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DISSERTATION

Remineralization of Artificially Demineralized Dentin In Vitro

zur Erlangung des akademischen Grades
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Von

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DEDICATION

This thesis is

Dedicated to my beloved

Parents who have always been a source of

Inspiration, encouragement and stamina

To undertake my higher studies and

To face the eventualities of life

With zeal, enthusiasm

and more of

love.

To my wounded homeland, Yemen

Inhalt

This Thesis builds on the Publication “Glass hybrid, but not calcium hydroxide, remineralized artificial residual caries lesions in vitro. **A Al-Abdi**, S Paris, F Schwendicke. Clin Oral Investig. 2017. PMID: 27033226

1. Abstract

Objectives: Based on new concepts on the pathogenesis of dental caries, the management of carious lesions in proximity to the dental pulp has been largely changed. Ultraconservative excavation or removal is usually advised to avoid pulpal damage. The remaining demineralized tissue near the pulp, however, comes with challenges. One such challenge is to remineralize the residual lesion, for example using lining or restorative materials. In the present study, we tried to compare the remineralization activity of two different materials, calcium hydroxide and glass hybrid, on artificial residual lesions, in a pulpal fluid simulation system in vitro.

Methods: On human dentin discs (n=20), artificial residual lesions (median mineral loss ΔZ [25^{th}/75^{th} percentiles]=1643 [1301/1858] vol%×µm) were chemically induced using demineralization solution without bacterial invasion. The dentin discs were divided into five parts, one of them served as baseline sample. The remaining four parts were used as experimental groups, with each being covered with a different material or material combination (n=20/group): Flowable composite (CO) without liner (control), setting and non-setting calcium hydroxide covered with flowable composite (CH-S, CH-NS), glass hybrid (GH). Samples were mounted in a dual-chamber device allowing pulpal fluid simulation. Pulpal surfaces were subjected to simulated pulpal fluid at 2.94 kPa, while coronal surfaces were exposed to artificial saliva, and once weekly rinsed with 200 ppm NaF. Solutions were changed regularly every 14 day. After 12 weeks, mineral loss differences compared to baseline were evaluated using transversal microradiography. Fluoride and strontium concentrations in exemplary samples were analyzed using Field Emission Electron Probe Micro-Analyzer (FE-EPMA).

Results: Mineral gain in CO (negative control) was ΔΔZ=372 (115/501) vol%×µm. This was not significantly different from CH-S (ΔΔZ=317 [229/919] vol%×µm) or CH-NS (ΔΔZ=292 [130/579] vol%×µm), ($p>0.05$/Wilcoxon-test), but while mineral gain was significantly higher in GH (ΔΔZ=1044 [751/1264] vol%×µm, $p<0.001$). GH samples showed fluoride and strontium enrichments deep into the dentin. Such enrichment was not found in CO or CH samples.

Conclusion: Within the limitations of this study, glass hybrid, but not calcium hydroxide provided coronal remineralization of residual carious lesions.

Clinical relevance: Glass hybrids might provide additional remineralization of residual carious lesions.

Keywords: dental caries; demineralization; lining; remineralization; pulp; pulpal fluid

2. Zusammenfassung

Ziel: Basierend auf neuen Konzepten zur Pathogenese der Dentalkaries hat sich das Management kariöser Läsionen in der Nähe der Zahnpulpa weitgehend geändert. In der Regel wird zu einer ultrakonservativen Exkavation oder Entfernung geraten, um Schäden an der Pulpa zu vermeiden. Das verbleibende demineralisierte Gewebe in der Nähe der Pulpa bringt jedoch Herausforderungen mit sich, u.a. die Remineralisierung der verbleibenden Läsion, zum Beispiel durch Liner- oder Restaurationsmaterialien. In der vorliegenden Studie haben wir die Remineralisierungsaktivität zwei verschiedener Materialien, Calciumhydroxid und Glashybrid, auf künstlichen Residualläsionen in vitro verglichen.

Methoden: Auf humanen Dentinscheiben (n=20) wurden künstliche Residualläsionen (medianer Mineralverlust ΔZ [25./75. Perzentile] =1643 [1301/1858] Vol.%×µm) chemisch induziert. Die Dentinscheiben wurden in fünf Teile geteilt, von denen einer als Ausgangsprobe diente. Die restlichen vier Teile dienten als Versuchsgruppen, wobei jede mit einem anderen Material oder einer anderen Materialkombination beschichtet wurde (n=20/Gruppe): Fließfähiges Komposit (CO) ohne Liner (Kontrolle), abbindendes und nicht abbindendes Kalziumhydroxid, bedeckt mit fließfähigem Komposit (CH-S, CH-NS), Glashybrid (GH). Die Proben wurden in einem Zweikammergerät montiert, das eine Simulation von Pulpaflüssigkeit ermöglichte. Die Pulpaoberflächen wurden der simulierten Pulpaflüssigkeit bei 2,94 kPa ausgesetzt, während die koronalen Oberflächen einem künstlichen Speichel ausgesetzt und einmal wöchentlich mit 200 ppm NaF gespült wurden. Die Lösungen wurden regelmäßig alle 14 Tage gewechselt. Nach 12 Wochen wurden die Unterschiede im Mineralverlust im Vergleich zur Ausgangsprobe mittels transversaler Mikroradiographie ausgewertet. Die Fluorid- und Strontiumkonzentrationen in exemplarischen Proben wurden mittels Field Emission Electron Probe Micro-Analyzer (FE-EPMA) analysiert.

Ergebnisse: Der Mineralgewinn in CO (Negativkontrolle) betrug ΔΔZ=372 (115/501) Vol.%×µm, dies war nicht signifikant unterschiedlich zu CH-S (ΔΔZ=317 [229/919] Vol.%×µm) oder CH-NS (ΔΔZ=292 [130/579] Vol.%×µm), (p>0.05/Wilcoxon-Test), aber während der Mineralgewinn in GH signifikant höher war (ΔΔZ=1044 [751/1264] Vol.%×µm, p<0.001). GH-Proben zeigten Fluorid- und Strontiumanreicherungen tief im Dentin. Eine solche Anreicherung wurde in CO- oder CH-Proben nicht gefunden.

Schlussfolgerung: Innerhalb der Grenzen dieser Studie bewirkte Glashybrid, nicht aber Calciumhydroxid, eine koronale Remineralisierung von kariösen Residualläsionen.

Klinische Relevanz: Glashybride könnten eine zusätzliche Remineralisierung von kariösen Residualläsionen bewirken.

3. Introduction

The conventional management of dental caries has been substantially altered given new insights on caries pathology and as well as the availability of adhesive restorations [1]. Previously, the caries process was thought to be a continuous irreversible series of events, starting with enamel demineralization followed by protein (collagen) degradation, both induced by an infection with cariogenic bacteria. The logical management was complete excision of the diseased tissue and replacement with an artificial restorative material [2]. Nowadays, it is recognized that dental caries is defined as a pathophysiological process in the dental biofilm, leading to an imbalance in the demineralization–remineralization equilibrium , in which the tooth structure alternately loses and gains calcium and phosphate ions, depending on the microenvironment [3], giving rise to the final loss of tooth minerals progressing to a cavity [4].

For management of deep carious lesions, i.e., those extending into the pulpal third of dentin, removal of carious tissue bears significant risks for the pulp. If excavating until only hard dentin is left, pulp exposure and post-operative complications are frequent [5, 6]. Therefore, ultraconservative excavation is usually recommended to avoid excessive removal of both sound and carious tissues [7-10] where demineralized and bacterial-contaminated dentin is deliberately left in proximity to the pulp, whereas in the areas away from the pulp conventional excavation until firm dentin remains is performed. The remaining lesion and the associated bacteria are sealed beneath the subsequent adhesive restoration, and thereby inactivated due to deprivation from dietary carbohydrates [11, 12]. Additional antibacterial treatments of the residual lesions are so far of unclear efficacy [13].

The residual dentin lesion remaining after selective removal may extend hundreds of micrometers beneath the restoration surface [14, 15]. This dentin has a higher water content and therefore lower stiffness than sound dentin [16-19], and conventional adhesive materials achieve only low bond strengths to such demineralised or contaminated dentin, mainly as it can only partially be infiltrated by resin monomers. Such poorly resin-infiltrated dentin is highly susceptible to hydrolytic and/or enzymatic degradation, which may eventually compromise the durability of the adhesive restoration [20, 21]. Remineralization of this sealed demineralized dentin would therefore be desirable [22] and can occur via seed crystals, acting as a precursor nidi for nucleation of calcium and phosphate, and epitaxial crystal growth [23-25], which requires the presence of any residual minerals serving as seeds. Non-classical bio-mineralization allows remineralization of apatite-depleted dentin, i.e., in the absence of

seed crystals [26]. In this remineralization, collagen acts as template for hierarchical intra-fibrillar mineralization, with calcium and phosphate being stabilized in a fluid phase as pre-nucleation clusters by matrix proteins or their poly-anionic analogues. These clusters then diffuse into the collagen, creating nucleation there and maturating to nanocrystals, which grow to intra-fibrillar apatite crystallites alongside with mineralization between collagen fibrils [26].

It is not fully clear so far which relevance both mineralization types have clinically and also up to debate from where any kind of mineral gain might be stemming from: Various studies indicated that remineralization is supported from the pulp, either actively mediated by odontoblasts or passively provided by mineral-containing pulpal fluids [27-29]. Such mineral gain, however, has also been expected to happen via cavity liners or remineralizing restorative materials.

For example, calcium hydroxide (CH) has been shown to provide remineralization in vitro [30] and clinically [31, 32]. This and other potential advantages of CH (e.g., it is antibacterial and induces tertiary dentin development) could explain why this material is widely used in general dentistry [33-35]. It still unclear, however, if different formulations (e.g., setting, or non-setting) of CH have different ability of remineralization, or how the mediated mineral gains compare with the remineralization activity of other materials.

An alternative material to CH is glass ionomer cement (GIC), which has been widely used for its fluoride releasing and remineralizing ability [36-43]. Because of its mechanical limitations, GIC has been mainly employed for temporary restorations or as a cavity liner under other materials like amalgam or composite resins. In order to overcome such limitations and wide the indication spectrum of GIC, glass hybrids (GH) have been developed. These are a kind of reinforced glass ionomer, with a second, smaller and more reactive silicate particle and higher molecular weight acrylic acid molecules, which supposedly increase matrix cross-linking. This, in turn, is thought to improve the material's flexural strength. Covering these restorations with a nano-coated resin layer is supposed to further improve wear resistance and aesthetical appearance. In summary, pulpal remineralization of sealed residual lesions is likely, while the additional benefits of remineralizing liners like CH or restorative materials like GH remain unclear. We aimed to assess the comparative effects of four dental materials on artificial residual lesions, whilst mimicking pulpal remineralization in vitro. Our null hypothesis was that the employed remineralizing materials (setting and non-setting CH, GH) do not provide any significantly different mineral gain compared with only pulpal remineralization.

4. Materials and Methods

Mineral gain in artificial residual lesions provided via the pulp or by dental materials in a dual-chamber device [22, 44] was assessed.

4.1 Experimental design

Dual remineralization activity via both the pulp and four dental materials was evaluated. Human dentin discs were subjected to demineralization to induce artificial residual lesions. Coronal surfaces were restored using different materials. Baseline mineral losses were measured via transversal microradiography, and specimens mounted in a dual-chamber device. Coronal surfaces were exposed to artificial saliva and fluoride rinses, and pulpal surfaces to simulated pulpal fluid under static pressure of 2.94 kPa. Mineral loss differences were assessed after three months [43] (Fig. 1).

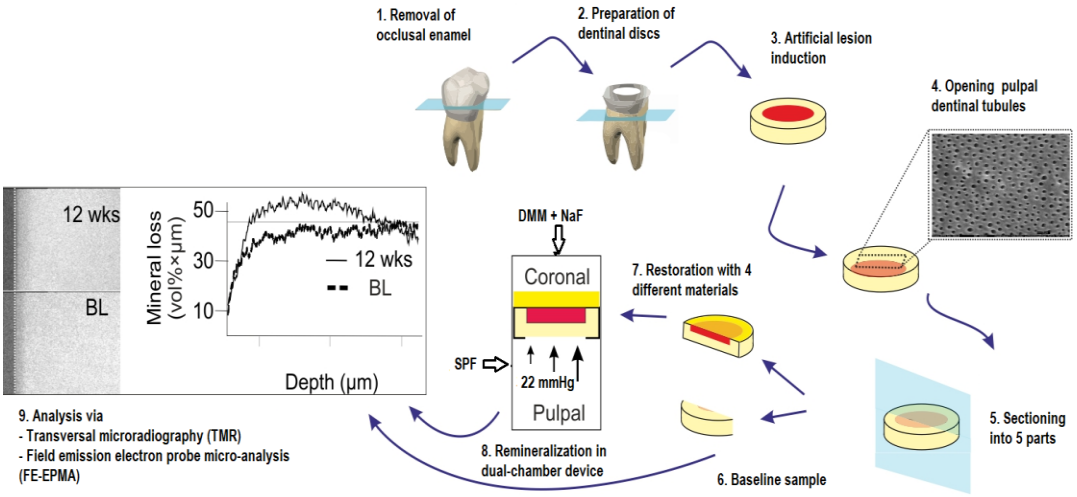

Figure 1: Samples preparation. Abreviations: Defined Mucin Medium (DMM), Sodium Fluoride (NaF), Simulated Pulpal Fluid (SPF) [43]. (Figure use permitted by Elsevier).

4.2 Specimens preparation

Twenty extracted human caries-free third molars devoid of any restoration were selected with informed consent based on an ethics approved protocol (EA4/102/14). Dentin discs from each tooth were obtained by sectioning both roots and coronal enamel of teeth parallel to the occlusal surface (Band Saw 300cl; Exakt Apparatebau, Norderstedt, Germany), the pulpal surface was flattened until the level of the pulp horns, and discs were plan-parallelized until a standardized thickness of 1.5 mm was

achieved. Coronal and pulpal dentin surfaces were then polished (Mikroschleifsystem 400 CS, abrasive Paper 1200, 2400 and 4000) and specimens covered with acid-resistant nail varnish (Maybelline, New York, NY, USA) except a round window (Ø=4mm) of coronal dentin, which was left uncovered. Artificial residual carious lesions were created in the unprotected area by storing the specimens in 5 l of a demineralizing solution (pH 4.95, 37 °C) containing 50 mM acetic acid, 3 mM $CaCl_2 \times H_2O$, 3 mM KH_2PO_2 and 6 mM methyl-hydroxydiphosphonate (Table1) for 14 d [43, 45]. No attempts of removing the smear layer before inducing the lesions were made. The pH of the solution was monitored daily (InLab micro, Mettler-Toledo, Giessen, Germany) and if necessary adjusted using HCl (Roth, Karlsruhe, Germany) or 10 M KOH. Each disc was divided into 5 parts, one of them served as baseline (control) sample for the analysis of mineral loss differences after the remineralization period. The remaining four parts were used as experimental specimens, each being covered with one different experimental material (N=20/group; (Figure 1).The pulpal surface was uncovered and submitted to 0.5 M EDTA (pH=5.0) for 2 min to remove the smear layer and open the dentinal tubules [46].The latter was controlled in a pilot-study on five discs using scanning electron microscopy (Figure 2, 3).

Table 1: Composition of demineralization solution.

Substance	**Manufacturer**	**g/mol**
$CaCl_2 \cdot 2H_2O$	Merck KGaA, Darmstadt, Germany	147.02
KH_2PO_4	Carl Roth GmbH, Karlsruhe, Germany	136.09
*MHDP	Merck KGaA, Darmstadt, Deutschland	176.00
CH_3COOH 100%	Merck KGaA, Darmstadt, Deutschland	60.05

The pH of solution was adjusted to 4,95 using KOH or HCl. *MHDP (Methylendiphosphoric acid)

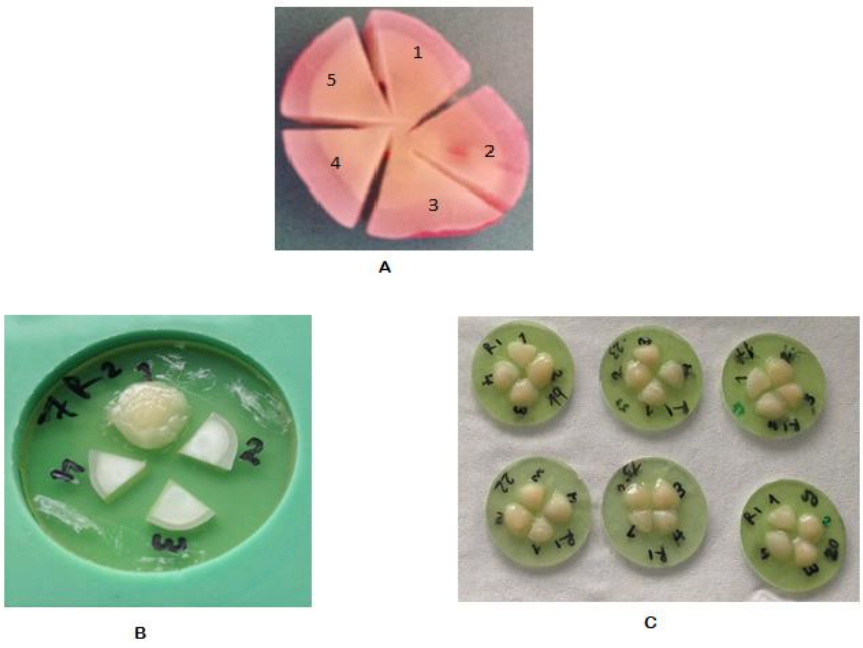

Figure 2: (A) Each dentin disc was divided into 5 parts; one part was used as control group and the other 4 parts were used as experimental groups. (B and C) Experimental specimens, each being covered with one different experimental material.

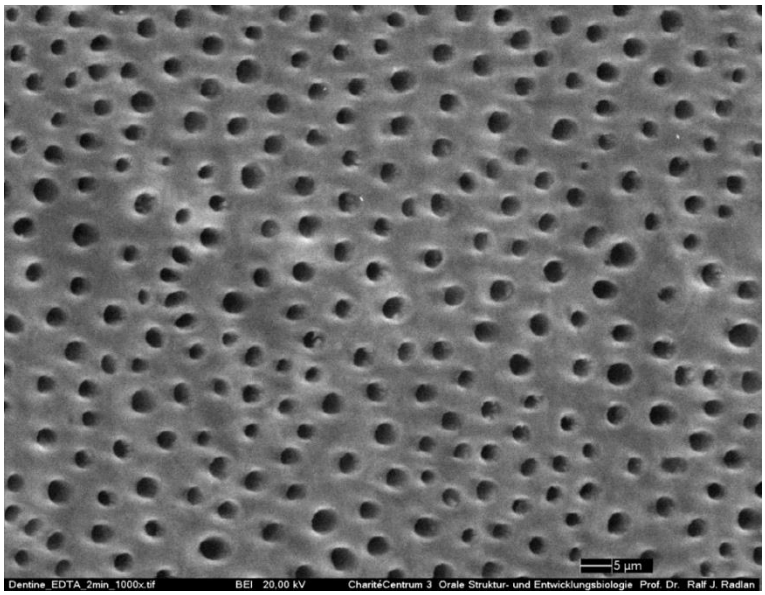

Figure 3: Scanning electron microscopic image showing the opening of pulpal dentinal tubules using 0.5 M EDTA (pH=5.0).

4.3 Restorative materials

The following materials were tested: (1) Flowable composite (Tetric EvoFlow, Ivoclar Vivadent, Schaan, Liechtenstein) without any liner served as negative control (no coronal, only pulpal remineralization, CO). (2) Setting calcium hydroxide (Dycal, Kerr, Scafati Salerno, Italy) covered with flowable composite (Tetric EvoFlow) (CH-S);(3)

Non-setting calcium hydroxide (UltraCal XS, Ultradent, Cologne, Germany) covered with flowable composite (Tetric EvoFlow) (CH-NS); (4) Glass hybrid (Equia Forte, GC, Tokyo, Japan) (GH) covered with a resin coating (Equia Coat, GC).

The restorative materials were applied according to the manufacturers' instructions, where CH was placed in a thin layer of about 0,5-1,0 mm and being lifted to set for 2 min before being covered with a composite restoration. The thickness of the applied restorations was 1.5-2.0 mm. A self-etch adhesive (iBond, Heraeus Kulzer, Hanau, Germany) was used for 15 s to bond the flowable composite to the peripheral dentin surface that was not covered with CH and was light-cured for 20s with an intensity of 950mW/cm2 (Smartlite, Dentsply Detrey, Konstanz, Germany). The same curing protocol was used for curing the composite resin. No conditioning was performed before applying GH restoration.

4.4 Pulpal fluid simulation system

The pulpal fluid simulation system was a custom-made dual-chamber device, with one chamber simulating the dental pulp, containing pulpal simulated fluid, and the other the mouth, containing defined mucine medium (DMM) [43, 47]. The pulpal chamber was covered by a Technovit lid, in which the dentin discs were fitted, with the pulpal surfaces directed toward the pulpal chamber and the coronal surfaces toward the coronal chamber.

4.5 Remineralization phase

Specimens were mounted on the lid of a custom-made dual-chamber system. The coronal chamber was filled with DMM (Table 2) to simulate the oral milieu[48] . Every 2 weeks, the coronal surfaces all the specimens were additionally rinsed with 200 ppm NaF solution for 5 min to allow possible fluoride recharge of the glass hybrid and to simulate a real scenario where toothpastes and/or mouth rinses are used. The pulpal chamber was immersed in simulated pulpal fluid [49] , containing hepes buffer, calcium, magnesium, phosphate, as well as albumin (Table 3), at a constant pulpal pressure of 2.94 kPa [43, 50, 51] using Sphygnomanometer for monitoring and adjusting the intrapulpal pressure. Chambers were stored at 37°C and under gentle agitation (70 rpm) for three months, with coronal and pulpal fluids being replaced every two weeks. The use of this system allows to simultaneous provide remineralization from coronally (induced by the materials) and pulpal mineral delivery, as is the case clinically [43, 52].

Table 2: Composition of Defined Mucin Medium (DMM).

Substance	Manufacturer	g/mol
KH_2PO_4	Carl Roth GmbH, Karlsruhe, Germany	136.08
K_2HPO_4	Carl Roth GmbH, Karlsruhe, Germany	174.18
NaCl	Carl Roth GmbH, Karlsruhe, Germany	58.44
KCl	Merck KGaA, Darmstadt, Germany	74.55
NH_4Cl	Merck KGaA, Darmstadt, Germany	53.49
$MgCl_2*6H_2O$	Merck KGaA, Darmstadt, Germany	203.3
$CO(NH_2)^2$ (Urea)	Merck KGaA, Darmstadt, Germany	60.06
Mucin , porcine stomach, Type III	Sigma-Aldrich, St. Louis, USA	-
$CaCl_2*2H_2O$	Carl Roth GmbH, Karlsruhe, Germany	147.02

Table 3: Composition of simulated pulpal fluid (SPF).

Substance	Manufacturer	g/mol
Hepes buffer	Carl Roth GmbH, Karlsruhe, Germany	30.00
CaO	Carl Roth GmbH, Karlsruhe, Germany	0.93
MgO	Carl Roth GmbH, Karlsruhe, Germany	0.60
NaCl	Carl Roth GmbH, Karlsruhe, Germany	77.60
H3PO4	Carl Roth GmbH, Karlsruhe, Germany	107.80
Albumin 20%	Carl Roth GmbH, Karlsruhe, Germany	50g/250ml distilled water

For SPF, 20% albumin solved in distilled water was added. The pH of both SPF and DMM solutions were adjusted to 7.4 using KOH or HCl.

4.6 Transversal microradiography (TMR)

Thin (100 µm) plan-parallel sections were prepared perpendicular to the surface (Band Saw 300 CLV; Exakt Apparatebau, Norderstedt, Germany) and polished (Mikroschleifsystem 400 CS, abrasive Paper 1200, 2400 and 4000) as described above during samples preparation. Baseline mineral loss (ΔZ) was determined by means of TMR. The same was applied with the experimental samples after remineralization period of three months. From each microradiograph, three different areas were selected to estimate mineral loss. Mineral loss differences (ΔΔZ) were calculated for each area, the collected data were analyzed (TMR 2000 Program) with positive differences indicating mineral gains and means for each sample being used for statistical analysis [43].

A nickel-filtered copper X-ray source (PW 1730/10, Philips, Eindhoven, The Netherlands) operating at 20 kV and 10 mA with a vertical tube (PW 2213/20, Panalytical, Kassel) and a 280 mm radiation-to-film distance was used to obtain radiographs. Films (Fuji fine 71337, Fujifilm, Tokyo, Japan) were exposed for 5s and developed under standardized conditions according to the manufacturer's recommendations. The microradiographs were analyzed with a digital-image-analyzing system (XC 77 CE, Sony, Tokyo, Japan) interfaced with a universal microscope (Axioskop2 60318, Zeiss, Oberkochen, Germany) and a personal computer (TMR for Windows 2.0.27.2, Inspector Research, Amsterdam, The Netherlands).

4.7 Field Emission Electron Probe Micro-Analysis (FE-EPMA)

The Electron Probe Micro-Analyzer ("EPMA") is an analytical instrument used to non-destructively determine the chemical composition of small volumes of solid materials, by irradiating electron beams onto the substance surface and measuring the characteristic X-ray that is generated. FE-EPMA in our study was utilized to measure fluoride and strontium concentrations in three exemplary samples. EMPA works by bombarding a micro-volume of a sample with a focused electron beam and collecting the X-ray photons emitted by the various elemental species. Because the wavelengths of these X-rays are characteristic for the emitting species, the sample composition can be identified by recording WDS spectra (Wavelength Dispersive Spectroscopy). WDS spectrometers are based on the Bragg's law and use various moveable, shaped monocrystals as monochromators. For FE-EPMA, three post-experimental samples (2 GH, 1 CO) were randomly chosen, resin-embedded, cross-sectioned, grinded and polished with 0.05 µm alumina suspension. Subsequently, a conductive carbon layer

(thickness: ~10 nm) was deposited on the cross-section surface and assessed via FE-EPMA (JEOL JXA-8530F). Quantitative line profiles were measured through the whole cross-section with a step size of 25 µm and a measurement time of 50 s (peak) and 20 s (background) for each step. An acceleration voltage of 15 kV and a beam current of 200 nA were used. Fluoride was measured with the LDE1-crystal. Strontium was measured with the TAP-crystal.

4.8 Statistical analysis

Statistical analysis was performed using SPSS 20 (IBM, Armonk, USA). Data distribution was assessed via Shapiro-Wilk-test. Group-wise comparisons were performed using Wilcoxon signed-ranked test, with Bonferroni correction for multiple significance testing. Level of significance was set at $p<0.05$ and all tests were performed two-sided.

5. Results

The microradiography analysis showed that the simulated artificial carious lesions had a median mineral loss of ΔZ [25th/75th percentiles]=1643 [1301/1858] vol%×µm) and lesion depths of 300 (264/330) µm. The mineral gain in CO was ΔΔZ=372 (115/501) vol%×µm (Fig. 4). This was not significantly different compared to CH-S (ΔΔZ=317 [229/919] vol%×µm) or CH-NS (ΔΔZ=292 [130/579] vol%×µm) (p>0.05/Wilcoxon/Bonferroni). In contrast, mineral gains were significantly higher in GH (ΔΔZ=1044 [751/1264] vol%×µm, p<0.001). This final mineral gain was not only due to remineralization of the carious lesion, but also hypermineralization of sound dentin (Figure 5). Moreover, FE-EPMA showed that the samples in GH showed fluoride and strontium enrichments deep into the dentin. Such enrichment was not found in the exemplary CO sample.

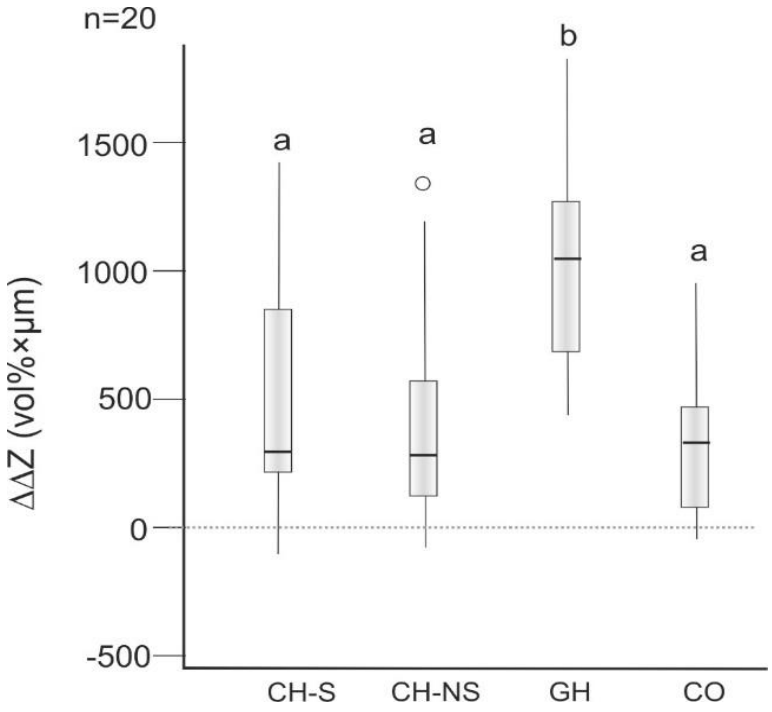

Figure 4: Mineral loss differences (ΔΔZ) after 3 months remineralization. Specimens were restored with four different restorative materials; setting calcium hydroxide liner (CH-S), non-setting calcium hydroxide liner (CH-NS), glass hybrid (GH), and composite without any liner (control, CO). Positive differences indicate mineral gain. GH showed significant mineral gain, whereas, other material were showed no significant differences compared to the control. Significant differences (p<0.05; Wilcoxon/Bonferroni) are indicated by different letters. Box and line: Interquartile range

and median, whiskers: minimum/maximum, circles: outliers. N: sample size [43]. (Figure use permitted by Springer, License No.5031420192108)

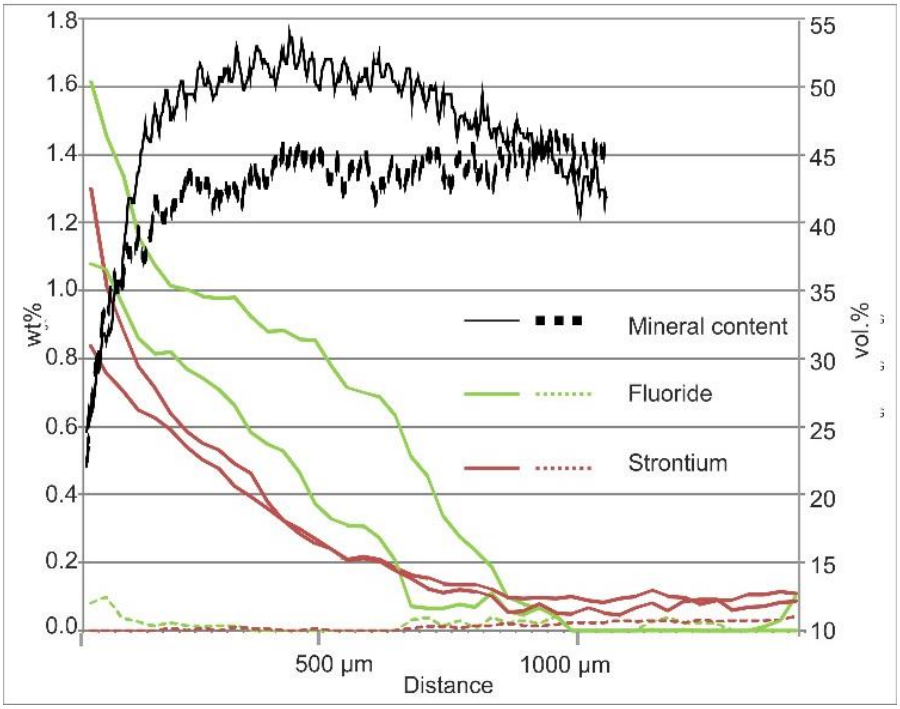

Figure 5: FE-EPMA was used to evaluate fluoride and strontium concentrations (left y-axis, weight %) in cross-sectioned GH and CO samples from the coronal surface (left) to the pulpal surface (right). Fluoride (green line) and strontium (red line) were determined in 2 GH samples (solid lines) and 1 CO sample (dotted lines). GH samples showed enrichment of fluoride and strontium in comparison to CO. Mineral content (in vol×%, right y-axis) was assessed via microradiography and is exemplarily shown for one GH sample (solid black line) and CO sample (dotted black line). TMR addressed that GH gained more mineral than CO. Note that mineral loss was assessed only for the coronal 2/3, not the full thickness of the dentin disc [43]. (Figure use permitted by Springer, License No.5031420192108)

6. Discussion

Remineralization of residual dentin lesions remaining after selective removal would allow to biologically mitigate certain limitations routed in this minimal-invasive management approach, possibly increasing restorative longevity and increasing dentists' acceptance of selective removal. In the present study we aimed to compare different restorative materials for their remineralization activity in a pulpal fluid simulation system in vitro. We found that GH, but not CH provided significant mineral gain, and demonstrated mineral diffusion deep into dentin.

6.1 Pulpal remineralization

In the present study we used a dual remineralization protocol with remineralization via simulated pulpal fluid and restorative dental materials. When confronted with external dental injury or irritations, i.e. dental caries, a complex defense mechanism is initiated involving the migration of dental pulp mesenchymal stem cells that differentiate into new odontoblast-like cells that secrete a form of tertiary (reparative) dentin [53-56].This newly secreted dentin acts as a mineralization barrier that walls off the pulp from bacterial infection. This natural healing process has been investigated in clinical trials in an attempt to learn how to pharmacologically trigger it for promoting reparative dentine formation [54]. It could be found that mineral gain and dentin remineralization are provided by the dental pulp without any additional trigger or material needed [28, 47, 57].

Theoretically, this could mean that cavity liners or remineralizing restoration materials are not required [27]. Notably, though, it is unclear how this mineral gain is realized. Such dentin remineralization could work via precipitation of minerals within dental tubuli, as has been found within the transparent zone of carious dentin. Such precipitation (mainly of whitlockite), however, does not seem to repair the physical properties of dentin [58]. Another mechanism could be epitaxial growth of remaining apatite seed crystallites [59] or spontaneous nucleation of minerals in the remaining collagen scaffold and its residual matrix proteins [59, 60]. Since the generated artificial carious lesions in our study were not completely mineral-depleted and collagen was not actively degraded (e.g. enzymatically) both mechanisms are theoretically possible.

In our study a simulated pulpal fluid was used [43]; various *in vitro* studies have shown possible effects of such simulated dentinal fluid on remineralization of artificial lesions [61, 62]. We confirm this mineral gain. Notably, the "quality of dentin" is determined by the overall tissue's characteristics that affect its competence: microstructure, mineral

density, and mineral position within the organic matrix [63]. Mechanical properties values, in comparison to mineral density measures, take into account both of these variables, since tissue strength is measured by mineral content, architecture and, mineral matrix binding.

6.2 Restorative remineralization

For deep residual lesions, it is unclear if pulpal remineralization is sufficient. First, a possible obliteration of dentinal tubules and the development of sclerotic or reparative dentin may limit pulpal remineralization activity. Second, for very deep lesions an effective mineral depletion in the outer aspects of the lesion are unlikely. To overcome this issue, restorative or lining materials for remineralization are needed. A range of *in vitro* systems have been developed to test the remineralization of dentin using various materials [22, 47, 64]. Here, we employed a system that allows concomitant pulpal and coronal, material-induced remineralization [43].

For the treatment of deep carious lesions cavity liners such as CH as are widely used [35]. CH releases calcium and hydroxyl ions which are supposed to have antibacterial effects [65], while any remineralization effect remains debatable [28, 29, 66-70]. There are also doubts as to the clinical efficacy of CH lining [31, 71]. Based on the present in vitro evaluation, CH showed no additional benefit in mineral gain compared with no lining; this was not different between setting and non-setting CH. For CH, we hence accept our null hypothesis. Our findings were inconsistent with a previous study that found that non-setting CH releases a higher amount of calcium and hydroxyl ions than setting CH may be due to the presence of undissolved calcium salicylate chelate formed after mixing of the base and catalyst pastes of the setting material [72]. Other study was revealed that CH releases different quantities of ions at different pH and subsequently exerting different effects [73].

GICs materials are commonly used in atraumatic restorative treatment of class I or smaller class II lesion but its use in extended and deep cavities as a restorative is still not common, mainly because of its poor physical properties. To overcome this issue, GH was produced with better mechanical properties. In this study, we found that GH provided significant mineral gains compared with the control [43]; we hence reject our null hypothesis for this material. *In vitro* [37-39, 74, 75] and *in vivo* [41, 42, 76, 77] studies showed that GIC remineralized carious dentin, mainly via release of calcium, phosphate as well as fluoride and strontium [36, 67]. As laid out, the delivery of minerals might not be sufficient to repair apatite-depleted dentin [28, 67, 78-80]. Moreover, an

effective mineralization of deeper lesions could be impeded by high concentrations of minerals being laid down at the material-dentin interface, mainly as accumulated minerals reduce any diffusion-driven mineral transport into the depth of the lesion [39, 81]. Interestingly, we found deep mineral accumulation, with increased fluoride and strontium concentrations in depths of around 500–700 µm. This is even more remarkable as the supplied pulpal pressure may have slowed down any coronal mineral diffusion from the GH material into the depth of the lesion. Additionally, we found accumulation of both fluoride and strontium (via EPMA) and, general mineral accumulation (via TMR) in sound dentin as well. This is in agreement with previous studies [41, 43, 76, 77, 82] that showed such effects of glass ionomers and the accumulation of minerals above the expected concentration in sound dentin associated with high fluoride concentrations [81, 83].

6.3 Study limitations and recommendations

This study comes with a range of limitations. First, we found mineral accumulation and were also able to demonstrate fluoride and strontium depositions. However, we could not assess the functional value of such mineral gain. The fluoride and strontium could be integrated into existing apatite crystals [84], but it remains speculative to where the exact crystallization (which would follow the classical, top-down approach) occurred. It might be that the observed “remineralization” in microradiography is mainly an accumulation of radiopaque ions within the dentin, which does not necessarily mean that any mechanical behavior is restored [18]. More important would be an intra-fibrillar mineralization [17, 18, 63, 85], something which may not be achieved by remineralization using GIC or GH. While some studies approved some kind of “re-hardening”, often measured via micro-hardness of mainly dry dentin, this does not necessarily indicate that the elastic behavior of moist dentin is restored [18, 74, 75], and the observed hardness gain has not been confirmed unambiguously [79, 86]. Future studies should employ nano-hardness assessment to relate mineral gain to the elastic behavior of dentin.

Second, we used a model to study mineral gains in residual lesions under simulation of both oral and pulpal effects. From coronally, artificial saliva and fluoride rinses were provided. The supply of fluoride was by design to allow the “recharge” of the GH, something which has been found to maintain the bioactivity of this material class [87-89]. From pulpally, a simulated pulpal fluid was used, which was supersaturated with minerals [47], while it is unclear if this fluid has the same effects of natural pulpal fluid.

Moreover, pulpal pressure as applied, while this pressure may vary from person to person, or change according to pulpal health. This may affect the effectiveness of any pulpal mineral delivery.

Third, we used standardized dentin discs and uniform, chemically induced artificial carious lesions. Chemically induced lesions have been shown to allow deeper remineralization than natural lesions and higher mineral gain [39]. Moreover, natural lesions have been subject to active pulpal reactions, i.e., tubular sclerosis, and bacterial degradation, which will affect remineralization speed and capacity.

Fourth, the decomposition of the organic dentin backbone by any carious attack has not been simulated. Remineralization of degraded dentin will differ, as laid out. Generally, the observed mineral gains may not be replicated in bacterially loaded, mineral-depleted dentin, as it has no seed crystals and allow classical remineralization [79]. Notably, and as discussed, there are many attempts to remineralize dentin via the non-classical pathway [85] .

Finally, this study investigated the mineral gains caused by different materials in addition to a passive mineralization from the pulp. However, such passive diffusion-determined remineralization is unlikely to occur clinically; instead, it will be mediated by cellular mechanisms. Further attempts should be made to combine cellular-based models for studying pulpal cell reactions and remineralization.

7. Conclusions

In conclusion, glass hybrid, but not calcium hydroxide, provided significant additional mineral gains in residual carious lesions when compared to pulpal mineral provision only. The relevance of this effect should be investigated further both with regards to cellular-mediated mineralization as well as the functional implications for residual carious lesion mechanics.

8. Compliance with Ethical Standards

Funding statement: FS received a grant from the German Research Foundation (SCHW 1766/2-1). This study was co-funded by GC Europe, Leuven, Belgium. The funders had no role in design, conduct, evaluation or interpretation of the study, or writing the manuscript.

Conflict of Interest: This study was co-funded by GC Europe, Leuven, Belgium. The funders had no role in design, conduct, evaluation or interpretation of the study, or writing the manuscript.

Ethical approval: All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. Extracted human permanent molars were obtained with informed consent based on an ethics approved protocol (EA4/102/14).

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10. Eidesstattliche Versicherung und ausführliche Anteilserklärung

10.1 Statutory Declaration (Eidesstattliche Versicherung)

“I, **Allam Al-Abdi**, by personally signing this document in lieu of an oath, hereby affirm that I prepared the submitted dissertation on the topic: **Glass hybrid, but not calcium hydroxide, remineralized artificial residual caries lesions in vitro** independently and without the support of third parties, and that I used no other sources and aids than those stated.

All parts which are based on the publications or presentations of other authors, either in letter or in spirit, are specified as such in accordance with the citing guidelines. The sections on methodology (in particular regarding practical work, laboratory regulations, statistical processing) and results (in particular regarding figures, charts and tables) are exclusively my responsibility.

Furthermore, I declare that I have correctly marked all of the data, the analyses, and the conclusions generated from data obtained in collaboration with other persons, and that I have correctly marked my own contribution and the contributions of other persons (cf. declaration of contribution). I have correctly marked all texts or parts of texts that were generated in collaboration with other persons.

My contributions to any publications to this dissertation correspond to those stated in the below joint declaration made together with the supervisor. All publications created within the scope of the dissertation comply with the guidelines of the ICMJE (International Committee of Medical Journal Editors; www.icmje.org) on authorship. In addition, I declare that I shall comply with the regulations of Charité – Universitätsmedizin Berlin on ensuring good scientific practice.

I declare that I have not yet submitted this dissertation in identical or similar form to another Faculty.

The significance of this statutory declaration and the consequences of a false statutory declaration under criminal law (Sections 156, 161 of the German Criminal Code) are known to me.”

Date Signature

10.2 Declaration of your own contribution to the top-journal publication for a PhD or MD/PhD degree (Ausführliche Anteilserklärung)

Allam Al-Abdi contributed the following to the below listed publication:

Publication 1:

Authors: Allam Al-Abdi, Sebastian Paris & Falk Schwendicke**.**

Title: Glass hybrid, but not calcium hydroxide, remineralized artificial residual caries lesions in vitro**.**

Journal: *Clin Oral Invest 21:389–396*

Year of Publication: 2016

The contributions in detail: The sole first author

Allam Al-Abdi:

- I participated in developing the study, formally analyzing and interpreting the data.
- I developed the methodology and experimental setup, particularly the remineralization model.
- I have prepared the samples for microradiograph and recorded all results.
- I have generated the tables 1, 2 and 3 and figures 1, 2, 3, 4 and 5.
- I have written the manuscript together with with FS.

FS, SP:

- They have participated in the development of the study, analyzed and interpreted and organized the data.
- They have contributed in acquisition of the financial support for the project leading to this publication.
- FS has co-authored the manuscript. Both authors revised it and agreed to it.

_____

Signature, date and stamp of first supervising university professor / lecturer

_____

Signature of doctoral candidate

11. Journal summary list

Selected JCR Year: 2015
Selected Editions: SCIE,SSCI
Selected Categories: 'DENTISTRY, SURGERY & MEDICINE'
Selected Category Scheme: WoS
Gesamtanzahl: 89 Journale

Rank	Full Journal Title	Total Cites	Journal Impact Factor	Eigenfactor Score
1	PERIODONTOLOGY 2000	3,376	4.949	0.004420
2	JOURNAL OF DENTAL RESEARCH	16,401	4.602	0.023310
3	ORAL ONCOLOGY	7,291	4.286	0.014940
4	Clinical Implant Dentistry and Related Research	3,161	4.152	0.007570
5	DENTAL MATERIALS	10,324	3.931	0.013230
6	JOURNAL OF CLINICAL PERIODONTOLOGY	11,767	3.915	0.013800
7	CLINICAL ORAL IMPLANTS RESEARCH	11,968	3.464	0.016610
8	JOURNAL OF DENTISTRY	6,199	3.109	0.011020
9	Molecular Oral Microbiology	577	3.061	0.002620
10	JOURNAL OF ENDODONTICS	12,519	2.904	0.016160
11	JOURNAL OF PERIODONTOLOGY	14,268	2.844	0.013060
12	INTERNATIONAL ENDODONTIC JOURNAL	5,253	2.842	0.008760
13	JOURNAL OF OROFACIAL PAIN	1,280	2.824	0.001940
14	OPERATIVE DENTISTRY	2,593	2.819	0.003360
15	International Journal of Oral Science	678	2.595	0.002510
16	JOURNAL OF PERIODONTAL RESEARCH	3,662	2.474	0.004930
17	Journal of Oral & Facial Pain and Headache	102	2.444	0.000400
18	European Journal of Oral Implantology	648	2.328	0.002390
19	CARIES RESEARCH	3,746	2.278	0.004540
20	COMMUNITY DENTISTRY AND ORAL EPIDEMIOLOGY	3,686	2.233	0.004050
21	Clinical Oral Investigations	3,250	2.207	0.009030
22	ORAL DISEASES	3,099	2.000	0.005750
23	JOURNAL OF ORAL REHABILITATION	4,600	1.926	0.005280
24	DENTOMAXILLOFACIAL RADIOLOGY	2,072	1.919	0.003540
25	INTERNATIONAL JOURNAL OF ORAL & MAXILLOFACIAL	8,038	1.859	0.009620

12. Ausgewählte Publikation

Allam Al-Abdi & Sebastian Paris & Falk Schwendicke

Glass hybrid, but not calcium hydroxide, remineralized artificial residual caries lesions in vitro. Clin Oral Invest (2017) 21:389–396

https:// doi.org/10.1007/s00784-016-1803-6

13. Lebenslauf

Mein Lebenslauf wird aus datenschutzrechtlichen Gründen in der elektronischen Version meiner Arbeit nicht veröffentlicht.

14. Publikationsliste

1. Pulpal Remineralisation of Artificial Residual Caries Lesions in vitro. Falk Schwendicke , **Allam Al-Abdi**, Hendrik Meyer-Lückel, Sebastian Paris. Caries Res. 2015;49(6):591-4.

2. Glass hybrid, but not calcium hydroxide, remineralized artificial residual caries lesions in vitro. **A Al-Abdi**, S Paris, F Schwendicke. Clin Oral Investig. 2017. PMID: 27033226

3. Remineralization effects of conventional and experimental ion-releasing materials in chemically or bacterially-induced dentin caries lesion. Falk Schwendicke **Allam Al-Abdi**, Agustín Pascual Moscardó , Alvaro Ferrando Cascales , Salvatore Sauro, Dent Mater. 2019 May;35(5):772-779.

4. In vitro performance of the DIAGNOcam for detecting proximal carious lesionsadjacent to composite restorations. Elhennawy K, Askar H, Jost-Brinkmann PG, Reda S, **Al-Abdi A**, Paris S,Schwendicke F. J Dent. 2018 Mar 8.

5. Secondary Caries Adjacent to Bulk or Incrementally Filled Composites Placed after Selective Excavation in vitro. Haitham Askar, **Allam Al-Abdi**, Uwe Blunck, Gerd Göstemeyer, Sebastian Paris and Falk Schwendicke. Materials 2021, 14, 939.

15. Wissenschaftliche Poster/Vorträge

1. ***Allam Al-Abdi****, Paris, S., Schwendicke, F****.***
   Glass hybrids, but not calcium hybrid remineralized artificial residual lesions in vitro. **IADR General Assembly** 2016, Abstract 661.

2. *Schwendicke, F.,* ***Al-Abdi, A****. Paris, S.*
   Glass hybrid, but not calcium hydroxide remineralized residual lesions in vitro. **Postervortrag, IADR General Session**, 2016, 22.06.2016, Seoul, Korea

3. H. Askar, **A. Al-Abdi**, U. Blunck, G. Göstemeyer, S. Paris, F. Schwendicke
   Secondary caries adjacent to bulk or incrementally filled composites placed after selective excavation in vitro.
   European Organisation for Caries Research (ORCA) Online Congress 7-10 July 2021

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Any omission in this brief acknowledgment does not mean lack of gratitude.

Thanking you

Allam Ahmed Mosleh Al-Abdi