Dentistry Section

An Evaluation of the Colour Stability and Surface Roughness of High Translucency Zirconia Dental Ceramic after Immersion in Different Acidic Media: An In-vitro Study

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ABSTRACT

Introduction: Demand for increasingly appealing metal-free ceramic restorations drives research. With its exceptional mechanical and biological properties, zirconia crown outperforms other traditional ceramic materials. With outstanding mechanical and biological qualities, zirconia has several therapeutic applications. Temperature, environment, diet, and smoking habits all affect colour and surface roughness of dental restorations.

Aim: To investigate the colour stability and surface roughness of high translucency monolithic zirconia following immersion in various acidic solutions.

Materials and Methods: This in-vitro study was conducted at Yenepoya Dental College, Mangalore, Karnataka, India, from December 2019 to June 2020. Thirty rectangular samples from a Computer-Aided Design (CAD)/Computer Aided Manufacturing (CAM) machined Zirconia blank with 10×8×1 mm dimensions were sintered at 1350°C, tested for colour stability (E) and surface roughness prior to immersion (R). Groups were classified into CO (coffee), TO (tobacco) and CA (citric acid medium). Colour stability and surface roughness were re-evaluated postimmersion on all samples using spectrophotometers and profilometer, respectively. A significant difference in surface roughness and colour stability between the test groups were assessed using the paired-t test. The data was analysed using Statistical Package for Social Sciences (SPSS) version 24.0.

Results: Colour stability and surface roughness variations between the baseline value and three groups were found to be unaltered by different acidic media. The intergroup comparison of spectrophotometric analysis between the three groups had a standard deviation of 0.422 for citric acid medium, 0.316 for Coffee medium and 0.422 for Tobacco medium with an overall p-value of 0.804. The intergroup comparison of profilometric analysis had a standard deviation of 0.316 for Citric acid medium, 0 for Coffee medium and 0 for Tobacco medium with an overall p-value of 0.381. The paired t-test study showed that immersion in different acidic media had little effect on surface roughness of samples with a p-value of 0.343 but it was under clinically acceptable range.

Conclusion: According to the present study, high translucency monolithic zirconia had greater colour stability when treated with citric acid, followed by tobacco and then with coffee media, whereas, zirconia in coffee media had greater surface roughness, followed by tobacco, and then with citric acid media. However, both the results were clinically acceptable, indicating a 10 year lifespan when properly glazed.

Keywords: Colour change, Mechanical property, Optical property, Surface quality, Zirconia with high translucency

INTRODUCTION

The rising demands for aesthetic restorations among patients encourage researchers to develop materials with better physical and mechanical properties. Since, its introduction in the field of fixed prosthodontics, zirconia dental ceramic has surpassed traditional dental ceramics in every aspect. Crowns and Fixed Dental Prosthesis (FDP) fabricated using newer zirconia dental ceramics are aesthetically pleasing and bear excellent mechanical, physical, and biological properties [1-3]. For dental prosthesis, zirconium oxide is used as a core (framework) in a tetragonal phase (t-ZrO₂). It has been introduced as a monolithic ceramic to be used without veneering ceramic [4]. CAD/CAM monolithic zirconia restorations provide great flexural strength, less opposed wear, good aesthetics, shorter laboratory time, and fewer dental visits [5-7]. Yttria-stabilised Tetragonal Zirconia Polycrystal ceramics (Y-TZP) is the most often used zirconia because of its excellent aesthetics, biocompatibility, and fracture resistance [8-11]. For aesthetic purposes, the greater translucency of the zirconia-based all-ceramic system without metal substructure proved effective [12].

Oral restorations are subject to colour variations and surface roughness owing to factors such as temperature, humidity,

beverages, food, and smoking habits. Staining may be exogenous or endogenous. Particle size, hardness, and oxidation of unreacted carbon double bonds induce endogenous staining [13]. When zirconia is exposed to water or water vapors, it may spontaneously change phase [14-16]. This situation generates surface roughness, particle movement, and microfracture nucleation [17-20]. Water enters the material, speeding up degradation and failure [21,22]. Multiple studies [23-27] have been conducted to evaluate various mechanical properties of dental zirconia, but there is a scarcity of studies evaluating the colour stability and surface roughness of this aesthetically pleasing material. As a result, the goal of this study was to assess the colour stability and change in surface roughness of high translucency zirconia material when exposed to acidic environments. The null hypothesis was that acidic materials had no effect on the colour stability and surface roughness of high translucency zirconia.

MATERIALS AND METHODS

This in-vitro study was conducted at Yenepoya Dental College, Mangalore, Karnataka, India, for a duration of 6 months from December 2019 to June 2020. Permission to conduct the study was obtained from Institutional Research Committee and Institutional Ethics Committee (181/19.11.2019/YEC2).

Inclusion criteria:

- Standard Tessellation Languages (STL) files with the specified dimensions (10×8×1 mm).
- Only high translucency zirconia material was used to mill the block.
- Only coffee, tobacco and citric acid medium are included.

Exclusion criteria:

- STL files with undesirable dimensions (10×8×1 mm).
- Materials other than high translucency zirconia.
- Apart from coffee, tobacco, and citric acid media, all different media were excluded.

Sample size calculation: The sample size was calculated using the G*Power software (version 3.1.9.4, 2019, Heinrich Heine University Düsseldorf, Germany) under the assumption of a three-group comparison. It was determined that a sample size of 30 was appropriate, with 10 samples in each group.

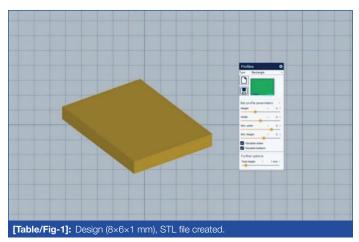
Materials included in the present study were:

- CAD/CAM Zirconia blank (16 mm) (Ceramill Zolid HT+, Amann Girbach Ceramill®, Amann Girrbach AG, Herrschaftswiesen, Koblach, Austria);
- 2) Immersion acidic media: (a) Citric acid medium; (b) Coffee medium; (c) tobacco medium.

Study Procedure

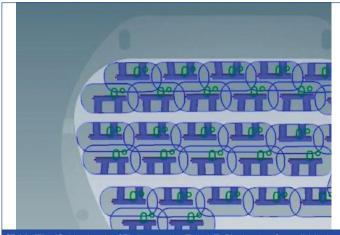
Thirty rectangle shaped zirconia specimens (10×8×1 mm) were milled out of zirconia blank and were sintered at 1350°C. To determine the impact of exposure on the colour stability and surface roughness, these specimens were divided randomly into three experimental groups of ten each. The groups were then immersed in three different acidic conditions.

Designing the STL file and CAD/CAM milling: A Standard Tessellation Languages (STL) file with the dimensions 8 mm in length, 6 mm in width, and 1 mm in thickness was produced using Tinker CAD software (version 1.3, Autodesk, Inc., San Rafael, CA, USA), yielding a rectangular form [Table/Fig-1]. Before being scanned with a CAD/CAM scanner (Amann Girbach Ceramill® map400 scanner), this STL file was loaded into CAM software (Ceramil mind programme, version 3.0, engine build 7783, think vision, Herrschaftswiesen, Koblach, Austria). Zirconia samples were sent using the Ceramill Mind application in STL format [Table/Fig-2].



As per manufacturer's instruction to compensate for shrinkage, the specimens designed were bigger in size (from 24.5 to 25%) [23] and these were nested onto a 16 mm Ceramill Zolid. HT+Zirconia blank [Table/Fig-3]. To create the specimens with the required dimensions, the blank was dry milled using an Amann Girbach Ceramill motion2, manufactured in 2014, Serial No. AAB75621, Herrschaftswiesen,

Koblach, Austria [Table/Fig-4]. Following milling, the zirconia samples were de-dusted, and the sprue attachment marks were polished using abrasives. The sintering process was place with the specimens inside the Ceramill Therm (Amann Girrbach) sintering furnace at 1350°C for two hours at a heat rate of 12°C/min [Table/Fig-5]. Specimens were cleaned in ultrasonic cleaner with 80% ethanol solution. Spectrophotometric analysis was performed before treatment to evaluate the baseline value [Table/Fig-6]. All samples collected after sintering were kept in an incubator at 37°C (to replicate the oral environment) for five days in artificial saliva, with the artificial saliva being replaced everyday [Table/Fig-7].



[Table/Fig-2]: Nesting the STL file onto the Zolid HT+Blank using Ceramill Mind Software.



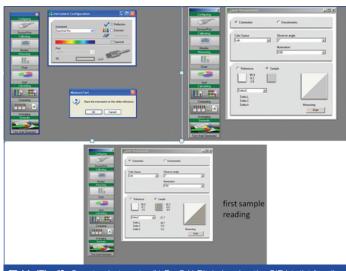
[Table/Fig-3]: Zolid HT+Blank before the milling process

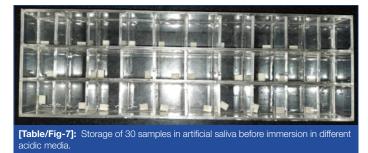


[Table/Fig-4]: Zolid HT+Blank after the milling process



[Table/Fig-5]: Samples in sintering tray, after completion of sintering cycle.



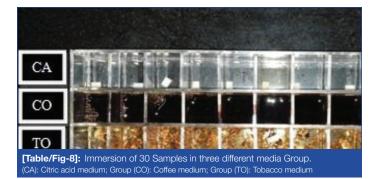


Sample grouping and randomisation: After storage, specimens were randomly divided into three groups (n=10) for immersion in the selected acidic media. For the distribution of specimens among the groups, Microsoft Excel 2016's RAND function (Redmond, WA, USA: Microsoft Corporation) was utilised. The three acidic media used for immersing the specimens used in this study are:

- a) Group 1 (CA)- Citric acid medium (pH around 3-6) [27]
- b) Group 2 (CO)- Coffee medium (pH around 5.6-6.3) [26]
- c) Group 3 (TO)- Tobacco medium (pH around 5-6) [25]

The specimens in each group were assigned numbers ranging from 1 to 10 and were referred to as CA1-CA10, CO1-CO10, and TO1-TO10 [Table/Fig-8].

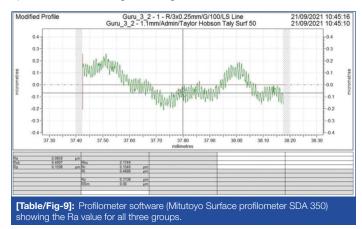
Assessment of colour and surface roughness at the baseline: All colour and surface roughness measurements were carried out by one operator who was not aware of the groups to remove operator bias. A reflectance spectrophotometer (I1 Pro® X-Rite) was used to record the initial colour measurements. The average of the three



readings- one from the centre and two from randomly selected offcentric areas- were taken against a white background. The CIELAB colour standard was used to specify the colour coordinates [13]. A contact profilometer (Mitutoyo Surface Profilometer SDA 350) was used to capture the initial surface roughness. One reading was taken from the centre and the other two were drawn at random from off-centric regions, and the average of the three readings were utilised [24].

Immersion media preparation and immersion procedure: The Tobacco medium (TO) was prepared by crushing 20 gm of tobacco leaves in 100 ml of water [25]. For Coffee medium (CO) 11.7 gm of coffee powder (Nescafe classic) was mixed in 200 mL of water at 55°C and then the solution was allowed to cool down [26] and Citric Acid media (CA) was prepared by taking 300 mL of fresh lime juice [27]. All the procedures were carried out by one operator. All of the procedures were completed at 37°C to prevent temperature from having an impact on the colour and surface grit of the examined materials. The specimens from each group were immersed in their respective immersion media for five days [Table/Fig-8] and were placed in an incubator at a constant temperature of 37°C. The solutions were replenished every 24 hours, and the immersion was continued to 120 hours. Based on study reported by Szalewski L et al., a five day immersion period will simulate around four years of conditioning of ceramic in the patients' oral cavity [28].

Assessment of colour and surface roughness after immersion in the tested media: The samples were gently washed with distilled water for 10 minutes and air-dried before each measurement. The same procedure that was applied for the colour and surface roughness measurement at the baseline was employed to record the final colour and surface roughness measurements once the immersion process was complete [Table/Fig-6,9]. Contact type reflectance spectrophotometer (I1 Pro® X-Rite) with geometric 20/0 observer curve and D50 light and the CIE L*a*b* colour space was used to represent the colour. It is feasible to assess the degree of visible colour change in each specimen using the CIE L*a*b* measurements. Values for CIE L*a*b* were obtained using the spectrophotometer. The CIE L*a*b* output of the spectrophotometer is based on a 28 standard observer and D65 illumination. For each specimen, the average reading for the three measurements was



computed. Change in colour before and after immersion with different acidic media was evaluated using the formula:

 $\Delta E^* = \{(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2\}^{1/2}$

Where L*is lightness, a* is green-red, b* is blue-yellow

The colour difference ΔE >3.3 was considered clinically unacceptable [29].

All the samples were then checked for surface roughness using Mitutoyo surface profilometer. SDA 350 and then compared with the values obtained at the baseline and compared among different acidic media [Table/Fig-9]. Change in surface roughness (in Micro mm) was calculated using the formula: Δ Ra=Raf-Rai. Where=Raf is the surface roughness after immersion and Rai is the surface roughness at the baseline [24].

STATISTICAL ANALYSIS

A Microsoft Excel spreadsheet (version 1910, 2019) from Microsoft Inc., Redmond, Washington, USA, was used to tabulate the data, and SPSS version 24.0 was used for statistical analysis (IBM SPSS Statistics for Windows, Version 24.0., 2016, IBM Corp., Armonk, NY, USA). The paired-t test was performed to examine if the test group's surface roughness and colour stability differed significantly from the control group.

RESULTS

The paired t-test study [Table/Fig-10] showed that surface treatment had no effect on colour stability variations between the baseline and various groups. For the group CA (Citric acid medium) and for the group CO (Coffee medium); the mean difference and standard deviation between baseline and after immersion was negligible with a p-value of 1. For group TO (Tobacco medium), the mean difference between baseline and after immersion was -0.1, with a standard deviation of 0.106 and a p-value of 0.594.

	Pretreatment		Post-treatment		
Group	Mean	SD	Mean	SD	p-value
Group 1	21.80	0.422	21.80	0.422	1.00
Group 2	21.90	0.316	21.90	0.316	1.00
Group 3	21.90	0.316	21.80	0.422	0.594
[Table/Fig-10]: Mean difference of spectrophotometric analysis between three different groups.					

[Table/Fig-11] showed the intergroup comparison of spectrophotometric analysis had a standard deviation of 0.422 for Citric acid medium, 0.316 for Coffee medium and 0.422 for Tobacco medium with an overall p-value of 0.804.

	Post-tre	eatment				
Group	Mean	SD	F	p-value		
Group 1	21.80	0.422				
Group 2	21.90	0.316	0.220	0.804		
Group 3	21.80	0.422				
[Table/Fig-11]: Intergroup comparison of spectrophotometric analysis between three different groups.						

The paired t-test study [Table/Fig-12] showed that surface treatment had no effect on surface roughness between the baseline and various groups. The mean difference between baseline and immersion for the CA (Citric acid medium) group was 0.10, and the standard deviation was 0.316, with a p-value of 0.343. For the group TO (tobacco medium) and the group CO (coffee medium), the mean difference between baseline and after immersion was insignificant.

[Table/Fig-13] showed the intergroup comparison of profilometric analysis had a standard deviation of 0.316 for Citric acid medium, 0 for Coffee medium and 0 for Tobacco medium with an overall p-value of 0.381.

	Pretreatment		Post-tre			
Group	Mean	SD	Mean	SD	p-value	
Group 1	0	0	0.10	0.316	0.343	
Group 2	0.10	0.316	0	0	0.343	
Group 3	0	0	0	0	-	
[Table/Fig-12]: Mean difference of profilometric analysis between three different						

groups.

	Post-treatment				
Group	Mean	SD	F	p-value	
Group 1	0.10	0.316			
Group 2	0	0	1.000	0.381	
Group 3	0	0			
[Table/Fig-13]: Intergroup comparison of profilometric analysis between three different groups.					

DISCUSSION

The colour stability and surface roughness of high translucency monolithic zirconia was tested after immersing in three acidic media namely citric acid, coffee and tobacco media. No significant differences were seen between groups or between pretreatment and post-treatment findings for both the parameters. Ceramics are popular because of their unexceptional chemical stability. Prior study has shown that acidic foods, coffee, tea, soft drinks, alcoholic beverages, and even fluoridated water can have an effect on the quality of restorative materials. The chemical, mechanical, and other properties of restorative materials affect the colour stability of these beverages. Because, it is present in many different foods and drinks, such as lemon, lime, orange, grapefruit, kiwi, strawberry, apple, pear, cherry, and raspberry, as well as in vegetables including mushrooms, potatoes, tomatoes, peas, and asparagus, the solution of citric acid was chosen for this investigation [27].

Demirel F et al., in their study immersed 2% concentrated citric acid solution for about eight hours at 37°C, in order to simulate approximately two years in-vivo condition. In the current study, the samples were immersed in various acidic media for approximately 120 hours which is equivalent to approximately 10 years of aging [27]. Artificial saliva was used over distilled water in this study to simulate the oral environment and offer data that is more realistic and comparable to clinical conditions. Coffee, a common beverage in the diet that is known to stain restorative materials, was used in research by Harianawala HH et al., on the colour stainability of restorative materials [30]. A properly glazed surface enhances stain resistance and colour stability, as evidenced by the fact that all of the samples included in this study were glazed in accordance with the manufacturer's instructions. An untrained eye can see colour changes below $\Delta E=1$, while those beyond $\Delta E=3.3$ need a trained eye. Because the materials were intended for aesthetic restorations, ΔE =3.3 was chosen as the clinical acceptability level. However, all groups had clinically acceptable ΔE [27]. In comparison to other acidic beverages like coca cola, citric acid has superior titrability (the speed at which saliva neutralises acidity) [31]. So, after treating the samples with citric acid, the surface roughness slightly increased (p-value=0.343) but was under clinically acceptable range not more than 0.2 µm. Researchers El Sokkary A et al., found that stored in citric acid (pH 2), glazed vita suprinity samples had a rougher surface than those maintained in artificial saliva [32]. Ra values for ceramics vary greatly depending on material composition, manufacturing process, measurement technique, and surface treatment [33-35].

Cigarette smoke has been shown to stain dental materials that are commonly used now-a-days [36]. Wasilewski MD et al., observed that tobacco was the main cause of discolouration and surface roughness changes [37]. These stains are caused by nicotine and tar, a sticky black residue that may embed in the sample surface. In this investigation, neither colour nor surface roughness was altered. This may be owing to the usage of crushed tobacco leaves that produce less heat. For protection from media penetration, all samples were glazed. Coffee medium may discolour resin-based items [31]. This study used zolid HT+high translucency monolithic zirconia, which contains 4 mol% Yttria, smaller grain sizes, and greater colour stability. There is a link between load type and coffee dissolving capacity at 55°C [31]. Using zolid HT+high translucency zirconia, Gawriołek M et al., found improved colour stability [38]. Extrinsic coffee stains were effectively removed by prophylaxis paste polishing on CAD/CAM Zirconia blocks.

According to Flavia AS et al., CAD-CAM lithium disilicate ceramics degraded when exposed to ordinary beverages [39]. Regardless of surface preparation, drinks lowered microhardness and changed colour. The following table represents the comparison between similar studies with the present study. In this study, the

glazed surface of high translucency monolithic zirconia remained unchanged following treatment. The coffee medium's surface roughness slightly showed a change in surface roughness with a p-value of 0.343 in the present study. Other media are created without heat, thus the preparation of coffee medium by diluting coffee powder at 55°C may be the cause. According to Sarac D et al., this may have induced glazing degradation and increased roughness [40].

In the present study, the colour stability of CAD-CAM manufactured high translucency monolithic zirconia zolid HT+restorative material was not affected by acid exposure. Coffee, but not other acidic drinks, decreased surface roughness of the CAD-CAM manufactured high translucency monolithic zirconia zolid HT+restorative material. In contrast to the many studies previously mentioned, colour changes occur after materials are immersed in various media, but the degree of colour changes is primarily influenced by the materials utilised [Table/ Fig-14] [27,31-33,36,37,39,41-43].

S. No.	Author's name and year	Place of study	Sample size	Solutions used	Parameters assessed	Conclusion
1	Demirel F et al., 2005 [27]	Hacettepe University, Ankara, Turkey	60	Citric acid (2%) and acidulated phosphate fluoride (1.23%)	Atomic force microscopy was used to measure the roughness.	Both autoglazed and overglazed specimens were significantly roughened by acidulated phosphate fluoride.
2	Mundim FM et al., 2010 [33]	Ribeirão Preto Dental School, University of São Paulo, Ribeirão Preto, SP, Brazil	45	Distilled water, coffee and cola drink	Colour change and effect of repolishing on the colour stability	Immersion in coffee generated more colour change in all types of composite resins examined in this investigation, and repolishing helped to reduce staining to clinically acceptable E values.
3	Jain C et al., [31]	Hitkarini Dental College, Jabalpur, India	90	Coffee, coca cola, orange juice, tea and water	Colour and surface roughness of these samples were measured using stereomicroscope and surface roughness tester	Of the five test fluids, Coffee caused the most discolouration of the ceramic, although Orange Juice caused the most surface roughness (1.48 m), possibly due to its high titratable acidity.
4	El Sokkary A et al., 2018 [32]	Egyptian Russian University, Egypt	76	2% citric acid solution and artificial saliva	Surface roughness and colour stability	The colour stability and surface roughness of Vita Suprinity were both negatively impacted by ageing in acidic pH medium (Citric acid solution). The surface roughness of Vita Suprinity was considerably affected by surface polish.
5	Schelkopf S 2019 [36]	University of Illinois at Chicago	100	Cigarette smoke	Colour stainability	When exposed to cigarette smoke, the CAD/CAM materials zirconia, lithium disilicate, and telio are all susceptible to discolouration. The polished finish does not make the material more stain-resistant as compared to the glazed surface of e.max lithium disilicate. The polished finish does not make zirconia more stain-resistant as compared to its glazed surface. All examined restorative materials exhibit less discolouration when they are brushed with a toothbrush after being exposed to cigarette smoke.
6	Wasilewski MD et al., 2010 [37]	Pontifical Catholic University of Parana, Curitiba, Parana, Brazil.	50	Effect of cigarette smoke and whiskey	Colour stability	According to the findings, the discolouration process had an impact on the colour stability of dental composites and was material and shade-dependent.
7	Flavia AS et al., 2019 [39]	Federal University of Ceará (UFC), Fortaleza, Ceará, Brazil.	160	Distilled water and distilled water plus brushing; coffee and coffee plus brushing; black tea and black tea plus brushing; red wine and red wine plus brushing; and cola and cola plus brushing.	Surface roughness, microhardness, and colour stainability	Regardless of surface preparation, beverages changed colour and reduced microhardness. Surface roughness increased, with glazed groups displaying greater diversity. Brushing the teeth did neither increase nor decrease the effects that were seen. The findings generally demonstrated that a surface with suitable qualities can be created through effective mechanical polishing.
8	Alsilani RS et al., 2021 [41]	Cairo University, Egypt	30	Coffee, Coca-cola	Colour stability and surface roughness	The most stable material in terms of colour and surface roughness was IPS e.max CAD, while Vita Enamic and PEEK displayed colour change that was above what was considered clinically acceptable.
9	Colombo M et al., 2017 [42]	University of Pavia, Pavia, Italy	40	Coffee, Coca-cola	Colour stability	Although colour integrity is unaffected by interaction with acidic beverages, the ∆E of CAD/CAM zirconia ceramics after immersion in coffee differed amongst products.
10	Al Amri MD et al., 2021 [43]	King Saud University, Riyadh, Saudi Arabia	80	Coffee	Translucency and colour stability	Comparing the newly introduced Crystal Ultra PICN material to resin nanoceramics and lithium disilicate glass-ceramic, the translucency was low. In contrast to resin nanoceramics, the Crystal Ultra material demonstrated superior colour stability, but more colour change when compared to Vita Enamic PICN and lithium disilicate glass-ceramic CAD/CAM materials.

11	Present study	Yenepoya dental college, Mangalore	30	Citric acid, coffee and tobacco media	Colour stability and surface roughness	High translucency monolithic zirconia had greater colour stability when treated with citric acid, followed by tobacco, and then with coffee media, whereas coffee media had greater surface roughness, followed by tobacco, and then with citric acid media. Both results, however, were clinically acceptable, indicating a 10-year lifespan when properly glazed.
[Table/Fig-14]: Comparative evaluation of similar studies [27,31-33,36,37,39,41-43].						

Limitation(s)

The material is attached to a tooth structure in a clinical setting and is only partially exposed to solutions and light. The fact that this study was an in-vitro experiment in which the material was stained on both sides sets it up for failure. Clinical trials can be utilised to validate this investigation's conclusions because it was an in-vitro research. While the specimen colour was matched with the backdrop, which was grey, the colour coordinate values may vary when using various backgrounds. The staining and surface roughness effects of various drinks and their combinations on high translucency monolithic zirconia materials must be investigated in more detail through clinical and in-vitro research.

CONCLUSION(S)

From this in-vitro investigation, it can be concluded that sample's colour stability was not affected by immersion in various acidic conditions, and no significant differences were seen between groups or between pretreatment and post-treatment findings. Immersion in different acidic media had little effect on surface roughness of samples with a p-value of 0.343 but it was under clinically acceptable range. According to this study, high translucency monolithic zirconia had greater colour stability when treated with citric acid, followed by tobacco, and then with coffee media, whereas coffee media had greater surface roughness, followed by tobacco, and then with citric acid media. Both results, however, were clinically acceptable, indicating a 10-year lifespan when properly glazed.

The oral environment presents far more challenges and a far more complex environment than the one used in this study to simulate it, with constant changes in temperature, pH, and different types of abrasive food, all of which can affect the materials colour and surface topography. Additionally, the role of saliva in reducing the pH value of the solutions was not taken into consideration. Hence, more clinical studies are needed to assess the behaviour of high translucency monolithic zirconia ceramic samples. In the present study, surface roughness was checked with the profilometer but more research is needed to assess the surface morphology of high translucency monolithic zirconia ceramic samples using Scanning Electron Microscope (SEM) analysis.

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