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Original Article

Assessment of remineralisation potentials of bioactive dental composite using an in-vitro demineralised dentine model

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الملخص

أهداف البحث: يشجع مفهوم الحد الأدنى من التدخل الجراحي لطب الأسنان على تجريف التسوس السني التحفظي وإعادة تمعدن أنسجة الأسنان المتبقية. ومع ذلك، يعتبر إعادة تمعدن العاج أكثر صعوبة من إعادة تمعدن الميناء ويرجع ذلك بسبب الاختلاف في تركيبتهم. يهدف هذا البحث إلى تقييم إمكانية إعادة التمعدن لحشوة -اكتيفا المنشط الحيوي الترميمي و بيتوفل-2 على عينات العاج المنزوعة المعادن، ثم مقارنتها بحشوة المتماثرات الزجاجية باستخدام جهاز مطيافية تشتت الطاقة بالأشعة السينية وجهاز الصلادة.

طرق البحث: تم خلع عشرة أضراس سليمة، وتقسيمها إلى نصفين ونزع المعادن جزئيا منها باستخدام ٣٧٪ حمض الفوسفوريك لمدة ٦٠ ثانية. تمت معاينة جميع العينات قبل وضع الحشوة باستخدام جهاز مطيافية تشتت الطاقة بالأشعة السينية وجهاز الصلادة. وبعد ذلك تم تقسيم العينات إلى أربع مجموعات تحتوي كل مجموعة على ٥ عينات. المجموعة ١ تم استخدام حشوة - اكتيفا المنشط الحيوي الترميمي، المجموعة ٢ استخدم فيها حشوة بيتوفل-2، المجموعة ٣ تم استخدام حشوة المتماثرات الزجاجية والمجموعة الأخيرة مجموعة التحكم. بعد وضع الحشوات والتخزين، تم تقييم العينات باستخدام جهاز مطيافية تشتت الطاقة بالأشعة السينية وجهاز الصلادة.

النتائج: أظهرت النتائج أن بروتوكول انتزاع المعادن باستخدام ٣٧٪ حمض الفوسفوريك حقق انخفاض كبير في نسبة الكالسيوم الى الفوسفات وتغيرات جهاز الصلادة. حدثت إعادة التمعدن في جميع العينات، ولكن لوحظت أعلى نسبة تغيير في نسبة الكالسيوم الى الفوسفات و جهاز الصلادة في مجموعة اكتيفا المنشط الحيوي الترميمي (٢٠,٦٦٪ ، ٨٢,٠١٪) على التوالي، يليها مجموعة بيتوفل-2 ثم مجموعة المتماثرات الزجاجية وأخيرا مجموعة التحكم.

الاستنتاجات: تعتبر حشوة اكتيفا المنشط الحيوي الترميمية الأكثر تفوقا لإعادة تمعدن العاج من مجموعة بيتوفل-2 و المتماثرات الزجاجية لترميم الأسنان.

الكلمات المفتاحية: حشوة اكتيفا المنشط الحيوي الترميمية؛ مركب بيتوفل؛ جهاز مطيافية تشتت الطاقة بالأشعة السينية؛ جهاز الصلادة؛ إعادة تمعدن العاج.

Abstract

Objective: Minimally invasive dentistry encourages conservative caries excavation and remineralisation of the remaining dental tissues. However, dentine remineralisation is more difficult than enamel remineralisation due to the differences in their composition. This study aims to assess the remineralisation potential of Activa BioActive-Restorative and Beautifil II restoration on demineralised dentine samples, and compares it with glass-ionomer (GIC) restoration using energy dispersive X-ray (EDX) and Knoop hardness number (KHN).

Methods: Non-carious extracted molar teeth were used, a total number of ten teeth were sectioned into halves and partially demineralised using 37.0% phosphoric acid for 60 s. All samples are assessed using EDX and KHN prior to restorations. The samples are then subdivided into four groups (n = 5). Group 1 was restored with Activa BioActive-Restorative, Group 2 received Beautifil II, Group 3 was restored with GIC, and the last group was used as a negative control. After storage, the samples were analysed using EDX and KHN.

Results: The demineralisation protocol with 37.0% phosphoric acid significantly decreased the calcium:-phosphate (Ca:P) ratio and KHN. Remineralisation occurred in all groups, but the highest percentage change in Ca:P ratio and KHN was observed in the Activa BioActive-Restorative group (20.7%, 82.0%,

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respectively), followed by the Beautifil II group, glass ionomer group, and the control group, in that order.

Conclusion: Activa BioActive-Restorative restoration presents superior remineralisation compared to Beautifil II and glass-ionomer dental restorations.

Keywords: Activa BioActive-Restorative; Beautifil composite resin; Dentine; Energy dispersive X-Ray spectroscopy; Knoop hardness number

Introduction

Dental caries is a multi-factorial disease that occurs through the action of bacteria and their by-products, which dissolve the mineral content of enamel and dentine.[1] Over the past few decades, there has been a rapid increase in the prevalence of caries and it is considered the most common cause of extraction in primary teeth.[2] To reduce these numbers, dentists should aim to prevent, detect, and treat caries early by following the concept of minimally invasive dentistry.

Cariology studies have focused on the pathological mechanism of caries to develop different strategies that help in managing caries and its effect.[3] This includes minimising tooth cutting to the outer necrotic zone.[3,4] With the development of minimally invasive dentistry (MID), conventional dental caries removal principles have shifted to a more conservative intervention. Cavity preparation should end with a good peripheral seal that can be achieved by selective caries removal of caries-infected dentine, which contain the highest amount of bacteria, leaving only caries-affected dentine as it has a higher potential for remineralisation.[5] However, dentine remineralisation is more challenging than enamel remineralisation due to the differences in their composition. Therefore, various emerging bioactive dental materials that promote dentine remineralisation have been introduced and withdrawn in the past few years.[6]

The development of therapeutic bio-interactive materials results in tissue remineralisation, reduces the susceptibility to tooth mineral loss, and recovers its mechanical properties.[7]

Glass ionomer, Activa BioActive-Restorative, and Beautifil II are examples of flouride releasing restorative materials available in the market. The largest amount of fluoride is released from conventional glass-ionomer dental cement materials. It is considered to be a bioactive synthetic material that releases physiologically active ions (fluoride, calcium/strontium, and silicate) into the surrounding tissue.[8] In addition, it induces remineralisation depending on pre-existing nucleation and forms fluoro-apatite crystals that are more acid resistant than hydroxyl-apatite.[4,9] Giomers are a true hybrid of two compounds, glass ionomer and composite resin. It contains pre-reacted glass (PRG) filler particles within a resin matrix. PRG particles are responsible for the high amount of fluoride released and recharged from the giomer. Beautifil II is classified as a giomer restorative material that is a good choice for aesthetic restorations. Using S-PRG technology, many excellent characteristics and advantages of both glass ionomer and composite can be achieved in the material.[10] On the other hand, a new generation of bioactive materials with remineralising features is Activa BioActive (Pulpdent), which was made commercially available in 2013. It has been claimed that calcium, phosphate, and fluoride ions are continuously and passively diffused from the restorative material.[11] Moreover, it provides a guarantee to reverse tooth caries, prevent recurrent caries, and is capable of regaining lost minerals.[12]

Multiple methods are currently used to evaluate the occurrence of remineralisation, one of which is energy dispersive X-ray (EDX) microanalysis. Elemental analysis is used to identify the presence and amount of specific elements.[13] In this study, EDX was used to measure the mineral content and to evaluate the remineralisation ability of the materials. Moreover, microhardness testing has been used in many studies to reflect the demineralisation and remineralisation of tissues based on their hardness using the Knoop hardness number (KHN).[14] It is considered a valuable method to record the changes in specific specimens as it has been correlated with the amount of minerals in the samples, which may confirm mineral gain or remineralisation.[6,15]

The aim of this experiment was to evaluate a dentine demineralisation model that provides internal control segments with a relatively simple demineralisation protocol. Second, we assessed the interaction between this model and three different ion-releasing materials: Activa BioActive-Restorative Pulpdent, USA), Beautifil II restoration (SHOFU Dental GmbH, Japan), and a glass-ionomer cement (GIC) Fuji IX (GC Corporation. Tokyo, Japan) in terms of the relative effects of mineral deposition on the KHN of the underlying tissues.

Materials and Methods

Non-carious molar teeth were collected and used in the current study Total N = 10. For each tooth, the roots were cut to create a horizontal reference line. The crowns were sectioned vertically into two halves.

Preparation of demineralised dentine samples

Each sample was divided into demineralised and sound sections. First, a reference point was created using a fissure diamond bur (Figure 1); then, they were polished with 1200-grit carborundum papers using (MetaServ 250 Grinder-Polisher with Vector Power Head-Buehler-USA) under

running water. Each half was then covered with tape, leaving a 2 mm wide window for demineralisation. To demineralise the area, 37.0% phosphoric acid (Meta Etchant, Meta Biomed, Korea; lot MET1704271) applied for 60 s (Figure 1).[16,17] Samples were then washed, dried, and cleaned in an ultrasonic bath with deionised water for three minutes. For the baseline record, the Ca:P ratio was measured using EDX analysis in both sound and demineralised sections, with EDX (Oxford Instruments, England).[18] Afterwards, KHN was recorded using microhardness (MicroMet 6040).

Sample preparation for the evaluation of dental restorations

All samples were subdivided into four groups: five halves of teeth were restored with Activa BioActive-Restorative (Pulpdent, USA; lot 190619), following the manufacturer's instructions. The material was placed on the sample using a plastic instrument and then light-cured with a 5 Watt LED (E-Morlit, Apoza, China) for 20 s. Samples in Group 2 were restored with Beautifil II restoration (SHOFU Dental GmbH, Japan; lot 081971) following the manufacturer's instructions. The material was applied using a plastic instrument and then light-cured for 10 s using an LED light cure unit. For Group 3, Glass-ionomer FujiIX (GC Corporation, Japan; lot 1104061) was used to restore the samples following the manufacturer's instructions. The last group was used as a negative control and stored without restoration. All samples were stored in separate glass vials containing 7.0 ml of phosphate buffered saline (PanReac AppliChem ITW Reagents, USA) solution for four weeks. The storage solutions were replaced every two days, as shown in Figure 1.[19] The groups were blinded and numbered by group (1, 2, 3, 4,) to minimise bias as the observers were unaware of the identity or treatment group of their subjects while conducting research.

After storage, samples were sectioned horizontally at the reference mark to clearly show sound and demineralised dentine (Figure 1). Samples were then scanned with EDX and KHN was recorded to evaluate the changes in mineral content and KHN.

Statistical analysis

Data were explored for normality using Kolmogorov–Smirnov and Shapiro–Wilk tests, data exhibited parametric (normal) distribution. To test the demineralisation model, the differences between sound and demineralised dentine were compared using an independent samples t-test separately for different techniques (Ca:P ratio and KHN). The mineral content and hardness changes were examined before and after storage for comparison. The percentage change after ageing was calculated using the following equation $((Xa - Xb)/Xb) \times 100\%$, where Xb is the value before ageing and Xa is the value after ageing. A One-Way ANOVA test was performed to compare the groups. Tukey's post-hoc test was used to detect the differences between groups. The significance level was set at $p \leq 0.05$. Statistical analysis was performed with IBM® SPSS® (IBM Corp. Released 2011. IBM SPSS Statistics for Windows, Version 20.0. Armonk, NY: IBM Corp).

Results

Demineralisation protocol

The results confirm that demineralisation has occurred before applying any materials to the samples. It was found that the Ca:P ratio exhibited a significant decrease; the ratio was 1.70 in the sound dentine, and decreased to 1.45 in the demineralised dentine ($P = 0.344$) after application of 37% phosphoric acid. In addition, the KHN was measured and a significant decrease was recorded after demineralisation protocol. It was 32.7 in the sound sections and dropped to 17.3 in the demineralised dentine ($P = 0.001$) (Figure 2). This decrease in the Ca:P and KHN confirms that dentin demineralisation occurred following the application of 37.0% phosphoric acid.

Energy dispersive X-ray microanalysis before and after storage

EDX microanalysis was used to measure the Ca:P ratio in the demineralised sections for all four groups. First, the percentage changes in the Ca:P ratio were calculated before and after the storage period (four weeks). The changes relating to the analysed demineralised tissues are outlined as follows: the highest percentage change in Ca:P ratio was observed in the Activa BioActive-Restorative group (22.7%), followed by the Beautifil II group (18.3%), the GIC restoration group (15.1%), and the last group was the control group (5.93%) (Figure 3). However, the differences were insignificant between Activa BioActive-Restorative, GIC restoration, and Beautifil II restoration. Significant differences were observed only in the demineralised tissues compared to the control group with Activa BioActive, Beautifil II, and glass-ionomer groups ($P = 0.001$). However, comparing the material groups, there were no significant differences (P-values = 0.071, 0.101, 0.253, respectively).

Microhardness test before and after storage

Changes in the KHN were recorded for all four groups. The highest percentage change in KHN was found in the Activa BioActive-Restorative group (82.0%), followed by GIC restoration (72.5%), Beautifil II restoration (66.0%), and the control group (1.16%), in that order (Figure 4). There were significant differences when comparing restorative material groups and the control group ($P = 0.001$). However, there was no significant difference between restorative materials when compared to each other (P-values = 0.742, 0.541, 0.223, respectively).

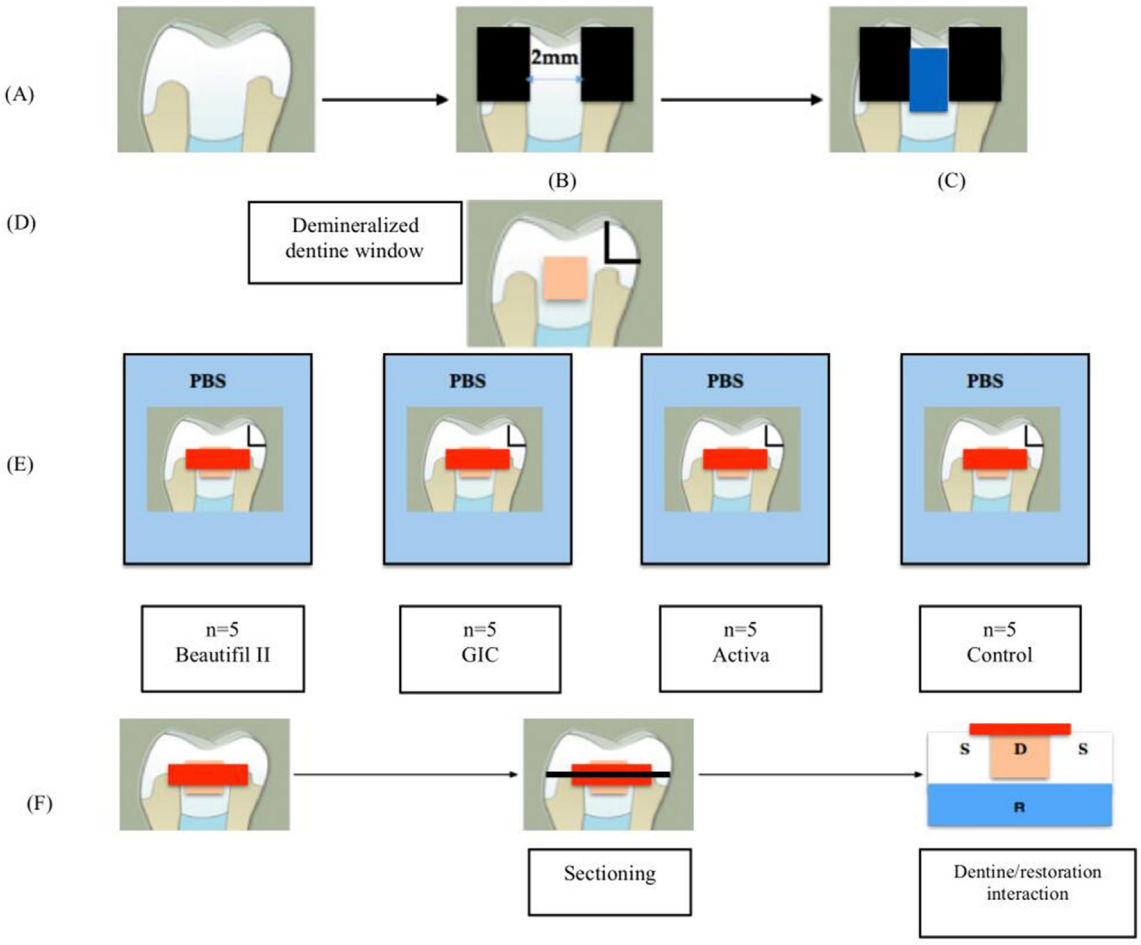

Figure 1: (A) Ten extracted sound teeth sectioned into halves. (B) Part of the dentine is covered with tape. (C) Demineralisation using 37% phosphoric acid for 60 s. (D) Twenty demineralised samples were selected as test samples. (E) Five halves received Activa BioActive, five halves received Beautifil II restoration, five halves received GIC, and the remaining five halves were used as control samples; all samples were stored in PBS storage media. (F) Samples were sectioned at the reference mark and placed in resin base. (S) sound dentine (D) demineralised lesion (R) resin base.

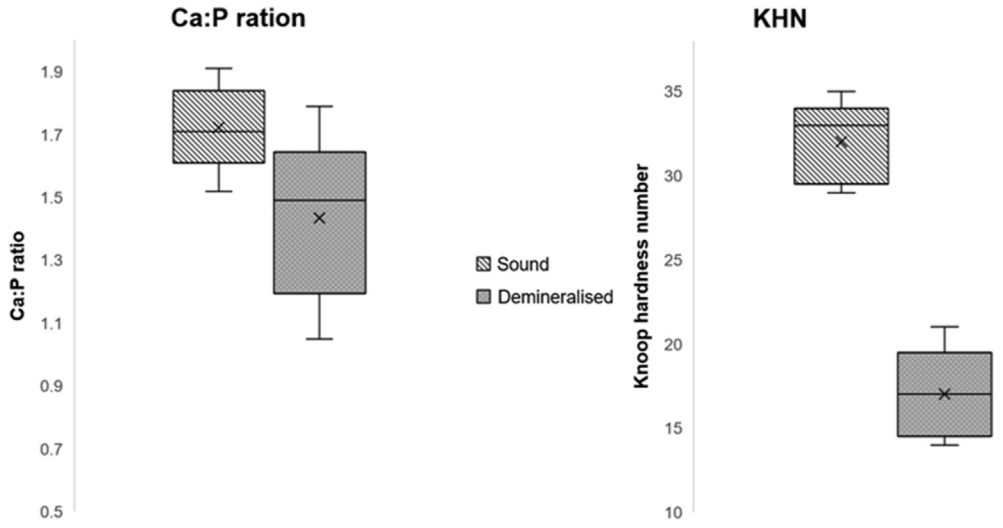

Figure 2: Decrease in Ca:P ratio from 1.70 to 1.45 and KHN from 32.65 to 17.31 after demineralisation with 37% phosphoric acid.

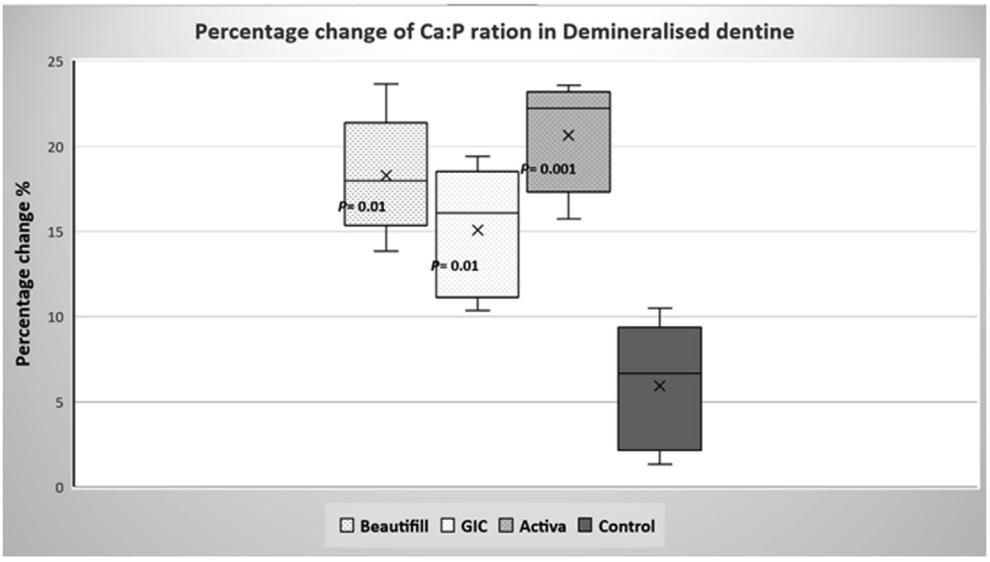

Figure 3: Percentage changes of Ca:P ratio in the demineralised dentine in the Activa BioActive, Beautifil II, glass ionomer, and control groups. Activa BioActive presents the highest percentage change of 20.66.

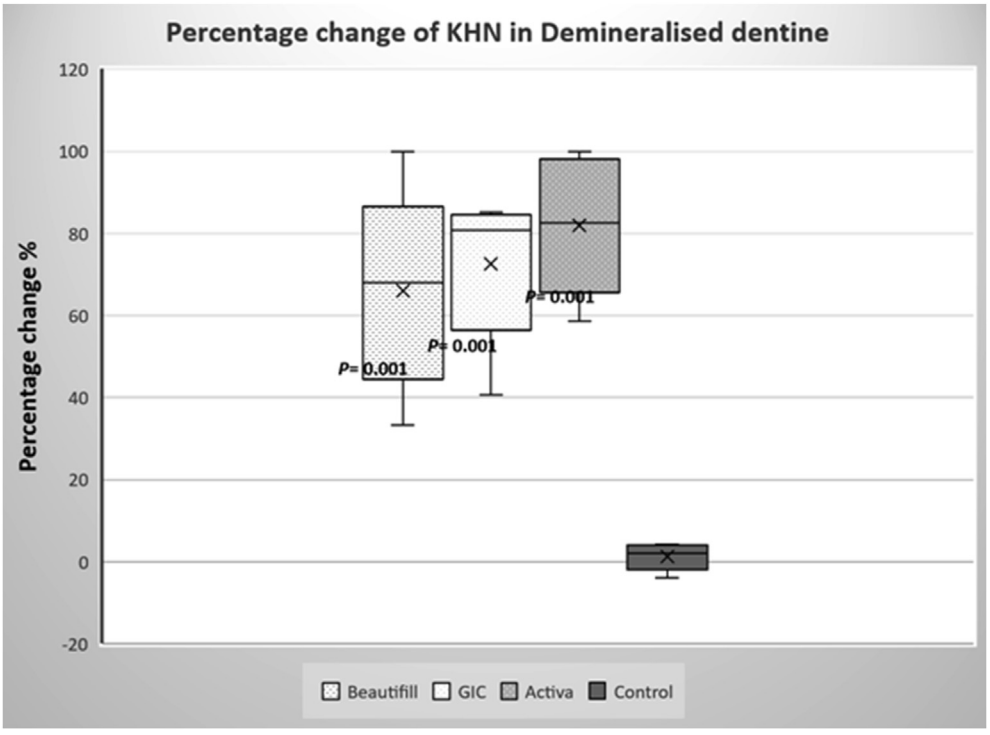

Figure 4: Percentage changes of KHN in the demineralised dentine in the Activa BioActive, Beautifil II, glass ionomer, and control groups. Activa BioActive presents the highest percentage change of 82.01.

Discussion

Dentine mainly consists of inorganic materials, hydroxyapatite, and non-crystalline amorphous calcium phosphate.[20] These minerals dissolve when exposed to acids from bacteria leading to dentine demineralisation.[21] Advancements in understanding the caries process and the biology of the accompanying dentine-pulp defence and regenerative responses have encouraged the application of minimal caries removal rather than a more aggressive traditional excavation approach. This approach relies on accurate diagnosis, then identifying the excavation end point to include only the irreversibly caries-infected dentine. This management technique enables healing of remineralisable caries-affected dentine and

avoids pulp injury. However, there is still no obvious clinical demarcation of the caries excavation limit that will ensure a good quality restoration and at the same time preserve the tooth structure from unnecessary cutting.[22,23] Schwendicke et al. listed strong recommendations that support selective caries removal by leaving sound peripheral margins for good seal and demineralised affected dentine on the pulpal floor for remineralisation and to avoid pulp exposure.[24]

In line with these advancements in managing dental caries, the search for the "ideal" material that could support the application of such an approach has been a subject of major interest in the dental and biomaterial research communities. The assessment of these materials' interactions with dental tissues and their therapeutic potential is essential to determine their efficacy and clinical applications.

In the current study, two emerging materials (Activa BioActive and Beautifil II) were evaluated for their remineralisation potentials using a demineralised dentine model to simulate clinically caries-affected dentine. The demineralisation protocol was applied using 37% phosphoric acid for 60 s.[16] Many protocols were applied in previous studies to perform in-vitro dentine demineralisation, the most common and easiest one being using phosphoric acid. An in-vitro study used 37% phosphoric acid for 15 s to induce partial demineralisation in dentin, and the results revealed a decrease in both nanohardness (Hi) and modulus of elasticity (Ei). In addition, they found less hydroxyapatite in demineralised dentine that was able to help in the remineralisation process.[25] Therefore, the dentine surface in this study was partially demineralised using 37% phosphoric acid for 60 s, which ensured the exposure and not destruction of the collagen fibres that are needed for remineralisation. As presented in the results section, this protocol was validated by EDX and microhardness, which confirmed that demineralisation decreased the Ca:P ratio and KHN.

EDX is one of the best techniques for the detection of mineral deposition in the tissue X because it has characteristic X-rays that show the presence of elements in the samples.[13] Previous studies have utilised EDX to evaluate remineralisation in dentine by quantitatively measuring the mineral content within the samples before and after applying the materials.[26,18] Moreover, the current study adopted microhardness to record the changes in the dental hard tissue by measuring the KHN). This technique is considered useful and commonly used in dental hard tissue studies to indirectly measure the amount of mineral content. Additionally, it is used to detect the changes in the tissues after applying any treatment in-vitro by measuring the mineral loss and gain in the demineralisation and remineralisation process.[6,27] Moreover, microhardness can be used as a gold standard to differentiate between sound dentine and caries zones.[6]

Regarding the investigated materials in this study, bioactive composite (Activa BioActive), giomer (Beautifil II), and glass-ionomer restorations were used as bioactive dental materials. These materials have similar clinical indications and related compositions, which makes them comparable. The remineralisation process of these materials depends on the classical approach, which relies on the epitaxial growth of residual crystals. Therefore, the presence of pre-existing crystals is essential as they cannot induce remineralisation on totally demineralised dentine.[4] Thus, partial demineralisation was performed. Activa BioActive-Restorative exhibited the best increase in both the Ca:P ratio and microhardness, although non-significantly. This material releases fluoride, calcium, and phosphate to promote remineralisation and prevent secondary caries.[11] It is characterised by the release of fluoride ions to form fluorapatite depending on the residual crystals, similar to GIC. The utilisations of fluoride include a "classical" ion-based, crystallisation concept.[28] However, the ionisation procedure depends on water, so hydrogen ions released from these groups are substituted by calcium in tooth structure. Additionally, biomineralisation could be stimulated by releasing Ca^{2+} and OH^{-} ions from Activa BioActive similar to the pulp capping materials.[12] Another benefit of this material has recently been reported, the continuous release of fluoride, calcium, and phosphate was found to promote the apatite formation and remineralisation result of seals the margins and reduce postoperative sensitivity.[29] Moreover, Activa BioActive-Restorative consists of a resilient, patented resin matrix combined with energy-absorbing elastomeric elements and acting like a fluoride reservoir.[30] A recent study found that that phosphate ions increased over time more than glass ionomer.[31] These findings are in agreement with our results and may explain the highest reading recorded of Ca:P ratio and KHN in the Activa BioActive-Restorative group, which may translate into providing the highest remineralisation potential, among other materials.

On the other hand, Beautifil II restoration is a second-generation giomer. It has surface PRG-ionomer (S-PRG) particles that are found to be a reservoir for fluoride that can release and recharge fluoride ions.[32,33] There is a shortage of studies confirming the release of calcium and phosphate ions from Beautifil II restoration. According to reports, fluoride released from giomers was higher than that of composite resins, but lower than that of GICs.[34] Glass-ionomer restorations were found to release more fluoride than giomer (Beautifil II) and other compomers.[35] Moreover, glass-ionomers have also exhibited a higher hardness number than compomer and other fluoride-containing composite restorative materials.[36] These findings support the results of this study, as glass-ionomers have a higher percentage change in KHN than the Beautifil II and control groups. This may be due to the formation of fluoro-apatite, which is harder and more acid resistant than hydroxyapatite, in higher amounts from GIC restorations compared with Beautifil II restoration.[37] Another report found that Beautifil II restoration has greater long-term fluoride releasing ability than Gradia Direct X and Tetric EvoCeram dental restorations.[33]

One of the limitations of the present study is the sample size, and a large number of samples are needed to record the differences between the groups. Moreover, as few studies have shown increased release of phosphate ions from Activa BioActive materials over time, a longer storage time of up to six months may be more useful. Further clinical trials are suggested to evaluate dentine remineralisation using Activa BioActive and Beautifil II restorations on carious teeth and using other techniques that could measure the actual presence of apatite, such as micro-Raman spectrometry.

Conclusion

Within the limitations of the current in-vitro study, it can be concluded that Activa BioActive-Restorative showed a non-significant but higher percentage change in the Ca:P and KHN. Therefore, such restoration may have a superior ability to remineralise demineralised dentine than Beautifil II dental and glass-ionomers. Further studies are required to expand on these findings.

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Conflict of interest

The authors have no conflict of interest to declare.

Ethical approval

Ethical approval was obtained from the Research Ethics Committee, Faculty of Dentistry, King Abdulaziz University, Jeddah, Kingdom of KSA (Number: 126-09-19; Date 24-9-2019).

Authors contributions

BA and LA conceived and designed the study, conducted research, provided research materials, collected and organised data, and wrote the initial and final drafts of the article. SS analysed and interpreted the data and provided logistic support. All authors have critically reviewed and approved the final draft and are responsible for the content and similarity index of the manuscript.

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