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# COLOR STABILITY AND CYTOTOXICITY OF RESIN CEMENT WITH COLOR INDICATOR VS CONVENTIONAL RESIN CEMENT (IN-VITRO STUDY)

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## ABSTRACT

**Aim:** This in vitro study was conducted to compare Color stability and Cytotoxicity of resin cement with color indicator vs conventional resin cement.

**Methods:** Sixty cement samples were prepared; 30 for Group I; Conventional resin cement (Maxcem Elite™ ) and 30 for Group II; Resin cement with color indicator (Maxcem Elite Chroma™). Each group was divided into two subgroups; A: for color stability test(n=20) and B: for cytotoxicity test(n=10). Color stability was measured by vita easyshade spectrophotometer; half of samples were measured before and after immersion into distilled water and other half before and after1000 thermal cycles (TC). Cytotoxicity was measured by the methyl-thiazol-tetrazolium (MTT) colorimetric assay using WI-38 fibroblast cell after 24 and 48h. Data was collected and IBM® SPSS® Statistics Version 20 was used for analysis. The significance level was established at $P \leq 0.05$.

**Results:** For color stability; higher mean of control group II (3.08) than group I (2.98) with no statistically significant difference between them (p=0.790) and higher mean of thermocycled group I (3.46) than group II (1.65) with statistically significant difference between them (p=0.014). For cytotoxicity; A statistically significant difference between Group I and Group II, with higher mean of group II (2.414% and 1.626%) than group I (1.764% and 0.166%) at 24 and 48 hours respectively.

**Conclusions:** After Tc Maxcem Elite Chroma™ more color stable than Maxcem Elite™ and more cytotoxic which decreased after 48h.

**KEYWORDS** Color stability, Cytotoxicity, resin cement with color indicator, conventional resin cement.

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## INTRODUCTION

The application of all-ceramic restorations in clinical dentistry has grown because they offer superior mechanical performance along with excellent aesthetics. The establishment of a strong bond between ceramic restoration and tooth structure is a critical factor in determining their clinical success. Both traditional and resin-based adhesive techniques can be used to lute all-ceramic restorations, however adhesive cementation is preferred in order to produce a strong and stable bond. **Lima et al., 2019**[1]

With the development of adhesive dentistry, restorative procedures may be carried out with less tooth preparation, protecting the dental substrate and increasing the lifespan of the restoration. By enhancing retention and lowering solubility, resinous luting agents have enhanced indirect restoration, so strengthening the restoration and the tooth structure. **Miotti et al., 2020**[2]

Self-adhesive resin cements bond to the teeth without the need for an additional adhesive or etchant. Acid functional monomer is an essential ingredient in self-adhesive resin cements. Although these cements cannot completely dissolve or demineralize the smear layer to achieve mechanical retention, they can effectively form a chemical bond with the tooth through the acid-base reaction. When compared to other multistep resin cements, the effects of self-adhesive resin cements were satisfactory. **Sun et al., 2018**[3]

Both the removal of excess cement and the timing of that removal are crucial. It might be challenging to judge when it is best to remove excess cement, however some manufacturers provide a color cleanup indicator to help with this decision. Any excess material can be safely removed once the indicator's color has faded, and light curing will then produce the ultimate bond strength between the tooth and restoration. **Niemi et al., 2020**[4]

Color stability has a direct effect on the success of aesthetic restorative materials. The ADA recommends using the CIELAB (Standard Commission Internationale de l'Éclairge) color system, which expresses color in terms of luminosity and chromaticity. The aesthetics of restorations are determined by color, surface texture light reflectance and transmittance, translucency and opacity. **Bhattacharya et al., 2020**[5]

Artificial aging variables such as temperature, humidity and ultraviolet light have been utilized to assess the stability of color in dental restorations under mimicked clinical situations. Thermocycling is a popular in vitro aging technique that simulates temperature changes in a humid oral cavity. **Yamali et al., 2023**[6]

Biocompatibility is the ability of the material to effectively elicit a favorable host response following a particular implantation. Therefore, in addition to strength, aesthetics and clinical manipulation, biocompatibility must be considered in the development of any dental biomaterial. The substances that leached from the material may have harmful or allergic side effects at the clinical or subclinical level. **GERÇEK et al., 2023**[7]

The study aimed to compare color stability and cytotoxicity of resin cement with color indicator vs conventional resin cement.

### Null hypothesis

No difference between color stability and cytotoxicity of all types of cements.

Thermocycling not affect on the color stability of all types of cements.

## MATERIALS AND METHODS

### Ethical consideration

This study was approved by Ethics Committee of Faculty of Dentistry, Minia University RHDIRB2017122004 under the protocol number (803), 2023. All steps of the study were caried out regarding to this protocol.

### Sample size calculation

Using G power statistical power Analysis program (version 3.1.9.4) [8] for sample size determination, A total sample size of 60 were determined. That was been sufficient to detect a large effect size (d) =1.4 for color stability test and (d) =2.24 for cytotoxicity test, with an actual power (1-β error) of 0.8 (80%) and a significance level (α error) 0.05 (5%) for two-sided hypothesis test.

### Grouping and Samples preparation

Thirty samples of each type of dental resin cement were made, for a total of sixty samples. Group I for Conventional resin cement and group II for Resin cement with color indicator. Based on the test, each group was split into two subgroups; subgroup A for color stability test(n=20) and subgroup B for cytotoxicity test(n=10). The materials, brand name, description, Composition, manufacturer and lot number were represented in (**Table 1**).

### Samples preparation:

Sixty disc-shaped specimens of the two-resin cement (n =30) were constructed according to the recommendations of ISO 4049:2019 by filling a split metal mold (15 mm in diameter and 1.2 mm in thickness) for color stability test and according to ISO 10993-5:2009 by filling a split metal mold (4 mm in diameter and 2 mm thickness) for cytotoxicity test **figure1** to prevent porosities within the material, the mold was filled with cement, the sample's surface was covered with a transparent plastic matrix strip and the sample was gently pressed with a microscope glass. All of the samples' polymerization was done by a light-emitting diode source for 10 seconds on each sample. After taking the sample out of the metal mold, silicon carbide papers were used to eliminate any excess flash. **Mazzitelli et al.,2023**, **Kashi et al.,2022**[9,10]

### Color Stability measurement:

Samples were subdivided into two subgroups (n=10) regarding thermocycling, subgroup without thermocycling and only immersion in distilled water for 7 days that was changed each 24h, at room temperature and subgroup was placed in a thermocycler apparatus and exposed to 1000 thermal cycles at 5°C and 55°C in water with a dwelling time of 30s.

TABLE (1) Materials used in this study

| Brand name | Description | Composition | Manufacturer | Lot No. |
|---|---|---|---|---|
| **Maxcem Elite™** Universal Resin Cement | A dual cure, self-etch/ self-adhesive cement. | Methacrylate esters, GPDM, UDMA, HEMA, Mineral fillers and ytterbium fluoride (filler load 69% weight), activators, and stabilizers. | Kerr dental Corporation, California, USA. | **9526700#** |
| **Maxcem Elite™ Chroma** Universal Resin Cement with Cleanup Indicator | A dual cure, self-etch, adhesive cements. Dispenses pink before fading at the gel state indicating the optimal time to clean up excess cement. | Methacrylate ester monomers, GPDM, 1,1,3,3-tetramethylbutyl hydroperoxide (TEGDMA), HEMA, GDM, UDMA, and proprietary self-curing redox, camphorquinone, fluroaluminosilicate glass filler, silica, barium glass filler, (filler load 67% weight) activators, stabilizers. | Kerr dental Corporation, California, USA. | **9259309#** |

**GPDM:** ***Glycerol phosphate dimethacrylate,*** **UDMA:** ***Urethane dimethacrylte,*** **HEMA:** ***Hydroxyethyl methacrylate,*** **TEGDMA:** ***Triethyleneglycol dimethacrylate,*** **GDM:** ***Glycerol dimethacrylate.***

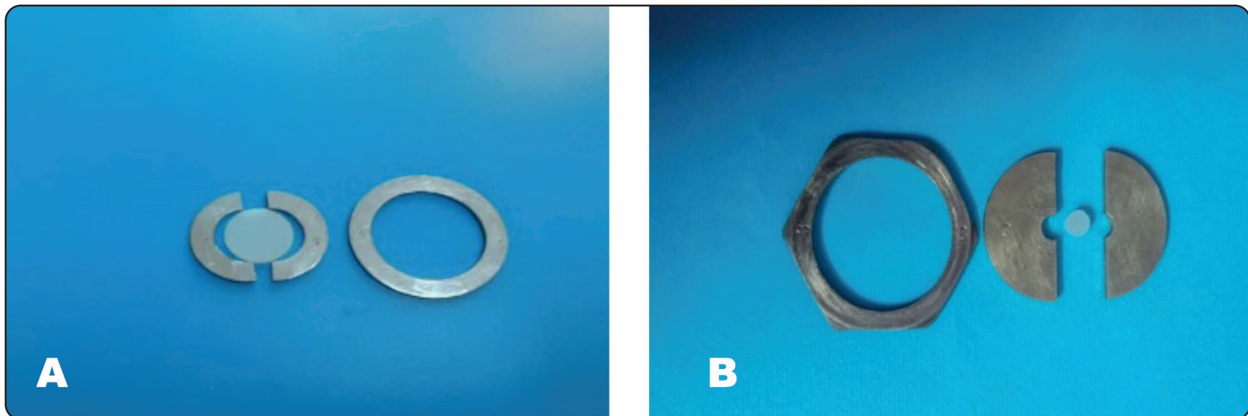


Fig. (1) A split metal mold A: for color stability test sample and B: for cytotoxicity test sample.

The change in color of the tested groups after immersion and thermocycling was measured. A spectrophotometer (VITA easy shade spectrophotometer, Bad Sackingen, Germany) was used to record color measurements of all disks and compared with the control group before immersion and thermocycling process. After cleaning and gently drying each disk with tissue paper, calibration was carried out in accordance with the manufacturer's instructions as follow; After the instrument probe was inserted into the calibration block holder, the probe tip was flush with and perpendicular to the calibration block, which depressed the calibration block. Next, a successful calibration was completed at each disc's top surface by positioning the probe against a white background, flush with the disc surface and making a small mark with a graphite pencil to repeat the readings away from that mark. **Kalantari et al., 2017**[11]. After all specimens were measured the mean value of three measurements ΔL, Δa and Δb was recorded and after immersion and thermocycling process all these measurements were repeated.

Color stability was measured by color change assessment and the following formula was used:

$$\Delta E = [(L^*_1 - L^*_2)^2 + (a^*_1 - a^*_2)^2 + (b^*_1 - b^*_2)^2]^{1/2}$$

Before (1) and after (2) water aging, L* represents brightness ranging from 0 = black to 100 = white, a* denotes redness/greenness ranging from -a = green to +a = red and b* ranging from −b = blue to +b = yellow. **Kumah et al., 2019**[12]

**Cytotoxicity measurement:**

The cement sample was incubated in maintenance medium for 24 hours, after which the media was assayed as a sample in three replicates. To create a complete monolayer sheet, 1 X 105 WI-38 fibroblast cells / ml (100 ul / well) were added to a 96-well tissue culture plate. The plate was then incubated at 37°C for 24 hours. Once a confluent sheet of cells had formed, the growth material was removed from the 96-well micro titer plates and the cell monolayer was twice washed with wash media. Three wells were left as controls, with only maintenance medium and different wells were evaluated using 0.1 ml of filtrate. Plate was tested after being incubated at 37°C. Physical signs of cytotoxicity, such as shrinkage, rounding and granulation were examined. A 5 mg/ml MTT solution was made in sterile phosphate-buffered saline (PBS) (BIO BASIC CANADA INC). To each well, 20ul of MTT solution was put. The media shake at 150 rpm for five minutes to fully incorporate the MTT. Incubated for four hours at 37°C with 5%

$CO_2$ to facilitate the metabolism of MTT. Discard the media. Formazan, an MTT metabolic product, can be redissolved in 200 $\mu$L Dimethyl sulfoxide solvent (DMSO). Position at 150 rpm on a shaking table for 5 minutes, to thoroughly mix the formazan into the solvent. Read optical density at 560nm and subtract background at 620nm. [13,14,15]

MTT used to detect cell viability using the optical density and then the cytotoxicity measured after subtracted the viability from 100.

**Viability** = (OD Test/OD control) × 100. **Kashi et al.,2022**[10]

### Statistical analysis

Data was collected and analyzed with IBM® SPSS® Statistics Version 20 for Windows. After the data were checked for normality using the Shapiro-Wilk and Kolmogorov-Smirnov tests, a parametric (normal) distribution was found. In unrelated samples; the independent sample t-test and the one-way ANOVA test were used to compare the two groups and more than two groups respectively. In related samples; the paired sample t-test and Two-way ANOVA were used to compare two groups and to assess the effect of the interactions between different variables respectively. A significant level P<0.05 was established.

## RESULTS

### Color stability:

Comparing the statistical results of both groups; I (Maxcem Elite™) and II (Maxcem Elite™ Chroma) the data revealed that there was no statistically significant difference between control (Group I) and (Group II) where (p=0.790). The mean value of control Group II (3.08±0.71) higher than mean value of control Group I (2.98±0.49) and there was a statistically significant difference between thermocycled (Group I) and (Group II) where (p=0.014). The mean value of thermocycled Group I (3.46±1.12) higher than mean value of thermocycled Group II (1.65±0.63).

Within each group; Maxcem Elite™ (Group I); the statistical results revealed that (ΔE) value was (2.98±0.49) for control sub group and (3.46±1.12) for thermocycled sub group. There was no statistically significant difference between (Control) and (Thermocycled) where (p=0.404). Regarding Maxcem Elite™ Chroma (Group II); the statistical results revealed that (ΔE) value was (3.08±0.71) for control sub group and (1.65±0.63) for thermocycled sub group. There was a statistically significant difference between (Control) and (Thermocycled) where (p=0.010). as shown in **Table 2** and **Figure 2**.

TABLE (2) The Color change (ΔE) values of control and thermocycled of group I and II.

| Variables | Color change (ΔE) | | | | p-value |
|---|---|---|---|---|---|
| | Group I | | Group II | | |
| | Mean | SD | Mean | SD | |
| **Control** | 2.98 | ±0.49 | 3.08 | ±0.71 | 0.790ns |
| **Thermocycled** | 3.46 | ±1.12 | 1.65 | ±0.63 | 0.014* |
| ***p-value*** | 0.404ns | | 0.010* | | |

***; significant (p<0.05)     ns; non-significant (p>0.05)***

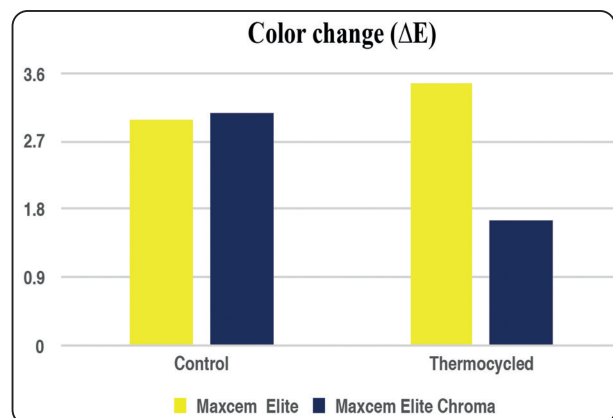


Fig. (2) Bar chart shows color change (ΔE) values of Group I and Group II

**Cytotoxicity:**

Comparing the statistical results of both groups I and II the data revealed that; at 24 hours: A significantly higher value was recorded in Group II (2.414±0.316%) in comparison to Group I (1.764±0.062%). The difference between groups was statistically significant (p=0.009) and at 48 hours: A significantly higher value was recorded in Group II (1.626±0.207%) in comparison to Group I (0.166±0.021%). The difference between groups was statistically significant (p=0.000).

Within each group Maxcem Elite™ (Group I); the statistical results revealed that higher value was recorded at 24 hours (1.764±0.062%), in comparison to 48 hours (0.166±0.021%). The difference between observation times was statistically significant (p=0.000). Regarding Maxcem Elite™ Chroma (Group II); the statistical results revealed that higher value was recorded at 24 hours (2.414±0.316%), in comparison to 48 hours (1.626±0.207%). The difference between observation times was statistically significant (p=0.012). as shown in **Table 3** and **Figure 3**.

TABLE (3) The Cytotoxicity values of group I and II after 24 and 48 hours.

| Variables | (Cytotoxicity%) | | | | |
|---|---|---|---|---|---|
| | Group I | | Group II | | p-value |
| | Mean | SD | Mean | SD | |
| **After 24hrs** | 1.764 | ±0.062 | 2.414 | ±0.316 | 0.009* |
| **After 48hrs** | 0.166 | ±0.021 | 1.626 | ±0.207 | 0.000* |
| ***p-value*** | 0.000* | | 0.012* | | |

*; significant (p<0.05)

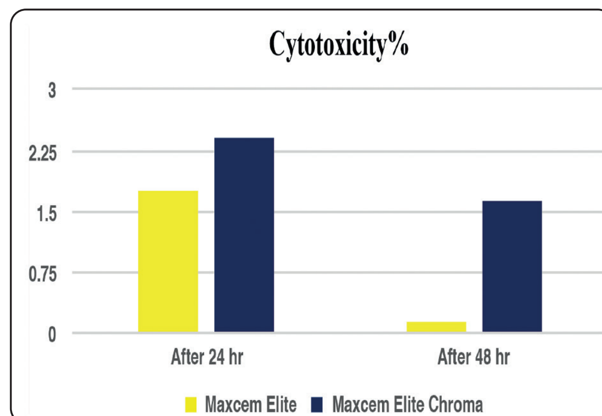


Fig. (2) Bar chart shows color change (ΔE) values of Group I and Group II

## DISCUSSION

Agreeing with the null hypothesis no statistically significant difference between control group I and II where (p=0.790). with control II (Maxcem Elite™ Chroma) showed a higher **Δ**E (3.08) than Group I (Maxcem Elite™) (2.98).

Disagreeing with the null hypothesis there was statistically significant difference between thermocycled group I and II where (p=0.014). there was increased in **Δ**E value of Group I (Maxcem Elite™) (3.46) and decreased **Δ**E value of Group II (Maxcem Elite™ Chroma) (1.65) where Group I (Maxcem Elite™) showed a higher **Δ**E than Group II (Maxcem Elite™ Chroma).

The result of Group I (Maxcem Elite™) showed that with thermal aging, ΔE value were increased (3.46) and the value of ΔE considered clinically unacceptable (ΔE >3.3), that attributed to temperature and humidity cause oxidation of tertiary amines used in self-part of dual cured resins, which are necessary for initiating polymerization but may cause color changes in cements during aging, as more likely to oxidize in line with **Pissaia et al.,2015**[16], **Haralur et al.,2017**[17], **Prieto et al.,2018**[18], **Alkurt et al.,2018**[19], **Atay et al.,2019**[20].

However, the majority of studies consider ΔE≤3.3 to be clinically acceptable. According

to another study, 2 is the gold standard for ΔE. In general, ΔE > 3.3 is clinically unsatisfactory, the human eye cannot detect ΔE values below 1, and values between 1 and 3.3 may be considered clinically acceptable. **Hoorizad et al.,2021**[21]

Result might be attributed to amine-based co-initiators found in dual-cure resin cements, that made it chemically unstable and carry out hydrolytic degradation, due to the resin monomer's capacity to absorb water; influence the mechanical properties, resulting in filler-matrix interface degradation and material discoloration in agreement with **Mazzitelli et al.,2023**[9]

The result of Group II (Maxcem Elite™ chroma) showed that; there was decreased **Δ**E value after thermocycling (1.65) might be attributed to the smaller size of filler of the Maxcem Elite™ chroma as; the amount of water absorbed by the composites matrix decreases with the small filler. This produces the highest color stability outcomes. This is because the presence of smaller particles gives rise to a more homogenous structure than the polymeric matrix produced by larger particle in agreement with **Yang et al.,2022**[22]

The results are not in line with the results of a study carried out by **Alghazzawi et al.,2024**[23] were showed that high concentrations of monomers are released into aquatic environments by TEGDMA-based resins, which eventually cause discoloration. Increased camphorquinone (CQ) in TEGDMA formulations may be the cause of a resin's gradually become yellowish.

Disagreeing with the null hypothesis there was statistically significant difference between Groups I and II, the data revealed that; at 24 hours: A significantly higher value was recorded in Group II (Maxcem Elite™ Chroma) (2.414%), in comparison to Group I (Maxcem Elite™) (1.764%) where (p=0.009). At 48 hours: A significantly higher value was recorded in Group II (Maxcem Elite™ Chroma) (1.626%), in comparison to Group I (Maxcem Elite™) (0.166%) where (p=0.000). levels of cytotoxicity were decreased over time.

The results indicated that the cytotoxicity of the investigated cements varied and eventually declined, with Maxcem Elite™ Chroma being more cytotoxic cement. This can be explained by the cements' distinct compositions, which are mostly connected to the nature of the matrix monomers. TEGDMA that present in the Maxcem Elite™ Chroma present severe cytotoxicity. TEGDMA released from Maxcem Elite™ Chroma increased over time (24 and 48 h), reduced levels of glutathione, cause oxidative stress stimulated ROS, decreased cell viability in fibroblasts and induce cytotoxicity in contrast to fibroblasts that were not subjected. This observation consistent with **Tumscitz et al.,2017**[24] and **Moralez et al.,2023**[25]

Cements show the highest degree of cytotoxicity within the first twenty-four hours, according to ISO 7405. In order to determine the relationship between the amount of cytotoxicity and the time, the cytotoxicity of cements was assessed after 24 and 48 hours in the current investigation. The findings indicated that the cytotoxicity reduced with time, which might be related to the self-neutralizing mechanism of self-adhesive cements. This mechanism hindered the release of unreacted monomers by preventing additional component hydrolysis in line with **Oguz et al.,2020**[26] and **Jain et al.,2024**[27].

The outcomes conflict with the findings of a study carried out by **Klein-Júnior et al.,2018**[28] which showed that according to the 7-day analysis, cement cytotoxicity increased over time while cell viability decreased. This was explained by the residual monomers that are released when monomers are transformed into polymers. However, uncured resin cement debris—which comprises monomers, degradation products, initiators, activators, and stabilizers—is what causes cellular cytotoxicity.

Regarding the effect of time, **Şişmanoğlu et al.,2020**[29] found that, in contrast, cements' level

of cytotoxicity has grown with time. Possible explanation might be extended times for the extraction procedure and **Kashi et al.,2022[10]** claimed that the cytotoxicity of each type of cement was unaffected by time. This might be related to there were three different kinds of interactions that occurred between the monomers in the cement formulation: synergistic effects, which increase the effects of each monomer, additional effects, which added to the effects of each monomer and antagonist effects, which decrease the effects of each other. The majority of the antagonist interaction takes place in the first 24 to 48 hours. Additive and synergistic responses often occur after 48 hours.

## CONCLUSIONS

Within the limitations of this study, these could be concluded:

1- There was no difference in ($\Delta$E) between control Maxcem Elite™ and Maxcem Elite™ Chroma.

2- Maxcem Elite Chroma™ more color stable than Maxcem Elite™ after thermocycling. (acceptable $\Delta$E=1.65)

3- Maxcem Elite Chroma™ more cytotoxic than Maxcem Elite™.

4- Cytotoxicity was improved by time.

## RECOMMENDATIONS

Further investigations are recommended to evaluate cytotoxicity over a period more than 48h, translucency and water sorption and solubility of the cement.

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