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Surface and mechanical properties of adhesives with calcium phosphates challenged to different storage media

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Aim: To evaluate the behavior of experimental dental adhesives with hydroxyapatite (HAp), alpha-tricalcium phosphate (α-TCP) or octacalcium phosphate (OCP) after storing them in three different media: dry storage, distilled water, or lactic acid. Methods: An experimental adhesive resin was formulated with bisphenol A glycol dimethacrylate, 2-hydroxyethyl methacrylate, and photoiniciator/co-initiator system. HAp (G_{HAp}), α-TCP ($G_{\alpha\text{-TCP}}$), or OCP (G_{OCP}) were added to the adhesive resin at 2 wt.%, and one group remained without calcium phosphates to be used as a control (G_{Ctrl}). The adhesives were evaluated for surface roughness, scanning electron microscopy (SEM), and ultimate tensile strength (UTS) after storing in distilled water (pH=5.8), lactic acid (pH=4) or dry medium. Results: The initial surface roughness was not different among groups ($p>0.05$). G_{HAp} showed increased values after immersion in water ($p<0.05$) or lactic acid ($p<0.05$). SEM analysis showed a surface variation of the filled adhesives, mainly for $G_{\alpha\text{-TCP}}$ and G_{HAp}. G_{HAp} showed the highest UTS in dry medium ($p<0.05$), and its value decreased after lactic acid storage ($p<0.05$). Conclusions: The findings of this study showed that HAp, OCP, and α-TCP affected the physical behavior of the experimental adhesive resins in different ways. HAp was the calcium phosphate that most adversely affected the surface roughness and the mechanical property of the material, mainly when exposed to an acid medium.

Keywords: Dentin-bonding agents. Calcium phosphates. Acids. Polymers. Tensile strength.

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Introduction

Recurrent caries at the tooth-restoration interface is one of the major causes of restoration replacement over time[1]. This outcome is related to materials' hydrolytic and enzymatic degradation when in contact with the oral environment, leading to higher surface roughness, biofilm accumulation at the margin, and caries development[2]. Moreover, restorative materials may not completely seal the tooth interface. Mainly over time, the sealing ability still is a concern since gaps are prone to caries development[3]. Restorative resin-based materials have been modified to decrease polymerization shrinkage, hydrolysis degradation, and to decrease gaps formation at the interface via a biomimetic remineralization approach[4].

Bioactive fillers have been added to resins to provide them bioactivity with the ultimate purpose of reducing the incidence of caries around the restoration's margin. Calcium orthophosphates (CaP) are the most representative fillers able to release calcium and phosphate ions, which are retained in the oral biofilm and induce dental remineralization[5-8]. CaP present different molecular forms, crystalline structures, and solubility values[9,10]. Previous studies evaluated CaP as fillers in experimental adhesive resins showing promising results such as increased bond strength[11,12] and mineral deposition at the tooth[6,8,11].

Currently, studies that evaluate ion-releasing bioactive materials, such as those filled with bioglasses and CaP, stored them in water[13,14], ethanol[13], artificial saliva[15] or simulated body fluid[5]. However, the release of ions may increase materials' roughness over time[16], especially when they are exposed to acid medium, which could occur in the presence of an acidogenic biofilm[17]. The results of a recent *in vitro* study suggest that low pH increases the surface roughness and alter the superficial topography of resin-based orthodontic adhesives[18]. The change in the roughness may indicate modifications not only in the morphology of the material but also in its chemical and physical stability[19,20]. The bioactive material must maintain its mechanical and chemical properties to seal the cavity adequately[21].

In a previous study, the authors formulated an experimental adhesive resin composed of bisphenol A glycol dimethacrylate, 2-hydroxyethyl methacrylate, and a photoinitiator/co-initiator system[11]. The material was filled with different calcium orthophosphates at 2 wt.%: hydroxyapatite (HAp; $Ca_5(PO_4)_3(OH)$), alpha-tricalcium phosphate (α-TCP; $Ca_3(PO_4)_2$), or octacalcium phosphate (OCP; $Ca_8H_2(PO_4)_6 5H_2O$)[11]. The filled adhesives were compared to the base resin without CaP (control group), and they showed a higher degree of conversion. Furthermore, the α-TCP group showed a high microshear bond strength compared with the other groups. The α-TCP and HAp groups induced mineral deposition at the tooth-resin interface, suggesting that these fillers could be an alternative to formulate bioactive dental resins.

Despite these findings, the effect of different storage media on the behavior of adhesives composed of α-TCP, HAp, or OCP was not investigated so far. The aim of this study was to evaluate the behavior of experimental adhesive resins with HAp, α-TCP, or OCP after storing them in three different storage media. The null hypotheses to be tested are: (1) there are no differences among the adhesives formulated with different CaP regarding their surface and mechanical properties; (2) different storage media do not influence the surface and mechanical properties of the adhesives.

Materials and Methods

In this study, the dependent variables analyzed are surface roughness, surface morphology, and ultimate tensile strength. Two independent variables were analyzed: (1) the variable "addition of CaP", with four different adhesives composed solely by the base resin or with 2 wt.% of hydroxyapatite (HAp), alpha-tricalcium phosphate (α-TCP), or octacalcium phosphate (OCP); (2) the variable "storage medium", in which the adhesives were immersed: dry storage, distilled water, or acidic solution (Figure 1).

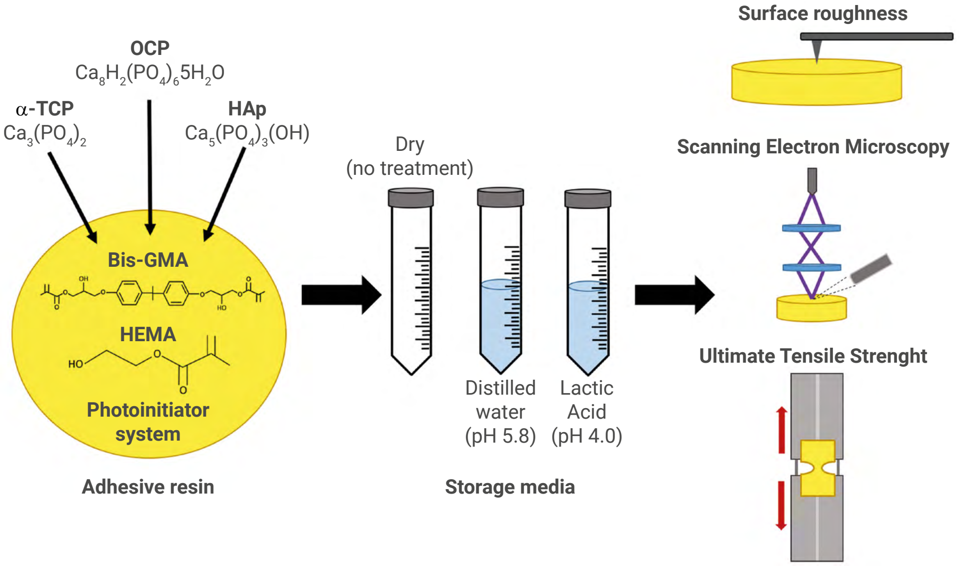

Figure 1. Representative illustration that summarizes the materials and methods of the study.

Experimental Adhesive Resin Formulation

The experimental adhesive resin was formulated mixing 66.66 wt.% bisphenol A glycol dimethacrylate and 33.33 wt.% 2-hydroxyethyl methacrylate. Camphorquinone and ethyl 4-(dimethylamino) benzoate were added as a photoinitiator system at 1 mol%. Butylated hydroxytoluene was added at 0.01 wt.% as polymerization inhibitor. These reagents were purchased from Aldrich Chemical, St Louis, MO, USA. Three CaP were previously synthesized, and they were added in this base resin at 2 wt.%: α-TCP ($G_{\alpha\text{-TCP}}$, 6.03 µm)[22], OCP (G_{OCP}, 4.94 nm)[23] and HAp (G_{HAp}, 26.7 nm)[24]. A group without CaP was used as control (G_{Ctrl}), totaling four groups. The mixture (resin/particles) was hand-mixed for 5 min, sonicated for 180 s, and hand-mixed again for 5 min.

Surface Roughness (Ra)

Five samples per group were prepared (10 mm diameter X 1 mm thickness) using a polyvinylsiloxane mold. The uncured adhesive resins were inserted in the molds between two transparent Mylar strips. The samples were light-cured for 30 s on each side (Radii Cal, SDI; Bayswater, Victoria, Australia, 1200 mW/cm^2). The top of each sample was marked and divided in half. Four measurements of surface roughness were performed immediately on one half using a profilometer (Mitutoyo, Surftest SJ–201P, Chicago, USA) with a tracing length of 2 mm and 0.25 mm cut-off. Then, each sample was submerged and stored in individual-hermetic containers for 3 days at 37 °C[17], in 10 mL[25,26] of different media as distilled water or lactic acid with pH at 5.8 and 4[17], respectively. The samples were placed vertically so that the surfaces to be tested were kept exposed to the different media. The pH of these media was evaluated along with the study via a digital pH meter (DM-22, Digimed, São Paulo, SP, Brazil). After 3 days of storage, four new measurements were performed on the other half on the top of each sample. The initial roughness (Ra1), final roughness (Ra2), and roughness variation (ΔRa) were recorded for each group.

Surface morphology via scanning electron microscopy (SEM)

The surface morphology of three samples per group used in the roughness assessment (stored in distilled water and lactic acid) was evaluated via SEM. Other three samples per group were prepared and stored in a dry environment inside a desiccator with silicon dioxide at 37 °C for 3 days to be also analyzed via SEM. The samples were placed on metallic stubs and gold-sputter coated (15–25 nm) (SDC 050, Baltec, Vaduz, Liechtenstein). SEM analysis (SEM, JSM 6060, JEOL, Tokyo, Japan) was performed under 7 kV, at 5,000´ and 8,000´ magnification.

Ultimate Tensile Strength (UTS)

Thirty samples per group were prepared in a metallic matrix with an hourglass shape (8 mm long, 2 mm wide, 1 mm thickness, and 1 mm^2 at constriction area) after photoactivation for 30 s on each side[27]. After photoactivation, the samples were measured with a digital caliper (Mitutoyo, Kawasaki, Kanagawa, Japan; accuracy of 10 μm) to obtain the constriction area of each one. Then, the thirty samples from each group were divided and submerged into 1 mL of the three media of storage (n = 10, dry environment in a desiccator, distilled water, or lactic acid) to be sat for 3 days at 37 °C. The specimens were fixed in metallic jigs with cyanoacrylate resin to be tested for tensile strength. The tests were performed in a universal testing machine (EZ Test EZ-SX, Shimadzu, Japan) at a crosshead speed of 1 mm/min. The values were obtained in newtons, and the final UTS was expressed in megapascals (MPa) using the constriction area of each sample.

Statistical Analysis

The data were analyzed using the software SigmaPlot®, version 12.0 (Systat Software, Inc., San Jose, CA, USA). Data distribution was evaluated using the Shapiro–Wilk test. One-way analysis of variance (ANOVA) was used to compare groups for initial surface roughness. Paired t test was used for each group to evaluate the difference between immediate and final surface roughness. Kruskal-Wallis was used to compare ΔRa among groups in both media, and Dunn's was used as post-hoc after immersion in lactic acid. Two-way ANOVA was used to compare groups for UTS dry medium, distilled water or lactic acid. A significance level of 0.05 was considered.

Results

The results of surface roughness before (Ra1) and after (Ra2) immersion of the experimental adhesive resins, as well as the ΔRa, in distilled water and in lactic acid are shown in Table 1. The authors did not perform other statistical analysis such as three-way ANOVA, split-plot two-way ANOVA, or two-way ANOVA with repeated measures to analyze this data because there is a dependency within the same group (the same group was tested before and after the storage in the liquids). Moreover, the samples tested for immersion in water or lactic acid are not the same since this is a destructive method and the same sample could not be immersed in both liquids one after the other. Statistical analysis with repeated measures considering different immersions (in water or lactic acid) should not be applied. In this context, the one-way ANOVA revealed no statistically significant differences among groups for Ra1 ($p>0.05$). After immersion in distilled water, G_{HAp} roughness increased ($p<0.05$), while the other groups showed no statistically significant differences ($p>0.05$). Moreover, there was no difference among groups for ΔRa after immersion in water ($p>0.05$). On the other hand, the materials presented different behavior after immersion in lactic acid solution. While there was no difference among groups for Ra1 ($p>0.05$), the roughness of $G_{α\text{-}TCP}$ and G_{HAp} increased after immersion in lactic acid ($p<0.05$) and G_{Ctrl} and G_{OCP} had no differences between Ra1 and Ra2 ($p>0.05$). When comparing ΔRa after immersion in lactic acid, G_{HAp} showed the highest variation among groups, with statistical difference in comparison to G_{Ctrl} ($p<0.05$).

Table 1. Results of surface roughness of the experimental adhesive resins with different calcium phosphates before and after the immersion in distilled water or lactic acid.

Group	Storage in distilled water			Storage in lactic acid		
	Roughness before immersion	Roughness after immersion	ΔRa (%)	Roughness before immersion	Roughness after immersion	ΔRa (%)
	(Ra1, μm)	(Ra2, μm)		(Ra1, μm)	(Ra2, μm)	
G_{Ctrl}	0.09 (±0.01) ^{Aa}	0.12 (±0.05) ^{a}	47.93 (±70.28) ^{A}	0.11 (±0.04) ^{Aa}	0.13 (±0.03) ^{a}	24.74 (±49.88) ^{B}
G_{OCP}	0.10 (±0.03) ^{Aa}	0.15 (±0.07) ^{a}	59.35 (±76.50) ^{A}	0.10 (±0.04) ^{Aa}	0.16 (±0.10) ^{a}	59.07 (±75.10) ^{AB}
$G_{α\text{-}TCP}$	0.08 (±0.03) ^{Aa}	0.13 (±0.03) ^{a}	78.85 (±97.40) ^{A}	0.10 (±0.03) ^{Aa}	0.21 (±0.06) ^{b}	116.94 (±83.58) ^{AB}
G_{HAp}	0.07 (±0.01) ^{Aa}	0.21 (±0.08) ^{b}	173.91 (±96.28) ^{A}	0.08 (±0.02) ^{Aa}	0.30 (±0.06) ^{b}	285.64 (±110.34) ^{A}

Different capital letters indicate statistically significant difference in the same column ($p<0.05$).

Different small letters indicate statistically significant difference in the same row within the same medium of storage (distilled water or lactic acid) ($p<0.05$).

The images from SEM analyses corroborate the findings of surface roughness measurement. Few differences can be observed in the surface of G_{Ctrl} between dry storage and distilled water storage (Figure 2 A–D). Higher irregularities are identified for G_{Ctrl} when it was stored in lactic acid (Figure 2 E, F). As well as observed for G_{Ctrl}, almost no differences are observed among images of dry storage and distilled water for G_{OCP} (Figure 3 A–D). When exposed to lactic acid, more irregularities are observed (Figure 3 E, F). Compared with G_{Ctrl}, G_{OCP} showed higher defects when exposed to lactic acid. The surface of $G_{\alpha\text{-TCP}}$ stored in distilled water, and lactic acid (Figure 4 C–F) showed larger irregularities than G_{Ctrl}. G_{HAp} showed the highest difference on the surface between dry storage and distilled water storage (Figure 5 A–D) compared with G_{Ctrl}, G_{OCP}, and $G_{\alpha\text{-TCP}}$. In addition, after storing in lactic acid (Figure 5 E, F), G_{HAp} presented higher irregularities, with larger cracks and cavities with irregular borders distributed on an irregular surface.

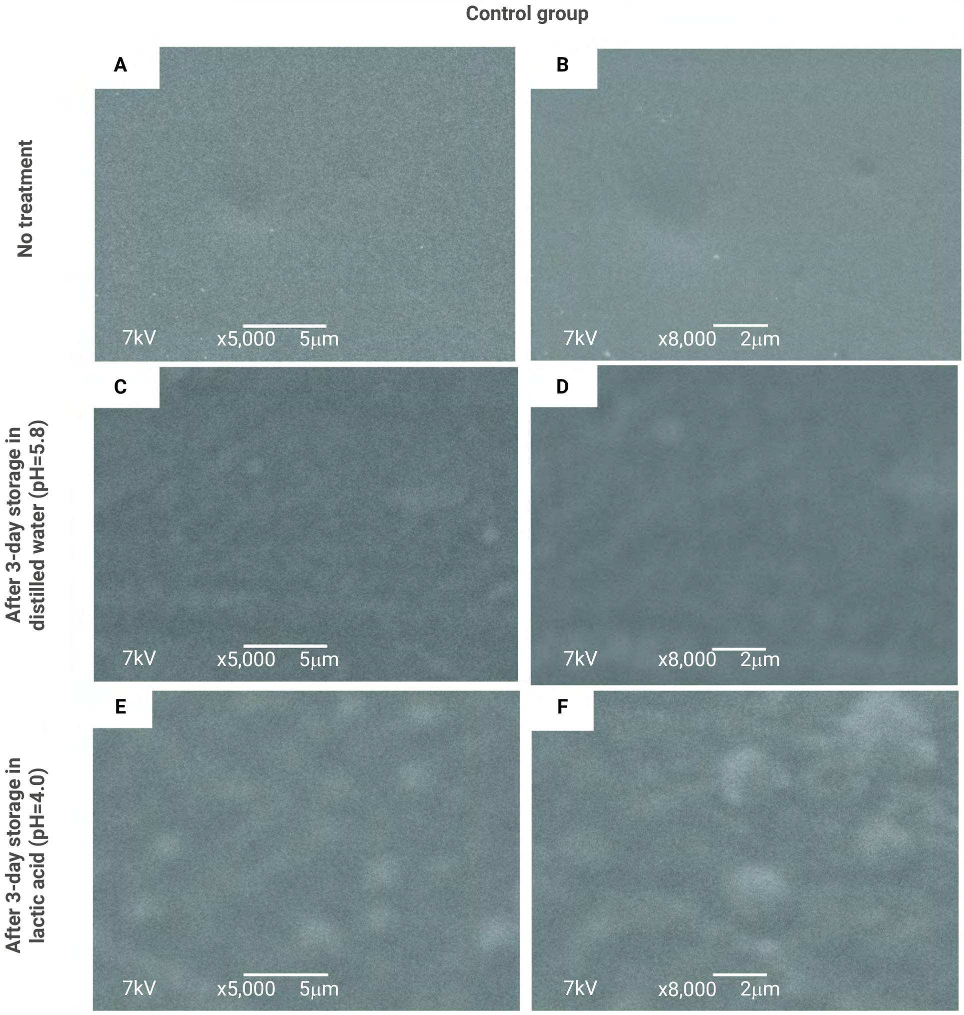

Figure 2. Scanning electron microscopy of G_{Ctrl} at dry (no treatment), distilled water, or lactic acid storage. Few differences are observed for this group without calcium phosphates addition when the surface is exposed to dry storage (A and B) compared to that after water storage (C and D). After the exposition to lactic acid, G_{Ctrl} presents higher irregularities (E and F).

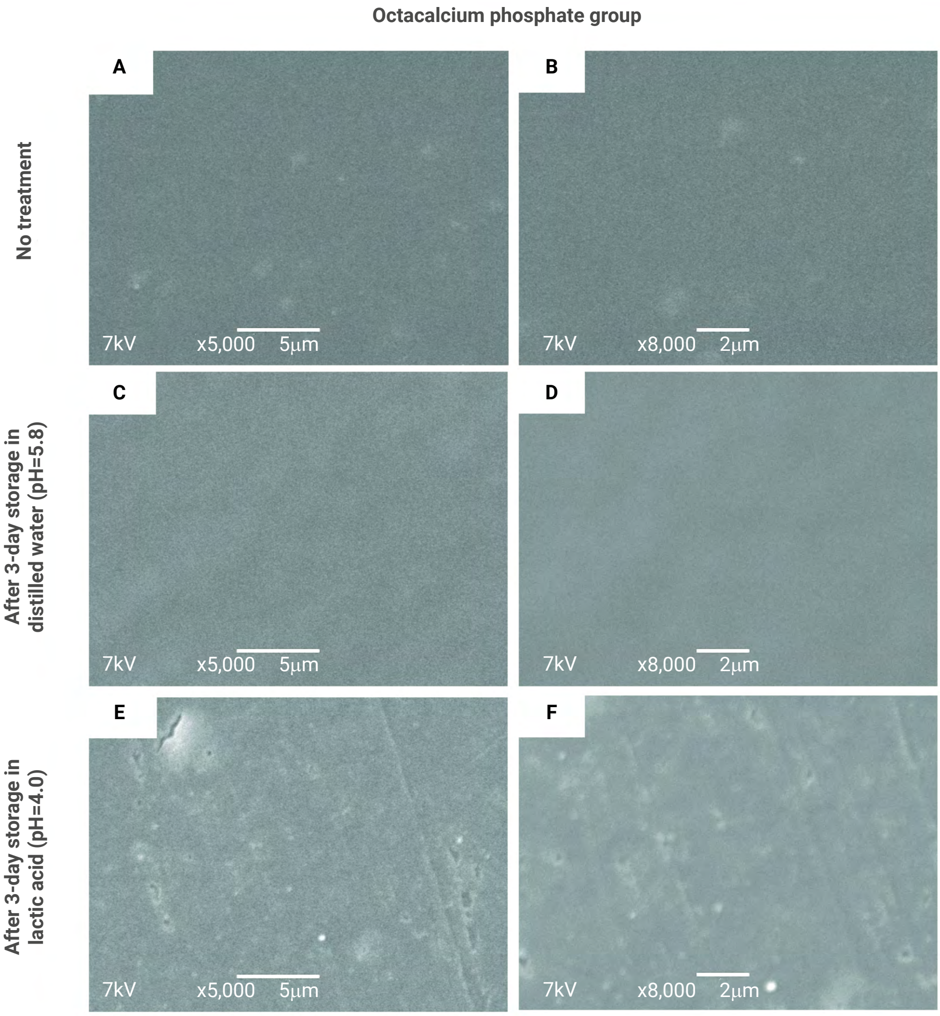

Figure 3. Scanning electron microscopy of G_{OCP} at dry (no treatment), distilled water, or lactic acid storage. Few differences are observed for G_{OCP} when the surface is exposed to dry storage (A and B) compared to that after water storage (C and D). After the exposition to lactic acid, this group showed higher irregularities (E and F).

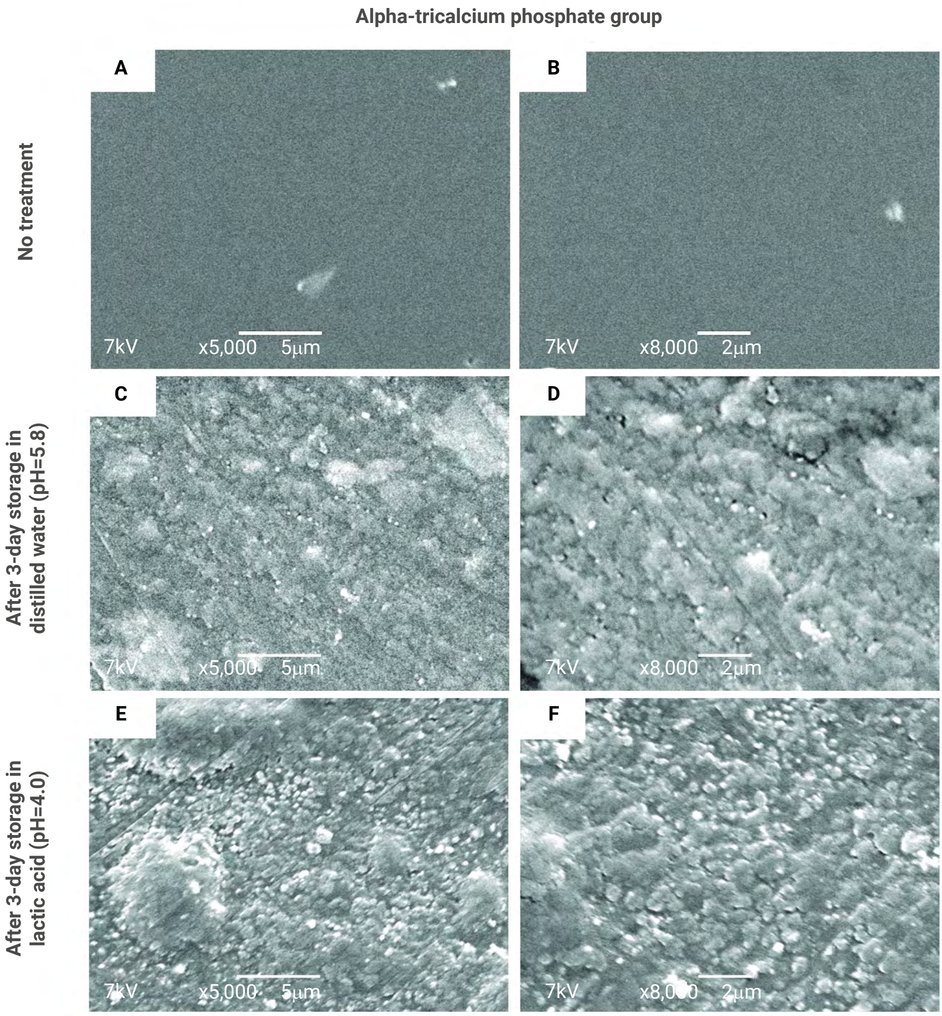

Figure 4. Scanning electron microscopy of $G_{\alpha\text{-TCP}}$ at dry (no treatment), distilled water, or lactic acid storage. High differences are observed within this group when "no treatment" (A and B) is compared to the surfaces after water (C and D) or lactic acid (E and F) exposition. Observe that $G_{\alpha\text{-TCP}}$ shows a much more irregular surface after water or lactic acid storage in comparison to G_{Ctrl} and G_{OCP}.

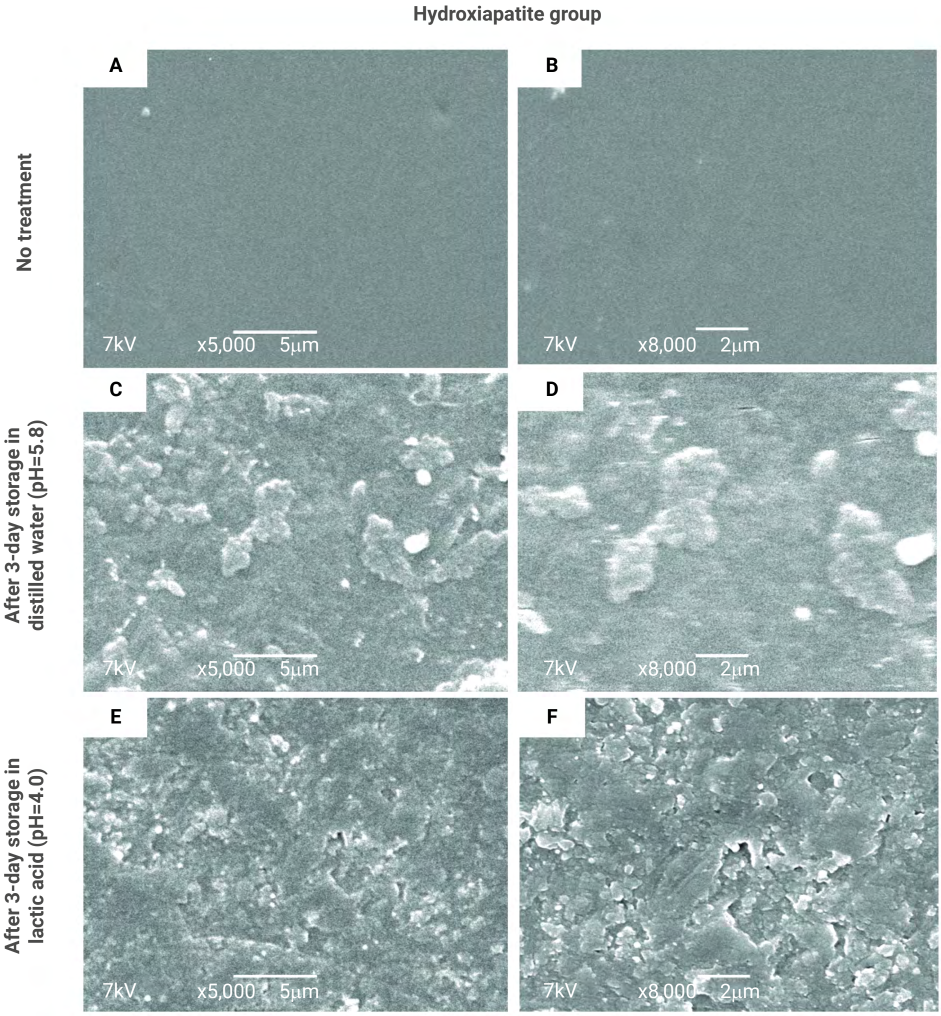

Figure 5. Scanning electron microscopy of G_{HAp} at dry (no treatment), distilled water, or lactic acid storage. High differences are observed within this group when "no treatment" (A and B) is compared to the surfaces after water (C and D) or lactic acid (E and F) exposition. Observe that, mainly after the lactic acid exposition, G_{HAp} shows more irregularities than the other adhesive resins, areas with large cracks and cavities surrounded by irregular borders.

The results of the UTS of the experimental adhesive resins exposed to different media are shown in Table 2. After dry storage, the values ranged from 28.69 (±10.93) MPa for G_{Ctrl} to 46.34 (±10.72) MPa for G_{HAp}, with a statistically significant difference between G_{Ctrl} and G_{HAp} ($p<0.05$). The values of UTS after distilled water storage ranged from 30.53 (±6.07) MPa for G_{OCP} to 39.26 (±9.44) MPa for G_{Ctrl}, without differences among groups ($p>0.05$). After lactic acid storage, the values of UTS ranged from 29.56 (±6.43) MPa for G_{HAp} to 33.87 (±11.93) MPa for G_{Ctrl}, also without differences among groups ($p>0.05$). G_{HAp} was the only group that presented a statistically significant difference among the different storage media, with lower UTS values after lactic acid storage (29.56 ± 6.43 MPa) than dry medium (46.34 ± 10.72 MPa) ($p<0.05$).

Table 2. Results of ultimate tensile strength of the experimental adhesive resins with different calcium phosphates after their storage in different media: dry, distilled water or lactic acid.

Group	Dry (MPa)	Water (MPa)	Lactic Acid (MPa)
G_{Ctrl}	28.69 (±10.93) ^{Ba}	39.26 (±9.44) ^{Aa}	33.87 (±11.93) ^{Aa}
G_{OCP}	35.96 (±17.19) ^{ABa}	30.53 (±6.07) ^{Aa}	32.61 (±6.93) ^{Aa}
$G_{\alpha\text{-TCP}}$	34.43 (±11.48) ^{ABa}	38.14 (±7.88) ^{Aa}	30.36 (±13.22) ^{Aa}
G_{HAp}	46.34 (±10.72) ^{Aa}	34.60 (±9.89) ^{Aab}	29.56 (±6.43) ^{Ab}

Different capital letters indicate statistically significant difference in the same column ($p<0.05$).
Different small letters indicate statistically significant difference in the same row ($p<0.05$).

Discussion

Bioactive materials with ion-releasing fillers such as CaP have been investigated to induce the remineralization process of dental tissues[7]. Studying the behavior of bioactive materials when exposed to different media could assist in understanding their physical properties. In this study, adhesive resins with HAp, α-TCP, or OCP were tested regarding their physical properties after storing in distilled water, lactic acid, or dry medium. There were significant differences among the adhesives with different CaP, leading to the rejection of the first null hypothesis. Furthermore, the storage media influenced the behavior of the adhesives, which led us also to reject the second null hypothesis.

Dental materials are susceptible to suffering chemical and physical modifications in the oral environment due to hydrolysis and to bacterial enzymes, leading to their degradation over time[28]. High surface roughness contributes to the attachment of microorganisms and biofilm development[28], besides making it more difficult to maintain hygiene[28]. In 1990, an *in vivo* study using fluorethylenepropylene or cellulose acetate strips suggested that the surface roughness of Ra = 0.2 μm was a threshold value for bacterial retention in intraoral surfaces[29]. Moreover, it is suggested that when the values are lower than 0.2 μm, the materials' chemical properties may be more important for biofilm formation than the surface roughness.

Currently, lower values up to 0.1 μm are recommended for polishing resins with inorganic particles to reduce biofilm accumulation[2]. $G_{\alpha\text{-TCP}}$ and G_{HAp} presented Ra higher than 0.2 μm after immersing in lactic acid, and G_{HAp} showed values above 0.2 μm even after distilled water storage. In addition to inducing remineralization, ion-releasing materials have been suggested to inhibit biofilm formation by increasing the pH around them and delaying bacterial colonization[30]. However, the exposed CaP on the materials' surface, accompanied by the increase of surface roughness, was shown not to decrease bacterial adhesion[17]. In this study, as well as in the previous report[17], the samples were not subject to pH cycles, which could lead to different results and, maybe, lower surface roughness differences. However, this method is a way to evaluate the material over an extreme situation.

Besides the surface roughness measurement, the surface morphology of the experimental adhesive resins was evaluated via SEM, which supported the results observed for Ra. In distilled water, $G_{\alpha\text{-TCP}}$ and G_{HAp} showed larger holes interspersed with small prominences on an irregular surface compared with α-TCP or HAp in a dry medium. A uniform pattern over the entire surface of $G_{\alpha\text{-TCP}}$ and G_{HAp} was observed, probably due to a slight hydrolytic effect on the resin matrix[31]. After lactic acid storage, the variation of surface integrity was more pronounced for CaP groups than for G_{Ctrl}. This result corroborated the values found for ΔRa, mainly for G_{HAp}, which should statistically significant difference for G_{Ctrl} after immersion in acid. We could also observe that the G_{OCP} showed small grooves and holes scattered on the surface after lactic acid storage, while $G_{\alpha\text{-TCP}}$ presented a similar pattern to $G_{\alpha\text{-TCP}}$ immersed in distilled water but with cracks in greater quantity.

The group containing HAp showed larger cavities with irregular limits distributed over a slightly smooth surface. It is possible that these cavities were created due to the release of HAp agglomerates because low values of surface area were found for HAp previously synthesized by the same method[24]. Nanoparticles are prone to agglomeration due to their high surface energy. In composite resins, agglomerates of nanoparticles presented lower adhesion to the organic matrix compared with microparticles, detaching over time[32]. These agglomerates jeopardize the composite resins compared to microparticles, making the material more susceptible to mechanical failure and surface wear[33]. This process could occur with HAp in the experimental adhesive resin because the small molecules of lactic acid could diffuse through pores among HAp agglomerates and produce faster dissolution[34].

In the mechanical analysis, the immediate UTS increased with HAp incorporation compared with G_{Ctrl}, without differences for $G_{\alpha\text{-TCP}}$ and G_{OCP}. In distilled water, there were no differences in UTS, neither among groups nor between the same group comparing dry storage and distilled water storage. On the other hand, the mechanical performance was different after immersion in lactic acid solution, with G_{HAp} showing reduced UTS compared with G_{HAp} in dry storage. This group also presented the highest surface roughness variation after exposure to the lactic acid solution. These results suggest that, even without statistically significant differences among $G_{\alpha\text{-TCP}}$, $G_{HAp,}$ and G_{OCP} after storing in lactic acid, the UTS could be jeopardized for G_{HAp} over time in acid conditions.

The pH of the medium and the type of filler determine the release rate of the ions[7], altering materials' mechanical properties. HAp is soluble in acid solutions[9], insoluble in alkaline solutions, and distilled water, while α-TCP and OCP are more soluble than HAp at neutral pH[9]. Even so, there were no differences for G_{Ctrl}, $G_{OCP,}$ and $G_{\alpha\text{-TCP},}$ depending on the storage media. The rationale for that may be a better distribution of OCP and α-TCP within the polymer, leading to lower CaP–resin interfaces to be exposed and to react with lactic acid. Another important factor related to the solubility of CaP is the size of the particles, in which the decrease to a nanoscale level can increase their dissolution[35]. Furthermore, CaP stability decreases with the increase of impurities' presence[9] and the method used to synthesize the HAp[24] leads to the presence of carbonates in the final powder, which may have favored its dissolution[9].

Here we observed the different behavior of bioactive resin-based restorative materials depending on the type of CaP incorporated into them. Interestingly, the physical response of the materials when facing various storing media depended on the CaP added. Therefore, further evaluations are encouraged *in situ* and *in vivo* to deeply understand the biological effects of these bioactive materials in patients with different risks of caries.

In conclusion, the findings of this study showed that HAp, OCP, and α-TCP affected the physical behavior of the experimental adhesive resins in different ways. HAp was the CaP that most adversely affected the surface roughness and the mechanical property of the material, mainly when exposed to an acid medium.

Acknowledgments

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