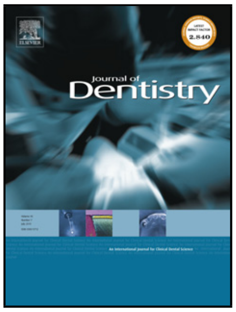

Mineral exchange within restorative materials following incomplete carious lesion removal using 3D non-destructive XMT subtraction methodology

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PII: S0300-5712(20)30135-4
DOI: https://doi.org/10.1016/j.jdent.2020.103389
Reference: JJOD 103389

To appear in: *Journal of Dentistry*

Received Date: 1 May 2020
Revised Date: 21 May 2020
Accepted Date: 28 May 2020

Please cite this article as: { doi: https://doi.org/

Mineral exchange within restorative materials following incomplete carious lesion removal using 3D non-destructive XMT subtraction methodology

Short title: Assessment of cavity liners following incomplete carious lesion removal with XMT

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Abstract

Objectives. The objective of this study was to quantify the changes in mineral and selected element concentrations within residual carious dentine and restorative materials following incomplete carious lesion removal (ICLR) using different cavity liners, with non-destructive subtraction 3D-X-ray Microtomography (XMT, QMUL, London, UK).

Materials and Methods. A total of 126 extracted teeth with deep dental caries were assessed using International Caries Risk and Assessment (ICDAS). Eight teeth were subsequently selected after radiographic evaluation. Each lesion was removed, leaving a thin layer of leathery dentine at the deepest part of cavity. Different cavity lining materials were placed; Mineral Trioxide Aggregate (MTA), calcium hydroxide, ($Ca(OH)_2$), resin-based material (RBM). For each, the restorative material was an encapsulated glass ionomer (GIC) and the control group had a GIC restoration alone. Each tooth was immediately placed in Simulated Body Fluid (SBF). All samples were then imaged using XMT at baseline, and three weeks after treament. The XMT images were then subtracted to show the mineral concentration changes three weeks after treatment.

Results. There were significant increases in mineral concentrations within the residual demineralised dentine in individual teeth treated with $Ca(OH)_2$, MTA, RBM, and GIC following immersion in SBF for three weeks. GIC group without any liners showed the greatest increase in mineral concentration, followed by MTA and $Ca(OH)_2$.

Conclusion. Mineral changes in demineralised dentine and within restorative materials are quantifiable using non-destructive 3D-XMT subtraction methodology. This laboratory study suggested that calcium, phosphate and strontium ion-exchange occurs with GIC, MTA and $Ca(OH)_2$ in deep dentinal lesions following ICLR.

Clinical relevance. In clinical practice, incomplete carious lesion removal could be performed to avoid the dental pulp exposure. 3D non-destructive XMT subtraction methodology in a laboratory setting is advantageous to provide evidence for different restorative materials on deep carious lesions prior to clinical

investigations.

Keywords: Dental caries, calcium hydroxide, MTA, GIC, resin-based material, XMT

Introduction

Dental caries is one of the most prevalent diseases worldwide affecting billions of people globally and causing significant health care costs.[1] However, there have been significant challenges for the management of deep caries lesions due to the risk of dental pulp exposure. Currently, there are three treatment options for deep carious lesions; total carious lesion excavation/ complete caries excavation (TCLE), stepwise excavation (SWE) and incomplete carious lesion removal (ICLR). Nowadays, TCLE for deep carious lesions is not recommended due to the potential indirect dental pulp damage. This could be from the irritation going through the residual demineralised dentine and/or from the unnecessary weakening of tooth structure integrity.[2,3] However, the aim of the ICRL technique, especially for the deep carious lesions, is to arrest the progression of dental caries and to maximise the remineralisation of the demineralised residual dentine thus maintaining the dental pulp vitality.

The introduction of minimally invasive dentistry (MID) has changed the conventional concepts for management of dental caries into a more conservative approach.[4] Despite this, it should be noted that in most countries, many clinicians still would prefer restorative intervention in cases where the carious lesions are clinically and radiographically confined to enamel, or, only just extended into dentine. Interestingly, this invasive approach was strongly influenced by the caries risk assessments that were performed for patients especially with high caries risk profile.[3]

In clinical practice, cavity liners are commonly used for deep carious lesions due to their sealing ability; being antimicrobial, having remineralisation potential, and also providing thermal or electric insulation.[5] Calcium hydroxide ($Ca(OH)_2$) has been used in direct, and in indirect pulp capping, as the “gold standard” lining material.[6] Mizuno et al.,[7] reported that $Ca(OH)_2$ promotes the formation of reparative dentine by; cellular differentiation, matrix secretion, and subsequent remineralisation. Favourable clinical outcomes for deep carious lesions have also been observed with the use of $Ca(OH)_2$.[8] However, the effect of Mineral Trioxide Aggregate (MTA) in reparative dentine formation for the management of deep carious lesion is not fully understood.

Glass ionomer restorative materials have the ability to adhere to moist enamel and dentine surfaces, with increased wear resistance and have anti-cariogenic properties due to their long-term fluoride release. Whereas, glass-ionomer cements are synthetic materials, which are inherently bioactive, contributing to the release of biologically active ions (fluoride, calcium/strontium and silicate) into the surrounding tissue.[9]

Bioactive dental materials are becoming increasingly popular in clinical dentistry, and are able to have a biological effect or be biologically active, forming a mechanical and/or a chemical bond with the hard tissues (*i.e.,* dentine/enamel). Many glass ionomer cements contain strontium which lies below Ca in Group II of the Periodic Table and is similar chemically to calcium. For example, fluoro-alumino-silicate glass has the ability to degrade and the carboxylic acid groups chelate calcium ions in the hydroxyapatite of dentine, a process that is considered to be bioactive.[10]

It has been reported that when the ICLR technique was carried out (whilst leaving some residual carious dentine), there was a higher tooth survival rate compared to stepwise excavation.[11] In addition, Singh et al.[12] recently reported that the success rate (clinical and radiographic) using the ICLR technique and composite resin with or without cavity liners ($Ca(OH)_2$/resin modified GIC liner) in deep carious lesions in permanent teeth was 96% after 12 months (96.8% for the $Ca(OH)_2$ group, 94.6% in the composite resin only group and 96.5% in the RMGIC group). Interestingly, the success rates of these restorations were independent of the cavity liners used in the study. However, there were still seven dental pulpal failures and to date, there is a lack of evidence concerning the reasons why some resin-based restorations fail in deep carious lesions.

With this respect, Kuhn et al.[13] provided evidence that dentine reorganisation and mineral changes were not dependent on the lining material and composite resin used to restore the deep carious lesions by step-wise excavation and indirect pulp capping techniques. It was reported that the carious arrestment (95% success rate) might be due to a host-driven process rather than the effects of dental materials.

These clinical studies used composite restorations either with or without the lining materials to restore deep carious lesions. In addition, there were limiations *i.e.*, small sample size, lack of control for assesssors' bias and lack of classifications for the severity of carious lesions present that made the outcomes questionable.

In the literature, there is still a lack of data to demonstrate and validate the efficacy of different restoration materials (using a reinforced GIC restoration either with or without MTA/resin based liner) and the potential ion exchange between these materials and residual demineralised dentine following the removal of deep carious lesions.

Non-destructive 3D X-ray Microtomography (XMT) has been used to measure mineral concentrations in dental hard tissues for a range of studies.[14-16] The MuCAT XMT system developed at Queen Mary University of London (QMUL), employs time-delay integration for enhanced contrast resolution, and incorporates accurate beam-hardening correction and calibration; all to obtain accurate linear absorption coefficient (LAC) measurements. Calibration is performed after every scan to characterise the X-ray spectrum to allow LACs to be measured in each image voxel with a high degree of accuracy, and, using published X-ray absorption coefficient data, to be converted to mineral concentration values if the chemical composition is known. Alignment of XMT images before and after each treatment regimen allows the migration of chemical ions to be quantified. This is achieved by subtracting the aligned XMT images, visualising and quantifying the changes in the region of interest identified at the subtracted XMT images.

The objective of this study was to quantify the changes in mineral and selected element concentrations within carious dentine and restorative material following incomplete carious lesion removal with different lining materials, using non-destructive 3D X-ray Microtomography (XMT, QMUL, London, UK). The null hypothesis of this study is that there is no difference between different lining materials used in the changes of minerals within the residual carious dentine and also within restorative material following incomplete carious lesion removal using 3D XMT.

Materials and Methods

126 freshly extracted teeth with dental caries were collected from the Oral Surgery Department (Ethical approval No. ORECNI, 16/NI/0101).

Clinical and radiographic assessments of each tooth were initially carried out to determine the extent of carious lesions. Eight teeth were then selected after applying the following criteria: teeth with coronal caries with ICDAS Score 5 (distinct cavity in opaque or discoloured enamel with visible dentine.[17] The teeth had cavitated extensive lesions close to the dental pulp. The dental probe with a blunt end is used to confirm the presence of the cavity within dentine. This was achieved by sliding the blunt end along the suspect pit or fissure and a dentin cavity was detected if the probe entered the opening of the cavity and the base was in dentine. The selected teeth were then stored in 1% thymol solution. A pre-treatment XMT scan with a voxel size of 30 µm was performed to ensure sufficent depth of the carious lesions into the dentine without any pulp exposure.

Study design

The eight extracted teeth with deep carious lesions (ICDAS Score: 5) were divided into three test groups and one control group as shown in Table 1.

All teeth were prepared using the PCR technique, leaving behind a thin layer of affected dentine, and then restoring with a dental material.[18] Following the application of each dental pulp capping material ($Ca(OH)_2$, MTA or RBM), hybrid encapsulated GIC restorations were placed into the cavity according to the manufacturers' recommendations. Immediately, each restored tooth was mounted into a clear soft wax with 5 mm thickness (6969 from PothHille&Co Ltd, Rainham, Essex, UK) leaving the crown exposed, and located in a clear small container (10ml internal diameter; Sterilin, UK) containing 1% thymol solution. Each container was mounted onto the XMT movable kinematic stage, ensuring the long axis of the tooth was parallel to the XMT rotational axis.

Sample storage

Two storage media were prepared; a 1% thymol solution for antibacterial and anti-fungal function (this was only used for the initial storage of extracted teeth prior to the placement of restorations), and simulated body fluid (SBF), prepared in containing; NaCl (136.82 mM), $NaHCO_3$ (3.72 mM), KCl (3.06 mM), $K_2HPO_4 \cdot 3H_2O$ (1.00 mM), $MgCl_2 \cdot 6H_2O$ (1.61 mM), $CaCl_2 \cdot 2H_2O$ (2.50 mM), Na_2SO_4 (0.50 mM), 1N-HCl (40 mL) and Tris $(CH_2OH)_3CNH_2$ (50 mM).[19] The pH was adjusted to 7.25 with KOH using a pH meter (Oakton Instruments, Nijkerk, The Netherlands) at 36.5°C. The SBF solution was stored in a sterilised polyethylene bottle and kept in an orbital shaker incubator at a speed of 60 rpm (Ks 4000 i control IKA) at 36.5°C. Following the baseline XMT scan, the thymol solution in each container was replaced with SBF using a pipette.

XMT Scans

The X-ray generator was operated at 90 kV and 180 µA. The geometry of the QM XMT apparatus was set for 15 µm voxel dimension. For each image, 1107 X-ray projections were recorded over a period of approximately 20 hours (the long scan time was required for high contrast resolution). A calibration carousel was automatically scanned immediately thereafter.[14]

Following the placement of restorations, each container was immediately XMT scanned again in order to obtain the baseline scan for each tooth. Each container was then filled with SBF and then located in an orbital shaker incubator for three weeks.[14] Following this, the final XMT scans for each tooth were performed.

Substraction XMT Image analysis

Each XMT scan was corrected for beam-hardening (at equivalent 40 keV monochromatic energy)[20] and reconstructed to obtain a 3D image. Then, the final image of each sample was aligned with the corresponding baseline image using in-house developed alignment software running under IDL (Harris Geospatial Solutions, Inc). This alignment software allows the user to select and view three orthogonal slices through the specimen and makes the adjustments to the rotation in x, y and z-axis as well as offset in the same axes and scale (7 degrees of freedom). After an initial approximate manual alignment, a more precise automatic alignment is made using a simplex optimisation method that adjusts all 7 degrees of freedom whilst measuring the edge alignment in the three selected slices.[14]

The baseline image was then subtracted from the final image using in-house developed routines in IDL. This 3D subtraction is used to image the linear attenuation coefficient (LAC) change in the demineralised area. To quantify this LAC change, the average LAC value in a total of 15 randomly selected 3×3×3 voxel regions in the baseline image (all at a depth of approximately 200 µm within the residual dentine) was compared with the corresponding average LAC value measured from the 15 voxel regions in the same locations obtained from the final XMT image.

Furthermore, if the chemical ions leached from the restorative material is known, and it is assumed to be one only, then it is possible to convert the measured LAC changes to “leached ion concentration” changes. This was calculated from published mass attenuation coefficient values of the corresponding elements.[21, 22] It should be noted that this is not possible with fluoride, since the mass attenuation coefficient of fluorine is too low to be detected by the XMT system.

$$\boldsymbol{\Delta} C = \boldsymbol{\Delta}\mu/\mu_m$$

Where,

ΔC = change in concentration (g cm^{-3}) of leached ions

$\Delta \mu$ = change in linear attenuation coefficient (cm^{-1})

μ_m= mass attenuation coefficient (attenuation per unit area) of element/ion (cm^2g^{-1}); values are as follows:

Sr= 9.8 cm^2/g

Ca= 1.8 cm^2/g

F = 0.28 cm^2/g

Results

The LAC changes and leached ion concentration changes for each test and control samples according to different minerals are presented in Table 2.

Test group 1. $Ca(OH)_2$ and GIC group

Figure 1 shows an increase in the LAC of the residual demineralised dentine in the subtracted image for both Note, the bright curved line in Tooth 1 within the GIC, near the boundary, represents a void that was filled with solution on the second scan, creating a relatively large difference in LAC; not to be confused with ion transfer.

Test group 2. MTA and GIC group

Figure 2 shows a marked increase in the LAC within the residual demineralised dentine just beneath the MTA material in Teeth 4 and 6. Interestingly, both teeth demonstrated mineral gain within the GIC following the incomplete carious lesion removal and restoration using MTA and GIC.

Test group 3. Bioactive resin-based lining and GIC group

Figure 3 shows no detectable change in LAC within the residual demineralised dentine in Tooth 7. However, there was a small increase in the LAC above the residual demineralised dentine in Tooth 5 after three weeks. Both resin based liners had an optimum marginal integration with the demineralised residual dentine as shown in Figure 3. However, there was a discrepancy between the GIC and the resin matrix (shown in the subtracted images in Figure 3),

Control Group with GIC treated only

Figure 4 shows an increase in the LAC above the residual demineralised dentine, and a decrease in the LAC at the GIC restoration site for both Tooth 2 and Tooth 3.

Discussion

This study demonstrates the applicability of non-destructive subtraction 3D X-ray Microtomography (XMT) to image and quantify the changes in LAC within carious dentine following incomplete carious lesion removal with different restorative materials. It could be speculated that this reported ion exchange would be favourable within the residual carious lesion either with a cavity liner and GIC restoration or GIC alone to contribute to the remineralisation process and would potentially protect the health of the dental pulp. This study is the first to show these ion exchanges using the 3D subtraction methodology within GIC with or without different cavity liners and also within the residual carious lesion to protect the vitality of dental pulp.

Davis et al.,[14] previously demonstrated small changes in the LAC one week after GIC restoration using ART technique and these authors reported that the continuing ion transfer needs to be observed over a longer period of time to detect significant changes in the LAC over time. It should also be noted that the significant changes in the LAC from the subtracted images is valuable for the assessment of remineralisation in laboratory based studies prior to the controlled randomised clinical studies. Therefore, in this XMT study, the samples were stored in the SBF for a period of three weeks.

In this study, the calcium hydroxide lining material with hybrid GIC restoration (Group 1) showed a layer formation on the surface of the residual dentine adjacent to the restoration. This might be associated with the release of calcium ions from the cement reacting with phosphate from the SBF to form amourphous calciumphosphate.[23]

In the MTA and hybrid GIC group (Group 2), the presence of bismuth oxide increases the radio-opacity, which could clearly be seen in the XMT images. It has been reported that there is an immediate release of ions ie., calcium, which could be due to the high solubility of MTA and its water sorption properties.[24,25] However, the marginal adaptation with MTA at the base of the cavity was suboptimal in both cases. It should be noted that the leaching of ions from the material into the demineralised dentine and also within the GIC was clearly visible, which might be due to the reaction of tri-calcium silicate with water, producing calcium silicate and hydroxide.[24-26] It has been reported that this material has promoted dentine remineralisation in the presence of SBF.[27]

In the resin-based material group (Group 3), there was evidence of optimum marginal integrity on the base of the tooth surface, providing a good adaptation with the dentine without any marginal gaps between the tooth surface and material. However, the subtracted XMT images showed only weak evidence of ion exchange in the demineralised residual dentine after the three-week immersion period. In addition, there was a noticeable gap between the GIC and resin matrix (shown in the subtracted images in Figure 3), which could be related to the lack of integration between these two materials since resin material is hydrophobic, whilst GIC has hydrophilic nature.

Lastly, the GIC (Group 4) showed the greatest increase in LAC in the demineralised residual dentine (about 20%) and about the same reduction in the GIC in the period of three weeks, with evidence of remineralisation within the demineralised dentine. However, the increase in LAC might be due to strontium, (Sr) which has a mass attenuation coefficient over five times that of calcium. Intrestingly, there was the appearance of a thin radiolucent line above the partially demineralised dentine and within the GIC. It might be related to the ion exchanges from the GIC into the residual demineralised dentine which was a corresponding loss of the mass attenuation coefficient within the cement. With this respect, the ion exchanges from the restorative material became the gain for the demineralised dentine. The GIC also showed good integration with the tooth surface. Interestingly, the surface of this material in the occlusal region demonstrated loss of minerals, which could be due to the immediate interaction of this GIC material after the placement of restoration within the storage medium. A previous semi-quantitative study investigated remineralisation of dentinal carious lesions and their strontium uptake using electron probe micro-analyser on cut sections, and found Sr within the lesions.[28,29] However, there is a risk that sectioning and polishing might have had an impact on components of the glass ionomer restoration over the surface of teeth being examined in these studies. The subtraction XMT technique descibed here is non-destructive, and directly quantitative, and therefore the quantitative results reported in this study are in agreement with the indirect semi-quantitative measurements of the previous study.

The small sample size could be a limitation of this study. However, the non-destructive nature of XMT coupled with the alignment procedure means that mineral changes are measured in the same sample at the start, and at the end of the three-week post-operative period. Therefore, the samples are their own controls[30] reducing the need for a large sample size required to account for variability in baseline mineral concentration.

It should also be noted that although the XMT image alignment is algorithm accurate, some dimensional change is inevitable over time that makes perfect alignment impossible.[13] When digital image subtraction is performed, even a shift of one tenth of a voxel results in a clearly visible line in images with enhanced contrast. This should not be confused with the presence of a marginal gap. Similarly, voids that are empty in one scan and filled with water in another also show up very clearly in the contrast-enhanced images. In this study, results are therefore taken from voxels that are selected away from any boundaries to avoid errors caused by misalignment.

Finally, a slower rate of mineralisation over three weeks in the RBM group was reported when compared to the other groups. It should be noted that the ability of the QMUL XMT technique to detect fluorine is not possible, since the QMUL XMT technique is sensitive to Sr^{2+} because of its high mass attenuation coefficient (9.8 cm^2/gm at 40 keV) and, to a lesser extent, Ca^{2+} (1.8 cm^2/gm), however not to fluorine.[14] For ions that cannot be detected in XMT, and to verify the concentration of those that can, Energy Dispersive X-ray analysis (EDX) could be used, however since this would involve sectioning the teeth, it would not be possible to obtain results at different time points. Therefore, it should be noted that in the case of calcium and fluorine, there is no way of knowing how much was initially present.

In conclusion, within the limitations of this unique laboratory based study with different cavity liners using non-destructive subtraction 3D X-ray Microtomography (XMT) was able to quantify the increased/decreased mineral concentrations within carious dentine following incomplete carious lesions removal. The restorative materials; GIC alone, MTA, calcium hydroxide, and resin based material showed a potential for releasing ions into residual demineralised dentine. However, further histological and microbiological investigations in addition to the use of XMT are required to analyse the remineralised carious lesions to determine the nature of the mineral formed.

CRediT author statement

Zain Syed: Investigation, Data curation, Software, Writing- Original draft preparation

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Aylin Baysan: Conceptualization, Supervision, Methodology, Investigation, Validation, Writing-Reviewing and Editing,

Declaration of interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

The authors express their gratitude to Miss Ting Yu Cheng (5th Year Undergraduate Dental student), Dr David Mills (XMT laboratory) and Ms Shameem Shariff (Dental Physics Laboratory) for their assistance throughout the study.

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Figure 1. (a)-Aligned XMT of Tooth 1 (left) and Tooth 8 (right) after three weeks following the placement of $Ca(OH)_2$. (b) Subtracted images of Tooth 1 and Tooth 8 with 8x enhanced contrast showing radio-opacity (white arrow) around the residual demineralised dentine and black arrow demonstrates mineral loss from the restorative material.

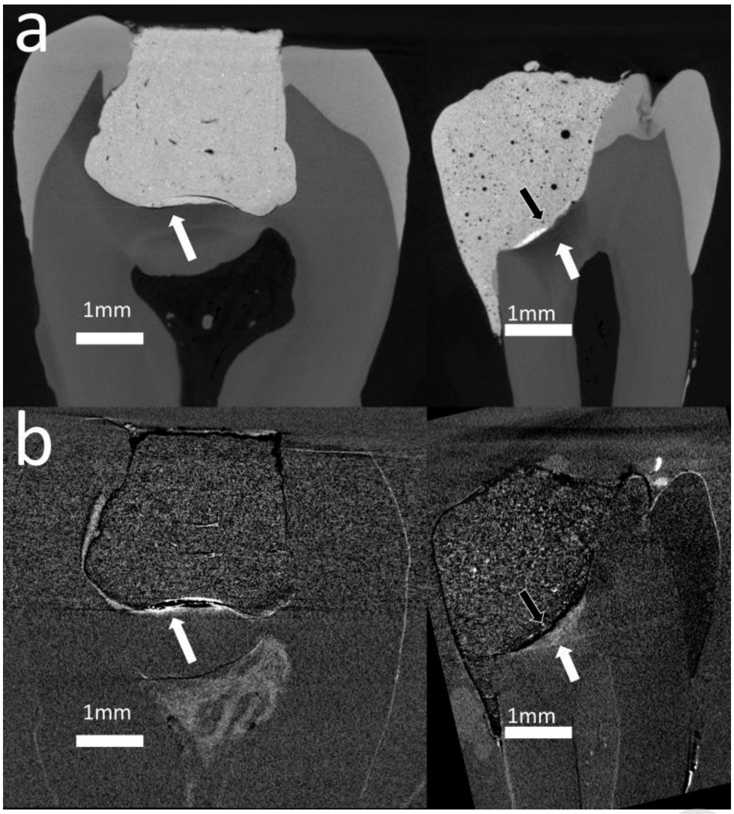

Figure 2. (a)-Tooth 4 (left) and Tooth 6 (right) after the restorations with MTA+ (GIC).

(b)- Subtracted images of Tooth 4 and Tooth 6 after three weeks with 8x enhanced contrast following the placement of restorations showing is a marked drop in mineral concentration in the MTA, with the increase in mineral concentration going deep into residual demineralised dentine (White arrow). Interestingly, there also appears to be an increase in mineral concentration within the GIC.

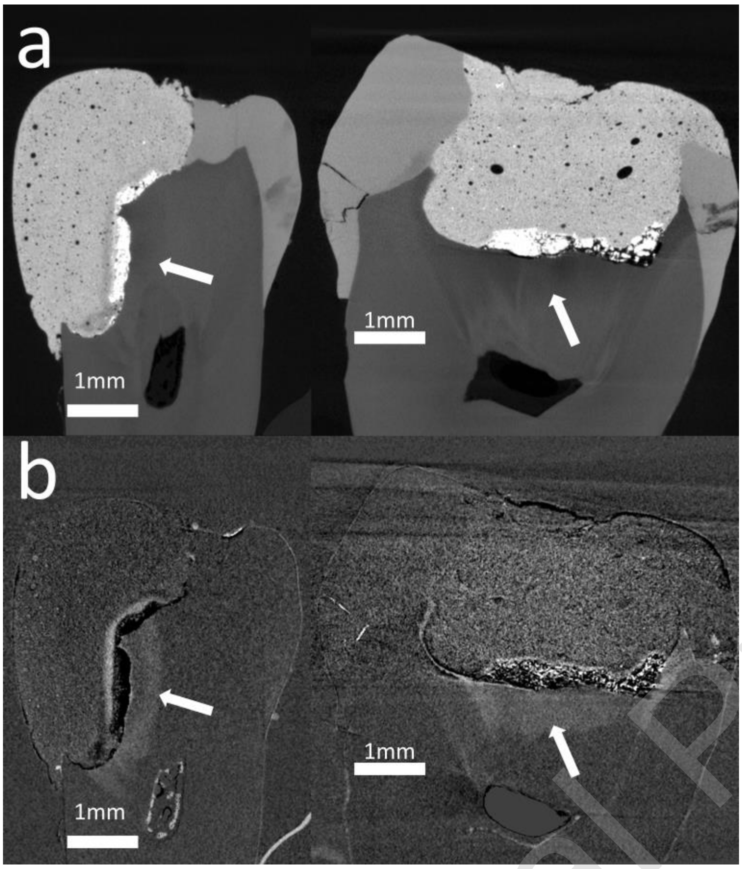

Figure 3. (a)-Aligned XMT of Tooth 5 (left) and Tooth 7 (right) after three weeks following the placement of resin-based material. (b)-subtracted images of Tooth 5 and Tooth 7 with 8x enhanced contrast and black arrow demonstrates the discrepancy between the GIC and the resin matrix .

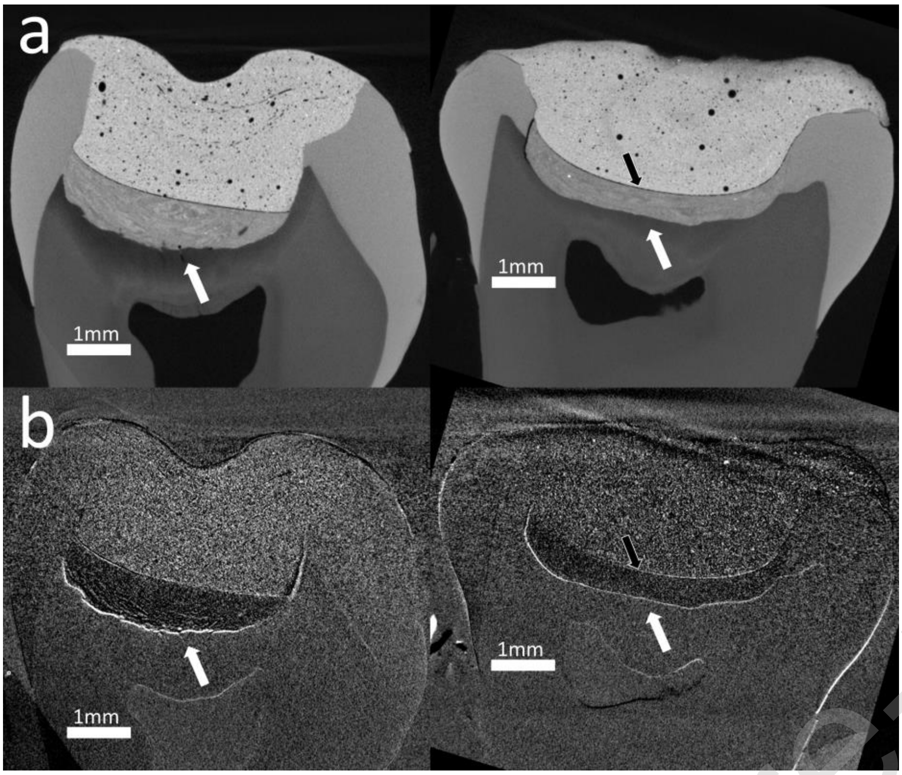

Figure 4. (a) Aligned XMT of Tooth 2 (left) and Tooth 3 (right) with the reinforced encapsulated GIC. (b) Subtracted images of Tooth 2 and Tooth 3, showing both increase in LAC in the residual demineralised dentine (radio-opaque area) (white arrow) and decreased LAC in the GIC restoration (radiolucent area) (white arrow) with 8x enhanced contrast.

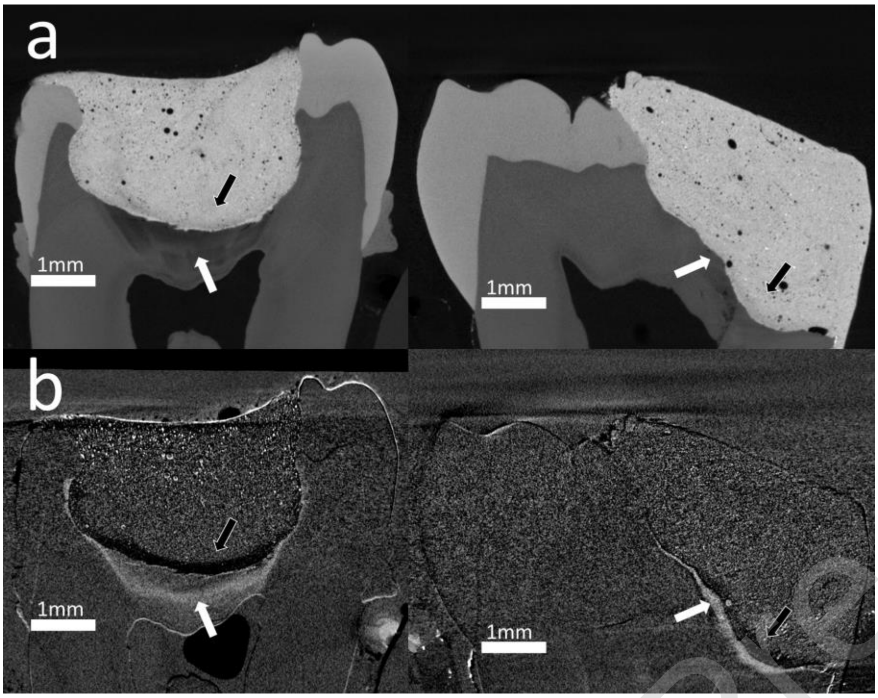

Table 1. Restorative materials used in this study

Group 1.	$Ca(OH)_2$ (Life, Kerr USA) + reinforced encapsulated GIC (EQUIA FORTE, GC Japan)
Group 2.	MTA (Angelus, Brazil) + reinforced encapsulated GIC (EQUIA FORTE, GC Japan)
Group 3.	Resin based Liner (Activa, Pulpdent, USA) + reinforced encapsulated GIC (EQUIA FORTE, GC Japan)
Group 4.	Encapsulated GIC alone (EQUIA FORTE, GC Japan)

Table 2. LAC and concentration differences for each test and control samples according to different minerals

Groups	**LAC Difference (cm^{-1})**	**ION**	**Concentration difference ($mg\ cm^{-3}$)**
Group 1. $Ca(OH)_2$	0.149 (Tooth 1)	Calcium	81.60
	0.168 (Tooth 8)	Calcium	91.80
Group 2. MTA	0.050 (Tooth 4)	Calcium	27.78
	0.071 (Tooth 6)	Calcium	38.97
Group 3. **Resin based Liner**	0.058 (Tooth 5)	Fluoride	Unknown
	0.034 (Tooth 7)	Fluoride	Unknown
Group 4. **Encapsulated GIC alone**	0.153 (Tooth 2)	Strontium	15.61
	0.209 (Tooth 3)	Strontium	21.31