethacrylate (Dentsply, NC, USA) was mixed according to the manufacturer's instructions and poured into PVC cylinders (20mmX12mm). The prepared teeth were subsequently mounted into the auto-polymerizing resin and placed in a simulated tooth-brushing

Table I - Materials used in the study

Group	Brushing Agent	Ingredients	Manufacturer
1	Distilled Water		
2	Active Wow	Purified Water, Xylitol, Diatomite Diatomaceous Earth, Baking Soda, Activated Charcoal, Tea Tree Oil, Organic Coconut Oil, Xantham Gum, Citric acid, Natural Flavors	Active Wow Toronto, Ontario, Canada
3	Crest 3D White with Charcoal	Sodium Fluoride 0.243%, Water, Sorbitol, Hydrated Silica, Disodium Pyrophosphate, Sodium Lauryl Sulphate, Flavor, Cellulose Gum, Sodium Hydroxide, Sodium Saccharin, Carbomer, Charcoal Powder, Polysorbate 80, Mica, Titanium Dioxide	Procter & Gamble, Cincinnati OH, USA
4	Curaprox	Water, Sorbitol, Hydrated Silica, Glycerin, Charcoal Powder, Aroma, Decyl Glucoside, Cocamidopropyl Betaine, Sodium Monofluorophosphate, Tocopherol, Xanthan Gum, Maltodextrin, Mica, Hydroxyapatite (NANO), Potassium Acesulfame, Titanium Dioxide, Microcrystalline Cellulose, Sodium Chloride, Potassium Chloride, Citrus Limon, Peel Oil, Sodium Hydrochloride, Zea Mays, Starch, Amyloglucosidase, Glucose	Curaden USA Inc Mesa, Arizona, USA
5	Colgate Essentials with Charcoal	Sodium Monofluorophosphate 0.76%, Water, Hydrated Silica, Sorbitol, Calcium Pyrophosphate, Glycerin, PEG-12, Pentasodium Triphosphate, Tetrapotassium Pyrophosphate, Flavor, Sodium Lauryl Sulphate, Cellulose Gum, Sodium Saccharin, Charcoal Powder, Xanthan Gum, Cocamidopropyl Betanine, Blue 1, Red 40, Yellow 5	Colgate-Palmolive Company, New York, NY, USA

machine (Toothbrushing Simulator MEV 4X-3D, Odeme Dental Research, Brazil) and subjected to brushing for 25,000 cycles using a soft, circular bristled toothbrush (Colgate Extra-Clean Full Head, Colgate Palmolive Company, NY, USA) at 2 strokes per second with a load of 200 g. All specimens in each of the five groups were brushed for 25,000 cycles. Mounted specimens were brushed perpendicular to the direction in which baseline Ra readings were taken. The entire surface of each specimen was exposed to toothbrush bristles and toothpaste slurry (1:1 ratio with distilled water by volume) at all times during simulated brushing. Specimens were thoroughly washed with distilled water, lightly blotted and Ra readings were immediately taken using the same profilometric standards used for baseline measurements.

All the specimens were vacuum desiccated (Ted Pella Inc, Redding, CA, USA) for 30 minutes, mounted on aluminum stubs (Pelco, Redding, CA, USA), and sputter coated (Denton Vacuum LLC, Moorestown, NJ, USA) with a homogenous coating of gold. Micrographs were obtained using a scanning electron microscope (Philips SEM 515, Eindhoven, Netherlands) operating at 30kV and at an operating distance of 12mm. Micrographs were taken at various magnifications. Micrographs with at least 3 of these distinctive grooves were chosen for analysis. Imaging software (Image J, National Institutes of Health, Stapleton, NY) was used to attain linear horizontal measurements from the middle of each crest to the middle of the adjacent crest, the Crest-to-Crest distance (C-C distance). Prior to quantifying linear measurements, the scale bar for each micrograph was used to calibrate the ImageJ software as it related to linear measurement in micrometers of that micrograph. Only one C-C measurement was taken for each distinctive groove. Three measurements were randomly taken of each selected micrograph for each specimen.

Using SPSS (Statistical Package for Social Sciences) Version 24 (IBM, Chicago) the means and standard deviations of C-C (Crest to Crest) distance and Ra (Average Roughness) were calculated for each tested toothpaste. Scatter plots followed by tests of correlation (Spearman-Rank) were used to look for an association between the two quantitative measurements. A Bland-Altman plot was used to look for agreement between mean Ra values and mean C-C distance.

RESULTS

Mean Ra and C-C distance together with their standard deviations can be seen in Table II. Micrographs of the dentine surfaces brushed with various charcoal containing toothpastes are shown in Figures 1-5. The characteristic grooves

Table II - Mean (S.D.) of Dentine Roughness and Crest to Crest Distance

Toothpaste Brand	Mean (S.D.) Ra Value	Mean (S.D) Crest to Crest Distance
Distilled (Control)	0.31 (0.05)	37.81 (6.04)
Active Wow	3.22 (0.83)	92.36 (4.01)
Crest 3D White with Charcoal	4.03 (1.66)	147.85 (5.10)
Curaprox	4.86 (1.21)	276.57 (13.02)
Colgate Essentials with Charcoal	4.13 (0.63)	173.69 (18.11)

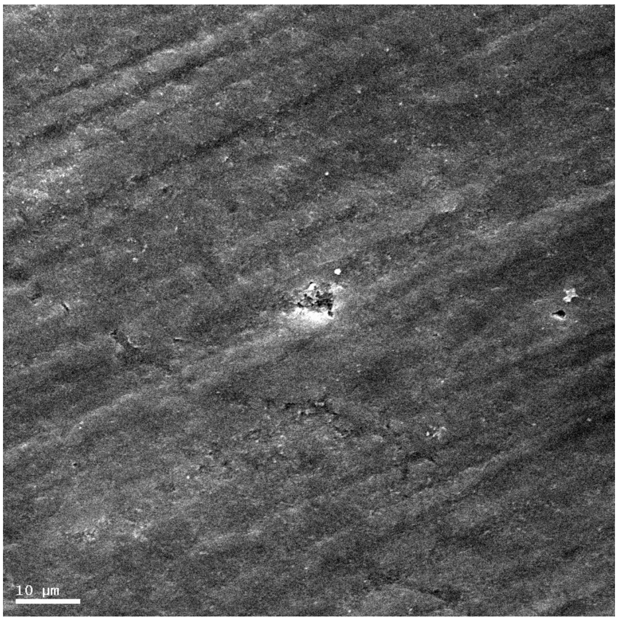

Figure 1 - SEM of dentine brushed with distilled water (control) X125.

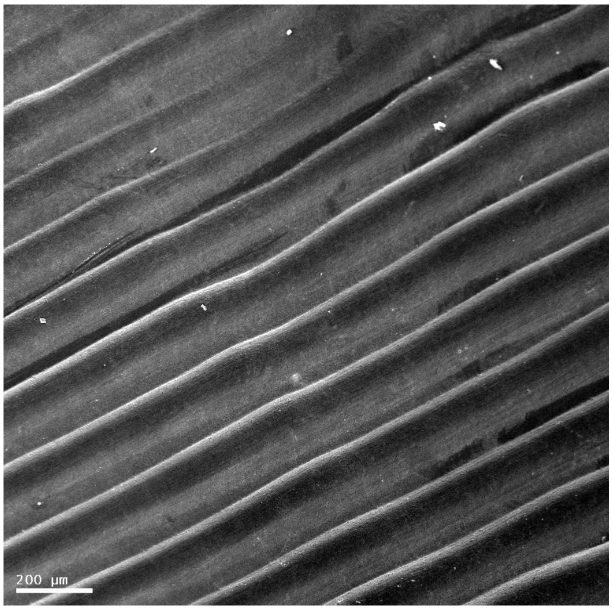

Figure 2 - SEM of dentine brushed with Active Wow Charcoal Toothpaste X 75.

were notably absent from the control dentine samples brushed with distal water only, as shown in Figure 1. Dentine samples brushed with the various toothpaste brands look machined in appearance as shown in Figures 2 to 5.

Figure 6 shows the scatter plot of the average Ra versus C-C distance. From the graph, it can be noted that there is a low positive correlation between the two measurements. Data was normally distributed for Ra, however, C-C distances were not normally distributed, therefore a Spearman-Rank test was used. However, when Spearman Rank tests were performed, the correlation between average Ra and C –C measurements was 0.709 at $p < 0.01$. Figure 7 shows the Bland-Altman plot for average Ra versus C-C Measurements. Even though correlation values were above 0.5, the results of this plot revealed that there was not good agreement between the two measured tests in assessing tooth brushing abrasion.

DISCUSSION

Profilometric methods are very popular in the assessment of tooth surface loss due to abrasion. Profilometric measurements also can examine the complexity of tooth brushing abrasion where the interplay of factors such as behavioral, mechanical, chemical, and biological aspects are important in individual toothbrushing [10].

Linear profilometry was the methodology employed in the larger study that examined the abrasive effect of charcoal containing toothpastes on exposed dentine at varying time points i.e. brushing cycles. The use of SEM in qualifying the appearance of abraded dentine happened at the terminal point of experimentation since surfaces had to be sputter coated. For this reason, SEM images were obtained of abraded dentine at the end of a cumulative abrasive challenge of 25,000 cycles.

In linear profilometric analysis, the vertical displacement of the contact stylus across the abraded or roughened surface provides various roughness parameters with average Ra being the mean of such deflections over the profile length [11]. Profilometry measures the crest to valleys of grooves that are formed because of the abrasive action of toothbrushing with toothpaste. In attempting to use SEM to quantify the abrasive change a horizontal crest to crest measurement was utilized. The authors compared vertical

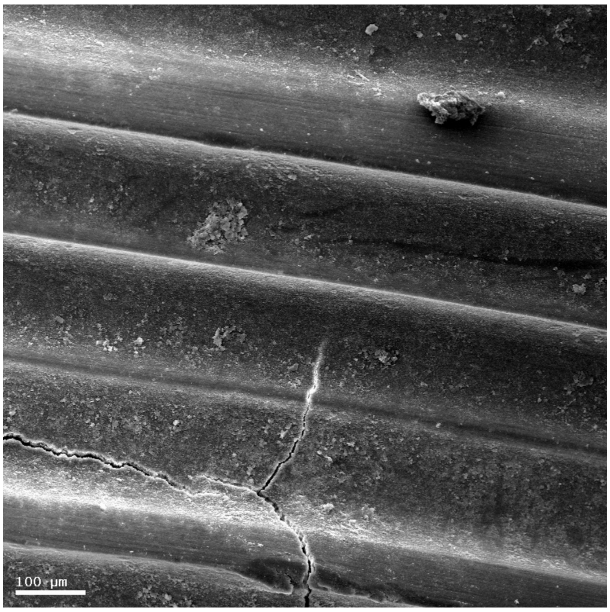

Figure 3 - SEM of dentine brushed with Crest 3D white with Charcoal X137.

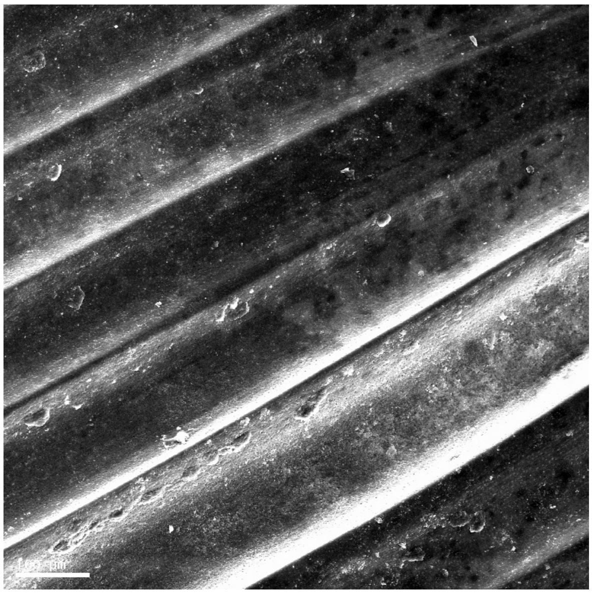

Figure 5 - SEM of dentine brushed with Colgate Essentials with Charcoal X 150.

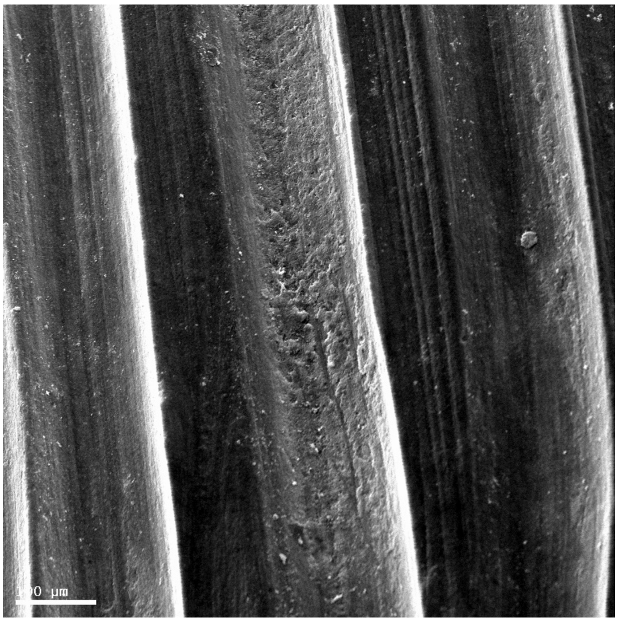

Figure 4 - SEM of dentine brushed with Curaprox X156.

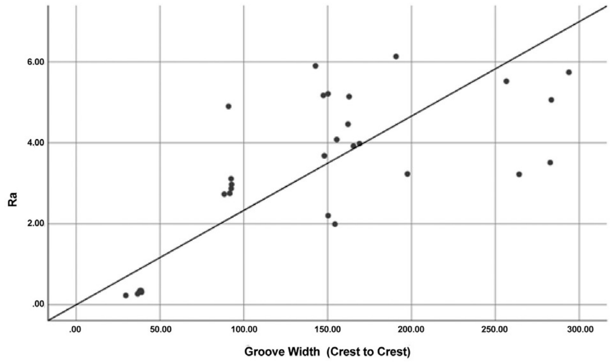

Figure 6 - Scatter plot of Ra versus Crest to Crest distance.

dentine loss with horizontal dentine loss within the characteristic groves that were formed by simulated toothbrushing.

While a comparison of the methods showed a positive correlation with a coefficient ratio for the Spearman-Rank statistical tests above 0.5, correlation values approaching 1 do not necessarily imply good agreement between the two methods for measuring the effect of toothbrushing on exposed dentine. In statistical methods, agreement measures the level of concordance between two or more sets of measurements [12]. Agreement is often used to demonstrate if newer methods or techniques to assess a measurement parameter can be substituted for known or proven methods. In this instance, the authors wanted to understand the use of quantifying crest-to-crest distance may be an appropriate method for assessing dentine roughness caused by toothbrushing.

A Bland and Altman plot is a meaningful way to assess bias between mean differences between the two methods and to estimate an agreement interval within which 95% of the differences of the second method compared to the first one fall [13]. A Bland-Altman plot is attained by plotting the difference between the methods versus the mean of method A and method B (Figure 7). Bland and Altman concluded that a high correlation between any two methods designed to measure

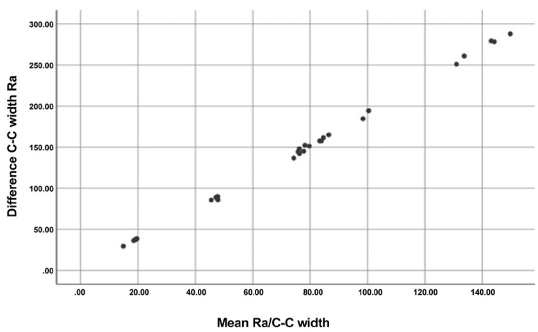

Figure 7 - Bland Altman Plot of Ra versus Crest to Crest distance.

the same property could be attributed to the effect of widespread sampling [14].

In this study, a typical Bland Altman plot showing agreement was not achieved despite good correlation and only three readings taken for each of the C-C readings. What is not yet clear is if there would be both good correlation and agreement between the two methods at earlier assessment periods less than 25000 cycles of brushing or if additional C-C readings across each crest would produce agreement.

Further work would see an expansion of the methodology in an attempt to validate the use of SEM to quantitatively measure dentine roughness due to tooth-brushing abrasion. This would involve a large sample being brushed with a single toothpaste and removing a fixed number of samples at each time point to be subjected to both profilometric and SEM analysis until the endpoint of the experiment. In this way, data could be obtained at each time-point for comparison of the methods. This can yield useful results on whether these characteristic grooves are produced at earlier time points and whether they can be easily measured and positively correlated with Ra compared with the grooves produced with longer brushing cycles as seen in this work.

This proposed method holds promise for the assessment of dental tissue where the combined effect of erosion and abrasion challenges are being evaluated where contact profilometry can introduce error into the methodology because of softened dental tissue caused by the erosive challenge [15]. Future work will also involve the use of this method in quantifying changes in dentine caused by combined erosive and abrasive challenges.

A cost analysis on the use of SEM compared to profilometry in quantifying roughness caused by abrasion alone or combined abrasive/erosive challenges should be considered before widespread use and acceptance of this method for the in vitro studies of tooth wear. However, those institutions that already have a scanning electron microscope may find this method a useful methodological adjunct in assessing tooth wear.

CONCLUSIONS

Within the limitations of this study the following conclusions may be drawn:

(1) The combination of tooth brushing with toothpastes produced defects with characteristic grooves on exposed dentine, which were visualized using SEM;

(2) Specimens of dentine brushed with water alone did not show these characteristic grooves;

(3) The null hypothesis was rejected since a positive correlation was noted between the measured C-C distances and Ra;

(4) Poor agreement, between average roughness (Ra) and C-C distance underscores the need for further validation of the study's findings.

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Author's Contributions

SMM was involved in project conceptualisation and development of the methodology. KB and AR were involved in data curation under the supervision of SMM. MI completed the formal analysis. SMM produced the original draft of the manuscript. All authors reviewed and edited the final draft of the manuscript.

Conflict of Interest

The authors declare that they have no conflicts of interest with respect to this research.

The authors did not use AI or AI-assisted writing technologies in the writing process for this manuscript.

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Regulatory Statement

This study was conducted in accordance with all the provisions of the local human subjects oversight committee guidelines and policies of The University of the West Indies, St. Augustine. The approval code for this study is CREC-SA.0181/02/2020.

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