In vitro Effect of Occlusal Loading on Cervical Wall Lesion Development in a Class II Composite Restoration

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Abstract
The aim of this study was to determine the effect of simulated occlusal loading on wall lesion development in cervical gaps of class II composite restorations in vitro. Sixty-four extracted human molars received standardized (4.0 × 4.2 × 3.0 mm) box preparations. The teeth were randomly assigned to one of two restoration groups: restoration with a normal or a low E-modulus composite material (CLEARFIL AP-X: E-modulus 16.8 GPa or CLEARFIL MAJESTY ES Flow: E-modulus 6.6 GPa). A metal matrix was placed at the bottom of the box for each restoration, creating a cervical gap of about 100 μm wide. Samples were exposed to simulated caries lesion development in a lactic acid solution (pH 4.8) for 8 weeks in a Rub&Roll device. Half of the samples were subjected to 90 N cyclic loading. After demineralization, the teeth were sectioned. Wall lesion development was measured using microradiography (transversal wavelength-independent microradiography) in two different locations (location 1: 1,000 μm and location 2: 1,600 μm from the gap entrance) and recorded in lesion depth (LD) (μm) and mineral loss (μm × vol%). Linear regression modeling was used to estimate the effect of loading and material on wall lesion development. Mean wall LD in location 1 across all groups was 150.83 μm with a standard deviation (SD) of 61.83 μm. In location 2, mean overall wall LD was 102.98 μm with an SD of 64.92 μm. Linear regression showed no significant effect of either loading or material on wall lesion development. Occlusal loading had no significant effect on secondary caries lesion development in composite class II restoration in this in vitro study.

Introduction
In general dental practices, secondary caries is of great influence on the survival of restorations. It is the most important reason for restoration failure, especially after the first 2 years [Mjör, 2005; Qvist, 2008; Kopperud et al., 2012]. Patient risk factors play an important role in the development of secondary caries lesions [Opdam et al., 2010]. As secondary caries lesions share the etiology of primary caries lesions, good oral hygiene and a diet low in carbohydrates are important for prevention of second-
ary caries. Local factors studied in in vitro and in situ studies such as marginal integrity, cavity disinfection, restorative material, and adhesive material also influence the development of simulated secondary caries lesions [Hollanders et al., 2018; Coelho et al., 2020; Rechmann et al., 2021]. The clinical significance of these local factors remains unclear as long-term clinical studies are scarce [Askar et al., 2020].

In in vitro models, the presence of an interfacial gap between restorative and tooth material was shown to increase secondary caries lesion presence and depth [Maske et al., 2017; Lehmensiek et al., 2018]. With an interfacial gap present, two specific lesion areas have been described: a wall and a surface lesion. The wall lesion develops along the interfacial gap, whereas the surface lesion occurs on the outside surface [Hals and Nernae, 1971]. The minimum gap width necessary for wall lesion development seems to be smaller than 30 μm [Nassar and Gonzalez-Cabezas, 2011; Maske et al., 2017]. However, the clinical relevance of gaps remains unclear as microleakage does not seem to be correlated with poorer clinical performance [Heintze, 2007].

For the development of the wall lesion, the diffusion rate into and out of the gap may be a limiting factor in small gaps, and increased hydrodynamic flow through loading may lead to increased lesion development [Kuper et al., 2013; Askar et al., 2017]. Occlusal forces deform a restoration and lead to expulsion of gap volume. With a force of 50 N on the restoration with a rigid cusp, the expected volume change is only 0.5% in a 100 μm wide gap [Hollanders et al., 2020]. However, if this volume change occurs frequently, such as in chewing and swallowing, it might have an impact on the rate of diffusion.

Materials with a higher elastic modulus show less deformation than materials with a low elastic modulus when the same force is applied. In the context of approximal restorations, this could mean that a restorative material with a lower elastic modulus leads to more expulsion of gap volume under the same load and consequently to deeper wall lesions next to it. A previous in vitro study into the effect of loading and material properties found the opposite effect [Askar et al., 2017]. Most in vitro wall lesion studies have used a very simplified model or a model focusing a gap opening, possibly overestimating the effect of loading [Kuper et al., 2013; Khvostenko et al., 2015; Askar et al., 2017].

The aim of this study was to determine the effect of simulated occlusal loading on wall lesion development in cervical gaps of realistically modeled class II composite restorations in vitro. The working hypothesis was that occlusal loading has a promoting effect on the depth of wall lesions.

Materials and Methods

Sample Size Calculation

A sample size calculation was performed for the lesion depth (LD) in wall lesions between loaded and unloaded groups. We expect wall LDs of around 100 μm in the control group. The expected standard deviation based on similar studies is around 50 μm [Kuper et al., 2013; Maske et al., 2017]. Assuming a significance level of 5% and a power of 80%, 16 samples per group are necessary to show a difference of 50 μm between the groups [Sealed Envelope Ltd., 2012]. A factorial design was chosen along with a multivariable analysis, to make the best possible use of the available samples.

Sample Preparation

Extracted human molars, stored in water at 4°C, were embedded into methacrylate at the roots using a standardized mold with the occlusal surface parallel to the bottom of the mold at a set distance of 27 mm. Standardized box preparations of 4.0 × 4.2 × 3.0 mm were cut into the mesial or distal side of the teeth using a cylindrical bur in a high-speed handpiece with constant water cooling (see Fig. 1). Subsequently, the 64 teeth were randomly assigned to one of four groups.

The samples in groups A and B were restored using CLEARFIL AP-X composite (Kuraray Noritake), material A. The samples in groups C and D were restored using CLEARFIL MAJESTY E-S flow (Kuraray Noritake), material M. These materials were chosen for their different elastic moduli. CLEARFIL AP-X has an elastic modulus of 16.8 GPa, whereas CLEARFIL MAJESTY Flow has an elastic modulus of 6.6 GPa, according to the manufacturer’s information. Groups A and C were unloaded, while groups B and D were loaded.

At the bottom of each box, a metal matrix was placed to create a gap of ±100 μm wide at the cervical outline. Restorations were made using a conventional bonding procedure according to manufacturer’s instructions. Etching gel (LOT 212341; DMG) containing 37% phosphoric acid was applied onto the sides of the box for 20 s, followed by a thorough rinse with water and air spray for 10 s. SA Primer (LOT 950020; Kuraray) was applied onto the sides of the box but not air dried. CLEARFIL PHOTO BOND (LOT 5D0043 and 9C0048; Kuraray) was applied onto the sides of the box in a very thin layer using a microbrush. These layers were not air dried to prevent the adhesive material from reaching the bottom of the box but not air dried. CLEARFIL PHOTO BOND (LOT 5D0043 and 9C0048; Kuraray) was applied onto the sides of the box in a very thin layer using a microbrush. These layers were not air dried to prevent the adhesive material from reaching the bottom of the box below the matrix. The adhesive layer was light cured using a Bluephase 20i curing light (Ivoclar Vivadent) for 20 s (wavelength 380–515 nm, max 1,100 mW/cm²). Next, the resin composite was applied in 3–4 layers of up to 1.5 mm thickness. The first layer of composite was cured for 40 s, whereas subsequent layers were cured for 20 s each. The samples were photographed under a light microscope at ×40 magnification. A ruler was photographed at the same magnification to calibrate measurement of the gap entrance width.
Caries Simulation
All molars were exposed to 0.1 M lactic acid solution (pH corrected to 4.8), containing 0.5 ppm fluoride in a Rub&Roll device [Ruben et al., 2014]. The device contains 4 metal rods covered by a rubber tube. The molars were placed in a cylinder rotating at 20 rpm. In Figure 1d, we present an image of the samples positioned in the device. The tubes were positioned so that they just touch the cusps of the molars. The loaded samples were positioned 1 mm higher using a small rubber stop. In this way, a load of approximately 90 N was put on each loaded sample (unpublished results). A graph showing the relation between the thickness of the rubber shim and the resulting load is found in online supplementary Figure 1 (see www.karger.com/doi/10.1159/000522589 for all online suppl. material). The lactic acid solution and the rubber tube sections were changed weekly. After 4 weeks, one sample of each group was taken out for measurement of LD. The lesions in these pilot samples were analyzed in different locations to determine the definitive measurement locations. The intended time of demineralization was adjusted to 8 weeks to obtain a lesion of approximately 100 μm in the control group.

Microradiography and Image Measurements
After exposure to the artificial carious lesion model, each sample was cut into a slab in the mesial-distal direction in the middle of the molar. The slabs were ground down to 1 mm thickness using a 400-grit sandpaper. Transversal wavelength-independent microradiography images of the samples were made using the method of Thomas et al. [2006]. This method was previously validated using TMR as the gold standard [Thomas et al., 2006]. The settings for the microradiography were 40 kV and 25 mA with an exposure time of 8 s. A step wedge with the same absorption coefficient as tooth material (94% Al/6% Zn alloy) was photographed along with each sample to allow quantification of LD and mineral loss (ML).

After X-ray exposure the films were developed (10 min), fixed (7 min), rinsed, and dried. A light microscope (Leica Microsystems, Wetzlar, Germany) with a magnification of ×10 was used together with a fixed digital camera (Canon EOS 50D; Tokyo, Japan) to obtain a digital image. The digital images were opened in Adobe Photoshop (Creative Cloud 2020; Adobe Systems, San Jose, CA, USA). If the composite was still present in the image, the area of the composite was selected and colored black (R = 0, G = 0, B = 0), so gap size was not measured as LD. The software was calibrated using a photograph of a small ruler made with the exact same microscopic settings. Then, the thickness of the enamel along the gap was measured. To allow for LD and ML measurement at consistent locations, lines were placed at set distances of 1,000 μm and 1,600 μm from the gap entrance in Adobe Photoshop.

Then, the edited image was opened in custom software developed in our lab (WIM software [Thomas et al., 2006]). The step wedge was selected first, allowing the software to calibrate the mineral content for each specific image. The software uses the step wedge calibration to correspond the gray values in the image with the actual mineral content since the step wedge has a known absorption coefficient. Using the lines drawn, LD (μm) and ML (μm × vol%) were measured in different locations (see also Fig. 1). The wall lesion locations were thought to be of most interest regarding the working hypothesis. However, to place the lesion progression speed in the model in perspective, surface lesions were measured as well.
• Location 1: wall lesion; 1,000 μm from the gap entrance, perpendicular to the tooth-restoration interface, a bar of 50 μm wide was selected from air to healthy dentin.
• Location 2: wall lesion; measured 1,600 μm from the gap entrance, perpendicular to the tooth-restoration interface, a bar of 50 μm wide was selected from air to healthy dentin.
• Location 3: surface lesions were measured 500 μm from the enamel-cementum junction perpendicular to the root surface, a bar of 50 μm wide was selected from air to healthy dentin.

Statistical Analysis
Samples with an enamel thickness >950 μm were excluded due to inability to measure both wall lesion locations. Descriptive statistics were performed. The effect of loading and restorative material on LD/ML was analyzed by linear regression. The possible confounding variables gap width and enamel thickness were included in the analysis as independent variables. A separate model was used for each location and for each outcome variable. Statistical analyses were performed in R statistical program. The significance level was set at 0.05.

Results
Five samples were excluded from the analysis because of the enamel thickness being too large (samples 1A, 2C, 9A, 9D, and 12C). One pilot sample from each group was sacrificed after 4 weeks to check the simulated wall lesion development. Therefore, 55 samples were included in the analyses. The average gap width in these 55 samples was 106.3 μm, with a standard deviation of 33.8 μm. The average gap width, enamel thickness, wall LD, and ML per group can be found in Table 1. ML and LD measurements for each location were strongly correlated.

The mean LD for both wall locations is presented in Figure 2. At wall location 1, lesions were slightly deeper in the unloaded groups, although this difference was not statistically significant. At wall location 2, lesions were equally deep or slightly deeper in loaded groups, though this difference was also not statistically significant. For ML, these results are similar (see Fig. 3).

Results from the linear regression analysis can be found in Table 2. There were no statistically significant differences between the different materials or loading conditions. Neither gap width nor enamel thickness had an effect on the lesion formation (LD or ML) in this study.

Discussion/Conclusion
In this study, we found no statistically significant differences between the loaded and unloaded samples. Mean differences that were observed were small and probably not clinically relevant, and there appeared to be an opposite trend for the two locations, therefore showing no overall effect when averaging the two locations. This could imply a difference in shape of the lesions due to the loading and potentially increased hydrodynamic flow.

A previous in vitro study by Askar et al. [2017] showed increased ML for wall lesions in loaded samples compared to unloaded samples. However, the sample design in that study was very different than the one used in this study. The study by Askar et al. [2017] used an in vitro biofilm model, while this study used an acidic gel. Biofilm models more closely approximate the clinical process of caries lesion development. However, in that model, smaller rectangular slabs of dentin with a thinner composite layer of 2-mm thick were used, likely allowing for more deformation of the gap area. Our model used a...
clinically relevant restoration design, with a much greater material thickness. The current model is more likely to reflect realistic composite dimensions and shows no effect of occlusal loading. However, with higher loads, such as occur in bruxism or in restorations with more substantial defects, such as debonded bridge abutments, there may still be an effect.

The load of 90 N was distributed by the rubber tube over the occlusal surface. Although this protocol has been shown to produce clinically relevant wear [Ruben et al., 2014; Ruben et al., 2018], gap deformation has only been shown in a finite elements model [Hollanders et al., 2020]. A force of 50 N spread out through a rubber tube led to a change in gap volume of only 0.15% in a 100-μm wide gap in that simulation. Such a small deformation may not lead to clinically relevant diffusion increase. The current literature shows that the maximum voluntary bite force at the location of the first molar is around 500 N [Rohrle et al., 2018]. If the maximum bite force is spread out over multiple teeth, the force on each molar will be less than

Table 1. Mean and SD of gap width, enamel thickness, LD, and ML for all locations and loading/material conditions

| Group                        | Material A                      | Material M                      |
|------------------------------|---------------------------------|---------------------------------|
|                              | unloaded (n = 13)               | loaded (n = 15)                 | unloaded (n = 13)               | loaded (n = 14)               |
|                              | mean   | SD     | mean   | SD     | mean   | SD     | mean   | SD     |
| Gap width, μm                | 83.6   | 34.8   | 100.7  | 24.5   | 113.8  | 26.3   | 126.4  | 36.0   |
| Enamel thickness, μm         | 318.2  | 286.1  | 579.3  | 254.5  | 467.0  | 165.9  | 541.6  | 303.0  |
| LD, μm                      |                                  |                                  |                                  |                                  |
| Wall location 1              | 147.4  | 75.1   | 133.1  | 59.0   | 174.9  | 55.6   | 150.7  | 55.7   |
| Wall location 2              | 90.3   | 64.1   | 108.3  | 62.4   | 103.3  | 73.6   | 108.7  | 65.5   |
| Surface location 3           | 353.6  | 124.8  | 312.7  | 166.5  | 285.4  | 143.7  | 331.9  | 156.8  |
| ML, μm × vol%                |                                  |                                  |                                  |                                  |
| Wall location 1              | 1,929  | 1,260  | 1,867  | 1,271  | 2,693  | 1,234  | 1,805  | 1,052  |
| Wall location 2              | 1,490  | 730    | 1,806  | 812    | 1,970  | 937    | 1,852  | 805    |
| Surface location 3           | 7,180  | 2,988  | 6,657  | 3,508  | 5,675  | 3,263  | 7,248  | 3,052  |

SD, standard deviation.

Table 2. Results of linear regression

| Area of analysis | Variable                    | LD, μm | 95% CI of effect | ML, μm × vol% |
|------------------|-----------------------------|--------|------------------|---------------|
|                  |                             | effect | p value          | effect        | p value        |
|                  |                             | lower  | upper           | lower         | upper         |
| Wall location 1  | Intercept                   | 139.3  | –               | 198.8         | 1,889          | 719            | 3,058         |
|                  | Loading                     | –8     | 0.729           | –59.5         | 41.9           | 58             | 0.907          | –938           | 1,054         |
|                  | Material^1                  | 25.8   | 0.324           | –26.3         | 78.0           | 772            | 0.137          | –253           | 1,796         |
|                  | Enamel thickness            | –0.0   | 0.302           | –0.1          | 0.0            | –1             | 0.344          | –2             | 1             |
|                  | Gap width                   | 0.2    | 0.428           | –0.4          | 0.8            | 3              | 0.609          | –9             | 15            |
|                  | Loading: material^2         | –15.7  | 0.647           | –84.3         | 52.8           | –934           | 0.17           | –2,281         | 413           |
| Wall location 2  | Intercept                   | 87.2   | –               | 151.7         | 1,542          | –742           | 2,342         |
|                  | Loading                     | 11.5   | 0.677           | –43.5         | 66.5           | 400            | 0.244          | –282           | 1,082         |
|                  | Material^1                  | 10.8   | 0.702           | –45.7         | 67.4           | 511            | 0.149          | –189           | 1,213         |
|                  | Enamel thickness            | 0.0    | 0.432           | –0.0          | 0.1            | –0             | 0.425          | –1             | 1             |
|                  | Gap width                   | –0.1   | 0.812           | –0.7          | 0.6            | 1              | 0.838          | –7             | 9             |
|                  | Loading: material^2         | –7.4   | 0.842           | –81.7         | 66.9           | –500           | 0.281          | –1,422         | 421           |

^1 Material A was used as the reference. ^2 Interaction of independent variables loading and material.
that total maximum. Furthermore, bite force during chewing has been measured previously at 37% of the maximum voluntary bite force [Gibbs et al., 1981]. Therefore, the forces in this model may have been slightly lower than clinically occurring forces but appear to be in a similar range. More research about the clinical situation is necessary before deeming occlusal forces irrelevant in secondary caries formation.

Several limitations are present in this study. In our goal to produce clinically relevant restorations, the biological variation between samples was substantial. Because different molars had different crown heights and enamel thickness, choosing a standard measurement location was challenging. Because of very thick enamel at the bottom of the box, 5 samples had to be excluded. The differences in shape between teeth may also have contributed to the differences in LD and ML by moderating loads differently. The lesion measurement method used thick sections (1 mm) of the samples to analyze LD and ML. This method has been previously validated using TMR as the gold standard [Thomas et al., 2006]. The advantage of the thicker sections is that a thicker (larger) part of the lesion is measured, instead of a very thin and possibly not representative part of the lesion. However, the downside of this technique is slight blurring and less precise measurements. The results from measuring a thicker section present more of an average over the lesion region, instead of a cross-section at one specific location, as produced by TMR. Since this technique is still destructive, it cannot be used to longitudinally assess lesion development in the same sample.

In conclusion, this study showed no significant effect of either loading or material on wall lesion development. With the currently available data, the likelihood of a relevant effect of occlusal loading on secondary caries lesion development in composite class II restoration gaps appears small. However, clinical data are required to confirm that preliminary conclusion.

Statement of Ethics

Extracted human teeth were collected anonymously as part of routine medical care. Ethical approval for use of these samples for research purposes was not required for this study in accordance with local and national guidelines. Written informed consent from participants was not required in accordance with local and national guidelines.

Conflict of Interest Statement

The authors have no conflicts of interest to declare.

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Author Contributions

Conception and design of the work: A.C.C.H., J.L.R., N.K.K., and M.-C.D.N.J.M.H.; data acquisition and analysis: A.C.C.H. and J.L.R.; and writing and revising the work: A.C.C.H., J.L.R., N.K.K., and M.-C.D.N.J.M.H.

Data Availability Statement

After publication, the raw and edited data as well as the syntax used for the analyses will be available through the DANS EASY archive.

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