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ORIGINAL ARTICLE

Effective use of an extraoral vacuum in preventing the dispersal of particulate matter from metal dental materials

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Abstract

Objectives: When dentists adjust prostheses at the dental chairside, particulate matter (PM) from the dental material is dispersed. Little is known about the effective use of an extraoral vacuum (EOV) in preventing PM dispersal. This study aimed to evaluate the effective use of an EOV in preventing the dispersal of metal PM in dental offices.

Methods: The following experimental conditions were planned: the distance from the EOV to the metallic materials (50, 100, 150, and 200 mm), the horizontal angle between the long axis of the micromotor handpiece and EOV (0°, 45°, and 90°), and the operating time of the EOV (during grinding, during grinding and 1 min thereafter, during grinding and the preceding 1 min, 1 min before and after grinding, and during grinding). Carborundum and silicone points were used for grinding and PM dispersal. Diameters (0.3, 0.5, 1.0, and 3.0 μm) were measured using a laser particle counter.

Results: Depending on the instrument used to grind, there were undetectable PMs of different diameters (Carborundum point: 0.3 and 0.5 μm, Silicone point: 0.3 μm). PMNs were reduced as the distance from the EOV to metal materials decreased. Operating the EOV before grinding along the long axis of the micromotor handpiece was effective in reducing the PMNs.

Conclusion: PMNs dispersed when grinding metals were effectively reduced by positioning the EOV closer to the grinding surface (within 150 mm) along the long axis of the micromotor handpiece and operating the EOV before grinding.

KEYWORDS
dental metal, dental offices, extraoral vacuum, particulate matter, pneumoconiosis

1 | INTRODUCTION

Aerosol was defined as "Sol in which the dispersed phase is a solid, a liquid, or a mixture of both and the continuous phase is a gas (usually air)," and within which particles with equivalent diameters usually between 0.01 and 100 μm are specified.[1]

During dental treatments, aerosols of various particle diameters are generated as various materials and teeth are ground and polished in dental offices. Some of the aerosols generated in the dental offices are solid (dental material alone [i.e., dental metal or resin], liquid [i.e., saliva, blood, and liquid containing bacteria, viruses, and other pathogens], and a mixture of both [liquids adhering to solid as a nucleus]).[2,3]

During the COVID-19 pandemic, many reports focused on aerosol "infection" in the dental office[4-6]; however, we should give attention to the inhalation of aerosols of solids ("particulate matter," PM) and mixtures of solids and liquids with solids as nuclei (PM) into the body. The particulate matter-induced acute phase response increases the risk of PM-induced cardiovascular disease (i.e., myocardial infarction, arrhythmia, and thrombosis), which identifies cardiovascular diseases as occupational diseases.[7,8]

Dental technicians often suffer from respiratory diseases such as pneumoconiosis, pulmonary fibrosis, and asthma.[9-13] Moreover, PM originating from dental material has been detected in histopathological examinations of the lungs of dentists and dental technicians.[14] These reports indicate that dental professionals are at high risk of PM inhalation due to the grinding (cutting and polishing) of dental material. PM generated during prosthetic adjustment, the same as that generated during tooth preparation and scaling, is suspected to remain suspended in the dental office for a long time.[15,16] Some studies have suggested that aerosols with diameters of less than 5 μm are widely distributed in the long term, and both patients and dental professionals inadvertently inhale them in dental offices.[3,17]

Hence, collecting them and preventing their dispersal in the dental office is important. In recent years, the use of extraoral vacuums (EOVs) has been recommended to prevent the dispersal of aerosols during dental treatment.

There have been reports of the use of local exhaust ventilation to reduce the dispersal of PM[18-20] in the dental laboratory. However, little is known about the effective use of local exhaust ventilation (e.g., EOV) in reducing the dispersion of "PM" at the chairside in the dental office.

This study aimed to evaluate the effective use of EOV to prevent the spread of PM generated at the dental chairside in the dental office.

2 | MATERIALS AND METHODS

The number of PM particles (PMNs) per cubic meter (m^3) was measured at the dental division of Tokushima University Hospital. The measurements of conditions are shown in Figure 1. An EOV (Free Arm Altio-T, Tokyo Giken; air pressure of 3.0 kPa at an air volume of 3.0 m^3/min) with a hood (Altio N Food [φ100 mm × D74 mm]) was used. Although hoods for EOVs have various forms (e.g., box-type hoods specialized for chairside laboratory work, scoop-type L-hoods), the N-hood, which is the most versatile and most frequently used by many of our dentists, was used in the study.

A plate-like grinding specimen (10 × 10 × 35 mm) was fabricated using a cast gold-silver-palladium alloy (12% Au, 20% Pd, 46% Ag, 20% Cu: Castwell M.C., GC, Tokyo) via the lost wax method and finally shaped by grinding.

The grinding was performed by one prosthodontist (with 12 years of relevant work experience) using a dental micromotor handpiece (TORQTECH ST-DH, J. MORIT CORP) with carborundum (Shofu Carborundum Point HP13, Shofu) or silicone (Shofu Silicone Point M2, Shofu) points. The dentist was asked to grind a 10 × 10-mm surface of the specimen at a frequency of 1 s^{-1}, and the maximum rotational speed of the micromotor handpiece during grinding was set to 30 000 min^{-1} without water injection. The prosthodontist was trained to grind with a contact pressure of 0.49–0.69 N, which was the average contact pressure applied by three prosthodontists (average relevant work experience: 10.3 years) in the free grinding of the metal crown's approximal surface without notice in a preliminary experiment. Furthermore, previous reports did not identify the force applied when adjusting the metal crown's approximal surface, and the grinding contact pressure was determined with reference to reports showing that the formation of living teeth by skilled dentists ranged from 0.49 N to 0.98 N.[21]

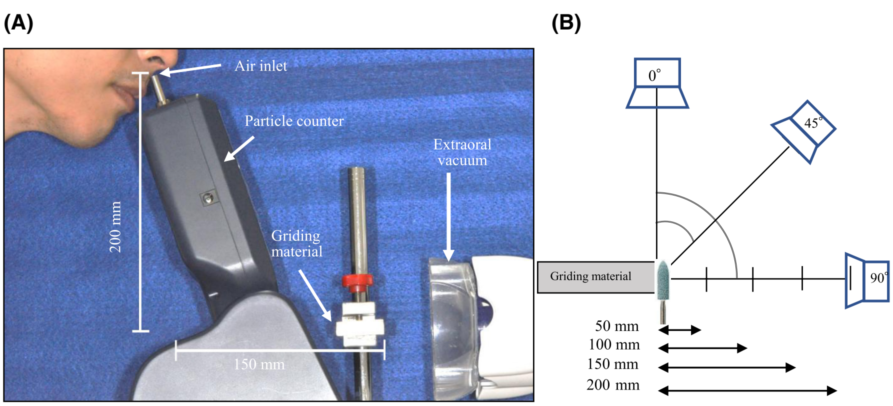

FIGURE 1 Measurement conditions. (A) Positional relationship of the grinding material, air inlet of the particle counter, and extraoral vacuum. (B) Setup conditions for the distance and angle between the grinding material and the extraoral vacuum.

A laser particle counter (suction flow rate: 2.83 L/min; MODEL 3889, KANOMAX) was used to measure PMNs of six different sizes (0.3, 0.5, 1.0, 3.0, 5.0, and 10.0 μm in diameter). Although the particles had diameters of ≥5 μm, which are widely distributed over the long term, we focused on the smaller particle diameters of 0.3, 0.5, 1.0, and 3.0 μm, which are considered more harmful to the human body. The total duration of measurement was 8 min, and grinding of the specimen was performed for 2 and 3 min after initiating measurement. The PMNs were measured 17 times over 10 s at 20-s intervals.

The inlet of the particle counter was set 200 mm above and 150 mm behind the grinding surface of the specimen and 1200 mm above the floor (Figure 1A). This position of the inlet was determined in the preliminary experiment, in which three prosthodontists ground the specimen. Each of the three prosthodontists performed a preliminary experiment for 3 days (on different days), grinding under the same conditions as the experimental times to determine.

The measurements were initiated at least 5 h after the closing of the dental office, with the air conditioner switched off to eliminate the influence of floating PM and aerosols generated during the day's dental treatments. Furthermore, for the optimal elimination of the PMNs floating in the dental office room, the average PMNs between the initiation of measurement and the initiation of grinding were defined as the baseline for each measurement. The measurements were taken under the following three conditions to determine the appropriate use of the EOV.

1. Distance from the grinding surface to the EOV.

The angle of the suction surface of the EOV relative to the long axis of the grinding metal specimen was fixed at 90°. The operating time of the EOV was fixed at 2 min during grinding, and the distances from the grinding surface to the EOV were set at 50, 100, 150, and 200 mm (Figure 1B).

2. Angle between the long axis of the micromotor handpiece and the EOV.

The distance from the grinding surface of the metal specimen to the EOV was fixed at 200 mm. The operating time of the EOV was fixed at 2 min during grinding, and three angles of the long axis of the micromotor handpiece relative to the suction surface of the EOV were 0°, 45°, and 90° (Figure 1B).

3. Operating time of the EOV.

The angle between the long axis of the grinding metal specimen and the EOV was fixed at 90°, and the distance from the grinding surface of the metal specimen to the EOV was fixed at 200 mm. The operating time of the EOV was set for four different durations as follows: (i) 2 min only while grinding the metal specimen, (ii) 2 min during grinding and 1 min after grinding, (iii) 2 min during grinding and 1 min before grinding, and (iv) 2 min during grinding and 1 min each before and after grinding.

2.1 | Statical analysis

The PMNs were measured three times for every condition. The differences in the PMNs at different distances, angles, and the duration of operation of the EOV were compared using Dunnett's test. Statistical analyses were performed using IBM SPSS Statistics 25 (IBM Japan).

3 | RESULTS

Table 1 shows the concentration of particle matter before, during, and after grinding under conditions in which no extraoral vacuum was used. (1) Patterns in which the concentration did not increase during grinding and did not change after grinding (carborundum points: 0.3, 0.5 μm, silicon point: 0.3 μm), (2) Patterns in which the concentration increased during grinding and decreased after grinding (carborundum points: 1.0, 3.0 μm, silicon point: 0.5, 1.0, 3.0, 5.0, and 10.0 μm), and (3) patterns that did not change during grinding but increased in concentration after grinding (carborundum point: 5.0 and 10.0 μm). The upper row shows the raw data of the average value, and the lower row shows the percentage increase/decrease of the average value during and after grinding, as well as before grinding.

Figure 2 shows the time-dependent changes in the PMNs with diameters of 1.0 μm when the specimens were ground using a silicone point at the four distances from the grinding surface of the specimen to the EOV. When the EOV was used, the PMNs gradually increased from the initiation of grinding of the metal specimen, reached a peak 1 min after the initiation of grinding, then decreased and immediately returned to the baseline level (before grinding) at the end of grinding. The change in the pattern of PMNs over time was almost identical regardless of the distance between the grinding surface and the EOV, and the peak of the PMNs decreased as the distance decreased. When the EOV was not used, the peak occurred approximately 1.5 min after the initiation of grinding, and the PMNs did not return to the baseline levels for a while. However, there was little change in the 0.3-μm and 0.5-μm-diameter PMNs when grinding with the carborundum point and the

TABLE 1 Particulate matter concentration without using the extraoral vacuum.

	Particle size (µm)	0 min ≦ Time < 3 min (Before grinding)	3 min ≦ Time < 5 min (During grinding)	5 min ≦ Time ≦ 8 min (After grinding)
Carborundum points	0.3	$5.92\times10^{7}\pm4.31\times10^{6}$	$5.94\times10^{7}\times4.17\times10^{6}$ (0.33%)	$5.92\times10^{7}\pm4.16\times10^{6}$ (0.00%)
	0.5	$5.70\times10^{6}\pm2.88\times10^{6}$	$5.94\times10^{6}\pm2.93\times10^{5}$ (4.21%)	$5.81\times10^{6}\pm1.87\times10^{5}$ (1.93%)
	1.0	$5.78\times10^{5}\pm2.43\times10^{4}$	$9.59\times10^{5}\pm3.31\times10^{5}$ (65.92%)	$6.21\times10^{5}\pm7.49\times10^{4}$ (7.44%)
	3.0	$2.88\times10^{4}\pm1.02\times10^{4}$	$3.52\times10^{5}\pm2.49\pm10^{5}$ (1122.22%)	$7.93\times10^{4}\pm7.18\times10^{4}$ (175.35%)
	5.0	$1.08\times10^{4}\pm4.25\times10^{4}$	$1.05\times10^{4}\pm3.73\times10^{3}$ (-2.78%)	$1.19\times10^{4}\pm4.38\times10^{3}$ (10.19%)
	10.0	$3.43\times10^{3}\pm1.32\times10^{3}$	$3.73\times10^{3}\pm1.06\times10^{3}$ (8.75%)	$4.24\times10^{3}\pm1.10\times10^{3}$ (23.62%)
Silicone points	0.3	$6.72\times10^{7}\pm5.23\times10^{5}$	$6.62\times10^{7}\pm1.80\times10^{6}$ (-1.49%)	$6.73\times10^{7}\pm7.66\times10^{5}$ (0.15%)
	0.5	$6.07\times10^{6}\pm1.93\times10^{5}$	$8.18\times10^{6}\pm2.19\times10^{6}$ (34.76%)	$6.67\times10^{6}\pm8.51\times10^{5}$ (9.88%)
	1.0	$6.16\times10^{5}\pm7.04\times10^{4}$	$1.65\times10^{6}\pm1.04\times10^{6}$ (167.85%)	$1.01\times10^{6}\pm6.12\times10^{5}$ (63.96%)
	3.0	$2.95\times10^{4}\pm8.10\times10^{3}$	$2.47\times10^{5}\pm3.17\times10^{5}$ (737.29%)	$9.61\times10^{4}\pm1.01\times10^{5}$ (225.76%)
	5.0	$7.18\times10^{3}\pm3.47\times10^{3}$	$5.29\times10^{4}\pm6.63\times10^{4}$ (636.76%)	$2.06\times10^{4}\pm2.47\times10^{4}$ (186.91%)
	10.0	$2.71\times10^{3}\pm2.43\times10^{3}$	$6.18\times10^{3}\pm4.93\times10^{4}$ (147.97%)	$4.74\times10^{3}\pm2.61\times10^{3}$ (74.91%)

Note: The upper row shows the raw data of the average value and the lower row shows the percentage of increase/decrease ratio of the average value during and after grinding, as well as before grinding.

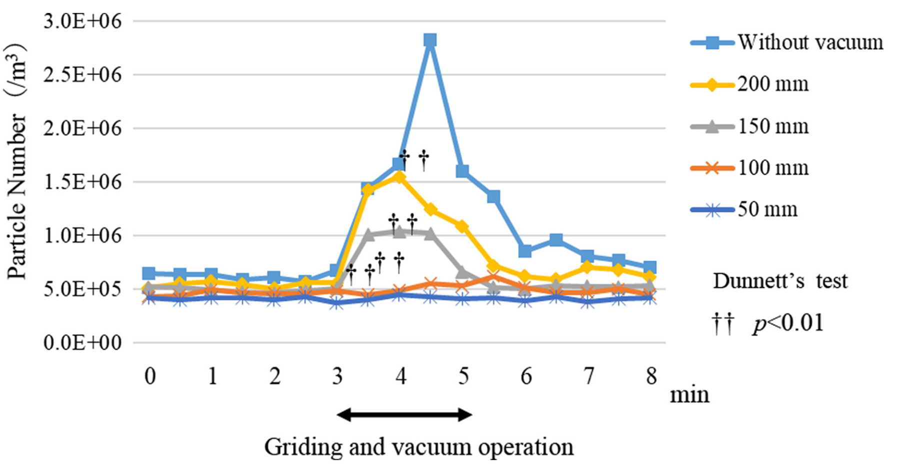

FIGURE 2 Typical time course of changes in the amount of particulate matter (silicone point; PMNs measuring 1.0 µm in diameter [d]; distance: 200 mm, angle: 90°).

0.3-µm-diameter PMNs when grinding with the silicone point; therefore, they were excluded from the statistical analysis.

Figure 3 shows the decrease in the ratios of the maximum PMNs at four different distances between the grinding surface of the metal specimen and the EOV. The reduction ratio refers to the maximum PMN with the use of the EOV under each condition/the maximum PMN without the use of the EOV. The maximum PMNs decreased with the use of the EOV, and the decrease was almost inversely proportional to the distance between the grinding surface of the metal specimen and the EOV. At a distance of 100 mm, the maximum PMNs decreased by 83.7%–89.2% ($P<.01$) on grinding with the carborundum point and by 66.2%–97.3% ($P<.01$) on grinding with the silicone point. The 3.0-µm-diameter PMNs also quickly decreased at a distance of 200 mm on grinding with the silicone point.

Figure 4 shows the decrease in the ratios of the maximum PMNs at three different angles between the long axis of the micromotor handpiece and the EOV. The maximum PMNs were affected by the angle, and the reduction ratio was at its highest when the angle was 0°, in which the EOV was positioned along the extended line of the long axis of the micromotor handpiece. The reduction ratios were 82.6%–97.7% ($P<.01$) using the carborundum point and 91.9%–99.1% ($P<.01$) using the silicone point. When we used the carborundum point, the reduction ratios were significant under all conditions for 1.0-µm and 3.0-µm-diameter PMNs ($P<.01$: Only 0.5 µm at 90° were $P<.05$). When the silicone point was used, the reduction ratios were significantly higher for 1.0-µm and 3.0-µm-diameter PMNs ($P<.01$) but were not significantly higher at 90° for 0.5-µm-diameter PMNs ($P=.382$).

Figure 5 shows the reduction ratios of the maximum PMNs for four different operating durations of the EOV.

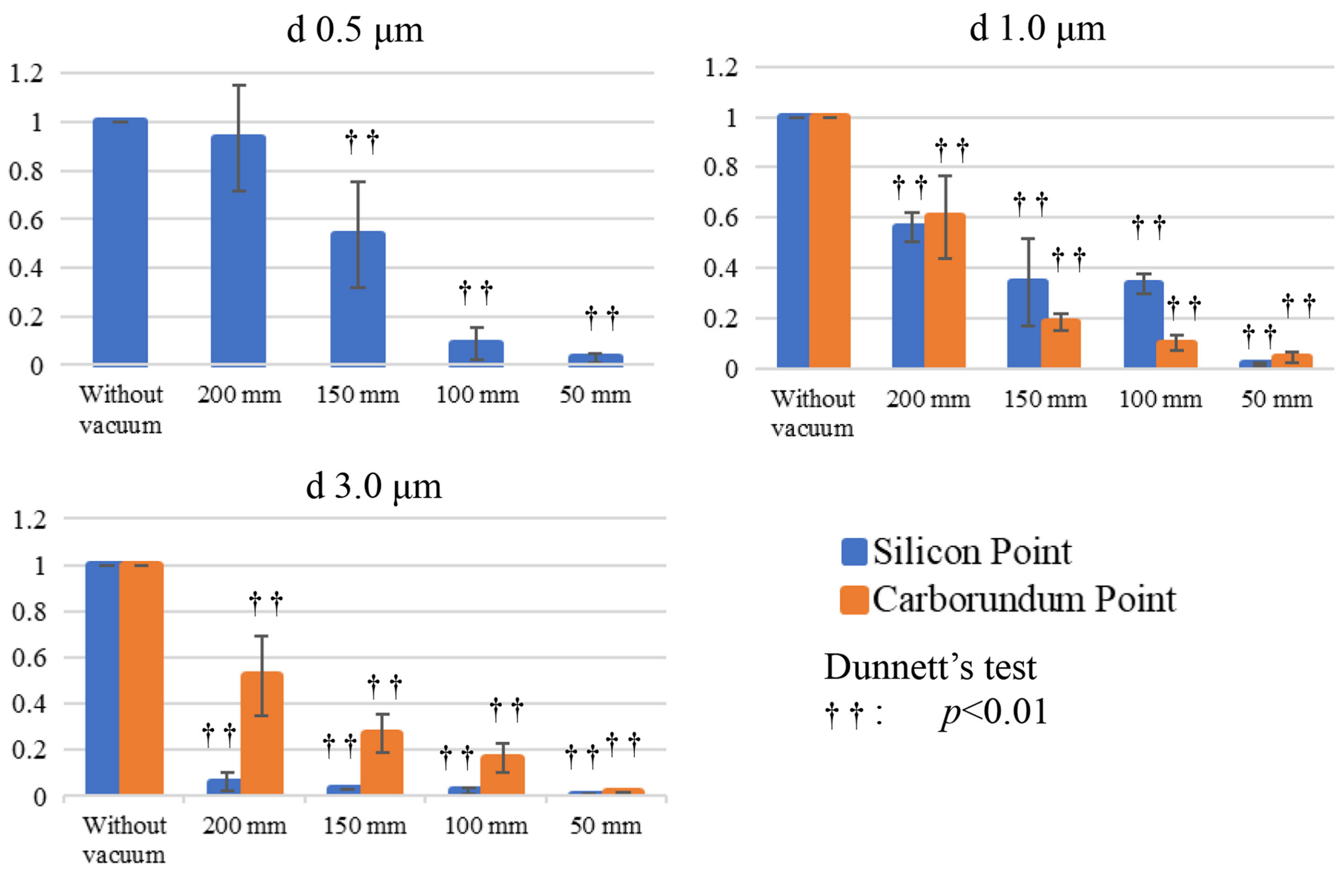

FIGURE 3 Reduction ratios: Influence of the distance between the grinding surface and extraoral vacuum on the maximum amount of particulate matter.

For PM of all sizes, the reduction ratio when the EOV was operated 1 min before grinding was higher than that when the EOV was used at the time of initiation of grinding. The pattern of change in the PMNs with time was not affected regardless of the duration of operation of the EOV and showed a similar increase and decrease pattern.

4 | DISCUSSION

There have been some reports which focused on "infection" regarding the prevention of the dispersion of aerosol generated from the oral cavity during dental treatments in dental offices, and reports which focused on "particulate matter" generated in the dental laboratory.[19,20] According to a previous report, the prevalence of pneumoconiosis among dental technicians varied from 4.6% in 12.5 years[22] to 50.0% in 12.2 years.[23] This difference is reportedly due to the working environment (self-employment or not; i.e., the availability or nonavailability of facilities for ventilation and the use or nonuse of toxic materials).[24] Froudarakis et al.[9] concluded that the presence of adequate ventilation facilities affected the prevalence of pneumoconiosis among dental technicians. On the other hand, the effect of local exhaust ventilation (EOVs) on the PM generated at the dental chairside during the adjustment of metal dental prostheses has not been reported. Although the use of digital technology such as CAD/CAM has become widespread, dentists still polish and adjust metal dental prostheses by the chairside in the dental office; therefore, they are highly likely to inhale the dental materials of PMs during dental practice, and more likely to suffer from respiratory diseases such as pulmonary fibrosis, silicosis, allergic pneumoconiosis, asthma, and lung cancer. There have been reports of dental pneumoconiosis thought to be caused by PMNs.[25,26] It is important to examine the effective use of the EOV because the EOV is the only local exhaust ventilation when dentists adjusted dental prostheses in dental offices.

The novel coronavirus infection has reaffirmed the usefulness of masks in preventing the spread of infections. Dental staff (dentist, dental hygienist, dental assistants, etc.) have been taking "infection control" measures such as the use of surgical masks and handwashing to prevent infection, even before the spread of the novel coronavirus infection. However, dental staff were less aware that the metal dental material "PMs" generated by grinding and polishing dental materials discussed in this report induced respiratory

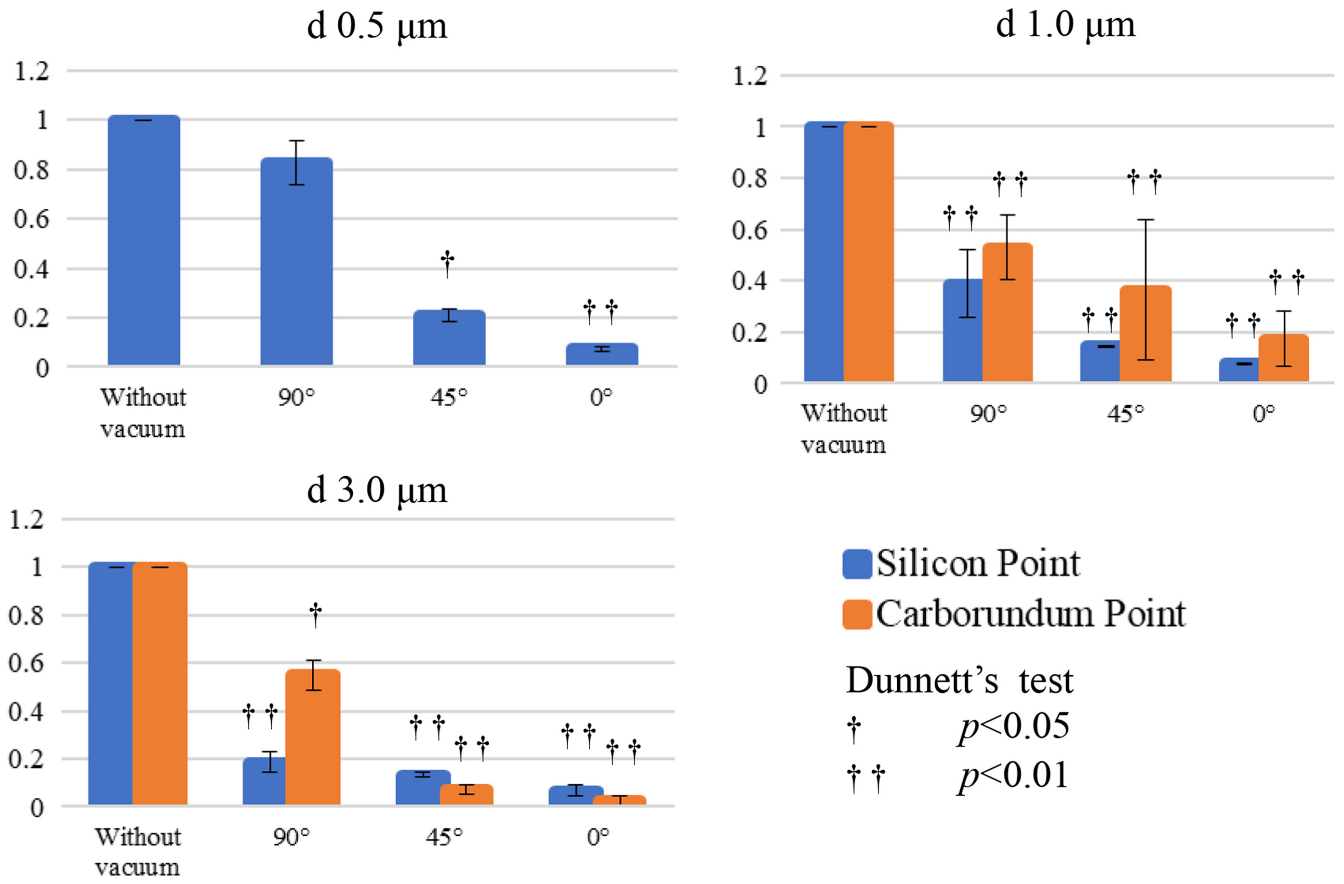

FIGURE 4 Reduction ratios: Influence of the angle between the grinding surface and extraoral vacuum on the maximum amount of particulate matter.

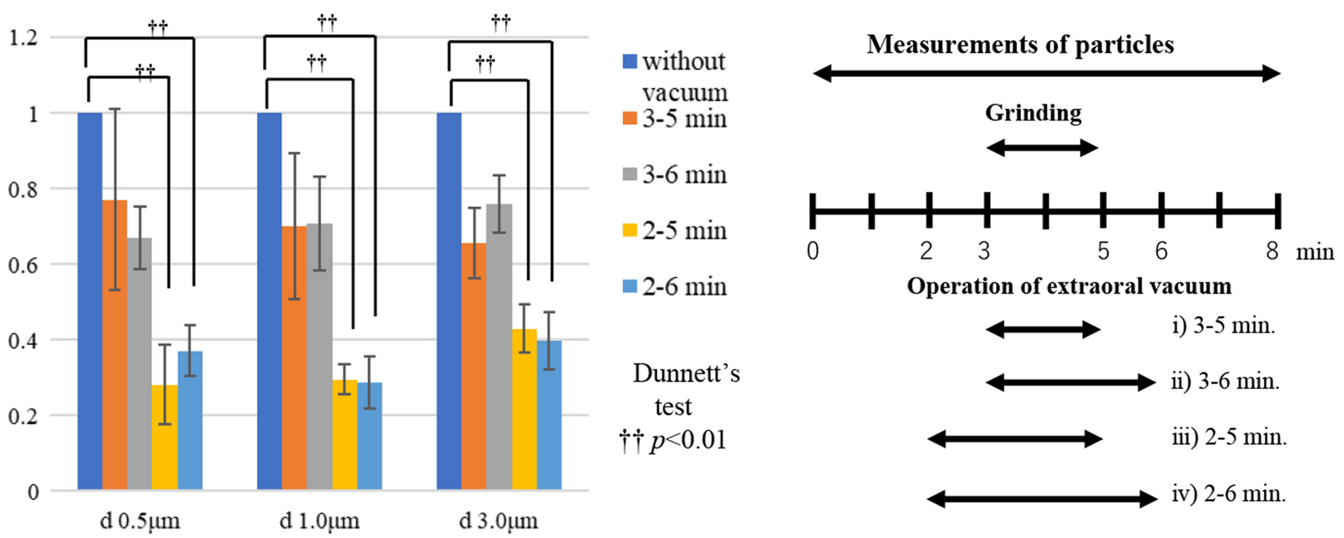

FIGURE 5 Reduction ratios: Influence of the operation time of the extraoral vacuum on the maximum amount of particulate matter: (silicone point; PMNs with the following diameters [d]: 0.5, 1.0, and 3.0 µm; distance: 200 mm, angle: 90°).

disease (i.e., pneumoconiosis) or cardiovascular disease (i.e., microvascular plaque). In the case of aerosols, masks made of combinations of different fabrics and multiple layers can block up to 90% of particles measuring 0.5–10.0 µm.[27] The presence of gaps between the skin surface and the mask decreases the overall filtration effect. With small-sized aerosols measuring less than 2.5 µm or a relative leak area of 1%,[28] the filtration efficiency decreased by 50%. The use of a mask cannot completely prevent the inhalation of PMs dispersed in the air because PMs of the size focused on in this report are smaller than the pore size of the mask.[29,30] Additionally, small PMs can enter the inside of the mask through the gap between the skin surface and the mask.[31-33] Therefore, personal protective equipment such as masks cannot completely prevent aerosol inhalation.

In this study, we detected PMs measuring 0.5, 1.0, and 3.0 (0.3 μm was not detected) which can still be inhaled even when using masks. Therefore, it is important to determine the usefulness of the EOV as a local exhaust in the dental office room.[34,35]

Although aerosols measuring ≤0.3 μm are generated in dental practices such as scaling or tooth formation, this study assumes infection by bacteria attached to the prosthetic metal; so, we did not consider 0.3 μm at the silicon point, where generation by grinding is considered extremely small. Therefore, we excluded 0.3 μm at the silicon point, 0.3 μm at the carborundum point, and 0.5 μm at the carborundum point. The performance of the local exhaust ventilation system is determined by the volume and air velocity of the exhaust air and the shape of the air food. The exhaust air volume is widely evaluated using the airflow calculation equation (Dalla Valle's equation) proposed by Dalla Valle[36]; however, the calculated value does not conform to the theoretical values due to various factors such as the size and shape of the hood's opening and the turbulence of airflow around the hood. Therefore, regression equations obtained from experimental data in the industry are available[37,38]; however, regression equations using dental EOVs have not been presented. Thus, it is more practical to evaluate dental local exhaust ventilation systems using actual reduction rates of the number of PMs rather than using theoretical equations. However, effective methods of preventing PM dispersal using the dental EOV have not yet been reported.

The results of this study also showed that the PMNs decreased as the distance between the grinding surface of the specimen and the EOV decreased, and the decrease was approximately inversely proportional to the distance. This result is consistent with the finding that the wind velocity near the front of the hood increases more rapidly near the hood-opening surface but decreases as one leaves this surface.

Table 1 shows the rate of increase in particles when the EOV was not operated. The rotation of the cutting tool of the handpiece scattered the chips toward the surgeon, and they settled down after grinding but were still suspended in the air. On the other hand, the 5.0-μm and 1.0-μm particles showed almost no increase during grinding; however, they increased after grinding. This suggests that the particles generated from the grinding were blown away by the wind generated from the tip of the handpiece and did not reach the surgeon's position; however, they were suspended and measured after grinding.

Similarly, at 0.3 μm, the microparticle concentration did not change, suggesting that no microparticles were generated by the grinding process. At 0.5, 1.0, 3.0, 5.0, and 10.0 μm, the rotating cutting tool of the handpiece scattered the particles toward the surgeon, and although they settled after grinding, it is presumed that they were still suspended in the air. From these facts, it can be inferred that the materials generated by the cutting of the carborundum point and the silicon point were different.

The reduction ratio of PMNs was at its highest when the EOV was positioned on the extended line along the long axis of the micromotor handpiece. Because compressed air is injected from the tip of the dental micromotor handpiece, the PM generated during grinding will be dispersed in the direction of the long axis and efficiently aspirated by the EOV placed along the same direction. Furthermore, the exhaust airflow is found to be at its highest on the long axis of the orientation of the local exhaust food in free space, which is consistent with the findings of this study.

Under the conditions in which the EOV was not operational, PMNs produced and the pattern of increase/decrease differed, even for same-size particles, depending on the tool used for grinding. The difference is thought to be due to differences in the generated particles themselves. Since the same flow rate and velocity were used for the EOV in this experiment, it is assumed that this difference is due to differences in the physicochemical properties (e.g., density, shape, surface area, electrostatic charge state, etc.) of the generated particle matter.

With respect to the time of operation of the EOV, the use of the EOV before grinding the metal specimen was very effective for all sizes of PMs, and the continuous operation of the EOV after grinding was also effective in suctioning scattered PM; however, the effect was limited. Using the EOV before grinding generates airflow in advance; hence, PMs would be efficiently removed at the initiation of grinding. Even when grinding at the farthest distance from the EOV, a significant effect was observed if the EOV was operated for 1 min before grinding; so, it is effective to generate airflow by operating the EOV 1 min before grinding.

Since the measurements in this report were performed while grinding the metal specimen only at the dentist's position, there is a possibility that PM could disperse in other directions, such as toward the patients and dental assistants. In addition, the materials dentists use include not only metal but resin, plaster, and impression materials with varying levels of toxicity. Therefore, the dispersal time and removal rates are different for different types of dental materials. Further studies on the effects of personal protection as well as general and local exhaust ventilation are necessary.

4.1 | Limitations in this study

1. In this report, the effectiveness of local exhaust ventilation (EOV) was only measured in the horizontal direction. A vertical effective position is necessary because rotating cutting instruments may also scatter PM in the vertical direction.
2. Because the airflow was not measured in this report, the effect of different local exhaust systems or different air foods cannot be mentioned. Theoretical values cannot be calculated because the compressors are not universal, and we have 100 dental chairs in our dental office running extraoral and intraoral vacuums.
3. Because grinding with a fixation device would reduce the grinding pressure due to the grinding of the specimen, the cutting was performed by a well-trained dentist (12 years of relevant work experience). There was no significant difference in the measured values, and we considered that the specimens were ground at a certain pressure within the setting; however, the object pressure value during grinding cannot be shown.

5 | CONCLUSION

The use of the EOV to prevent the spread of PM while grinding dental metal specimens in the dental office was effective when the EOV was placed closer to the grinding surface (at least within 150 mm), in the direction of the long axis of the micromotor handpiece and began operating at least 1 min before the grinding.

AUTHOR CONTRIBUTIONS

Hideki Suito conceived this study; Hideki Suito, Keiko Fujimoto, Takaharu Goto, and Kan Nagao collected and analyzed the data; Hidehiko Hosoki and Tetsuo Ichikawa provided their expert opinion on the subject; Hideki Suito wrote the first draft of the manuscript; Tetsuo Ichikawa critically reviewed the manuscript. All authors interpreted the study results, reviewed the manuscript, and approved its final version.

ACKNOWLEDGMENTS

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CONFLICT OF INTEREST STATEMENT

The local exhaust ventilation system used in this study was not provided by Tokyo Giken, and all other instruments and materials were purchased and placed at the expense of our hospital. Therefore, the authors have no conflicts of interest to declare.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

DISCLOSURES

Ethical approval: The study protocol was approved by the Ethics Committee of Tokushima University Hospital (Approval No. 3515), and the study was conducted as per the ethical guidelines of this hospital and the 1964 Helsinki Declaration and its later amendments or comparable ethical standards. *Informed consent*: Consent was obtained from all researchers who participated in this study. *Registry and the registration no. of the study/trial*: N/A. *Animal study*: N/A.

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