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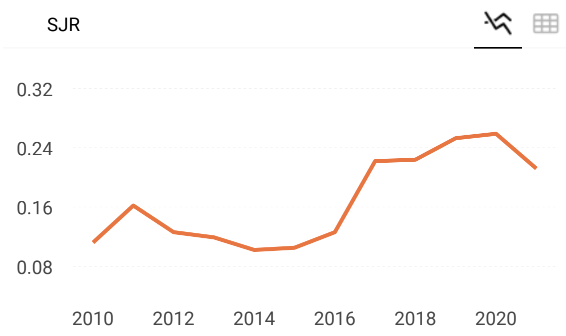

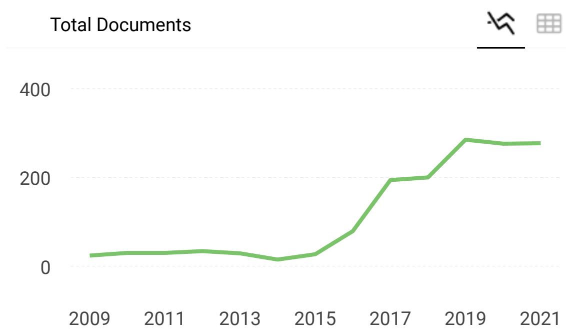

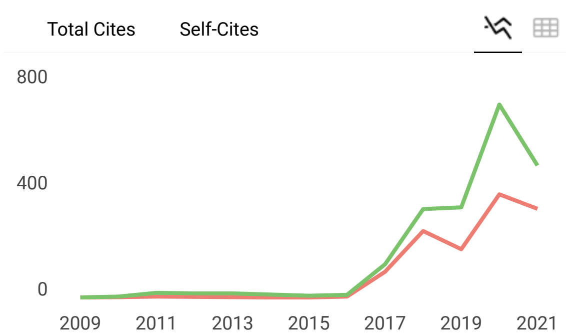

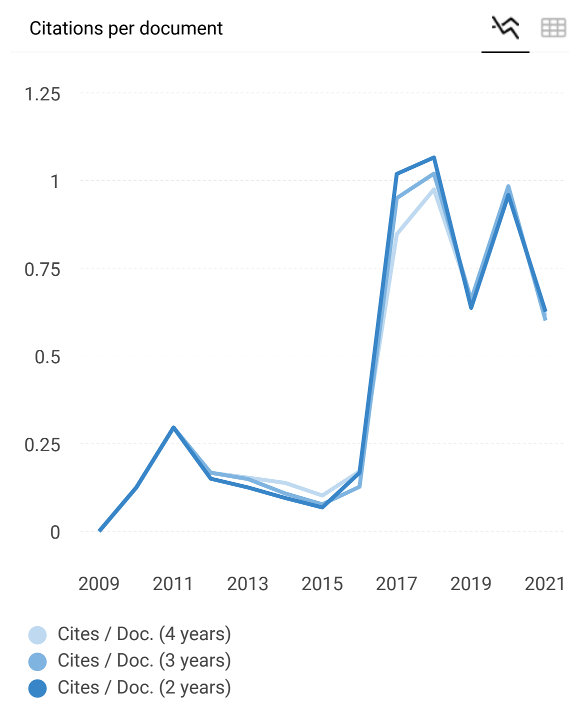

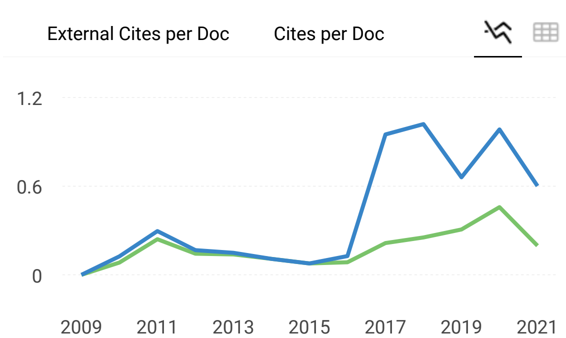

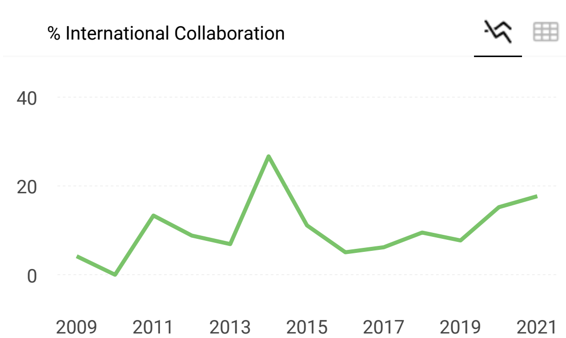

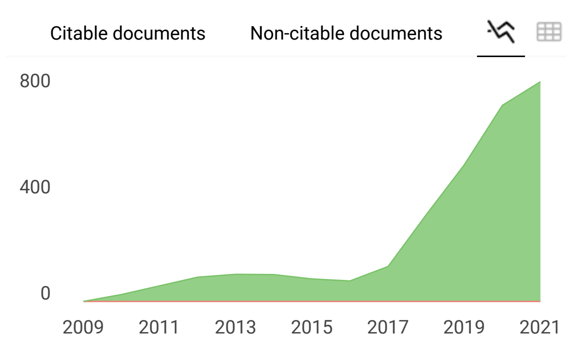

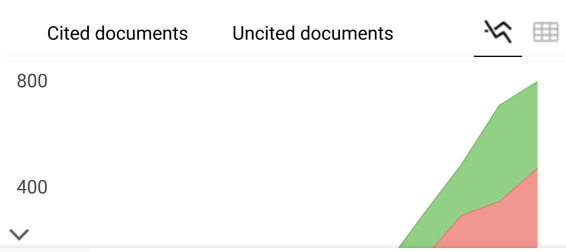

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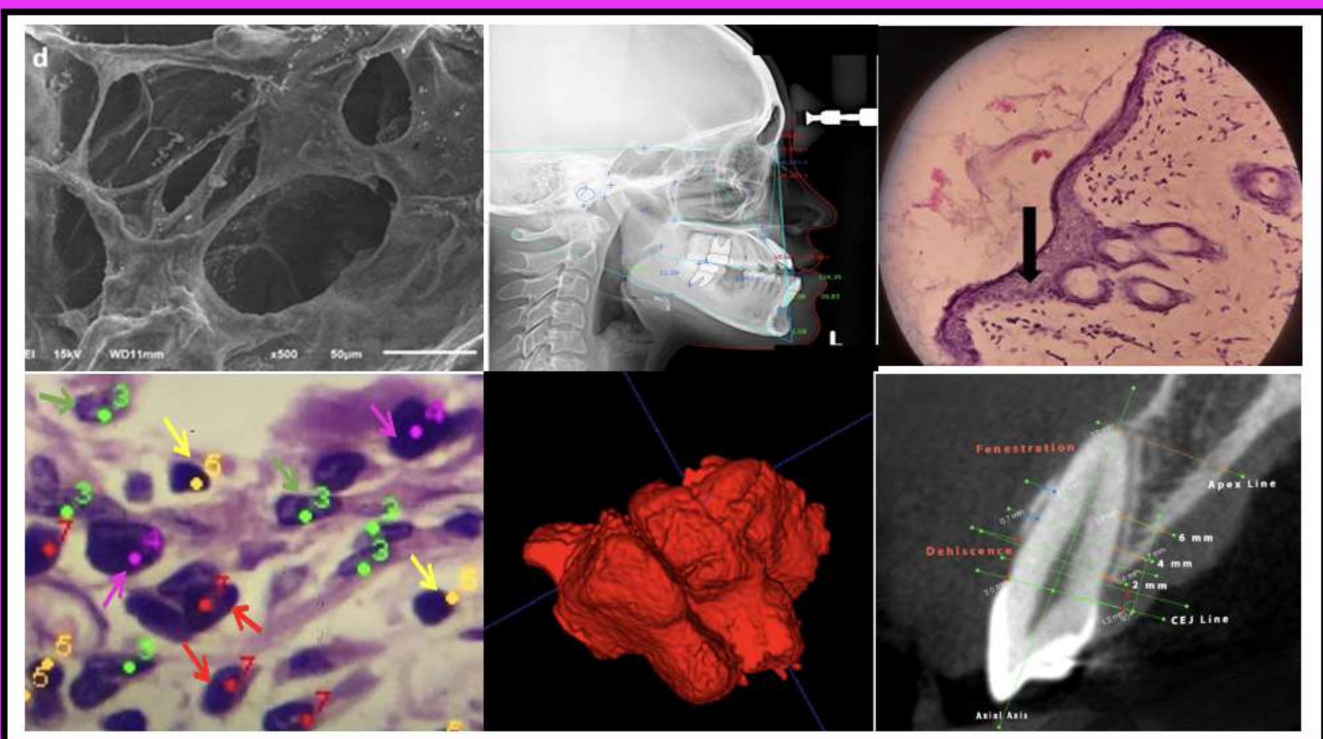

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The Effect of Tooth-Brushing Activity, Temperature, and pH to Acrylic and Composite Resin Microplastic Release

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Abstract

The use of acrylic and composite resin in daily dental practice is an essential material. Both contain plastic particles and are released by several oral activities. To assess the effect of Tooth-brushing activity, temperature, and pH changes of acrylic and composite resin microplastic release.

The samples were designed in a particular size and divided into three groups allocated randomly, soaked with and without artificial saliva for the above activities. The total time for each treatment was designed for 120 hours. The identification of microplastic released after treatment used a 10 µg/mL working concentration of Nile red and weighed in grams. The result was analyzed using one-way ANOVA ($P<0.05$) and paired t-test.

The study found that the acrylic and composite resin microplastics were identified differently in total artificial saliva after the treatment. The paired t-test showed significant differences for acrylic resin ($p=0.015$) in pH changes treatment and composite resin ($p=0.035$) in brushing activity. No significant differences were found between brushing activity, temperature, and pH changes treatment for acrylic ($p=0.298$) and composite resin ($p=0.293$) microplastic release. The result concluded that brushing activity, temperature, and pH changes might cause acrylic and composite resin microplastic release.

Experimental article (J Int Dent Med Res 2021; 14(4): 1394-1400)

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Introduction

Microplastics are synthetic solid particle or polymeric matrix, with regular or irregular shape with size ranging from one µm to five mm, of either primary or secondary manufacturing origin, and water insoluble. A recent study has been included the nano size into the microplastic definition.[1] In dentistry, materials containing plastic are usually used, such as dental polymers for cavity filling, sealants, dentures, and abrasion in dental polish.[2]

Dental health care behavior is closely related to tooth-brushing and consumption of food and beverages.[3] Tooth-brushing activity was one of the most oral daily routines for maintaining oral hygiene. On the other side, tooth-brushing activity has an abrasive effect due to several factors such as inappropriate frequency, duration, and technique of tooth-brushing.[4] Some food and beverages might also cause pH and temperature changes that may influence the tooth surface.[5,6] When dental materials such as composite resin are placed as a tooth restoration and acrylic resin for dental prostheses, they are constantly exposed by tooth-brushing activity, pH, and temperature changes, and other factors that lead to an unfavorable effect on dental material.[6,7] The dental material may undergo degradation caused by various factors in the oral cavity, such as mechanical load, temperature, pH, and tooth-brushing activity.[8] The degradation of dental material assumed it may release microplastics. Microplastics can be carried into the human body through inhalation and ingestion from the oral cavity. Microplastics particles (<130 µm) can translocate into human tissues then trigger a localized immune response.[9]

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Chronic inhalation of composite microparticles

(<5 μm) and nanoparticles may provoke local and systemic toxicity. Nano-particles (<100nm) may also enter the blood or lymphatic system resulting in systemic toxicity.[10] Ingested microplastics can cause physical damage such as erosion or ulcers in the digestive tract.[11] Prata investigates the risk of airborne microplastics to human health then concluded that airborne microplastics could cause airway and interstitial lung diseases.[12] Mak et al. investigated the effect of microplastics in the intestine, and the result showed that microplastics accumulated in the intestine could increase the expression of cytochrome p450. Increased expression of cytochrome p450 is associated with modulation of metabolism when exposed to microplastics.[13]

Various methods for microplastics identification have been developed, ranging from the simplest method visually and separated manually without a microscope to the aid of fluorescent dye. Nile red was the commonly used fluorescent dye for microplastic identification.[14] Recent studies have supported the use of Nile red as an accurate stain for the rapid detection and quantification of microplastics. Maes *et al*. validated the use of Nile red with analysis using Fourier-Transform Infrared Spectrometer (FTIR) to verify the polymeric content of fluorescing particles, then concluded that Nile red might be used for rapid detection microplastic without the need for additional spectroscopic analysis. Maes *et al*. also suggested that Nile red alone is sufficient to identify a particle as polymeric.[15,16]

The degradation of dental material assumes may release microplastics, but to date, there is still a lack of evidence acrylic and composite resin release microplastic after tooth-brushing, temperature, and pH treatment. Therefore, this study was conducted to assess the effect of between tooth-brushing activity, temperature, and pH to acrylic and composite resin microplastic release.

Materials and methods

An experimental laboratory study was carried out with a sample size of 20, divided equally between acrylic and composite resins groups. Tooth-brushing activity simulated with toothbrush simulator, temperature simulated with 5°C and 55°C cycle, and pH simulated with pH 4 and pH 7 cycle. In this study, a brushing simulator carried out using Oral-B 3D White electric toothbrush (Oral-B, USA). Temperature simulation carried out using refrigerator (LG, Korea) for 5°C and incubator (JISICO, Korea) for 55°C. pH simulation carried out using buffer solution pH 4 and pH 7 (Merck, Germany)

Sample preparation

Both acrylic and composite resin were made in sizes 10 mm x 10 mm x 3 mm. Three specimens of acrylic and composite resin were mounted with dental stone into a beaker glass for fixation, then pouring 10 mL of artificial saliva for brushing activity treatment. Three specimens of acrylic and composite resin were put in beaker glass then pouring 10 mL of artificial saliva for temperature treatment. Three specimens of acrylic and composite resin were put in beaker glass for pH treatment.

The remaining samples of acrylic and composite resin were scraped using a scalpel. The particles obtained were then kept into a beaker glass before adding 10 mL of artificial saliva, and was treated as a positive control.[18]

Tooth-brushing experiment

Seven Oral-B 3D White electric toothbrushes (Oral-B, USA) used. Each toothbrush work on one specimen. The toothbrush's handle holds a universal table vise (Krisbow, Indonesia) to ensure the toothbrush stays in place. A force of two Newton was applied for tooth-brushing. The cleaning force was generated using a 200 g Chrome Plating Calibration tied with dental floss (P&G, USA).

Specimen were randomly allocated. Three samples of acrylic and composite resin were assigned to each toothbrush. The remaining toothbrush was assigned to be the negative control sample. Negative control was the mounted dental stone into a beaker glass filled with 10 mL of artificial saliva without any acrylic or composite resin sample. The total of tooth-brushing stroke was equivalent to five years of tooth-brushing, 120 seconds twice a day of all teeth.[17] The total-brushing time was then designed as 120 hours.

Temperature treatment

Specimen were randomly allocated. Three samples of acrylic and composite resin were put in the refrigerator at 5°C treatment for 60 hours along with the negative control sample. The negative control sample was the beaker glass with 10 mL artificial saliva without any acrylic or composite resin sample. After 60 hours, the sample was moved into the incubator set at 55°C for 60 hours.

pH treatment

Specimen were randomly allocated. Buffer solution with pH 4 was poured 10 mL into the prepared three samples of acrylic and composite resin for 40 hours. After 40 hours, move the sample into another beaker glass then pour 10 mL pH 7 buffer solution for 80 hours. The negative control sample was the beaker glass with 10 mL artificial saliva without any acrylic or composite resin sample.

Microplastic identification and measurement

After the treatment is completed, the artificial saliva of three acrylic resin samples was collected and done the same thing as composite resin samples for each treatment. The microplastics identification was conducted by dripping Nile red solution (TCi, Japan) into the artificial saliva (working concentration 10 g/mL using n-Hexane solvent) and then incubated for 30 minutes. After the incubation period, artificial saliva contained Nile red is filtered using filter paper (Whatman, grade 934-AH, 55 mm diameter, 1,5 mm pore, GE Healthcare, USA), and the filter paper was air-dried for five minutes and examined under a fluorescent microscope (Zeiss Axio Vert A1, Germany) [Figure 1].[16,19] Microplastic measurement was done by weighing the filter papers in grams.

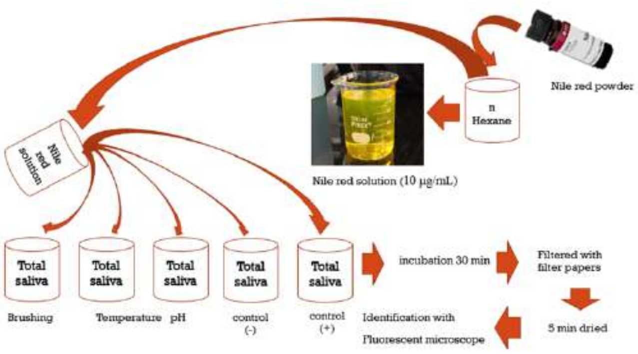

Figure 1. Microplastic identification steps.

Statistical Analysis

All statistical analysis was performed using statistical software SPSS for Windows version 22 (IBM, USA). After treatment, the among treatments were compared using a one-way ANOVA test. Inter-treatment comparison was conducted using a pair sample t-test. For all test, the level of significance chosen was $p < 0.05$.

Result

Identification microplastics was carried out using a fluorescent microscope on blue light filters (excitation wavelength 365 nm and emission wavelength 445 nm) and green light filters (excitation wavelength 450 nm and emission wavelength 515 nm). First, identification was conducted for the positive control sample and negative control sample in artificial saliva. The result showed that microplastics are found on the positive control sample, while no microplastics are found on the negative control sample. The Nile red-stained acrylic and composite microplastics particles were visible in both blue and green fluorescence [Figure 2].

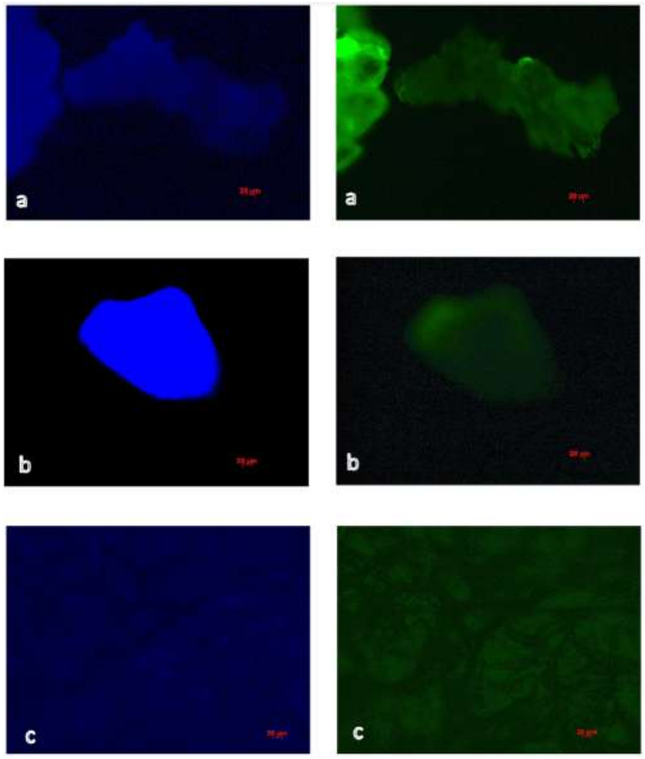

Figure 2. Microplastics identification using a fluorescence microscope with different filters (blue and green light) on positive and negative control. (a) positive control acrylic resin, (b) positive control composite resin, (c) negative control.

Microplastics found on positive control confirmed that Nile red could identify the acrylic and composite resin in artificial saliva. Furthermore, acrylic and composite resin samples of total artificial saliva were conducted after the tooth-brushing, temperature, and pH treatment. The result has shown that acrylic and composite resin microplastics were found in total artificial saliva after the tooth-brushing, temperature, and pH treatment [Figure 3].

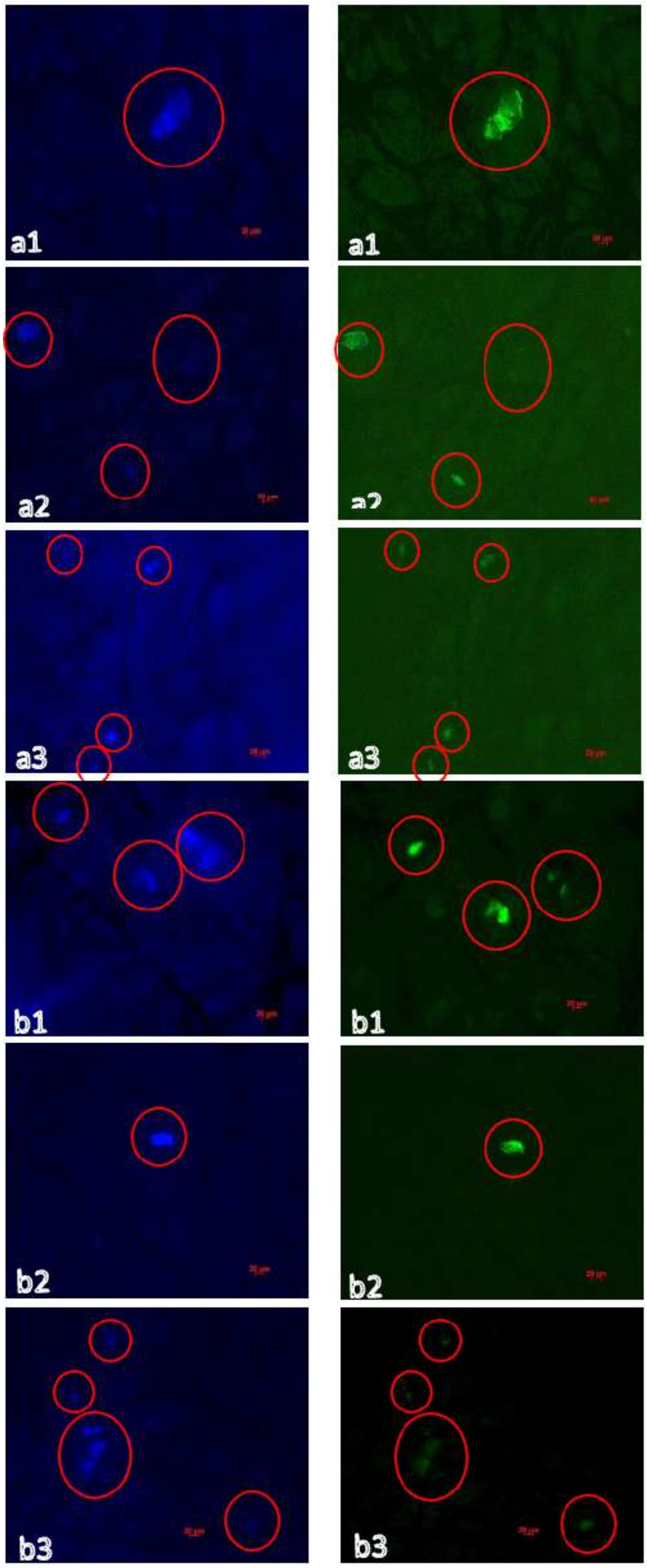

Figure 3. Microplastics identification using fluorescence microscope on samples with different filters (blue and green light). (a1) acrylic resin microplastics after brushing treatment, (a2) acrylic resin microplastics after temperature treatment, (a3) acrylic resin microplastics after pH treatment, (b1) composite resin microplastics after brushing treatment, (b2) composite resin microplastics after brushing treatment, (b3) composite resin microplastics after brushing treatment.

Acrylic and composite resin sample were weighed before and after the tooth-brushing, temperature, and pH treatment. The result shown there is differences in the weight of acrylic and composite resin before and after treatment, it indicates acrylic and composite resin sustained degradation after treatments and release the microplastic [Figure 4].

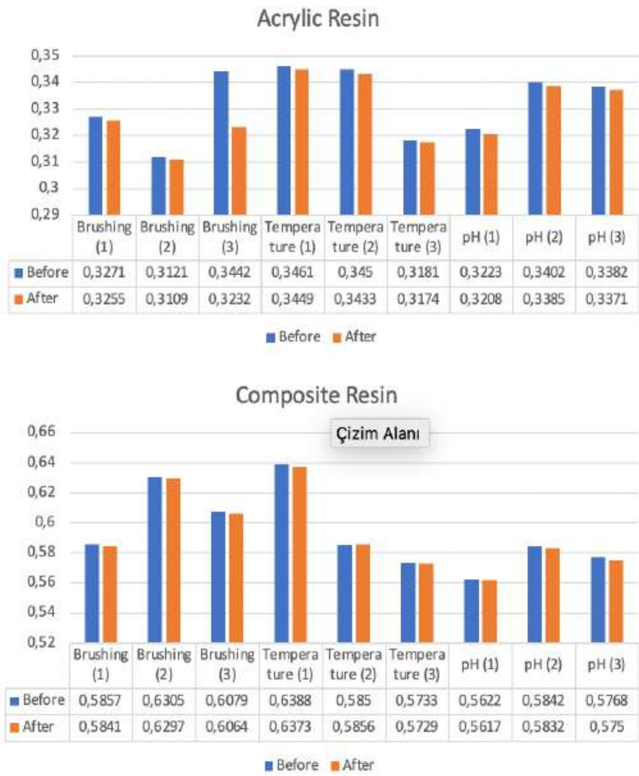

Figure 4. Acrylic and composite resin samples weight (in grams).

Data from each treatment were analyzed using paired t-test and showed significant differences only at pH treatment for acrylic resin and brushing treatment for composite resin (Table 1). The significant differences show there are differences in the amount of microplastic released after the treatment.

Sample	Treatment	Mean		SD		p
		Before	After	Before	After	
Acrylic resin	Brushing	0.3278	0.3198	0.0160	0.0078	0.349
Acrylic resin	Temperature	0.3364	0.3352	0.0158	0.0154	0.053
Acrylic resin	pH	0.3335	0.3321	0.0098	0.0098	0.015*
Composite resin	Brushing	0.6080	0.6067	0.0224	0.0228	0.035*
Composite resin	Temperature	0.5990	0.5986	0.0349	0.0341	0.549
Composite resin	pH	0.5744	0.5733	0.0111	0.0108	0.101

Table 1. Paired t-test among variables.

To determine the significant differences between treatments, the one-way ANOVA test was carried out. It was found that there were no significant differences between the treatments (p=0.298 for acrylic resin and p=0.293 for composite resin). Quantification of the microplastics were done by weighing the dried filter papers to obtain the amount of microplastic release from acrylic and composite resin. The result has showed that acrylic resin released 0.0595 g microplastics after brushing treatment, 0.0419 g microplastics after temperature treatment, 0.0439 g microplastic after pH treatment, Composite resin released 0.0337 g microplastics after brushing treatment, 0.0472 g microplastics after temperature treatment, 0.0442 g microplastics after pH treatment.

Discussion

Acrylic and composite resins were plastics-based dental materials.[2] Social *et al*. state that brushing treatment on composite resin will cause surface degradation.[20] Szczesio-Wlodarczyk *et al*. state that various factors, such as saliva, mastication, brushing activity, temperature, and pH, will cause degradation to acrylic resin and release toxic components resulted from degradation.[8] This study reveals acrylic and composite resin encounter weight loss after the brushing, temperature, and pH treatment in line with the above statement. This finding indicated acrylic and composite resin sustained degradation after brushing treatment and release the microplastic.

This study successfully found microplastic released from the acrylic and composite resin after brushing, temperature, and pH treatment with Nile red identification. The result was confirming our notion that the degradation of plastic base dental material will release microplastic. The microplastics found in this study are secondary. Secondary microplastics are plastics particles that result from wear, tear, abrasion, breakdown, and degradation of large plastic debris.[21]

Utilization of Nile red dyes to identify microplastic in this study in line with the research conducted by Shim *et al*., which identified the microplastic using the Nile red and fluorescence microscope.[22] Generally, microplastics can be identified using a conventional microscope. However, this method has low reliability, especially on small, transparent, and fiber-type particles.[23,24]

Micron-sized microplastics are usually identified using Raman spectroscopy and Fourier transform infrared spectroscopy. Both methods require repeated experiments to obtain reliable results because of the small and wet particle size, expensive equipment, and time-consuming.[20,25] Nile red has good reliability and sensitivity for microplastic identification. Gagne *et al*. stated that Nile red could detect microplastics up to 50-100nm nanoparticles in his research using transparent polystyrene material.[26] Maes *et al*. also suggested that microplastic identification using Nile red alone was sufficient.[15]

Our study found the highest amount of microplastic released after the treatments were 0.0595 g. According to a recent study, an estimated 80 g per day of microplastic entered the human body. Microplastic can also be found in food and drink (either tap water or bottled water). Approximately 0.44 MPs/g of nano and microplastics were found in sugar, 0.11 MPs/g in salt, 0.03 MPs/g in alcohol, and 0.09 MPs/g in bottled water.[27] Furthermore, microplastics could enter the human body through inhalation.[28] Because of the many sources of microplastics that can enter the human body, expectantly microplastics will receive more attention to be evaluated regarding the risks of microplastics to the environment and human health.

The effect of acrylic and composite resin microplastic on the environment and human health is unknown. However, generally microplastics consumed in the body can cause tissue inflammation, cell proliferation, necrosis, and compromise of immune cells.[29] Yong *et al*. stated lack of in vivo data on the effects of microplastics on humans to date, but it is known that microplastics in fish and mice caused inflammation, oxidative stress, and metabolic changes, and in fish, the microplastic could cause changes in the brain.[28] Prietl *et al*. stated that 20 nm microplastic was toxic to the human monocytic cell line (U937) and human monocytic cell line (THP-1) and stimulated IL-8 and caused an increase in oxidative stress in THP-1.[30] Dong *et al*. stated that microplastics cause cytotoxicity, oxidative stress, and inflammatory responses in human lung epithelial cells (BEAS-2B) and increase the risk of chronic obstructive pulmonary disease (COPD).[31] Poma *et al*. stated that microplastics with a size of 100 nm at 5-75 g/mL can stimulate the production of reactive oxygen species (ROS) are genotoxic and cause DNA damage in human fibroblast cells (Hs27).[32]

The limitation of this study was the brushing treatment conducted in this study significantly depends on the battery lifespan. Therefore, the future specific tools could be used to overcome the limitation.

Conclusion

Based on the result of this study, it can be concluded that the tooh-brushing activity, temperature, and pH changes might cause microplastic release in different number and there were no significant differences among treatment groups in microplastic release from the acrylic and composite resin. Further study to evaluate the acrylic and composite resin microplastics in vitro is recommended.

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Declaration of Interest

The authors report no conflict of interest.

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The Effect of Tooth-Brushing Activity, Temperature, and pH to Acrylic and Composite Resin Microplastic Release

by Joko Kusnoto

The Effect of Tooth-Brushing Activity, Temperature, and pH to Acrylic and Composite Resin Microplastic Release

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Abstract

The use of acrylic and composite resin in daily dental practice is an essential material. Both contain plastic particles and are released by several oral activities. To assess the effect of Tooth-brushing activity, temperature, and pH changes of acrylic and composite resin microplastic release.

The samples were designed in a particular size and divided into three groups allocated randomly, soaked with and without artificial saliva for the above activities. The total time for each treatment was designed for 120 hours. The identification of microplastic released after treatment used a 10 µg/mL working concentration of Nile red and weighed in grams. The result was analyzed using one-way ANOVA (P<0.05) and paired t-test.

The study found that the acrylic and composite resin microplastics were identified differently in total artificial saliva after the treatment. The paired t-test showed significant differences for acrylic resin (p=0.015) in pH changes treatment and composite resin (p=0.035) in brushing activity. No significant differences were found between brushing activity, temperature, and pH changes treatment for acrylic (p=0.298) and composite resin (p=0.293) microplastic release. The result concluded that brushing activity, temperature, and pH changes might cause acrylic and composite resin microplastic release.

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Introduction

Microplastics are synthetic solid particle or polymeric matrix, with regular or irregular shape with size ranging from one µm to five mm, of either primary or secondary manufacturing origin, and water insoluble. A recent study has been included the nano size into the microplastic definition.[1] In dentistry, materials containing plastic are usually used, such as dental polymers for cavity filling, sealants, dentures, and abrasion in dental polish.[2]

Dental health care behavior is closely related to tooth-brushing and consumption of food and beverages.[3] Tooth-brushing activity was one of the most oral daily routines for maintaining oral hygiene. On the other side, tooth-brushing activity has an abrasive effect due to several factors such as inappropriate frequency, duration, and technique of tooth-brushing.[4] Some food and beverages might also cause pH and temperature changes that may influence the tooth surface.[5,6] When dental materials such as composite resin are placed as a tooth restoration and acrylic resin for dental prostheses, they are constantly exposed by tooth-brushing activity, pH, and temperature changes, and other factors that lead to an unfavorable effect on dental material.[6,7] The dental material may undergo degradation caused by various factors in the oral cavity, such as mechanical load, temperature, pH, and tooth-brushing activity.[8] The degradation of dental material assumed it may release microplastics. Microplastics can be carried into the human body through inhalation and ingestion from the oral cavity. Microplastics particles (<130 µm) can translocate into human tissues then trigger a localized immune response.[9]

Chronic inhalation of composite microparticles

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(<5 µm) and nanoparticles may provoke local and systemic toxicity. Nano-particles (<100nm) may also enter the blood or lymphatic system resulting in systemic toxicity.[10] Ingested microplastics can cause physical damage such as erosion or ulcers in the digestive tract.[11] Prata investigates the risk of airborne microplastics to human health then concluded that airborne microplastics could cause airway and interstitial lung diseases.[12] Mak et al. investigated the effect of microplastics in the intestine, and the result showed that microplastics accumulated in the intestine could increase the expression of cytochrome p450. Increased expression of cytochrome p450 is associated with modulation of metabolism when exposed to microplastics.[13]

Various methods for microplastics identification have been developed, ranging from the simplest method visually and separated manually without a microscope to the aid of fluorescent dye. Nile red was the commonly used fluorescent dye for microplastic identification.[14] Recent studies have supported the use of Nile red as an accurate stain for the rapid detection and quantification of microplastics. Maes *et al*. validated the use of Nile red with analysis using Fourier-Transform Infrared Spectrometer (FTIR) to verify the polymeric content of fluorescing particles, then concluded that Nile red might be used for rapid detection microplastic without the need for additional spectroscopic analysis. Maes *et al*. also suggested that Nile red alone is sufficient to identify a particle as polymeric.[15,16]

The degradation of dental material assumes may release microplastics, but to date, there is still a lack of evidence acrylic and composite resin release microplastic after tooth-brushing, temperature, and pH treatment. Therefore, this study was conducted to assess the effect of between tooth-brushing activity, temperature, and pH to acrylic and composite resin microplastic release.

Materials and methods

An experimental laboratory study was carried out with a sample size of 20, divided equally between acrylic and composite resins groups. Tooth-brushing activity simulated with toothbrush simulator, temperature simulated with 5°C and 55°C cycle, and pH simulated with pH 4 and pH 7 cycle. In this study, a brushing simulator carried out using Oral-B 3D White electric toothbrush (Oral-B, USA). Temperature simulation carried out using refrigerator (LG, Korea) for 5°C and incubator (JISICO, Korea) for 55°C. pH simulation carried out using buffer solution pH 4 and pH 7 (Merck, Germany)

Sample preparation

Both acrylic and composite resin were made in sizes 10 mm x 10 mm x 3 mm. Three specimens of acrylic and composite resin were mounted with dental stone into a beaker glass for fixation, then pouring 10 mL of artificial saliva for brushing activity treatment. Three specimens of acrylic and composite resin were put in beaker glass then pouring 10 mL of artificial saliva for temperature treatment. Three specimens of acrylic and composite resin were put in beaker glass for pH treatment.

The remaining samples of acrylic and composite resin were scraped using a scalpel. The particles obtained were then kept into a beaker glass before adding 10 mL of artificial saliva, and was treated as a positive control.[18]

Tooth-brushing experiment

Seven Oral-B 3D White electric toothbrushes (Oral-B, USA) used. Each toothbrush work on one specimen. The toothbrush's handle holds a universal table vise (Krisbow, Indonesia) to ensure the toothbrush stays in place. A force of two Newton was applied for tooth-brushing. The cleaning force was generated using a 200 g Chrome Plating Calibration tied with dental floss (P&G, USA).

Specimen were randomly allocated. Three samples of acrylic and composite resin were assigned to each toothbrush. The remaining toothbrush was assigned to be the negative control sample. Negative control was the mounted dental stone into a beaker glass filled with 10 mL of artificial saliva without any acrylic or composite resin sample. The total of tooth-brushing stroke was equivalent to five years of tooth-brushing, 120 seconds twice a day of all teeth.[17] The total-brushing time was then designed as 120 hours.

Temperature treatment

Specimen were randomly allocated. Three samples of acrylic and composite resin were put in the refrigerator at 5°C treatment for 60 hours along with the negative control sample. The negative control sample was the beaker glass with 10 mL artificial saliva without any acrylic or composite resin sample. After 60 hours, the sample was moved into the incubator set at 55°C for 60 hours.

pH treatment

Specimen were randomly allocated. Buffer solution with pH 4 was poured 10 mL into the prepared three samples of acrylic and composite resin for 40 hours. After 40 hours, move the sample into another beaker glass then pour 10 mL pH 7 buffer solution for 80 hours. The negative control sample was the beaker glass with 10 mL artificial saliva without any acrylic or composite resin sample.

Microplastic identification and measurement

After the treatment is completed, the artificial saliva of three acrylic resin samples was collected and done the same thing as composite resin samples for each treatment. The microplastics identification was conducted by dripping Nile red solution (TCi, Japan) into the artificial saliva (working concentration 10 g/mL using n-Hexane solvent) and then incubated for 30 minutes. After the incubation period, artificial saliva contained Nile red is filtered using filter paper (Whatman, grade 934-AH, 55 mm diameter, 1,5 mm pore, GE Healthcare, USA), and the filter paper was air-dried for five minutes and examined under a fluorescent microscope (Zeiss Axio Vert A1, Germany) [Figure 1].[16,19] Microplastic measurement was done by weighing the filter papers in grams.

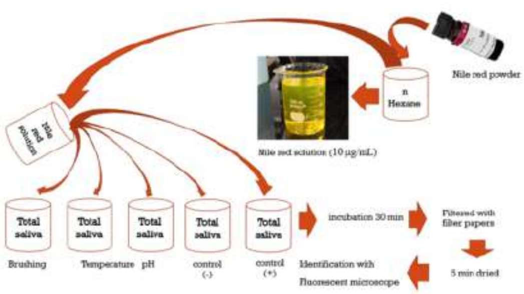

Figure 1. Microplastic identification steps.

Statistical Analysis

All statistical analysis was performed using statistical software SPSS for Windows version 22 (IBM, USA). After treatment, the among treatments were compared using a one-way ANOVA test. Inter-treatment comparison was conducted using a pair sample t-test. For all test, the level of significance chosen was $p< 0.05$.

Result

Identification microplastics was carried out using a fluorescent microscope on blue light filters (excitation wavelength 365 nm and emission wavelength 445 nm) and green light filters (excitation wavelength 450 nm and emission wavelength 515 nm). First, identification was conducted for the positive control sample and negative control sample in artificial saliva. The result showed that microplastics are found on the positive control sample, while no microplastics are found on the negative control sample. The Nile red-stained acrylic and composite microplastics particles were visible in both blue and green fluorescence [Figure 2].

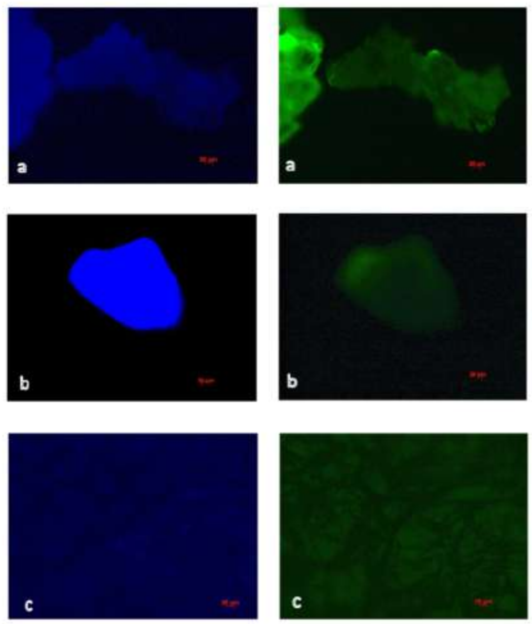

Figure 2. Microplastics identification using a fluorescence microscope with different filters (blue and green light) on positive and negative control. (a) positive control acrylic resin, (b) positive control composite resin, (c) negative control.

Microplastics found on positive control confirmed that Nile red could identify the acrylic and composite resin in artificial saliva. Furthermore, acrylic and composite resin samples of total artificial saliva were conducted after the tooth-brushing, temperature, and pH treatment. The result has shown that acrylic and composite resin microplastics were found in total artificial saliva after the tooth-brushing, temperature, and pH treatment [Figure 3].

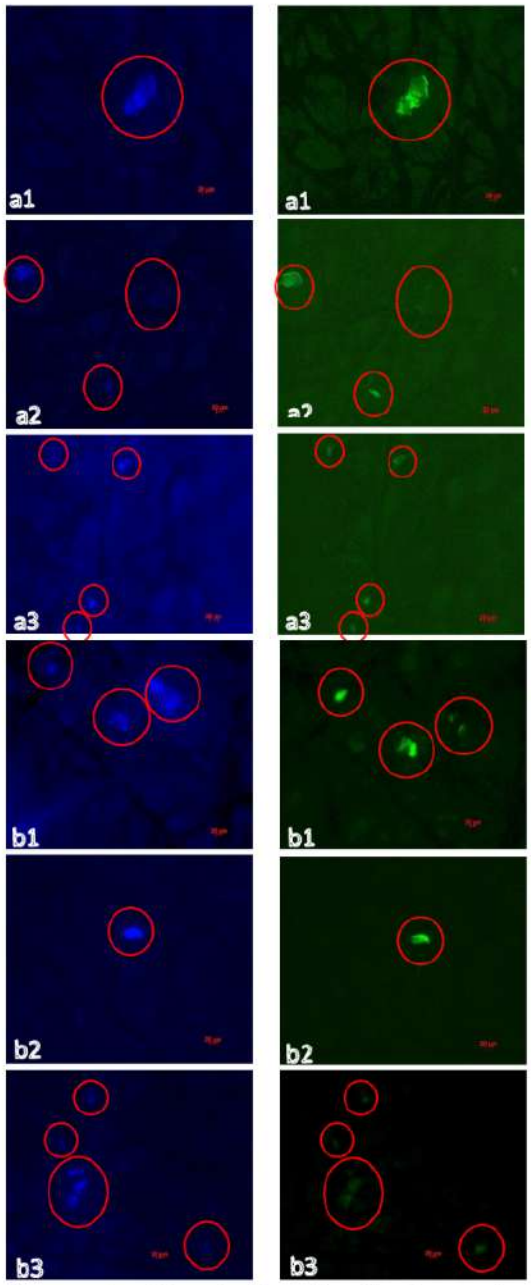

Figure 3. Microplastics identification using fluorescence microscope on samples with different filters (blue and green light). (a1) acrylic resin microplastics after brushing treatment, (a2) acrylic resin microplastics after temperature treatment, (a3) acrylic resin microplastics after pH treatment, (b1) composite resin microplastics after brushing treatment, (b2) composite resin microplastics after brushing treatment, (b3) composite resin microplastics after brushing treatment.

Acrylic and composite resin sample were weighed before and after the tooth-brushing, temperature, and pH treatment. The result shown there is differences in the weight of acrylic and composite resin before and after treatment, it indicates acrylic and composite resin sustained degradation after treatments and release the microplastic [Figure 4].

Figure 4. Acrylic and composite resin samples weight (in grams).

Data from each treatment were analyzed using paired t-test and showed significant differences only at pH treatment for acrylic resin and brushing treatment for composite resin (Table 1). The significant differences show there are differences in the amount of microplastic released after the treatment.

Sample	Treatment	Mean		SD		p
		Before	After	Before	After	
Acrylic resin	Brushing	0.3278	0.3198	0.0160	0.0078	0.349
Acrylic resin	Temperature	0.3364	0.3352	0.0158	0.0154	0.053
Acrylic resin	pH	0.3335	0.3321	0.0098	0.0098	0.015*
Composite resin	Brushing	0.6080	0.6067	0.0224	0.0228	0.035*
Composite resin	Temperature	0.5990	0.5986	0.0349	0.0341	0.549
Composite resin	pH	0.5744	0.5733	0.0111	0.0108	0.101

Table 1. Paired t-test among variables.

To determine the significant differences between treatments, the one-way ANOVA test was carried out. It was found that there were no significant differences between the treatments (p=0.298 for acrylic resin and p=0.293 for composite resin). Quantification of the microplastics were done by weighing the dried filter papers to obtain the amount of microplastic release from acrylic and composite resin. The result has showed that acrylic resin released 0.0595 g microplastics after brushing treatment, 0.0419 g microplastics after temperature treatment, 0.0439 g microplastic after pH treatment, Composite resin released 0.0337 g microplastics after brushing treatment, 0.0472 g microplastics after temperature treatment, 0.0442 g microplastics after pH treatment.

Discussion

Acrylic and composite resins were plastics-based dental materials.[2] Social *et al*. state that brushing treatment on composite resin will cause surface degradation.[20] Szczesio-Wlodarczyk *et al*. state that various factors, such as saliva, mastication, brushing activity, temperature, and pH, will cause degradation to acrylic resin and release toxic components resulted from degradation.[8] This study reveals acrylic and composite resin encounter weight loss after the brushing, temperature, and pH treatment in line with the above statement. This finding indicated acrylic and composite resin sustained degradation after brushing treatment and release the microplastic.

This study successfully found microplastic released from the acrylic and composite resin after brushing, temperature, and pH treatment with Nile red identification. The result was confirming our notion that the degradation of plastic base dental material will release microplastic. The microplastics found in this study are secondary. Secondary microplastics are plastics particles that result from wear, tear, abrasion, breakdown, and degradation of large plastic debris.[21]

Utilization of Nile red dyes to identify microplastic in this study in line with the research conducted by Shim *et al.*, which identified the microplastic using the Nile red and fluorescence microscope.[22] Generally, microplastics can be identified using a conventional microscope. However, this method has low reliability, especially on small, transparent, and fiber-type particles.[23,24]

Micron-sized microplastics are usually identified using Raman spectroscopy and Fourier transform infrared spectroscopy. Both methods require repeated experiments to obtain reliable results because of the small and wet particle size, expensive equipment, and time-consuming.[20,25] Nile red has good reliability and sensitivity for microplastic identification. Gagne *et al*. stated that Nile red could detect microplastics up to 50-100nm nanoparticles in his research using transparent polystyrene material.[26] Maes *et al*. also suggested that microplastic identification using Nile red alone was sufficient.[15]

Our study found the highest amount of microplastic released after the treatments were 0.0595 g. According to a recent study, an estimated 80 g per day of microplastic entered the human body. Microplastic can also be found in food and drink (either tap water or bottled water). Approximately 0.44 MPs/g of nano and microplastics were found in sugar, 0.11 MPs/g in salt, 0.03 MPs/g in alcohol, and 0.09 MPs/g in bottled water.[27] Furthermore, microplastics could enter the human body through inhalation.[28] Because of the many sources of microplastics that can enter the human body, expectantly microplastics will receive more attention to be evaluated regarding the risks of microplastics to the environment and human health.

The effects of acrylic and composite resin microplastic on the environment and human health is unknown. However, generally microplastics consumed in the body can cause tissue inflammation, cell proliferation, necrosis, and compromise of immune cells.[29] Yong *et al*. stated lack of in vivo data on the effects of microplastics on humans to date, but it is known that microplastics in fish and mice caused inflammation, oxidative stress, and metabolic changes, and in fish, the microplastic could cause changes in the brain.[28] Prietl *et al*. stated that 20 nm microplastic was toxic to the human monocytic cell line (U937) and human monocytic cell line (THP-1) and stimulated IL-8 and caused an increase in oxidative stress in THP-1.[30] Dong *et al*. stated that microplastics cause cytotoxicity, oxidative stress, and inflammatory responses in human lung epithelial cells (BEAS-2B) and increase the risk of chronic obstructive pulmonary disease (COPD).[31] Poma *et al*. stated that microplastics with a size of 100 nm at 5-75 g/mL can stimulate the production of reactive oxygen species (ROS) are genotoxic and cause DNA damage in human fibroblast cells (Hs27).[32]

The limitation of this study was the brushing treatment conducted in this study significantly depends on the battery lifespan. Therefore, the future specific tools could be used to overcome the limitation.

Conclusion

Based on the result of this study, it can be concluded that the tooh-brushing activity, temperature, and pH changes might cause microplastic release in different number and there were no significant differences among treatment groups in microplastic release from the acrylic and composite resin. Further study to evaluate the acrylic and composite resin microplastics in vitro is recommended.

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Declaration of Interest

The authors report no conflict of interest.

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