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Dr. Ritam Kundu
MDS, Department of Dentistry, Chanchal Sub-division Hospital, West Bengal, India

Dr. Nandana Bose
MDS, Consultant, Prosthodontist, H. Bose Clinic, West Bengal, India

Dr. Debjyoti Mandal
MDS, Consultant Periodontist, Privater Practice, West Bengal, India

Corresponding Author:
Dr. Ritam Kundu
MDS, Department of Dentistry, Chanchal Sub-division Hospital, West Bengal, India

Evaluation of the polymerization shrinkage of different resin cements under the zirconia crowns, using stereomicroscope and Scanning electron microscope

Dr. Ritam Kundu, Dr. Nandana Bose and Dr. Debjyoti Mandal

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Abstract
The goal of this *in vitro* investigation was to assess the microleakage caused by polymerization shrinkage of four different resin cements, which were used for cementation of monolithic zirconia crown on prepared maxillary and mandibular first premolar extracted for orthodontic purpose. The microleakage was analysed by dye staining, followed by evaluation via optical method using stereomicroscope and scanning electron microscope. Following statistical analysis of the results, it was discovered that all of the tested luting cements exhibit a sizable degree of polymerization shrinkage. The depth of dye penetration also showed high degree of correlation with polymerization shrinkage. However, the magnitude of correlation varied among the different groups.

Keywords: Resin cements, polymerization shrinkage, zirconia crown, stereomicroscope

Introduction
The clinical considerations in fixed prosthodontics have expanded as a result of the latest generation of particle-filled and high strength ceramics being introduced over the past ten years, which has opened the door to a wide range of dental materials. Also, the demand from patients for non-metallic materials for crown and bridge prosthesis has dramatically increased in recent years. Zirconia is one of the newest materials that has caught the interest of academic studies. Since the last decade, the use of zirconia-based ceramics as a biomaterial for dental crowns and implants in dentistry, has risen significantly, due to its properties like, high mechanical strength, biocompatibility, high wear resistance and superior aesthetics. Different aspects related to its clinical implication has caught the attention of the researchers as well.
Different clinical studies by researchers like, Gunge *et al*., (2017) [1], Shahdad *et al*., (2018) [2], Ortorp *et al*. (2009) [3] and many others have been conducted to assess the longevity of the zirconia crown. From these aforementioned studies, it can be considered that one of the most important elements in determining a crown's long-term success is, the retention of the crown and selection of the luting cement has a significant role in this regard. Nevertheless no proven definitive cementation process for zirconia ceramics has been validated yet. It is possible to use adhesive cementation as well as conventional cementation and both of these have their own advantages & disadvantages. Resin cements offer better esthetics, higher flexural and compressive strength. Resin cements are favoured in a variety of clinical settings because they have higher shear and tensile bond strengths than other cement types like glass ionomer cement and zinc phosphate cement. The adhesive nature of the resin cements produces crowns with higher retention and fracture resistance in addition to improved physical features. Adhesive cementation also results in reduced microleakage and lower water solubility.
Chemical bonding to metal oxides occurs when resin cements containing phosphate esters of acidic monomers are used. The most advised conditioning techniques for non-metallic zirconia crowns, however, are various air-blasting protocols coupled with chemical primers, such as formulations containing MDP monomers or silane coupling agents, followed by luting with dual-cured resin cements.

These self-adhesive resin-based dental cements facilitate the cementing process by not requiring the pretreatment of tooth material. While adhesive resin cements are great for bonding to base metal alloys, the absence of a surface oxide layer makes them less chemically reactive to noble alloy surfaces [4]. The retention resulting from the micro-mechanical and/or chemical bonding of the resin luting cements to the tooth and the prosthesis is one of the key variables in determining the clinical effectiveness of zirconia crowns [5]. When luting with phosphate base resin cements, certain investigations have found that zirconia prostheses and tooth surfaces have a sufficient bond strength [6, 7].

One of the important considerations with resin is the polymerization shrinkage. Typically, dental composites used in restorative procedures exhibit volumetric shrinkage ranging from less than 1% up to 6%, depending on the formulation and curing conditions. Resin cements exhibit at least similar or even higher polymerization shrinkage values [8]. As consequence of polymerization shrinkage and shrinkage stress, there is development of debonding along the restoration/tooth interface or at the restoration margins, resulting in internal and marginal gaps, micro-cracking of either or both the resin cement and tooth structure [8]. The aforementioned situation, clinically corresponds to formation of microleakage, i.e. a space between the tooth and the restoration, leading to the initiation of secondary caries, postoperative tooth sensitivity or pulpitis and discoloration of the tooth.

The monomer composition, filler content, and curing mode of the material as well as the characteristics of the substrate surfaces, such as dentin and the inner surface of the prosthesis, all have an impact on the bonding efficiency and microleakage associated with an adhesive luting cement.

There are different methods to evaluate polymerization shrinkage of resin cement in relation to tooth and or restoration, such as water and mercury dilatometers, transducer method, Bioman method for measuring shrinkage force etc. However, in this present study, dye staining followed by evaluation via optical method using a stereomicroscope was used for assessment of depth of penetration of dye within the tooth and restoration interface and scanning electron microscope (SEM) was used for quantitative assessment of the value of marginal gap and microleakage. This optical method was used over the other techniques due to its technical simplicity, ease of reproducibility and to avoid examiner's bias. The data obtained in this study was computer generated, hence the chances of possible error due to examiner's manual error could be avoided.

This present *in vitro* study was planned to assess the microleakage due to the polymerization shrinkage of four different resin cements, which were used for cementation of monolithic zirconia crown on prepared maxillary and mandibular first premolar, extracted for orthodontic purpose. The microleakage was analysed by dye staining, followed by evaluation via optical method using stereomicroscope and scanning electron microscope (SEM).

Materials and Methods

This study was conducted in the Department of Research and Development, Indian Institute of Science Education and Research, Kolkata, Mohanpur. The duration of the study was one year. A total of 48 freshly extracted permanent human maxillary premolars were selected. The teeth were immediately kept in a 10% formalin solution at room temperature for a week. With a scaler, calculus and periodontal tissue were eliminated (UDS-P Ultrasonic Scaler, Woodpecker). The teeth were then gently polished using a rotating brush and pumice at 6000 rpm. The roots of the teeth were embedded in auto-polymerizing acrylic resin cylinders (20 mm in height and 15 mm in diameter) up to 2 mm below the cemento-enamel junction and positioned with the long axis of the teeth perpendicular to the floor. (Fig:1).

The teeth were prepared manually for full coverage monolithic zirconia crown with the help of individual silicone (Fig:2) indexes by using a high-speed instrument under water-cooling with a diamond bur set. All preparations were done by a single operator. 1 mm wide rounded shoulder preparations were made at the cementoenamel junction. The occlusal surface was reduced by 2 mm. complete angle of convergence was targeted to be 6°. The preparations were rounded and smoothened, except for the gingival margins. The preparations were finished using fine and extra-fine diamond burs. Following which, all the prepared specimens were sent to the dental laboratory to fabricate the zirconia frameworks via Computer – Aided Design (CAD) and Computer- Aided Milling (CAM) device (Fig: 3). After that, the surface of sintered zirconia blocks was sandblasted perpendicularly for 10 seconds using 50 µm Al2O3 particles at 1 bar according to manufacturer's instruction. Then they were cleaned with 95% ethanol in an ultrasonic bath for 5 min before bonding. Later during the group allocation all these specimens were divided into four groups (n=12).

Regarding the cementation of crowns, manufacturing guidelines were followed. On the intaglio surface of 12 zirconia crowns, a universal primer (Monobond N) was applied using a disposable brush for Multilink N resin cement and then gently air-blown for 5 seconds. Via an automated mixing tip, resin cement was blended and applied to the surface. The extra cement was then scraped off after the bonded specimen was light-cured for 3 seconds on 4 surfaces. At four surfaces, light curing was kept up for further 20 seconds. For RelyX U200, equal volume of base and catalyst pastes were dispensed and then mixed for 10 seconds. It was followed by application on the inner aspect of the zirconia crowns, which were then luted on the prepared tooth and then cured for 40 seconds. For Para Core resin cement the chemical cured Para Bond adhesive was applied onto the prepared crown and then Para Core resin cement was applied directly into the intaglio surface of the zirconia crown. Cementation of the final crown was done with excess Para Core material being removed afterwards and then cured for 30 seconds on each side of the tooth. Similarly, for SoloCem resin cement, a small amount of Solo Cem dentine was placed on a mixing block to ensure homogeneous mixing and then Solo Cem was applied into the crown using the mixing tip which was then pressed onto the tooth and checked for correct seating. After brief curing for 2 to 3 seconds the excess was removed and then each side of the tooth was cured for 30seconds.

The specimens were kept in distilled water at room temperature for a week after the luting process. They were then thermocycled between 5°C to 55°C for 1000 cycles with a 20-second dwell time.

To prevent dye from penetrating other regions of the specimens after thermocycling, the root surfaces were covered with two layers of acrylic fingernail polish, and the root apices were sealed with a light-cured resin composite. A 1 mm window was created, situated below the crown margin. The teeth were stored at room temperature and submerged in a 2% methylene blue dye solution for 24 hours. The teeth were then dried after the methylene blue dye solution had been removed. After that, each tooth was sectioned longitudinally through the centre of the restoration, bucco-ligually or mesio-distally with a water-cooled low-speed diamond disc. Embedded root portion was thus removed with the help of high-speed air rotor.

The extent of dye penetration within each surface (i.e., either buccal and lingual or mesial and distal) of the sections was evaluated microscopically and recorded by one operator. With a computer-aided stereomicroscope (Euromex Trinocular, Holland Model-SB-1903-P) set to 40X magnification, scoring was done in accordance with the linear penetration of dye from the external margin of the luting agent (Fig. 4-A, 4-B).

• The dye penetration depths were scored according to Shah *et al*. [10]. as follows

0 - No micro leakage
1 - Microleakage to 1/3rd of axial wall
2 - Microleakage of 2/3rd of axial wall
3 - Microleakage of full length of axial wall
4 - Microleakage over the occlusal surface

Further estimation of polymerization shrinkage was conducted using scanning electron microscope and the values were expressed in µm.

The microleakage in the area of the tooth-cement interface was defined as linear penetration of methylene blue dye and it was determined with a stereomicroscope. The marginal gaps between tooth-cement interface (Fig:5) and crown-cement interface were defined as the perpendicular measurement by Scanning Electron Microscope (SEM). To avoid the electronic charge, the sectioned surfaces of the selected specimens were sputtered with gold (15-20 nm thick), followed by dehydration in the desiccator. Subsequently, gold - sputtered specimens were mounted on the specimen stub and then placed under the Scanning Electron Microscope (Supra 55 VP-4132 Carl Zeiss). Finally, the data obtained were tabulated MS Excel Spreadsheet and statistical analysis were performed.

Results

The data obtained was statistically evaluated by SPSS (version 24.0; SPSS Inc., Chicago, IL, USA) and GraphPad Prism version 5. Data has been summarized as mean and standard deviation for numerical variables and count for categorical variables. Shapiro Wilk test was administrated to check the normality for the data. Normally distributed 4 data sets were evaluated by one-way analysis of variance test (ANOVA) followed by Tukey's test. Not-normally distributed four data sets were compared by Kruskal Wallis' non parametric ANOVA followed by Dunnett's test. Pearson's or Spearman's correlation coefficient was used to find out significant correlation between two data sets. A value of $p < 0.05$ was considered as statistically significant. If the calculated p-value is below the threshold ($p<0.05$), then the null hypothesis is rejected in favour of the alternative hypothesis.

This study was based on the null hypothesis that no correlation exists between polymerization shrinkage of different resin cements and microleakage in monolithic zirconia crowns.

Analysis of the Depth of Dye Penetration Values:

Considering the results from the mean depth of dye penetration noticed in Group A was 1.1245, in Group B the mean depth of dye penetration was 3.525. In Group C, the mean depth of dye penetration was 1.4355 and in Group D, the mean depth of dye penetration value was noted as 2.08. (Table 1) One-way ANOVA test was performed to find for inter-group comparison of the data and it was found to be statistically significant in all the groups, as represented in (Chart. 1).

Considering the results from Table No. 2, the minimum amount of polymerization shrinkage noticed in Group A was 6.22 µm and the maximum amount of shrinkage in the Group A was– 16.09 µm. Similarly, in Group B the minimum and maximum polymerization shrinkage value was – 12.88 µm and 210.7 µm respectively, in Group C, minimum and maximum polymerization shrinkage value was as following: 5.89 µm and 33.87 µm and in Group D, minimum and maximum value was: 13.59 µm and 39.08 µm respectively. One-way ANOVA test was performed for inter-group comparison of the data and it was found to be statistically significant in all the groups, as represented in Chart. 2.

Correlation analysis of Depth of dye penetration and SEM data: Pearson's correlation coefficient was used to find out significant correlation between the depth of penetration and value of polymerization shrinkage. The polymerization shrinkage is presented in Y axis and the depth of penetration of dye is represented in X axis. In Group A, the p-value was 0.012, and $r = 0.708$. This denotes high degree of correlation. It is presented in the Chart No 3.

Fig 1: Embedded tooth

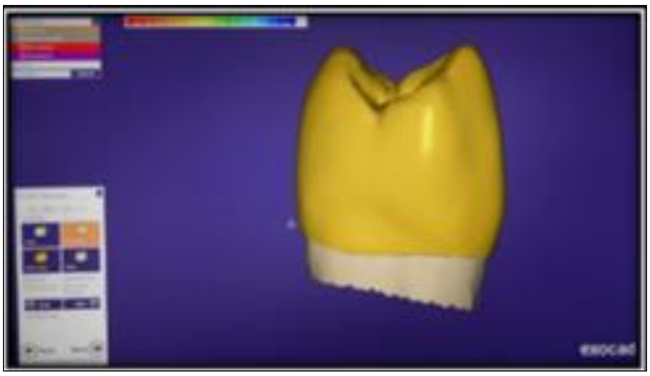

Fig 2: Tooth preparation

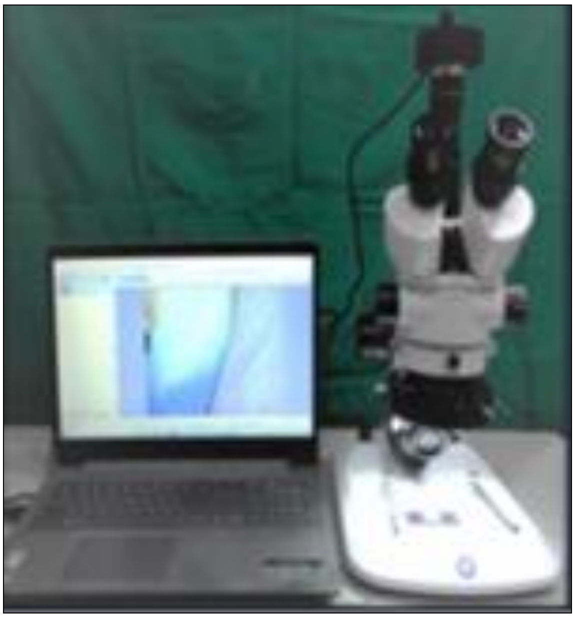

Fig 3: CAD-CAM crown preparation

Fig 4a, b: Stereomicroscopic evaluation

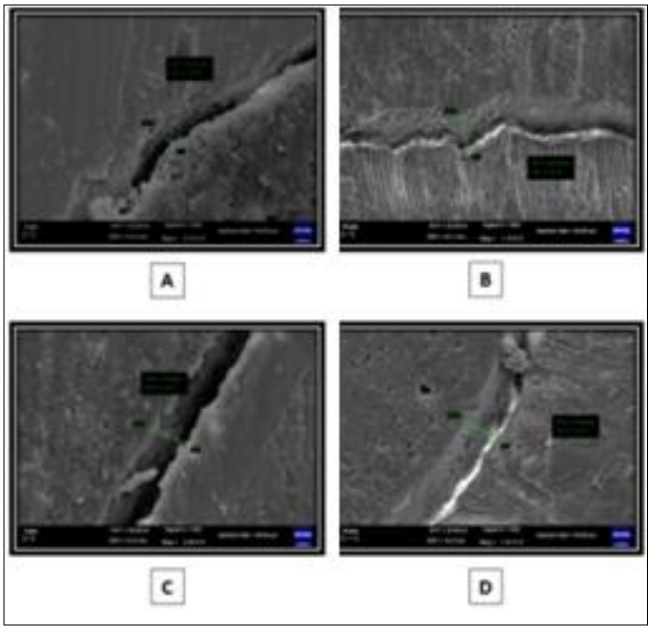

Fig 5: Scanning electron microscopic evaluation

Table 1: Depth of Dye Penetration

Value	Group A	Group B	Group C	Group D
Mean	1.1245	3.5205	1.4355	2.08
Median	1	4	2	2
Standard deviation	0.144	0.079	0.229	0.152

Table 2: Polymerization shrinkage

Value (in vm)	Group A	Group B	Group C	Group D
Maximum	16.09	210.7	33.87	39.08
Minimum	6.22	12.88	5.89	13.59
Mean	8.697	55.453	14.243	25.182
Median	7.605	62.945	13.780	25.42
Standard deviation	0.739	8.974	1.762	2.254

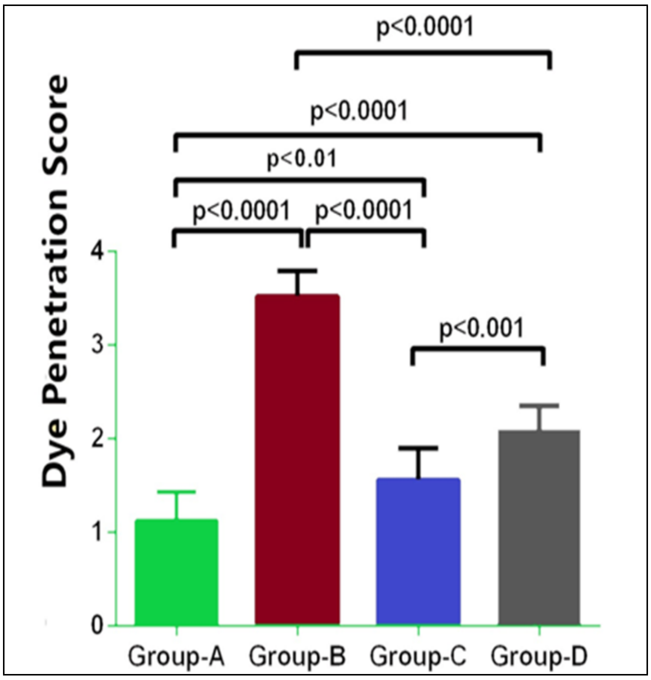

Chart 1: Depth of Dye Penetration

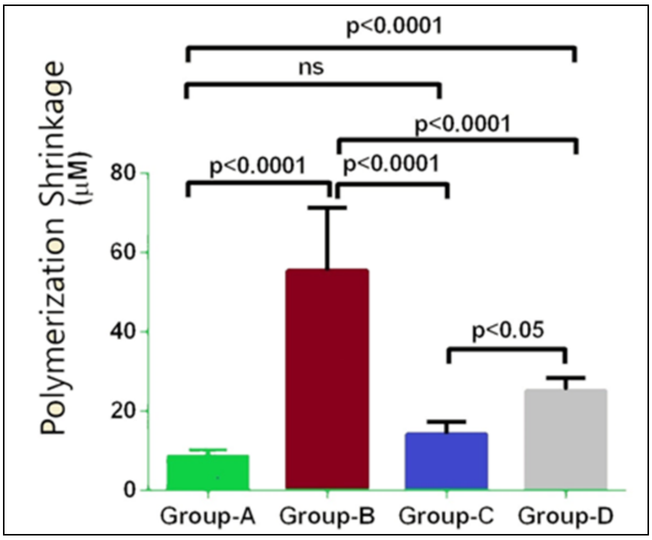

Chart 2: Polymerization shrinkage

Table 3: Correlation between the depth of penetration and value of polymerization shrinkage

	Number	12
Group A	Pearson correlation (r)	0.708
	p-value	0.012
Group B	Number	12
	Pearson correlation (r)	0.927
	p-value	<0.0001
Group C	Number	12
	Pearson correlation (r)	0.465
	p-value	0.128
Group D	Number	12
	Pearson correlation (r)	0.329
	p-value	0.293

Discussion

The present study was conducted for comparative analysis of microleakage in monolithic zirconia crowns associated with the polymerization shrinkage, when luted with four different resin cements. In this study, the teeth specimens were divided into four groups, namely: Group A (Specimens cemented with Multilink N), Group B (Specimens cemented with ParaCore), Group C (Specimens cemented with RelyX U200), Group D (Specimens cemented with SoloCem). Following the crown cementation with the group specific luting cement, the specimens were immersed in the dye solution. The specimens were then sectioned and evaluated. For extent of dye penetration, evaluation was carried under stereomicroscope and further estimation of polymerization shrinkage was conducted using scanning electron microscope. The method used in this study is in accordance with that done by Korkut *et al*. [9], Shah *et al*. [10] In this *in vitro* study, premolars, that were extracted for orthodontic purpose, were selected as test specimen. Tooth preparations were completed by a single operator and the monolithic zirconia crowns were made using the CAD-CAM method to ensure standardization in terms of prosthesis dimension. This was done to reduce the effect of different confounding factors on the results, obtained in this study.

Following the statistical analysis of the results obtained, it was found that there is a significant amount of polymerization shrinkage evident with all the tested luting cements. The depth of dye penetration also showed high degree of correlation with polymerization shrinkage. However, the magnitude of correlation varied among the different groups.

During the fabrication of zirconia crowns, the thickness of zirconia was maintained at 1 mm to 2 mm. Authors like Cardash (1993) [11] proposed that while using resin cements for luting, 2 mm ceramic thickness ensures better physical properties of the resin cement, as, such amount of thickness would allow the cement to adequately absorb light. However, Marginal discrepancies within the range of 100 µm can be considered as clinically acceptable [12, 13]. All the zirconia crowns tested in this study were within this value. i.e., 85.6±8.32 µm, which is in accordance with the findings of Gonzalo *et al*. [13]. The space required for the luting agent was digitally adjusted. With the consideration of impact of the die spacing factor on marginal discrepancy [13], all the zirconia crowns were fabricated with a die space of 40 µm. Following fabrication, the zirconia crowns were air abraded using alumina particle of 50 µm size. The restoration surface's roughness strengthens micromechanical interlocking by enhancing surface energy and wettability. This, in turn, allows cement to flow into the microporosities and improves micromechanical retention. Following cementation of the oxide ceramic restorations, the bond strength between the adhesive cement and the ceramic surface is enhanced by both the chemical and micromechanical retention. Four luting agents were used to lute the zirconia crowns to the prepared teeth. In Group A, Multilink N, Group B – ParaCore, Group C – RelyX U200 and Group D – SoloCem were used.

In this study, the specimens underwent thermocycling and dye staining, 7 days after the crown cementation. This process was clinically relevant, as the patients are usually asked for a follow up visit, usually 7 days after crown fixation. Resin cements used in this study undergo dual polymerization, i.e., chemical and physical. 24 hours following the crown fixation, the crowns were immersed in 2% methylene blue dye solution. *In vitro* microleakage studies can be considered as an initial screening method to evaluate the microleakage of a resin cement *in vivo*. In this present study 2% Methylene blue dye was used. Dye penetration is a convenient test for assessment of microleakage at tooth-luting agent-prosthesis interface. Following the staining, the specimens were sectioned using diamond discs for assessment of marginal gap and polymerization shrinkage. The problem that was encountered during sectioning the specimen, was the possible dissolution and removal of dye staining, during cutting with water-cooled diamond saws. There are various ways to measure the marginal gap and polymerization shrinkage. In the current study, the cross-sectional method was used to measure the marginal fit of cemented prosthesis. Stereomicroscope was used for qualitative assessment of specimen. The extent of the stains was identified and scored. The Scanning Electron Microscope (SEM) was used to quantify the shrinkage value, i.e., to measure the linear value of polymerization shrinkage (as expressed in µm). The measurements were done in captured image. To do this, software was used. As a result, measurements may be regarded as being devoid of observer errors. The microscopic marginal analysis offers the advantage of the possibility of long-term evaluation due to non-destructiveness of the specimen, as well as permitting evaluation of the whole restoration margin. However, this method is associated with drawbacks like unavailability of information about the quality of the prosthesis-cement-tooth- interface beyond the superficial surface. All microleakage studies have shown that a seamless marginal seal in dentin is impossible to achieve even under optimal laboratory conditions as no technique or adhesive system has proven to be 100% effective at dentinal margins. Some degree of error is unavoidable in any of the method employed for such a study.

From the result of the present study, the least amount of dye penetration was observed in Group A (i.e. Multilink N Group), the mean score was 1.125. The mean scoring of the rest of the groups (in ascending order) are. Group C (RelyX U200): 1.563, Group D (SoloCem): 2.084, Group B (ParaCore): 3.521. The inter group comparison of data revealed, statistically significant difference of values among all the groups. These findings also corroborated with the results of evaluation of polymerization shrinkage, using SEM. The smallest degree of microleakage (both at the cement-crown and tooth-cement interfaces) was obtained by the Multilink N self-adhesive resin cement and Monobond N primer (i.e. in Group A), the mean value in Group A was 8.697 µm, followed by RelyX U200 (Gr. C): 14.243 µm, then SoloCem (Gr D): 25.183 µm and ParaCore showed highest degree of microleakage, i.e. 55.453 µm. The inter group comparison of data revealed, statistically significant difference of values among all the groups, except Group A and Group C. There was no statistical significance of difference in values in these two groups. This finding can be attributed to the compositional similarity between these two resin cements. However, while performing the correlation analysis among the stereomicroscope and SEM data, highest degree of correlation was observed in Group B (r value 0.927), followed by Group A (r=0.708), Group C (r=0.465) and Group D (r=0.329). Although, the ParaCore resin cement contains high amount of glass particle as fillers, the high degree of dye penetration and polymerization shrinkage in this group can be attributed to absence of phosphoric acid monomer group in this cement, which aid in bonding of the cement to zirconia surface. From the result of this study, the utility of ParaCore resin cement in luting Zirconia crowns, requires further evaluation owing to its unfavorable findings. The findings of this study could not be corroborated directly with any other studies, as no single studies could be identified to the best of the knowledge, where these particular resins cements were assessed for polymerization shrinkage with relation to Zirconia crowns. However, the studies where other properties of these resin cements (i.e. shear bond strength, degree of conversion etc.) were assessed were indirectly correlated with the findings.

A dual-cured (chemical and photoinitiated) redox reaction for polymerization of the resinous phase and another acid-base reaction that results in the formation of calcium phosphates can be thought of as two simultaneous reactions that occur during the setting reaction of self-adhesive and dual-cured luting agents like Multilink N, Rely X U200, and SoloCem. Ionized phosphoric acid methacrylate of the monomer forms a bond with dentin. Ionization occurs in situ using either the water found in dentin or the water created when the phosphate monomers react with the basic filler to neutralise them [14, 15]. Due to their various interactions, Multilink N, Rely X U200, and SoloCem appear to be able to provide self-adhesion to tooth surfaces, sealing the tooth-cement interface effectively[14] and resulting in the lowest microleakage values. In this investigation, the microleakage at the cement-prosthesis interface and the cement-tooth interface were both equivalent. Unlike to other investigations, this one found that the tooth-cement interface was primarily where microleakage occurred.

In this study, one of the limitations that can be considered is the lack of evaluation of the effect of nonaxial loading and lateral movement. The study's inability to distinguish between distinct microleakage sites was another drawback. The cement-tooth interface and/or the cement-restoration interface may both experience microleakage. The cement-tooth contact has been identified as the weakest link because, in a stressed condition, the weakest link breaks first. Instead than focusing on the microleakage's exact location, this study concentrated on how much of it there was, as shown by how much staining there was. The interface at which the microleakage started cannot be identified by the method used. It is advised to conduct additional research to determine the weakest interface and its potential impact on microleakage. Another factor is the brief period of the trial, which makes it impossible to assess any potential bond weakening over time.

By conducting more research and developing various elements that strengthen the bond, it will be possible to further increase the adhesive capacity and compatibility of resin cements to zirconia ceramics. In addition, larger sample sizes and longer study durations are required for randomised control trials in order to confirm the *in vitro* study's findings, draw conclusions about oral conditions, and ultimately adopt a more definitive protocol for ensuring the bonding of zirconia restorations that will have minimal microleakage.

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