



# Contamination of Resin-Matrix Composites on Chairside Handling Using Latex or Nitrile Gloves: An In Vitro Study

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## Abstract

The aim of this study was to evaluate the effect of different gloves and clinical environment on the contamination of resin-matrix composites for restorative dentistry. Specimens of nano-hybrid resin-matrix composite ( $n = 6$ ) were divided in groups regarding the handling with (A) clinical spatula; (B) latex gloves; (C) latex or (D) nitrile gloves with human saliva; (E) latex or (F) nitrile gloves with human blood. After light curing, groups of specimens were analyzed by optical microscopy at magnification ranging from x30 up to x500 and by scanning electron microscopy at different magnification ranging from x50 up to x8000. Handling of resin-matrix composites with unpowdered nitrile gloves or clinical spatulas avoided the presence of contaminants. However, agglomerates of the resin-matrix composite itself became entrapped leading to a heterogenous morphological aspect. SEM images revealed the presence of corn-derived starch released from the powdered gloves. Also, the formation of micro-spaces (voids) occurred after handling with powdered latex gloves. Specimens handled with both type of gloves contaminated with saliva showed a conditioning layer composed of glycoproteins rolls and compounds including calcium-based chlorides, phosphates, and carbonates. Also, blood products were transferred from the contaminated gloves to the resin-matrix composites after handling. Thus, resin-matrix composite restorations are susceptible to contamination with debris from powdered gloves. Also, saliva or blood debris become adsorbed and entrapped in the resin-matrix composites during clinical handling leading to the presence of defects such macro- and micro-scale voids or contaminant agglomerates.

**Keywords** Resin composite · Contamination · Saliva · Blood · Gloves · Handling

## Introduction

Resin-matrix composites are commonly used in dental practice to directly restore damaged teeth taking into account shape, physicochemical, and esthetic features [1–3]. On dental restorations, resin-matrix composites are clinically handled and placed in the damaged tooth site. Clinical guidelines request the use of clean spatulas and gloves to avoid the presence of residues from patient saliva or blood as well as the environment or other previous materials [4–7]. Clinicians often perform a handling of increments using gloves followed by spatula placement to compact the resin-matrix composites into the damaged teeth site. Such handling procedure increase the risks of contamination of the resin-matrix composites whether the gloves and instruments are not hermetically cleaned. The products from saliva and blood can be embedded in the resin-matrix that affect the physicochemical properties of the material and then the long-term performance of the dental restoration. Also, the presence of pore-like defects or micro-scale voids into the

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resin-matrix composite microstructure can occur during handling [4, 5, 8].

Resin-matrix composites for dental restorations are traditionally composed of a methacrylate-based polymeric matrix involving photo-initiators compounds and silanized inorganic fillers. The organic matrix can be mainly composed of Bisphenol A-Diglycidyl Methacrylate (Bis-GMA) and other methacrylate-based molecules such Triethylene Glycol Dimethacrylate (TEGDMA) and Urethane Dimethacrylate (UDMA) for controlling viscosity and overall physical properties. Bis-GMA can be replaced by Ethoxilated Bisphenol A-Diglycidyl Methacrylate (Bis-EMA) considering similarity in molecular mass and physical properties although the viscosity of Bis-EMA is lower than that recorded for Bis-GMA [9–15]. Additionally, fillers are industrially added in the chemical composition to enhance the physical properties of the composite such as wear resistance, strength, elastic modulus, viscosity, color, radiopacity, translucency, etc. [9–12].

Commercially available resin-matrix composites reveal a broad range of different size and type of particle-like fillers. The size of filler particles incorporated in the resin matrix has continuously decreased over the years from the traditional to the submicron- and nano-structured composite materials [10–13]. Thus, a homogenous filler distribution within the organic matrix can be achieved by varying the size of the inorganic particles from micro- to nano-scale dimensions since the small nano-fillers are able to occupy the spaces between the larger particles. In this way, the filler content can be increased leading to an enhancement of the mechanical properties of the resin-matrix composites. Also, the decrease in the volume of the organic matrix causes a decrease in polymerization shrinkage, an increase in the degree of conversion of monomers, and a decrease in the material loss on wear [9, 13, 2].

Resin-matrix composites have viscoelastic properties that allow the clinician to compact and shape the material into the damaged tooth site prior to their polymerization [1] [8]. The restoration technique consists in the step-by-step compaction of multiple increments with thickness of around 2mm on the tooth site to ensure complete polymerization of large restorations and guarantee optimal physical properties. Incremental restoration techniques have also been recommended due to the polymerization shrinkage of the polymeric matrix of resin-matrix composites [3, 14]. The light-curing mode varies depending on the clinical case, materials, and clinician skills. However, most of resin-matrix materials used for dental restorations can be polymerized under a wavelength of around 470 nm and 400–600 mW/cm<sup>2</sup> intensity for 40 s. That depends on the photo-initiator (i.e., canforquinone) in the chemical composition of the organic matrix. Although the incremental filling technique is desirable, the longer time

required for placement and polymerization of increments provide a high probability of contamination in a clinical environment [14]. The major concern in the restoration procedure is related to the risks of contamination when contacting water, saliva, blood, or other materials [8]. Also, some clinicians use powdered gloves with starch granules which can be transferred to the resin-matrix composites [14]. The presence of defects in the material's microstructure such pores and debris negatively affect the mechanical properties of the resin-matrix composites and the long-term clinical success of the restoration [14]. A few studies have been performed involving contamination of oral and restorative surfaces although there is a lack of information on contamination between resin-matrix composite increments [14].

The aim of this study was to evaluate the contamination of resin-matrix composites during clinical handling after contacting powdered gloves, saliva, and blood. It was hypothesized that organic debris and minerals from human saliva or blood are adsorbed onto the surface of resin-matrix composites after handling using latex or nitrile gloves.

## Materials and Methods

### Preparation of the Medium

Human saliva was collected from a single participant with 22 years old. The participant was in good dental and oral health, with no history of antibiotic treatment over the previous 6 months to the saliva harvesting. The participant did not suffer from any systemic or salivary gland disease that could affect salivary secretion. The exclusion criteria of the participant involved history of periodontitis and a probing depth higher than 6 mm at the marginal teeth soft tissues. Saliva was stimulated by using paraffin wax pellets' (Sigma-Aldrich, USA) as chewing gum which was previously immersed in deionized water for 24 h. The stimulated saliva was discarded over the first minute, then 3 mL was harvested as a source of contamination on the resin-matrix composite specimens [20, 21]. Also, the human blood was harvested by using a needle-prick to alcohol wiped forefinger at the time of experiment. It has been shown that freshly drawn capillary blood is more suitable in laboratory experiments involving blood contamination than heparinized blood [7].

The use of human blood or saliva was approved by the Human Research Ethics Committee at the University Institute of Health Sciences (IUCS), cod. 13/CE-IUCS/CESPU/2022, that is in accordance with the Helsinki declaration of 1964. The volunteer signed the informed consent

prior to inclusion in the study since the purpose of the study was described.

### Preparation of Specimens

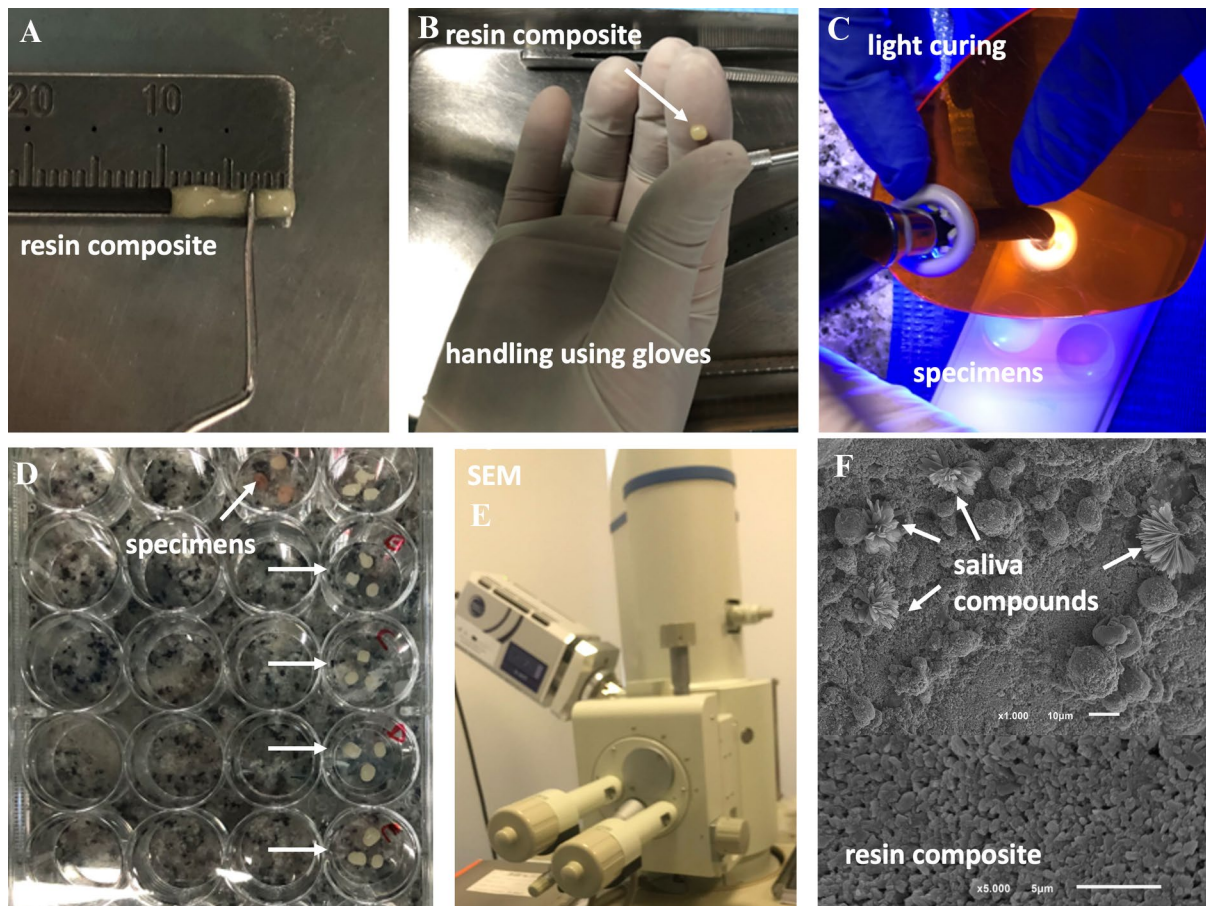
This *in vitro* study involved a completely randomized and blinded design, considering the effect of different contamination conditions of latex or nitrile gloves for clinical procedures used to handle a resin-matrix composite. The following groups were assessed: (A) control group, without handling or contamination, (B) powdered-free nitrile gloves, (C) powdered latex gloves, (D) powdered-free nitrile gloves coated with saliva, (E) powdered latex gloves coated with

saliva, (F) powdered-free nitrile gloves coated with human blood, and (G) powdered latex gloves coated with human blood. Forty two specimens ( $n=6$ ) were prepared from a light-cured nano-hybrid resin-matrix (Ceram.X Spectra™ ST HV, Dentsply, USA), according to the specifications of each test. Details on the resin-matrix composite are described in Table 1.

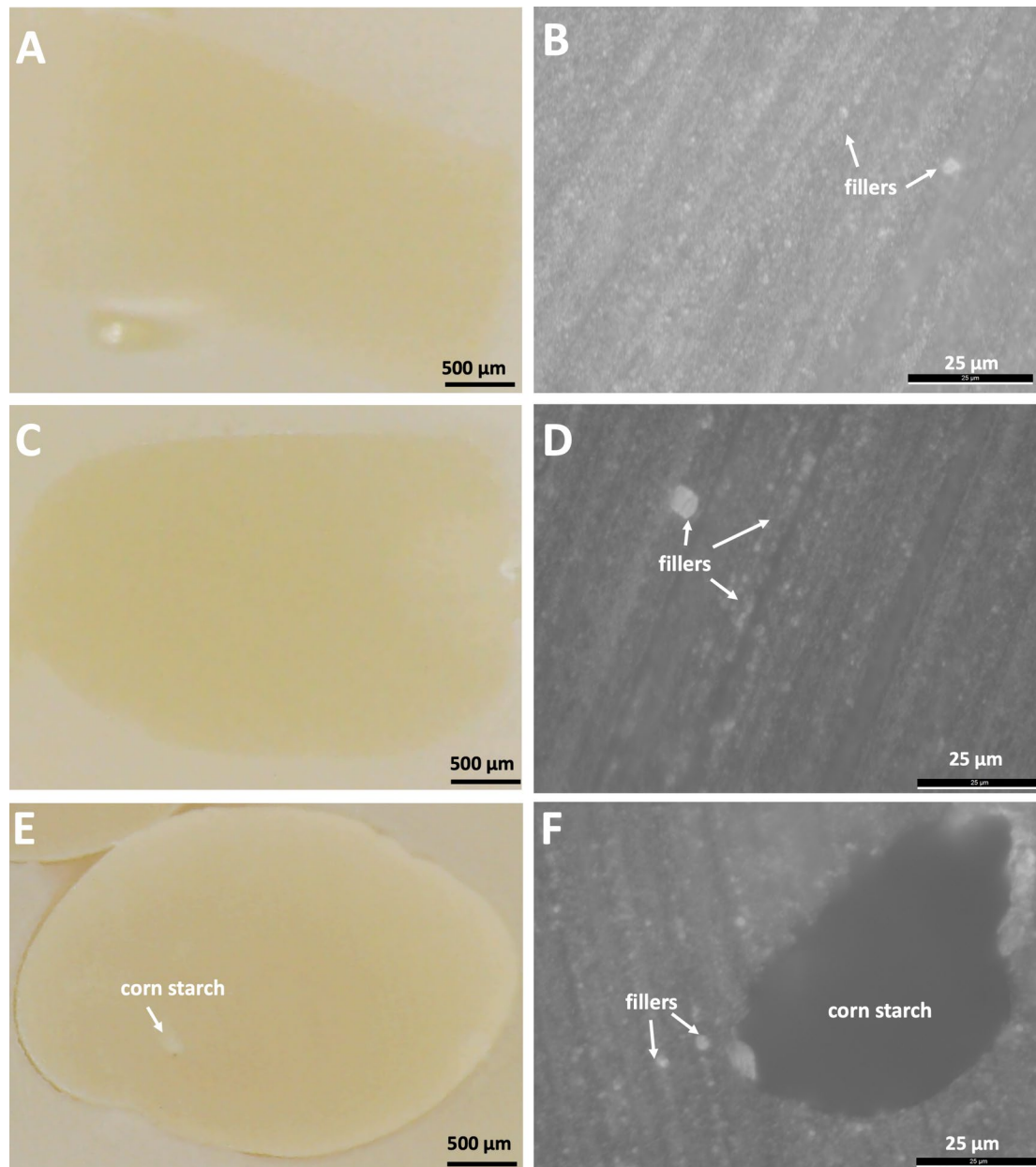
Powdered disposable latex gloves (Sensitive Latex Gloves Rubbergold™, Raclac S.A., Portugal) and nitrile gloves (Nitrile Docworld™, Raclac S.A., Portugal) were used in this study. The specification of the gloves was given by the manufacturer. In the control group, the resin-matrix composite was removed from with a Heidemann's clinical

**Table 1** Details of the resin-matrix composites

Manufacturer	Photoinitiators/shade	Fillers	Elastic modulus (GPa)	Flexural strength (MPa)	Organic matrix
Dentsply Sirona, Germany	CQ, A2	78–80 (wt%) 60–62 (v%)	8.5	135	Bis-EMA, UDMA, TEGDMA



**Fig. 1** Preparation of specimens. **A** Measuring 2mm-thick specimens for **B** handling, **C** light-curing, and **D** storage. **E** Picture of the scanning electron microscopy (SEM) used for the **F** analyses

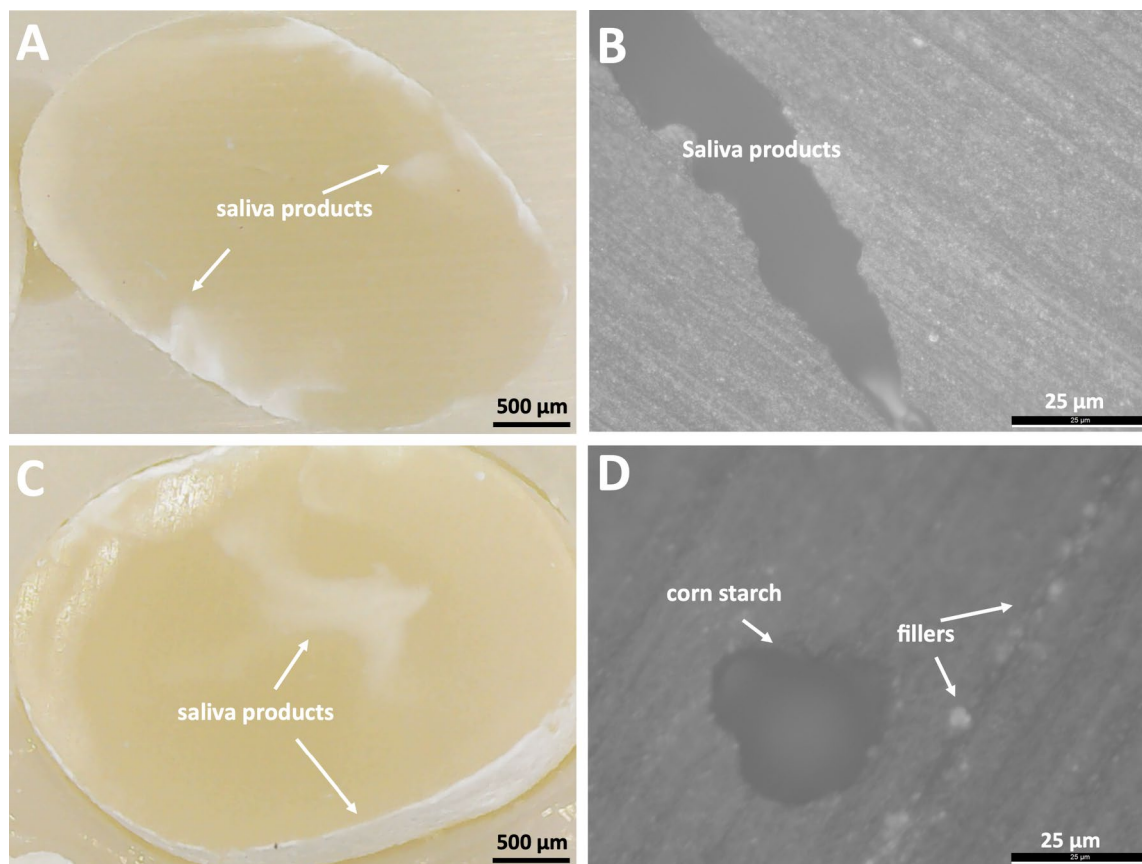


**Fig. 2** Optical microscopy (OM) images of the cross-sectioned resin-matrix composites retrieved from capsules (**A, B**) without handling, (**C, D**) after handling using nitrile gloves (free of powder) or (**E, F**)

powdered latex gloves. **A, C, E** OM images at  $\times 30$  and **B, D, F** at  $\times 1000$  magnification

spatula and placed into the molds without any handling or contamination [4] (Fig. 1). Heidemann's spatula is a clinical tool commonly used for chair-side handling the resin-matrix composite for direct restorations. On the glove contamination, powdered latex glove (group B) or nitrile glove (group C), were removed from its respective pack and immediately used without contamination with human blood or saliva. Specimens of 2 mm-thickness were handled for 15 s (on all groups with both gloves) to achieve a round shape for the incremental restoration [4] (Fig. 1A and B).

Gloves contaminated with human blood were used to handle the specimens and then dried carefully with oil-free compressed air for 5 s at a distance of 10 cm away from the surface (Fig. 1B). Caution was taken into account to maintain a layer of dry blood on the top of the specimens [7]. At last, groups of gloves were contaminated with stimulated human saliva and then dried at room temperature in a sterile polystyrene well-plate (Fig. 1D) [4]. Specimens were polymerized using a light-curing unit (Bluestar, Microdont, Brazil) at  $400 \text{ mW/cm}^2$  for 40 s, as seen in Fig. 1C. The wavelength



**Fig. 3** Optical microscopy (OM) images of the cross-sectioned resin-matrix composites handled using (A, B) nitrile gloves (free of powder) or (C, D) powdered latex gloves after contacting human saliva. A, C OM images at  $\times 30$  and B, D at  $\times 1000$  magnification

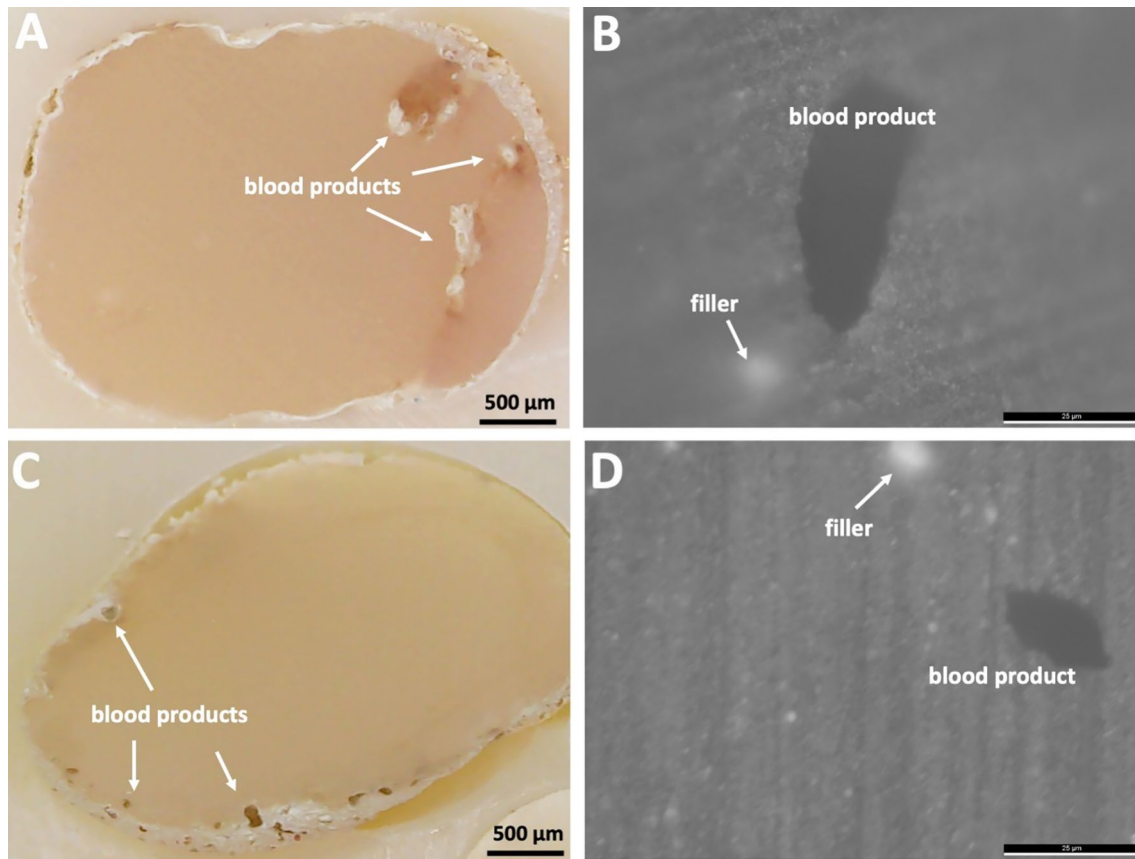
of the light-curing instrument was set in the range of 450 and 500nm. The light-curing unit was calibrated using a traditional radiometer (ProclinicExpert, Montellano, Lisbon) prior to the polymerization procedure.

### Microscopic Analyses

Groups of randomly resin-matrix composite specimens were embedded in autopolymerizing polyether modified resin (Technovit 400™; Kulzer GmbH, Germany) and then cross-sectioned at  $90^\circ$  relative to the plane of the long axis. Surfaces were wet ground down to 2400 Mesh using SiC abrasive papers and then polished with  $1\mu\text{m}$   $\text{Al}_2\text{O}_3$  particles. Surfaces were ultrasonically cleaned in isopropyl alcohol for 10 min and then in distilled water for 10 min. Cross-sectioned specimens were inspected by optical microscopy at magnification ranging from  $\times 10$  up to  $\times 500$ . Microstructural analyses were performed using an optical microscope (Leica DM 2500 MTM, Leica Microsystems, Germany) connected to a computer to image processing, using Leica Application Suite software program (Leica Microsystems, Germany). A number of six micrographs were acquired at  $\times 500$

magnification, for each specimen ( $n = 18$ ). The software program Adobe Photoshop (Adobe Systems Software, Ireland) was used to analyze black and white images, with the black representing the pores and the white the bulk material.

Before SEM analysis, other groups of specimens of each condition were conditioned in 2.5% glutaraldehyde solution for 5 min. Then, specimens were washed three times in distilled water and dehydrated through a series of graded ethanol solutions (50, 60, 70, 80, 90, and 100%). Then the specimens were sputter-coated with AgPd thin layer for scanning electron microscopy (SEM) analyses by using SEM unit (JSM-6010 LV, JEOL, Japan) coupled to energy dispersive X-Ray spectrometer (EDX) (Fig. 1E). The surface and microstructure of the specimens were evaluated by (SE) secondary and (BSE) backscattered electrons at magnification ranging from  $\times 50$  up to  $\times 8000$  and at an accelerated voltage of 15 kV (Fig. 1F). SEM images were recorded at three different areas for each specimen ( $n = 9$ ). The dimensions and morphological aspects of the biological components and materials were analyzed using the ImageJ 1.51 software program (NIH, Bethesda, Maryland, USA). The chemical analysis of the materials was performed by EDX



**Fig. 4** Optical microscopy (OM) images of the cross-sectioned resin-matrix composites handled using (A, B) nitrile gloves (free of powder) or C, D powdered latex gloves after contacting human blood. A, C OM images at  $\times 30$  and (B, D) at  $\times 1000$  magnification

under BSE mode using a silicon drift detector energy-dispersive spectrometer (SDD-EDS). The characteristic X-Rays emitted from the atoms are harvested by the SSD, that evaluated the signal considering standard computational data to estimate the atomic fraction.

## Results

Optical microscopic (OM) images of the cross-sectioned resin-matrix composites without handling are shown in Fig. 2A, B while OM images of the cross-sectioned resin-matrix composites handled with nitrile gloves (free of powder) and powdered latex gloves are shown in Fig. 2C–F.

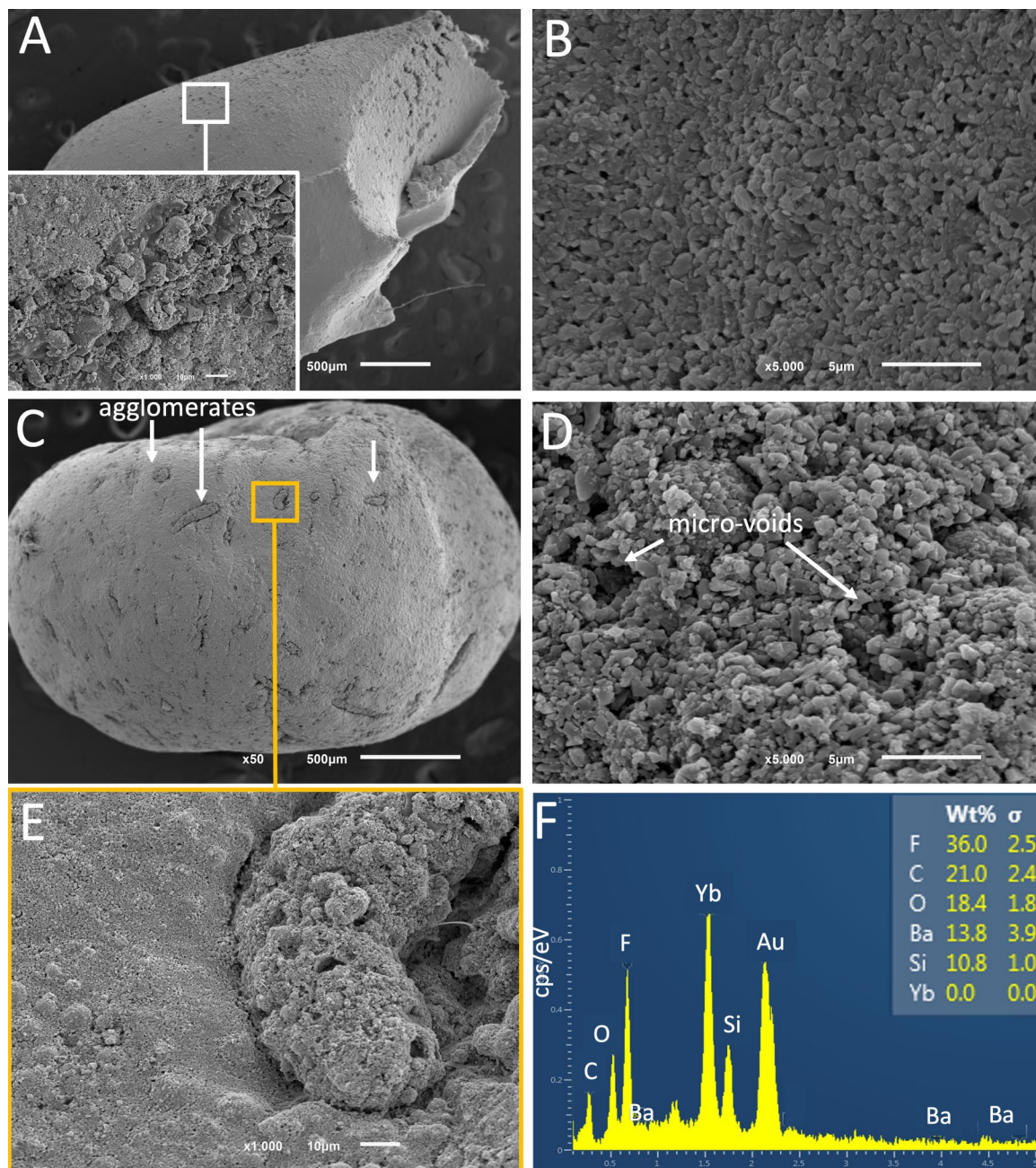
Optical microscopy images at  $\times 30$  magnification (Fig. 2A, C, and E) showed the morphological aspects of the resin-matrix composites while OM images at  $\times 1000$  magnification (Fig. 2B, D, and F) revealed the micro-scale fillers. As seen in Fig. 2E and F, a powder product can be detected in the material handled with powdered latex gloves. The powder product consists of corn starch

and its size around 20–30  $\mu\text{m}$  can be confirmed at  $\times 1000$  magnification (Fig. 2F).

Optical microscopic images of the cross-sectioned resin-matrix composites handled with nitrile gloves (free of powder) and powdered latex gloves after contacting human saliva are shown in Fig. 3A–D.

Optical microscopy images revealed entrapped saliva products in the resin-matrix composites at  $\times 30$  or  $\times 1000$  magnification (Fig. 3). As seen in Fig. 3C and D, saliva products can be detected in the material handled with nitrile or powdered latex gloves. Considering the dimensions, a potential corn starch debris can be noted in Fig. 3D.

Optical microscopic images of the cross-sectioned resin-matrix composites handled with nitrile gloves (free of powder) and powdered latex gloves after contacting human blood are shown in Fig. 4A–D. Optical microscopy images also revealed entrapped blood products in the resin-matrix composites at  $\times 30$  or  $\times 1000$  magnification (Fig. 4). A detailed view of the blood products is shown in Fig. 4C and D.



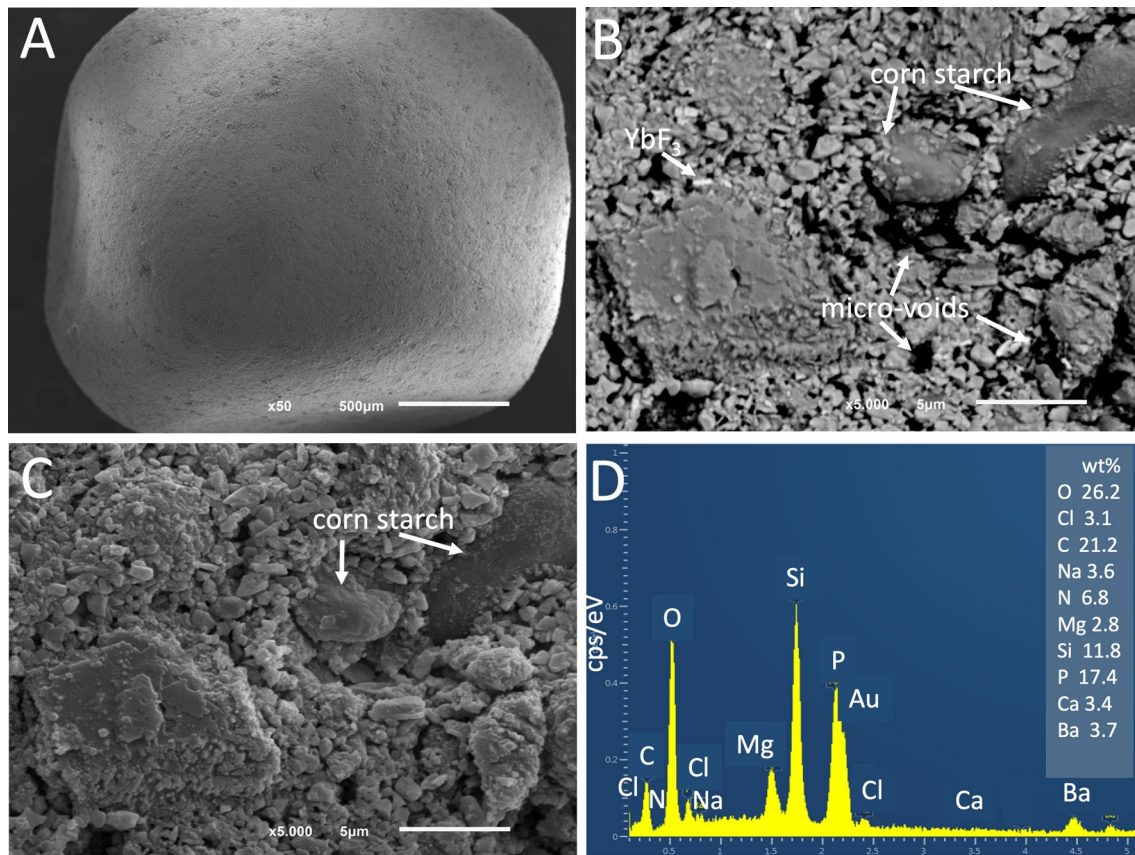
**Fig. 5** SEM images of the resin-matrix composites retrieved from capsules (**A**, **B**) without handling and **C**, **D** after handling using nitrile gloves (free of powder). **A**, **C** SEM images on SE mode and at

$\times 50$  and  $\times 1000$  magnification (zoom in) images. **B**, **D**, **E** SEM images at  $\times 5000$  magnification. **F** Chemical analysis on EDX spectra

SEM images of the resin-matrix composites without handling and handled with nitrile gloves (free of powder) are shown in Fig. 5.

SEM images at  $\times 50$  magnification showed the morphological aspects of the resin-matrix composites. Resin-matrix composites retrieved from the capsules showed irregular morphological aspects from the cutting procedure (Fig. 5A) although a compact microstructure can be noticed in Fig. 5B.

Handling of the resin-matrix composites with nitrile gloves free of powder avoided the presence of debris. However, agglomerates of the resin-matrix composite itself became entrapped leading to a heterogenous morphological aspect as seen in Fig. 5C. Additionally, micro-spaces (voids) were detected on the resin-matrix composites as seen in Fig. 5D and E. The elemental analysis by EDX is provided in Fig. 5E revealing the presence of F, Yb, Si, and Ba as the main compounds of the inorganic fillers.



**Fig. 6** SEM images of the resin-matrix composites retrieved from capsules and handled with powdered latex gloves. SEM images on SE mode and at **A**  $\times 50$  and **B**  $\times 1000$  magnification. **B**, **C** Presence of

corn starch (arrows) from the powdered gloves. **B** SEM on BSE mode showing micro-voids (arrows). **B**, **C** Images at  $\times 5000$  magnification. **D** Chemical analysis on EDX spectra

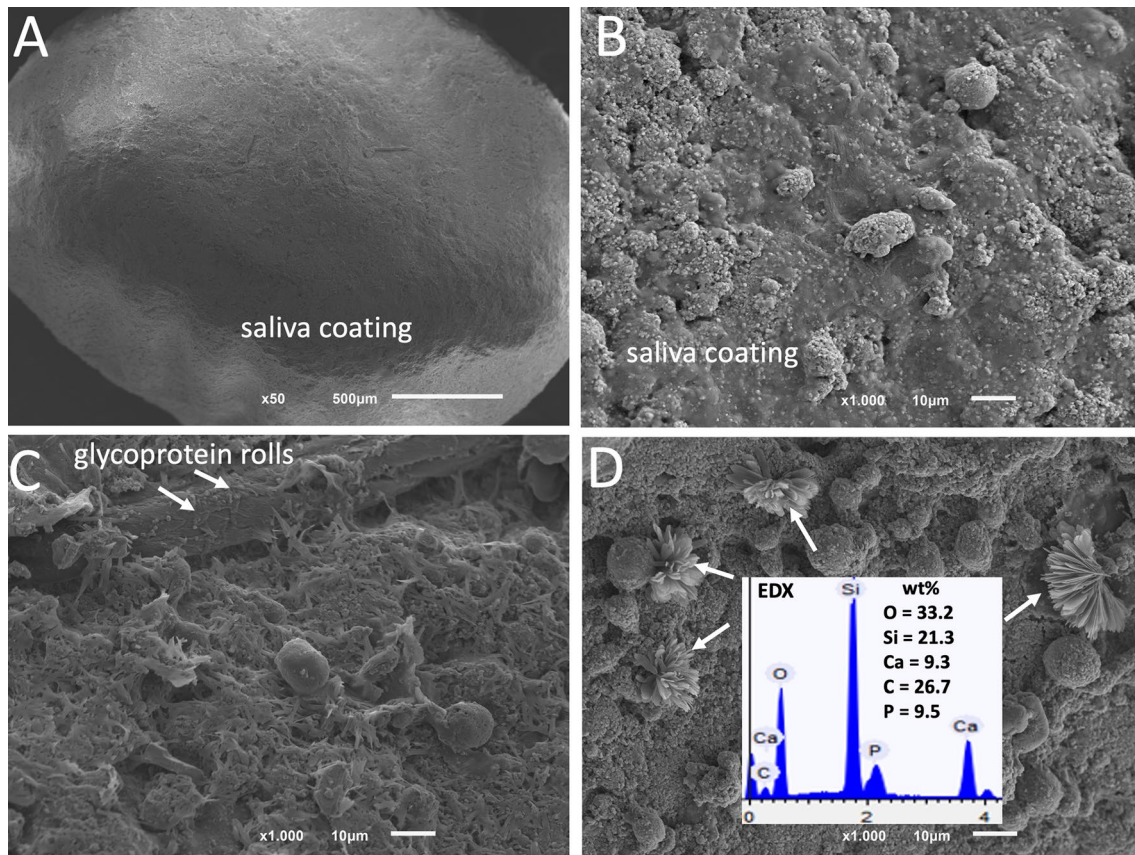
SEM images of the resin-matrix composites handled with powdered latex gloves are shown in Fig. 6. As seen in Fig. 6, SEM images on secondary electrons (SE) mode at  $\times 1000$  or  $\times 5000$  magnification revealed the presence of corn starch (white arrows) released from the powdered gloves. SEM images at back-scattered electrons (BSE) mode (Fig. 6B) confirmed the presence of the contaminants (corn starch) and the formation of micro-spaces (voids) after handling. The presence of N and C is shown in the EDX spectra (Fig. 6D).

SEM images of the resin-matrix composites handled with nitrile gloves contaminated with human saliva are shown in Fig. 7. A conditioning film of human saliva coated the resin-matrix composites as shown in Fig. 7. SEM images on SE mode at  $\times 1000$  magnification revealed the presence of glycoproteins (Fig. 7C). The presence of Ca, Cl, C, P, and O on EDX spectra suggested the formation of calcium-based chlorides, phosphate, and carbonates from the human saliva (Fig. 7D). It should be highlighted the formation of glycoproteins' rolls due to the handling procedure. Probably, the plastic deformation of the glycoproteins (i.e., mucin) was

augmented by the raise of temperature during the friction under handling with gloves.

SEM images of the resin-matrix composites handled with nitrile gloves contaminated with human saliva are shown in Fig. 8. A conditioning film of human saliva was also detected on the resin-matrix composites (Fig. 8B). SEM images on SE mode at  $\times 1000$  magnification revealed the presence of the human saliva coating (Fig. 8B, C) as well as the corn starch released from the powdered gloves (Fig. 8D).

SEM images of the resin-matrix composites handled with nitrile or powdered gloves contaminated with human blood are shown in Fig. 9. SEM images at  $\times 50$  magnification showed irregular morphological aspects of the resin-matrix composites (Fig. 9A, C). The presence of glove powder was not detected after handling with nitrile gloves although blood products can be noticed by SEM-EDX (Fig. 9B). SEM images on SE mode at  $\times 1000$  magnification revealed the presence of blood products coating (Fig. 9D-F) and corn starch released from the powdered latex gloves (Fig. 9F).



**Fig. 7** SEM images of the resin-matrix composites retrieved from capsules and handled with nitrile gloves contaminated with human saliva. SEM Images on SE mode and at **A**  $\times 50$  and **B–D**  $\times 1000$  mag-

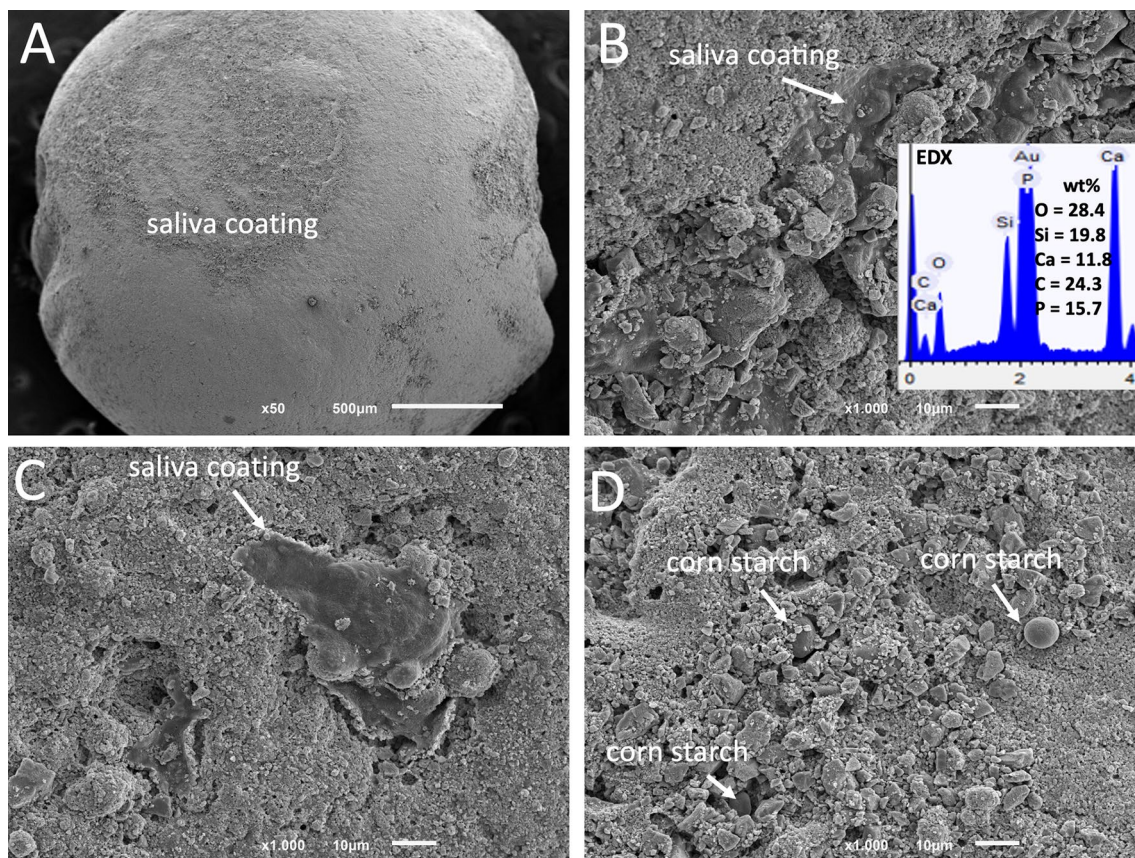
nification. **B** Saliva conditioning film and **C** formation of glycoproteins' rolls (arrows). **D** calcium-based chloride, phosphates, and carbonate compounds (arrows) were identified by SEM–EDX

## Discussion

In this study, increments of resin-matrix composites were handled using nitrile and latex powdered gloves contaminated with saliva or blood to simulate clinical conditions. The results of the present study support the hypothesis that debris from gloves as well products from human saliva and blood do adsorb onto the surface of the resin-matrix composites during clinical handling. They showed significant differences on the specimens that were handled or not with powdered or unpowdered gloves. Thus, powdered gloves coated with saliva and blood products do alter the surface of the resin-matrix composite. Debris can become entrapped in the resin-matrix composites due to the clinical handling.

On related findings reported in literature, a bibliographical search carried out on PubMed identified 21 previous studies regarding the contamination of resin-matrix composites by contacting gloves, saliva or blood. Only 10 previous

studies [4, 5, 7, 14, 22–27] were selected for comparison of results with those recorded in the present study taking into account the methods. Of the 10 selected previous studies, nine studies were performed in laboratory since saliva or blood was harvested from human participants. Only one study was performed in human participants and published as a case report [23]. Five previous in vitro studies evaluated the effect of the contamination of resin-matrix composites by using powdered gloves or human saliva on the mechanical properties and incremental layer debonding [4, 5, 14, 25, 26]. A previous in vitro study evaluated the shear bond strength of resin-matrix composite to glass fiber-reinforced composite after contamination with water or human saliva [22]. Three in vitro previous studies evaluated the shear bond strength of resin-matrix composites after saliva and blood decontamination [7, 24, 27]. A previous clinical report discussed the advantages and limitations of the application technique of direct shaping by occlusion for large composite restorations involving the entire occlusal surface [23]. In such technique, the final increment of resin composite was shaped by letting the patient occlude before the light-curing procedure. Special care was considered for moisture control



**Fig. 8** SEM images of the resin-matrix composites retrieved from capsules and handled with powdered latex gloves contaminated with human saliva. SEM Images on SE mode and at **A** 50× and

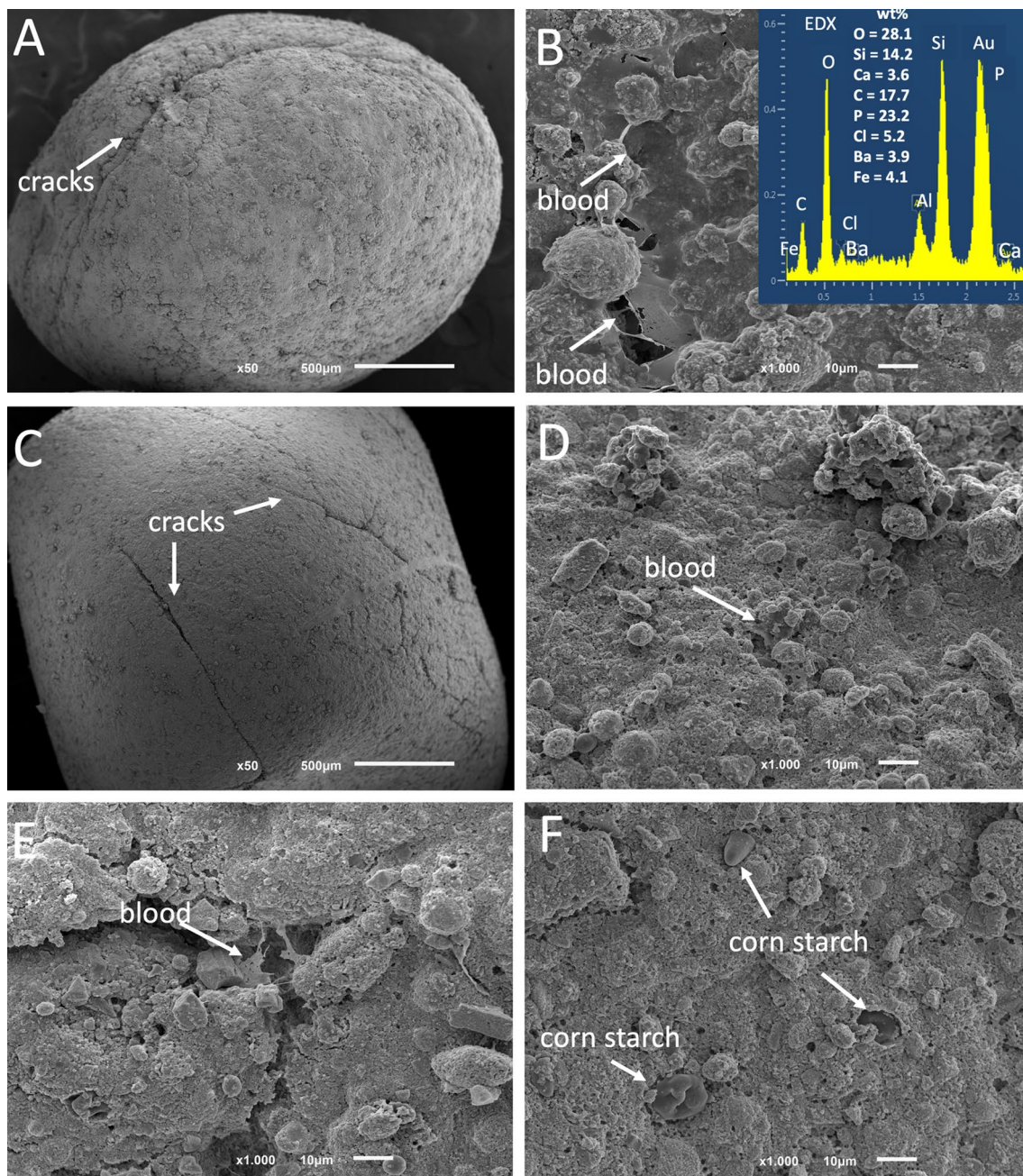
**B–D** ×1000 magnification. **B**, **C** Saliva conditioning film and **D** the presence of corn starch released from the latex gloves as identified by SEM–EDX

and handling of contamination concerning the sensitivity of the technique [23].

In the present study, a group of specimens was prepared by using only the Heidemann's clinical spatula that revealed the absence of debris contaminants, although irregular morphological aspects such as fissures and fragments were noted due to the cutting procedure of increments (Figs. 2 and 5). However, the spatula can be used to smoothen the surface of the resin-matrix composite increments as recommended by standard clinical guidelines [1, 8]. The absence of debris also occurred on the specimens prepared by handling using nitrile gloves. Even though the handling promoted spherical-shape aspect of the resin-matrix composite increments, agglomerates of the material itself became entrapped into irregular regions leading to a heterogeneous morphological aspect (Figs. 2 and 5). Considering the clinical handling can be sensitive to the operator handling, voids, fissures, and pores at macro- and micro-scale can take place into the microstructure of the material as seen in Fig. 5. There are some evidences that the technique used for composites placement can directly affect the incorporation of pores and debris into

the structural material [1, 8]. Voids, fissures, and pores at macro- and micro-scale can be spots for concentration of stresses and further fracture by cracking propagation under fatigue. Also, those defects are retentive regions for the accumulation of dyes, organic debris, and compounds from dietary and oral cavity leading to the change of color and translucency of resin-matrix composites [8, 28].

Regarding the microstructural analyses of the resin-matrix composites, inorganic fillers were identified within a size range below 3 µm as also reported in literature [29–31]. Such size of fillers allows the homogenization of the material on handling [32]. However, SEM and EDS analyses of specimens handled with powdered latex gloves confirmed the presence of the corn starch (corn starch) entrapped in the resin-matrix composite (Fig. 6B). Also, macro- and micro-scale defects were detected as seen in Fig. 6C. The presence of debris from the gloves can directly decrease the strength of the bulk material and the interface to tooth or resin-matrix composite increments [4, 5, 24]. In addition, the presence of corn starch debris can trap oxygen molecules which interfere with the polymerization of the resin-matrix composite increment layer. Corn starch establishes a cross-linking



**Fig. 9** SEM images of the resin-matrix composites retrieved from capsules and handled using **A, B** nitrile or **C–F** powdered latex gloves contaminated with human blood. SEM Images on SE mode and at **A, C**  $\times 50$  and **B, D–F**  $\times 1000$  magnification. **B, D–F** Human

blood products covered the surfaces as identified by SEM–EDX. **F** The presence of corn starch released from the powdered latex gloves

to epichlorohydrin containing not more than 2% MgO as a dispersive agent. Epichlorohydrin, which renders the corn starch absorbable, also is used as a solvent in natural and synthetic resin-matrix materials. The presence of any residual epichlorohydrin possibly could promote a partial dissolution of the resin-matrix composite leading to defects and poor mechanical properties [5].

Thus, the contamination with human saliva can bring other debris such glycoproteins, minerals, and water, as seen in Fig. 7. Saliva is mostly composed of water (99.4%), inorganic compounds including  $\text{Ca}^{2+}$ ,  $\text{K}^{2+}$ ,  $\text{PO}_4^-$ , and  $\text{Cl}^-$ , and organic compounds such as proteins, amino acids, and glycoproteins (i.e., mucin). In our study, the inorganic compounds were detected by EDS on the specimens surfaces after contact with human saliva. On

handling the resin-matrix composite, the temperature usually increases up to 47°C that is enough for the denaturation of proteins [33]. Therefore, the handling movement on friction promotes the plastic deformation of glycoproteins forming rolls, as seen in Fig. 7C. In fact, all those inorganic and organic compounds adsorbed on the surfaces can negatively affect the strength of the bulk material and the interface to tooth or resin-matrix composite increments [4, 14, 22, 24]. Some studies have also revealed that conditions of contamination with saliva significantly increased microleakage. The micro- and nano-gaps may engender staining, marginal breakdown, hypersensitivity, secondary caries, and the development of teeth pulp inflammatory reactions [6, 14, 34].

Blood contamination can also occur during the restoration procedures using adhesives and resin-matrix composites [24, 27]. Although the clinicians could visible clean (macro-scale view) the gloves with alcohol, a thin micro-scale layer of blood products can be enough for the contamination of the resin-matrix composite increments. In this study, the SEM images reveal a complex layer composed of blood products (i.e., red cells and platelets) and corn starch from the gloves. It can also be seen the presence of fissures and voids at macro- and micro-scale. In Fig. 4, it is noticed that the resin-matrix composite increment became colored with blood that would influence the esthetic behavior of the restoration. Some studies proved that proteins from blood could impair the adhesion and copolymerization of the resin-matrix composite increments layers leading to failures [7, 24, 27].

## Conclusion

Clinical handling of the resin-matrix composites provides a heterogeneous surface with macro- and micro-scale defects such as fissures, pores, and voids. Microscopic examination revealed that blood, saliva, and powder of latex gloves might remain entrapped in the resin-matrix composite after clinical handling. That can negatively affect the polymerization and adhesion of the resin-matrix composite. In this way, unpowdered gloves are required in the restoration procedure with resin-matrix composites. Also, gloves should be replaced after any potential contamination with saliva or blood. Further studies should perform chemical analysis of different brands of gloves before and after contamination and/or cleaning. Also, the contamination of the gloves and spatulas can occur after contacting other restorative materials or surfaces and therefore a careful cleaning procedure of the spatulas must be performed prior to the restoration.

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**Author Contributions** JCMS, MO, and OT designed the study while IC, RF-P, and OT performed the experimental study. IC, OT, and JCMS wrote the main manuscript text. BH, and RF-P, prepared the graphs and Figures. MO and JCMS formatted the manuscript text and improved the English language. JCMS, MO and BH provided the financial support for the present study. All the authors revised the final version of the manuscript and agreed with the submission for publication.

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**Data Availability** The data that support the findings of this study are available on request from the corresponding author [J.C.M.S]. The data are not publicly available due to them containing information that could compromise research participant privacy.

## Declarations

**Competing interests** The authors declare that they have no conflict of interests. The authors do not have any financial interest in any of the manufacturers of the test materials reported in this paper.

**Ethical approval** All procedures performed involving human participants followed the ethical standards of the research committee of the University Institute of Health Sciences (IUCS, Portugal) and therefore with the 1964 Helsinki declaration and its later amendments or comparable ethical standards (Ethical Protocols Number 13/CE-IUCS/2022). Informed consent was not necessary since all data were processed anonymously.

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