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Remineralization of human natural caries and artificial caries-like lesions with an experimental whisker-reinforced ART-composite

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Abstract

To compare the remineralization of human natural caries and artificial caries-like dentin lesions from a novel whisker-reinforced experimental composite resin to a resin-modified glass ionomer cement (RM-GIC) as control. Ten molars with moderate natural dentin caries were prepared (N). Artificial caries-like dentin lesions were prepared in occlusal dentin of ten caries-free molars and demineralized at pH=4.3 for 48 h (A). The cavities were restored with ART-composite or RM-GIC. All restored teeth were sliced into 120- μ m sections. Transverse microradiography combined with digital image analysis was performed to analyze the change in mineral density at the same position of the specimens before, after 4 weeks and 8 weeks remineralization/demineralization treatment. The mean percent remineralization $\pm$ standard deviation after 4 weeks and 8 weeks are: N: ART-composite: 27 $\pm$ 9, 46 $\pm$ 14; RM-GIC: 18 $\pm$ 6, 36 $\pm$ 11; A: ART-composite: 48 $\pm$ 9, 66 $\pm$ 11; RM-GIC: 50 $\pm$ 13, 62 $\pm$ 11. For the remineralization of natural caries, there was a significant difference between ART-composite and RM-GIC ($p<0.05$). For both restoratives there were significant differences between remineralization of natural and artificial caries ($p<0.001$). ART-composite and RM-GIC remineralized natural and artificial caries differently most likely due to differences in microstructure and composition of caries dentin.

KEY WORDS

Remineralization; Natural caries; Artificial caries; ART-composite

1. INTRODUCTION

Atraumatic Restorative Treatment (ART) is a preventive and restorative approach for managing carious teeth. The remineralizing ability of restorative materials is very important to the arrest and repair of carious dentin lesions in ART. Due to their desirable handling, adhesiveness, and anticariogenic and physical properties, glass-ionomer filling materials, including glass-ionomer cements and resin-modified glass ionomer cements, have been used in ART. However, their relatively low mechanical strengths suggest that glass-ionomer filling materials are inadequate for bulk restoratives [1,2].

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Recently a novel experimental whisker-reinforced fluoride-containing calcium phosphate (Ca-PO_4) composite resin [3] has been developed for ART (ART-composite) and compared to a resin-modified glass ionomer cement (RM-GIC; GC Fuji II LC, GC America Corp). Because of the strengthening effect of silicon carbide whiskers, this ART-composite showed higher mechanical strength [4,5] than RM-GIC, and similar wear resistance to that of RM-GIC [6]. This whisker-reinforced ART-composite, releasing calcium, phosphate, and fluoride ions, has shown promising *in-vitro* remineralization effects towards artificial demineralized dentin lesions [7].

However since there are substantial structural and histological differences between artificial and natural dentin lesions, it is not a priori applicable to transfer findings from artificial lesions to natural lesions. Compared to artificially demineralized dentin lesions, natural dentin lesions have rather complicated micro-structural patterns, which reflect the patho-morphological reaction of dentin to the caries attack in each tooth. The pathological and micro-structural differences exist among carious teeth due to the different speed and location of caries progression. In order to provide more clinically relevant data about the remineralization capability of remineralizing materials in ART, it is necessary to use a natural caries dentin lesion model for further evaluation of the remineralization of dental materials. To date, no study comparing the remineralization of natural carious teeth and artificial caries teeth is available as most remineralization studies have been performed on artificial dentin lesions.

Therefore, the objectives were to compare the remineralization effects of a whisker-reinforced ART-composite and a RM-GIC as control on artificial carious lesions and to examine a more clinically relevant model using natural carious dentin lesions. The hypotheses are that there is no difference in the remineralization pattern between natural and artificial carious lesions; and that there is no quantitative difference in the remineralization from the ART-composite and the RM-GIC for natural carious or artificial dentin lesions.

2. MATERIALS and METHODS

Preparation of specimens

Ten human molars with moderate active-like natural dentin carious lesions (N) and ten human molars with artificial dentin carious lesions (A) were used for this study. Based on the clinical characteristics of color, hardness, and wetness of the remaining dentin after the initial caries excavation, ten teeth with moderately active occlusal lesions were selected by one examiner for this study. According to staining by caries detecting dye¹ (Pulpdent Corp., Watertown, USA) during preparation, natural carious dentin lesions in this study are comprised of two distinct layers: blue color zone, and light blue color zone [8,9]. In this study, the blue colored soft carious dentin was removed leaving behind the light blue color zone on top of which was placed the restoration.

For the artificial caries-like lesions, one oval cavity (1 mm in depth $\times$ 3 mm in width $\times$ 6 mm in length) was drilled into the occlusal dentin surface of each of ten caries-free molars using an end mill. The teeth, except the cavities, were coated with acid resistant nail polish and incubated at 37°C for 48 h in 20 mL demineralizing solution (pH = 4.3), which consisted of an aqueous solution of 0.075 mol/L glacial acetic acid, 0.002 mol/L Ca (from CaCl_2), and

¹Disclaimer: Certain commercial equipment, instruments, or materials are identified in this paper to specify the experimental procedure adequately. Such identification is not intended to imply recommendation or endorsement by the National Institute of Standards and Technology or the American Dental Association Foundation, nor is it intended to imply that the materials or equipment identified are necessarily the best available for the purpose.

0.002 mol/L P (from KH_2PO_4). [10]. After demineralization, the cavities of artificial caries teeth were prepared for restoration.

Then the teeth were cut longitudinally through the middle of the cavities. The half of the tooth without filling was cut into 120- μm thick slices and the slice adjacent to the first cut was immediately put into fixation solution for TEM preparation (without being treated with remineralization/demineralization cycles). The other half was restored with ART-composite ($n = 5$) or a resin-modified glass ionomer cement (RM-GIC; GC Fuji II LC, GC America INC., Alsip, IL) ($n = 5$), and were light cured for 2 min. The restored teeth for both natural caries and artificial caries teeth were sliced into thin sections having a thickness of 120 μm . Approximately 3–5 slices could be sectioned from one restored half tooth. Two sections were treated with remineralization/demineralization cycles for 8 weeks to determine the amount of remineralization.

Light microscopy and transmission electron microscopy (TEM) examination

The 120 μm -thick sections from unrestored natural and artificial carious teeth were observed with a Stereoptical microscope (MZ 16, Leica, Heerbrugg, Switzerland) and then prepared for TEM examination. Sections were immersed into 2.5 %² glutaraldehyde in phosphate buffered saline (PBS) for 4 h and then post-fixed with 1 % osmium tetroxide for 2 h at room temperature. The osmium-fixed specimens were thoroughly rinsed three times with PBS, then dehydrated in an ascending ethanol series from 30 % to 90 %, in 10 % increments, and then in 96 % and 100 % twice in each solution for 10 min each time. After dehydration, the specimens were immersed in a mixture of propylene oxide and araldite epoxy resin (1:1) as a transition fluid for 24 h, and then embedded in pure epoxy resin in a 60 °C oven for 48 h. Semi-thin sections about 90 nm-110 nm thick were prepared with an ultramicrotome (Reichert Ultracut E, Reichert-Jung, Vienna, Austria) using a diamond knife. These sections were stained with Oolong tea extract (OTE, Ted Pella, Inc. Redding, USA) for 20 min and lead citrate for 5 min, and examined with a transmission electron microscope (TEM 201, Phillips, Eindhoven, the Netherlands).

Transverse Microradiography (TMR)

To determine the relative remineralization of slice sections after exposing them to the remineralizing restorative materials, TMR combined with digital image analysis was performed to compare the mineral density at the same position of the sections before, after 4 weeks, and after 8 weeks remineralization/demineralization cycling treatment. These sections used for transverse microradiography analysis were adjacent to the sections prepared for the TEM examination. Briefly, after taking contact microradiographs [11] and implementing digital image analysis to determine the “mineral-loss-before”, each section was sandwiched between a layer of parafilm and a plastic cover slip, and then wrapped with parafilm exposing only the restoration edge. The sections were incubated in 25 mL of artificial saliva solution (pH = 7.0) [12] with one hour incubation in 0.1 mol/L lactic acid demineralizing solution (pH = 4.3) per day at 37 °C for 4 weeks. Contact microradiographs of each section were retaken, and the mineral loss under the restoration after treatment (termed “mineral-loss-after”) was determined. The sections were incubated following the same remineralization/demineralization cycle condition for another 4 weeks, and contact microradiographs were retaken and the mineral loss under the restorations after 8 weeks incubation was determined. A copper grid was attached to each section to reposition the analysis box and determine the same position before and after treatment.

²All percentage notations are mass fraction percent unless stated differently.

Digital image analysis and calculation of relative remineralization (%)

Image J software (NIH, Bethesda, MD) was used to analyze quantitatively the change of mineral density at the same positions of the specimens before, after 4 weeks, and after 8 weeks exposure to the remineralization/demineralization process, and to calculate the relative remineralization in % from $(1 - \text{“mineral-loss-after”}/\text{“mineral-loss-before”}) \times 100$. One section from each tooth and for each section, four or more boxes at the cavity bottom were analyzed.

Statistical analysis

The effects of the type of dentin lesion (artificial and natural), filling materials (RM-GIC and ART-composite), and incubation time on the remineralization of dentin lesions were analyzed by analysis of variance (ANOVA). The threshold for statistical significance was set at $\alpha = 0.05$.

3. RESULTS

The light microscopy showed a superficial partially demineralized dark zone (DDZ) – the caries-affected dentin, a hypermineralized translucent zone (HTZ), and a normal dentin zone (NDZ) (Fig. 1a). In contrast, artificial lesions showed a homogeneous demineralization pattern (Fig. 1b). TEM results showed that compared to normal dentin (Figs. 2a – 2c) the caries-affected dentin has greater porosity (Fig. 2d and 2e) due to the demineralization, and some dentin tubules are blocked with mineral deposit (Fig. 2f). Sections with artificial lesions showed a homogeneous demineralization pattern (Figs. 2g – 2i) and a lower mineral content (Fig. 2i) compared to sections with natural caries lesions (Fig. 2f). Sections shown are typical examples of the average demineralization processes.

The microradiographs and mineral density profiles after quantitative digital image analysis of sections from the experimental groups before, after 4 weeks, and after 8 weeks remineralization/demineralization cycling treatment are shown in Fig. 3 to 4.

Fig. 3 shows representative sections of natural caries lesion (Fig. 3a–c) and of artificial caries lesions (Fig. 3e–g) restored with the ART-composite. The resulting mineral density profiles of the sections after quantitative digital image analysis (Figs. 3d) indicated that the demineralization depth in natural carious lesion was about 650 μm . For the ART-composite section (profiles **a–c** in Fig. 3d), the normalized mineral density of the superficial demineralized dentin is 0.37 (with a range from 0.3 to 0.45). The normalized mineral density at the same position increased to 0.57 after 4 weeks (ranging from 0.40 to 0.63), and to 0.66 after 8 weeks (ranging from 0.58 to 0.75 in this group). The remineralization process started from the interface between restorative and demineralized dentin and extended towards the pulp (see block arrow in Figs. 3b and 3c).

In contrast, the demineralized dentin depth in the artificial carious dentin (profiles **e–g** in Fig. 3d) is about (200–250) μm (profile **e** in Fig. 3d). The normalized mineral density of superficial demineralized dentin is at 0.07 before treatment (ranging from 0.05 to 0.08 in this group). However, after 4 and 8 weeks (Fig. 3d, profiles **f** and **g**, respectively) the normalized mineral densities at the same position are still less than 0.1. The remineralization process started adjacent to the normal dentin and extended towards the restorative/cavity floor interface (see block arrow in Figs. 3f and 3g).

Fig. 4 shows representative sections of natural caries lesion (Fig. 4a–c) and of artificial caries lesions (Fig. 4e–g) restored with RM-GIC. The demineralized dentin depth in the natural caries section (**a–c** in Fig. 4d) is about 650 μm (profile **a** in Fig. 4d). Before treatment, the normalized mineral density at the interface between restorative and

demineralized dentin is about 0.45 (ranging from 0.38 to 0.5 in this group). After 4 weeks, the normalized mineral density of the same position increased to 0.56 ranging from 0.5 to 0.6 in this group (profile **b** in Fig. 4d), and to 0.63 after 8 weeks ranging from 0.58 to 0.71 in this group (profile **c** in Fig. 4d). The remineralization process started at the interface between restorative and demineralized dentin and extended towards the pulp (see block arrow in Figs. 4b and 4c).

In contrast, the demineralized dentin depth in the artificial carious dentin (profiles e-g in Fig. 4d) is about 250 μm (e in Fig. 4d). The normalized mineral density of superficial demineralized dentin is at 0.03 before treatment (ranging from 0.05 to 0.09 in this group). However, after 4 weeks (profile f in Fig. 4d) and 8 weeks (profile g in Fig. 4d), the normalized mineral density of the same position are still lower than 0.1. The remineralization process started adjacent to the normal dentin and extended towards the restorative/cavity floor interface. (see block arrow in Figs. 4f and 4g).

The mean percentage of remineralization and standard deviations in parenthesis as a measure of the standard uncertainty for group natural carious dentin lesions and group artificial dentin lesions from ART-composite and RM-GIC after 4 and 8 weeks are: N: ART-composite: 27 ± 9 , 46 ± 14 ; RM-GIC: 18 ± 6 , 36 ± 11 ; A: ART-composite: 48 ± 9 , 66 ± 11 ; RM-GIC: 50 ± 13 , 62 ± 11 (Fig. 5). Three-way ANOVA revealed that there were no significant interactions among the three factors: type of dentin lesion (artificial and natural), filling materials (RM-GIC and ART-composite), and incubation time. However, significant interactions on the remineralization were detected between filling materials and type of dentin lesion (artificial and natural). Therefore, two-way ANOVA was done for each filling material and each dentin carious lesion separately. Two-way ANOVA results indicated that for the remineralization of natural carious dentin lesions, there is a statistically significant difference between ART-composite and RM-GIC ($p < 0.05$), and between 4 and 8 weeks ($p < 0.05$); for artificial dentin lesions, there is a statistically significant difference between 4 and 8 weeks ($p < 0.05$), but not between ART-composite and RM-GIC ($p > 0.05$). For ART-composite, there is a statistically significant difference between 4 and 8 weeks ($p < 0.05$), and between natural and artificial dentin lesions ($p < 0.05$); for RM-GIC, there is a statistically significant difference between 4 and 8 weeks ($p < 0.05$), and between natural carious and artificial dentin lesions ($p \leq 0.001$).

4. DISCUSSION

Our TEM and microradiograph results indicate that the demineralization degrees and demineralization pattern of the two kinds of carious lesions (natural & artificial) are quite different (see Figs. 1 and 2). In the current and previous studies [11–13], artificial dentin lesions were created by treating dentin with a demineralizing solution to develop caries-like demineralized dentin. In this way, the demineralized dentin was about (200 to 250) μm thick (see Figs. 3 and 4), generally showing demineralized intertubular dentin and exposed dentin tubule orifices. Compared to artificial dentin lesions, natural carious lesions in dentin have rather complicated micro-structural patterns. Natural caries lesions can be classified into a superficial partially demineralized dark zone including caries infected dentin and caries affected dentin, a hypermineralized translucent zone, and an apparently normal dentin zone (Figs. 2) [9,14,15]. The demineralization pattern of artificial lesions was not the same as that of natural lesions, with a significant difference in the Ca and P content between demineralized dentin in artificial dentin lesions and caries affected dentin in natural carious dentin lesions [15]. These different zones with different Ca and P contents [15] reflect the natural patho-morphological reaction of dentin to the caries attack and are the consequence of demineralization/remineralization during the caries progress. In general, the precipitation of mineral deposition is driven by local factors such as pH, the presence of seed crystals in

collagen matrix, and the surface area available for crystal growth. Therefore, artificial dentin lesions and the caries affected dentin, with the underlying hypermineralized translucent dentin, provided different remineralizable conditions.

In dental practice, ART technique is usually employed on frank cavitated lesions which may or may not be active. Comparing to active-like caries, more complicate remineralization processes (from the reaction of pulp to caries or from saliva or clinic treatment) have been involved into the development of the inactive natural caries. Since there are differences in the micro-morphological structure and micro-composition of two types of natural caries, the remineralization of two types of natural caries reacting to the remineralizing materials may be different. The purpose of this initial study is to investigate the remineralization of our experimental materials in natural and artificial caries. So only active-like carious teeth were used in this study. According to previous studies the soft blue colored outer layer, determined as caries infected dentin [8,9,16], is highly contaminated with cariogenic bacteria and shows complete denaturation of the dentin collagen network into a gelatinized mass of microfibrils [16]. Therefore, this very soft carious dentin [9] was completely removed during excavation. The light blue colored inner layer, regarded as caries affected dentin, is only partially demineralized since apatite crystals are still bound to crosslinked collagen fibers having distinct cross banding similar to those in normal dentin [16]. So, the collagen fibrils of this layer are believed to be physiologically remineralizable and lost mineral and apatite crystals can be reincorporated [17] according to caries pathology. This kind of dentin probably presents favorable precipitation conditions with its large surface area of crystallites in collagen fibrils in saliva-like solution (pH = 7).

For both ART-composite and RM-GIC, artificial dentin lesions show statistically higher remineralization than do natural carious teeth (Fig. 2), and a different remineralization process was found between natural dentin lesions and artificial dentin lesions (see Fig. 3 and 4). Starting from the interface between restorative and demineralized dentin and extending towards the pulp in natural dentin lesions contrasts with the remineralization process in artificial dentin lesions, which started adjacent to the normal dentin and extended towards the restorative/cavity floor interface.

In teeth with artificial caries, the normalized mineral densities at the interface (same position) remained at the original level even after 4 and 8 weeks of treatment, while the mineral densities adjacent to the normal (unaltered) dentin increased greatly approaching the level of unaltered dentin. That is, the remineralization process started adjacent to the normal dentin and extended towards the restorative/cavity floor interface. This is opposite from the remineralization process in natural carious dentin lesions, which have much higher mineral content adjacent to the cavity floor than artificial lesions.

In a previous *in-vivo* study [18], the deep etched dentin containing a high mineral content showed considerable remineralization adjacent to the sound dentin in vital teeth, and no remineralization was observed in the superficial etched dentin. The reason for much more remineralization in the deep decalcified layers in that study was the reaction of the pulp in vital teeth without exposure to saliva or capping materials such as $\text{Ca}(\text{OH})_2$. The same remineralization process occurred in our *in-vitro* study, using extracted teeth that we restored with ART-composite or RM-GIC. This means that after exposure to materials the vital pulp reaction is not the only reason for the different remineralization process. The amount of mineral content present serving as seed crystals in collagen matrix may be a key factor influencing the remineralization process. This is confirmed by the remineralization process in natural dentin carious lesions starting from the interface between restorative and demineralized dentin which has sufficient hydroxyapatite present that act as seed crystals inducing to mineral deposition in the remineralizable collagen matrix.

Our TEM results show that natural carious dentin lesions have more mineral crystal seeds and surface area available for crystal growth than artificial dentin lesions. Therefore, it is speculated that in natural carious dentin lesions, the mineral crystal within the collagen network may hinder the ability of the mineral ions to diffuse into deep dentin. In contrast, in artificial dentin lesions with low mineral content throughout the lesion, the mineral ions can rapidly diffuse through the demineralized collagen fibrils to reach the mineral-rich area, adjacent to the sound dentin and precipitate. However, after 4 weeks of fast mineral deposition, the remineralization in artificial dentin lesions seems to slow down and shows less remineralization than in natural carious dentin lesions. As a result, the hypothesis that there is no difference in the remineralization between natural and artificial dentin lesions for ART-composite or RM-GIC has to be rejected.

In this study, the experimental ART-composite resin is an experimental whisker-reinforced fluoride releasing calcium phosphate (Ca-PO₄) composite [7]. The unsilanized inorganic fillers consist of tetracalcium (TTCP) and dicalcium phosphates (DCPA) which can form hydroxyapatite [Ca₁₀(PO₄)₆(OH)₂] and sodium hexafluorosilicate. Therefore, this experimental ART-composite releases calcium, phosphate, and fluoride ions similar to the resin-reinforced calcium phosphate cements [12] in amounts to produce supersaturation with respect to the formation of hydroxyapatite and fluorapatite [11]. Further, the precipitation of calcium and phosphate might be promoted by the fluoride release from the sodium hexafluorosilicate additive to form FAp, which has been shown to effectively remineralize the demineralized tooth hard tissue *in-vitro* [19]. Therefore, the formulation of this ART-composite provides the necessary ion release for the remineralization of the targeted dentin lesions, in agreement with the results of the current study showing that ART-composite has a significant remineralization effect on both natural and artificial dentin lesions. In addition, since our previous study indicates the bonding agent negatively influenced the remineralizing function of ART composite [11], the PMGDM with self-etching and self-adhesive properties is also important for the adhesion of our ART experimental composite material to carious dentin.

Fluoride plays an important role in the inhibition and reversal of the caries process due to its actions including the reduction of demineralization, the enhancement of remineralization, and the inhibition of microbial growth and metabolism [20]. The ion-leachable filler glass used in RM-GIC is based on SiO₂-Al₂O₃-CaF₂-AlPO₄-Na₃AlF₆ composition with the fluoride content being up to more than 20 %. On the other hand, although there are sufficient F ions released from RM-GIC, there are only limited amounts of Ca ions released from RM-GIC, and the amount of PO₄ ions supplied by the saliva is low. While supersaturation does not necessarily predict to what extent the minerals will precipitate [11], calculations of saturation levels of ions after dissolution from RM-GIC showed supersaturation with respect to hydroxyapatite and fluorapatite. Yet, the saturation levels for ART-composite were substantially higher, which may explain the statistically lower remineralization of natural dentin lesions observed for RM-GIC ($p < 0.05$). The hypothesis that there is no difference in the remineralization of ART-composite and RM-GIC for natural dentin carious lesions or artificial dentin lesions has to be rejected.

5. SIGNIFICANCES

Our study indicates that remineralization of natural carious and artificial caries-like dentin lesions seem to follow different mechanisms due to differences in microstructure and dentin composition. These differences may have caused RM-GIC and ART-composite to have similarly high remineralization in artificial dentin lesions, but not in natural dentin lesions. Artificial dentin lesions can be used as a simplified standard remineralization model to evaluate the *in-vitro* remineralization potential of dental remineralizing materials, but this

model does appear to lead to higher values. The current study demonstrates the feasibility to develop a natural dentin lesion model for providing more clinically relevant information in predicting the real remineralization exerted by remineralizing materials. Also, the experimental whisker-reinforced fluoride-containing calcium phosphate composite developed for ART has shown promising *in-vitro* potential to repair natural dentin lesions.

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Fig. 1.

Stereoptical photographs of a tooth section with natural caries (Fig. 1a) and a tooth section with artificial caries (Fig. 1b). Natural caries lesions can be classified into a superficial partially demineralized dark zone (DDZ) - caries affected dentin, a hypermineralized translucent zone (HTZ), and a normal dentin zone (NDZ) (Fig. 1a). In contrast, the demineralized dentin (DD) in artificial lesions showed a homogeneous demineralization pattern (Fig. 1b).

Fig. 2.

TEM photographs of normal dentin (2a – 2c), natural carious dentin (2d – 2f), and artificial carious dentin (2g – 2i). Compared to normal dentin (Figs. 2a – 2c) the caries-affected dentin shows more porosity (Fig. 2d and 2e) due to the demineralization, and some dentin tubules are blocked with mineral deposit (Fig. 2f). Sections with artificial lesions showed a homogeneous demineralization pattern (Figs. 2g – 2i) and a lower mineral content (Fig. 2i) compared to sections with natural carious lesions (Fig. 2f).

Fig. 3.

Comparison of microradiograph images and mineral density profiles (3d) of specimens in the ART group before remineralization, and after 4 weeks and 8 weeks of remineralization/demineralization treatment. Images and corresponding profiles **a-c** are from a natural caries specimen and **f-g** from an artificial caries specimen. Accordingly the mineral profiles of natural carious specimens after quantitative digital image analysis are **a** (before treatment), **b** (after 4 weeks treatment) and **c** (after 8 weeks treatment) in Fig. 3d, and the mineral profiles of artificial carious specimen are **e** (before treatment), **f** (after 4 weeks treatment) and **g** (after 8 weeks treatment) in Fig. 3d. For natural caries specimens (**a-c**), the remineralization process started from the interface between restorative and demineralized dentin and extended towards the pulp (see block arrow in Figs. 3b and 3c). For artificial caries specimens (**f-g**), the remineralization process started adjacent to the normal dentin and extended towards the restorative/cavity floor interface (see block arrow in Figs. 3f and 3g). Scale bar = 200 μm .

Fig. 4.

Comparison of microradiograph images and mineral density profiles (3d) of specimens in the RM-GIC group before remineralization, and after 4 weeks and 8 weeks of remineralization/demineralization treatment. Images and corresponding profiles a-c are from a natural caries specimen and f-g from an artificial caries specimen. Accordingly the mineral profiles of natural carious specimens after quantitative digital image analysis are **a** (before treatment), **b** (after 4 weeks treatment) and **c** (after 8 weeks treatment) in Fig. 4d, and the mineral profiles of artificial carious specimen are **e** (before treatment), **f** (after 4 weeks treatment) and **g** (after 8 weeks treatment) in Fig. 4d. For natural caries specimens (**a-c**), the remineralization process started from the interface between restorative and demineralized dentin and extended towards the pulp (see block arrow in Figs. 4b and 4c). For artificial caries specimens (**f-g**), the remineralization process started adjacent to the normal dentin and extended towards the restorative/cavity floor interface (see block arrow in Figs. 4f and 4g). Scale bar = 200 μ m

Fig. 5.

The mean percent relative remineralization with standard deviations of groups natural caries dentin lesion (N) and artificial caries dentin lesion (A) restored with one experimental whisker-reinforced ART composite (ART) and resin modified-glass ionomer cement (RM-GIC) after 4 weeks and 8 weeks. Statistical difference ($p < 0.05$) was marked with stars.

Table 1

General composition of the ART experimental composite resin (ART composite) and resin modified glass ionomer cement (RM-GIC).

ART Composite Resin	Resin modified glass ionomer cement (RM-GIC)
Resin <ul style="list-style-type: none"> • PMGDM • EBPADMA Fillers: 75 mass fraction% <ul style="list-style-type: none"> • TTCP • SiC/DCPA • DCPA • sodium hexafluorosilicate Cure initiator system <ul style="list-style-type: none"> • Benzoyl peroxide • Tertiary amine • Camphorquinone 	Liquid <ul style="list-style-type: none"> • Distilled water • Polyacrylic acid • 2-Hydroxyethyl methacrylate • Urethane dimethacrylate • Camphorquinone Powder <ul style="list-style-type: none"> • (Fluoro) Alumino silicate glass