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Aging Reduces the Anticaries Effect of Antibacterial Adhesive – An In Vitro Biofilm Study

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**Purpose:** This in vitro study investigated whether aging different restorative materials influences secondary caries development using a short-term in vitro biofilm model, hypothesizing that the antibacterial adhesive employed may lose its effect over time.

**Materials and Methods:** Sixty enamel-dentin blocks were divided into 6 groups with n = 10 per group. The groups were restored with three different restorative materials, of which each sample contained an artificial gap: composite with conventional adhesive (CCA; negative control), composite with an antibacterial adhesive (CAA), and amalgam (A; positive control). Half of the groups were prepared fresh and half of the groups were submitted to an aging protocol consisting of water storage, thermocycling, storage in human saliva, and storage in 0.9% saline solution. All specimens were subjected to an intermittent 1% sucrose biofilm model for 20 days to create artificial caries lesions. Lesion progression in the enamel and dentin next to the different materials was measured as lesion depth (LD) and mineral loss (ML), using transverse wavelength independent microradiography (T-WIM). Regression analysis was used to evaluate the effect of aging on LD and ML per restorative material, corrected for gap size.

**Results:** In the amalgam group, aging led to shallower lesions and less mineral loss. Fresh amalgam samples showed an average lesion depth of 156.65 ± 39.18 μm at wall dentin locations. Aged amalgam samples had an average lesion depth of 73.42 ± 73.50 μm. Fresh CAA samples showed lower average surface mineral loss values (9104 ± 2631 μm•vol%) than did fresh CCA samples (13166 ± 4769 μm•vol%). After aging, this effect was absent, and the average mineral loss in the CAA group was 13382 ± 5586 μm•vol%, while in the CCA group it was 15518 ± 9283 μm•vol%.

**Conclusion:** Aging can influence secondary caries development either positively or negatively depending on the kind of restorative material. Antibacterial adhesives may lose their effectiveness over time.

**Keywords:** adhesives, aging, amalgams, antimicrobial monomers, antibacterial adhesive, bacterial challenge, adhesives, composite restorations, secondary caries.

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Secondary caries refers to a caries lesion developing close to the margins of an existing restoration.\textsuperscript{19} The presence of a restoration may promote or slow the caries process in several ways. The surface texture of a restorative material can promote plaque retention and the formation of secondary caries.\textsuperscript{4} Furthermore, the presence of an imperfect interface between tooth and restorative material increase the risk of secondary caries development.\textsuperscript{11}
The mere presence of a restorative material close to demineralizing enamel or dentin may already influence the rate of caries progression. When enamel or dentin demineralize in the absence of a restorative material, it releases dissolution products into the surrounding fluid until saturation is reached. However, when enamel or dentin demineralize in proximity to a restoration, a local lack of dissolution mineral is created by the non-dissolving restorative material. At the interface with the restorative material, a greater plaque volume is available for diffusion of dissolution products away from the surface of enamel or dentin, which enhances caries progression.

Specific properties of restorative materials can also influence the caries process. Some restorative materials release ions which can be used in the remineralization process, eg, fluoride in glass-ionomer cement. New composites and adhesives have been developed with the addition of antibacterial components, which are claimed to show an anti-caries effect. Amalgam is thought to reduce caries development due to bacteriostatic properties of the material and the formation of oxides in the tooth-amalgam interface which help to seal the margins. Several clinical studies have shown that composite restorations are correlated with a higher failure rate due to secondary caries than amalgam restorations in high caries-risk patients.

The development and improvement of composite materials is ongoing. Adhesives containing antibacterial components have been shown to reduce secondary caries formation in vitro. However, the examination of fresh samples may be of limited value if information is lacking on the behavior over time in a clinical environment. It is well known that the oral environment can degrade restorative materials. Antibacterial adhesives or composites may lose their efficacy clinically by dilution or washout of unbound antibacterial components.

The adhesive interface of composite restorations seems to become more unstable after aging, which may increase the risk of secondary caries. Therefore, the aim of this study was to investigate whether the cariostatic properties of an antibacterial adhesive remain effective after an aging process. A conventional adhesive was used as a negative control, while samples restored with amalgam served as a positive control.

### MATERIALS AND METHODS

#### Preparation of the Samples

Secondary caries development in gaps next to three restorative materials was evaluated in vitro. The three restorative materials used were:

- **Composite with conventional adhesive (CCA):** Clearfil AP-X composite + Clearfil SE Bond (Kuraray Noritake; Tokyo, Japan) (n = 20)
- **Composite with antibacterial adhesive (CAA):** Clearfil AP-X composite + Clearfil Protect Bond (Kuraray) (n = 20)
- **Amalgam (A):** Dispersalloy amalgam (regular set, Dentsply International; Milford, DE, USA) (n = 20)

Of each restorative material, block samples (3.2 x 3.2 x 2.0 mm) were prepared according to the method of an earlier study. The composite and adhesive were condensed into a putty mold (3.2 x 3.2 x 2.0 mm) and light cured for 20 s. Primer was applied into the mold first, followed by the adhesive, resembling the clinical situation with primer toward the side of the tooth substrate. After polymerization of the adhesive, composite material was condensed and light cured.

The amalgam was mixed for 17 s and condensed in the mold with a green compacting handpiece. Excess amalgam was removed by grinding with 800-grit paper one day after setting.

Dentin-enamel block samples (3.2 x 3.2 x 2.0 mm) were cut from bovine incisors and polished.

Per group, half of the restoration block samples were prepared fresh (n = 10) before subjecting them to a biofilm model, and the other half of the samples were submitted to an aging protocol first (Table 1).

<table>
<thead>
<tr>
<th>Group sample sizes by material and aging condition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Composite with conventional adhesive</td>
</tr>
<tr>
<td>Aged</td>
</tr>
<tr>
<td>Fresh</td>
</tr>
</tbody>
</table>

The aging protocol consisted of 4 weeks of storage in water at room temperature (renewed weekly), followed by:

- 4 weeks of thermocycling (31,063 cycles, 10 s in 55°C distilled water, 20 s of draining, 10 s in 5°C distilled water, 20 s draining), followed by
- 4 weeks of storage in human saliva renewed every two days (saliva was collected from one volunteer in the morning before oral hygiene and eating), followed by
- 8 weeks of storage in 0.9% saline solution renewed every two days, followed by
- 8 weeks of storage in water at room temperature, renewed weekly.
After aging, the restorative block samples and the dentin-enamel block samples were placed together on a polystyrene bar with flowable composite. A matrix of 100 μm thickness was placed between the restorative block and the dentin-enamel block to create a large gap. Exact gap sizes were measured with a WF10X lens (Future-Tech: Tokyo, Japan) of a microhardness tester (Microhardness Tester FM-700, mfc; Tokyo, Japan) bearing further lenses (M10/0.25 210/0, Future-Tech). The width of the gap was measured on top of the samples, as it was the entrance for bacteria and nutrients.

Samples were sterilized by gamma radiation (total dose: 4.08 KGy) at the Regional Center of Oncology/Radiotherapy Service, Faculty of Medicine in Pelotas, Brazil. A microradiographic image of the configuration of the samples is shown in Fig 1.

**Biofilm Model**

The specimens were subjected to the biofilm model described by Van de Sande et al. Human saliva was used as the inoculum and the enamel-dentin blocks were the substrate. The nutrient growth medium used for the experi-
ment was chemically Defined Medium enriched with Mucin (DMM), which was pH of 6.8. Biofilms were grown under intermittent sucrose exposure.

After approval by the local Research Ethics Committee (School of Dentistry, Federal University of Pelotas, Pelotas, RS, Brazil) under protocol No. 1.634.686/2016, 24 ml of fresh stimulated saliva was collected from one healthy subject (a 43-year-old female without caries activity). Saliva was stimulated by paraffin film and collected in the morning (during fasting), after the volunteer had abstained from oral hygiene measures for 24 h. The sterilized specimens were aseptically transferred into sterile wells (24-well tissue culture plate) and 0.4 ml of homogenized saliva was placed onto each specimen with utmost care taken to cover the gap. After 1-h incubation at 37°C, 1.8 ml of DMM 1% sucrose was added. After 6 h, the growth medium was replaced for DMM without sucrose. DMM renewal was performed up to the end of the experiment, alternating mediums with (6 h) and without sucrose (18 h). The biofilms were incubated anaerobically with increased CO₂ using the Anaerobac system (Probac do Brasil produtos Bacteriológicos; Santa Cecilia, SP, Brazil) in anaerobic jars for 20 days at 37°C.

At the end of the experiment, samples were cleaned by immersing them first in 0.9% saline solution and subsequently removing the visible biofilms with a piece of gauze soaked in distilled water.

**Transversal Wavelength Independent Microradiography (T-WIM)**

T-WIM images of the samples were take before exposure to the biofilm model at baseline (T₀) and after 20 days (T₂₀) using the method of Thomas et al.²⁸ The settings for microradiography were 60 kV and 30 mA for an exposure time of 8 s. Lesion depth and mineral loss were measured by subtracting the values at baseline from the values recorded for the same sample after 20 days.

T-WIM is a microradiographic method for measuring mineral content in a transversal geometry with thick tooth sample sections (≤ 3.2 mm). In contrast to other methods, T-WIM samples are not destroyed after analysis and therefore caries lesion development can be monitored over time. T-WIM samples are imaged on film with polychromatic x-rays together with an aluminium/zinc stepwedge (94% Al / 6% Zn alloy). The film is calibrated by means of the stepwedge, as the relationship between the absorption of x-rays by aluminium and the tooth material is known. The grey values of the stepwedge are related to the thickness of the stepwedge. Subsequently, the mineral content of the tooth sample with a certain thickness can be calculated by these grey values. In sound enamel, the mineral content should be around 85 vol% and ca 47 vol% in sound dentin. In areas where mineral loss has occurred due to a caries lesion, visible as a radiolucency compared to sound enamel or dentin, the mineral loss can be quantified in integrated mineral loss (μm·vol%) or lesion depth (μm).

**Film Processing and Image Measurements**

After exposure, the films were developed (10 min), fixed (7 min), rinsed and dried. A digital image of each sample was recorded with a light microscope at 10X (Leica Microsystems; Wetzlar, Germany) and a CMOS camera (Canon EOS 50D; Tokyo, Japan). Microradiographs were quantitatively assessed for the presence of wall lesions and surface lesions. A lesion with a progressing front parallel to the outer surface of the tooth sample was considered an outer surface lesion. A wall lesion was defined as a lesion progressing perpendicularly to the restoration-tooth interface. LD and ML for T-WIM were defined as the distance on the microradiograph between the thresholds of 8 vol% and 78.3 vol% mineral for enamel and between 8 vol% and 43.2 vol% mineral for dentin.²⁸ Each sample was measured at four locations using a software program developed in our laboratory (School of Dentistry, Federal University of Pelotas): 1. for surface lesions in enamel (surface), 400 μm from the tooth restoration gap; 2. for wall lesions in enamel (wall enamel), 200 μm above the enamel-dentin junction (EDJ); 3. for wall lesions in dentin (wall dentin₁) 200 μm below the EDJ, and for wall lesions in dentin (wall dentin₂) 800 μm below the EDJ. The measurements in dentin were averaged to create one wall dentin location.

Baseline measurements (T₀) were subtracted from measurements after 20 days (T₂₀) in order to estimate true lesion depth and mineral loss at T₂₀. The differences were used in the statistical analysis.
**Table 2a** Results of multiple linear regression analysis using amalgam (positive control group)

<table>
<thead>
<tr>
<th>Location</th>
<th>Variable</th>
<th>Mineral loss (μm•vol%)</th>
<th>Lesion depth (μm)</th>
<th>95% CI effect</th>
<th>95% CI effect</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Effect</td>
<td>p</td>
<td>Lower</td>
<td>Upper</td>
</tr>
<tr>
<td>Surface</td>
<td>Constant</td>
<td>7960</td>
<td>0.165</td>
<td>-3662</td>
<td>19582</td>
</tr>
<tr>
<td></td>
<td>Aging</td>
<td>-785</td>
<td>0.763</td>
<td>-6226</td>
<td>4656</td>
</tr>
<tr>
<td></td>
<td>Gap</td>
<td>14</td>
<td>0.351</td>
<td>-17</td>
<td>45</td>
</tr>
<tr>
<td>Wall enamel</td>
<td>Constant</td>
<td>8056</td>
<td>0.245</td>
<td>-6139</td>
<td>22250</td>
</tr>
<tr>
<td></td>
<td>Aging</td>
<td>-2667</td>
<td>0.406</td>
<td>-9312</td>
<td>3978</td>
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<tr>
<td></td>
<td>Gap</td>
<td>5</td>
<td>0.780</td>
<td>-33</td>
<td>43</td>
</tr>
<tr>
<td>Wall dentin</td>
<td>Constant</td>
<td>893</td>
<td>0.513</td>
<td>-1855</td>
<td>3640</td>
</tr>
<tr>
<td></td>
<td>Aging</td>
<td>-1260</td>
<td>0.055</td>
<td>-2546</td>
<td>26</td>
</tr>
<tr>
<td></td>
<td>Gap</td>
<td>10</td>
<td>0.007</td>
<td>3</td>
<td>17</td>
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</tbody>
</table>

Values in italics are statistically significant.

**Table 2b** Results of multiple linear regression analysis for group CCA: APX + SE

<table>
<thead>
<tr>
<th>Location</th>
<th>Variable</th>
<th>Mineral loss (μm•vol%)</th>
<th>Lesion depth (μm)</th>
<th>95% CI effect</th>
<th>95% CI effect</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Effect</td>
<td>p</td>
<td>Lower</td>
<td>Upper</td>
</tr>
<tr>
<td>Surface</td>
<td>Constant</td>
<td>4087</td>
<td>0.620</td>
<td>-13311</td>
<td>21486</td>
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<td>Aging</td>
<td>186</td>
<td>0.964</td>
<td>-8599</td>
<td>8972</td>
</tr>
<tr>
<td></td>
<td>Gap</td>
<td>33</td>
<td>0.255</td>
<td>-27</td>
<td>92</td>
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<tr>
<td>Wall enamel</td>
<td>Constant</td>
<td>3885</td>
<td>0.524</td>
<td>-8919</td>
<td>16689</td>
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<tr>
<td></td>
<td>Aging</td>
<td>-1837</td>
<td>0.550</td>
<td>-8303</td>
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<tr>
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<td>Gap</td>
<td>23</td>
<td>0.285</td>
<td>-21</td>
<td>66</td>
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<tr>
<td>Wall dentin</td>
<td>Constant</td>
<td>-102</td>
<td>0.950</td>
<td>-3365</td>
<td>3162</td>
</tr>
<tr>
<td></td>
<td>Aging</td>
<td>-1158</td>
<td>0.161</td>
<td>-2806</td>
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</tr>
<tr>
<td></td>
<td>Gap</td>
<td>13</td>
<td>0.022</td>
<td>2</td>
<td>24</td>
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</tbody>
</table>

Values in italics are statistically significant.

**Table 2c** Results of multiple linear regression analysis for group CAA: APX + PB

<table>
<thead>
<tr>
<th>Location</th>
<th>Variable</th>
<th>Mineral loss (μm•vol%)</th>
<th>Lesion depth (μm)</th>
<th>95% CI effect</th>
<th>95% CI effect</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Effect</td>
<td>p</td>
<td>Lower</td>
<td>Upper</td>
</tr>
<tr>
<td>Surface</td>
<td>Constant</td>
<td>14407</td>
<td>0.001</td>
<td>6475</td>
<td>22339</td>
</tr>
<tr>
<td></td>
<td>Aging</td>
<td>5386</td>
<td>0.016</td>
<td>1114</td>
<td>9658</td>
</tr>
<tr>
<td></td>
<td>Gap</td>
<td>-20</td>
<td>0.150</td>
<td>-47</td>
<td>8</td>
</tr>
<tr>
<td>Wall enamel</td>
<td>Constant</td>
<td>-2603</td>
<td>0.485</td>
<td>-10296</td>
<td>5089</td>
</tr>
<tr>
<td></td>
<td>Aging</td>
<td>1183</td>
<td>0.555</td>
<td>-2960</td>
<td>5325</td>
</tr>
<tr>
<td></td>
<td>Gap</td>
<td>42</td>
<td>0.004</td>
<td>15</td>
<td>69</td>
</tr>
<tr>
<td>Wall dentin</td>
<td>Constant</td>
<td>1374</td>
<td>0.238</td>
<td>-949</td>
<td>3696</td>
</tr>
<tr>
<td></td>
<td>Aging</td>
<td>-862</td>
<td>0.171</td>
<td>-2113</td>
<td>389</td>
</tr>
<tr>
<td></td>
<td>Gap</td>
<td>11</td>
<td>0.011</td>
<td>3</td>
<td>19</td>
</tr>
</tbody>
</table>

Values in italics are statistically significant.
**Statistical Analysis**

Multiple linear regression analysis was performed to evaluate the effect of aging on the LD and ML for each restorative material and measurement location, corrected for gap size. The data were corrected for clustering. CCA and CAA groups were further compared with an independent samples t-test for each location and aging condition. p-values < 0.05 were considered statistically significant.

**RESULTS**

Of the 60 samples, 6 (n = 1 aged amalgam, n = 1 fresh amalgam, n = 2 aged CCA, n = 2 fresh CCA) were discarded due to fracture of the enamel-dentin block off the polystyrene bar. The mean gap size was 309 μm (± 76 μm) and the overall range of gap sizes was 176 to 527 μm. The mean values of LD (lesion depth) and ML (mineral loss) (Fig 2) of each material at the three different locations are shown in a bar chart per material (aged/fresh) at each measurement location. Figure 3 shows an example of a microradiograph of a surface and wall lesion.

Amalgam demonstrated lower LD and ML after aging at all locations. CAA showed a trend for higher LD and ML in surface and wall enamel locations after aging, but similar values for fresh and aged samples at wall dentin locations. CCA exhibited a trend for higher LD and ML at the surface location after aging but similar values for fresh and aged samples at wall dentin locations. The bar graphs show raw data which are not corrected for differences in gap size.

Tables 2a through 2c display the results of multiple linear regression analysis conducted for each material and each location. Gap size had a significant effect on lesion depth and mineral loss in the wall dentin groups of all materials, and larger gaps consistently led to larger wall lesions (0.001 < p < 0.024). Aging had a significant inhibitory effect on the depth of wall lesions next to amalgam, as seen in the wall dentin location (p = 0.055 for mineral loss, p = 0.016 for lesion depth). Aged amalgam samples on average had a lesion depth 53.57 μm lower than their fresh counterparts. Also, aging significantly influenced surface lesion development next to CAA, leading to higher lesion depth and mineral loss in the aged vs fresh group (p = 0.016 for mineral loss, p = 0.029 for lesion depth). On average, aged CAA samples had wall dentin lesion depths that were 72.38 μm higher than their fresh counterparts.

To take a closer look at the effect aging had on CAA samples, CCA and CAA groups were compared before and after aging at all locations. The results of the independent samples t-tests can be found in Tables 3a and 3b. Fresh CAA samples showed significantly shallower lesions and less mineral loss at the surface lesion than did CCA samples (p = 0.035 for mineral loss, p = 0.06 for lesion depth). After aging, this difference was absent, and CAA and CCA groups performed similarly.

**DISCUSSION**

In this in vitro study, we investigated whether an antibacterial adhesive would remain effective after artificial aging. Deeper lesions and more mineral loss were observed in aged samples that had been restored with composite with antibacterial adhesive, compared to fresh samples. Aged samples restored with amalgam showed shallower lesions and less mineral loss than fresh samples.

There is no consensus on how to age restorative materials and simulate wear. In an overview by Lambrechts et al., different wear methods were investigated; the authors concluded that there is no method which exactly replicates the oral environment with all its biological variations. The total aging time in the present study was 6 months. According to Kermanshahi et al., biodegradation of the composite-dentin interface occurs within 7 days after immersion in esterase solution. According to an in vitro study by Mahler et al., a gap in a high-copper amalgam restoration immersed in a 1.0% NaCl solution can become sealed with corrosion products within 8 weeks. These time periods were used as a benchmark for an aging protocol of 6 months with different methods.

Restorative materials and tooth samples were prepared as blocks due to the analytical method used (T-WIM). This method allows radiographic imaging without the destroying the samples. However, this technique is limited because the samples must be placed perfectly parallel to the direction of the x-ray beam, or it will cause a shadow that can be mistaken for a lesion. For that reason, blocks of restorative material and dental material were placed together on a polystyrene bar with a gap of 100 μm parallel to the direction of the x-ray beam. The gap size of 100 μm was chosen to allow some biofilm formation in this space, as opposed to wall lesion formation relying solely on microakkage in gaps as narrow as 30 μm.

A pilot study showed that aging the samples fixed together on the polystyrene bar was not feasible, due to differences in temperature and water uptake between the polystyrene bars and the restorative materials. This caused all restoration blocks to detach from the polystyrene bars during thermocycling, and therefore the samples (dentin blocks and restorative material) were put together after aging. Amalgam corrodes over time, and during aging it releases cariostatic agents, such as Ag, Cu, and Zn ions, which inhibit bacterial growth and caries lesion formation. The fact that some influence of aging with amalgam was observed despite the lack of opportunity for its corrosive products to fill the gap during the aging process is possibly related to amalgam releasing cariostatic agents after corrosion on the surface, or a difference in surface texture.

Aging had a significant effect on the surface lesions in the CAA group. This effect was the opposite of that which aging had on amalgam, i.e., aging promoted lesion formation in the antibacterial adhesive group. Clerfil Protect Bond (used in the CAA group) is an adhesive containing the antibacterial monomer 12-methacryloyloxydodecylpyridinium bromide (MDPB). Clerfil Protect Bond exerts an anticario-
genic effect by the release of this unpolymerized mono-
mer.\textsuperscript{9,12,23} During aging, the unreacted MDPB may leak out, so that the anticariogenic effect of Clearfil Protect Bond decreases with aging, resulting in larger surface lesions.

In the group of samples restored with composite and conventional adhesive, aging did not show any effect on secondary caries lesion formation. CAA samples restored with Clearfil Protect Bond performed better than CCA samples restored with Clearfil SE Bond when fresh. After aging, CCA and CAA groups behaved similarly, reinforcing the hypothesis that unreacted MDPB may have leaked out during aging, making a protective effect of Clearfil Protect Bond evident in the fresh samples but absent after aging.

Clinically, amalgam seems to have a slight advantage over resin composite regarding secondary caries due to the aging process. If the goal is to improve resin materials with releasable non-bound antibacterial agents, the effect would be more beneficial if the release were controlled over time.

Improving the performance of composite materials in high caries-risk patients could provide such materials with an advantage. At the moment, however, Clearfil Protect Bond may not provide sufficient protection in long-term models. Clinically investigating this material’s performance in high caries-risk patients would be an interesting topic for further research.

\textbf{CONCLUSION}

Within the limitations of the present study, aging influenced caries development next to restorations. Samples restored with Clearfil Protect Bond showed smaller surface lesions than samples restored with Clearfil SE Bond in fresh conditions. After aging, this protective effect was no longer present. Aging decreased wall lesion development next to amalgam, in line with previous data and expectations.

\begin{table}
\centering
\caption{Comparison of CCA and CAA groups for lesion depth (μm) using the independent samples t-test}
\begin{tabular}{|l|c|c|c|c|}
\hline
Location & Fresh & & Aged & \\
\hline & CCA & CAA & CCA & CAA \\
\hline Surface & Mean ± SD & 183.2 ± 73.1 & 130.9 ± 33.2 & 210.8 ± 124.2 & 197.7 ± 80.7 \\
\hline p-value & & p = 0.060 & & p = 0.791 \\
\hline 95% CI difference & & -2.4 – 106.9 & & -89.5 – 115.7 \\
\hline Wall enamel & Mean ± SD & 229.2 ± 78.1 & 169.7 ± 40.9 & 175.0 ± 91.3 & 233.0 ± 189.6 \\
\hline p-value & & p = 0.053 & & p = 0.440 \\
\hline 95% CI difference & & -1.0 – 119.9 & & -213.3 – 97.4 \\
\hline Wall dentin & Mean ± SD & 115.7 ± 56.7 & 129.9 ± 50.0 & 111.7 ± 52.6 & 123.5 ± 58.1 \\
\hline p-value & & p = 0.429 & & p = 0.532 \\
\hline 95% CI difference & & -50.4 – 21.9 & & -49.8 – 26.2 \\
\hline
\end{tabular}
\end{table}

\begin{table}
\centering
\caption{Comparison of CCA and CAA groups for mineral loss (μm • vol\%) using the independent samples t-test}
\begin{tabular}{|l|c|c|c|c|}
\hline
Location & Fresh & & Aged & \\
\hline & CCA & CAA & CCA & CAA \\
\hline Surface & Mean ± SD & 13166 ± 4769 & 9103 ± 2631 & 15518 ± 9283 & 13382 ± 5586 \\
\hline p-value & & p = 0.035 & & p = 0.553 \\
\hline 95% CI difference & & 321 – 7804 & & -5338 – 9611 \\
\hline Wall enamel & Mean ± SD & 10143 ± 5235 & 8717 ± 3457 & 9798 ± 5557 & 12265 ± 6339 \\
\hline p-value & & p = 0.497 & & p = 0.400 \\
\hline 95% CI difference & & -2923 – 5776 & & -8510 – 3576 \\
\hline Wall dentin & Mean ± SD & 3554 ± 2704 & 4242 ± 2131 & 3268 ± 1528 & 3980 ± 1775 \\
\hline p-value & & p = 0.399 & & p = 0.213 \\
\hline 95% CI difference & & -2325 – 948 & & -1850 – 427 \\
\hline
\end{tabular}
\end{table}
ACKNOWLEDGEMENTS

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REFERENCES

17. Maske TT, Kuper NK, Cenci MS, Huysmans M. Minimal gap size and dentin wall lesion development next to resin composite in a microcosm biofilm model. Caries Res 2017;51:475–481.

Clinical relevance: Secondary caries development can be influenced by the aging of restorative materials.