Microbial contamination of resin composites inside their dispensers: An increased risk of cross-infection?

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Short title: Microbial contamination of resin composite materials

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ABSTRACT

Objectives: To evaluate the effects of microorganisms' contamination inside the dispensing syringes of different types of resin-based composites (RBCs).

Methods: This study encompassed two sections. First, an anonymous electronic survey was submitted *via* Google forms to Italian dentists to acquire information about composite handling during clinical procedures. Then, a bench test was performed on nanohybrid RBCs differing in matrix chemistry and fillers [Filtek™ Supreme XTE (3M™); Venus Pearl (Kulzer GmbH); Admira Fusion x-tra (Voco)] to evaluate the microbial viability on their surfaces with/out photocuring. Uncured RBCs were exposed to standardized inocula of *Streptococcus Mutans, Candida Albicans, Lactobacillus Rhamnosus,* or mixt plaque in an *in vitro* model reproducing clinical restorative procedures. Half of the RBC specimens were cured after exposure. Microbial viability was assessed using an MTT-based test. Statistical analysis included three-way ANOVA and Tukey's tests ($p<0.05$).

Results: Among 300 dentists completing the survey, the majority declared to use the spatula to carry the RBCs from the syringe to the dental cavity (50% same spatula; 35% two spatulas). However, 80% of respondents had personal feelings that using one spatula could be a source of cross-contamination. *In vitro* results using one spatula showed microbial contamination of all RBCs after one hour of storage. The contamination levels depended on the used strain and RBC type ($p<0.0001$), but photocuring did not reduce contamination ($p=0.2992$).

Conclusions: Microbial species' viability on uncured RBCs and after photocuring shows the existence of a considerable risk of cross-infection. Clinical procedures in Restorative Dentistry need to acknowledge and to reduce such risk during RBCs handling.

Clinical significance: Dentists must be aware of the possibility of cross-infection during restorative procedures, especially when the same spatula is repeatedly used for placing RBC in the cavity.

INTRODUCTION

Cross-contamination control in the dental setting is an ever-current and relevant topic for healthcare workers and patients' biosafety. The infection control issue has constantly aroused the interest of the international scientific community, and in the aftermath of the Covid-19 pandemic it has increasingly taken over the shape of a global problem [1,2]. Several hundred microbial species can be found in saliva and constitute oral biofilms. Indeed, saliva and detached amounts of dental plaque can be considered as possible sources of cross-infection. Considering the oral environment where dentists operate, close attention to biosecurity and cross-infection prevention is crucial in dentistry. Negligence or operative difficulties can interrupt the well-established preventive measures usually adopted in dental practice. These circumstances can expose both dental healthcare providers (DHCP) and their patients to the risk of cross-contamination [3] due to the possibility for operators and patients to come into contact, directly or indirectly, with saliva, blood, and respiratory secretions of other patients [4,5].

Restorative treatments are the most represented procedures performed in every dental clinic worldwide. In this field, an increase in patients' esthetic requests, compliance with the principles of minimally invasive dentistry, and advances in dental materials science contributed to making resin composites the gold standard in anterior and posterior restorations [6,7]. The unceasing improvement in adhesive methodologies has expanded the use of resin-based composite (RBC) materials, even replacing indirect restorations in wide cavities with cuspal involvement [8,9]. Extensive cavity reconstructions, therefore, require sequential layering of RBCs.

Virtually all manufacturers provide dental RBCs with two delivery systems, screw-type syringes, and disposable, single-use compules. The latter contains a significantly lower amount of material and is supposed to be used on a single patient, virtually eliminating this cross-contamination pathway. However, such a system involves considerable disadvantages in terms of higher costs and disposal/reprocessing of material and plastics. Furthermore, information from dental vendors indicates that compules are mainly used in the US, while in the European market, syringes are predominant.

Considering the use of RBC syringes, relatively low amounts of material have to be repeatedly picked up from the syringe. In most cases, small chunks of material are picked up with a spatula until the restorations are completed. The use of the same spatula to carry the material from the composite package into the patient's mouth leads to the contamination of the RBC inside its package, raising concerns about the material as a possible source of cross-contamination. From this point of view the oral environment of the patient represents a huge source of microorganisms, as well as the cavity

itself. Even after proper isolation (use of dental dam), residual contamination is not eliminated by the drilling and cleaning procedures. Some of the most common species found are *Streptococcus mutans*, Lactobacilli spp., and *Candida albicans*.

Very few studies have dealt with this problem, and no evidence-based guidelines exist. Previous preliminary data indicated RBC photopolymerization as a possible way to significantly decrease the bacterial load of a contaminated RBC [10]. It is also well-known that RBCs differ in their compositions, such as the type of photoinitiator and the resin blend, which could differently influence microbial viability.

Therefore, this study investigated resin composite handling during common dental clinical procedures and evaluated the potential for cross-contamination by composite packaging *in vitro*. For this purpose, a first observational study was performed in a survey to explore RBC materials' handling during their routine restorative treatments. After that, an *in vitro* test aimed to assess microorganisms' survival inside the packaging of different resin-based composite materials while in contact with the uncured material. The first null hypothesis was that no viable microorganism could be detected on the uncured materials as evidence for an absence of risk for cross-contamination from such restorative procedures. The second null hypothesis was that no viable microorganism could be detected on the cured materials.

MATERIALS AND METHODS

Part 1. Survey

For the first part of the study, an anonymous electronic survey was submitted *via* Google forms (Alphabet, Mountain View, CA, USA) to Italian DHCPs. A reminder to participants was sent if DHCPs did not respond within two weeks. The survey consisted of a single-choice format, and some questions were endowed with the possibility for written comments. Participation in the survey was voluntary without providing any remuneration.

The survey was divided into four domains: professional status, restorative treatment procedure, resin composite handling, perception of the cross-infection potential of the handling procedure (Fig. 1). No information regarding the specific proprietary material was asked.

Part 2. Microbial viability

Composites

A total of four syringes for each of three different properly-stored RBC materials were used for microbiological evaluation: 1) Filtek Supreme XTE (FS; Body, 3M ESPE, St. Paul, MN, USA); 2) Venus Pearl (VP; Kulzer GmbH, Hanau, Germany); 3) Admira Fusion x-tra (AF; Voco GmbH, Cuxhaven, Germany). The shade of the RBCs was the same (A2). All RBCs were previously tested not to spontaneously react with any reagent of the biochemical test used to evaluate viable microbial biomass. Compositions and batch numbers of the materials are shown in Table 1.

Microorganisms

The culture media and reagents were obtained from Becton– Dickinson (BD Diagnostics-Difco, Franklin Lakes, NJ, USA). An artificial saliva medium (ASM) simulating the average electrolyte composition of human whole saliva was prepared from 0.1 L of 150 mM $KHCO_3$, 0.1 L of 100 mM NaCl, 0.1 L of 25 mM K_2HPO_4, 0.1 L of24 mM Na_2HPO_4, 0.1 L of 15 mM $CaCl_2$, 0.1 L of 1.5 mM $MgCl_2$ and 0.006 L of 25 mM citric acid. The volume was made up to 1 L, and pH was adjusted to 7.0 by pipetting 4 M NaOH or 4 MHCl solutions under vigorous stirring [11].

Pure suspensions of either *Streptococcus mutans* strain ATCC 35668 or *Lactobacillus rhamnosus* GG strain ATCC 53103 in brain–heart infusion broth (BHI) were obtained after overnight incubation at 37 °C in a 5% supplemented CO_2 environment. Cells were harvested by centrifugation (1.500 g at 19 °C for 5 min), washed twice with sterile ASM, and resuspended in the same medium. The cell suspensions were subsequently subjected to sonication (Sonifier model B-150; Branson, Danbury, CT, USA; operating at 7W energy output for 30 s) to disperse bacterial chains, then each suspension adjusted to 0.5 McFarland. A pure suspension of *C. albicans* strain ATCC 90028 was obtained after a 24 h incubation following the above procedure and adjusted to 0.5 McFarland in ASM. Mixed oral flora was obtained from two experimenters who refrained from oral hygiene procedures for 36 h, did not take any medication in the month previous to the experiment, and did not smoke. Samples were collected from the buccal surfaces of upper and lower molars and premolars using sterile spatulas, harvesting the cells and resuspending in ASM, then adjusting to 0.5 McFarland.

Experimental procedures

An *in vitro* procedure was set up to mimic oral contamination during restorative procedures. The working parts of sterile Heidemann spatulas (one for each tested strain and RBC) were contaminated with 100 µl of microbial suspension and immediately used to evenly distribute a fixed amount of uncured RBC along the inner surfaces of a well in 96-well, flat-bottom, tissue culture-

treated transparent plates (Corning™ 3370 Microtiter plates, Thermo Scientific Italy, Rodano, MI, Italy). This procedure left a free volume of about 100 µl inside each well.

A total of seven wells were obtained for each strain and RBC. Thus, each well simulated the tip of a composite syringe that was contaminated by the modeling instrument. The inner surfaces of additional empty wells (n=7 for each strain) were touched with the spatulas to obtain the controls. The plates containing the uncured contaminated RBCs were kept at room temperature in light-proof conditions for one hour to reproduce a clinically appropriate time interval between using the same RBC syringe on consecutive patients. After that, the residual viable biomass in each well was assessed using a biochemical colorimetric test [12].

Briefly, two starter stock solutions were prepared by dissolving, respectively, 5 mg/ml of 3-(4,5)-dimethylthiazol-2-yl-2,5- diphenyltetrazolium bromide (MTT), or 0.3 mg/ml of N-methylphenazinium methyl sulfate (PMS) in sterile phosphate-buffered saline (PBS). The solutions were stored at 2°C in light-proof vials until the day of the experiment when a measurement solution (MS) was made by mixing 1ml of MTT stock solution, 1ml of PMS stock solution, and 8ml of sterile PBS. A lysing solution (LS) was prepared by dissolving 10% v/v of sodium dodecyl sulfate and 50% v/v of dimethylformamide in distilled water.

After one hour of storage, a total of 100 µl of MS was pipetted in each well of the contaminated plates, left to react in light-proof conditions for 1 h, then 90 µl were carefully transferred from each well to new 96-well plates, where 90 µl of LS were added. After 30 min in an orbital shaker, the solution was read using a dual-wavelength spectrometer (550nm and 630nm, Genesys 10-S, Thermo Spectronic, Rochester, NY, USA), subtracting the second reading from the first one. In this way, turbidimetry increases by elution of leachates from the RBCs were prevented from influencing readings.

Additional 96-well black plates with transparent flat-bottom (Corning™ 3340) were used to repeat the experimental procedures described above, photopolymerizing the RBCs inside each well (Spectrum 800, Dentsply International Inc., York, PA, USA, 800mW/cm^2, 20 s) before pipetting the MS. A total of seven wells were obtained for each strain and RBC. The inner surfaces of additional empty wells (n=7 for each strain) were touched with the spatulas to obtain the controls. The light-curing unit had a built-in radiometer by which the light-curing power was checked every 10 specimens; no Turbo tip was used. Each well was cured by applying the tip of the light-curing unit directly to the transparent bottom of the plate. Light curing was repeated, placing the tip of the curing unit over each well opening, avoiding direct contact. A perfect correspondence of the light-curing

tip's diameter to the transparent bottom and the walls prevented the over-polymerization of neighboring wells from occurring.

Statistical analysis

All analyses were performed using statistical software (JMP 14.0, SAS Institute, Cary, NC, USA). Survey data were analyzed using a Chi-square test ($p < 0.05$).

For the microbial viability analysis, after verification of the normal distribution of the data (Shapiro-Wilk's test) and homoscedasticity (Levène's test), a three-way ANOVA was run considering the factors photocuring (yes *vs.* no), RBC (Filtek Supreme XTE; Venus Pearl; Admira Fusion x-tra), and strain (*S. mutans*, *L. rhamnosus*, *C. albicans*, mixt oral flora). Tukey's test was used to identify significant differences between groups. The significance level was set at a two-sided $p < 0.05$.

RESULTS

Survey

Of the 700 questionnaires sent, a total of 300 dentists completed the survey. Most of the DHCP answering the questionnaire were dentists with a Master's degree (38.2%), followed by general practitioners (30.2%), dentists attending a Master course in Restorative and Esthetic Dentistry (14.2%), dentists with post-graduate specialty (not specified, 6.8%) and Doctorate Students (10.6%). Most respondents reported using rubber dams during their daily restorative procedures (82%), even though nearly 12.9% of the interviewed declared to use it only occasionally and 5.1% declared they never use it. When managing the composite paste in form of screw-syringe, the preferred technique for handling the material during restorative treatments was reported to be the use of a spatula (64% against 19% of those who used alternative methods and 17% who used the spatula occasionally) (Fig. 1). Among DHCPs who preferred to use a spatula to carry the composite material from the syringe to the dental cavity, 50% used the same spatula, and 35% used different spatulas (one to take the necessary amount of material, and another to carry the material and fill the cavity). The remaining 15% of the respondents stated to pick up a single mass of composite all of once and place it on a surface (paper or plastic plate or mixing container, covered or not). In the latter case, 42.3% admitted to taking an excessive amount of material compared to that necessary to finalize the restoration. Among those who use the spatula to withdraw the composite from the syringe, 40% of them asserted that they are not in the habit of cleaning the spatula in the passage between the syringe and the dental cavity and vice versa (Fig.1). The majority of the DHCPs (80%) stated that they had at least once the concern that the repeated use of the same spatula from the composite tube to the dental cavity, and

vice versa, could be a vehicle for infection. The trend of composite handling and protocols varied among the different educational levels (Fig. 2)

Microbial viability

All tested strains maintained high viability after contamination of all tested RBCs. The photocuring process did not influence this outcome.

Taking into account ANOVA analysis, the factors "strain" and "RBC" were highly significant, as well as their interaction (all $p<0.0001$). This outcome means that the tested microorganisms showed peculiar responses (streptococci and lactobacilli exhibited increased metabolism compared to the yeast and the mixed flora model) and that, depending on their composition, RBCs influenced the viability and metabolic response of microorganisms (Figure 3). The factor "photocuring" was not significant ($p=0.1136$). A highly significant interaction among all factors ($p=0.0009$) meant that they could not be considered alone, so the post-hoc analysis was performed on an unsplit dataset (Table 2).

Considering the tested microorganisms' viability on uncured RBCs (Table 2), *S. mutans* showed significantly higher viability on AF than VP and FS ($p = 0.0013$ and $p < 0.0001$, respectively). The viability levels on AF were comparable with the control ($p = 0.7294$). *L. rhamnosus* viability levels on all tested RBCs were significantly lower than the control ($p < 0.0005$). Viability levels of *C. albicans* and mixed oral flora were similar between all RBCs and the control.

Considering the tested microorganisms' viability on cured RBCs, *S. mutans* showed significantly lower viability than the control ($p<0.0001$). No significant difference in viability levels was shown between all tested RBCs and the control in the other three microbiological models, namely *L. rhamnosus, C. albicans,* and mixed oral flora.

All cured RBCs showed comparable viability to uncured ones ($p=0.2992$).

DISCUSSION

Cross-contamination control is paramount in the dental setting, including restorative procedures. A critical point in the procedure has been identified in the RBC syringe that may come into contact, during tooth restoration procedures, with patients' saliva, blood, and respiratory secretions [1,2].

The present study was structured to reproduce clinical behavior by laboratory research and provide outcomes in the laboratory that can be used to improve clinical procedures. For this reason, the study encompassed two phases: preliminary information about DHCPs' clinical practice that can constitute potential sources of contamination in Restorative dentistry was collected. Then, the contamination potential of the most commonly used restorative procedure was studied *in* vitro. A secondary outcome of the second phase was to assess if different RBC types, or photocuring the material, influence the amount of contamination.

Resin composites are the election materials in Restorative Dentistry. Composites are available in different shades, to ensure the greatest possible naturalness of the restoration, and with different delivery modes (syringes, compules and blisters) [13-15]. The choice of how to dispense the material is usually left to the practice of the clinician, with a particular tendency in some countries to use one delivery rather than others. In Europe, and in Italy in particular, the composite in the form of a syringe is widely used. For this reason, the questionnaire of this study aimed at collecting information about the method of composite pick up from the syringes by a sample of Italian dentists. It should be mentioned that the sample interviewed in the questionnaire represents only a small part of the total number of dentists in Italy, thus placing limits of interpretation on these results. The survey found that, among DHCPs, the most used method to remove an amount of composite from its syringe is to apply a single spatula which is then used to insert the material into the cavity to be restored (Fig.1). It is noteworthy that the repeated use of the same spatula between the material's syringe and the dental cavity is at high risk of contamination of the inner or external surface of the syringe [16,17]. Even though biosafety precautions during resin composite handling cannot be neglected, very little information is still present on the possibility of cross-contamination with improper or negligent handling of the material during restorative procedures. Furthermore, according to the results obtained from the survey, clinicians commonly believe that the repeated use of the spatula to carry the RBC from the syringe to the tooth cavity can be a potential risk of cross-contamination.

The bench test results (second phase) indicated that, no matter what RBC may be used among the tested ones, all tested microbial strains and the mixed oral flora showed high viability rates, confirming the risk of cross-infection. Accordingly, the first null hypothesis was rejected. The tested materials differ in terms of composition, particle size, filler content, and photoinitiators. The type of initiator can influence the elution of leachable into the oral cavity, resulting in oral bacterial biofilm growth that can be affected in opposite ways [18]. In fact, the literature provides mixed data regarding the influence of RBC leachates and unpolymerized resin compounds on microbial viability, colonization, and proliferation. Literature data show that unpolymerized monomers can stimulate, decrease, or even be of no influence on microbial viability and growth [19-21].

This complex situation is a consequence of resin monomers providing, often at the same time depending on the concentration and pH, cytotoxic effects while acting as a source of nutrients and stimulating growth. Some monomers even express hormone-like activity [22].

For these reasons, when a microorganism comes in contact with the unpolymerized surface of an RBC, its behavior strongly depends on the resin blend. We used a microorganism with a relatively high metabolism and the capacity to degrade methacrylate surfaces (*S. mutans*) and a microorganism with a much lower metabolism, such as *C. albicans* [23-25].

The mixed oral flora is the model that most closely resembles the complexity expressed by the oral biofilm and, as confirmed by our results, has lower metabolism compared to *S. mutans* or *L. rhamnosus* monocultures. Our results show that, contrarily to the other two tested microbial models, these monocultures were affected by the RBC type, showing a significant decrease in viability when in contact with Filtek Supreme XTE (Fig. 3). Differences in the resin blend between tested RBCs can explain such behavior. The resin blend of Filtek Supreme XTE contains Bis-GMA and urethane-based dimethacrylates, and TEGDMA; Venus Pearl has a Bis-GMA-free blend while Admira Fusion x-tra is an ORMOCER hybrid system based on Bis-GMA-free and TEGDMA-free urethane resin. Our results, therefore, imply that Bis-GMA and TEGDMA containing blends reduced the viability of *S. mutans* and *L. rhamnosus*, while urethane-based blends had no, or little effect on their viability.

In the present study, the photocuring procedure did not influence the viability rates, independent of the initiator system contained, indicating that such a procedure did not help mitigate cross-contamination risks. The second null hypothesis was therefore rejected.

The latter finding contrasts with the study by Pauletti *et al* [10], who found that photocuring of the tested RBC (Opallis FMG, Joinville, Brazil) resulted in no contamination. On the contrary, microorganism contamination (*Bacillus spp.*, *Staphylococcus sp.* and *Candida sp*) was observed on the non-photocured RBC specimens [10]. Then again, the differences in the experimental setup may have been responsible for the different outcomes between the studies. It is known that the light-curing unit can develop significant heat to the irradiated target [26-28], but it is unlikely to provide a bactericidal effect at its operational envelope.

In this scenario, any measure that can reduce microbial contamination of the operative site, including the dental dam, is considered extremely useful and was demonstrated to reduce cross-contamination risk significantly [29]. Indeed, the use of a dental dam is mandatory whenever possible during restorative procedures for the patient and DHCP's biological safety [29].

Regarding the microorganisms tested in the present study, *S. mutans* was chosen as it is a well-known bacterium primarily associated with cariogenic risk. It can be found in a higher prevalence on the surfaces of resin composites than those of some other restorative materials, or natural hard tissues [30,31]. *L. rhamnosus* GG is one of the most well-known probiotic bacteria. *C. albicans* is an opportunistic microorganism that is most frequently isolated from fungal infections [32]. The adhesion capability of this yeast to restorative materials has been demonstrated in several *in vitro* studies [33-35]. These microorganisms were selected both for safety reasons and for being commonly found in the carious lesions. They show good colonization abilities and resistance capacity in the external environment. Regarding the use of mixed plaque, a full-grown oral biofilm is an ecological unit formed by several different microbial species This microbial community is known to express virulent and persistence characteristics that are superior to those of the single species, thanks to synergisms between different species and the presence of the extracellular matrix that effectively protects its inhabitants [36,37].

Samples were collected from the buccal surfaces of upper and lower molars and premolars using sterile spatulas. This latter method has been shown to provide artificial oral microcosms showing a good correlation with the microbial composition of supragingival plaque, in contrast with other collection methods based on stimulated or unstimulated human saliva [38]. Therefore, this model can be regarded as the one that more closely approaches the microbial composition of contaminated RBC syringes.

In the bench test, it was decided not to reproduce clinical behaviors regarding cleaning the spatula. It is noteworthy that only 3% of the DHCPs using one spatula applied a possibly effective cleaning protocol, such as using ethanol. However, the latter procedure cannot be considered sufficient for decontamination of any instrument coming into direct contact with a patient, where standard sterilizing procedures must be applied [13]. Cleaning with a piece of absorbent paper may be compared with the decontamination efficacy of a wiping cloth on hard surfaces. A study found a significant efficacy of cleaning cloths in reducing surface contamination even without the addition of an antimicrobial agent [39]. Future studies may address the impact that such a procedure may have on the amount of contamination of the spatula surfaces, evaluating its impact on the cross-contamination risk.

In conclusion, within the limits of the study, our results indicated that operative procedures involving the use of the same spatula to carry the material from its syringe to the restoration site provide contamination of RBCs left inside the syringe for at least one hour. This time can be more

than sufficient to cross-contaminate the following patient. Procedures breaking the contact between the oral cavity and the RBC syringe should be implemented in routine restorative dental practice.

REFERENCES

[1] L. Meng, F. Hua, Z. Bian, Coronavirus disease 2019 (Covid-19): emerging and future challenges for dental and oral medicine, J. Dent. Res. 99 (2020), 481-487. https://doi: 10.1177/0022034520914246.

[2] M. Banakar, L.K. Bagheri, D. Jafarpour, S. Moayedi, M.H. Banakar, A. MohammadSadeghi, COVID-19 transmission risk and protective protocols in dentistry: a systematic review. BMC Oral Health. 20 (2020) 275. https://doi: 10.1186/s12903-020-01270-9.

[3] E. Laneve, B. Raddato, M. Dioguardi, G. Di Gioia, G. Troiano, L. Lo Muzio, Sterilisation in dentistry: a review of the literature, Int. J. Dent. 15 (2019) 6507286. https://doi:10.1155/2019/6507286

[4] A. Amato, M. Caggiano, M. Amato, G. Moccia, M. Capunzo, F. De Caro, Infection Control in Dental Practice During the COVID-19 Pandemic, Int. J. Environ. Res. Public. Health. 17 (2020) 4769. https://doi: 10.3390/ijerph17134769.

[5] A. Ionescu, M.G. Cagetti, J.L. Ferracane, F. Garcia-Godoy, E. Brambilla, Topographic aspects of airborne contamination caused by the use of dental handpieces in the operative environment. J. Am. Dent. Assoc. 151 (2020) 660-667. https://doi: 10.1016/j.adaj.2020.06.002.

[6] C. Yanikian, F. Yanikian, D. Sundfeld, R. Lins, L. Martins, Direct composite resin veneers in nonvital teeth: a still viable alternative to mask dark substrates, Oper. Dent. 44 (2019) E159-E166. https://doi: 10.2341/18-220-T.

[7] A.M. da Veiga, A.C. Cunha, D.M. Ferreira, T.K. da Silva Fidalgo, T.K. Chianca, K.R. Reis, L.C. Maia, Longevity of direct and indirect resin composite restorations in permanent posterior teeth: a systematic review and meta-analysis, J. Dent. 54 (2016) 1-12. https://doi: 10.1016/j.jdent.2016.08.003.

[8] S. Scolavino, G. Paolone, G. Orsini, W. Devoto, A. Putignano, The simultaneous modeling technique: closing gaps in posteriors, Int. J. Esthet. Dent. 11 (2016) 58-81.

[9] S. Deliperi, D.N. Bardwell, Clinical evaluation of direct cuspal coverage with posterior composite resin restorations, J. Esthet. Restor. Dent. 18 (2006) 256-65. https://doi: 10.1111/j.1708-8240.2006.00033.x.

[10] N.A. Pauletti, L.P.S. Girotto, F.H.S. Leite, D.N. Mario, Effect of photoactivation on the reduction of composite resin contamination, Eur. J. Oral Sci. (2017) 1-4. https://doi: 10.1111/eos.12345.

[11] A. Ionescu, E. Brambilla, S. Hahnel, Does recharging dental restorative materials with fluoride influence biofilm formation? Dent. Mater. 35 (2019) 1450-1463. https://doi: 10.1016/j.dental.2019.07.019.

[12] A. Ionescu, E. Wutscher, E. Brambilla, S. Schneider-Feyrer, F.J. Giessibl, S. Hahnel, Influence of surface properties of resin-based composites on in vitro Streptococcus mutans biofilm development, Eur. J. Oral. Sci. 120 (2012) 458-65. https://doi: 10.1111/j.1600-0722.2012.00983.x.

[13] L.G. Lopes, A.A. Máximo, C. T. Taveira, J. B. Souza, F.C.L. Moreira, R.B. FonsecaP.C.F. Rodrigues, F.C. Pimenta, How to avoid cross contamination during handling resin composites with spatulas. Rev. Odontol. Bras. Central 25 (2016), 94-97.

[14] A. Porenczuk, B. Górski, W. Bielas, D. Gozdowski, A. Mielczarek, The application of restorative material packed in blisters in cross infection control in restorative dentistry. Nowa Stomatologia 1 (2019), 27-33.

[15] B.W. Nilsen, M. Mouhat, A. Jokstad, Quantification of porosity in composite resins delivered by injectable syringes using X-ray microtomography. Biomater. Investig. Dent. 7 (2020): 86-95.

[16] R.O. Ferreira, N.C.M. Caires, Assessment of contamination of composite resins tubes in Dental School Clinics in a private university at Manaus-AM. J. Health Sci. Inst. 30 (2012) 206–210.

[17] J.C.F. Almeida, A.K.S. Prado, W.C. Silva, S.F. Pedrosa, M.A.O. Moura, R.M. Chaves, L.G. Lopes, Contamination of composite resin at dentistry offices, Rev. Odontol. Bras. Central. 19 (2010) 211–215.

[18] J.L. Nancy, K. Courtney, M.K. Alison, Y. Jing, L.G. Sheng, Effect of dental monomers and initiators on Streptococcus mutans oral biofilms, Dent. Mater. 34 (2018) 776-785. https://doi: 10.1016/j.dental.2018.02.003.

[19] K. Kawai, M. Torii, Y. Tuschitani , Effect of resin components on the growth of Streptococcus mutans. J Osaka Univ. Dent. Sch. 28 (1988)161–170.

[20] P. Khalichi, D.G. Cvitkovitch, J.P. Santerre, Effect of composite resin biodegradation products on oral streptococcal growth. Biomaterials 25 (2004) 5467-5472.

[21] I. Nedeljkovic, K. Yoshihara, J. De Munck, W. Teughels, B. Van Meerbeek, K.L. Van Landuyt, No evidence for the growth-stimulating effect of monomers on cariogenic Streptococci. Clin. Oral Investig. 21(2017) 1861-1869.

[22] M. Goldberg, In vitro and in vivo studies on the toxicity of dental resin components: a review. Clin. Oral Investig. 12(2008) 1-8.

[23] H.H. Chao, X.F. Zhang, B. Chen, B. Pan, L.J. Zhang, L. Li et al. Bisphenol A exposure modifies methylation of imprinted genes in mouse oocytes via the estrogen receptor signaling pathway. Histochem. Cell. Biol. 137 (2012) 249-259.

[24] M.D. Bourbia, D.G. Cvitkovitch, J.P. Santerre, Y. Finer, Cariogenic bacteria degrade dental resin composites and adhesives. J. Dent. Res. 92(2013) 989-994.

[25] E. Brambilla, A.C. Ionescu, G. Cazzaniga, M. Ottobelli, L.P. Samaranayake, Levorotatory carbohydrates and xylitol subdue Streptococcus mutans and Candida albicans adhesion and biofilm formation. J. Basic Microbiol. 56(2016) 480-492.

[26] K.D. Jandt, R.W. Mills, A brief history of LED photopolymerization, Dent. Mater. 29 (2013) 605-617. https://doi: 10.1016/j.dental.2013.02.003.

[27] F.A. Rueggeberg, M. Giannini, C.A.G. Arrais, R.B.T.Price, Light curing in dentistry and clinical implications: a literature review, Braz. Oral. Res. 28 (2017) e61. https://doi: 10.1590/1807-3107BOR-2017.

[28] M. Cadenaro, T. Maravic, A. Comba, A. Mazzoni, L. Fanfoni, T. Hilton, J. Ferracane, L. Breschi, The role of polymerization in adhesive dentistry, Dent. Mater. 35 (2019) e1-e22. https://doi: 10.1016/j.dental.2018.11.012.

[29] M.A. Cochran, C.H. Miller, M.A. Sheldrake, The efficacy of the rubber dam as a barrier to the spread of microorganisms during dental treatment, J. Am. Dent. Assoc. 119 (1989) 141-144. https://doi: 10.14219/jada.archive.1989.0131.

[30] I. Nedeljkovic, J. De Munck, A.A. Ungureanu, V. Slomka, C. Bartic, A. Vananroye, C. Clasen, W. Teughels, B. Van Meerbeek, K.L. Van Landuyt, Biofilm-induced changes to the composite surface, J. Dent. 63 (2017) 36-43. https://doi: 10.1016/j.jdent.2017.05.015

[31] H. Chen, B. Zhang, M.D. Weir, N. Homayounfar, G.G. Fay, F. Martinho, L. Lei, Y. Bai, T. Hu, H.H.K. Xu, S. mutans gene-modification and antibacterial resin composite as dual strategy to suppress biofilm acid production and inhibit caries, J. Dent. 93 (2020), 103278. https://doi: 10.1016/j.jdent.2020.103278.

[32] E. Ueta, T. Tanida, S. Doi, T. Osaki, Regulation of Candida albicans growth and adhesion by saliva, J. Lab. Clin. Med. 136 (2000), 66-73. https://doi: 10.1067/mlc.2000.107304.

[33] T. Pereira, A.A. Cury, M.S. Cenci, R.C. Rodrigues-Garcia, In vitro Candida colonization on acrylic resins and denture liners: influence of surface free energy, roughness, saliva, and adhering bacteria, Int. J. Prosthodont. 20 (2007), 308-310.

[34] S.A. Klotz, D.J. Drutz, Z.E. Zajic, Factors governing adherence of Candida species to plastic surfaces, Infect. Immun. 50 (1985), 97-101. https://doi: 10.1128/IAI.50.1.97-101.1985.

[35] R. Bürgers, W. Schneider-Brachert, M. Rosentritt, G. Handel, S. Hahnel, Candida albicans adhesion to composite resin materials, Clin. Oral Investig. 13 (2009), 293-299. https://doi: 10.1007/s00784-008-0226-4.

[36] E. Brambilla, M. Gagliani, A. Ionescu, L. Fadini, F. García- Godoy, The influence of light-curing time on the bacterial colonization of resin composite surfaces, Dent. Mater. 25 (2009) 1067-107. https://doi: 10.1016/j.dental.2009.02.012.

[37] S.M. Michalek, J. Katz, N.K. Childers, M. Martin, D.F. Balkovetz, Microbial/host interactions: mechanisms involved in host responses to microbial antigens, Immunol. Res. 26 (2002) 223-234. https://doi: 10.1385/IR:26:1-3:223.

[38] S. Gomar-Vercher, A. Simón-Soro, J.M. Montiel-Company, J.M. Almerich-Silla, A. Mira, Stimulated and unstimulated saliva samples have significantly different bacterial profiles, PLoS One. 13 (2018) e0198021. https://doi: 10.1371/journal.pone.0198021.

[39] S.M. Masuku, D. Babu, E. M. Martin, O.K. Koo, C. A. O'Bryan, P. G. Crandall, S. C. Ricke, Cleaning and decontamination efficacy of wiping cloths and silver dihydrogen citrate on food contact surfaces. J. Appl. Microbiol. 113 (2012), 89-95.

Figure 1. Questions presented in the survey regarding the use of rubber dam and the resin composite handling during routine restorative treatments. Representative charts after dentist's feedback on the management approach of RBCs during restorative procedures. A great number of interviewed declared to use rubber dam during restorative treatments (1). Among the 300 DHCP respondents, the majority of them stated to use a single spatula to pick-up the composite from the syringe and transport it into the dental cavity and vice-versa (2 and 3). The spatula is usually not cleaned during layering procedures (4). Most of the interviewed reported the self-perception that using only one spatula during restorative procedures could represent a possible means of infection (5).

Figure 2. Schematic representation and percentage of the responses obtained for each educational level. DS: Doctorate students (N=20); SD: Dentist with specialty (N=30); MS: Master student (N=20); GD: General dentist (N=110); MD: Master degree (N=120). A similar trend in composite handling with one spatual was observed among DS, SD and MS, with a decrease in its use among GD and MD. DS resulted the educational level that more than others use one spatula for restorative procedures. The tendency of not cleaning the spatula during the repeated restorative tretaments differed among the clinicians interviewed.

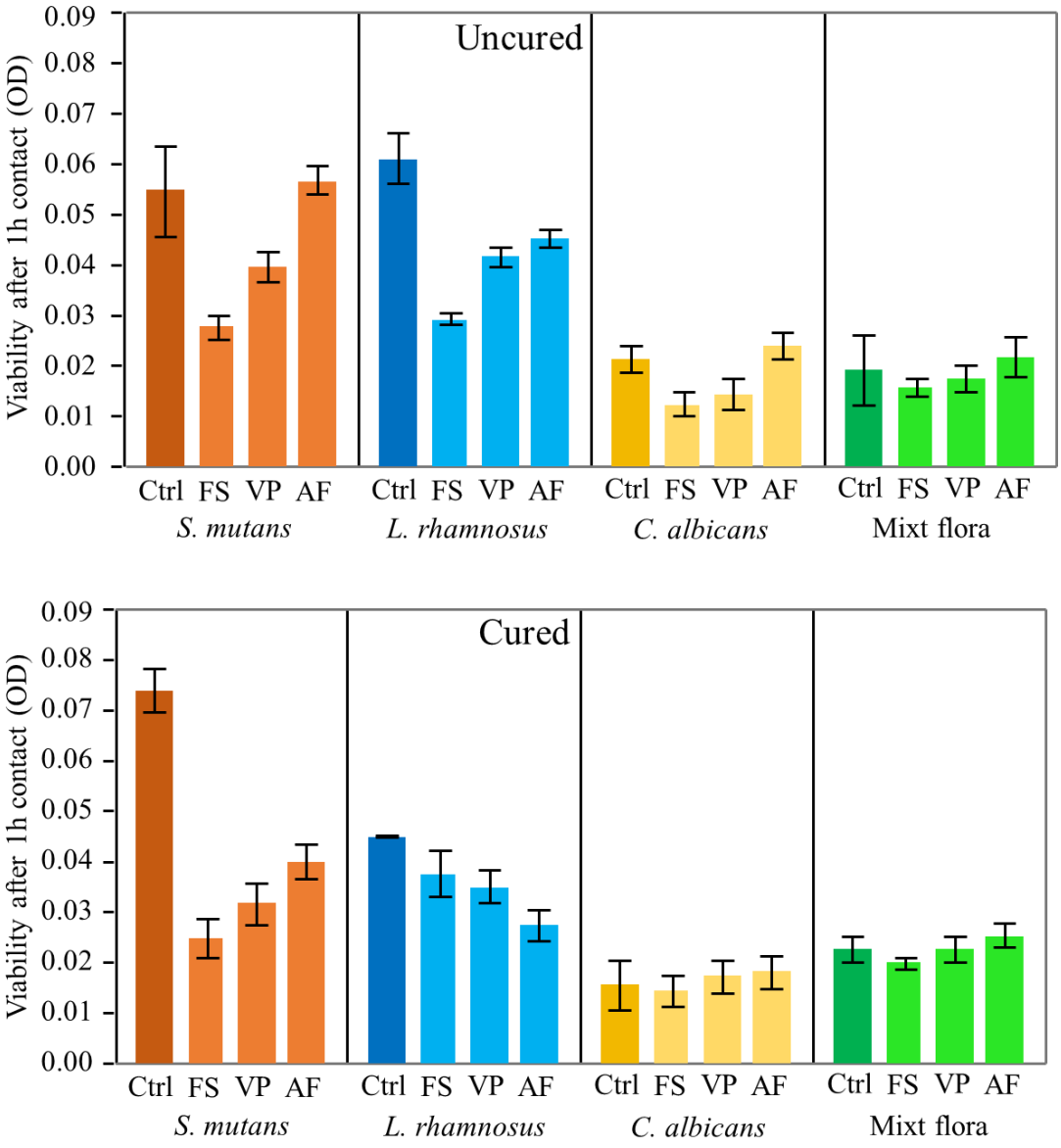
Table 1. Composition of the tested resin-based composites (RBCs). All materials are light-curing direct resin composites with nanohybrid fillers.

RBC	Composition
Filtek™ Supreme XTE (3M) LOT: N803720 Shade: A2B	Resin, Bis-GMA, UDMA, TEGDMA, bis-EMA, PEGDMA. Filler, combinazioni of non-agglomerate/non-aggregated 20 nm silica, non-agglomerated/non-aggregated 4 to 11 nm zirconia, aggregated zirconia/silica cluster.
Venus Pearl (Kulzer GmbH). LOT: 010033A Shade: A2	Resin, TCD-Urethaneacrylate, UDMA, TEGDMA. Filler, Barium Aluminium Boro Fluor Silicate Glass, Silica, Titanium Dioxide, Fluorescent pigments, Metallic Oxide Pigments, Organic Pigments, Aminobenzoicacidester, BHT, Camphorquinone.
Admira Fusion x-tra (Voco). LOT: 1722221 Shade: A2	Resin, ORMOCER (Urethane-based Organically-modified Ceramic). Filler, Glass ceramics, silica nanoparticles, pigments.

Table 2. Results of the microbial viability assessment. Means and standard deviation are given for each group. Different letters indicate significant differences between groups (Tukey's test, $p<0.05$). Results are grouped by the specimens' processing protocol (uncured vs. cured RBCs), the tested strain, and the tested RBC (FS, Filtek Supreme XTE; VP, Venus Pearl; AF, Admira Fusion x-tra). "Ctrl" labels control specimens where microorganisms were distributed on the surfaces of the tissue culture-treated plates so that 100% viability is expected.

Polymerization	Microorganisms	RBC	Viability, mean OD(±1SD)
Uncured	*S. mutans*	Ctrl	0,054636(0,015478)a,b,c
Uncured	*S. mutans*	FS	0,027614(0,006572)e,f,g,h,i,j,k,l
Uncured	*S. mutans*	VP	0,039468(0,007967)c,d,e,f,g
Uncured	*S. mutans*	AF	0,056732(0,007961)a,b
Uncured	*L. rhamnosus*	Ctrl	0,061066(0,008455)a
Uncured	*L. rhamnosus*	FS	0,029339(0,00338)d,e,f,g,h,i,j,k
Uncured	*L. rhamnosus*	VP	0,041617(0,005251)b,c,d,e
Uncured	*L. rhamnosus*	AF	0,045332(0,004602) b,c,d
Uncured	*C. albicans*	Ctrl	0,021241(0,004604)e,f,g,h,i,j,k,l
Uncured	*C. albicans*	FS	0,012367(0,006713)l
Uncured	*C. albicans*	VP	0,014363(0,008151)k,l
Uncured	*C. albicans*	AF	0,023951(0,007065)f,g,h,i,j,k,l
Uncured	Mixt flora	Ctrl	0,019085(0,01207)g,h,i,j,k,l
Uncured	Mixt flora	FS	0,015669(0,004884)j,k,l
Uncured	Mixt flora	VP	0,017409(0,006849)j,k,l
Uncured	Mixt flora	AF	0,021731(0,010348)h,i,j,k,l
Cured	*S. mutans*	Ctrl	0,073783(0,007485)a
Cured	*S. mutans*	FS	0,024837(0,009922)f,g,h,i,j,k,l
Cured	*S. mutans*	VP	0,031542(0,01085)d,e,f,g,h,i,j
Cured	*S. mutans*	AF	0,039947(0,009109)c,d,e,f,g
Cured	*L. rhamnosus*	Ctrl	0,044858(0,000388)b,c,d,e,f
Cured	*L. rhamnosus*	FS	0,037509(0,011918)c,d,e,f,g,h
Cured	*L. rhamnosus*	VP	0,03499(0,00818)c,d,e,f,g,h,i
Cured	*L. rhamnosus*	AF	0,027506(0,007902)e,f,g,h,i,j,k,l
Cured	*C. albicans*	Ctrl	0,015468(0,008674)i,j,k,l
Cured	*C. albicans*	FS	0,014445(0,008172)k,l
Cured	*C. albicans*	VP	0,017252(0,008562)j,k,l
Cured	*C. albicans*	AF	0,018107(0,008825)j,k,l
Cured	Mixt flora	Ctrl	0,022646(0,004809)e,f,g,h,i,j,k,l
Cured	Mixt flora	FS	0,019815(0,002625)i,j,k,l
Cured	Mixt flora	VP	0,02258(0,006458)h,i,j,k,l
Cured	Mixt flora	AF	0,025393(0,006153)f,g,h,i,j,k,l

Figure 3. Results of the microbial viability assessment (MTT). Optical density (OD) values (means±1 SE) are representative of viable and metabolically active microbial cells. FS = Filtek supreme XTE; VP = Venus Pearl; AF = Admira Fusion x-tra.



Figure

Figure 1.

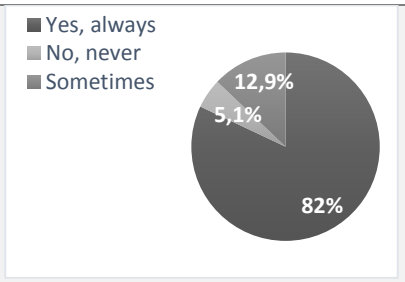
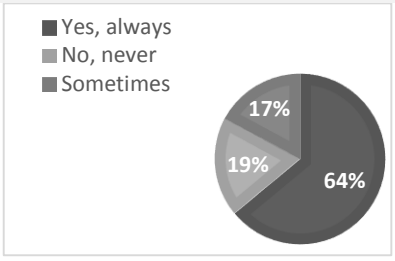
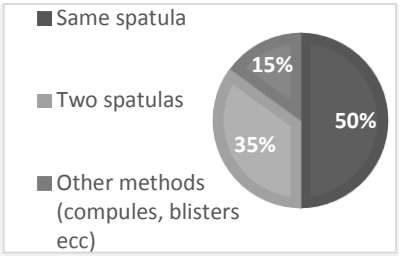
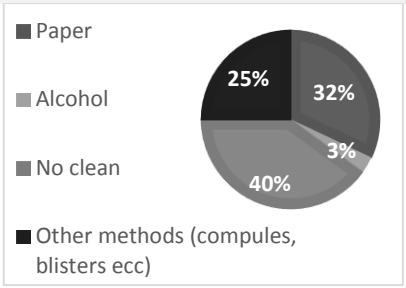
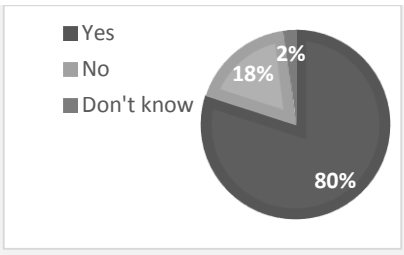
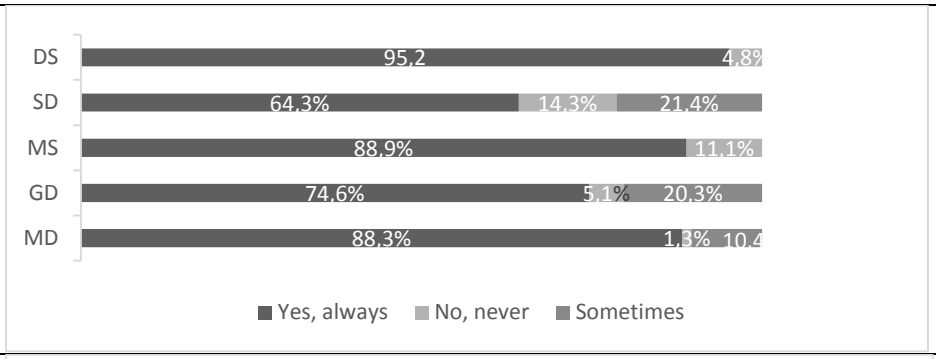
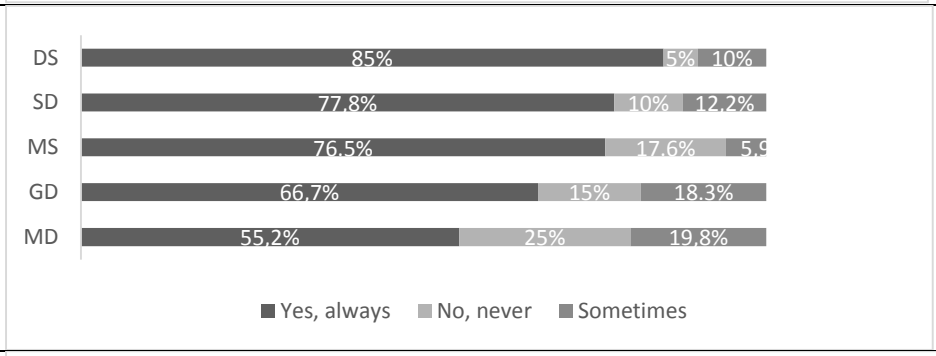
	QUESTION	ANSWERS	CHARTS
1.	Do you use the rubber dam during restorative procedures?	A. Yes, always; B. No, never; C. Sometimes.	
2.	Do you use a spatula to take the RBCs from the syringe and carry it in the dental cavity during your routine restorative procedures?	A. Yes, always; D. No, never; E. Sometimes.	
3.	If yes	A. I always use the same spatula; B. I use two separate spatulas; C. Other methods (compules. blisters ecc).	
4.	In case you use the same spatula	A. I clean the spatula every step with a paper; B. I clean the spatula with alcohol; C. I do not clean the spatula; D. Other methods (compules, blisters ecc).	
5.	Have you ever had the feeling that the recurrent use of the same spatula between the syringe and the dental cavity could be a source of cross-contamination?	A. Yes; B. No; C. Don't know.	

Figure 2

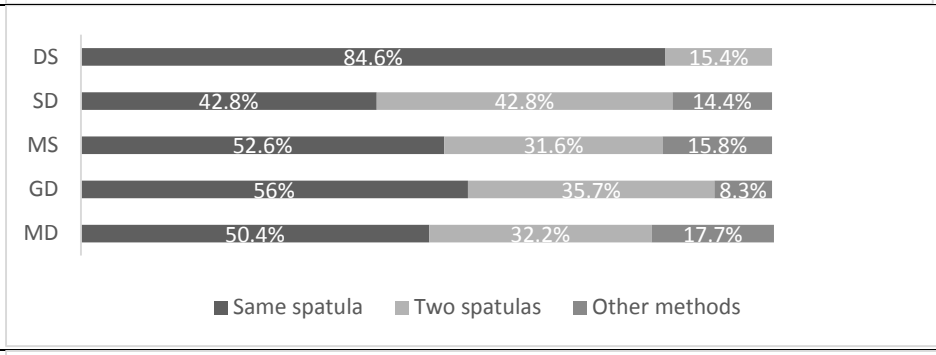
1. Do you use rubber dam during restorative procedures?



2. Do you use a spatula to take the RBCs from the syringe and carry it in the dental cavity during your routine restorative procedures?



3. If yes



4. In case you use the same spatula

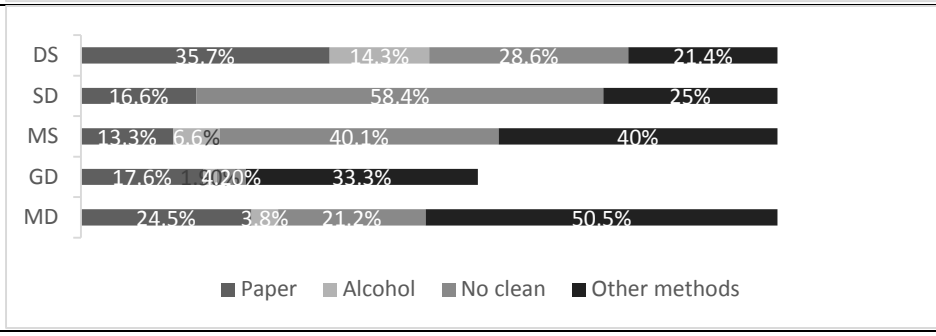
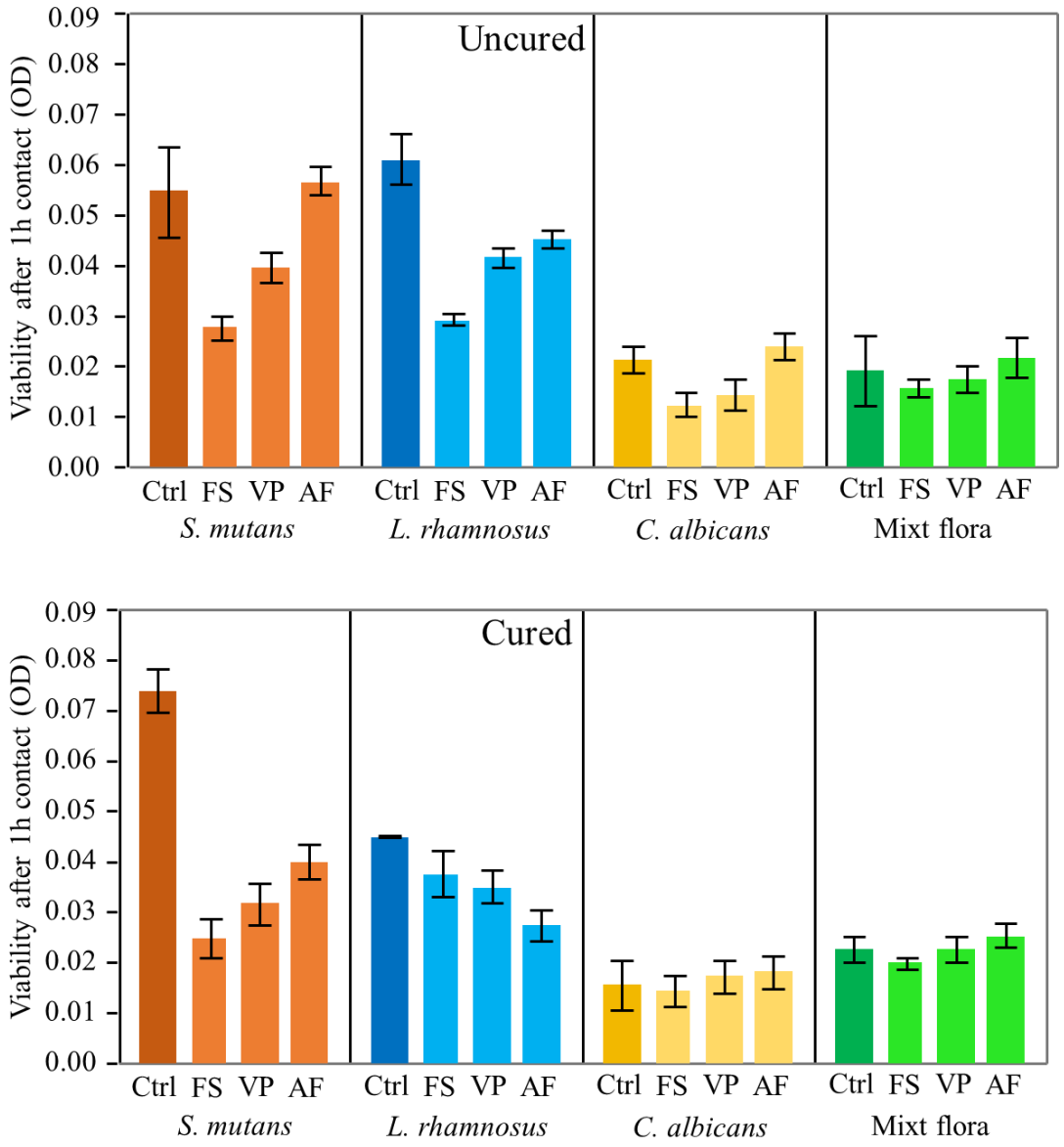


Figure 3


Uncured
Cured
Viability after 1h contact (OD)
0.09
0.08
0.07
0.06
0.05
0.04
0.03
0.02
0.01
0.00
Ctrl FS VP AF
S. mutans
L. rhamnosus
C. albicans
Mixt flora

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CRediT

“Microbial contamination of resin composites inside their dispensers: an increased risk of cross-infection?

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Claudia Mazzitelli: Conceptualization, Writing original draft, Investigation; **Andrei Ionescu**: Conceptualization, Investigation Writing original draft; **Uros Josic**: Data curation, Investigation; **Eugenio Brambilla**: Supervision, Visualization; **Lorenzo Breschi**: Project administration, Supervision; **Annalisa Mazzoni**: Supervision, Writing Review & Editing, Visualization.