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The effect of low pH on surface hardness, volume, surface area and morphology of direct dental restorative materials

An in vitro pilot study

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Table of content

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Both the practical and theoretical work have been equally distributed between the three authors. Selection and assessment of the references in the thesis was collectively performed.

Abstract

Background: Dental erosions as a disease is an increasing problem today, in particularly among adolescents. They may be caused by intrinsic factors such as gastric juice, frequently present in the oral cavity of bulimic patients. Direct dental restorative materials may be used to treat erosions, but the data regarding the effects of low pH on volume changes of these materials are limited.

Objective: To investigate the effect of a hydrochloric acidic solution with a pH of 1.2 on surface hardness, volume, surface area and morphology of three direct dental restorative materials, using microhardness test, micro computed tomography (micro-CT) and scanning electron microscopy (SEM).

Material and methods: Using Tetric EvoCeram, Tetric EvoFlow and GC Fuji II LC, 13 specimens of each material were made. The prepared specimens were thereafter randomly allocated within their respective material group and exposed to a hydrochloric acidic solution (pH 1.2) for 6 and 48 hours (equivalent to 2 and 16 years of vomiting, respectively), before they underwent microhardness test, micro-CT and SEM. The tests were performed both before and after acidic exposure. Statistical analyses were performed using a significance level set to 5%.

Results: A reduction in microhardness was seen in all the materials, but only Tetric EvoCeram had a significant reduction in Knoop hardness number (KHN) ($P = 0.039$) after 48 hours of acidic exposure (mean values from 29.73 to 27.13 KHN). Significant volume reductions were seen after 48 hours for Tetric EvoFlow ($P = 0.039$) (mean values from 56.04 to 54.17 mm^3) and GC Fuji II LC ($P = 0.023$) (mean values from 63.86 to 55.11 mm^3). No significance was found regarding surface area. SEM showed varying results regarding surface morphology; several images exhibit an apparent increase in roughness with acidic exposure, while others did the opposite.

Conclusion: In general – in the absence of mechanical stimulus – the microhardness of the materials may be reduced, there may be a loss of volume, and differences in surface area and morphology may appear after acidic exposure. The observed changes were small, and few were significant. Consequently, this indicates that dental restorative materials may have a protective effect against erosions on non-loaded areas of the dentition. However, there are several limitations of our study; a low sample size, multiple clinical factors we did not take

into account, and uncertainty about the validity of the exposure times used. This complicates the drawing of any certain conclusions, making further investigations required to obtain complementary results.

Keywords: Erosion, bulimia, direct dental restorative materials, resin-based composites, resin-modified GIC, acidic exposure, microhardness, micro-CT, SEM.

1 Introduction

1.1 Dental erosion

Dental erosion is a major contributor to tooth wear, defined as an irreversible chemical decomposition of dental hard tissue without the impact of bacteria (1-3). Both extrinsic and intrinsic factors cause dental erosions, such as acidic foods and drinks, and gastric juice, respectively (1, 2). These risk factors are the most dominating etiological factors for erosions (2). Lately, studies have also shown a possible hereditary component for developing dental erosions, which can contribute to explain variations in occurrence and severity among patients with similar acidic exposure (2). Therefore, some individuals may be more vulnerable to developing erosions due to a lower resistance towards the earlier mentioned risk factors (2).

Within dentistry, dental erosion is one of the most widespread diseases (1). There is an increasing prevalence regardless of age, but in particular among children and adolescents (1). In 2019, Mulic et al. found the prevalence of dental erosion among Norwegian 16- and 18-year-olds to be as high as 32-64% (2). In addition to causal and preventive treatment of erosions such as diet guide and usage of fluoride mouth rinses, erosive lesions may be treated by dental restorations (2, 4). These restorations may be affected by an occasional low pH of the oral cavity, possibly reducing their clinical performance and durability (5, 6).

1.2 Dental erosion and bulimia

Gastric juice is a causative factor for dental erosion (2). Some reports have shown that gastric juice – with a pH fluctuating from 1.0-3.0 – can cause just as severe erosions as strong dietetic acids (1, 7). A higher frequency of acid regurgitation corresponds to an increased risk of developing dental erosions (1). Bulimia (bulimia nervosa) is an eating disorder where one of the characteristics is overeating followed by vomiting, which is why bulimic patients are prone to erosions (1, 8). Oral manifestations found in bulimic patients can include enlargement of parotid gland, reduced saliva secretion, xerostomia and dental erosion, where the latter is the main oral clinical finding (1, 9). In a study by Uhlen et al. from 2014, they found the prevalence of dental erosion to be almost 70% among the 66 investigated patients experiencing self-induced vomiting (10).

1.3 Distribution

Early stages of enamel erosions can be difficult to discover as the patient seldom has any complaints or symptoms, and because they usually do not cause remarkable changes in tooth

morphology (2). The distribution of dental erosions generally shows a predominance on occlusal surfaces of the mandibular 1st molars and on the palatinal surfaces of the anterior maxillary teeth, of which the latter typically indicates an intrinsic cause such as gastric juice (1, 2). In 2014, Jaeggi & Lussi reviewed several articles regarding the distribution of erosions among different age groups (11). Children aged 2-4 years had the following distribution: 33% in 1st molars, 18% in 2nd molars, 18% in canines, 16% in lateral incisors and 15% in central incisors (11). The enamel of the deciduous dentition is more susceptible to severe erosions compared to the enamel of the permanent dentition due to lower hardness and thickness (11). It is – on the other hand – uncertain whether the deciduous dentition in general is more vulnerable towards acidic exposure compared to the permanent dentition, as the mechanical forces of a child's mouth are weaker (11). Regarding children and adolescents, Jaeggi & Lussi also found the most affected surfaces to be palatinally of the incisors in the maxilla and occlusally on the 1st molars in the mandible (11). The prevalence of dental erosion increases with age, and some studies find erosions more frequently among men compared to women as their consumption of acidic beverages is higher, and men have stronger masticatory forces than women (2, 11, 12).

1.4 Treatment

Conservative and preventive treatment is preferred when treating early stages of dental erosion (2, 13). This can include determination of causal factors, dietary guidance, use of fluoride supplements, and counselling regarding oral hygiene (2, 3). The initial minimally invasive treatment is sealing the tooth with for example bonding, fissure-sealant, flowable composite or glass ionomer cement (GIC), preventing further adverse development (2, 14, 15). When the dentine is exposed and the erosions become more extensive, restorative treatment may be needed as dentine dissolves more rapidly than enamel due to a content of more soluble minerals (15, 16). However, the difference in dissolution between enamel and dentin is less striking than what was formerly believed (16). Nonetheless, excessive acidic demineralization can potentially lead to functional, aesthetic and sensitivity issues (3). Formerly, the treatment of extensive erosions solely consisted of indirect restorative treatment such as crowns and bridges, but recently, direct restoration materials such as dental composites have been suggested for this purpose as well (4). These materials are appropriate for treating erosions; not only is direct restorative treatment less invasive, less time consuming and a more affordable treatment compared to indirect methods, but the materials are also easy to use due to their adaptive ability (17-19). Dental composites have also shown

good longevity; a study comparing composites and crowns found no significant difference in 10 years of survival (19, 20).

1.5 Direct dental restorative materials

As mentioned, two types of direct dental restorative materials that may be used for treating erosions are resin-based composites and glass ionomer cements (2).

Resin-based composites

Polymers are macromolecules, made of smaller molecules called monomers (21). The process where monomers transform into polymers is called polymerization, which takes place during curing (21). Monomers or polymers combined with other components resulting in favourable qualities is known as a resin (21). Dental resin-based composites consist of three parts: the matrix, fillers and coupling agents (21). The matrix is made of cross-linked polymers, consisting of methacrylate monomers like bis-GMA and UDMA, as well as TEGDMA (21). The filler particles consist of for example glass, silica, quartz, crystalline or metal oxide, enhancing the physical properties of the matrix (21, 22). The filler size and loading contribute to determine the viscosity of the resin-based composites; more filler increase the viscosity (21). Coupling agents bind fillers to the matrix, strengthening the matrix (21).

Resin-based composites contain different types of bonds prone to hydrolysis, such as ester bonds found in methacrylate monomers, and oxane bonds between coupling agents and fillers (22). Hydrolysis is caused by water and is a slow process in the normal conditions of the oral cavity, but acids may catalyse the reaction (22). For ester bonds, hydrolysis may even occur at physiological pH due to their high susceptibility to nucleophilic attack (22). Water may also erode the filler surface, but the most used filler materials – being silica and quartz – are comparatively inert (22, 23). Barium glass is – on the other hand – not particularly inert, as the filler surface quickly dissolves when in contact with water (24). The weakest element of the resin-based composites, however, seems to be the interface between the filler and matrix (22).

Glass ionomer cements

Glass ionomer cements (GICs) are materials based on the reaction of glass powder and polyacrylic acid, consisting of a powder component and a liquid component (21). When mixing the two, the acid-base reaction starts (21). There are various GIC formulas available,

depending on the clinical purpose (21). As conventional GICs have limitations, resin-modified GICs were developed (25). For these materials, the powder component contains fluoroaluminosilicate glass particles and initiators, and the liquid component contains methacrylate modified polyacrylic acid and a polyacrylic acid water solution (21). The polymerization of resin-modified GIC can be light activated or chemically activated, depending on the material (21).

The components of GIC vulnerable to acid are the glass particles. In an article by Perera et al. from 2020 investigating several GICs, they found GC Fuji® IX Extra to show a weak resistance towards acids, likely due to more reactive glass particles (26). Furthermore, they found highly reactive glass particles to facilitate a larger diffusion of ions from the cured GIC, causing a decrease in mass (26). The mass decrease is accompanied by the polyacrylic acid functioning as an anode, buffering the acidic environment while simultaneously dissolving the GIC-filling (26). In general, the surfaces of GICs have been sealed with a varnish, protecting the surface from its surroundings (27). However, this protection is only temporary as the layer wears away (27).

Direct dental restorative materials and acidic exposure

Former studies have found all direct dental restorative materials to show signs of degradation when exposed to acid (2). These signs include an increase in wear and roughness of the materials, as well as a decrease in surface hardness (2). Conventional GICs and resin-modified GICs show an increased degradation compared to composites, but none of the dental restorative materials are superior to enamel (2). Despite this, acidic environments have limited effects on composites (2).

Several studies regarding erosion on direct dental restorative materials have evaluated the changes in microhardness and surface roughness after acidic exposure (7, 28, 29). However – to the authors' knowledge – there are no published studies of the same topic that examines the volume changes of the materials in addition to the other parameters.

1.6 Objective and hypothesis

Objective

The objective was to investigate the effect of a hydrochloric acidic solution with a pH of 1.2 – simulating the pH of gastric juice (7) – on surface hardness, volume, surface area and

morphology of three direct dental restorative materials, using microhardness test, micro computed tomography (micro-CT) and scanning electron microscopy (SEM).

Hypotheses

There are differences in microhardness, total volume and surface area of the materials after exposure to a hydrochloric acidic solution with a pH of 1.2.

In line with the hypothesis, these null hypotheses were formulated:

1) There are no differences in microhardness among the restorative materials after exposure to a hydrochloric acidic solution with a pH of 1.2.
2) There are no differences in total volume among the restorative materials after exposure to a hydrochloric acidic solution with a pH of 1.2.
3) There are no differences in surface area among the restorative materials after exposure to a hydrochloric acidic solution with a pH of 1.2.

2 Material and methods

In this study, we investigated surface hardness, volume, surface area and morphology of three different direct dental restorative materials; an ordinary composite (Tetric EvoCeram, Ivoclar Vivadent AG, Schaan, Liechtenstein), a flowable composite (Tetric EvoFlow, Ivoclar Vivadent AG, Schaan, Liechtenstein) and a resin-modified GIC (GC Fuji II LC Capsule, GC Europe N.V, Tokyo, Japan). An overview of the materials used in this study are presented in Table 1.

Table 1 *Description of materials used in this study (30-34). Application procedures are according to the instructions for use.*

Material	Specification	Composition	Application procedure
Tetric EvoCeram – A2 Ivoclar Vivadent AG (Schaan, Liechtenstein) **Group A**	Light-curing, nano-hybrid composite	Bis-GMA, urethane dimethacrylate, ethoxylated Bis-EMA, barium glass filler, ytterbiumtrifluoride, mixed oxide, prepolymers, additives, catalysts, stabilizers, pigments. Filler: 75-76 wt.% / 53-55 vol.%.	Apply layers of maximum 2 mm for optimal result. Light-cure for 20 seconds with a light intensity of ≥ 500 mW/cm^2.
Tetric EvoFlow – A2 Ivoclar Vivadent AG (Schaan, Liechtenstein) **Group B**	Light-curing, flowable nano-hybrid composite	Bis-GMA, urethane dimethacrylate, decandioldimethacrylat, barium glass filler, ytterbiumtrifluoride, mixed oxide, highly dispered silica, prepolymers, additives, catalysts, stabilizers, pigments. Filler: 57.5 wt.% / 30.7 vol.%.	Apply layers of maximum 2 mm for optimal result. Light-cure for 20 seconds with a light intensity of ≥ 500 mW/cm^2.
GC Fuji II LC Capsule – A2 GC Europe N.V (Tokyo, Japan) **Group C**	Light-curing reinforced glass ionomer restorative in capsules	Fluoroaluminosilicate glass, polyacrylic acid, water, HEMA.	Before activation, shake the capsule to loosen the powder. Push the plunger to activate. Place the capsule into the GC Capsule Applier and click once. Then, place the capsule into a mixer (+/- 4000 RPM) and mix for 10 seconds. Place the capsule into the GC Capsule Applier and click twice, then extrude. Light-cure for 20 seconds (470 nm wavelength). Apply a finishing coat of GC Fuji COAT LC, and light cure for 10 seconds.

2.1 Preparation of specimens

The three authors worked with the experiments collectively. Each author had the responsibility for one material each to ensure consistency of the application technique (Appendix 7.1, Image 1). A total of 39 specimens were made, 13 specimens per group of the three direct dental restorative materials (Table 1). The specimens were made with an approximate diameter of 10 mm and a thickness ranging between 1.06-1.14 mm for Tetric EvoCeram, 0.79-0.89 mm for Tetric EvoFlow, and 0.94-1.07 mm for GC Fuji II LC. To standardize the finishing of the samples, a plastic strip and a glass plate were placed on top of the uncured material and pressed down with finger pressure (35) (Appendix 7.1, Image 2). Then, the samples were light-cured through the plastic strip according to the manufacturers' instructions (Table 1 and Appendix 7.1, Image 3) with Bluephase® G2 (Ivoclar Vivadent, Schaan, Liechtenstein). To ensure optimal intensity output, the intensity of the LED lamp was tested five times prior to the curing and five times after. We used Teflon Tape (article number 30-8129, Clas Ohlson®) on the floor of the mould to simplify removal of the specimens after curing, ensuring that they were not damaged during the procedure. Sof-Lex™ discs were used to even out irregularities on the back and sides of the specimens (Appendix 7.1, Image 4). The prepared specimens were thereafter randomly allocated within their respective material group using www.randomizer.org.

2.2 Storage and analyses

After allocation, the specimens were individually stored in a 9 mL glass container covered with 5 mL deionized water. They were placed in a static incubator (Memmert, INB400, Germany) at 37°C for at least 24 hours to complete curing (28). The specimens were stored in deionized water for different amounts of time, ranging from approximately 1-30 days. The specimens constituted our baseline group at this point, as a control group. Before the different tests were performed, they were rinsed with deionized water and gently patted dry with a paper tissue. As mentioned, there was a total of 13 specimens per material. Six of these specimens underwent micro-CT, one of them underwent SEM, and microhardness test was performed on the remaining six. As SEM excludes the opportunity of performing further tests on the specimen, it was excluded from further examination. We chose to make specimens exclusively for microhardness testing, as we considered the indents to potentially interfere with the results from the micro-CT and SEM.

The baseline specimens for each material were then divided into two groups. The specimens in one of the groups were exposed to the acidic solution for 6 hours, the other ones for 48 hours. The acidic solution used in this study is a hydrochloric acid with a pH of 1.2 (HCl (Riedel-DeHaen/Sigma-Aldrich), H_2O). The specimens were covered with 5 mL of the acidic solution and stored in the static incubator at 37°C. A digital pH-meter (inoLab Multi 9310 IDS, Avantor, Radnor, USA) was used to measure the pH value of the acidic solution both before and immediately after immersion to examine a potential change. After acidic exposure, the specimens were rinsed with deionized water and gently patted dry with a paper tissue before examination. Micro-CT was performed on three of the specimens in each group, and microhardness test was performed again on the same specimens as earlier. SEM was performed on one of the specimens that underwent micro-CT in each of the groups.

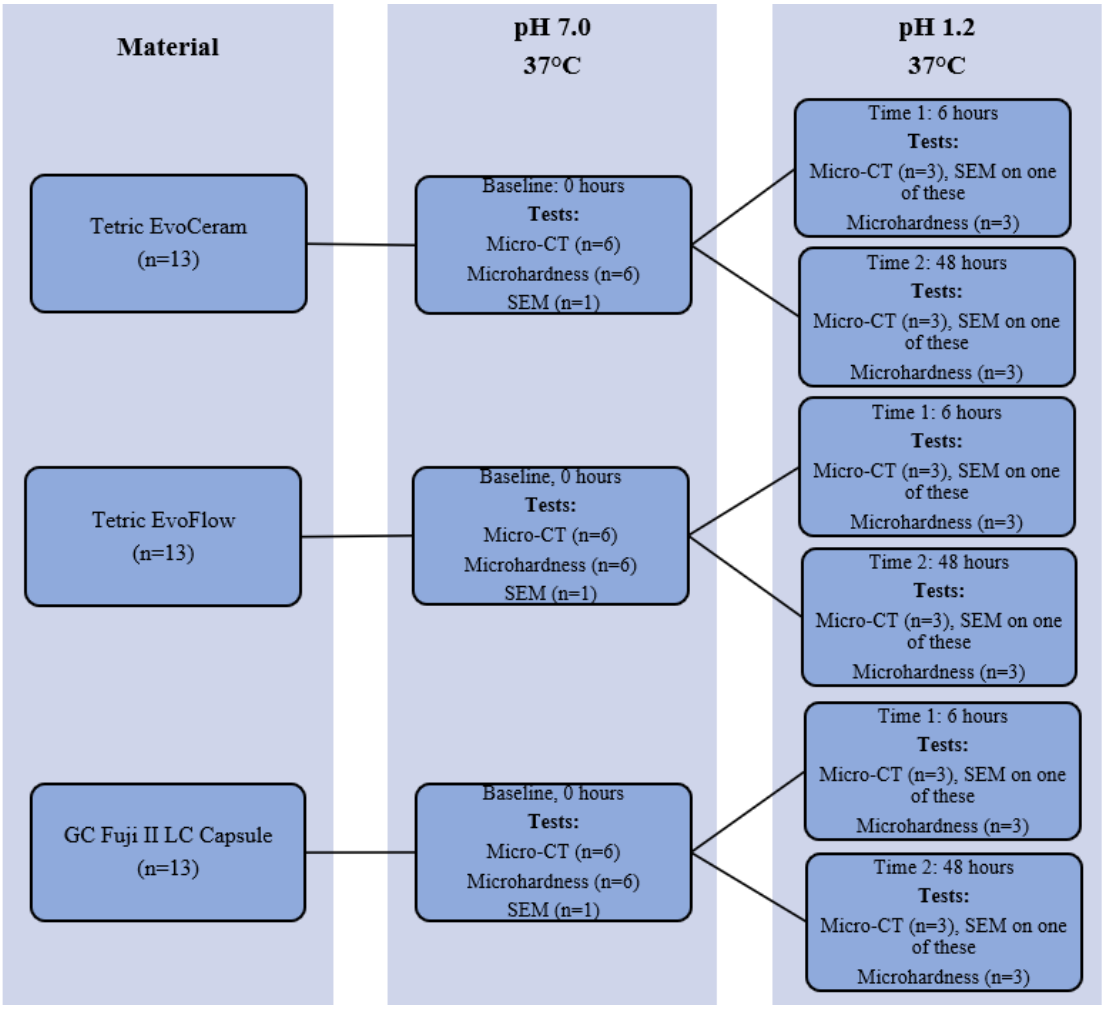

Figure 1 *Overview of the materials, pH, temperature, exposure times and tests.*

Microhardness test

A microhardness tester can be used to decide alterations in hardness of eroded surfaces of teeth and restorative materials by indenting a diamond tip onto the specimens (4). The length of the indentations is measured with a microscope, and the Knoop or Vickers hardness number is calculated (4).

The microhardness tester (ZHVμ-A, Indentec Hardness Testing Machines, Stourbridge, UK) performed five indentations on each specimen, using a Knoop diamond with a 200 g load and a 15 second dwell time. These parameters were selected after testing on pilot specimens. The indentations were randomly placed by the authors. We examined the smooth surface of the specimens, being the light-cured side. The analyses were done by at least two of the authors to ensure correct execution of the procedure.

Micro computed tomography (micro-CT)

Micro-CT is a method utilising the inequalities in intensity of x-rays before and after passing through an object, forming an image using the differences in x-ray attenuation (36). This is a non-invasive method, not harming the specimens during the analyses (37).

The volume and surface measurements were obtained by the use of micro-CT (Skyscan 1272, Bruker, Kontich, Belgium). Scans were performed by Berit Tømmerås, and calibration was performed by Berit Tømmerås and Napat Limchaichana Bolstad. The following settings were used: *image pixel size 8.00 µm, filter 0.11 mm Cu, source voltage 100 kV, source current 100 µA, exposure 2500 ms, 180° rotation for Tetric EvoCeram and GC Fuji II LC, 360° rotation for Tetric EvoFlow, rotation step 0.200° for Tetric EvoCeram and GC Fuji II LC, rotation step 0.400° for Tetric EvoFlow, scan duration Tetric EvoCeram 3h:4m:43s, scan duration Tetric EvoFlow 2h:52m:39s, scan duration GC Fuji II LC 3h:4m:52s.* Tetric EvoFlow had a 360° rotation as this setting gave the best results for these specimens. Image reconstruction was performed using NRecon (version 1.7.5.4) (InstaRecon Inc, IL, USA / Bruker, Kontich, Belgium). The micro-CT scans were analysed using CTAn (version 1.20.3.0) (Bruker-microCT). To ensure that the program interprets material and void correctly, the grayscale values were visually selected for each specimen by the use of binary selection. Too low a grayscale value leads to a larger white area of the scans, making the program incorrectly interpreting a larger volume, and vice versa. Selection of the area analysed was done manually for some of the specimens to exclude misinterpretation, as a smaller selection of our

scans had artifacts resembling white rays outside of the material. The selection of area analysed excluded these artifact areas, making the analyses more correct. Selection of grayscale values and area analysed was done prior to the analyses. The analyses were done by at least two of the authors to ensure correct execution of the procedure.

Scanning electron microscopy (SEM)

To evaluate surface morphology, SEM scans the surface of the samples with a beam of electrons to generate an image (38). SEM scans were performed by Tom-Ivar Eilertsen by the use of Zeiss Sigma HV. The specimens were sputter-coated with gold-palladium before investigation, and the examination was performed using a spot size of 2.00 kV with a WD of 2.7-3.6 mm. Two images were made for each specimen, using two different magnifications; 3.0K X and 10.0K X. The images were qualitatively assessed for surface alterations by the authors.

2.3 Statistical analyses

Excel (version 2203) (Microsoft, USA) was used for the initial data analyses and graphs. The statistical analyses were obtained by the use of Sigmaplot 14 (Systat. Software, San Jose, CA, USA).

To ensure normality of the data set, Shapiro-Wilk test was performed in advance of the paired t-tests. All the tests except for one passed, and Wilcoxon Signed Rank test was therefore used for this test. To investigate the difference between the means of two pairs of measurements done on the same sample, paired t-test was performed. The significance level was set to 5%.

The normality of the data set, as well as the equality of group variances, was tested in advance of the One Way ANOVA by the use of Shapiro-Wilk test and Brown-Forsythe test, respectively. One Way ANOVA was used to compare data between groups, using a significance level of 5%.

3 Results

3.1 Microhardness

The microhardness results of the direct dental restorative materials are shown in Figure 2 and Table 2. Tetric EvoCeram showed the highest Knoop hardness overall, followed by Tetric EvoFlow and GC Fuji II LC, respectively. When the microhardness results after 48 hours of acidic exposure was tested against each other, Tetric EvoCeram had a significantly higher hardness compared to the other two materials ($P < 0.001$). Only one material had a statistical change in hardness; Tetric EvoCeram showed a significant reduction in hardness after 48 hours of acidic exposure ($P = 0.039$).

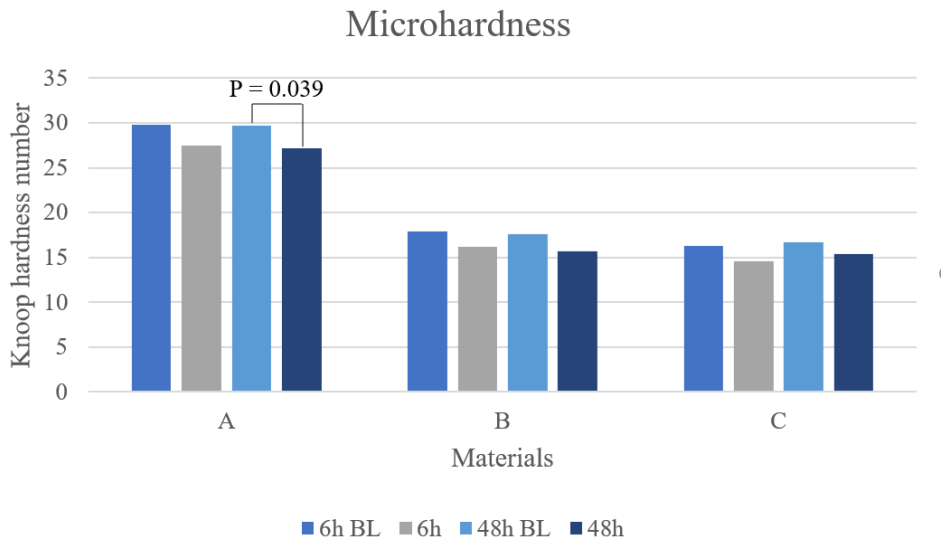

Figure 2 *Presentation of the microhardness results. The significant reduction is presented in the figure. Abbreviations: A: Tetric EvoCeram, B: Tetric EvoFlow, C: GC Fuji II LC. 6h BL: baseline specimens before 6 hours of acidic exposure, 6h: specimens after 6 hours of acidic exposure, 48h BL: baseline specimens before 48 hours of acidic exposure, 48h: specimens after 48 hours of acidic exposure.*

Table 2 *Presentation of the microhardness results. The values are represented in Knoop hardness number (KHN).*

Microhardness

	A 6h BL	A 6h	A 48h BL	A 48h	B 6h BL	B 6h	B 48h BL	B 48h	C 6h BL	C 6h	C 48h BL	C 48h
Minimum value	29.20	25.20	28.80	25.20	17.00	15.80	17.00	14.40	14.40	13.40	15.60	14.60
Maximum value	31.00	29.60	31.20	28.80	18.60	16.80	18.00	16.80	19.20	16.80	17.60	15.80
Mean value	29.80	27.47	29.73	27.13	17.93	16.20	17.60	15.67	16.27	14.60	16.73	15.33
Standard deviation	1.04	2.20	1.29	1.81	0.83	0.53	0.53	1.21	2.57	1.91	1.03	0.64

3.2 Micro computed tomography (micro-CT)

The results from the micro-CT regarding volume are presented in Figure 3 and Table 3. Overall, CG Fuji II LC had the largest reduction in volume both after 6 and 48 hours of acidic exposure compared to Tetric EvoCeram and Tetric EvoFlow. GC Fuji II LC and Tetric EvoFlow both had a significant volume reduction after 48 hours, with P-values of 0.023 and 0.039, respectively. There were no other significant volume reductions.

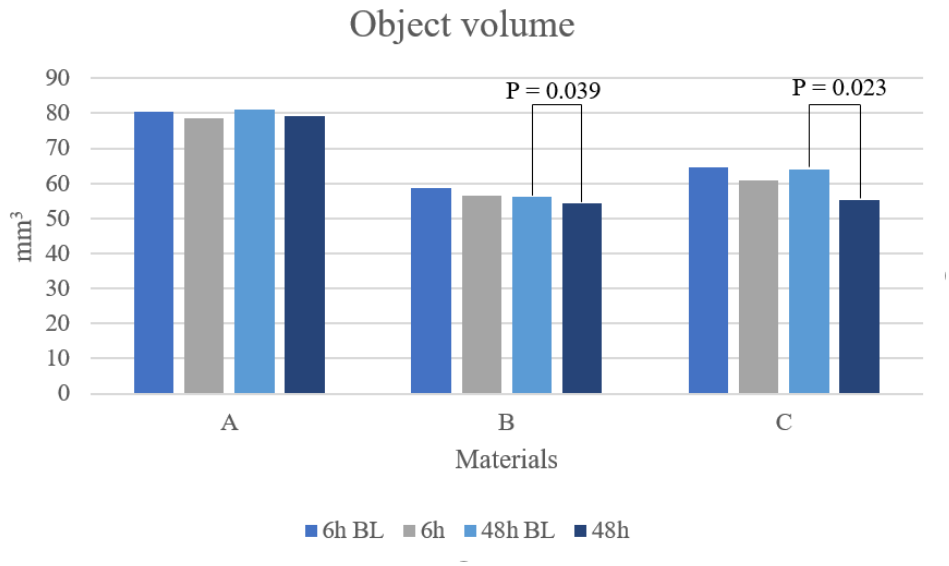

Figure 3 *Presentation of the micro-CT results regarding volume. The significant reductions are presented in the figure. Abbreviations: A: Tetric EvoCeram, B: Tetric EvoFlow, C: GC Fuji II LC. 6h BL: baseline specimens before 6 hours of acidic exposure, 6h: specimens after 6 hours of acidic exposure, 48h BL: baseline specimens before 48 hours of acidic exposure, 48h: specimens after 48 hours of acidic exposure.*

Table 3 *Presentation of the micro-CT results regarding volume. The values are represented in* mm^3.

Object volume

	A 6h BL	A 6h	A 48h BL	A 48h	B 6h BL	B 6h	B 48h BL	B 48h	C 6h BL	C 6h	C 48h BL	C 48h
Minimum value	79.00	76.47	80.23	77.98	57.04	53.00	54.56	51.94	60.67	58.90	62.06	52.26
Maximum value	81.77	80.99	81.50	80.63	61.29	58.42	58.02	56.42	66.71	62.46	66.23	59.90
Mean value	80.56	78.66	80.99	79.10	58.78	56.33	56.04	54.17	64.52	60.96	63.86	55.11
Standard deviation	1.42	2.26	0.67	1.37	2.23	2.92	1.78	2.24	3.35	1.84	2.14	4.18

The results from the micro-CT regarding surface area are presented in Figure 4 and Table 4. None of the tests were significant. However, the results indicate an increase in surface area for Tetric EvoCeram and Tetric EvoFlow, but a decrease was seen for GC Fuji II LC.

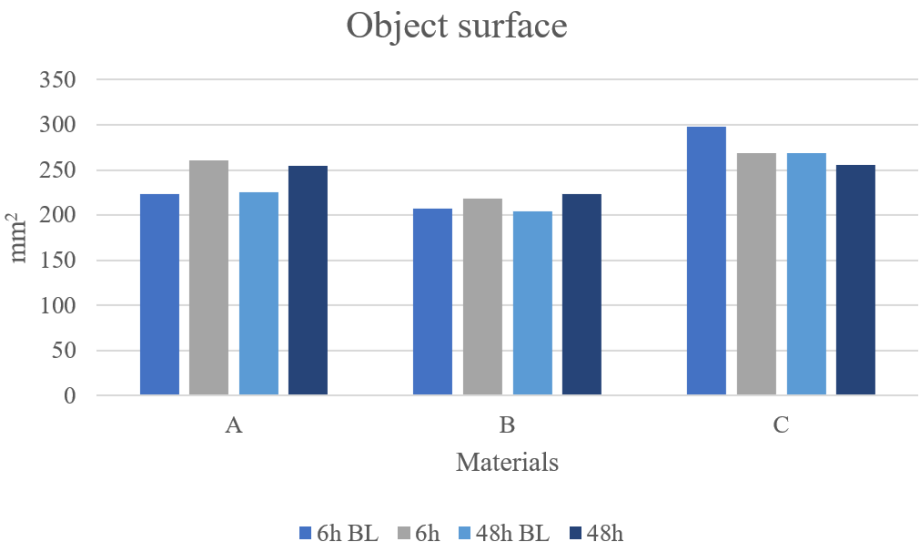

Figure 4 *Presentation of the micro-CT results regarding surface area. Abbreviations: A: Tetric EvoCeram, B: Tetric EvoFlow, C: GC Fuji II LC. 6h BL: baseline specimens before 6 hours of acidic exposure, 6h: specimens after 6 hours of acidic exposure, 48h BL: baseline specimens before 48 hours of acidic exposure, 48h: specimens after 48 hours of acidic exposure.*

Table 4 *Presentation of the micro-CT results regarding surface area. The values are represented in* mm^2.

Object surface

	A 6h BL	A 6h	A 48h BL	A 48h	B 6h BL	B 6h	B 48h BL	B 48h	C 6h BL	C 6h	C 48h BL	C 48h
Minimum value	206.27	215.39	215.71	210.26	199.52	201.79	199.52	201.09	241.46	241.46	262.44	219.74
Maximum value	251.54	302.98	231.83	284.02	210.69	237.26	212.97	255.21	380.59	324.83	275.89	325.58
Mean value	223.21	260.62	225.17	254.27	206.80	218.74	204.65	222.93	297.91	268.85	268.28	255.52
Standard deviation	24.69	43.86	8.42	38.89	6.30	17.78	7.27	28.53	73.18	48.49	6.90	60.68

3.3 Scanning electron microscopy (SEM)

Tetric EvoCeram

Figure 5 presents the SEM images for Tetric EvoCeram at baseline, and after 6 and 48 hours of acidic exposure. Regarding both magnifications, Tetric EvoCeram appears to be quite

smooth at baseline. The filler particles appear to have an even distribution. After 6 hours, there seems to be an increase in roughness, and the filler particles are more unevenly distributed. The images after 48 hours are more similar to the baseline images, looking smoother and more even than the images after 6 hours of acidic exposure.

Tetric EvoCeram

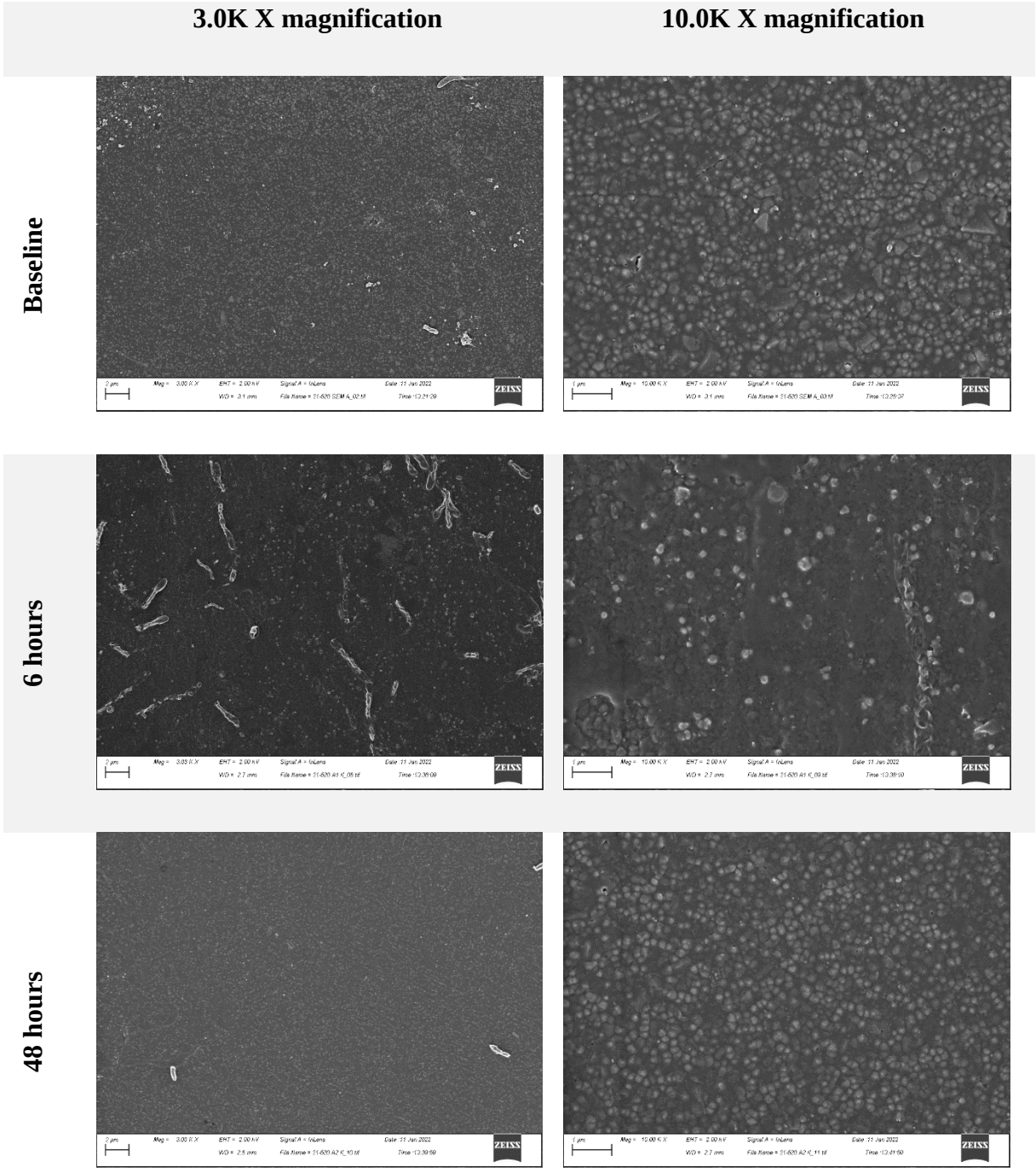

Figure 5 *Presentation of the SEM images for Tetric EvoCeram at baseline, and after 6 and 48 hours of acidic exposure.*

Tetric EvoFlow

Figure 6 presents the SEM images for Tetric EvoFlow at baseline, and after 6 and 48 hours of acidic exposure. When looking at the images with a magnification of 3.0K X, the baseline image appears to be smooth with a few larger pores. After 6 hours of exposure, the pores seem to have disappeared, giving it a more homogenous appearance. The same can be seen after 48 hours of exposure. In other words, the roughness of Tetric EvoFlow seems to decrease with acidic exposure. However, when looking at the images with a magnification of 10.0K X, the roughness seems to increase as the filler particles appear to be more evident with acidic exposure. The dark pores of the baseline image cannot be seen neither after 6 nor 48 hours of exposure.

Tetric EvoFlow

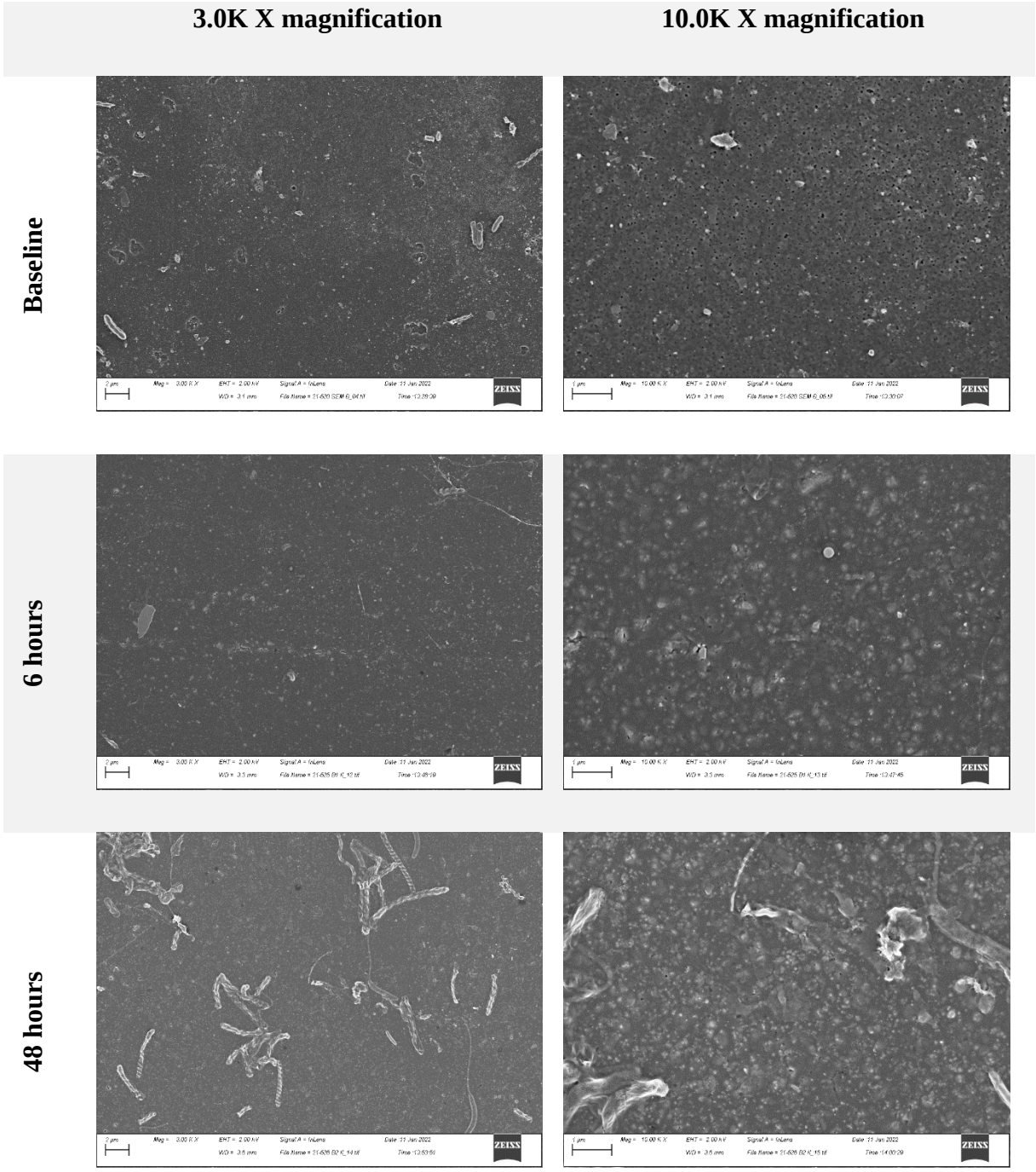

Figure 6 *Presentation of the SEM images for Tetric EvoFlow at baseline, and after 6 and 48 hours of acidic exposure.*

CG Fuji II LC

Figure 7 presents the SEM images for GC Fuji II LC at baseline, and after 6 and 48 hours of acidic exposure. Regarding both magnifications, GC Fuji II LC exhibit a less rough surface

from baseline to 6 hours of exposure, but a rougher surface from 6 hours to 48 hours of exposure. The images after 48 hours of exposure appear to be the roughest overall, with a less homogenous appearance compared to the other images. A fracture line can be seen on these, enhancing the non-homogenous appearance.

GC Fuji II LC

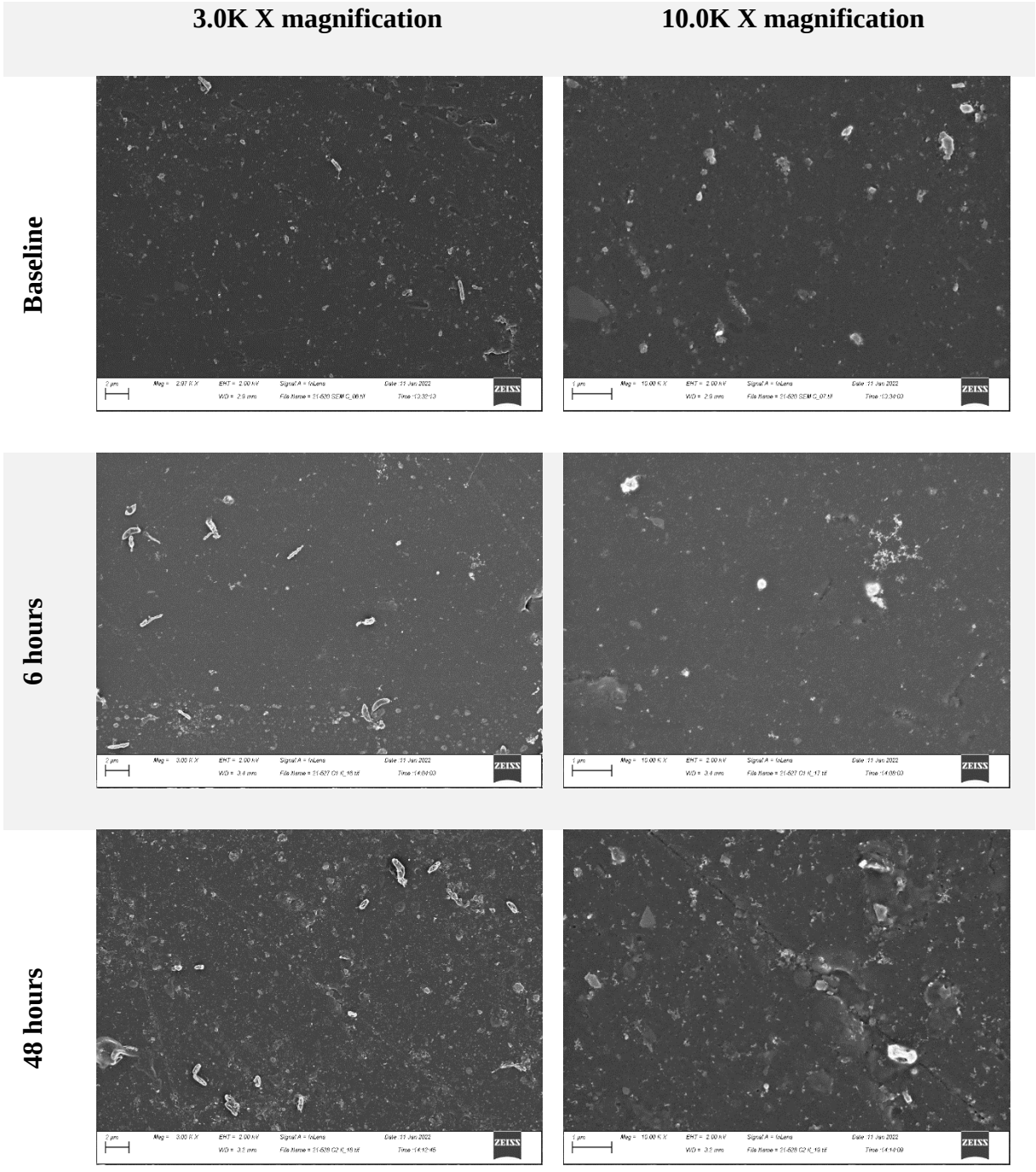

Figure 7 *Presentation of the SEM images for GC Fuji II LC at baseline, and after 6 and 48 hours of acidic exposure.*

4 Discussion

4.1 Discussion of results

The objective was to investigate the effect of a hydrochloric acidic solution with a pH of 1.2 – simulating the pH of gastric juice (7) – on surface hardness, volume, surface area and morphology of three direct dental restorative materials, using microhardness test, micro-CT and SEM. We hypothesized changes in microhardness, total volume, and surface area of the materials after exposure to the acidic solution. Based on our results, two of the three null hypotheses could be partly rejected; there were some significant changes in microhardness and total volume, but no significant changes regarding surface area.

Microhardness

The null hypothesis regarding microhardness can be partly rejected, as differences before and after acidic exposure were seen, although only one was significant. Tetric EvoCeram had a significant reduction in hardness after 48 hours of acidic exposure. However, a pattern could be seen; all the materials showed a reduction in hardness after acidic exposure, regardless of the length of exposure.

A study immersing two composite materials in simulated gastric juice with a pH of 1.2 for 6 and 24 hours did not find any significance in reduction in microhardness (7), which is similar to our results, as our only significant microhardness reduction is seen after 48 hours. A study by Briso et al. from 2011 found that GICs have the lowest microhardness values in general compared to composites, also similar to our results (28). This study investigated 60 specimens of composites and 30 specimens of GC Fuji II LC immersed in HCl with a pH of 1.6 for five weeks, among other things. They saw a significant reduction in microhardness for all these materials.

An advantage of using the microhardness test, is that it is an analysis method widely used for studies regarding acidic impact on dental restorative materials, making it easier to compare our results with similar studies.

Micro computed tomography (micro-CT)

To the authors' knowledge, there are no previous publications analysing the volume differences with micro-CT in direct dental restorative materials before and after acidic

exposure, which is why we wanted to include micro-CT in our study. This – however – also makes it difficult to find scientific evidence to compare our results with.

The null hypothesis regarding total volume can be partly rejected. GC Fuji II LC had a significant volume loss after 48 hours. This despite being covered with a protective layer of coat. Even though GC Fuji II LC is a resin-modified GIC, it was not an unexpected finding as conventional GICs have proven to be unsuitable to use in acidic conditions given that they easily decompose in such environments (15, 18). It also showed a larger volume loss compared to the two resin-based composites, which was expected as composites in general have proven to have good longevity in acidic environments (4). Also, GC Fuji II LC had the lowest microhardness among the tested materials.

Tetric EvoFlow also had a significant volume reduction after 48 hours, but Tetric EvoCeram did not. This was an unanticipated finding as we thought Tetric EvoFlow would be less affected by an acidic exposure than Tetric EvoCeram due to a lower content of fillers. Our theory is based on the matrix protecting the fillers from acidic contact, as the matrix – according to the literature – may be less vulnerable to acids compared to filler particles (22, 23). This will, theoretically, make a filler-to-matrix ratio of a flowable composite favourable in an acidic environment. The theory is supported by the constituents of resin-based composites vulnerable to acid; the interface between the filler and matrix appears to be the weakest element, and the filler surface may have a potential of being eroded (22). Our findings do not correlate with this theory. This could be due to a small sample size and inaccuracies regarding grayscale values chosen prior to the micro-CT analyses.

The null hypothesis concerning surface area was not rejected as none of the tests showed a significant difference, although differences were seen. We expected to see an increase in surface area in all the materials after acidic exposure as the erosion likely causes more pores. This result was seen for Tetric EvoCeram and Tetric EvoFlow. Regarding GC Fuji II LC, the surface area showed a decrease after acidic exposure. This might be explained by an erosion of the finishing coat, leaving a smooth surface of the underlying GIC. This is not inconceivable as the coat may be vulnerable to acid due to its content of up to 50% methyl methacrylate, containing ester bonds prone to hydrolysis (22, 39). This situation is – however – conceivably not transferable to the oral cavity as the coat likely wears away due to abrasion. Abrasion did not take place in our in vitro study.

A disadvantage with micro-CT is the visually selected grayscale values and analysed areas, making the method operator dependent. If a grayscale value was set too low, a larger area of the specimens would be white, leading to the analysis interpreting a larger area of the specimen to be material. And vice versa: if a grayscale value was set too high, a larger area of the specimens would be black, leading to the analysis interpreting a larger area of the specimen to be void. This could have a large impact on our results, as we wanted to investigate volume changes and surface area using micro-CT. Incorrect values would directly affect our results, giving incorrect analyses and therefore results of low value. Even though the analyses were conducted by at least two of the authors, these potential sources of error are difficult to exclude. In retrospect, we could have weighed the specimens before and after acidic exposure to check for volume changes. These results could have been compared to the micro-CT results, contributing to confirm whether the seen volume changes between these two methods correlate or not.

Micro-CT is a method widely used withing dentistry (37). Throughout our study, it has proven to be a valuable method of analysis to use when investigating the effect of low pH on direct dental restorative materials, as it provides exact numbers representing different parameters such as volume and surface area – among several others. This provides the opportunity to perform quantitative statistical analyses. One can also do qualitative analyses using micro-CT, as the scans give visual images that may provide useful information. Furthermore, it is a non-invasive method (37). However, there are – as formerly mentioned – clear disadvantages with this method as well, such as the manual selection of grayscale values.

Scanning electron microscopy (SEM)

SEM was included in the study to visualise the changes in surface morphology. We expected the roughness of the surfaces of all the materials to increase with acidic exposure, but no evident pattern could be seen for either of the three materials. We also expected the magnifications of the same specimen to correlate regarding surface morphology, but this was not seen either, complicating the interpretation of the images. It is – however – worth mentioning that this was a qualitative investigation; no quantitative measurements of surface roughness were done. Also, when analysing the SEM images, numerous bacteria could be seen. This contributed to complicating the interpretation of the images.

It was difficult to choose the most representative areas for analysing. The images were also challenging to analyse due to their variation in appearance, both between and within the groups. A plausible cause could be that every image shows different specimens; one specimen was not followed from baseline to 48 hours as SEM is a destructive method, excluding the opportunity of performing further tests on the same specimen and analysing it again later. This is an evident disadvantage with SEM.

The use of multiple methods of analysis

Microhardness, micro-CT and SEM are methods of analysis investigating different parameters that should be collectively considered when investigating the effect of low pH on direct dental restorative materials. We observed all of these parameters under the same conditions, which we consider to strengthen our study.

4.2 Discussion of methods

Selection of materials

Ordinary and flowable composites are widely used direct dental restorative materials, which is why we chose to investigate these. Resin-modified GICs were developed as conventional GICs have limitations, being more aesthetic, adhering better to tooth substance and having improved mechanical qualities (25). Thus, we also wanted to investigate a resin-modified GIC. Specifically, we chose Tetric EvoCeram, Tetric EvoFlow and GC Fuji II LC as they are familiar materials to the authors and widely used at Universitetstannklinikken in Tromsø. In addition, there are – to the authors' knowledge – few studies within this topic investigating an ordinary and a flowable composite with a somewhat similar filler-to-matrix ratio, making it reasonable to investigate the acidic effects as a function of this ratio.

Selection of exposure time

Our selection of exposure time was based on an article by Backer et al., where they exposed specimens made of CAD/CAM resin composites to an acidic solution with a pH of 1.2 for 6 and 24 hours in total (7). This corresponds – according to them – to 2 and 8 years of vomiting, respectively (7). We found two studies basing their exposure times on these intervals (7, 40). However, it is somewhat unclear how the exposure times were determined, and it is therefore uncertain whether they are fully representative for the clinical situation. We chose to expose our specimens for 6 and 48 hours. 6 hours of exposure is – as mentioned – equivalent to 2

years of vomiting, and Backer et al. found changes in surface roughness after this amount of exposure. They could, however, not find changes in surface hardness in their specimens neither after 6 hours of exposure, nor 24 hours. Therefore, we wanted to expose our specimens for 48 hours – equivalent to 16 years of exposure – to see whether this is a sufficient amount of exposure time for changes in surface hardness of our chosen dental restorative materials to appear (7). To ensure differences in certain qualities of our filling materials after 48 hours of acidic exposure, we read several articles regarding clinical endurance of various filling materials. An article by Burke & Lucarotti found that only approximately 34% of the studied resin composite fillings remained clinically satisfactory after 15 years (41). Regarding GICs, Scholtanus & Huysmans found that the percentage of clinically satisfactory fillings was as low as 60% after 6 years (42). Hence, we considered an acidic exposure of our specimens equivalent to 16 years of vomiting to be appropriate.

At first, we planned to expose the specimens to the hydrochloric acidic solution for a substantially longer period of time compared to the chosen exposure times. In retrospect, this could have been beneficial as it could have improved our results. This is due to the hydrochloric acidic solution presumably penetrating the resin matrix more profoundly with a longer exposure time.

Selection of solutions

Originally, our objective was to investigate the effect of both intrinsic and extrinsic acid on the direct dental restorative materials. It is conceivable that we would have obtained a fuller understanding of how the materials work in different acidic environments if multiple acidic solutions would have been used. Unfortunately, we were unable to do so due to limitations regarding the amount of time and the size of the thesis. As a result, we chose to investigate the effect of a hydrochloric acid with the pH of 1.2, using deionized water with a neutral pH as a baseline. A pH of 1.2 corresponds to the pH of gastric juice, which can be found in the oral cavity of a bulimic patient repeatedly (7). Hence, we wanted to use an acidic solution that resemble the pH of this endogenic acid, as it may affect dental restorative materials used in the oral cavity.

Preparation

There are multiple factors that may have affected the specimens during preparation. The three authors made the specimens for one material each, meaning that slight differences between

the materials cannot be excluded. There is for example a difference in thickness of the materials, despite our standardised preparation technique using a glass plate to extrude the excess material, and the formerly mentioned testing of the LED lamp securing an adequate output for each light curing. Tetric EvoFlow was the material with the thinnest specimens. This – however – may be explained by the low filler content and hence low viscosity and large polymerization shrinkage (21). We had the option to make thicker specimens. The main reason why we chose not to, was to assure homogenous specimens of GC Fuji II LC by only needing one capsule per specimen.

To ease the removal of the specimens from the mould, Teflon Tape was used. The tape was somewhat difficult to remove from the specimens, and a small amount remained even after the finishing using Sof-Lex™ discs. This is a factor that could have had an influence on volume loss, as it may protect the surface of the discs from the acidic solution. It was, however, such a small amount situated on the back of the specimens that we assume it did not affect our results.

Storage of specimens

The acidic solution was not changed daily due to such a short immersion time that we assumed it would not saturate the solutions. We measured the pH before and after immersion of the specimens and found that the pH levels were stabile for Tetric EvoCeram and Tetric EvoFlow, constantly being approximately 1.2. However, the pH increased slightly after immersion for GC Fuji II LC, with the highest measured value being 1.636. This could be explained by GICs' ion release in acidic environments, functioning as a buffer and gradually increasing the surrounding pH (28, 43).

The specimens were stored in glass containers immersed in deionized water for a prolonged amount of time compared to originally planned. Articles have shown that cured materials take up water, potentially affecting surface roughness, volume, and hardness over time (28, 29, 43). The articles insinuate an increased surface roughness for composites, and an increased volume and a reduced hardness for both composites and GICs. This could be seen in our results, as the baseline specimens for microhardness test and micro-CT tests of all the materials show a variance in hardness, volume and surface area, respectively. We cannot exclude the possibility that this have also affected the measured results after acidic exposure.

Another factor that may have had an influence, is the placement of the specimens in their respective glass containers. It is conceivable that the surface facing down is less affected by acid, impacting our results. In retrospect, it is possible that we could have benefitted from using a shaking incubator.

4.3 Clinical relevance

As for all in vitro studies, our results are not directly transferable to the clinic. It is challenging to simulate the exact conditions of the oral cavity, and there are several factors that affect dental restorative materials. These are factors such as saliva, temperature, pH, and different types of mechanical wear. Despite multiple factors affecting the dental restorative materials at once, it may be beneficial to investigate the isolated impact of one or few factors; this is an advantage with in vitro studies.

There are various types of mechanical wear in the oral cavity, such as occlusal forces, mastication of foods, abrasion from toothbrushing, and tongue movement. These factors were not considered in this study. As a consequence, our results could be limited to restorations in non-loaded areas of the dentition, such as palatal surfaces of maxillary incisors.

The temperature and pH of an oral cavity may fluctuate several times during the day, depending on the temperature and pH of ingested foods and beverages (44). It is hard to resemble these fluctuations in the laboratory. We found a temperature of 37°C to best simulate the environment of the oral cavity. The pH level is affected by salivary flow, which is beneficial in oral cavities affected by acidic substances as it dilutes, buffers and somewhat removes the threat from the surface of the teeth and restorations (4). It is important to address that bulimic patients may experience a lower saliva secretion, making them more vulnerable to acidic impacts (9). It is also worth mentioning that the pH of gastric juice is fluctuating to some degree, with a range from 1.0 to 3.0 (7). However, we found several other studies using a constant low pH, imitating gastric juice (7, 28). Aware of our parameters not fully representing the clinical situation, we still found a constant acidic impact with a low pH to be the most expedient to do when imitating a worst-case scenario in the oral cavity of a bulimic patient, investigating how an extensive acidic impact may affect direct dental restorative materials used in the mouth of such a patient.

5 Conclusion

Within the limitations of the present study, our conclusions are as follows:

In the absence of mechanical stimulus, the microhardness of the materials may be reduced, there may be a loss of volume, and differences in surface area and morphology may appear after acidic exposure. The observed differences were small, and only a few of the tests were significant. This indicates that dental restorative materials may have a protective effect against erosions on non-loaded areas of the dentition. Due to the small sample size, multiple clinical factors we were unable to simulate and uncertainty about the validity of the exposure times used, it is not certain that our results are representative for the actual clinical situation. Therefore, we consider our study to be a pilot study; further investigations are required to obtain complementary results.

6 References

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7 Appendix

7.1 Images – material and methods

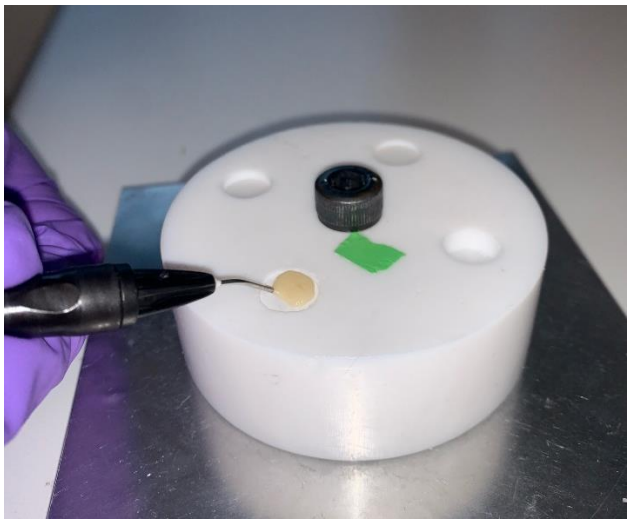

Image 1 *Application of Tetric EvoFlow in the mould.*

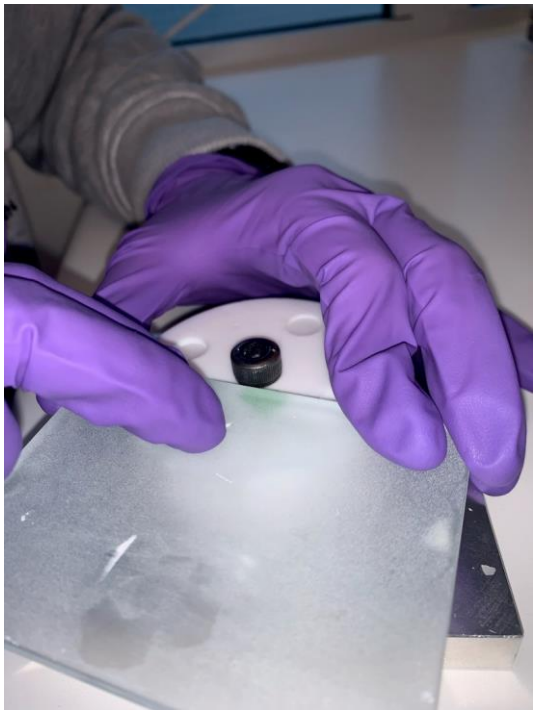

Image 2 *Pressing with finger pressure to standardize the finishing of the sample.*

Image 3 *Light-curing with a Bluephase® G2 LED lamp.*

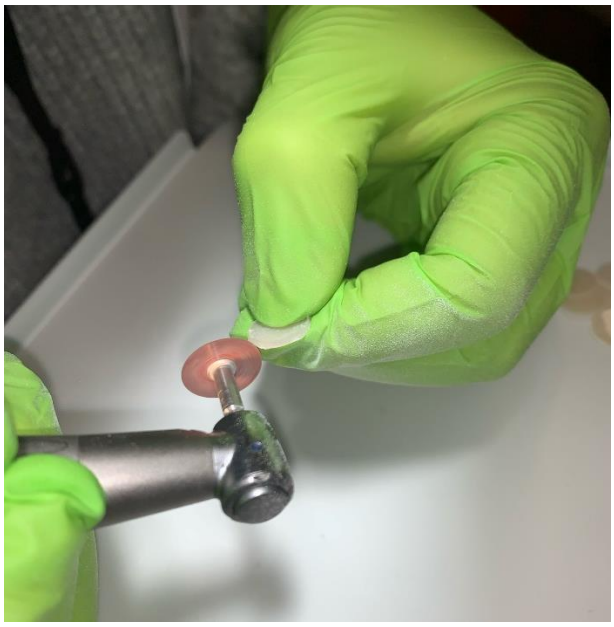

Image 4 *Finishing of the specimen using Sof-Lex™ discs.*