Particle size effects on abrasion, surface polishing and stain removal efficacy in a tooth model system

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Particle Size Effects on Abrasion, Surface Polishing and Stain Removal Efficacy in a Tooth Model System

Short title: Toothpaste Abrasive Particle Size Effects

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Highlights

- The existence of a critical particle size (CPS) for toothpaste abrasive has been demonstrated for the first time in a tooth model system.
- A linear relationship was observed between cleaning power and enamel abrasion.
- A linear association was shown between enamel abrasion and the number of toothbrush strokes.

Abstract

Four calcined alumina abrasive particles [ultrafine (0.05 μm), 3 μm, 9 μm and 20 μm] with defined sizes were investigated for their effects on toothbrush abrasion, surface polishing and stain removal *in vitro*. The existence of a critical particle size (CPS) was shown for the first time in a tooth model system and in the present study a CPS of ~2.3 μm for d10, 4.3 μm for d50 or 7.8 μm for d90 for the calcined alumina abrasives was apparent. The d10, d50 and d90 values indicate that 10%, 50% and 90% of the particles measured were less than or equal to the size stated. This dimension enabled maximum abrasive action on the tested specimens resulting in the largest wear depth, greatest surface polishing and best stain removal. The enamel wear depth decreased when brushed with abrasives above the critical particle size and became almost independent of further particle size increases, which is useful for minimising wear effects in the development of dentifrice. The findings provide new information on abrasive particle size for modification and control of toothpaste abrasivity and cleaning.

Keywords

Bovine enamel; Toothbrush abrasion; Surface polishing; Stain removal; Critical particle size

1. Introduction

Oral health is essential to general health and quality of life. Toothbrushing with toothpaste aims to remove the dental plaque biofilm, food debris and dental stain on accessible tooth surfaces [1]. Indeed, there is an increasing demand for cleaning products which improve dental aesthetics [2]. The potential adverse effect, however, of toothbrushing with toothpaste is tooth abrasion [3,4] and this can lead to dentine hypersensitivity, dental pain, and poor aesthetics [5,6]. Ideally, a toothbrushing regime should show excellent cleaning efficiency for dental plaque and stain removal, as well as for polishing ability, while exerting minimal tooth wear [7-9]. It is therefore important that we develop new toothpaste formulations and toothbrush designs which optimally "clean" whilst minimising dental hard tissue damage.

Dentifrices or toothpastes are formulated with dental abrasive particles [10] which are the key components that physically clean and polish the tooth surfaces [11,12]. Historically, the commonly used dental abrasives included silica, alumina, dicalcium phosphate, calcium carbonate, sodium carbonate, hydroxyapatite, perlite and diamond [8,13-17]. A significant number of studies have investigated the factors contributing to good oral hygiene, including stain removal and tooth wear. Data indicate that properties of the abrasive particles, including their hardness, shape, size, size distribution and concentration are key to cleaning performance [7,9,10,12,16-18].

The amount of toothpaste abrasives added in the toothpaste formulation varies with the abrasive properties used. The most commonly used abrasives are hydrated silica and calcium carbonate, and they are included in the range of 8 w/w% to 20 w/w%; alumina and perlite, however, are included at lower concentrations of 1 w/w% to 2 w/w%, due to their higher enamel abrasivity [16]. Furthermore, particle sizes and their size distributions are important factors in determining abrasivity and stain removal [12]. Generally the use of abrasives with relatively broad particle size ranges in dentifrices has previously precluded an understanding of the influence of particle size. However, the size effect is a well-known phenomenon in abrasion and it is understood that there is a minimum abrasive particle size or critical particle size (CPS) which allows maximum abrasive action [16,19,20]. Reportedly, wear or wear rate increases as abrasive particle size also increases up to a threshold or a CPS, after which the wear rate becomes almost independent of further particle size increases. This CPS is frequently reported as being approximately 100 μm [20], however this value is variable dependent upon the different substrates and abrasives used, e.g. 45 μm for copper on copper, and 90 μm for nylon on nylon [19]. Indeed, a particle size of ~40 μm is reported as optimal when metals, such as Silver Ag, Cadmium Cd and Molybdenum Mo or dental tissues (including enamel and dentine) abraded against SiC grits with a diameter range of 13-125 μm [1].

In a tooth model system, limited knowledge exists with regard to the influence of the abrasive particle size and size distributions on tooth wear and stain removal [16]. Data indicate, however, that toothpastes with larger particle sizes produce more wear than those with smaller particles [21-23, 24], although results in one recent report suggested no effect of the particle size on enamel erosion and abrasion [25]. Similarly there was a lack of association between average particle size and enamel wear when three sizes of commercially produced silicon carbide grit with a diameter range of 14-73 μm were used as abrasive particles [26]. Interestingly, a linear relationship has been found between abrasive particle size and enamel abrasion when enamel was brushed with bioactive glass containing toothpastes [23]. Larger particles also showed higher dentine abrasion effects when brushing was performed using calcium carbonate (two particle sizes with an average particle diameter of 7 µm and 15 µm, respectively) and aluminium hydroxide (two particle sizes with an average particle diameter of 8 µm and 13 µm, respectively). No impact of particle size on enamel erosion/abrasion was found when eroded enamel was brushed with commercial toothpastes consisted of abrasive particles above 40 µm [25].

To our knowledge there have been no studies which have explored the existence of a CPS in a tooth model system. Consequently, we hypothesised that there would exist a CPS in a tooth model system. Therefore, the aim of this study was to investigate the effect of calcined alumina abrasives with defined particle size and size distribution on toothbrush abrasion, polishing and stain removal.

2. Materials and methods

2.1. Characterisation of calcined alumina abrasives

Four calcined alumina abrasives (Almatis GmbH, Ludwigshafen, Germany) [designated as ultrafine (0.05 µm), 3 µm, 9 µm and 20 µm] with defined particle size were used in the present study. Particle size analysis was performed on a Malvern Mastersizer 2000 laser diffraction particle size analyser (Malvern Instruments Ltd, United Kingdom). Calcined alumina abrasives were added in distilled water to an agitated flask attached to the diffraction machine and the particle size distribution of the abrasives was determined. Particle distribution graphs are shown as average values of three measurements, error bars were negligible and were omitted.

The morphology of the calcined alumina abrasive particles was observed under Scanning Electron Microscopy (SEM, EVO MA10, Zeiss). The alumina abrasive particles were adhered to carbon tape on an aluminium stub (Agar Scientific Ltd., UK). Ultra-thin sputtered conductive coating (gold) (EMITECH K550X, Emitech, United Kingdom) was applied to the particles prior to SEM observations to prevent charging of the specimens. Representative images were captured under a range of magnifications.

2.2 *Preparation of bovine enamel samples*

Bovine permanent incisor teeth were collected and stored in 0.1% (w/w) thymol (Sigma-Aldrich, UK) solution at 4 °C prior to use. Tooth crowns were obtained from the dissection of bovine teeth using a custom-built diamond-edged saw with water cooling. Bovine enamel specimens (approximate 12mm × 18mm) were prepared following embedding tooth crowns in Ø25 mm blocks of epoxy resins (Buehler, UK). A Phoenix Beta Grinder/Polisher (Buehler, UK) was used with SiC abrasive discs (Buehler, UK) for sample preparation. Eight bovine enamel specimens (approximate 10mm × 10mm of the enamel surface was exposed) per treatment group were prepared to either: a) Polished surface group with 600-grit Silicon Carbide grinding paper (SiC) ground following 3 µm diamond finish for enamel abrasion, b) Partially roughened surface group with 400-grit SiC ground finish for surface polishing , or c) Roughened surface group with 280-grit SiC ground finish for *in vitro* stain removal. 5 minutes ultrasonication in tap water was applied following each treatment to remove any residual grinding/polishing materials.

2.3. *Tooth staining*

The tooth staining assay previously reported was used [27]. Freshly combined solutions 0.1% (w/w) tannic acid (ACS reagent, Sigma-Aldrich) and (0.1% (w/w) of diammonium iron (II) sulphate 6-hydrate (Sigma-Aldrich) form a dark colloidal iron (III) tannic acid complex (“ferric-tannate”) on contact with air, which mimics a dietary tannin staining. The fresh mixture was applied as successive layers on the enamel specimens for tooth staining, with the initial layer, a 40 µl aliquot of the mixture was pipetted onto each specimen and dispersed evenly over the specimen surface before air drying. For the subsequent 9 layers, 10 µl aliquots of the solutions were applied as described above. Each layer was dried at 40 °C in an oven (D-63450 Hanau, Kendro Laboratory Products Ltd, Germany) for 10 mins before application of the subsequent layer.

2.4. Toothbrushing protocol

The toothbrushing protocol used has previously been reported [27-29]. Eight bovine enamel specimens per treatment group were mounted in two brushing channels of an *in vitro* brushing simulator and the experimental setup has been previously reported [29]. Oral B P35 medium toothbrushes were used for the brushing. A test band of the enamel specimen was exposed when the un-brushed reference area was covered by ADA/ISO standard tape. Slurries were generated with the addition of 1% (w/w) calcined alumina abrasives in 10% (w/w) Glycerol (VWR International BVBA, Belgium) plus 0.5% (w/w) Hercules 7 MF Carboxymethyl Cellulose (Hercules Incorporated, USA). A brushing load of 150 g was applied and 150 g slurry was used in each channel [29]. Specimens were “brushed” at a brushing speed of 120 rpm for up to 10,000 strokes and a temperature of 20°C was maintained throughout the brushing procedure.

2.5. Surface profiles of enamel specimens

Surface profiles were obtained before and after brushing using a Talysurf Series 2 inductive gauge profilometer (Taylor-Hobson, UK). Linear profiles (2D) were obtained on the surfaces with a point spacing of 0.25 µm and at a measurement speed of 0.5 mm/s. The wear depth and arithmetic mean surface roughness (Ra) values were calculated (µltra version 5.1.14, Taylor-Hobson, UK). The inductive gauge profilometer uses a conical probe with 2 μm diamond tip to accurately measure surfaces at the sub-micron level, has a resolution of 16 nm and a 1 mm range in the z-axis.

2.6. Gloss measurements

A Novo-Curve small area glossmeter (Rhopoint Instruments Limited, UK) was used and gloss measurements were determined before and after brushing at intervals of 90 degrees rotation about the centre point of each specimen.

2.7. Colour evaluation

Changes in colour were determined as previously described [27]. All surfaces were consistently dried prior to colour measurements. A calibrated spectrophotometer (Minolta CM-2600d, Konica Minolta Sensing Americas, Inc, USA) was used to measure colour values (L*, a*, b*) for each tooth specimen before staining (=Initial), after 10 layers of stain application (=Stained) and after the brushing treatments (=Brushed). The a* and b* values represent two colour axes, with a* the red-green axis and b* the yellow-blue axis. The L* value represents the value of 'brightness/darkness' of a colour, such that a perfect reflecting diffuser has an L* values of 100 and the perfect black body has an L* value of zero. The stain removal was assessed using the following formula:

$$\% \, Removal = \frac{L^* \text{ (Brushed)} - L^* \text{(Stained)}}{L^* \text{ (Initial)} - L^* \text{(Stained)}} \times 100$$

Where L* (Initial), L* (Stained) and L* (Brushed) is the brightness before staining, after 10 cycles of stain application and after toothbrushing for the requisite number of strokes with alumina abrasive slurry, respectively.

Images of the enamel surfaces before staining, after 10 layers of stain and post-stain removal with 1,000 brush strokes were digitally captured (Nikon D7000 camera, Nikon Corporation) to visually demonstrate the effects of stain removal.

2.8. Statistical analyses of the data

Single factor ANOVA was used for the data analyses with a significance level of $p \leq 0.05$ applied. Pearson correlation coefficient was used to determine the linear relationship between two variables, and is denoted by r. It has a value between -1 and +1 inclusive, where -1 is perfect negative linear correlation, 0 is no linear correlation, and +1 is perfect positive linear correlation.

3. Results

3.1. Characterisation of calcined alumina abrasives

The particle size distributions of the alumina abrasives are shown in Figure 1 and Table 1. The volume distribution is defined as the distribution per volume of the particle sizes, shown as Volume %, a differential of total volume of all counts.

Representative SEM micrographs of the test calcined alumina abrasives are shown in Figure 1. Differences in particle size and range of morphologies for the four calcined alumina abrasives can be clearly observed. All the abrasive particles were irregular shape and consisted of a mixture of relatively small and large particles. SEM micrographs clearly demonstrated that ultrafine is the finest, then followed by 3 µm and 9 µm, and 20 µm is the largest in terms of particle sizes. For the ultrafine abrasive, agglomerates were observed due to its very fine particle size and individual particles were smaller than 1 µm.

3.2. Abrasivity of calcined alumina abrasives on polished enamel

Table 2 provides wear depth and surface finishing of the polished enamel specimens after brushing up to 10,000 strokes with the calcined alumina abrasives. Wear occurred for all the tested enamel specimens, and the wear depth increased as the number of toothbrushing strokes increased. A linear relationship (r^2=0.99) was identified between brushing strokes and wear depth (enamel loss) for the tested alumina abrasives (Figure 2). Statistically significant differences (p<0.05) were detected in wear depth between the four calcined alumina abrasives. The ultrafine caused the least wear (least abrasive), followed by the 9 µm and 20 µm abrasives (medium abrasive), while the 3 µm abrasive produced the most wear (most abrasive).

There was a decrease in gloss for all of the polished enamel surfaces after toothbrushing with all four calcined alumina abrasives. Statistically significant differences were detected for the decreases in gloss when the polished enamel specimens were brushed with the four alumina abrasives. There was a trend for the decrease in gloss to be greater when the polished enamel specimens were brushed with the larger size abrasive particles.

The polished enamel surfaces became roughened with the toothbrushing and there was an increase in the surface roughness for all of the polished enamel surfaces. The increase in surface roughness of the polished enamel specimens was lowest for the ultrafine abrasive, followed by the 3 µm and 20 µm particles and the 9 µm particles generated the greatest increase in surface roughness. The loaded abrasive particles acted by generating grooves in the polished enamel surfaces which resulted in a roughening of the polished enamel surfaces, hence, resulting in the surface roughness increase. Abrasive particles with different particle size and distribution impacted differently on the surface roughness increase, and no statistically significant differences in roughness increase was detected between the 9 µm and 20 µm alumina abrasives.

Changes in gloss and roughness were not linearly related with regards to the number of brush strokes although greater changes in gloss and roughness were found with an increase in brushing strokes used.

3.3. Polishing effect of calcined alumina abrasives on partially roughened enamel

Polishing effect results for the alumina abrasives on the partially roughened enamel specimens after brushing up to 10,000 strokes are shown in Table 3. There were no appreciable differences in wear depth during the first 3000 brushstrokes, especially for the least abrasive ultrafine particles. The ultrafine calcined alumina caused the least wear (least abrasive), with a ranking of 9 µm < 20 µm < 3 µm particles for the amount of wear produced.

There was an increase in gloss for all of the roughened enamel surfaces due to the wear to the partially roughened enamel specimens, and these specimens also showed a decrease in surface roughness. The wear depth, gloss increase, and roughness decrease were not linear in relation to the number of brush strokes and the greatest changes were seen during the initial 1000 brushstrokes applied. The relationships between these parameters and the number of brushstrokes showed differences amongst the different alumina abrasives. The most abrasive particles increased the gloss and decreased the surface roughness at a greater rate than the least abrasive particles.

Notably, there was a continual increase in gloss and decrease in surface roughness for roughened enamel surfaces brushed with the ultrafine particles throughout the entire brushing protocol, up to 10,000 strokes. In contrast, for the 3 µm abrasive particles there was only a marginal decrease in gloss and increase in surface roughness at 10,000 brush strokes compared with the use of 5000 brush strokes.

Compared with the 20 µm abrasive, the other three abrasives (ultrafine, 3 µm and 9µm particles) showed an enhanced performance on the finish of roughened enamel surfaces in terms of gloss increase and surface roughness decrease.

3.4. Stain removal on partially roughened enamel

The *in vitro* stain removal efficacy results are presented in Figure 3a. Data indicate that the 3 µm abrasive demonstrated the greatest cleaning power, followed by the 9 µm and 20 µm, and ultrafine abrasive which removed least stain. The images of enamel surfaces before staining and post-stain removal following application of 1000 brush strokes are shown in Figure 3b, relatively minimal stain was removed from the enamel surfaces after brushing when using the ultrafine abrasive. Increased stain was removed when brushing with the 20 µm and 9 µm abrasives, and the 3 µm abrasive removed the most stain from the stained surfaces.

4. Discussion

Both the individual particle sizes and the size distributions of the abrasive particles used in toothpastes are important factors in determining abrasivity and stain removal [12]. In the present study, the effect of calcined alumina abrasives with defined particle size and size distribution were investigated on toothbrush abrasion, polishing and *in vitro* stain removal. Results under these experimental conditions demonstrated that there exists a CPS for the tested calcined alumina abrasive particles. This is the first time that this has been demonstrated with a tooth model system and data support the hypothesis underpinning this study.

4.1. Critical particle size of the tested calcined alumina abrasives

4.1.1. Critical particle size for abrasivity of polished enamel

Size effect is a well-known phenomenon in abrasion with two-body wear a result of direct contact and three-body wear due to surfaces being abraded by an "intervening slurry of abrasive particles" [30]. Furthermore, there is reportedly a minimum abrasive particle size or CPS which allows maximum abrasive action [16,19,20]. The relationship between enamel abrasivity and particle size after 10,000 brush strokes are shown in Figure 4a. It is apparent that there is a CPS of ~2.3 µm for d10, 4.3 µm for d50 and 7.8 µm for d90 for the calcined alumina abrasives. Notably, no CPS has previously been studied or reported in the literature. A linear relationship was however found between particle size and enamel abrasion when enamel was brushed with bioactive glass-based toothpastes (particle size range of 5-65 µm) [23], calcium carbonate (median particle size range of 1-13 µm) and dicalcium phosphate (median particle size range of 1-16 µm) [21]. Larger particles were shown to have a higher dentine abrasion when brushed with calcium carbonate and aluminium hydroxide. Similar results were reported with smaller particles and narrow distributions of the particle size reducing the magnitude of enamel wear when silica abrasives with a range of 4-12 µm particle size were used. No impact of particle size on enamel erosion/abrasion was reported when eroded enamel was brushed with commercial toothpastes which contained abrasive particles above 40 µm in size [25]. Notably, no clear association between average particle size and average striation width (enamel loss) was shown when enamel was brushed with commercial silicon carbide grit with a particle size range of 14-73 µm [26].

4.1.2. Critical particle size effects on polishing and in vitro stain removal for partially roughened enamel

Unlike abrasivity and stain removal, a cleaning property of toothpaste that has received little attention is its polishing ability [7]. High enamel lustre is important as more highly polished enamel surfaces appear whiter than duller enamel surfaces and they do not accumulate extrinsic stain. These properties are also important for the aesthetics of the dentition. Furthermore and more importantly, smoothed and polished tooth surfaces are less receptive to the build-up and retention of dental plaque. Indeed, the effects of enamel surface finish on *in vitro* stain removal have confirmed that it requires fewer brush strokes to remove stain from polished compared with roughened enamel surfaces [27].

Dulled enamel specimens have also been used to study polishing effects [7,8] and previous work has used tooth specimens etched with acid prior to toothbrushing. In our study, partially roughened enamel specimens were used to analyse the polishing effects and results have now confirmed that a CPS also exists for the polishing effects of toothpaste abrasives using this approach (see Figure 4b). Indeed, a CPS of ~2.3 μm for d10, 4.3 μm for d50 and 7.8 μm for d90 for the calcined alumina abrasives is apparent.

Only one study has thus far investigated the potential for correlation between stain removal and particle size and no significant impact of particle size on tooth cleaning when brushing with perlite abrasive particles was detected [8]. However, we have now demonstrated CPS for *in vitro* stain removal of ~2.3 μm for d10, 4.3 μm for d50 and 7.8 μm for d90 for the calcined alumina abrasives (see Figure 4c).

4.2. Interrelationship between stain removal and tooth wear

Both the individual particle sizes and size distributions of the abrasive particles are important factors in determining abrasivity and stain removal. It is therefore not surprising that abrasivity and stain removal are correlated [12]. Normally more stain is removed when brushed with more abrasive particles. In the present study the 3 μm particles are the most abrasive, therefore it has greatest cleaning power and the maximum stain removal is at 3 μm. A linear relationship was detected under the present experimental conditions between enamel abrasivity (at 10,000 brush strokes) and *in vitro* stain removal efficacy (1000 brush strokes) (data not shown). Similar results have been reported elsewhere indicating the relationship between dentine abrasivity and stain removal [31,32]. Interestingly however exceptions to this have also been reported, and some data show that for a toothpaste with improved cleaning, increased dentine abrasivity is not a pre-requisite. This can potentially be explained due to the different influence on dentine and stains by factors such as abrasive type, particle surface and size, as well as the chemical influence of other toothpaste components [33].

4.3. Finishing and polishing mechanism

Results for the roughened enamel surfaces showed that brushing with alumina abrasives produced wear, increased gloss and decreased surface roughness. Gloss increased and surface roughness decreased with the increasing number of brushstrokes. Notably, there was a peak at which the increase in gloss and decrease in surface roughness was optimal. After that peak, further brushing resulted in the surface becoming rougher and exhibited a lower gloss finish. Interestingly, the number of brushstrokes required to reach the optimal peak of surface finish was different for the various abrasives used. When the partially roughened enamel surfaces were brushed with abrasive particles, there was removal of asperities through wear (abrasion) which reduced the size of the peaks and troughs and therefore smoothed the partially roughened surfaces thereby decreasing surface roughness. Different abrasive treatments impact the removal of asperities at different rates, therefore requiring different brushstrokes to reach the optimal peak of surface finish. Having more abrasive particles results in a requirement for a fewer number of brushstrokes.

This phenomenon may be explained by the stages described below.

Stage 1: There is a partially roughened enamel surface prior to the commencement of brushing.

Stage 2: The partially roughened surface would subsequently become smoother due to the increasing number of brushstrokes allowing for some of the peaks and troughs previously present on the roughened surface to be removed due to abrasion. Consequently, the gloss would increase and the surface roughness would decrease.

Stage 3: The finish and polish would reach a maximum effect with further brushing, and surface peaks and troughs would be removed resulting in a smoother surface. The gloss would therefore achieve the highest level and surface roughness would be at its lowest level.

Stage 4: The smooth surface would become gradually roughened due to further brushing. Consequently, compared with stage 3, the gloss would decrease, surface roughness would increase, as would wear depth.

Our data indicate that there are differences in the number of brushstrokes to reach each of the stages described above for the different abrasives used. Generally, the most abrasive particles would require fewer brushstrokes compared with the least abrasive particles, to reach each stage. Indeed, it was notable that there was a continual increase in gloss and decrease in surface roughness for partially roughened enamel surfaces brushed with the ultrafine calcined alumina particles throughout the whole brushing procedure up to 10,000 strokes. In contrast, for the 3 µm abrasive particles there was a marginal decrease in gloss and increase in surface roughness at 10,000 brushstrokes compared with data from brushing at 5,000 strokes.

5. Conclusions

Results from the present study have confirmed the existence of a critical particle size (CPS) for the first time in a tooth model system and a CPS of ~2.3 µm for d10, 4.3 µm for d50 or 7.8 µm for d90 for the calcined alumina abrasives was apparent. Abrasive particle size affected the wear of the polished enamel surfaces, the finish of the partially roughened enamel surfaces and the *in vitro* stain removal on the roughened enamel surfaces. This dimension identified enabled maximum abrasive action on the tested specimens resulting in the largest wear depth, greatest surface polishing and best stain removal. The novel findings provide new information on abrasive particle size for modification and control of toothpaste abrasivity and cleaning, supporting the development of new toothpaste formulations which can harness optimal abrasive particle size and size distributions for tooth cleaning.

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7. References

[1] Wright KHR. The abrasive wear resistance of human dental tissue. Wear 1969;14:263-284.

[2] Joiner A. Whitening toothpastes: A review of the literature. Journal of Dentistry 2010;38s:e17-e24.

[3] Dyer D, MacDonald E, Newcombe RG, Scratcher C, Ley F, Addy M. Abrasion and stain removal by different manual toothbrushes and brush actions: studies *in vitro*. Journal of Clinical Periodontology 2001;28:121-127.

[4] Wiegand A, Kuhn M, Sener B, Roos M, Attin T. Abrasion of eroded dentin caused by toothpaste slurries of different abrasivity and toothbrushes of different filament diameter. Journal of Dentistry 2009;37:480-484. https://doi.org/10.1016/j.jdent.2009.03.005.

[5] Sehmi H, Olley RC. The effect of toothbrush abrasion force on dentine hypersensitivity *in vitro*. Journal of Dentistry 2015;43:1442-1447. https://doi.org/10.1016/j.jdent.2015.10.014.

[6] West NX, Sanz M, Lussi A, Bartlett D, Bouchard P, Bourgeois D. Prevalence of dentine hypersensitivity and study of associated factors: A European population-based cross-sectional study. Journal of Dentistry 2013;41:841-851. https://doi.org/10.1016/j.jdent.2013.07.017.

[7] Dental Products Testing, Therametric Technologies, Inc, Health Science Research Centre, Indiana University-Purdue University. Abrasion, polishing, and stain removal characteristics of various commercial dentifrices *in vitro*. The Journal of Clinical Dentistry 2011;22:11-18.

[8] Wang B. Cleaning, abrasion, and polishing effect of novel perlite toothpaste abrasive. The Journal of Clinical Dentistry 2013;24:88-93.

[9] Power JM, Craig RG. Wear of dental enamel. Wear 1973;23:141-152.

[10] Seong J, Hall C, Young S, Parkinson C, Macdonald E, Bodfel Jones S, West NX. A randomised clinical *in situ* study to evaluate the effects of novel low abrasivity anti-sensitivity dentifrices on dentine wear. Journal of Dentistry 2017;57:20-25.

[11] Davies RM. What's in toothpaste and why. Dent Update 2004;31:67-71.

[12] Ashcroft AT, Joiner A. Tooth cleaning and tooth wear: a review. Proceedings of the Institution of Mechanical Engineer, Part J: Journal of Engineering Tribology 2010;224:539-549. https://doi.org/10.1243/13506501JET671.

[13] Lippert F. An introduction to toothpaste-its purpose, history and ingredients. Monograph in Oral Science 2016;23:1-14. https://doi.org/10.1159/000350456.

[14] Tawakoli PN, Becker K, Attin T. Abrasive effects of diamond dentifrices on dentine and enamel. Swiss Dental Journal SSO 2018;128:14-19.

[15] Sabrah AHA, Lippert F, Kelly AB, Hara AT. Comparison between radiotracer and surface profile methods for the determination of dentifrice abrasivity. Wear 2013;306:73-79. https://dx.doi.org/10.1016/i.wear.2013.07.001.

[16] Hara AT, Turssi CP. Baking soda as an abrasive in toothpaste: Mechanism of action and safety and effectiveness considerations. JADA 2017;148:27S-33S.

[17] Descartes S, Courtieux L, Berthier Y, Peditto F. Tribological study of oral care silica. Tribology International 2015;82:551-560. https://dx.doi.org/10.1016/j.triboint.2014.02.023.

[18] Lippert F, Arrageg MA, Eckert GJ, Hara AT. Interaction between toothpaste abrasivity and toothbrush filament stiffness on the development of erosive/abrasive

lesions in vitro. International Dental Journal 2017; 67(6):344-350.

[19] https://doi.org/10.1111/idj.12305.

Rabinowicz E, Mutis A. Effect of abrasive particle size on wear. Wear 1965;8:381-

[20] 390.

Gåhlin R, Jacobson S. The particle size effect in abrasion studied by controlled

[21] abrasive surfaces. Wear 1999;224:118-125.

Epstein S, Tainter ML. The relationship of particle size and other properties of
dentifrice ingredients to toothbrush-abrasion of enamel. Journal of Dental

[22] Research 1943;22:335-344.

De Boer P, Duinkkerke ASH, Arends J. Influence of tooth paste particle size and

[23] tooth brush stiffness on dentine abrasion *in vitro*. Caries Research 1985;19:232-
239.

Mahood A, Mneimne M, Zou LF, Hill RG, Giliam DG. Abrasive wear of enamel

[24] by bioactive glass-based toothpastes. The American Journal of Dentistry
2014;27:263-267.

Baig M, Cook R, Pratten J, Wood R. The effect of shape and size distribution of

[25] abrasive particles on the volume loss of enamel using micro-abrasion. Wear
2020;448-449:203212. https://doi.org/10.1016/j.wear.2020.203212.

Ganss C, Marten J, Hara AT, Schlueter N. Toothpastes and enamel

[26] erosion/abrasion-impact of active ingredients and the particulate fraction. Journal
of Dentistry 2016;54:62-67. https://doi.org/10.1016/j.jdent.2016.09.005.

Mass MC. Enamel structure and mirowear: An experimental study of the response

[27] of enamel to shearing force. American Journal of Physical Anthropology
1991;85:31-49.

[28] Wang CX, Lucas R, Smith AJ, Cooper PR. An in vitro screening assay for dental stain cleaning. BMC Oral Health 2017;17:Article number 37. https://doi.org/10.1186/s12903-016-0328-3.

[29] Parry J, Smith AJ, Sufi F, Rees GD. Effect of simulator design on *in vitro* profilometric assessment of toothpaste abrasivity. Wear 2012;278-279:34-40. https://doi.org/10.1016/j.wear.2011.12.018.

[30] Parry J, Harrington E, Rees GD, McNab R, Smith AJ. Control of brushing variables for the *in vitro* assessment of toothpaste abrasivity using a novel

[31] laboratory model. Journal of Dentistry 2008;36:117-124. https://doi.org/10.1016/j.jdent.2007.11.004.

Lambrechts P, Debels E, Landuyt KV, Peumans M, Meerbeek BV. How to

[32] simulate wear? Overview of existing methods. Dental Materials 2006;22:693-701.

Baxter PM, Davis WB, Jackson J. Toothpaste abrasive requirements to control

[33] naturally stained pellicle: The relation of cleaning power to toothpaste abrasivity. Journal of Oral Rehabilitation 1981;8:19-26.

Wilkinson JB, Pugh BR. Toothpastes - cleaning and abrasion. Journal of the Society of Cosmetic Chemists 1970;21:595-605.

Wülknitz P. Cleaning power and abrasivity of European toothpastes. Advances in Dental Research 1997;11:576-579.

Table 1 Particle size characteristics of the tested calcined alumina abrasives*

Abrasive	d10 [µm]	d50 [µm]	d90[µm]
Calcined Alumina ultrafine	1.1	2.2	5.2
Calcined Alumina 3 µm	2.3	4.3	7.8
Calcined Alumina 9 µm	4.4	7.4	11.9
Calcined Alumina 20 µm	8.2	14.0	23.2

* Data are average values of three measurements. The d10, d50 and d90 values indicate that 10%, 50% and 90% of the particles measured were less than or equal to the size stated.

Table 2 Surface finish and wear depth of polished bovine enamel (n=8, mean ± standard deviation)

Brush strokes	Parameters	ultrafine	3 µm	9 µm	20 µm
Before	Gloss, GU	107.3±1.1	108.1±1.0	108.4±1.1	108.7±0.7
	Roughness, µm	0.036±0.009	0.037±0.004	0.031±0.005	0.034±0.005
1000	Gloss, GU	101.2±3.2	89.7±3.2	81.0±6.8	73.5±5.0
	Gloss change, GU	-6.1±2.8	-18.4±2.8	-27.4±5.9	-35.3±4.8
	Roughness, µm	0.042±0.013	0.048±0.009	0.060±0.007	0.055±0.006
	Roughness change, µm	0.006±0.015	0.012±0.008	0.029±0.008	0.021±0.010
	Wear depth, µm	0.058±0.013	0.182±0.109	0.107±0.034	0.156±0.075
3000	Gloss, GU	100.9±4.1	85.6±4.2	74.3±8.3	65.7±4.2
	Gloss change, GU	-6.4±4.1	-22.5±3.6	-34.1±7.4	-43.0±4.1
	Roughness, µm	0.051±0.016	0.057±0.007	0.075±0.018	0.072±0.014
	Roughness change, µm	0.015±0.019	0.020±0.008	0.044±0.020	0.039±0.013
	Wear depth, µm	0.094±0.025	0.393±0.142	0.262±0.100	0.276±0.094
5000	Gloss, GU	101.0±4.4	83.4±3.6	73.5±7.7	64.5±6.3
	Gloss change, GU	-6.3±4.1	-24.7±3.3	-34.9±6.9	-44.2±6.0
	Roughness, µm	0.043±0.006	0.065±0.008	0.085±0.018	0.082±0.011
	Roughness change, µm	0.007±0.012	0.028±0.007	0.053±0.018	0.049±0.012
	Wear depth, µm	0.110±0.030	0.675±0.226	0.471±0.202	0.421±0.109
10000	Gloss, GU	102.5±4.9	78.3±7.1	69.2±9.6	60.2±6.6
	Gloss change, GU	-4.8±4.4	-29.8±6.5	-39.2±8.8	-48.6±6.4
	Roughness, µm	0.054±0.011	0.099±0.023	0.110±0.033	0.105±0.020
	Roughness change, µm	0.017±0.015	0.063±0.023	0.079±0.032	0.071±0.022
	Wear depth, µm	0.204±0.070	1.376±0.439	0.833±0.372	0.778±0.200

Table 3 Surface finish and wear depth of partially roughened bovine enamel (n=8, mean ± standard deviation)

Brush strokes	Parameters	ultrafine	3 µm	9 µm	20 µm
Before	Gloss, GU	17.6±5.8	22.0±6.8	17.3±6.0	18.0±4.9
	Roughness, µm	0.151±0.023	0.124±0.036	0.146±0.016	0.146±0.027
1000	Gloss, GU	46.2±6.9	76.8±10.7	62.3±6.7	47.9±6.5
	Gloss change, GU	28.6±3.6	54.7±6.1	45.1±6.0	30.0±3.1
	Roughness, µm	0.109±0.015	0.078±0.020	0.094±0.014	0.116±0.018
	Roughness change, µm	-0.043±0.011	-0.046±0.024	-0.052±0.016	-0.031±0.013
	Wear depth, µm	0.216±0.026	0.216±0.062	0.195±0.021	0.317±0.088
3000	Gloss, GU	55.6±2.4	84.6±7.8	73.6±5.0	57.2±5.2
	Gloss change, GU	38.0±5.5	62.6±6.7	56.4±4.8	39.2±4.1
	Roughness, µm	0.0928±0.0110	0.0697±0.0167	0.0828±0.0121	0.1111±0.0210
	Roughness change, µm	-0.0582±0.0155	-0.0539±0.0270	-0.0629±0.0107	-0.0351±0.0263
	Wear depth, µm	0.121±0.0222	0.3694±0.1684	0.2405±0.0566	0.4256±0.1380
5000	Gloss, GU	65.8±4.0	84.9±6.3	76.1±3.3	60.4±4.9
	Gloss change, GU	48.2±8.2	62.9±7.9	58.8±4.6	42.4±5.5
	Roughness, µm	0.083±0.008	0.071±0.015	0.082±0.008	0.104±0.012
	Roughness change, µm	-0.068±0.019	-0.053±0.031	-0.064±0.017	-0.043±0.022
	Wear depth, µm	0.213±0.038	0.619±0.257	0.380±0.147	0.492±0.186
10000	Gloss, GU	90.2±6.3	79.9±8.3	73.4±4.1	60.9±6.0
	Gloss change, GU	72.6±9.2	57.9±8.2	56.1±7.7	42.9±6.5
	Roughness, µm	0.065±0.005	0.102±0.027	0.098±0.008	0.108±0.019
	Roughness change, µm	-0.086±0.023	-0.021±0.040	-0.048±0.019	-0.038±0.023
	Wear depth, µm	0.223±0.050	1.226±0.584	0.707±0.401	0.696±0.312

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473 ***Figure legends***

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Figure 1 Characteristics of the tested calcined alumina abrasives.
Particle size distribution: Particle size distributions of the calcined alumina abrasives determined by using Malvern Mastersizer 2000 laser diffraction particle size analyser. Data are average values from three measurements. The volume distribution is defined as the distribution per volume of the particle sizes, shown as Volume %, a differential of total volume of all counts.
SEM observation: Representative SEM micrograph of the tested calcined alumina abrasives particles. a) ultrafine; b) 3 µm; c) 9 µm; and d) 20 µm. Differences in particle size and range of morphologies for the four calcined alumina abrasives can be clearly observed. For the ultrafine abrasive, agglomerates were observed due to its very fine particle size and individual particles were smaller than 1 µm. All the abrasives consisted of a mixture of relatively small and large particles

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Figure 2 Relationship between brush strokes and enamel loss (wear depth) obtained from polished bovine enamel up to 10,000 brush strokes for the abrasive particles studied. A linear relationship (r^2=0.99) was found between brushing strokes and wear depth (enamel loss, shown as mean and standard deviation) for the tested alumina abrasives.

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Figure 3 a) Stain removal efficacy for roughened bovine enamel specimens after 1000 strokes when brushed with calcined alumina abrasives; b) Representative images of enamel surfaces before staining, after staining and post-stain removal with 1000 brush strokes. (left to right): before stain; after staining; stain brushing with ultrafine abrasive; stain brushing with 3 µm abrasive; stain brushing with 9 µm abrasive; stain brushing with 20 µm abrasive.

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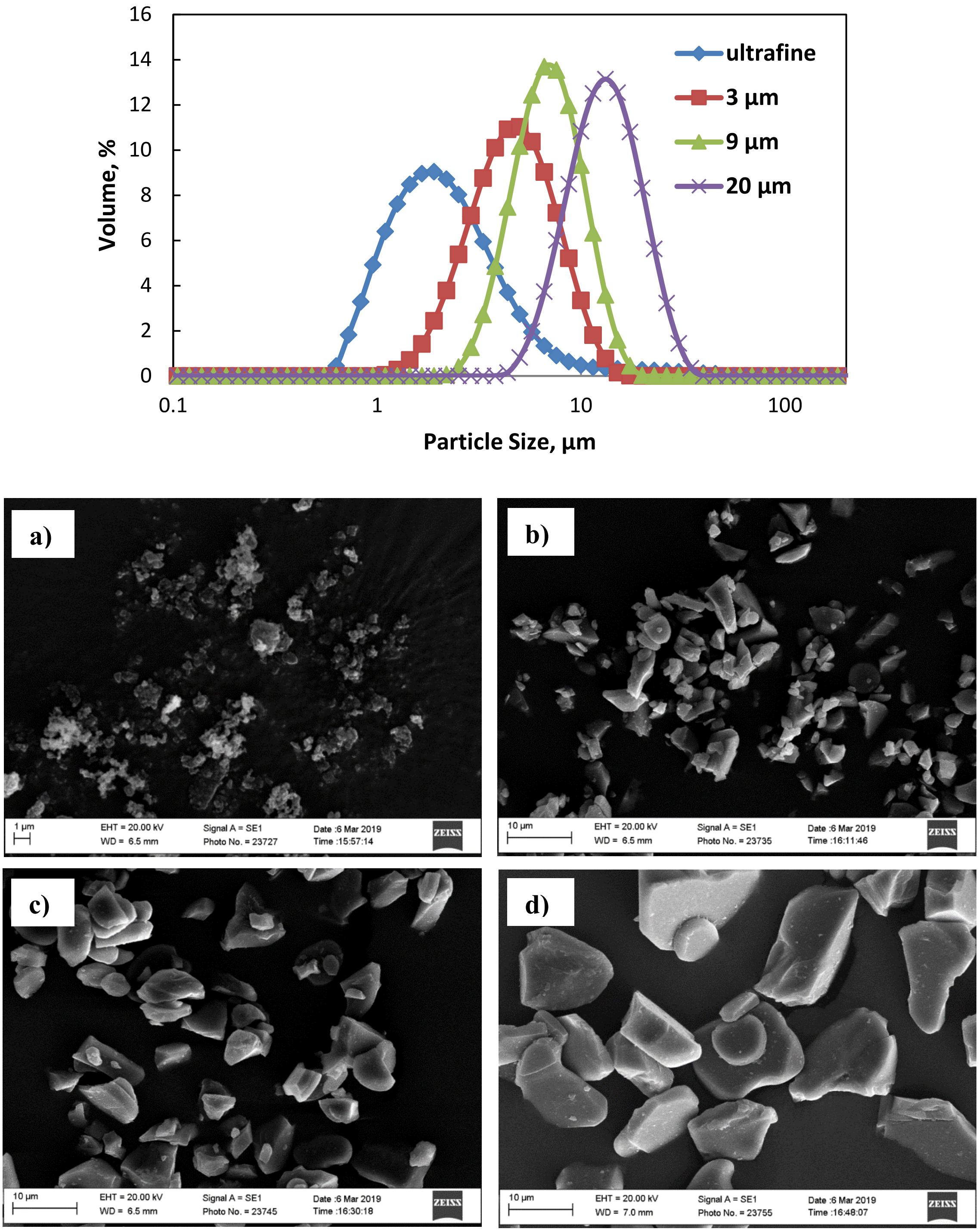
Figure 4 The relationship between enamel abrasivity, polishing capability (gloss change), and *in vitro* stain removal efficacy against abrasive particle size (d10, d50 and d90). The d10, d50 and d90 values indicate that 10%, 50% and 90% of the particles measured were less than or equal to the size stated.

a) Enamel wear depth of polished specimens after 10,000 brush strokes plotted against calcined alumina particle size (d10, d50 and d90);
b) Gloss change of partially roughened enamel specimen surfaces after 3,000 brush strokes plotted against calcined alumina particle size (d10, d50 and d90);
c) Stain removal efficacy of roughened enamel specimens after 1,000 brush strokes plotted against calcined alumina particle size (d10, d50 and d90).



16
14
12
10
8
6
4
2
0
Volume, %
0.1
1
10
100
Particle Size, µm
ultrafine
3 µm
9 µm
20 µm
a)
b)
c)
d)

Figure 1

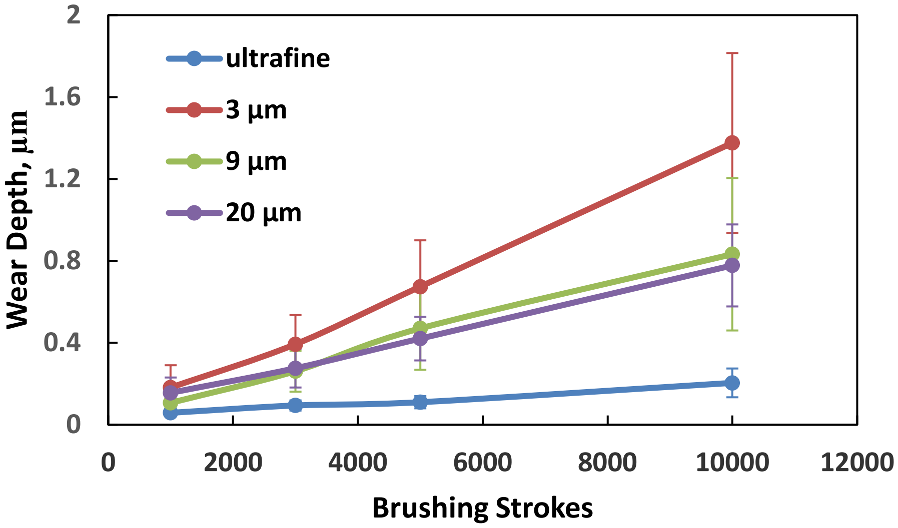

ultrafine
3 µm
9 µm
20 µm
Wear Depth, µm
2
1.6
1.2
0.8
0.4
0
0
2000
4000
6000
8000
10000
12000
Brushing Strokes

Figure 2

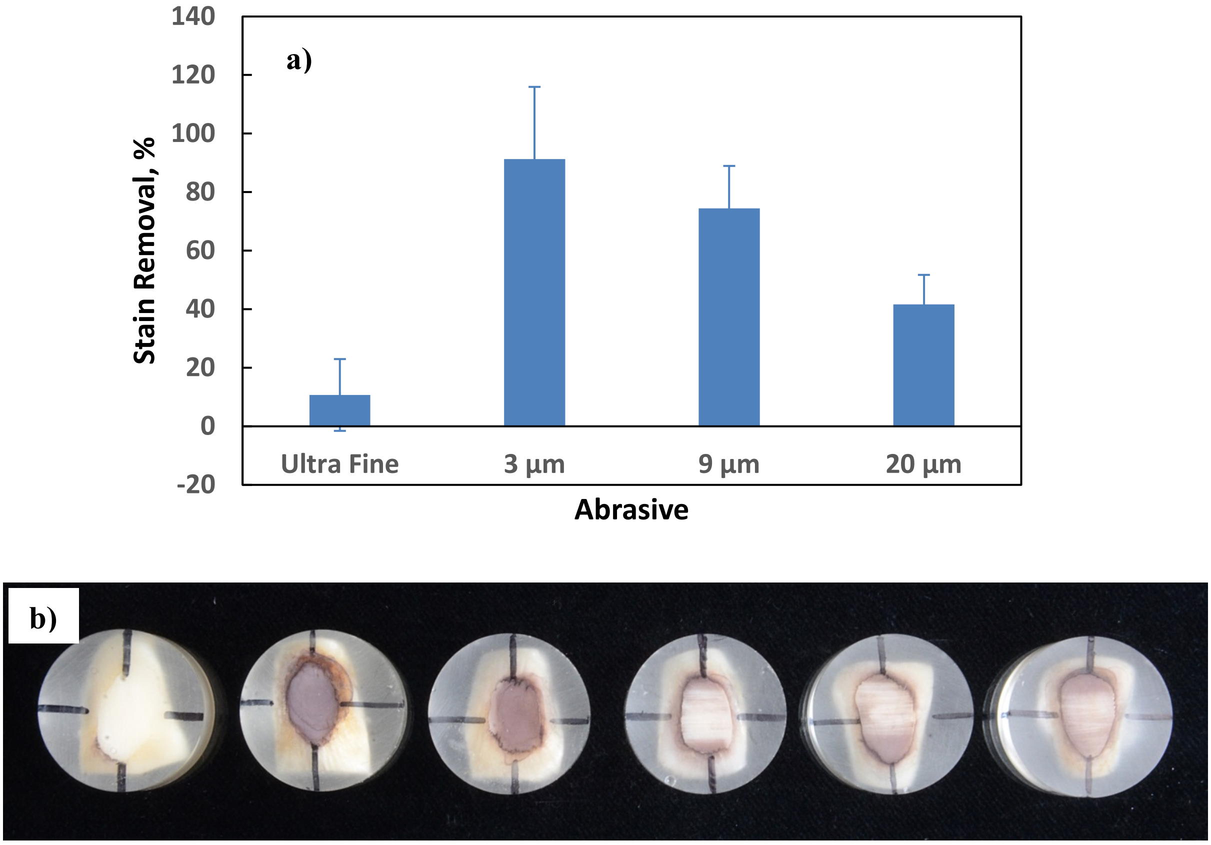

a)
Stain Removal, %
140
120
100
80
60
40
20
0
-20
Ultra Fine
3 µm
9 µm
20 µm
Abrasive
b)

Figure 3

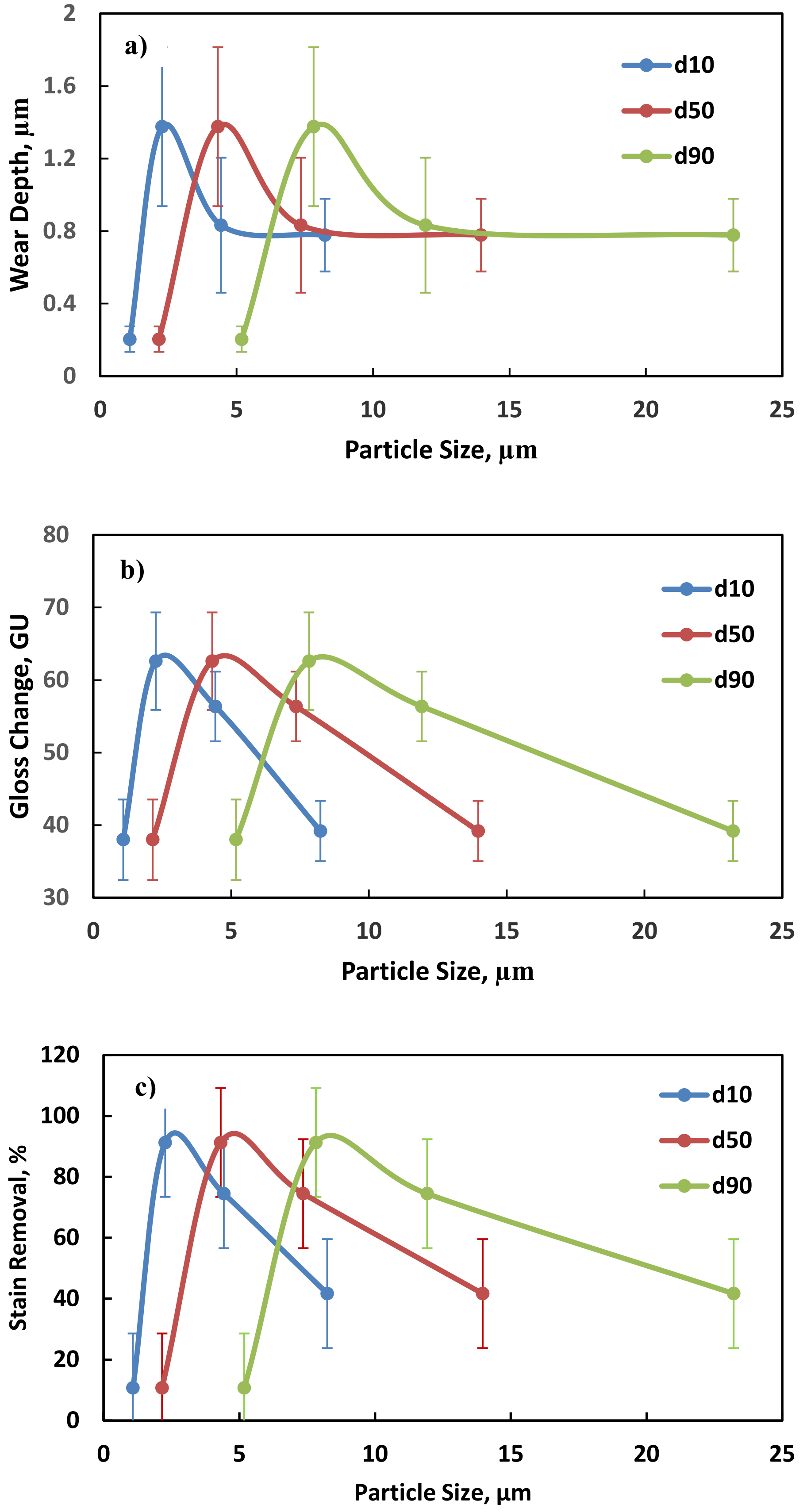

a)
Wear Depth, µm
2
1.6
1.2
0.8
0.4
0
0
5
10
15
20
25
Particle Size, µm
d10
d50
d90
b)
Gloss Change, GU
80
70
60
50
40
30
0
5
10
15
20
25
Particle Size, µm
d10
d50
d90
c)
Stain Removal, %
120
100
80
60
40
20
0
0
5
10
15
20
25
Particle Size, µm
d10
d50
d90

Figure 4