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Effect of handling, time of use and photoactivation on the contamination of dental composite resins

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ABSTRACT. This study evaluated the effect of contamination of composite resins (CRs) handled by undergraduate students during restorative procedures, varying the time (baseline, 30 days and 60 days) and experimental condition (before and after handling, contamination with saliva [positive control] and photoactivation). Eight CR tubes were randomly distributed at the dental clinic and the samples were organized into four groups: CR fragments collected before (GB) and after (GA) the restorative procedure; CR fragments contaminated with saliva (GS) and photoactivated (GP) both collected after the procedure. These 4 groups were evaluated in 3 different times: baseline (after sealing), 30 days and 60 days of use of the CR. Samples that had positive turbidity in Brain Heart Infusion (BHI) broth were sown in BHI and Sabouraud Dextrose (SB) agars for subsequent counting of Colony Forming Units (CFU mL^{-1}). The results showed that the handling was responsible for increasing contamination ($p < 0.05$) at the baseline (GB [n = 0] and GA [n = 3]), as well as after 30 (GB [n = 1] and GA [n = 6]) and 60 (GB [n = 1] and GA [n = 5]) days of use. Photoactivation was responsible for the reduction for microorganisms in T0 and T60. Additionally, the time use and conservation did not influence the contamination of CRs. Handling was responsible for the increase of contamination of CR, the photoactivation seems to reduce the number of viable microorganisms and the time of use seems not to potentiate the effect of tube contamination.

Keywords: composite resin; handling; contamination; photoactivation.

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Introduction

Composite resins (CRs) are widely used materials in dentistry and can be a source of cross-contamination if manipulation and disinfection protocols are not used correctly. It is a non-sterilizable material, which does not have a specific standard for disinfection or universal measures for its handling (Aleixo, Queiroz, Custódio, & Moura, 2010; Almeida et al., 2010; Ferraz, Rocha, Rocha, Martins, & Jacques, 2010; Werle, Santos, & Dotto, 2012; Pinheiro et al., 2016). Another aggravating factor concerns the technique recommended in the manufacture of CR restorations, which consists of the homogenization of the CR with the fingertips and subsequent gradual application of small portions within the cavity preparations. In this context, the insertion spatula is taken several times from the CR tube to the cavity preparation to be restored. All of this can lead to contamination and cross-infection, due to the use of the same glove and a single spatula during the procedure (Werle et al., 2012; Martins et al., 2015; Pauletti, Girotto, Leite, & Mario, 2017).

The professionals' awareness in relation to biosafety when handling CRs is essential to avoid any type of contamination. Thus, the tubes need to be disinfected always before, during and after use to reduce the spread of microorganisms and clean the spatula after each insertion by means of chemical agents, in order to reduce the chances of potential cross-contamination (Batista, Gomes, Freitas, & Alvarez-Leite, 2013).

Some studies in the literature have found bacterial contamination after handling by academics who perform intraoral procedures, both in the CR masses and outside the tubes that condition this material (Aleixo et al., 2010; Werle et al., 2012). Other studies show that handling of CR with fingertips introduces organic and inorganic impurities (Sheikh, Heymann, Swift, Ziemiecki, & Ritter, 2010; Oskoee et al., 2012) that leads to the formation of porosities within the material (Sarrett, 2005) and interferes with mechanical properties (Heck, Kina, Vieira, & Andrada, 2010; Sheikh et al., 2010; Oskoee et al., 2012; Martins et al., 2015).

Additionally, Heck et al. (2010) found that contamination with chemical components (C, O, Ba, Yb, Al, Si, Mg, Ni, Ca) could decrease the physical properties of CRs and compromise the longevity of restorations. After contamination, microorganisms can remain viable, even after polymerization of CR (Almeida et al., 2010; Ferraz et al., 2010), although other studies have demonstrated the effectiveness of light in modulating microorganisms (Donnelly, McCarron, Tunney, & Woolfson, 2007; Pauletti et al., 2017).

Ultimately, there is no well-established protocol for cleaning gloves in the literature that can reduce the potential for contamination in procedures involving restorative dentistry (Martins et al., 2015). Based on this assumption, it is essential that the professional knows the right way to manipulate the material and its applications in order to have success and longevity of the restorative procedures.

Thus, the aim of this study was to assess contamination in CRs handled by students during restorative procedures, varying the time (baseline, 30 days and 60 days) and experimental condition (before and after handling, saliva contamination and photoactivation). The null hypotheses were that no significant differences would be found among groups in contamination according to: (1) handling, (2) time of use and (3) photoactivation.

Material and methods

This prospective study assessed the potential of contamination of CRs used for a period of 60 days. Clinical phase consisted of the collection of the handled CR specimens by the students and the laboratory phase was referring to microbiological analysis. The study was approved by the institutional Ethics Committee (protocol number: 2642508) and the students, who agreed to participate in the research, signed the consent form.

Collection of samples

Eight CR tubes (Opallis, DA3, FGM Dental Products, Joinville, SC, Brazil) were initially identified by numerical marking on the tube plunger with a discretely rotating drill bit to allow a blind study. Later, the CR tubes were randomly distributed to be used in patient care.

During collection, fragments of 2 mm thick of CR were collected directly from the tubes using a sterile plastic spatula and placed in test tubes containing 3 mL of sterile Brain Heart Infusion Broth (BHI, Kasvi, São José dos Pinhais, PR, Brazil). All collection was performed within the fire safety area to avoid contamination.

Study groups

The study groups included CR specimens according to the time they were collected for analysis, as shown below. Additionally, the groups were evaluated at three different times: baseline (after opening the seal), 30 days, and 60 days of composite resin use. The CR tubes were used daily at the dental clinics for the 60 days of the research and stored in the refrigerator when they were not being used, according to the manufacturer's instructions.

- Group B (GB): CR fragment obtained before the restorative procedure.
- Group A (GA): CR fragment obtained after the restorative procedure.
- Group P (GP): Photoactivated CR fragment obtained after the restorative procedure.
- Group S (GS): CR fragment obtained after saliva contamination (positive control).

Microbiological evaluation

After 48 hours of incubation of the CR specimens in BHI broth, the qualitative assessment of contamination was performed according to the methodology described by Batista et al. (2013), based on the presence or absence of turbidity in the tubes with the BHI broth. Those culture media that remained clear and translucent for 48 hours were considered non-contamination. In cases where there was growth of microorganisms in the tubes (presence of turbidity), contamination levels were evaluated by sowing in specific solid media (Figure 1).

The tubes with positive turbidity were agitated with Vortex agitator (Phoenix® - AP56, Ind. E Com. de Equip. Araraquara, SP, Brazil) and 1.0 mL aliquot of each tube was sown in both Petri dishes (90 x 16 mm) containing Agar BHI (Kasvi, São José dos Pinhais, PR, Brazil) for the cultivation of bacteria, yeasts and moulds and Sabouraud Dextrose Agar (SD, Kasvi, São José dos Pinhais, PR, Brazil) for fungi, moulds and yeasts (Pauletti et al., 2017). Incubation was performed at 37°C, at 75 rpm for 48 hours (Badaró et al., 2020). After this period, the colony forming units (CFU mL^{-1}) were quantified.

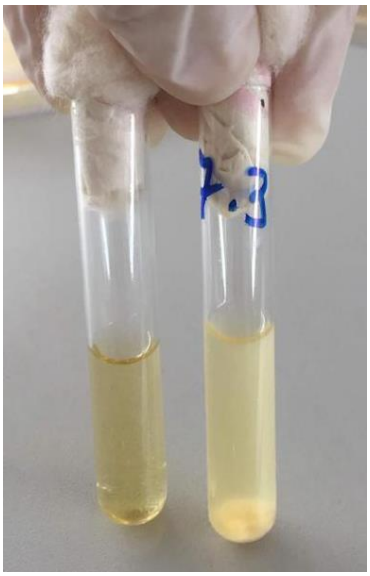

Figure 1. Representative image of the qualitative assessment of contamination. Culture medium with turbidity (right) and clear/translucent (left), showing contamination and non-contamination, respectively.

After 30 and 60 days using the CR tubes, the microbiological evaluation was again performed with new CR fragments obtained from the same tubes in use, according to the protocol already described. The analysis at different times aimed to determine the effect of the time of use on the contamination.

Statistical analysis

The data were initially described based on the quantification of CFUs in each sample. The quantitative variable 'contamination' was recategorized ordinally at 0 CFU mL^{-1} (without contamination), 1-300 CFU mL^{-1} (mild/moderate contamination) and +300 CFU mL^{-1} (intense contamination) to allow statistical analysis. Kruskal-Wallis (among groups) and Freedman (within groups) tests followed by the DSCF and Durbin-Conover tests respectively for multiple comparisons were performed. All analyses were performed with the Jamovi software (version 1.6.13) using a significance level of 5% ($\alpha = 0.05$).

Results

Table 1 shows the results of the visual turbidity assessment of CR tubes used in the survey. Table 2 explains the number of microorganisms using the BHI and SD Agar culture media. Table 3 shows the results of the statistical analysis according to time and experimental condition.

The results showed that handling with fingertips (GB vs. GA) was responsible for increased contamination at all times evaluated ($p < 0.05$), including baseline (GB [n = 0]; GA [n = 3]), after 30 days (GB [n = 1]; GA [n = 6]) and 60 days (GB [n = 1]; GA [n = 5]) of use and conservation of CR. Contamination levels of photoactivation group (GP) in T0 and T60 were similar to GB ($p > 0.05$), indicating some effect of light on the reduction of viable microorganisms. Additionally, the use and conservation time of the CRs does not seem to have influenced the contamination, because before manipulation in the two times evaluated (T30 and T60), only one CR was contaminated.

Table 1. Turbidity evaluation of samples grown in BHI Broth.

Resin	T0				T30				T60			
tube	GB	GA	GP	GS	GB	GA	GP	GS	GB	GA	GP	GS
1	-	-	-	+	-	+	+	+	-	-	-	+
2	-	+	-	+	+	+	+	+	-	-	-	+
3	-	+	+	+	-	-	-	+	-	+	+	+
4	-	-	-	+	-	+	+	+	-	-	+	+
5	-	+	-	+	-	-	-	+	+	+	+	+
6	-	-	-	+	-	+	+	+	-	+	+	+
7	-	-	-	+	-	+	-	+	-	+	-	+
8	-	-	-	+	-	+	+	+	-	+	+	+

no turbidity/non-contamination (-), with turbidity/contamination (+).

Table 2. Number of Colony Forming Units (CFU/mL) in Brain Heart Infusion (BHI) and Sabouraud Dextrose (SD) culture media according to time (Baseline [T0], after 30 [T30] and 60 [T60] days of RC use) and experimental condition (before [GB] and after [GA] of handling, photoactivation [GP] and contaminated with saliva [GS]) of analysis.

Resin Tube	Culture media	T0				T30				T60			
		GB	GA	GP	GS	GB	GA	GP	GS	GB	GA	GP	GS
1	BHI	-	-	-	>300	-	>300	>300*	>300	-	-	-	>300
	SD	-	-	-	0	-	>300	34	16	-	-	-	>300
2	BHI	-	>300	-	>300	>300	>300*	>300	>300	-	-	-	>300
	SD	-	0	-	0	0	12	0	>300	-	-	-	1
3	BHI	-	>300	>300	>300	-	-	-	>300	-	1	9	>300
	SD	-	0	0	0	-	-	-	61	-	0	12	>300
4	BHI	-	-	-	>300	-	>300*	>300	>300	-	-	>300*	>300
	SD	-	-	-	0	-	0	>300	>300	-	-	4	>300
5	BHI	-	100	-	>300	-	-	-	61	>300	>300	>300*	>300
	SD	-	0	-	0	-	-	-	0	>300*	>300	251	>300
6	BHI	-	-	-	>300	-	>300*	>300	>300	-	>300*	1	>300*
	SD	-	-	-	0	-	0	>300	21	-	34	1	>300
7	BHI	-	-	-	>300	-	>300*	-	>300	-	5	-	>300*
	SD	-	-	-	0	-	0	-	>300	-	1	-	>300
8	BHI	-	-	-	>300	-	>300	2	>300	-	>300*	1	>300
	SD	-	-	-	0	-	>300	0	2	-	12	-	>300

*Two different species of microorganisms observed.

Table 3. Absolute frequency (n) of contaminated resins after ordinal categorization in 0 $CFUmL^{-1}$, 1-300 $CFUmL^{-1}$ e +300 $CFUmL^{-1}$, according to time and experimental condition.

Group	T0		T30		T60		p[a]
GB	0 CFU: 8 0-300 CFU: 0 300 CFU: 0	Aa	0 CFU: 7 0-300 CFU: 0 >300 CFU: 1	Aa	0 CFU: 7 0-300 CFU: 0 >300 CFU: 1	Aa	0.368
GA	0 CFU: 5 0-300 CFU: 1 300 CFU: 2	Ba	0 CFU: 2 0-300 CFU: 0 300 CFU: 6	Ba	0 CFU: 3 0-300 CFU: 2 300 CFU: 3	Ba	0.157
GP	0 CFU: 7 0-300 CFU: 0 300 CFU: 1	ABa	0 CFU: 3 0-300 CFU: 1 300 CFU: 4	Bb	0 CFU: 3 0-300 CFU: 3 300 CFU: 2	ABb	0.034*
GS	0 CFU: 0 0-300 CFU: 0 300 CFU: 8	Ca	0 CFU: 0 0-300 CFU: 1 300 CFU: 7	Ca	0 CFU: 0 0-300 CFU: 0 300 CFU: 8	Ca	0.135
p[b]	<0.001*		0.007*		0.002*		

* Statistically significant difference. a Freedman test / Durbin-Conover test (pairwise comparisons). b Kruskal-Wallis test / DSCF test (pairwise comparisons).

Discussion

Dentists, assistants and patients are significantly exposed to bacteria, viruses and fungi responsible for the transmission of infectious diseases, since several potential sources of infection (saliva, blood and nasal secretions) are part of the professional's routine (Aleixo et al., 2010; Pinheiro et al., 2016). Contaminated hands are the biggest vehicles for contamination of surfaces (Molinari, 2003).

Some studies in the literature have found bacterial contamination both in the CR masses and in the external part of the tubes containing this material (Aleixo et al., 2010; Werle et al., 2012; Batista et al., 2013). The handling of CR with fingertips before their insertion in the cavities represents a routine procedure within the protocols of restorative dentistry. Such process facilitates the adaptation of the restorative material in the cavity, avoiding the formation of air bubbles that can compromise the resistance of the restoration. However, the results of this study showed that handling of CR with fingertips was responsible for the considerable increase in the contamination of CR fragments at all times evaluated. These findings, therefore, negate the first null hypothesis of the study.

Similar results were also observed in the literature and the authors emphasized the need for protocols to maintain the aseptic chain, such as performing absolute isolation of the operative field and disinfecting the spatulas always after the insertion of each increment of the CR (Aleixo et al., 2010; Werle et al., 2012; Martins et al., 2015; Pauletti et al., 2017). In addition, disinfection with 70% alcohol significantly decreases the

amount of microorganisms in the procedure gloves, more effectively reducing the risk of cross-infection (Aleixo et al., 2010).

Almeida et al. (2010) evaluated 55 CR tubes and found a high level of contamination, reaching 80% (n = 44) of the evaluated sample. Werle et al. (2012) observed contamination in 51% of CRs (n = 51), as well as a very strong correlation between the number of students in the clinics and the percentage of contamination (Clinic 1 [252 students] responsible for 72.5% of the contamination, while Clinic 2 [78 students] by 7.9% and Clinic 3 [84 students] for 19.6%). Disagreeing with these findings, Andrade, Silva Filho, Zimath and Galiassi (2017) evaluated 144 CR samples distributed in undergraduate dental clinics and found contamination of only 3, confirming that the use of correct biosafety protocols is effective in preventing cross-infection.

Another aggravating factor is that handling can contaminate the CR with impurities or other chemical components and significantly alter the surface microhardness and resistance to flexion as demonstrated by Heck et al. (2010). Additionally, an in vitro study evaluated the effect of handling of CRs with gloves contaminated with saliva and latex powder and found that saliva was responsible for a reduction in the mechanical properties of the CR, suggesting the need to clean the spatula and glove with alcohol 70% to prevent the negative effects of contamination Martins et al. (2015).

The objective of this research was also to verify the effect of photoactivation in the contamination levels of CR and a reduction in the number of microorganisms was found especially in baseline and T60, becoming statistically similar to GB. These results partially deny the second null hypothesis of the study. Similarly, a study evaluated the contamination of CRs used in dental clinics and the effect of photoactivation on the level of contamination. The authors found significantly less contamination in the photoactivated CR group, suggesting that light produces reactive oxygen species that act directly on microbial viability (Pauletti et al., 2017). The polymerization process of CRs requires the activation of initiator molecules (usually camphorquinone) by emitting light at a certain wavelength. The photoinitiator in the excited state reacts with a co-initiator, producing free radicals (Popovic & Dokic, 2009).

Regarding the time of use and conditioning of CRs over time (30 and 60 days), the results of this research showed that contamination does not seem to have a disseminating effect, thus confirming the third null hypothesis of the study. Most studies in the literature assessing the contamination of CRs are transversal, which makes it difficult to establish conclusions that justify this low potential for contamination of the resin mass in the long term. However, it is suggested that CRs may have certain components in their composition that prevent the spread of microorganisms in the CR mass, leaving only the surface contaminated by direct contact with the handling spatula. Another reason that can justify no contamination along the CR mass is the cytotoxic action on the monomers present in its constitution (Ferraz et al., 2010).

There is no well-established protocol in the literature for cleaning gloves that can reduce the potential for contamination in procedures involving restorative dentistry (Martins et al., 2015). Based on this assumption, it is essential that the student or professional be familiar with the right way to manipulate the material and its applications so that the restorative procedure is successful. In addition, establishing clinical protocols that aim to control infection to be performed correctly since graduation is required (Pimentel, Batista Filho, Santos, & Rosa, 2012).

Finally, it becomes relevant to assess the contamination of CRs that are used for a long time in the dental clinic to be sure of the risk of infection that patients and professionals are subjected. Future studies evaluating contamination of other surfaces and identification of microorganisms may also provide important subsidies for the establishment of conduct for clinical day-to-day activities.

Conclusion

Handling with fingertips was responsible for increasing the contamination of CRs in the undergraduate dental clinic showing the need to establish a biosafety protocol for the correct handling of these materials. Photoactivation seems to reduce the number of viable microorganisms and the time of use does not seem to enhance the contamination effect of the tube.

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