

Storing Tooth Segments for Optimal Esthetics

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Objective: A fractured whole crown segment can be reattached to its remnant; crowns from extracted teeth may be used as pontics in splinting techniques. We aimed to evaluate the effect of different storage solutions on tooth segment optical properties after different durations. **Study design:** Sixty central incisor crowns were divided into 6 groups ($n = 10$); Group 1 was kept dry; Groups 2, 3, 4, 5, and 6 were placed in an isotonic solution, water, milk, saliva, and casein-phosphopeptide–amorphous-calcium-phosphate (CPP–ACP), respectively, for 30 min, 12 h, 1 day, 1 week, and 3 weeks. Color values were measured using a colorimeter. Data were analyzed with Kruskal–Wallis tests, Mann–Whitney U-tests, and Friedman Wilcoxon tests with Bonferroni stepwise corrections ($p < 0.05$). **Results:** ΔE^* values varied from 0.3 to 15.3 over the 3 week period. Group 1 demonstrated the greatest color changes over all durations; Group 6 exhibited the least. L^* , a^* , b^* , and ΔE^* values varied between time periods in all groups; the differences were significant ($p < 0.01$), except for L^* and ΔE^* values in Group 2 and a^* values in Group 6 ($p > 0.01$). Comparing ΔE^* values, Group 6 was significantly different from the other groups for all durations ($p < 0.01$), except Group 4. **Conclusions:** A CPP–ACP complex solution seems a good choice for tooth fragment storage. Milk and saliva solutions may cause perceptible color changes if tooth fragments are stored for 3 weeks before use.

Key words: fractured tooth, optical properties, storage conditions

INTRODUCTION

Dental trauma is one of the most frequent injuries in daily life, and dental treatment approaches for fractured teeth are an important component of clinical dentistry^{1,2}. According to clinical research, 25% of individuals between 6 and 50 years of age suffer from a tooth injury, usually involving the maxillary incisors^{3–6}. The most popular treatment methods for fractured teeth involve reattaching them to their original remnants^{1–4}. This technique has

several advantages over other techniques because the morphology of the tooth is similar to its original form after treatment. Additionally, the amount of chair time required for a reattachment is less than that required for other restorations. It is also a reliable and low-cost method, allows for the maintenance of incisal function in the dental structure, and results in minimal tooth loss. However, all methods present some challenges to clinicians^{1–3}.

A multi-center clinical study⁶ evaluated the long-term survival of fragment bonding in the treatment of fractured crowns and indicated that tooth reattachment is a realistic alternative. Reattachment procedures may include adhesive techniques with or without various kinds of post materials^{7–13}. Regardless of the reattachment procedure selected by the dentist, it usually involves the storage and preparation of a fragment prior to its reattachment, and these procedures are important determinants of the overall clinical outcome. The results of several studies have shown that fragment discoloration results from the dehydration of dentin in the fragment and decreases the bonding strength between the tooth remnant and fragment^{9,10}. Accordingly, it is generally recommended that the fragment be kept moist until its reattachment to prevent the occurrence of such problems^{9–11}. Regarding the current literature, some of the tested storage media for avulsed teeth have included: tap water, saline, saliva, and milk¹⁴. Casein phosphopeptide–amorphous calcium phosphate (CPP–ACP) complexes are produced by the tryptic digestion of the milk protein casein by aggregation with calcium and phosphate ions and purification by ultrafiltration. The ability of CPP–ACP to localize amorphous calcium phosphate on

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the tooth surface helps to maintain a state of super-saturation with respect to tooth minerals¹⁵. CPP-ACP complexes have been shown to prevent demineralization and to promote remineralization of the enamel subsurface. A damaged enamel surface is also recommended to be recovered after bleaching for a long-lasting bleaching effect. Previous studies have reported that fluoride or CPP-ACP recovered a damaged enamel surface and prevented staining¹⁶⁻¹⁸. No previous studies have been performed to determine the possibility of using a CPP-ACP complex as a storage medium for fractured teeth.

It was¹⁹ previously reported that when a fractured tooth segment is dehydrated prior to treatment, the color difference between the crown fragment and fractured tooth was remarkable; the crown fragment was whiter than the fractured tooth because of the effects of dehydration. Before reattaching the fractured tooth segment, some clinical pretreatments such as a root canal treatment or gingivectomy might be necessary, and these treatments may take time. Even if stored in media, the optical properties of fractured tooth parts may change over different durations. In a conservative approach for restoring a single edentulous space, some research has recommend splinting the extracted tooth to adjacent teeth with the aid of grooves and fiber-reinforced composite²⁰. Therefore, in some cases, the crown parts of extracted teeth might be used as pontics after storage in a solution^{20,21}. Even though there are reattachment and splinting techniques, if the fractured portion has a color mismatch with the existing part of the tooth or adjacent teeth in the mouth, then even the adhesive success of the treatment will be debatable. However, searching the literature produced no scientific knowledge about how optical properties change during this period, or the most preferable storage solution for enhanced optical properties.

The aim of the study was to investigate the optical properties of tooth segments after storage in different solutions for 30 min, 6 h, 12 h, 1 day, 1 week, and 3 weeks. The hypotheses of the present study were that the optical properties of tooth segments would be affected by the type of storage solution and the storage duration.

MATERIALS AND METHOD

A total of 60 central incisors recently extracted for periodontal reasons were selected for the present study (Izmir Katip Celebi University Ethical Committee approved this study - number: 183). Teeth with any visible caries, cracks, or hypoplastic defects were excluded. All teeth were gently polished with a rubber cup and polishing paste under running water by 1 operator for 2 min to remove any residue or staining. Then, the crowns of the teeth were sectioned 2 mm apically to the cemento-enamel junction using double-faced diamond discs under running water (KG Sorensen, Barueri, Brazil). The pulp tissues of the crown were removed with hand instruments, cleaned before the experimental procedures, and then divided into 6 experimental storage solution groups (n = 10). The teeth were kept dry or immersed in various solutions as follows:

Group 1: Kept dry without any storage solution (Dry)

Group 2: 0.9% isotonic saline solution (SS)

Group 3: Water

Group 4: Milk

Group 5: Saliva (Artificial saliva was prepared according to the following formula: 1 g sodium carboxymethylcellulose, 4.3 g xylitol, 0.1 g potassium chloride, 5 mg calcium chloride, 40 mg potassium phosphate, 1 mg potassium thiocyanate, and 100 g deionized water.)

Group 6: CPP-ACP (GC Tooth Mousse, GC Corporation, Tokyo, Japan) was applied to all crown surfaces for 2 min, as recommended by the manufacturer's guidelines. Then, the specimens were kept in isotonic solutions during the experimental periods.)

Before the experimental procedures, the initial color values of all specimens were recorded (T₀). Specimens were stored in solutions for 30 min (T₁), 6 h (T₂), 12 h (T₃), 1 day (T₄), 1 week (T₅), and 3 weeks (T₆). Before the measurements were taken, each specimen was rinsed with distilled water for 30 s and gently cleaned with a soft toothbrush for 2 min and then ultrasonically cleaned in distilled water for 10 min.

The color values (L*, a*, b*) of each specimen were measured with a colorimeter (Shade Eye Ex; Shofu, Japan) in a viewing booth under standard illuminant D65 and the values were recorded according to the Commission Internationale de L'Éclairage (CIE) L*a*b* system. The L*a*b* color notations of the crowns were measured 3 times consecutively before the mean of the 3 readings was calculated to obtain the initial color of the specimen. L* (the lightness, brightness, or value) corresponded to the L* of the CIE L*a*b* system and represented the lightness/darkness of the color; a* was the measure of redness (positive) or greenness (negative); and b* was the measure of yellowness (positive) or blueness (negative). The CIE measurements enabled an evaluation of the degree of perceptible color change (ΔE*) based on the same 3 coordinates, L*, a*, and b*^{22,23}. The CIE color differences were calculated using the following equation between T₀ and T₁, T₂, T₃, T₄, T₅, and T₆.

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

Statistical Analysis

The L*, a*, b*, and ΔE* values were analyzed with Kruskal-Wallis tests and Mann-Whitney U-tests with Bonferroni stepwise corrections. A Friedman Wilcoxon test with a Bonferroni stepwise correction was also used to evaluate the differences in L*, a*, b*, and ΔE* values over time. The data analyses were evaluated at a significance level of p < 0.05 for all individual tests.

RESULTS

The means and standard deviations of the L*, a*, and b* coordinates before and after the experimental procedures are presented in Table 1, Table 2, Table 3, respectively. Table 4 shows the crown color changes (ΔE* values) after the experimental procedures.

Table 1 shows the L* values of the groups regarding to each tested period. In SS solution, there were no L* value changes statistically during the test periods. In dry conditions, every increasing tested period L* values are statistically different and getting higher.

Table 2 shows the a* values of the groups regarding to each tested period. In dry condition, there is no a* value changes statistically until 30 min but later there statistically differences between the time periods. There is no statistically difference between the a* values of the tested periods in CPP-ACP.

Table 3 shows the b* values of the groups regarding to each tested period. In saliva, there was no b* value change statistically until 12 h but later there is a slightly decrease in these values. In CPP-ACP, there is not b* value changes statistically between 12 h to 3 weeks'

Table 4 shows the ΔE* values which means the color changes of the groups until the each tested period. The color changes (ΔE* values) varied between 0.3 and 15.3 over the 3 week period. The

Table 1. L* values of the crowns before and after the experimental procedures

	L*0	L*1 (30 min)	L*2 (12 hours)	L*3 (1 day)	L*4 (1 week)	L*5 (3 weeks)
Dry	67.8±1.71	70.1±0.82	71.2±1.33	72.6±1.44	77.7±0.75	81.8±2.26
SS	71.8±1.21	69.3±0.81	68.1±1.81	68.6±0.61	69±0.51	68.4±11
Water	72.5±0.81	70.2±0.52	69±1.237	68.7±0.747	69.1±0.9578	68.6±0.9678
Milk	68.5±1.112	68.8±1.112	68.3±1.812	67.1±1.31	69.1±0.82	70.8±1.33
Saliva	70.4±0.44	69.4±114	68.7±1.912	68.6±1.8123	69±1.812	68.4±1.13
CPP-ACP	68.9±0.71	68.7±0.223	68.5±0.52	68.4±0.713	68.4±1.32	69.1±1.44

In each horizontal column (each tested time period), values with the same numbers indicate no significant difference ($p > 0.05$) between the experimental groups.

In SS solution, there were no L* value changes statistically during the test periods. In dry conditions, every tested period L* values have statistically different and getting higher.

Table 2. a* values of the crowns before and after the experimental procedures

	a*0	a*1 (30 min)	a*2 (12 hours)	a*3 (1 day)	a*4 (1 week)	a*5 (3 weeks)
Dry	0.5±0.31	0.6±0.21	1±0.32	1.5±0.33	1.8±0.24	2.4±0.45
SS	0.4±0.11	0.2±02	0.2±0.212	0.3±0.212	0±0.112	0.4±0.112
Water	-0.7±0.31	-0.3±0.21	-0.1±0.11	0.3±0.212	0±0.212	0.2±0.22
Milk	0.1±0.11	0±0.11	-0.1±0.21	-0.1±0.21	0.4±0.22	0.2±0.212
Saliva	0.4±0.113	0.3±0.21	0.2±0.11	0.3±01	0±0.12	0.5±0.13
CPP-ACP	-0.1±0.21	-0.1±0.11	-0.1±0.11	0±0.11	0±0.11	-0.1±0.11

In each horizontal column (each tested time period), values with the same numbers indicate no significant difference ($p > 0.05$) between the experimental groups.

In dry condition, there was no a* value change statistically until 30 min but later there became some differences between time periods

Table 3. b* values of the crowns before and after the experimental procedures

	b*0	b*1 (30 min)	b*2 (12 hours)	b*3 (1 day)	b*4 (1 week)	b*5 (3 weeks)
Dry	15.8±1.21	16.5±1.42	16.8±1.12	17.2±1.63	20±2.14	21.5±2.25
SS	11.6±0.81	11.5±1.31	11.4±1.21	11.2±1.11	10.8±12	10.1±0.93
Water	13.9±1.61	12.9±1.42	13±1.31	12.9±1.52	13.3±0.72	12.6±1.73
Milk	13.5±1.11	13.8±2.11	12.7±1.92	12.9±1.6123	13.1±1.812	11.7±1.63
Saliva	11.6±1.91	11.5±21	11.4±1.51	11.2±0.82	10.8±12	10.5±1.42
CPP-ACP	13.2±1.21	13.2±1.11	12.9±1.22	12.7±1.52	12.8±0.92	12.9±1.62

In each horizontal column (each tested time period), values with the same numbers indicate no significant difference ($p > 0.05$) between the experimental groups.

In saliva, there was no b* value change statistically until 12 h but later there is a slightly decrease in the values. In CPP-ACP, there is not statistically difference between 30 min to 3 weeks' b* values

Table 4. Crown color changes before and after the experimental procedures (ΔE^*)

	ΔE^*1 (30 min)	ΔE^*2 (12 hours)	ΔE^*3 (1 day)	ΔE^*4 (1 week)	ΔE^*5 (3 weeks)
Dry	2.9abe1	3.9ad2	5.2d3	11.1d4	15.3d5
SS	2.6ab1	3.1a1	3.2a1	3a1	3.7a1
Water	2.5b1	3.6acd2	4acd23	3.9ae12	4.1ac3
Milk	1.2ef12	1.1aef1	2aef12	2.4ef1	3ef2
Saliva	1.2abe13	1.7ae1	1.9ae34	1.8e13	2.3e4
CPP-ACP	0.3g1	0.6g12	0.8g23	0.8g23	0.7g3

In each column, values with the same superscript letters indicate no significant difference ($p > 0.05$). In each horizontal column (each tested time period), values with the same numbers indicate no significant difference ($p > 0.05$) between the experimental groups.

greatest color changes were exhibited by crowns that had been kept dry during all periods, while crowns kept in CPP-ACP complex solutions exhibited the least changes. The L^* , a^* , b^* , and ΔE^* values varied between the T_1 , T_2 , T_3 , and T_4 periods for all groups, and significant differences were found between them ($p < 0.01$), except for L^* and ΔE^* values of specimens kept in isotonic solutions and a^* values of specimens kept in CPP-ACP complex solutions ($p > 0.01$). Comparing ΔE^* values between the groups, the CPP-ACP complex solution group's color changes were significantly different from the other groups for all durations ($p < 0.01$), except for the specimens stored in milk. For all time periods, there were no significant differences between the isotonic solution and water or between milk and saliva ($p > 0.01$). At the end of 3 weeks, Group 1 was significantly different from Group 2 ($p = 0.001$), Group 3 ($p = 0.001$), Group 4 ($p = 0.001$), and Group 5 ($p = 0.001$); Group 1 exhibited the highest ΔE^* values. The only non-significant differences from Group 1 were with Group 2 ($p = 0.838$), Group 3 ($p = 0.398$), Group 4 ($p = 0.035$), and Group 5 ($p = 0.399$) for the T_1 period.

DISCUSSION

The hypotheses that the optical properties of tooth segments would be affected by the type of storage solutions and storage duration were validated. CPP-ACP resulted in the least color changes for fractured crowns after 30 min, 12 h, 1 day, 1 week, and 3 weeks of storage. The proposed CPP-ACP mechanism of action is that it acts as a calcium and phosphate reservoir and attaches to the tooth surfaces, thus buffering free calcium and phosphate ion activities²⁴. This provides a state of ionic super-saturation with respect to the tooth enamel, inhibiting demineralization and enhancing remineralization. Therefore, CPP-ACP might swiftly obliterate the dentinal tubules by the rapid precipitation of calcium phosphate crystals onto the surface, and also the insides of the dentinal tubules, increasing remineralization²⁴. More recently, a laboratory study has also shown a similar effect in dentin following a 5 minute application of CPP-ACP (GC Tooth Mousse) to dental plaque and tooth surfaces²⁵. From the results of the study, it seems that applying this agent also has a significant effect in stabilizing the optical properties of crowns until they are used.

Generally, the success of crown fragment reattachment is dependent upon the retrieval of the crown fragment at the time of injury. Discoloration of the crown fragment presumably results from dehydration of the underlying dentin. In 1 case²⁶, the crown fragment was kept in a dry condition for 12 days, and the authors reported that it became a matte white color and that it was difficult to mask the color disharmony between the fractured tooth and the reattached fragment with composite resin.

Dehydration may result in teeth appearing whiter by increasing enamel opacity, as light can no longer scatter from hydroxyapatite crystal to crystal²⁷. Dehydration mostly affects the main coordinate L^* , which represents lightness. This change is caused by the effects of dehydration that lead to increased opacity of the enamel. Dehydration of a test tooth results in the replacement of the water around the enamel prisms with air. A larger difference in refractive indices and greater scattering are produced at a dehydrated enamel-air interface than at an enamel-water interface²⁸. Dehydrated enamel demonstrates decreased translucency, causing greater reflection, and thus masking the underlying dentin shade, resulting in a lighter

appearance. Some studies have reported that color changes are more greatly influenced by the L^* and b^* parameters, while a^* values have been considered the axis with the lowest capacity to influence the process²⁹⁻³⁰. In the present study, the crowns that were kept dry showed higher L^* and b^* values than those kept in solutions. After 3 weeks of immersion, the groups stored in water and saliva demonstrated decreased L^* values, while the teeth kept dry and those in milk and CPP-ACP solutions became brighter. Additionally, they exhibited color changes (ΔE^* values) between 2.9 and 15.3. When the ΔE^* value of 2 colors is 0, the color difference is described as perfect; a value of 0.5 to 1.5 units is very good; 1 to 2 is good; 2 to 3.5 is clinically perceptible; and >3.5 is unacceptable³¹.

The results of this study show that if a tooth segment is kept dry for more than 30 minutes, it may result in an unacceptable color match, and the color change values increased with time. In all the experimental procedures of the present study, the color change values of the crowns were lower than the unacceptable limit during the first 30 min. Storing the crowns for up to 3 weeks in milk, saliva, or a CPP-ACP complex solution resulted in acceptable color changes. For the T_1 , T_2 , T_3 , and T_5 periods, there was no significant difference between milk and saliva, while CPP-ACP solution was significantly different, demonstrating the lowest ΔE^* values.

ΔE^* values were unacceptable for dry and water storage after 12 h and isotonic solution storage after 3 weeks; therefore, it may be speculated that the patient may find the color match between the fractured tooth segment and the existing portion of the tooth or that with adjacent teeth unacceptable. Tooth color was the primary reason for the 89.3% patient dissatisfaction rate in a survey of patient satisfaction in appearance²⁷. Therefore, the accurate measurement and matching of tooth color is integral to a successful esthetic outcome, especially for anterior restorations. It has also been reported in previous cases that the color values of teeth might eventually return to their baseline values, but at least 1 year of time was required for teeth to regain their baseline color in the mouth. However, no scientific data supported this knowledge, and having a mismatched crown color for a year might result in patient dissatisfaction. Using a CPP-ACP complex solution seems to be a good choice for fractured crown storage prior to use for optimal esthetics. Additionally, milk and artificial saliva solutions were within acceptable limits, and they could also be utilized for the storage of fractured crowns.

In further studies, CPP-ACP complex solution should be evaluated with *in vitro* studies on extracted teeth regarding color and bonding strength.

CONCLUSIONS

Within the limitations of the present study, CPP-ACP complex solution seems to be a good choice for the storage of tooth segments for durations of up to 3 weeks prior to use for optimal esthetics. There was a perceivable change in the color of teeth that were kept dry and in those placed in water after 12 h of storage. Storing tooth segments in milk or saliva solutions for up to 3 weeks also resulted in perceptible color changes. The tooth segment became lighter and yellowish when kept dry, and color changes increased with time.

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