EFFECT OF CITRIC ACID ON COLOR STABILITY AND SURFACE ROUGHNESS OF TRANSLUCENT MONOLITHIC ZIRCONIA WITH DIFFERENT STAINING TECHNIQUES

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ผลของกรดซิตริกต่อเสถียรภาพของสีและความหยาบพื้นผิวของโมโนลิธิคเซอร์โคเนียแบบใสที่ผ่านการเคลือบสีที่ต่างกัน

น.ส.ปาณิศา รินทอง

วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาวิทยาศาสตรมหาบัณฑิต
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Thesis Title	EFFECT OF CITRIC ACID ON COLOR STABILITY AND SURFACE ROUGHNESS OF TRANSLUCENT MONOLITHIC ZIRCONIA WITH DIFFERENT STAINING TECHNIQUES
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Field of Study	Prosthodontics
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ปาณิศา รินทอง : ผลของกรดซิตริกต่อเสถียรภาพของสีและความหยาบพื้นผิวของโมโนลิธิคเซอร์โคเนียแบบใสที่ผ่านการเคลือบสีที่ต่างกัน. (EFFECT OF CITRIC ACID ON COLOR STABILITY AND SURFACE ROUGHNESS OF TRANSLUCENT MONOLITHIC ZIRCONIA WITH DIFFERENT STAINING TECHNIQUES) อ.ที่ปรึกษาหลัก : ผศ. ทพญ. ดร.ปรารมภ์ ซาลิมี

ความสำคัญและที่มา โมโนลิธิคเซอร์โคเนียแบบใสถูกพัฒนาเพื่อแก้ไขปัญหาเรื่องความสวยงามของเซอร์โคเนียรุ่นดั้งเดิมที่มีความทึบแสง วัสดุนี้เหมาะกับการใช้งานในฟันหน้าซึ่งเมื่ออยู่ในช่องปากพื้นผิวของเซอร์โคเนียจะสัมผัสกับอาหารและเครื่องดื่มที่มีสภาวะกรด ซึ่งอาจมีผลต่อเสถียรภาพของสีและความหยาบพื้นผิวของวัสดุแต่ละชนิดที่ระดับแตกต่างกัน

วัตถุประสงค์ การศึกษานี้มีวัตถุประสงค์เพื่อศึกษาผลของกรดซิตริกต่อเสถียรภาพของสีและความหยาบพื้นผิวของโมโนลิธิคเซอร์โคเนียแบบใสด้วยเทคนิคการเคลือบสีที่ต่างกัน

วัสดุและวิธีการ การทดลองทำโดยใช้โมโนลิธิคเซอร์โคเนียแบบใส ขึ้นรูปโดย CAD-CAM software และตัดแบ่งเป็นแผ่นกลม เส้นผ่าศูนย์กลาง 14 ×1.5 มม. จำนวน 80 ชิ้น จากนั้นขัดชิ้นงานทั้งหมดให้เรียบด้วยหัวขัดหยาบชนิดหินเคลือบเพชรตามด้วยหัวขัดชุด VITA SUPRINITY® จากนั้นแบ่งเป็น 4 กลุ่ม กลุ่มละ 20 ชิ้น ตามขัดและเคลือบผิว ได้แก่ กลุ่มที่ไม่ได้ปรับแต่งพื้นผิว (NT), กลุ่มที่ผ่านการขัด (PO), กลุ่มที่เคลือบสีแล้วเผาตามด้วยเคลือบแก้ว (S-G), และกลุ่มที่เคลือบสีผสมเคลือบแก้วแล้วเผา (S+G) เคลือบด้วย VITA AKZENT Plus® STAIN and GLAZE จากนั้นแบ่งเป็น 2 กลุ่มย่อยกลุ่มละ 10 ชิ้น แยกตามสารละลายที่แช่ จากนั้นนำชิ้นงานทั้งหมดไปทำการแช่ในน้ำลายเทียมที่ 37 องศาเซลเซียสเป็นเวลา 14 วัน (กลุ่มควบคุม) และแช่ในกรดซิตริกความเข้มข้น 2% เป็นเวลา 8 ชั่วโมง เพื่อจำลองสภาวะ 2 ปีในช่องปาก ทำการวัดค่าความแตกต่างของสี (ΔE) และความหยาบพื้นผิว (Ra) ก่อนและหลังการแช่ด้วยเครื่องวัดความหยาบพื้นผิวแบบสัมผัส

ผลการศึกษา สำหรับการเปลี่ยนสีพบว่ากลุ่ม S+G และ S-G มีค่าเฉลี่ยสูงกว่ากลุ่ม NT และ PO อย่างมีนัยสำคัญในสารละลายทดสอบทั้งสอง อย่างไรก็ตามไม่พบความแตกต่างอย่างมีนัยสำคัญของ ΔE ระหว่างกลุ่ม S+G และ S-G ส่วนความหยาบพื้นผิวไม่มีความแตกต่างอย่างมีนัยสำคัญทางสถิติระหว่างความหยาบพื้นผิว (Ra) และการเปลี่ยนแปลงความหยาบพื้นผิว (ΔRa) ในกลุ่มการขัดแต่งพื้นผิวทั้ง 4 กลุ่มในสารละลายทั้งสอง

สรุปผลการศึกษา กรดซิตริกมีผลต่อเสถียรภาพของสีของการเคลือบเซอร์โคเนียแบบใส ทั้งสองเทคนิค แต่ไม่เกินเกณฑ์ที่สายตามองเห็นการเปลี่ยนแปลงได้ นอกจากนี้กรดซิตริกไม่ส่งผลกระทบต่อความหยาบพื้นผิวในทุกกลุ่ม

สาขาวิชา	ทันตกรรมประดิษฐ์	ลายมือชื่อนิสิต .....
ปีการศึกษา	2561	ลายมือชื่อ อ.ที่ปรึกษาหลัก .....

5975826732 : MAJOR PROSTHODONTICS
KEYWORD: Citric acid Color stability Surface roughness Surface finish Translucent monolithic zirconia
Panisa Rinthong : EFFECT OF CITRIC ACID ON COLOR STABILITY AND SURFACE ROUGHNESS OF TRANSLUCENT MONOLITHIC ZIRCONIA WITH DIFFERENT STAINING TECHNIQUES. Advisor: Asst. Prof. Prarom Salimee, Ph.D.

Background and rationale: Translucent monolithic zirconia is developed to solve the problem of esthetic in the conventional generation of zirconia with high opacity. When these materials are used for anterior restoration in the oral cavity, they can contact with foods and beverages that are acidic, which may affect the color stability and surface roughness of the material at different levels.

Objective: The objective of this study was to determine the effects of citric acid on color stability and surface roughness of translucent monolithic zirconia with different staining techniques.

Materials and Methods: A total of 80 disc specimens of VITA YZ® XT (14 mm in diameter and 1.5 mm in thickness) were designed with computer-aided design and computer-aided manufacturing (CAD-CAM) software then cut with a low-speed saw and sintered, according to the manufacturer's instruction. All sintered specimens were divided into 4 groups, including no treatment (NT), polishing (PO), stained then glazed (S-G) and mixing of stain and glaze (S+G). For the PO specimens, they were polished by diamond coated grinding bur and VITA SUPRINITY® polishing set. The S-G and S+G groups were subjected to glaze coated by VITA AKZENT Plus® STAIN and GLAZE. All specimens were separated into 2 subgroups (n=10). The first subgroup was immersed in artificial saliva at 37 °C for 14 days to simulate the exposure of saliva in the oral cavity for 2 years in vivo. It was also used as a control group. The other subgroup was immersed in 2% citric acid solution for 8 h to simulate 2-year exposure of citric acid in the oral cavity. The measurement of color change (ΔE) and surface roughness (Ra) before and after the immersion was conducted with a spectrophotometer and a contact type profilometer respectively.

Results: For the color change, the results showed that S+G and S-G groups had significantly higher mean values of ΔE than NT and PO groups in both solutions. When considering these 2 staining techniques, ΔE value in citric acid solution was significantly higher than artificial saliva. However, no significant difference of ΔE was observed between S+G and S-G groups. For the surface roughness, there was no statistically significant difference between surface roughness (Ra) and surface roughness change (ΔRa) among 4 surface finish groups in both solutions.

Conclusions: It can be concluded that citric acid had an unfavorable effect on the color stability of both staining techniques on translucent monolithic zirconia but did not exceed the perceptible threshold. However, citric acid did not affect the surface roughness in all surface finish groups.

Field of Study:	Prosthodontics	Student's Signature .....
Academic Year:	2018	Advisor's Signature .....

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CHAPTER I

INTRODUCTION

Background and Rationale

Zirconia restorations have been continually developed particularly in terms of its strength, which can serve a conservative preparation as PFM. The first generation of zirconia was used for the framework and then veneered with ceramic. The advantages of this generation include the high strength and superior of fracture toughness, the easiness of processing, less time consuming, less invasive preparation and less wear opposing structure when meticulously polishing. However, chipping of veneering porcelain and low-temperature degradation (LTD) were the main problems that urge the manufacturers to continue developing monolithic zirconia in order to avoid chipping of veneering porcelain (1). Even though it was developed into monolithic form, it is still limited to whitish colored shade which was limited to posterior restoration in the early period (2). The coloring strategies can be created by using a polychromatic shade that was initially mixed with zirconia powder or dyed through the infiltration of the colored liquid (3, 4) Further development is to modify translucent monolithic zirconia by microstructure modified process which can be used in anterior restorations with a decreasing in mechanical properties but more resistance to LTD. This can improve the esthetic in the patients with high occlusal forces (2, 4).

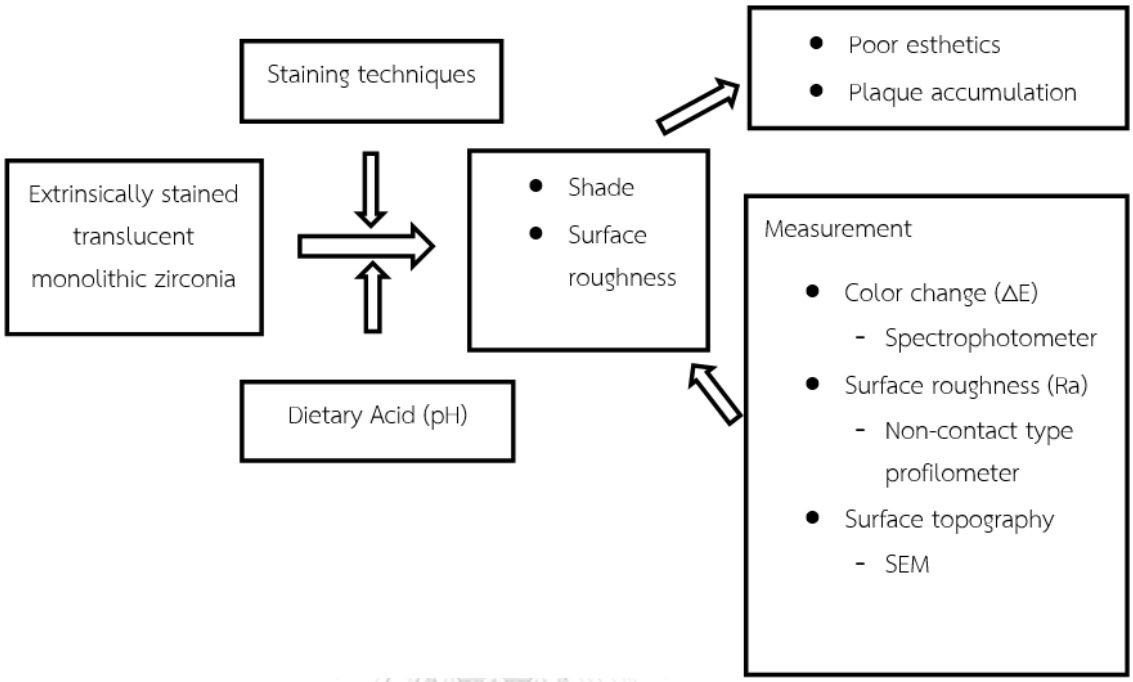
To imitate the variation of the natural tooth characteristic of dentine and enamel, the increase of translucence in the multicolor block might not be sufficient due to the complex esthetical components and characteristics. Therefore, surface characterization and external staining might be needed especially when adjacent natural teeth exists (5-7). To maintain long-lasting color stability, clinicians should pay attention to prevent changes in the shade and surface roughness of an extrinsically stained layer. Since the external surface is directly exposed to the complex environment of the oral cavity, many factors need to be considered for the degradation of the external ceramic surface; such as physical and chemical stress (8), especially chemical degradation from the daily intake of acidic foods and/or beverages. Generally,

hydroxyl organic acid such as citric acid is the major acid in fruit and vegetables which mostly found in daily diets (9). Several studies (10-12) have shown the effects of citric acid on ceramic materials with different results which are depended on the material's compositions. The acidic environment can increase the surface roughness in glass ceramic as receive auto-glazing or overglazing (10, 11). Moreover, it had a negative effect on surface roughness and color stability of zirconia-reinforced lithium silicate glass ceramic (ZLS) with polishing more than glazing (13). To date, none of studies has investigated the durability of the extrinsically stained translucent monolithic zirconia after exposed to a long-term acidic diet.

The aim of this study is to evaluate the changes in the color and surface roughness of extrinsically stained translucent monolithic zirconia when exposing citric acid and other different methods of applying the stain. This will help the clinicians to predict the long-term success in an aesthetic appearance of translucent monolithic zirconia by external staining.

The null hypotheses were that there were no statistically significant difference on color change (ΔE) or surface roughness change (ΔRa) in 4 different surface finishing techniques of translucent monolithic zirconia after artificial saliva or acid immersion.

Conceptual Framework



Research Question

Does citric acid immersion have an effect on color stability and surface roughness of extrinsically stained translucent monolithic zirconia with two different staining methods?

P: translucent monolithic zirconia

I: citric acid immersion

C: salivary immersion

O: color stability and surface roughness of extrinsic stain

Research Objectives

1. To evaluate the color stability of extrinsically stained translucent monolithic zirconia after acid immersion.
2. To evaluate the surface roughness of extrinsically stained translucent monolithic zirconia after acid immersion.

Research Hypotheses

1. Ho_1: Citric acid immersion does not have a significant effect on color change (ΔE) of translucent monolithic zirconia with 4 different surface finish methods.

   Ha_1: Citric acid does have a significant effect on color change (ΔE) of translucent monolithic zirconia with 4 different surface finish methods.
2. Ho_2: Citric acid does not have a significant effect on surface roughness (ΔRa) of translucent monolithic zirconia with 4 different surface finish methods.

   Ha_2: Citric acid have a significant effect on surface roughness (ΔRa) of translucent monolithic zirconia with 4 different surface finish methods.

Proposed Benefits

1. To provide recommendations for awareness of acidic food or beverage intake in patient who receive translucent monolithic zirconia restoration.
2. To provide guidelines for considering the application of extrinsically stain technique for translucent monolithic zirconia.

Keywords

1. Citric acid
2. Color stability
3. Surface roughness
4. Surface finish
5. Translucent monolithic zirconia

Type of research

Laboratory experimental research

CHAPTER II

Literature Review

Zirconia ceramic

Zirconia (ZrO_2) is a crystalline dioxide of zirconium with a mechanical property comparable to those metals, but zirconia's optical appearance is very similar to a natural tooth. Zirconia is polymorphic in three different phases: monoclinic (M) at room temperature, stable tetragonal (T) between 1,170 to 2,370°C and cubic (C) above 2,370°C. In general, the strength of the phase is tetragonal, cubic and monoclinic, respectively. From this situation, the monoclinic phase was compromised by mechanical properties from the reduction of particle cohesion and density. The unique property of this material is its "transformation toughening" that is spontaneous tetragonal transforming into a monoclinic phase. When stresses; such as, a crack propagate in the materials, the tetragonal grain transform into a monoclinic form with a 3-5% volume expansion of the grains, which resulted in compressive stress at the edge of the induced crack front in the microscopic level. This process is the basic superior high strength properties of Y-TZP with fracture toughness of 5-10 $MPa.m^{1/2}$ and flexural strength of 900-1,400 MPa. The most widely used form of dental zirconia is 3Y-TZP, which is achieved by adding 2-3% of yttrium oxide (Y_2O_3). This additive can reduce the transformation of tetragonal to the monoclinic phase during the cooling periods of 670-1,070°C (14-16).

Generally, the first generation of zirconia was opaque in nature. The technique for solving this esthetic compromised problem was veneering porcelain on the framework. However, many clinical studies have shown that the major problem is the chipping of the veneering porcelain up to 50% after one to seven years of observation. This chipping problem has driven manufacturers to use two alternative techniques of a hybrid-structured fixed partial denture by CAD-on or fired fusing and monolithic or a full contoured structure of Y-TZP. However, the development of new generation dental zirconia has been driven by the major goal of strong concurrent with esthetics (1, 15).

Development generations of dental zirconia ceramic

The growth in the dental zirconia industry has focused on improving esthetics to coincide with the mechanical strength of the monolithic type by modifying the additive dopants and sintering process(4). Many manufacturers have attempted to change the weakness of these materials by developing generations of current zirconia ceramics used for dental restoration. The conventional generation is 3Y-TZPs, which is used for the framework with veneering porcelain. The second generation of 3Y-TZPs monolithic zirconia reduces more of the alumina and eliminates the porosity in the high temperature sintering process. Although it has been improved to have acceptable translucence it still has unsatisfactory anterior restoration. The third generation is developed by incorporating some of the transparent phase; such as, the cubic phase into a 3Y-TZPs and combining it by increasing the yttrium content between 4-5% Y-PSZ. This modified process has gained more translucency but been compromised in the mechanical property. However, it has reduced the influence of low temperature degradation due to the cubic phase does not display the transformation of toughening. The latest generation of cubic containing zirconia consists of about 8-10 mol% of yttrium, and the cubic phase contains about 10-15% while the flexural strength is about 600-750 MPa (2). Although it increased in translucence it still lacks imitating some optical effects such as complexation of shade or multi-structures of crystalline enamel, so the shade modification and characterizations for monolithic zirconia are needed.

Shade modification and characterizations for monolithic zirconia

To approach coloring of monolithic zirconia, there are 2 methods of the applications: internal coloration and external coloration. The internal coloration is in the production stage that can be done in 2 application stages; the first type is the use of metal oxide mix with ZrO_2 powder, resulting in a pre-colored green stage block or the second type uses a specific colored liquid apply on green stage framework by dipping or brushing before the sintering process. The external coloration is applied after sintering of zirconia followed by traditional firing like traditional ceramic (1, 2, 4, 6, 17).

In clinical application, it is difficult to match human teeth, which have complex shades and multi-crystal structures. To achieve the esthetic appearance of monolithic zirconia, the application of external surface staining can be done with highly pigmented glaze. However, the main disadvantages are the low durability and the reduction of translucency (18). In general, these techniques are often used with two traditional recommendations which are the auto or overglazed technique (18-20). Most of the glaze should be 50 μm in thickness or more for adequate durability (21).

For the durability of the extrinsic stain, the study of Garza et al. compared the three different techniques of the extrinsic stain of lithium disilicate including glaze only, stain followed by glaze and mixing stain and glaze together, then subjected it to simulated brushing. The results found that combining the stain and glaze together significantly changed color difference and roughness of the lithium disilicate. The study suggested that the separation of stain followed by glaze was more durable than the mixed technique (22). This result was the same as the study of Chi et al. (23) in the roughness and weigh loss of leucite-reinforced glass. These indicated that type of material influenced durability of surface staining (24).

The acid reaction on surface roughness and color stability of dental ceramics

In clinical situations, dental ceramic restorations are always exposed to oral complex environments. It is degraded by mechanical force, a chemical attack or a combination effect. Generally, ceramic restorations can be usually stained on the external surface with shading porcelain for acceptable esthetic. If the stained layer is too thin, the colorant layer will be easily attacked by the external environment. The outcome from a chemical attack by an organic agent can weaken the structure by creating surface flaws and increasing the susceptibility of the ceramic to a future chemical attack (8).

In case of zirconia ceramic, it was known that conventional zirconia was difficult to be degraded by acid. Recently, the previous study (25) has shown that zirconia can be etched by hydrofluoric acid (HF) in an unusual condition which changes the surface roughness and induced phase transformation. According to Xie et. al, the surface change and roughness increased in HF but not seen in 10% of the acetic and 20% of

the citric acid at ambient temperature (26). However, Sokkary, Elguindy and Shihi investigated the effect of citric acid on zirconia reinforced lithium silicate glass ceramic (ZLS) which the results showed that citric acid aging had an effect on the surface roughness and color stability of different surface finishing methods (13). These results indicated that the acidity of dietary acids could destroy the surface properties of ceramic restorations with different outcomes depending on the types of acid and differences in ceramic material composition.

Common dietary acids in food and beverages and its reaction with surface staining of dental ceramic

There are two kinds of common acid that are usually found in food and beverage: hydroxy and non-hydroxy organic acid. Hydroxyl organic acids were mostly found in fruit and fruit-related products which are usually below pH 5; such as, malic, tartaric, lactic, acetic, oxalic acid and citric acid. Citric acid and malic acid are the major acids in fruit and vegetables with a ratio of 30:4. Citric acid shows many concentrations; such as, 0.3% (W/V) in ready-to-drink juice, 1% (W/V) in orange juice, and 6% (W/V) in lemon juice (9). Generally, the average exposure of citric acid is about 40 seconds per day (10). Non-hydroxy organic acid is often used to modify the flavor of food and beverage; such as, 0.1% (W/V) of phosphoric acid (9) and 0.3-0.6% (W/V) of carbonic acid (9).

Many studies have shown the effects of citric acid on ceramic materials with different results; for example, Kukiattrakoon et al. found a significant change in surface roughness when different types of ceramic exposed to citric and acetic acid, especially 4% acetic acid in four types different glass (12). Sokkary, Elguindy and Shihi showed the effect of citric acid on zirconia reinforced lithium silicate glass ceramic (ZLS) that had increase surface roughness and color difference when it was self-glazed (13). Moreover, the study of Demirel et al. showed a moderate change in the surface roughness of pressable ceramic in both auto-glazing and overglazing, but there was no statistical significance when exposed to 2% of citric acid for eight hours on atomic force measurement (11). However, Demirhanoglu and Şebnem showed that there was no surface roughness change in feldspathic porcelain on both self-glazing and

overglazing in the profilometer measurement. The study also recommended overglazing for a better result (10).

Physiological properties testing

Surface roughness measurement

The surface loss and change can be measured by surface roughness measurement. “Roughness” is a measure of surface texture. It is a quantifying measurement, which indicates the deviation of the surface from its ideal form. Although the roughness average (Ra) is still the main report measurement of surface change in dental studies, other surface parameters should be further reported to describe the meaning of the surface quality. There are many instruments which are used to measure the quantity and quality of surface loss and change both with micro and nanoscopic techniques. The qualitative surface measurement of SEM can be utilized because of the large depth of view and high resolution of 3D image (27).

Color measurement

Visual color measuring varies among individual variation and some problems include the fatigue of the receptor response (28, 29). Therefore, color measuring instruments are a representative of quantitative data for a valid color parameter. A criterion is established for color measurement to be indicated by color difference (ΔE), which is a standard for measurement of color stability in dentistry. However, there is no definite cut point of different values. Many previous studies suggested the value of ΔE was more than 3.3 or 3.7 as a noticeable change, while many researchers consider this to be unacceptable which ΔE value of 3.3 is intraoral perceivable (30-32). A value of ΔE which was more than 5 was considered to be clinically unacceptable, which requires restoration replacement (30, 32).

CHAPTER III

Material and Methods

Materials

1. Extra Translucent ZrO_2 shade A3 (VITA YZ® XT, VITA Zahnfabrik, Bad Säckingen, Germany)
2. BODY STAINS POWDER (VITA AKZENT plus, VITA Zahnfabrik, Bad Säckingen, Germany)
3. GLAZE POWDER (VITA AKZENT plus, VITA Zahnfabrik, Bad Säckingen, Germany)
4. Distilled water Grade3 (ISO 3696-1:1987)
5. Artificial saliva (Pharmaceutical division, faculty of dentistry of Chulalongkorn university)
6. Citric acid monohydrate (MERK®, Darmstadt, Germany)

Equipment

1. Silicon carbide paper 500-grit (Buehler CarbiMet®, Illinois, USA)
2. Polishing Machine (Minitech 233, Presi, Grenoble, France)
3. Plastic vacuum mold form
4. Plastic container
5. Apply staining brushes
6. Plastic forceps
7. Acrylic specimen holder plate
8. Digital vernier caliper (Mitutoyo series 500, Kawasaki, Japan)
9. Low speed saw (Isomet 1000, Buehler, Illinois, USA)
10. Ultrasonic bath (VGT-1990, QTD, China)
11. pH meter (Orion model 900A, Orion Research Inc., Massachusetts, USA)
12. Temperature incubator 37 °c (model ES-20, Orbital Shaker-Incubator, Biosan, Latvia)
13. A contact stylus profilometer (Talyscan 150, Taylor Hobson, Leicester, England)
14. Spectrophotometer (UltraScan XE, Hunter lab, Virginia, USA)

Table 1 Material details and physical properties of Translucent zirconia blank, stain and glaze paste materials

Material	Manufacturer	Type	Composition	Process	Flexural strength (MPa)	Young's modulus (GPa)	Enlargement factor	Compatible materials	CTE	Finishing	Application
VITA YZ XT	VITA Zahnfabrik H. Rauter GmbH & Co. KG	Extra translucent ZrO_2	ZrO_2 86.4 - 90.5 (wt%); Y_2O_3 8 – 10 (wt%); HfO_2 1.5 - 2.5 (wt%),Other oxides	Soft milling; dry milling	>600 MPa	210 GPa	Material shrinking about 20%, Enlargement factor 1.2345	VITA VM 9; VITA YZ® XT SHADE LIQUID Coloring; AKZENT Plus stains and glaziers	10 (20-500°C) 10^{-6}/K	Glaze firing with VITA AKZENT® Plus	Stains and Glaziers; high gloss polish of the contact surfaces
VITA AKZENT Plus	VITA Zahnfabrik H. Rauter GmbH & Co. KG	Internal and external painting, translucent, marking, fluorescent	-	-	-	-	-	Metal ceramics, pressed ceramics, titanium ceramics, ZrO_2 ceramics, all-ceramics, VITABLOCS, lithium silicate ceramics, VITA SUPRINITY PC, VITA VM 11	CTE-wide and independent		Can be used universally for all VITA veneering ceramics, imitation of natural discoloration effects, stable and color-stable, model of the color palette are effects occurring in the natural tooth, can be mixed with each other

Figure 1 Extra Translucent zirconia blank

Figure 2 Body stain and glaze powder

Figure 3 Citric acid and artificial saliva solutions

Part I: Specimen preparation

1. Sample size calculation

According to previous study of Sokkary, Elguindy and Shihi (13) about acid immersion as a reference, The calculation for number of specimens of color difference (ΔE) and surface roughness difference (ΔRa) using formula as follow, $\mu 1$ = 0.73, $\mu 2$ = 2.03, $\sigma 1$ = 0.11, $\sigma 2$ = 0.15, α = 0.05, and β= 0.20 sample size for each group n = 1. Moreover, the surface roughness difference (ΔRa) used $\mu 1$ = 2.25377 $\mu 2$ = 2.25245, $\sigma 1$ = 0.0009, $\sigma 2$ = 0.0010, α = 0.05, and β= 0.20 as the sample size for each group n = 9 then adjusted the sample size for each group to n = 10 per group.

$$n_1 = \frac{\left(z_{1-\frac{\alpha}{2}} + z_{1-\beta}\right)^2 \left[\sigma_1^2 + \frac{\sigma_2^2}{r}\right]}{\Delta^2}$$

$$r = \frac{n_2}{n_1}, \quad \Delta = \mu_1 - \mu_2$$

Figure 4 Sample size calculation formula modified from Bernard, R. (2000).

2. Specimen preparation

2.1. Specimen fabrication

Eighty translucent monolithic zirconia discs were prepared in the laboratory by using translucent zirconia blanks shade A3 (VITA YZ® XT – Extra Translucent ZrO_2; Lot No.75410, VITA Zahnfabrik, Bad Säckingen, Germany), designed with CAD-CAM software and milled into a cylinder shape with a diameter of 18x14 mm for 20 pieces. The specimens were sliced into 80 discs by the low-speed saw (Isomet 1000; Buehler, IL, USA) and polished with 500-grit silicon carbide paper. The sintering process was carried out as recommended by the manufacturer with a 20 percent shrinkage. After sintering, all specimens were measured using a digital vernier caliper (Mitutoyo series 500, Kawasaki, Japan) to ensure the mean size of 14 mm x 1.5 mm. The specimens were then cleaned using an ultrasonic cleanser

with deionized water for 10 min and were dried with absorbent paper. The crack and defection were microscopically examined using a 40X stereomicroscope (SZ61, OLYMPUS, Tokyo, Japan).

Figure 5 The specimen was designed and milled by CAD-CAM software

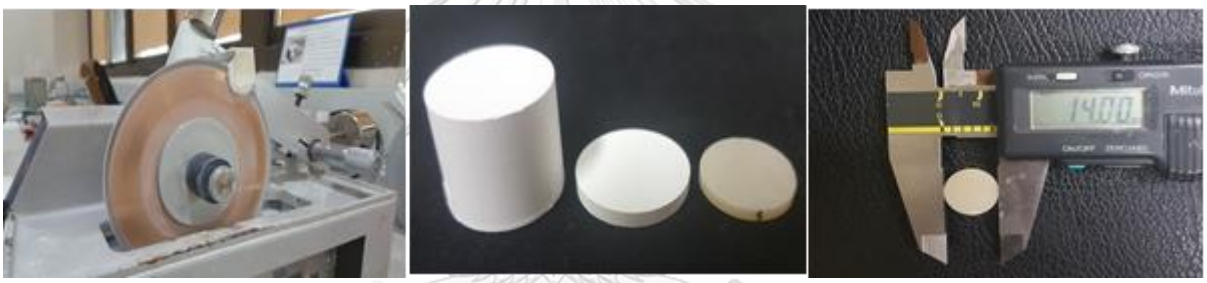


Figure 6 The specimen was cut by Isomet cutting machine and sintered following the manufacturer's instruction

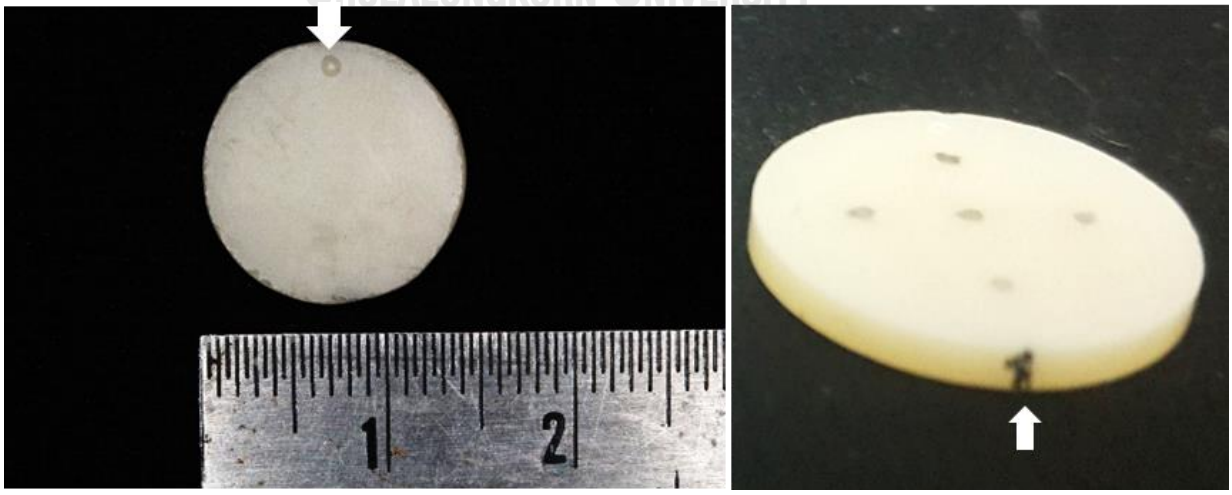


Figure 7 The specimen with the reference mark for measurement

2.2. Group division

All 80 specimens of extra-translucent monolithic zirconia were divided into 4 groups according to the type of surface finish including no treatment (NT), polishing (PO), stained then glazed (S-G) and mixing of stain and glaze (S+G). Each group was separated into 2 subgroups (n = 10) according to the type of immersion solutions of artificial saliva and 2% w/v of citric acid as depicted in the following diagram (Figure 8).

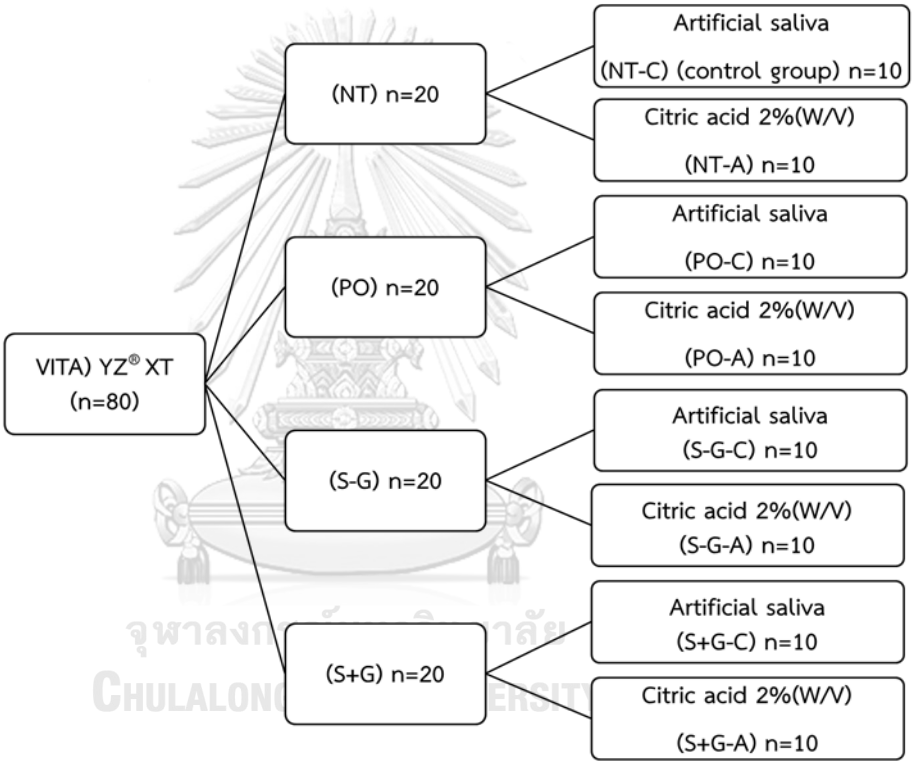


Figure 8 The diagram of the divided specimen groups

2.3. Surface finishing process

All specimens were randomly divided into 4 groups and then marked the specimen number of each subgroup opposite the fiducial marker. The NT group, as received no any surface treatment. To simulate clinical surface finish procedure, the other 60 specimens were ground for 15 sec with diamond coated grinding tools (EVE DIASYNT Plus® coarse, VITA Zahnfabrik) and were polished with VITA

SUPRINITY® Polishing set, as recommended by the manufacturer's instruction, with a low-speed handpiece for 60 sec per surface by the same operator following the protocol of Vichi et al. (34). The polishing process was carried out manually (with 40 g force) in unidirectional movements without water coolant.

The polished specimens were randomly divided into two subgroups of different staining techniques which were stained then glazed (S-G) and mixing of stain and glaze (S+G) with glazing powder of VITA AKZENT Plus® CHROMA A STAIN and GLAZE LT (VITA Zahnfabrik). The specimens were glazed in vacuum ceramic furnace (VITA Vacumat V60 i-Line Porcelain Furnace, VITA Zahnfabrik) according to the manufacturer's instruction (Table 2). The layer was approximately 0.067 ± 0.01 mm in thickness (Figure 9 and 10).

Table 2 The stains fixation and glaze firing temperature protocol of the specimens

Process	**Temperature/time**
Pre-dry	500°C
Pre-heating time	6 min
Heating time	5.37 min
Temperature rise rate	80°C/min
Ending temperature	950°C
Holding time for ending temperature	1 min

Staining techniques	N/A	N/A	Glaze ↑ Stain ↑	Stain + glaze ↑
2-steps polishing sets (60 s)	N/A	√	√	√
Diamond coated grinding tools (15 s)	N/A	√	√	√
Specimen groups				
	NT	PO	S-G	S+G

Figure 9 A schematic drawing of 4 groups of specimen fabrications with different surface finishing techniques

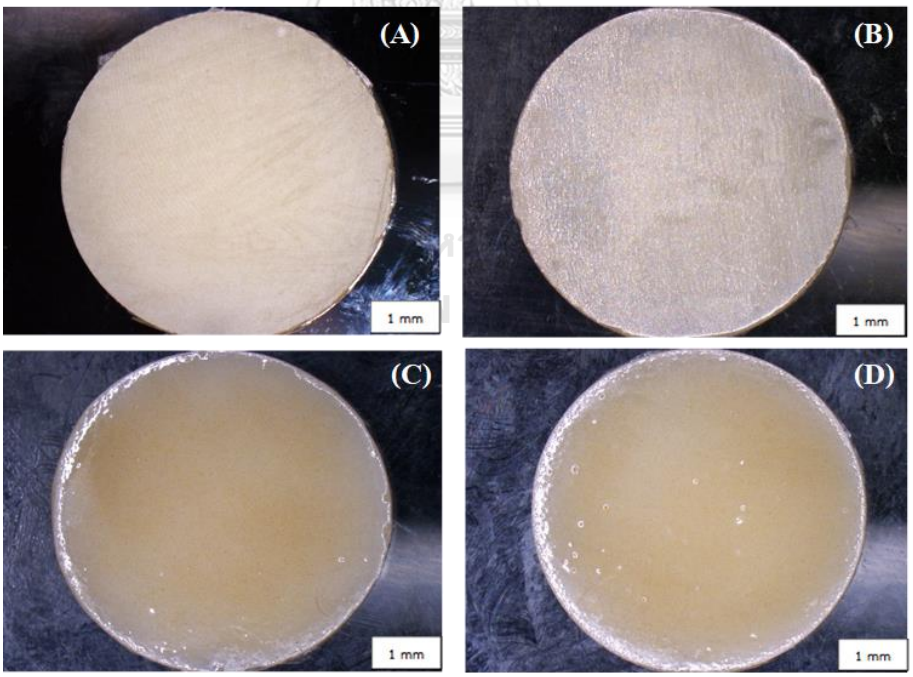


Figure 10 The specimens with different surface finishing techniques: (A) no treatment (NT), (B) polishing (PO), (C) stained then glazed (S-G), and (D) mixing of stain and glaze (S+G)

Part II: Immersion protocol

All specimens were initially immersed in deionized water at 37°C for 24 h before being immersed in an artificial saliva or citric acid. The specimens in artificial saliva (mean pH 6.5 ± 0.13) were immersed at 37 °C for 14 days as a control group with replacing of the saliva every 2 days, which the other were immersed in 25 ml of the 2% w/v of citric acid solution (mean pH 2.03 ± 0.01) for 8 h respectively to simulate the exposure of saliva and citric acid in the oral cavity for 2 years, following the protocol of Demirhanoglu and Sokkary et al. (10, 13). The pH value was measured 3 times by pH meter (Orion model 900A, Orion Research Inc., Massachusetts, USA). After immersion, all specimens were cleaned by the ultrasonic cleanser with deionized water for 10 min, dried with absorbent paper and subjected to color and surface roughness measurement, respectively.

Table 3 The immersion protocol of specimens

Group	Staining methods			
Solutions	NT	PO	S-G	S+G
Artificial saliva	10	10	10	10
2% citric acid	10	10	10	10
total	20	20	20	20

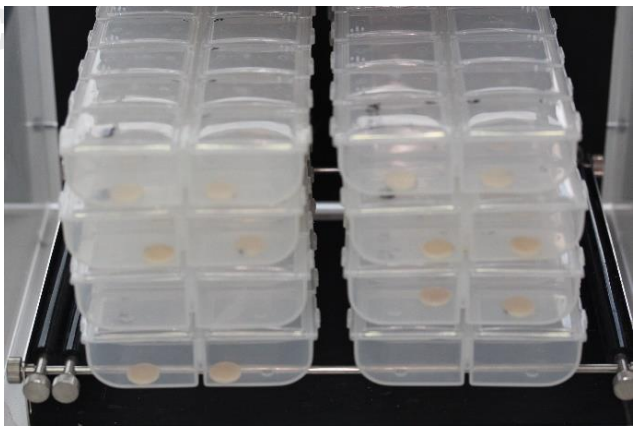


Figure 11 The immersion of specimens in artificial saliva and citric acid solution in the temperature control incubator

Part III: Physical properties measurement

1. Color measurement methods

The color difference (ΔE) was measured after storage in the solution using a spectrophotometer (UltraScan XE, Hunter lab, Virginia, USA) with the reflection mode of standard D65 illumination, two-degree observer, and 10 mm port under a white background. Each specimen was measured clockwise in five different areas included center and four quadrants with a customized holder. To determine the color difference of two colors the following CIE 1976 formula (36), it was calculated under computer software as

$$\Delta E_{ab^*} = [(\Delta L^*)^2+(\Delta a^*)^2+(\Delta b^*)^2]^{1/2}$$

Where ΔE refers to the color difference, L* for lightness, a* for redness to greenness, and b* for yellowness to blueness.

2. Surface roughness measurement methods

The surface roughness (roughness average, Ra) before and after immersing in the solutions was measured using a stylus contact type profilometer (Talyscan 150, Taylor Hobson, Leicester, England) with a resolution of 0.06 μm. The process was carried out with a diamond stylus radius 5 μm, angle of stylus 90° perpendicular to the specimens (area of 2 mm x 2 mm), a cutoff length of 0.25 mm, a force of 4 mN, and the rate of 500 μm/s. Roughness was measured at five different areas and the mean surface roughness measurement was calculated for each sample. The surface of specimens in each surface finishes after immersion was microscopically examined using scanning electron microscope (JSM-IT500, JEOL, Massachusetts, USA) before and after immersion in artificial saliva and citric acid.

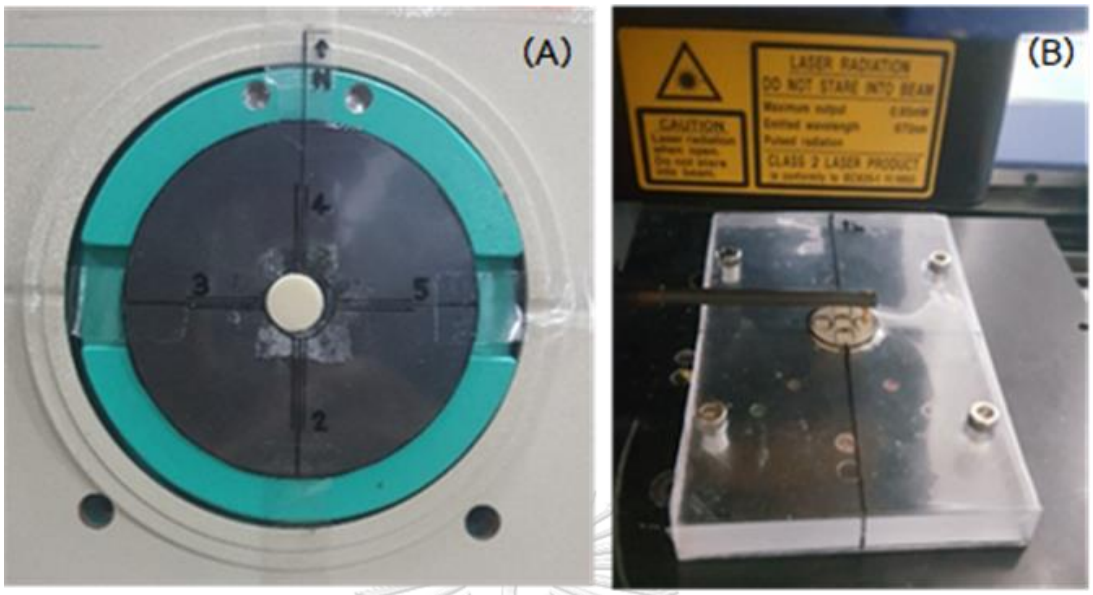


Figure 12 The sample position that was attached to the spectrophotometer (A) and a contact profilometer (B)

Statistical analysis

The data were analyzed using the statistics software (SPSS 22.0, SPSS Inc., New York, USA). The level of significance was set at *P*-value < 0.05. The data were tested for normal distribution by the Kolmogorov-Smirnov and Shapiro-wilk test. To determine the differences within groups, the ΔE, Ra and ΔRa values from before and after immersion were compared by t-test and one-way ANOVA with Tukey's HSD post-hoc analysis. Two-way ANOVA was used for analyzing the interaction between solutions and surface finish conditions based on the ΔE and ΔRa values.

CHAPTER IV
RESULTS

Results of color change

For the types of surface finish, the ΔE values in S-G and S+G groups were significantly higher than the NT and PO groups (P<0.01). However, there was no significant difference between S-G and S+G in both solutions (Table 4). Table 5 showed the ΔE in S-G and S+G groups immersed in artificial saliva was significantly lower than immersed in citric acid (*P*<0.001), no statistical differences between the PO and NT groups in saliva and citric acid were observed (*P*>0.05). The results of the two-way ANOVA analysis revealed that types of solutions (*P*=0.000), types of surface finish (*P*=0.000) and interaction between types of solutions and types of surface finish (*P*=0.017) had an influence on the color difference.

Table 4 Mean (SD) of color change (ΔE) between surface finish methods of translucent monolithic zirconia with different surface finish methods after immersed in artificial saliva and citric acid for 2 years simulation

Immersion solutions	Color change (ΔE)				*P*-value
	NT	PO	S-G	S+G	
Artificial saliva	0.58 (0.08) ^{a}	0.71 (0.08) ^{a,b}	0.73 (0.11) ^{a,b}	0.73 (0.18) ^{a,b}	0.018*
Citric acid	0.71 (0.29)^{A}	0.79 (0.11)^{A}	1.02 (0.14)^{B}	1.06 (0.11)^{B}	0.000*

The same superscript letters in the rows are not significantly different based on multiple comparisons Tukey's HSD test (*P*>0.05)

Table 5 Mean (SD) of color change (ΔE) between solutions of translucent monolithic zirconia with different surface finish methods after immersed in artificial saliva and citric acid for 2 years simulation

Immersion solutions	Color change (ΔE)			
	NT	PO	S-G	S+G
Artificial saliva	0.58 (0.08) [a]	0.71 (0.08) [b]	0.73 (0.11)	0.73 (0.18)
Citric acid	0.71 (0.29)[a]	0.76 (0.11) [b]	1.02 (0.14)	1.06 (0.11)
***P*-value**	0.191	0.221	0.000*	0.000*

The same superscript letters in the columns are not significantly different based on t-test (P>0.05)

According to the mean value of the color components, the specimens in S-G and S+G groups in artificial saliva and citric acid showed a higher significant difference change in b* value (*P*<0.001). For the PO group, it showed a significant higher value of L* and a* in citric acid (Table 6).

Table 6 Mean (SD) of CIE L*a*b*values among each subgroup for 2 years simulation in different immersion solutions

Surface finish		Solution media			
		Artificial saliva		Citric acid	
		Before immersion	After immersion	Before immersion	After immersion
L*	NT	78.67 (0.73)	78.10 (0.72)	78.67 (1.19)	78.79 (1.17)
	PO	75.89 (0.71)	76.68 (0.68)	78.98 (1.04)	78.83 (1.00)
	S-G	65.94 (1.47)	66.03 (1.51)	65.71 (1.36)	66.43 (1.48)
	S+G	67.71 (1.22)	67.87 (1.19)	66.82 (1.38)	67.34 (1.46)
a*	NT	2.15 (0.12)	2.08 (0.12)	2.12 (0.18)	2.11 (0.19)
	PO	1.97 (0.08)	1.98 (0.10)	2.18 (0.13)	2.28 (0.17)
	S-G	6.65 (0.48)	6.81 (0.49)	6.77 (0.55)	6.95 (0.53)
	S+G	6.03 (0.59)	6.15 (0.61)	6.08 (0.39)	6.29 (0.41)
b*	NT	18.58 (0.47)	18.12 (0.56)	18.08 (0.44)	25.47 (0.48)
	PO	18.00 (0.53)	17.77 (0.45)	18.16 (0.57)	25.95 (0.45)
	S-G	25.23 (0.41)	25.67 (0.42)	18.54 (0.76)	24.67 (0.59)
	S+G	24.92 (0.60)	25.16 (0.63)	17.58 (0.66)	25.33 (0.65)

L*, Lightness; a*, green-red; b*, blue-yellow.

Results of roughness change

With regard to the surface roughness, tables 7 and 8 showed the mean value of the Ra and ΔRa before and after the immersion. The one-way ANOVA indicated that there was no significant difference ($P>0.05$) between 4 different surface finishing techniques. For the t-test of ΔRa value, there was no significant difference ($P>0.05$) between artificial saliva and citric acid (table 9). The result of the two-way ANOVA revealed that types of solutions ($P=0.687$), types of surface finish ($P=0.958$) and interaction between types of solutions and types of surface finish ($P=0.410$) had no influence on ΔRa in each surface finish.

Table 7 Mean (SD) of surface roughness (Ra) between surface finish methods of translucent monolithic zirconia with different surface finish methods before and after immersed in artificial saliva and citric acid for 2 years simulation

Groups	Storage agent	Ra (μm)		*P*-value
		Before immersion	After immersion	
NT	Artificial saliva (control)	0.393±0.105	0.408±0.105	0.129
	Citric acid	0.446±0.129	0.448±0.128	0.855
PO	Artificial saliva	0.225±0.019	0.229±0.016	0.481
	Citric acid	0.268±0.026	0.255±0.017	0.092
S-G	Artificial saliva	0.106±0.026	0.105± 0.021	0.857
	Citric acid	0.084±0.013	0.085± 0.014	0.827
S+G	Artificial saliva	0.135±0.040	0.134± 0.032	0.860
	Citric acid	0.086±0.006	0.091± 0.009	0.80

Table 8 Mean (SD) of surface roughness change (ΔRa) between surface finish methods of translucent monolithic zirconia with different surface finish methods after immersed in artificial saliva and citric acid for 2 years simulation

Immersion solutions	Surface roughness change (ΔRa)				*P*-value
	NT	PO	S-G	S+G	
Artificial saliva	-0.000 (0.016)	0.005 (0.020)	-0.001 (0.019)	-0.004 (0.019)	0.803
Citric acid	-0.005 (0.034)	-0.007 (0.019)	0.001 (0.015)	0.005 (0.008)	0.557

Table 9 Mean (SD) of surface roughness change (ΔRa) between solutions of translucent monolithic zirconia with different surface finish methods after immersed in artificial saliva and citric acid for 2 years simulation

Immersion solutions	Surface roughness change (ΔRa)			
	NT	PO	S-G	S+G
Artificial saliva	-0.000 (0.016)	0.005 (0.020)	-0.001 (0.019)	-0.004 (0.019)
Citric acid	-0.005 (0.034)	-0.007 (0.019)	0.001 (0.015)	0.005 (0.008)
***P*-value**	0.703	0.177	0.952	0.208

For the scanning electron microscope photograph, the surface of specimens was microscopically examined using a scanning electron microscope at a magnification of 10,000X. Before immersion, all specimens showed a smooth surface with some bubbles from the glazing process. After the immersion in artificial saliva for 14 days and citric acid for 8 h, no significant changes were detected as shown in Figure 13.

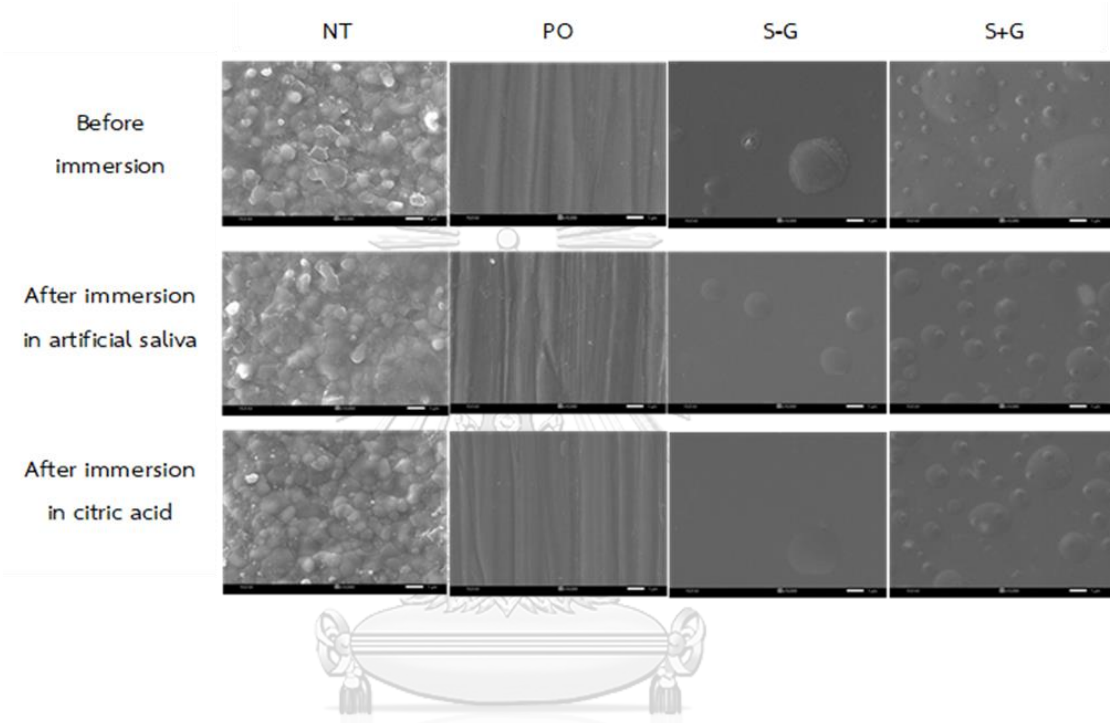


Figure 13 Scanning electron microscope photograph of the specimens at a magnification of 10,000X

CHAPTER V
DISCUSSION

Despite the structure development of zirconia to improve the translucency, it still needs the externally staining with surface coating using porcelain (2). However, this material is easily destroyed either by a mechanical or chemical attack, especially dietary citric acid (8, 9), resulting in the negative effects on Ra and ΔE of ceramic materials such as feldspathic and glass ceramic (12, 13). So, this study was done to simulate the change of surface with different staining techniques in the extra translucent zirconia material (VITA YZ® XT) to identify the effects of citric acid on color change and surface roughness of the material.

Color change

Based on the results of this study, the first null hypothesis for the color change was rejected, since there was a significant difference between S-G and S+G compared with NT and PO groups after acid immersion. However, the first two staining techniques had no significant difference. The color differences can be caused by types of materials, fabrication and shading techniques, exposure solutions, contributing to surface textures with distinct surface finishing appearances (3, 14, 22, 35, 36).

Concerning to the types of material, fabrication and shading techniques, the different of ceramic elements yield a variety of color changes. Grenza et al. had shown the higher ΔE in lithium disilicate than leucite-reinforced specimens with the mixing of stain and glaze more than stained then glazed group with simulated brushing for 12 years (22). Darafshi et al. had reported that feldspathic porcelain had more color change than translucent monolithic zirconia when soaking in the mouthwash every 2 minutes per day for 7 days (37), This study was consistent with our results showing a significant difference between S-G and S+G than NT and PO groups. The explanation might be due to that the S-G and S+G groups had been coated with the porcelain staining which consists of pigment metals in low fusing porcelain. Glazing materials consisted of low or ultra-low fusing ceramic material. These elements contained most of the glass phase (14).

For the change of color component, there was an alteration of b* value in the citric acid in glazing group (S-G and S+G group) that significant difference from baseline (before immersion) and saliva. Kim et al. (37) also showed that b* value of glazing was higher than polishing specimens, which meant that glazing group were yellower than polishing group. However, the present study found that the value of L* and a* of the polishing group were significantly higher than NT, S-G and S+G groups. This might because the use of extra-translucent monolithic zirconia which containing cubic phase. Shahmal et al. stated that the cubic phase in material might alter the reflective index of this material since the light is more diffused through the clear cubic particles which reduced light scattering in the smaller grain boundary area from the conventional zirconia (38). Nevertheless, b* value has been changed due to porosity in glazing specimens which increase light scattering as shown in SEM (fig.13). Therefore, these results were contrasted with the study of Kim et al. (37) which using non-translucent zirconia. Furthermore, the L* value of the glazing group was lower than the polishing group due to the tendency of increasing ΔRa of the glazing group. This was supported by previous studies of Kim et al. and Lee et al. which showed that the rough surface could reduce *L value (37, 39). Moreover, water sorption could be increased by citric acid which resulted in the penetration of aqueous solution into the bulk of the glazing material (13, 40).

Anusavice stated that water, aqueous solutions and chelating effect of citric acid can attack the glass. The destruction process occurred by exchanging hydrogen ions with the alkaline ions of glass. Then, the water molecules were inward diffusion in the bulk of the materials (8). This may cause color change in the coating layers of the S-G and S+G groups. This effect is different from NT and PO groups, which are plain zirconia surfaces that are structurally polycrystalline and are resistant to damage from chemicals for pre-shading by mixing metal oxide into pressing zirconia powder (3) in a regular temperature (38, 39). These, therefore, provide the color stability of NT and PO groups.

In case of color perception, this study found that the ΔE of zirconia in NT and PO groups ranged from 0.58 to 0.79. While a change in the color of S-G and S+G staining techniques ranged from 0.73 to 1.06. The S+G group has the highest color change

value in citric acid solution with the level of 1.06 ± 0.11 which could be detected by an eye of a trained person (41). The result corresponded with an in vitro study of Vichi et al. using spectrophotometer with gray card to measure the color change of resin composite (42) which showed that the ΔE values between 1 - 3.3 can be perceived by human eye, but clinically acceptable. Conversely, the study of Ishigawa-nagai et al. which measured all-ceramic crown with zirconia substructure indicated that ΔE at 1.6 could not be perceived by an eye. This clinical study used the intraoral spectrophotometer and measuring for six areas compared with the adjacent natural tooth (43). However, if ΔE was more than 3.3, it could be perceived by the patient and might be clinically unacceptable. This corresponded with a distinguished study of Johnston and Kao (32) which indicating the value of ΔE at 3.7 which evaluated the ΔE between a composite veneer and adjacent natural teeth. The value of ΔE at 6.8 was claimed as clinically unacceptable which the restoration should be changed (30, 32). This present study used a value of ΔE less than 3.3 as a clinical acceptable condition. Therefore, the maximum color change after acid immersion for 2 years in this study was still clinically acceptable.

Surface roughness change

The second null hypothesis was accepted for the surface roughness change since there is no significant difference of ΔRa among NT, PO, S-G and S+G groups after acid immersion. The surface roughness change could be caused by the surface texture that received different surface finishing, types of exposure solution and types of material (8, 12, 13, 22, 26, 44, 45).

For the surface texture, the polishing process of monolithic zirconia specimens in this study was carried out by manual polishing protocol without water coolant. The Ra of the PO group which received only the final polishing process ranged from 0.225 to 0.268 μm, while in S+G and S-G showed smoother surface which the Ra ranged from 0.135 to 0.084 μm after the immersion in citric acid. The results agree with the studies of Khayat and Chun et al. (44, 45), which showed that an appropriated polishing process led to the same level of smoothness and even more consistent compared to the glazing process. The Ra values in the PO group obtained from this study were more

than 0.2 μm which have been reported that to increase bacteria accumulation for resin composite (46), however, they were relatively smoother than human enamel (0.64± 0.25 μm) which evaluated by using the non-contact and replica mode (47). It could be claimed that the Ra in the PO, S-G and S+G groups in this study were clinically acceptable. However, the manual polishing process in this study which use a 40-g force polished in one direction resulted in the surface with grooves as shown in SEM (Fig.13). Further testing with other polishing methods with the increase of the force 1-2 N (48) or multidirectional movements may be advantages (44). In some cases, specimens ground under a diamond rotary cutting instrument especially for zirconia with some diamond pastes could deliver smoother surfaces (1). On the contrary, smoother surface were achieved from the polishing process using the VITA SUPRINITY® polishing set recommended by the manufacturer. In this study, we have obtained the surface roughness of the PO group from 0.230±0.017 to 0.255±0.026 μm using VITA SUPRINITY® polishing set. This value is lower than the Ra at 0.28 μm of a study by Caglar et al. using Meisinger® and EVE Diacera® polishing systems that are developed for use especially on monolithic zirconia (49). Although the thickness of the glazing layer in this study was thicker than those obtained from the laboratories, it was still in the acceptable range of 50-100 μm (1, 21).

The result of this study indicated that citric acid had no effect on surface roughness of translucent monolithic zirconia due to the characteristic of a weak acid of this solution, they existed as a mixture of hydrogen ions and citrate ions leading to the ability to form a complex with the calcium of teeth (50), which weakly degrade ceramic materials by chelating and ion leaching mechanism (8). Previous study (26) reported that 20 % of citric acid could not damage the low translucent zirconia surface in the ambient temperature for 7 and 14 days. However, the study of zirconia reinforced glass-ceramic indicated that 2% of citric acid could degrade the glazing surface of this material by increasing the Ra and ΔE value in 8 h of immersion (13). Citric acid from the acidic beverage was shown to increase the surface roughness on feldspathic, aluminous, leucite-reinforced and fluorapatite ceramic after 168 h of immersion (12). For the acidic property of artificial saliva with the mean pH of 6.5 ± 0.13, the Ra of the specimens immersed in citric acid was very closed to those

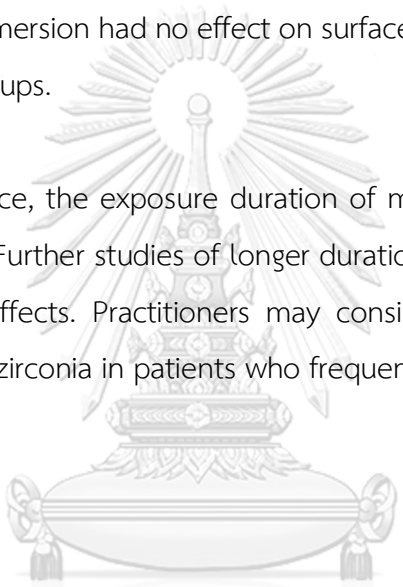
immersed in artificial saliva. This was in agreement with the studies of Demirhanoglu et al. and Demirel et al. (10, 11) which indicated that there was no significant difference in the surface roughness in fluoromica-based glass ceramics and glass ceramic after received auto or overglazing.

CHAPTER VI
CONCLUSIONS

After 2 years of acid immersion simulation of extra-translucent monolithic zirconia with different surface finish, it can be summarized as followed;

1. Citric acid immersion had an effect on color change of different surface finish, it had no effect on polishing group but affected both staining groups, while the 2 staining technique were not significantly different.
2. The acid immersion had no effect on surface roughness of neither polished nor stain groups.

In clinical practice, the exposure duration of materials in the oral cavity was longer than the study. Further studies of longer duration of immersion are needed to prove the significant effects. Practitioners may consider these factors when using translucent monolithic zirconia in patients who frequently consume citric acid in daily life.



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APPENDIX

Normality test and homogeneity of variance test in color difference(ΔE)

Tests of Normality

Group		Kolmogorov-Smirnov			Shapiro-Wilk		
		Statistic	df	Sig.	Statistic	df	Sig.
DT_E_2y	saliva NT	.219	10	.189	.880	10	.131
	saliva po	.194	10	.200*	.902	10	.232
	saliva s-g	.181	10	.200*	.891	10	.176
	saliva s+g	.153	10	.200*	.928	10	.424
	citric NT	.188	10	.200*	.929	10	.437
	citric po	.240	10	.106	.881	10	.133
	citric s-g	.210	10	.200*	.946	10	.616
	citric s+g	.189	10	.200*	.957	10	.754

*. This is a lower bound of the true significance.

a. Lilliefors Significance Correction

One-way ANOVA for color differences(ΔE) between surface finish groups in each solution

Solution 1: Artificial saliva

Test of Homogeneity of Variances

DT_E_2y			
Levene Statistic	df1	df2	Sig.
3.445	3	36	.027

ANOVA

DT_E_2y					
	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	.163	3	.054	3.839	.018
Within Groups	.509	36	.014		
Total	.672	39			

Multiple Comparisons

Dependent Variable: DT_E_2y							
(I) tx			Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
Tukey HSD	NT	po	-.12900	.05318	.090	-.2722	.0142
		s-g	-.14900*	.05318	.039	-.2922	-.0058
		s+g	-.15800*	.05318	.026	-.3012	-.0148
	po	NT	.12900	.05318	.090	-.0142	.2722
		s-g	-.02000	.05318	.982	-.1632	.1232
		s+g	-.02900	.05318	.947	-.1722	.1142
	s-g	NT	.14900*	.05318	.039	.0058	.2922
		po	.02000	.05318	.982	-.1232	.1632
		s+g	-.00900	.05318	.998	-.1522	.1342
	s+g	NT	.15800*	.05318	.026	.0148	.3012
		po	.02900	.05318	.947	-.1142	.1722
		s-g	.00900	.05318	.998	-.1342	.1522
Games-Howell	NT	po	-.12900*	.03610	.011	-.2310	-.0270
		s-g	-.14900*	.04244	.013	-.2697	-.0283
		s+g	-.15800	.06247	.103	-.3421	.0261
	po	NT	.12900*	.03610	.011	.0270	.2310
		s-g	-.02000	.04186	.963	-.1392	.0992
		s+g	-.02900	.06208	.965	-.2125	.1545
	s-g	NT	.14900*	.04244	.013	.0283	.2697
		po	.02000	.04186	.963	-.0992	.1392
		s+g	-.00900	.06597	.999	-.1998	.1818
	s+g	NT	.15800	.06247	.103	-.0261	.3421

		po	.02900	.06208	.965	-.1545	.2125
		s-g	.00900	.06597	.999	-.1818	.1998

*. The mean difference is significant at the 0.05 level.

Solution 2: Citric acid

Test of Homogeneity of Variances

DT_E_2y			
Levene Statistic	df1	df2	Sig.
8.099	3	36	.000

ANOVA

DT_E_2y					
	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	.952	3	.317	9.905	.000
Within Groups	1.153	36	.032		
Total	2.105	39			

Multiple Comparisons

Dependent Variable: DT_E_2y							
(I) tx			Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
Tukey HSD	NT	po	-.04900	.08004	.928	-.2646	.1666
		s-g	-.31200*	.08004	.002	-.5276	-.0964
		s+g	-.34800*	.08004	.001	-.5636	-.1324
	po	NT	.04900	.08004	.928	-.1666	.2646
		s-g	-.26300*	.08004	.012	-.4786	-.0474
		s+g	-.29900*	.08004	.003	-.5146	-.0834
	s-g	NT	.31200*	.08004	.002	.0964	.5276
		po	.26300*	.08004	.012	.0474	.4786

		s+g	-.03600	.08004	.969	-.2516	.1796
	s+g	NT	.34800*	.08004	.001	.1324	.5636
		po	.29900*	.08004	.003	.0834	.5146
		s-g	.03600	.08004	.969	-.1796	.2516
Games-Howell	NT	po	-.04900	.09747	.957	-.3408	.2428
		s-g	-.31200*	.10213	.040	-.6113	-.0127
		s+g	-.34800*	.09822	.019	-.6409	-.0551
	po	NT	.04900	.09747	.957	-.2428	.3408
		s-g	-.26300*	.05626	.001	-.4233	-.1027
		s+g	-.29900*	.04880	.000	-.4370	-.1610
	s-g	NT	.31200*	.10213	.040	.0127	.6113
		po	.26300*	.05626	.001	.1027	.4233
		s+g	-.03600	.05755	.922	-.1995	.1275
	s+g	NT	.34800*	.09822	.019	.0551	.6409
		po	.29900*	.04880	.000	.1610	.4370
		s-g	.03600	.05755	.922	-.1275	.1995

*. The mean difference is significant at the 0.05 level.

T-test for color differences(ΔE) between solution groups in each surface finish process

Treatment: NT

Group Statistics

Solution		N	Mean	Std. Deviation	Std. Error Mean
DT_E_2y	saliva	10	.5760	.08222	.02600
	citric	10	.7090	.28954	.09156

Independent Samples Test

		Levene's Test for Equality of Variances		t-test for Equality of Means						
		F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	95% Confidence Interval of the Difference	
									Lower	Upper
DT_E_2y	Equal variances assumed	18.623	.000	-1.397	18	.179	-.13300	.09518	-.33297	.06697
	Equal variances not assumed			-1.397	10.442	.191	-.13300	.09518	-.34386	.07786

Treatment: PO

Group Statistics

Solution		N	Mean	Std. Deviation	Std. Error Mean
DT_E_2y	saliva	10	.7050	.07920	.02504
	citric	10	.7580	.10570	.03343

Independent Samples Test

		Levene's Test for Equality of Variances		t-test for Equality of Means						
		F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	95% Confidence Interval of the Difference	
									Lower	Upper
DT_E_2y	Equal variances assumed	1.649	.215	-1.269	18	.221	-.05300	.04177	-.14075	.03475
	Equal variances not assumed			-1.269	16.683	.222	-.05300	.04177	-.14125	.03525

Treatment: S-G

Group Statistics

Solution		N	Mean	Std. Deviation	Std. Error Mean
DT_E_2y	saliva	10	.7250	.10607	.03354
	citric	10	1.0210	.14310	.04525

Independent Samples Test

		Levene's Test for Equality of Variances		t-test for Equality of Means						
		F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	95% Confidence Interval of the Difference	
									Lower	Upper
DT_E_2y	Equal variances assumed	1.412	.250	-5.255	18	.000	-.29600	.05633	-.41434	-.17766
	Equal variances not assumed			-5.255	16.596	.000	-.29600	.05633	-.41506	-.17694

Treatment: S+G

Group Statistics

Solution		N	Mean	Std. Deviation	Std. Error Mean
DT_E_2y	saliva	10	.7340	.17964	.05681
	citric	10	1.0570	.11245	.03556

Independent Samples Test

		Levene's Test for Equality of Variances		t-test for Equality of Means						
		F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	95% Confidence Interval of the Difference	
									Lower	Upper
DT_E_2y	Equal variances assumed	2.948	.103	-4.819	18	.000	-.32300	.06702	-.46380	-.18220
	Equal variances not assumed			-4.819	15.114	.000	-.32300	.06702	-.46576	-.18024

Two-way ANOVA for color differences(ΔE) and their interaction

Univariate Analysis of Variance

Tests of Between-Subjects Effects

Dependent Variable: DT_E_2y						
Source	Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared
Corrected Model	1.925[a]	7	.275	11.910	.000	.537
Intercept	49.377	1	49.377	2138.888	.000	.967
Solution	.810	1	.810	35.089	.000	.328
tx	.862	3	.287	12.453	.000	.342
Solution * tx	.252	3	.084	3.641	.017	.132
Error	1.662	72	.023			
Total	52.963	80				
Corrected Total	3.587	79				

a. R Squared = .537 (Adjusted R Squared = .492)

Normality test and homogeneity of variance test in surface roughness (Ra)

Tests of Normality

Group		Kolmogorov-Smirnov			Shapiro-Wilk		
		Statistic	df	Sig.	Statistic	df	Sig.
Ra_baseline	saliva NT	.220	10	.187	.913	10	.303
	saliva po	.210	10	.200*	.871	10	.102
	saliva s-g	.103	10	.200*	.969	10	.881
	saliva s+g	.194	10	.200*	.928	10	.425
	citric NT	.161	10	.200*	.975	10	.935
	citric po	.219	10	.189	.907	10	.263
	citric s-g	.231	10	.141	.935	10	.503
	citric s+g	.200	10	.200*	.858	10	.071
Ra_2y	saliva NT	.237	10	.119	.845	10	.050
	saliva po	.193	10	.200*	.927	10	.423
	saliva s-g	.214	10	.200*	.892	10	.178
	saliva s+g	.186	10	.200*	.941	10	.564
	citric NT	.157	10	.200*	.955	10	.727
	citric po	.235	10	.126	.899	10	.213
	citric s-g	.179	10	.200*	.919	10	.350
	citric s+g	.171	10	.200*	.947	10	.634

*. This is a lower bound of the true significance.

a. Lilliefors Significance Correction

Paired t-test for surface roughness change(ΔRa) between solution groups in each surface finish process

Treatment: Artificial saliva vs NT

Paired Samples Statistics

		Mean	N	Std. Deviation	Std. Error Mean
Pair 1	Ra_baseline	.3932300	10	.10539868	.03332999
	Ra_2y	.4078450	10	.10470497	.03311062

Paired Samples Correlations

		N	Correlation	Sig.
Pair 1	Ra_baseline & Ra_2y	10	.965	.000

Paired Samples Test

		Paired Differences					t	df	Sig. (2-tailed)
		Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference				
					Lower	Upper			
Pair 1	Ra_baseline - Ra_2y	-.01461500	.02763687	.00873955	-.03438523	.00515523	-1.672	9	.129

Treatment: Artificial saliva vs PO

Paired Samples Statistics

		Mean	N	Std. Deviation	Std. Error Mean
Pair 1	Ra_baseline	.2246180	10	.01877582	.00593744
	Ra_2y	.2291860	10	.01636524	.00517514

Paired Samples Correlations

		N	Correlation	Sig.
Pair 1	Ra_baseline & Ra_2y	10	.381	.277

Paired Samples Test

		Paired Differences					t	df	Sig. (2-tailed)
		Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference				
					Lower	Upper			
Pair 1	Ra_baseline - Ra_2y	-.00456800	.01964649	.00621277	-.01862225	.00948625	-.735	9	.481

Treatment: Artificial saliva vs S-G

Paired Samples Statistics

		Mean	N	Std. Deviation	Std. Error Mean
Pair 1	Ra_baseline	.1063370	10	.02647125	.00837094
	Ra_2y	.1051430	10	.02137798	.00676031

Paired Samples Correlations

		N	Correlation	Sig.
Pair 1	Ra_baseline & Ra_2y	10	.656	.039

Paired Samples Test

		Paired Differences					t	df	Sig. (2-tailed)
		Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference				
					Lower	Upper			
Pair 1	Ra_baseline - Ra_2y	.00119400	.02036672	.00644052	-.01337548	.01576348	.185	9	.857

Treatment: Artificial saliva vs S+G

Paired Samples Statistics

		Mean	N	Std. Deviation	Std. Error Mean
Pair 1	Ra_baseline	.1349370	10	.03965973	.01254151
	Ra_2y	.1335190	10	.03203564	.01013056

Paired Samples Correlations

		N	Correlation	Sig.
Pair 1	Ra_baseline & Ra_2y	10	.781	.008

Paired Samples Test

		Paired Differences					t	df	Sig. (2-tailed)
		Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference				
					Lower	Upper			
Pair 1	Ra_baseline - Ra_2y	.00141800	.02479524	.00784094	-.01631945	.01915545	.181	9	.860

Treatment: Citric acid vs NT

Paired Samples Statistics

		Mean	N	Std. Deviation	Std. Error Mean
Pair 1	Ra_baseline	.4460460	10	.12937678	.04091253
	Ra_2y	.4478520	10	.12812348	.04051620

Paired Samples Correlations

		N	Correlation	Sig.
Pair 1	Ra_baseline & Ra_2y	10	.972	.000

Paired Samples Test

		Paired Differences					t	df	Sig. (2-tailed)
		Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference				
					Lower	Upper			
Pair 1	Ra_baseline - Ra_2y	-.00180600	.03036345	.00960176	-.02352670	.01991470	-.188	9	.855

Treatment: Citric acid vs PO

Paired Samples Statistics

		Mean	N	Std. Deviation	Std. Error Mean
Pair 1	Ra_baseline	.2683770	10	.02557409	.00808724
	Ra_2y	.2546870	10	.01687239	.00533552

Paired Samples Correlations

		N	Correlation	Sig.
Pair 1	Ra_baseline & Ra_2y	10	.475	.165

Paired Samples Test

		Paired Differences					t	df	Sig. (2-tailed)
		Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference				
					Lower	Upper			
Pair 1	Ra_baseline - Ra_2y	.01369000	.02298817	.00726950	-.00275475	.03013475	1.883	9	.092

Treatment: Citric acid vs S-G

Paired Samples Statistics

		Mean	N	Std. Deviation	Std. Error Mean
Pair 1	Ra_baseline	.0836680	10	.01309188	.00414002
	Ra_2y	.0845110	10	.01382538	.00437197

Paired Samples Correlations

		N	Correlation	Sig.
Pair 1	Ra_baseline & Ra_2y	10	.612	.060

Paired Samples Test

		Paired Differences					t	df	Sig. (2-tailed)
		Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference				
					Lower	Upper			
Pair 1	Ra_baseline - Ra_2y	-.00084300	.01188038	.00375691	-.00934172	.00765572	-.224	9	.827

Treatment: Citric acid vs S+G

Paired Samples Statistics

		Mean	N	Std. Deviation	Std. Error Mean
Pair 1	Ra_baseline	.0859540	10	.00560575	.00177269
	Ra_2y	.0911650	10	.00862445	.00272729

Paired Samples Correlations

		N	Correlation	Sig.
Pair 1	Ra_baseline & Ra_2y	10	.371	.291

Paired Samples Test

		Paired Differences					t	df	Sig. (2-tailed)
		Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference				
					Lower	Upper			
Pair 1	Ra_baseline - Ra_2y	-.00521100	.00836161	.00264417	-.01119254	.00077054	-1.971	9	.080

Normality test and homogeneity of variance test in surface roughness change (ΔRa)

Tests of Normality

Group		Kolmogorov-Smirnova			Shapiro-Wilk		
		Statistic	df	Sig.	Statistic	df	Sig.
DT_Ra_2y	saliva NT	.217	10	.200*	.857	10	.070
	saliva po	.177	10	.200*	.903	10	.235
	saliva s-g	.179	10	.200*	.935	10	.499
	saliva s+g	.156	10	.200*	.950	10	.664
	citric NT	.200	10	.200*	.910	10	.283
	citric po	.177	10	.200*	.914	10	.307
	citric s-g	.179	10	.200*	.944	10	.593
	citric s+g	.172	10	.200*	.872	10	.106

One-way ANOVA for surface roughness (ΔRa) between surface finish groups in each solution

Solution1: Artificial saliva

Test of Homogeneity of Variances

DT_Ra_2y			
Levene Statistic	df1	df2	Sig.
.235	3	36	.871

ANOVA

DT_Ra_2y					
	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	.000	3	.000	.331	.803
Within Groups	.013	36	.000		
Total	.013	39			

Multiple Comparisons

Dependent Variable: DT_Ra_2y							
(I) Group			Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
Tukey HSD	saliva NT	saliva po	-.00483200	.00838528	.939	-.0274155	.0177515
		saliva s-g	.00028200	.00838528	1.000	-.0223015	.0228655
		saliva s+g	.00343900	.00838528	.976	-.0191445	.0260225
	saliva po	saliva NT	.00483200	.00838528	.939	-.0177515	.0274155
		saliva s-g	.00511400	.00838528	.928	-.0174695	.0276975
		saliva s+g	.00827100	.00838528	.758	-.0143125	.0308545
	saliva s-g	saliva NT	-.00028200	.00838528	1.000	-.0228655	.0223015
		saliva po	-.00511400	.00838528	.928	-.0276975	.0174695
		saliva s+g	.00315700	.00838528	.982	-.0194265	.0257405
	saliva s+g	saliva NT	-.00343900	.00838528	.976	-.0260225	.0191445
		saliva po	-.00827100	.00838528	.758	-.0308545	.0143125
		saliva s-g	-.00315700	.00838528	.982	-.0257405	.0194265
Games-Howell	saliva NT	saliva po	-.00483200	.00808640	.931	-.0277593	.0180953
		saliva s-g	.00028200	.00800392	1.000	-.0223985	.0229625
		saliva s+g	.00343900	.00804711	.973	-.0193706	.0262486
	saliva po	saliva NT	.00483200	.00808640	.931	-.0180953	.0277593
		saliva s-g	.00511400	.00871033	.935	-.0195046	.0297326
		saliva s+g	.00827100	.00875003	.781	-.0164593	.0330013
	saliva s-g	saliva NT	-.00028200	.00800392	1.000	-.0229625	.0223985
		saliva po	-.00511400	.00871033	.935	-.0297326	.0195046
		saliva s+g	.00315700	.00867387	.983	-.0213581	.0276721
	saliva s+g	saliva NT	-.00343900	.00804711	.973	-.0262486	.0193706
		saliva po	-.00827100	.00875003	.781	-.0330013	.0164593
		saliva s-g	-.00315700	.00867387	.983	-.0276721	.0213581

Solution2: Citric acid

Test of Homogeneity of Variances

DT_Ra_2y			
Levene Statistic	df1	df2	Sig.
8.861	3	36	.000

ANOVA

DT_Ra_2y					
	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	.001	3	.000	.702	.557
Within Groups	.016	36	.000		
Total	.017	39			

Multiple Comparisons

Dependent Variable: DT_Ra_2y							
(I) Group			Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
Tukey HSD	citric NT	citric po	.00257000	.00941386	.993	-.0227837	.0279237
		citric s-g	-.00478900	.00941386	.956	-.0301427	.0205647
		citric s+g	-.01007600	.00941386	.710	-.0354297	.0152777
	citric po	citric NT	-.00257000	.00941386	.993	-.0279237	.0227837
		citric s-g	-.00735900	.00941386	.862	-.0327127	.0179947
		citric s+g	-.01264600	.00941386	.542	-.0379997	.0127077
	citric s-g	citric NT	.00478900	.00941386	.956	-.0205647	.0301427
		citric po	.00735900	.00941386	.862	-.0179947	.0327127
		citric s+g	-.00528700	.00941386	.943	-.0306407	.0200667
	citric s+g	citric NT	.01007600	.00941386	.710	-.0152777	.0354297
		citric po	.01264600	.00941386	.542	-.0127077	.0379997
		citric s-g	.00528700	.00941386	.943	-.0200667	.0306407
Games-Howell	citric NT	citric po	.00257000	.01212375	.996	-.0326533	.0377933

		citric s-g	-.00478900	.01165070	.976	-.0391457	.0295677
		citric s+g	-.01007600	.01093004	.794	-.0434464	.0232944
	citric po	citric NT	-.00257000	.01212375	.996	-.0377933	.0326533
		citric s-g	-.00735900	.00760103	.769	-.0289213	.0142033
		citric s+g	-.01264600	.00644227	.253	-.0316582	.0063662
	citric s-g	citric NT	.00478900	.01165070	.976	-.0295677	.0391457
		citric po	.00735900	.00760103	.769	-.0142033	.0289213
		citric s+g	-.00528700	.00550056	.773	-.0212803	.0107063
	citric s+g	citric NT	.01007600	.01093004	.794	-.0232944	.0434464
		citric po	.01264600	.00644227	.253	-.0063662	.0316582
		citric s-g	.00528700	.00550056	.773	-.0107063	.0212803

T-test for surface roughness change(∆Ra) between solution groups in each surface finish process

Treatment: NT

Group Statistics

Solution		N	Mean	Std. Deviation	Std. Error Mean
DT_Ra_2y	saliva	10	-.0002640	.01636808	.00517604
	citric	10	-.0048650	.03353718	.01060539

Independent Samples Test

		Levene's Test for Equality of Variances		t-test for Equality of Means						
		F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	95% Confidence Interval of the Difference	
									Lower	Upper
DT_Ra_2y	Equal variances assumed	9.706	.006	.390	18	.701	.00460100	.01180109	-.02019216	.02939416
	Equal variances not assumed			.390	13.057	.703	.00460100	.01180109	-.02088231	.03008431

Treatment: PO

Group Statistics

Solution		N	Mean	Std. Deviation	Std. Error Mean
DT_Ra_2y	saliva	10	.0045680	.01964649	.00621277
	citric	10	-.0074350	.01857718	.00587462

Independent Samples Test

		Levene's Test for Equality of Variances		t-test for Equality of Means						
		F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	95% Confidence Interval of the Difference	
									Lower	Upper
DT_Ra_2y	Equal variances assumed	.195	.664	1.404	18	.177	.01200300	.00855042	-.00596076	.02996676
	Equal variances not assumed			1.404	17.944	.177	.01200300	.00855042	-.00596478	.02997078

Treatment: S-G

Group Statistics

Solution		N	Mean	Std. Deviation	Std. Error Mean
DT_Ra_2y	saliva	10	-.0005460	.01930579	.00610503
	citric	10	-.0000760	.01525270	.00482333

Independent Samples Test

		Levene's Test for Equality of Variances		t-test for Equality of Means						
		F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	95% Confidence Interval of the Difference	
									Lower	Upper
DT_Ra_2y	Equal variances assumed	.424	.523	-.060	18	.952	-.00047000	.00778048	-.01681618	.01587618
	Equal variances not assumed			-.060	17.085	.953	-.00047000	.00778048	-.01687913	.01593913

Treatment: S+G

Group Statistics

Solution		N	Mean	Std. Deviation	Std. Error Mean
DT_Ra_2y	saliva	10	-.0037030	.01948451	.00616154
	citric	10	.0052110	.00836161	.00264417

Independent Samples Test

		Levene's Test for Equality of Variances		t-test for Equality of Means						
		F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	95% Confidence Interval of the Difference	
									Lower	Upper
DT_Ra_2y	Equal variances assumed	8.322	.010	-1.329	18	.200	-.00891400	.00670494	-.02300056	.00517256
	Equal variances not assumed			-1.329	12.206	.208	-.00891400	.00670494	-.02349549	.00566749

Two-way ANOVA for surface roughness change(ΔRa) and their interaction

Univariate Analysis of Variance

Tests of Between-Subjects Effects

Dependent Variable: DT_Ra_2y						
Source	Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared
Corrected Model	.001a	7	.000	.484	.843	.045
Intercept	6.319E-05	1	6.319E-05	.159	.691	.002
Solution	6.516E-05	1	6.516E-05	.164	.687	.002
Tx	.000	3	4.092E-05	.103	.958	.004
Solution * Tx	.001	3	.000	.973	.410	.039
Error	.029	72	.000			
Total	.030	80				
Corrected Total	.030	79				

a. R Squared = .045 (Adjusted R Squared = -.048)

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