The effect of thermocycling on microleakage analysis of bulk filled base composite in comparison to incrementally placed nanohybrid composite in class II MOD restorations
(An in vitro study)

Lena A. Hassan, B.D.S. (1)
Adel F. Ibraheem, B.D.S., M.Sc. (2)

ABSTRACT
Background: The study aim was to evaluate thermocycling effect on microleakage of occlusal and cervical margins of MOD cavity filled with bulk filled composites in comparison to incrementally placed nanohybrid composite and to evaluate the difference in microleakage between enamel and dentin margins for the three materials groups.

Materials and method: Forty eight maxillary first premolars were prepared with MOD cavities. Samples were divided into three groups of sixteen teeth according to material used: Grandio: Grandio. SDR: SDR +Grandio. X-tra: X-tra base + Grandio. Each group was subdivided into two according to be thermocycled or not. After 24 hrs immersion in 2% methylene blue, samples were sectioned and microleakage was estimated.

Results: Thermocycling significantly increased microleakage at occlusal margin in Grandio group compared to other groups. SDR composite use before and after thermocycling significantly reduced microleakage at occlusal and cervical enamel margins compared to other groups. Grandio group had non significant difference to X-tra group in microleakage before thermocycling at occlusal and cervical enamel margins while it had a significant increase after thermocycling. No material had significantly reduced dentin margin microleakage before or after thermocycling. Only SDR group before thermocycling, significantly reduced microleakage in enamel margin compared to dentin margin.

Conclusion: Thermocycling did not increase microleakage in all the groups except for Grandio group in occlusal margin. SDR group showed reduced microleakage in occlusal and enamel margins in comparison to other groups. None of the materials reduced microleakage in dentin margin.

Keywords: Microleakage, thermocycling, bulk filled, nanohybrid.

INTRODUCTION
A perfect restoration should provide a permanent and perfect seal between the tooth structure and the restoration margins. Any discrepancy in the seal will create a microscopic gap permitting the passage of fluids with its contained bacteria, ions and molecules between the restoration and the tooth structure, named as microleakage (1). Microleakage is a significant problem associated with composite restorative materials and it happens due to multiple reasons: as one of the outcomes of polymerization shrinkage of composite resins, due to the mismatch between the thermal expansion coefficients of the tooth and the composite or the mismatch between the elastic moduli of the tooth and the restoration (1,2). Many techniques to reduce polymerization shrinkage and stress were investigated in in vitro studies as: light irradiation technologies like using quartz tungsten halogen (QTH) or light emitting diode (LED), using different irradiation techniques (conventional or soft-start QTH irradiation), placing composite resin with different placement techniques (bulk or incremental placement) or the use of an intermediate flowable layer (3-8).

One of these approaches to reduce stress is the incremental placement of composite as a way to reduce composite constraint and volume during curing. It has been suggested that the oblique incremental restorative technique could limit the effects of polymerization shrinkage at the cavosurface margin (9). However, the restoring of large MOD restorations with composite resins using this technique is time consuming (10).

There had been multi significant developments to reduce polymerization shrinkage stress either by using composite resin that uses nanotechnology in their fillers so that the filler loading of modern composite resins often exceeds 60% of the filler volume (10,11) or by using modified or non-methacrylate monomer resin formulations (12). The most recent development in composite resins are bulk filled flowable composite resins to be used under conventional composite resin materials as a liner or a base (13,14) with depth of cure exceeding 4 mm (15). The modified methacrylate resin has a slow polymerization rate through the use of a polymerization modulator (16), the filler content is reported as (68% wt.) for SDR and (75% wt.) for X-tra base (14,17). This means that the time consuming incremental technique for both mesial and distal proximal boxes in Class II cavities could be completed in a single increment with...
bulk filled flowable composite bases and the remaining increments for occlusal cavity would be performed as normal with conventional composite resins (18).

MATERIALS AND METHODS

Samples selection and mounting
Forty eight extracted upper first premolar teeth for orthodontic purposes belong to patients of age 18-24 years stored for no more than 3 months examined under magnifying lens to exclude any samples with caries and cracks. The roots of teeth were covered with a layer of wax short of the cervical line in 3mm and a base of cold cure acrylic was mounted.

Cavity preparation
A modified dental surveyor was used to standardize cavity preparations so the long axis of the bur was parallel to the long axis of the tooth during the preparation. Fissure bur (No: 835314009) was used and replaced after four preparations. The teeth received MOD cavity of 3mm width and 2mm occlusal depth measured from cavosurface margin. Mesial margin (0.5-1mm) below cemento-enamel junction with axial depth 3mm and distal margin (0.5-1mm) above cemento-enamel junction with axial depth 2mm. Length of gingival seat was 1.5mm for butt joints margins (1,2,19-22). Cavity depth and width was checked by digital caliper and periodontal probe.

Samples distribution and restorative technique
After teeth preparation matrix band and retainer were placed. Xeno Self-etch one step adhesive was applied following the manufacturer instructions and light cured from occlusal direction for 20 seconds. The teeth were randomly distributed and named according to the filling materials used into three groupseach contain sixteen samples as follows:

- **Group Grandio:** Restored with Grandio material placed with oblique incremental layering technique with 1mm thickness triangular wedges, by filling the mesial (dentin) box with 6 wedges and distal (enamel box) by 4 wedges followed by 4 triangular for the occlusal remaining cavity each in 1mm thickness layer measured with periodontal probe and cured for 20 seconds.

- **Group SDR:** Restored with SDR bulk filled low shrinkage base composite in 1 bulk placed layer that starts from the box toward the occlusal surface so that it extends 1mm short of the occlusal cavosurface margin and with thickness 4mm for the mesial box and 3mm for the distal box and cured for 20 seconds, then one occlusal layer of 1mm thickness of Grandio composite is applied and cured for 20 seconds.

- **Group X-tra:** Restored with X-tra base bulk filled low shrinkage base composite in 1 bulk placed layer that starts from the box toward the occlusal surface so that it extends 1mm short of the occlusal cavosurface margin with 4mm thickness from the mesial side and 3mm from the distal side and cured for 10 seconds, then one occlusal layer of 1mm thickness of Grandio composite is applied and cured for 20 seconds.

Light curing device was checked by digital radiometer before every curing of the samples to ensure 768 mW/cm² light intensity for each time. After curing of the restoration the band and retainer were removed and the occlusal surfaces were finished using low grit diamond finishing burs and polished with rubber points the proximal margins were finished with Sof-Lex flexible discs (4,16,20,23,24).

Samples storage and thermocycling of the samples:
All the specimens were stored for 24hrs and each group was subdivided into two sub groups of eight teeth each:

- **Initial:** for direct microleakage measurement.
- **Thermo:** to be thermocycled.

Thermocycling was carried out by soaking the specimens alternatively into (5-55 ±1-2°C) water bath chambers with 30 seconds immersion time in each bath and 10 seconds transition time (25). The thermocycling continued for (66.2 hours)all the 24 specimens were thermocycled in the same time (26,27).

The thermocycling machine
The thermocycler used in this study was fabricated (by Dr. Lena A. Hassan) following the design of Thermocycler 1000 (SD Mechatronik co. for dental research equipment, Germany) with modification of the original design (Fig.1).

Microleakage measurement:
All the samples were dried and two layers of nail varnish were applied short 1mm from the restoration margins. The root apices were sealed with glass-ionmer cement and immersed in 2% methylene blue dye for 24 hours. The samples had been washed under running water and dried. The samples were blocked with cold cure resin then the teeth were longitudinally cut in the center and perpendicular to the long axis of the sample into four pieces using microtome with a disk thickness of 0.01mm cutting at high speed with water coolant. The first cut dissected the tooth mesiodistally and the second cut was buccolingually. The presence of microleakage
was done by visualization by two observers using stereomicroscope at 40X magnification.

**Occlusal dye penetration scoring system** (20,22,28):
- 0: No dye penetration
- 1: Leakage not deeper than dentino-enamel junction.
- 2: Leakage deeper than dentino-enamel junction.
- 3: Leakage along occlusal and/or axial wall.
- 4: Leakage into dentinal tubules.

**Cervical dye penetration scoring system** (22,29):
- 0: No dye penetration.
- 1: Dye penetration not exceeding the middle of cervical wall.
- 2: Dye penetration exceeding the middle of cervical wall.
- 3: Dye penetration up to half the axial wall length.
- 4: Dye penetration along full the axial wall.

Statistical analysis of the results was done by using one way ANOVA test for non parametric (Kruskal Wallis test), Mann Whitney U-test and Wilcoxon signed rank test.

**RESULTS**

In order to see the effect of thermal aging on microleakage, Mann-Whitney U test was applied and it showed that at the occlusal margin there was significant difference in microleakage median score between initial and thermocycling phases in group Grandio (P=0.005), while there was a non significant difference between initial and thermocycling phases for SDR and X-tra groups. At the enamel and dentin margins all the groups showed a non significant difference in microleakage median scores between initial and thermocycling phases.

Kruskal-Wallis test was applied to show the difference in microleakage among the three groups of materials and the results were confirmed by Mann-Whitney U test. It showed that for the occlusal margin in the initial phase there was a significant difference in median scores among the three groups with the highest median score in both groups Grandio and X-tra (median score was the same=IV) and with non significant difference between them. While the lowest median score was in group SDR (SDR+Grandio) (median score=II) with a significant difference between SDR group and Grandio group and a significant difference between SDR group and X-tra group. In the thermocycling phase there was a significant difference in median scores among the three groups with the highest median score in both groups Grandio and X-tra (median score was the same=IV) and with non significant difference between them. While the lowest median score was in group SDR (median score=II) with a significant difference between SDR group and Grandio group and a significant difference between SDR group and X-tra group.

Wilcoxon-signed rank test was applied in order to locate the difference in microleakage between enamel and dentin margins for the three groups of materials at the initial and thermocycling phases and there was no statistical significant difference between enamel and dentin margins for group Grandio and group X-tra in both initial and thermocycling phases and in group SDR in the thermocycling phase. The exception was group SDR in the initial phase, there was statistically significant increased microleakage in dentin margin in comparison to enamel margin.

**DISCUSSION**

**The effect of thermal aging on microleakage**

At the occlusal margin for group SDR (Grandio+SDR) and X-tra (Grandio+X-tra base), the difference in microleakage median scores was non significant statistically between initial and thermocycling phases. This is in agreement with the results obtained by Aguiar et al. (30) (Fig. 2 B,C).

There was a significant difference in microleakage median scores between initial and thermocycling phases of group Grandio (Fig.2A). This means that the use of SDR and X-tra base had reduced the effect of thermocycling at the occlusal margin. The explanation might be although the occlusal most layer of all the groups were filled by Grandio material but the higher strain capacity and low elastic modulus of the two flowable base composites (SDR material in SDR and X-tra base material in X-tra groups), had provided an advantage to protect the restoration from thermal stress that could cause further damage to the tooth restoration interface combined with already applied polymerization
shrinkage stress that occurred in group Grandio\textsuperscript{(31-33)}. Microleakage increase in group Grandio might be also related to the effect of the restorative technique of the deep layers and its influence on the occlusal most layers. Versluis \textit{et al.} noted that oblique and horizontal incremental restorative techniques resulted in much higher coronal surface stresses than bulk curing technique \textsuperscript{(34)}. Another explanation to the increased microleakage after thermocycling in group Grandio is the voids formation at the margins that may lead to gross microleakage \textsuperscript{(35)}. The voids formation could be because of the voids formation ability of the material due to the high filler loading combined with restorative technique that may caused entrapment of air and voids between layers in addition to water storage and hydrolysis of the adhesive that increased the microleakage more than the thermal stress on Grandio material \textsuperscript{(36,37)}.

At the cervical margin the results showed there was statistically non significant increase in microleakage between initial and thermocycled phases for each of the three groups in enamel and dentin margins. This means that thermocycling or absence of it did not affect cervical margin sealing ability of the three groups of materials. This goes with agreement with a study conducted by Rossomando and Wendt and Aguiar \textit{et al.} \textsuperscript{(30,38)}. The explanation to the reduced microleakage in SDR group might be related to the lower shrinkage stress of EBPDMA in SDR material \textsuperscript{(40)}. While there was statistically non significant difference between median scores of groups Grandio and X-tra base (Fig.3 A).

At thermocycling phase, there was significant difference in microleakage median score among all the groups (Fig.3 A), with the highest in Grandio group. The cause is the same as in the initial phase but in thermocycling phase, the lower microleakage value of SDR and X-tra base is due to the higher strain capacity and low elastic modulus of the two flowable base composites, had provided an advantage to protect the restoration from thermal stress that could cause further damage to the tooth restoration interface combined with the already applied polymerization shrinkage stress \textsuperscript{(30-33)}. For Grandio's group the increased microleakage (combined the thermal stress with higher polymerization shrinkage stress of the material) might be related to the higher elastic modulus (due to high filler loading), stress build up from the restorative technique and the voids formation in addition to the effect of water storage and hydrolysis of the adhesive, increased the microleakage more than the effect of thermal stress alone on Grandio material \textsuperscript{(36,37)}.

The effect of materials and techniques on microleakage

The occlusal margin

At the occlusal margin in the initial phase, when comparing SDR and Grandio groups, SDR had statistically significant lower microleakage median score than Grandio. The explanation is that SDR had reduced the amount of stress applied at occlusal margin and reduced microleakage amount due to bond failure. This may be related to the stress reduction quality of SDR base material (due to the presence of the more flexible EBPDMA in its polymer matrix) with its low shrinkage stress (1.57 Mpa) and low elastic modulus combined with the use of bulk filling technique \textsuperscript{(39-40)}. In comparison the higher stress of Grandio material (2.7 Mpa) with the use of oblique incremental technique might increased the stress at the occlusal margin and led to the higher microleakage \textsuperscript{(35,41)}. When comparing SDR and X-tra base groups, SDR had statistically significant lower median score of microleakage than X-tra. This could be explained by the lower stress value of SDR that reduced the amount of stress applied at the occlusal margin, in comparison to the higher stress value of X-tra base (5.93 Mpa). This increase in stress in X-tra base material could be related to the effect of TEGDMA in its matrix that produce higher carbon double bonds concentrations leading to increased degree of conversion and increased shrinkage stress \textsuperscript{(4)} in comparison to the low shrinkage stress of EBPDMA in SDR material \textsuperscript{(40)}. The voids formation from thermal stress that could cause further damage to the tooth restoration interface combined with the already applied polymerization shrinkage stress \textsuperscript{(30-33)}. For Grandio's group the increased microleakage (combined the thermal stress with higher polymerization shrinkage stress of the material) might be related to the higher elastic modulus (due to high filler loading), stress build up from the restorative technique and the voids formation in addition to the effect of water storage and hydrolysis of the adhesive, increased the microleakage more than the effect of thermal stress alone on Grandio material \textsuperscript{(36,37)}.

At the enamel margin in both the initial and thermocycling phases, there was a non significant difference among the median scores of the three groups of materials. This result is with agreement with the results of Reis \textit{et al.} and Munroz-Viveros \textit{et al.} \textsuperscript{(21, 28)} (Figure 3 B).

At the enamel margin in both the initial and thermocycling phases, there was a non significant difference in microleakage median scores between Grandio group and X-tra base group. This result agrees with the results obtained by Moorothy \textit{et al.} \textsuperscript{(18)}. There was a significant difference between SDR group and Grandio, and between SDR group and X-tra base group, with the lowest microleakage median score in SDR group (Figure 3 C). This result disagrees with the results of Moorothy \textit{et al.} \textsuperscript{(18)}. This difference could be related to the differences in bond strength between the bonding systems (Xeno V* self etch was used in this study while they used All bond three step adhesive system) with the differences in response of the adhesive systems to the polymerization shrinkage stress applied by the three materials. The explanation to the reduced microleakage in SDR group might be related to the lower
polymerization shrinkage stress of SDR material (due to the modified monomer formulation) that allows more stress relief in SDR, with its low elastic modulus may applied less stress at the margin than the other two materials\cite{11,16,21}. Another explanation is the voids formation at the margins that may lead to gross microleakage \cite{35}. The voids formation in Grandio group could be related to the effect of oblique restorative technique used that may caused entrapment of air and voids formation between the layers or because of the ability of the material itself to form voids due to its high filler loading \cite{11,16,21,35}. While the increase in microleakage for X-tra base group might be also related to the high voids formation ability of X-tra base material combined with larger marginal gap formation ability\cite{11,16,21,35}. While the result is in agreement with the study conducted by Reis et al. \cite{11,16,21,35}. The better influence of SDR material on microleakage of class II composite restorations filled by using bulk, incremental and conventional curing techniques. J Adhes Dent 2003; 25: 13-427-38.

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Figure 2. The difference in microleakage median score between initial and thermocycling phases of the three groups of materials (A: at the occlusal margin, B: at the dentin margin, C: at the enamel margin).

Figure 3. The difference in microleakage median score among the three groups of materials in initial and in thermocycling phases (A: at the occlusal margin, B: at the dentin margin, C: at the enamel margin)