Study on Rehardening of Demineralized Dentin with Pulp-capping Agents

Using a New Hardness Determination System

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Abstract

Purpose

We measured the hardness of demineralized dentin over time using the Cariotester to determine the effectiveness of several types of pulp-capping agent for IPC.

Methods

Extracted human molars were used to prepare dentin samples with a diameter 10 mm and thickness of 2 mm. Sound dentin samples were immersed in lactic acid solution and were regarded as demineralized when the value obtained using the Cariotester was approximately 20 KNH. HY-Bond Temporary Cement Soft (Shofu), Neodyne-α (Neo Dental Chemical Products), Dycal (Dentsply), and Calcipex Plain II (Nippon Shika Yakuhin) with a 60% calcium hydroxide mixture were used as the pulp-capping agents in the present study. Each pulp-capping agent was applied to the surface of demineralized dentin and covered with base cement. After the base cement solidified, pulp-capped dentin samples and controls were divided into 2 groups: those placed in a container with 100% humidity and those immersed in remineralization solution, and stored at 37°C in a thermostatic chamber for 1 and 3 months. The hardness of the capping agent-applied region was then measured. Data were analyzed using a one-way analysis of variance and Tukey’s test (α=0.05). Pulp-capping agent-applied surfaces were also observed under SEM.
Results

The hardness of demineralized dentin increased with the application of Dycal, Calcipex plain II, and a 60% calcium hydroxide mixture, and mineralized substance-like aggregates were deposited on the surface of and between collagen fibrils. It was suggested that the remineralization of demineralized dentin depended on the calcium hydroxide concentration contained in a pulp-capping agent.

Conclusion

The hardness of demineralized dentin increased and remineralization features were noted following the application of a pulp-capping agent contained over 27% calcium hydroxide.

Key words: Knoop hardness, pulp-capping agent, remineralization
Introduction

The concept of minimal intervention (MI): the conservation of sound dentin to minimize invasion, has recently become widely pervasive in caries treatment. Terashima\textsuperscript{1} and Sato\textsuperscript{2,3} described the division of carious dentin into the outer layer, in which the tooth substance is demineralized by cariogenic bacteria, leading to the collapse of collagen fibrils, and the inner layer, in which the tooth substance is partially demineralized by the indirect influence of cariogenic bacteria. Caries detector solutions have been developed to distinguish between these two layers and are widely used for caries removal\textsuperscript{4,5}. The outer layer stained with caries detectors should be removed, whereas active conservation of the non-stained inner layer has been reported to induce physiological remineralization after restoration\textsuperscript{6-8}. However, pulpectomy is selected when caries progresses to deep dentin close to the pulp because the removal of all infected dentin exposes the pulp. \textit{Indirect pulp capping (IPC)} is recommended in these cases in an attempt to conserve dental pulp and avoid pulpectomy\textsuperscript{9}. In IPC, infected dentin near the pulp is intentionally left and a tannin-fluoride mixture-combined carboxylate cement or calcium hydroxide preparation is applied to promote the sterilization and remineralization of residually infected dentin and formation of tertiary dentin (reparative dentin). The remineralization of demineralized dentin via the application of a tannin-fluoride mixture-combined carboxylate cement and calcium hydroxide preparation has already been confirmed using various approaches, such as hardness and X-ray radiographical, bacteriological, and histopathological methods\textsuperscript{10-17}. Although hardness is a clinically important index, only a few studies have used it as an objective index.
A hardness measurement device for carious dentin, the Cariotester, has recently been designed by Shimizu\textsuperscript{18,19} et al. and released by SAMEIME co. Carious dentin hardness can be directly measured in the oral cavity using the Cariotester, which has facilitated the introduction of an objective index, hardness, into caries treatment.

In the present study, the hardness of demineralized dentin was measured over time using the Cariotester to investigate the effectiveness of several types of pulp-capping agents for IPC. In addition, Pulp-capping agents were applied to demineralized dentin prepared using a lactic acid solution and the Knoop hardness of the pulp-capping agent-applied surfaces was measured after 1 and 3 months. Pulp-capping agent-applied surfaces were also observed under SEM and the effectiveness of the agents tested was determined.

Materials and Methods

1. Experimental samples

The subject teeth were human molars that were extracted at the Department of Oral Surgery, Osaka Dental University Hospital, and stored in physiological saline at -40°C. They were defrosted under running water immediately before being used in the experiments. The occlusal surface of each tooth was macroscopically observed, and teeth with caries, white turbidity, coloration, and crack were excluded. This study was approved by the Medical Ethics Committee of Osaka Dental University (approval number: 110742).
2. Experimental methods

1) Hardness measurement

To investigate time-course changes in the hardness induced by application of a pulp-capping agent, using Cariotester SUK-971 (Cariotester, SANEIME co.), the hardness of sound dentin, demineralized dentin, and dentin 1 and 3 months after pulp-capping was measured following the manufacturer’s instructions. The measurement range was set at the center within a 3-mm diameter on the enamel side in the sound dentin samples, and a site within a 3-mm diameter from the center in the demineralized and pulp-capped dentin samples. The hardness was measured at 5 sites in each sample, and the mean of the 5 values was adopted. The number of samples was 3 for each condition.

2) Preparation of samples

The roots of the human molars were cut at 3 mm apical from the anatomical cervical line, the dental pulp was removed, and the coronal enamel and root dentin were cut vertically to the tooth axis using a model trimmer. The enamel side of the exposed dentin and the lateral side of the pulp cavity were polished using waterproof polishing paper #1000 to prepare a dentin sample with a diameter 10 mm and thickness 2 mm. The hardness was measured on the enamel side of the dentin sample using the Cariotester, and the tooth was regarded as a sound dentin sample when the value obtained was approximately 60 KNH.
3) Preparation of demineralized dentin

The major organic acid produced by cariogenic bacteria, lactic acid (Kishida), was used to decalcify sound dentin samples. The enamel side of sound dentin samples was immersed in 50 ml of 20 mM lactic acid solution for 10 hours while being aspirated from the lateral side of the pulp cavity at 0.01 MPa using an aspirator (MDA-006, Ulvac). The sound sample was then sufficiently washed with distilled water, and the hardness was measured on the enamel side using the Cariotester. Samples were regarded as demineralized when the value obtained was approximately 20 KNH.

4) Preparation of pulp-capped dentin samples

The pulp-capping agents and cement tested are shown in Table 1. As commercially available products, HY-Bond Temporary Cement Soft (Shofu), Neodyne-α (Neo Dental Chemical Products), Dycal (Dentsply), and Calcipex plain II (Nippon Shika Yakuhin) were tested following the manufacturers’ instructions. To investigate the influence of calcium hydroxide concentration on the remineralization of demineralized dentin, a 60% calcium hydroxide mixture was prepared by mixing 0.6 g of calcium hydroxide (Kishida) with 1 ml of distilled water, and tested as a pulp-capping agent. To prepare pulp-capped dentin samples, each pulp-capping agent was applied to the surface of demineralized dentin and by coverage with base cement (Shofu). Demineralized dentin samples covered with BC only and no pulp-capping agent were prepared as controls. After BC solidified, the pulp-capped dentin samples and controls were divided into 2 groups: those placed in a container with 100% humidity (DW group) and those
immers in remineralization solution (1.5 mmol/l CaCl₂, 0.9 mmol/l KH₂PO₄, 130 mmol/l KCl, 20 mmol/l HEPES) adjusted to pH 7.0 with KOH (RS group), and stored at 37°C in a thermostatic chamber for 1 and 3 months. After storage, BC and pulp-capping agents were removed from the pulp-capped dentin samples and controls, without touching the capping agent-applied region, and the hardness of the capping agent-applied region was measured.

3. Observation under SEM

To observe the surface of the pulp-capped dentin samples after hardness measurement, the samples were fixed and dehydrated with an alcohol series following the standard method, and then freeze-dried using a t-BuOH freeze dryer, VFD21S (VD). The samples were then subjected to gold vapor deposition using an ion sputtering device, E-1030 (Hitachi), and observed under a field-emission scanning electron microscope, S-4000 (Hitachi). The surfaces of the sound and demineralized dentin samples were similarly processed and observed.

4. Statistical analysis

Data were analyzed using a one-way analysis of variance and Tukey’s test (α=0.05).

Results

1. Hardness measurement

The hardness of samples, as measured using the Cariotester, is shown in Table 2.

1) Hardness of sound and demineralized dentin samples
The mean hardness levels of sound and demineralized dentin samples were 62.1±0.9 and 19.4±3.6 KNH, respectively. Hardness was significantly lower in all demineralized dentin samples than in sound dentin samples.

2) Hardness of pulp-capped dentin samples

(1) HB-applied dentin

Hardness levels after 1 and 3 months were 21.7±10.7 and 23.7±5.6 KNH in the DW group, and 29.4±7.3 and 14.1±7.8 KNH in the RS group, respectively. No significant differences were observed in hardness levels between demineralized dentin samples and DW or the RS groups at 1 or 3 months.

(2) NE-applied dentin

Hardness levels after 1 and 3 months were 15.5±4.1 and 20.5±6.1 KNH in the DW group, and 22.9±3.6 and 24.9±10.1 KNH in the RS group, respectively. No significant differences were observed in hardness levels between demineralized dentin samples and DW or the RS groups at 1 or 3 months.

(3) DY-applied dentin

Hardness levels after 1 and 3 months were 17.4±4.8 and 23.4±8.3 KNH in the DW group, and 20.8±3.3 and 29.9±7.8 KNH in the RS group, respectively. No significant differences were observed in hardness levels between demineralized dentin samples and the DW group at 1 or 3 months. No significant difference was observed in hardness levels between the RS group and demineralized dentin samples at 1 month, but was significantly higher in the RS group at 3 months.

(4) CA-applied dentin
Hardness levels after 1 and 3 months were 27.1±8.2 and 39.2±6.2 KNH in the DW group, and 30.1±12.2 and 33.6±6.7 KNH in the RS group, respectively. Hardness was not significantly different from that of the demineralized dentin samples in the DW or the RS group at 1 month, but it was significantly increased at 3 months.

(5) CH-applied dentin

Hardness levels after 1 and 3 months were 43.1±4.6 and 38.9±1.7 KNH in the DW group, and 40.9±5.4 and 39.1±7.3 KNH in the RS group, respectively. Hardness levels were significantly increased in both the DW and RS groups at 1 and 3 months than in demineralized dentin samples.

(6) Control

Hardness levels after 1 and 3 months were 22.4±1.2 and 22.1±8.2 KNH in the DW group, and 17.4±1.9 and 22.2±6.9 KNH in the RS group, respectively. No significant difference was observed in hardness levels between demineralized dentin samples and the DW or RS groups at 1 or 3 months.

2. Observation under SEM

1) Sound dentin samples

SEM observation of the sound dentin samples are shown in Figure 1 (left). The sample surface was densely covered with mineralized substances, such as hydroxyapatite, and no collagen fibril was exposed.
2) Demineralized dentin samples

SEM observation of the demineralized dentin samples are shown in Figure 1 (light). Collagen fibrils were exposed due to demineralization by lactic acid. Striation was observed on most collagen fibrils, but was absent on the others. The slight retention of mineralized substances was observed on the collagen fibril surface; however, no mineralized substance was present between collagen fibrils.

3) Pulp-capped dentin samples
(1) HB-applied samples

SEM observations of the dentin are shown in Figure 2. In the DW group, collagen fibrils were exposed after 1 month, similar to those in demineralized dentin samples, and no mineralized substance-like aggregates adhered to the collagen fibril surface. The slight deposition of mineralized substance-like aggregates was observed on the collagen fibril surface at 3 months. In the RS group, collagen fibrils were exposed at 1 month, similar to those in the DW group; however, mineralized substance-like aggregates were deposited on the collagen fibril surface. The deposition of aggregates on the collagen fibril surface had progressed at 3 months, and the space between collagen fibrils was more likely to be sealed by these aggregates.

(2) NE-applied samples

SEM observations of the dentin are shown in Figure 3. In the DW group, although collagen fibrils were exposed at 1 and 3 months, almost no deposition of mineralized substance-like aggregates was noted. In the RS group, similar to those in the DW group, collagen fibrils were exposed and almost no deposition of
mineralized substance-like aggregates was noted at 1 month. Some collagen fibrils were exposed at 3 months and no mineralized substance-like aggregate was deposited in some regions; however, the sample surface was more likely to be covered by mineralized substance-like aggregates.

(3) DY-applied samples

SEM observations of the dentin are shown in Figure 4. In the DW group, collagen fibrils were exposed at 1 month, and mineralized substance-like aggregates were deposited on the surfaces of some collagen fibrils. The striation of collagen fibrils was also noted. The deposition of aggregates on the collagen fibril surface was more extensive at 3 months than at 1 month, and the space between collagen fibrils was more likely to be sealed by the aggregates. In the RS group, the deposition of mineralized substance-like aggregates was observed on the collagen fibril surface at 1 month, and the space between collagen fibrils was more likely to be sealed by the aggregates. This deposition had progressed further at 3 months, the space between collagen fibrils was sealed by the aggregates, and the sample surface was densely covered.

(4) CA-applied samples

SEM observations of dentin are shown in Figure 5. In the DW group, mineralized substance-like aggregates were deposited on the collagen fibril surface and between collagen fibrils at 1 month. These depositions had further progressed at 3 months, and the sample surface was densely covered by the aggregates. In the RS group, similar to those in the DW group, mineralized substance-like aggregates were deposited on the collagen fibril surface and between collagen fibrils at 1 month, and had densely covered the sample surface.
by 3 months.

(5) CH-applied samples

SEM observations of the dentin are shown in Figure 6. In the DW group, mineralized substance-like aggregates were deposited on the collagen fibril surface and between collagen fibrils at 1 month, and had densely covered the sample surface by 3 months. In the RS group, similar to those in the DW group, mineralized substance-like aggregates were deposited on the collagen fibril surface and between collagen fibrils at 1 month, and had densely covered the sample surface by 3 months.

(6) Control (BC-applied samples)

SEM observations of the dentin are shown in Figure 7. In the DW group, similar to those in demineralized dentin samples, collagen fibrils were exposed at 1 and 3 months, and no mineralized substance-like aggregate had formed on the collagen fibril surface. In the RS group, the deposition of mineralized substance-like aggregates on the collagen fibril surface was slightly higher than that in the DW group at 1 month; however, most collagen fibrils were exposed, which was similar to that observed in the DW group. The deposition of mineralized substance-like aggregates was slightly more extensive at 3 months than at 1 month, but was still very limited.
Discussion

Although the remineralization of demineralized dentin after IPC is an important issue in caries treatment, it has not yet been fully elucidated. Although caries in extracted teeth are used as a material to investigate various problems, it is difficult to collect a large number of carious teeth with a similar grade of demineralized dentin. The artificial preparation of demineralized dentin similar to spontaneous caries may be very useful to solve the problems described above. Although many studies have attempted to prepare artificial carious dentin\textsuperscript{20-28}, caries was limited to the superficial layer in many of these reports, and the preparation of artificial carious dentin with a thickness similar to that of spontaneous caries has not yet been established. We used lactic acid as a solution to decalcify dentin because it is an organic acid produced at high levels by cariogenic bacteria\textsuperscript{29,30}. To prepare demineralized dentin with a thickness similar to that of caries, teeth were immersed in lactic acid solution aspirated from the lateral side of the pulp cavity using an aspirator, which reduced the Knoop hardness of the lateral side of the pulp cavity. We also investigated time-course changes in the Knoop hardness of the pulp-capping agent-applied surface. Similar investigations of the hardness of the lateral side of the pulp cavity may facilitate the preparation of demineralized dentin samples with a constant thickness similar to that of carious dentin, which would increase the significance of studies on the depth of remineralization of carious dentin. Pulp-capped dentin samples were stored in remineralization solution under conditions of 100% humidity. Dental pulp fluid has been shown to act as remineralization solution in vital teeth\textsuperscript{31}. Even though the composition of the remineralization solution used was
not completely consistent with that of dental pulp fluid, we used it to investigate the influence of Ca, contained in dental pulp fluid, on remineralization.

HY-Bond Temporary Cement Soft is a tannin-fluoride mixture (HY agent)-combined carboxylate cement that is mainly used for temporary cementation in dental practice; however, Nagamine\textsuperscript{13}, reported that HY agent-combined carboxylate cement promoted the remineralization of residual carious dentin. The guidelines for caries treatment established by the Japanese Society of Conservative Dentistry also recommend pulp-capping using HY agent-combined carboxylate cement in IPC\textsuperscript{9}. Thus, we tested this product as a pulp-capping agent. No increase was observed in the hardness of demineralized dentin in either the DW or RS group; however, the deposition of mineralized substance-like aggregates was observed over time in both groups on SEM. HY agents have been shown to strengthen organic substances and increase the acid resistance of collagen fibrils\textsuperscript{32}. These effects may have strengthened collagen fibrils and promoted the deposition of mineralized substance-like aggregates.

Although hardness was not improved during the application period in this study, the deposition of aggregates on collagen fibrils may be further promoted by prolonging the application period, thereby increasing hardness.

Neodyne-α is zinc oxide eugenol cement. The liquid component, eugenol, has superior analgesic sedative effects on dental pulp, and is used as a temporary sealing or lining material in dental practice for caries that reach the deep dentin and cause pulpitis. Since its powder component contains approximately 19% calcium hydroxide and the overall content may be approximately 15%\textsuperscript{33}, this product was tested as a pulp-capping agent. No increase was observed in the
hardness of demineralized dentin in either the DW or RS group, and no deposition of mineralized substance-like aggregates on the collagen fibril surface was noted in the DW group on SEM. However, the sample surface was mostly covered by mineralized substance-like aggregates in the RS group, although some collagen fibrils were exposed, which indicated that the surface was remineralized. In addition, the striation of collagen fibrils disappeared after 1 month but reappeared after 3 months, which may have been due to the inhibition of hydrolysis of collagen fibrils by the protein astringent action of zinc oxide\textsuperscript{34}. The presence or absence of the striation of collagen fibrils is considered to be related to the remineralization of dentin, with decreased remineralization due to damage to collagen fibrils being reported previously\textsuperscript{35}. The application of Neodyne to carious dentin may contribute to the long-term physiological remineralization of carious dentin by preventing the hydrolysis of collagen fibrils.

Dycal is a calcium hydroxide pulp-capping agent. Since the base, containing 56.7% titanium dioxide, and catalyst paste, containing 53.5% calcium hydroxide and 9.7% zinc oxide, are mixed\textsuperscript{36}, the overall calcium hydroxide content may be approximately 27%. An increase in hardness was not observed in the DW group, but was at 3 months in the RS group, and mineralized substance-like aggregates were densely deposited on the collagen fibril surface on SEM, which indicated that the application of Dycal and subsequent 3-month immersion in remineralization solution remineralized demineralized dentin. The deposition of mineralized substance-like aggregates on the collagen fibril surface was also noted in the distilled water group on SEM and was promoted over time, suggesting that hardness increases with the prolongation of the application
period.

Clacipex Plain II is a calcium hydroxide root canal filler mainly used in dental practice to fill the root canal. We tested it as a pulp-capping agent in the present study because it contains approximately 48% calcium hydroxide. An increase in hardness was observed after 3 months in both the DW and RS groups, and the deposition of mineralized substance-like aggregates on and between collagen fibrils was observed on SEM. These results suggest that demineralized dentin was remineralized 3 months after the application of a pulp-capping agent containing approximately 24% calcium hydroxide.

A 60% calcium hydroxide mixture was prepared by mixing 0.6 g of calcium hydroxide and 1 ml of distilled water as an experimental pulp-capping agent to investigate the influence of calcium hydroxide concentrations on the remineralization of demineralized dentin. An increase was observed in hardness after 1 month in both the DW and RS groups, and mineralized substance-like aggregate deposition was observed on and between collagen fibrils on SEM. These results indicated that demineralized dentin was remineralized 1 month after the application of a pulp-capping agent containing 60% calcium hydroxide. Therefore, the application of a pulp-capping agent containing approximately 60% or higher calcium hydroxide may increase hardness over the deep layer of thick demineralized dentin.

The base cement was a glass ionomer cement, which is used as a lining material in dental practice. The sustained release of fluoride ions is a characteristic of glass ionomer cement, and has been shown to promote remineralization. We tested the base cement as a pulp-capping agent-covering material in the present study.
Samples covered only with the base cement without a pulp-capping agent were also prepared as a control because the influence of fluoride ions slowly released from the covering base cement on demineralized dentin remineralization was considered. No increase was observed in hardness in either the DW or RS group. The deposition of mineralized substance-like aggregates on and between collagen fibrils was confirmed in the remineralization solution group after 3 months on SEM, which suggested that prolonging the application period may promote aggregate deposition and increase the hardness of demineralized dentin.

In caries treatment, demineralized dentin is mechanically removed using a spoon excavator or round bar, and the removal range is decided based on finger sensation. Histopathological studies have classified caries into the first and second layers of carious dentin, and this has facilitated the development of caries detectors, which are now widely used as an index for demineralized dentin removal. However, finger sensation and differentiation using a caries detector depend on the operator’s subjective judgment, such that demineralized dentin may be left in place. Thus, using an objective index, hardness, is necessary.

Dentin hardness has been measured using micro-hardness meters, such as Mohs, Vickers, and Knoop hardness meters. However, these studies were performed using extracted teeth; dentin hardness has not yet been measured in vital teeth in the oral cavity. Shimizu et al. recently developed a hardness measurement device for carious dentin, the Cariotester, which is now commercially available. The direct measurement of the Knoop hardness of carious dentin in the oral cavity using the Cariotester has facilitated the introduction of an objective index, hardness, into caries treatment.
Many cases in which dental caries has progressed to deep dentin close to the pulp have been reported in dental practice. Pulpectomy was previously indicated for these cases when caries caused pulp exposure. However, a recent study of dental pulp clarified its high healing capacity and the reversibility of dental pulp inflammation\textsuperscript{47,48}; therefore, the importance of dental pulp protection has been recognized and IPC is now recommended. Infected dentin near the pulp is intentionally left in IPC, even in caries progressing to deep dentin close to the pulp. This is then treated with a tannin-fluoride mixture-combined carboxylate cement or calcium hydroxide preparation; through which residually infected dentin is sterilized and remineralized and tertiary dentin (reparative dentin) formation is promoted, with the aim of dental pulp conservation. Although this is limited to cases with no pulp symptoms, favorable outcomes were obtained in most cases when IPC achieved restoration without pulp exposure. The effectiveness of IPC has been investigated using not only hardness, but also various other methods, such as X-ray radiography and bacteriological and histopathological examinations. However, these were performed using samples prepared from extracted teeth; studies using carious dentin in the oral cavity have not yet been conducted. If the presence or absence of the remineralization of residual demineralized dentin and dentin restoration can be confirmed by measuring the hardness of demineralized dentin in the oral cavity over time using the Cariotester, pulp exposure may be prevented at re-entry.
Conclusions

The hardness of various dentin samples was measured using a novel Knoop hardness measurement system, Cariotester, and the sample surfaces were observed using SEM. The following conclusions were obtained:

1. When only lining with base cement was applied without pulp-capping, the hardness of demineralized dentin did not increase, and no remineralization occurred.

2. When the calcium hydroxide concentration was low, the hardness of demineralized dentin did not increase, but mineralized substance-like aggregate formation was observed.

3. When a pulp-capping agent containing calcium hydroxide at or above a specific concentration was applied to demineralized dentin, the hardness of the demineralized dentin increased and features of remineralization were observed.

It was suggested that the remineralization of demineralized dentin depended on the calcium hydroxide concentration contained in a pulp-capping agent.

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Reference


18) Shimizu A, Honda K, Natsumi Y and Hasegawa M. Development and


Shuppan: Tokyo; 2010, 65. (in Japanese)


新規Knoop硬さ測定システムによる覆髄剤の有効性の検討

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覆髄剤が脱灰象牙質の硬さに与える影響について
抄録
目的

Minimal Intervention(MI)の概念に基づき、齲蝕が深部象牙質にまで進行し歯髄に近接する場合、歯髄に近接する深部齲蝕象牙質を保存し、露髄を回避する目的で暫間的間接覆髄法(IPC)が行われる。本研究では、新規Knoop硬度測定システムであるカリオテスターSUK-971（三栄エムイー）を用いて象牙質試料の硬度を測定し、覆髄剤が軟化象牙質へ与える影響を検討した。

材料および方法

ヒト抜去大臼歯から直径10mm、厚さ2mmの象牙質試料を作製し、象牙質試料の歯髄腔側からアスピレーターで吸引しながら、エナメル質側を20mM乳酸溶液に浸漬して、エナメル質側の硬さが20KNH程度となる脱灰象牙質試料を作製した。脱灰象牙質試料に、ハイボンドテンポラリーセメントソフト（松風）、ネオダイナミン-α（ネオ製薬）、ダイカル（デンツプライ三金）、カルシベックスプレーンⅡ（日本歯科薬品）、60％水酸化カルシウム混和物（キシダ）を貼付し、ベースセメント（松風）で被覆したものを覆髄試料、覆髄剤を貼付せずベースセメントのみで被覆したものをコントロールとして作製し、湿度100％容器中または石灰化溶液中で1か月間および3か月間保管後、脱灰象牙質の硬度を測定した。試料数は各条件につき3試料とし、得られた値は一元配置分散分析およびTukeyの検定にて統計解析を行った（α=0.05）。また、硬度測定後、覆髄剤貼付部のSEM画像の観察を行った。
結果および考察

ダイカル, カルシペックスプレーン II, 60%水酸化カルシウム混和物を貼付した脱灰象牙質試料では硬さが向上し, SEM 画像でも石灰化物の緻密な沈着が認められた。コントロール, ハイボンドテンポラリーセメントソフト, ネオダイン−α を貼付した脱灰象牙質試料では, 硬さは向上せず, SEM 画像でも石灰化物の緻密な沈着は認められなかった。このことから, 水酸化カルシウムを27%以上含有する覆髄剤を脱灰象牙質に応用することで, コラーゲン線維表面に石灰化物の沈着が起こり, 脱灰象牙質が硬化したと考えられる。また, カリオテスターを用いることで, IPC 後の象牙質の硬さの継続的変化をチェアーサイドで客観的に評価することが可能になると考えられる。

結論

覆髄剤貼付による脱灰象牙質の再石灰化は, 覆髄材に含有される水酸化カルシウムの含有濃度に影響されることが示唆された。

キーワード: Knoop 硬さ, 覆髄剤, 再石灰化
Figure legend

Table 1  Materials
The materials tested as pulp-capping agents in this study.

Table 2  Knoop hardness of dentin in each condition after 1 and 3 months
The unit is Knoop hardness of dentin in each condition. ( ) means SD of Knoop hardness. The number of samples was 3 for each condition. In each group, values within the same superscript letters are significantly different (α=0.05).

Fig. 1  SEM images of sound and demineralized dentin
The sample surface of sound dentin was densely covered with mineralized substances, such as hydroxyapatite, and no collagen fibrils were exposed. The collagen fibrils of demineralized dentin samples were exposed due to demineralization by lactic acid. Striation was observed on most collagen fibrils, but was absent on the others. The slight retention of mineralized substances was observed on the collagen fibril surface, but no mineralized substance was present between collagen fibrils.

Fig. 2  SEM images of dentin applied HB
In the DW group, collagen fibrils were exposed after 1 month, similar to those in demineralized dentin samples, and no mineralized substance-like aggregates adhered to the collagen fibril surface. The slight deposition of mineralized substance-like aggregates was observed on the collagen fibril surface at 3 months.
In the **RS** group, collagen fibrils were exposed at 1 month, similar to those in the **DW** group; however, mineralized substance-like aggregates were deposited on the collagen fibril surface. The deposition of aggregates on the collagen fibril surface had progressed at 3 months, and the space between collagen fibrils was more likely to be sealed by the aggregates.

**Fig. 3** SEM images of the dentin applied NE

In the **DW** group, collagen fibrils were exposed at 1 and 3 months, and almost no deposition of mineralized substance-like aggregates occurred.

In **RS** group, similar to those in **DW** group, collagen fibrils were exposed and almost no deposition of mineralized substance-like aggregates was noted at 1 month. Some collagen fibrils were exposed at 3 months and no mineralized substance-like aggregates were deposited in some regions; however, the sample surface was more likely to be covered by mineralized substance-like aggregates.

**Fig. 4** SEM images of the dentin applied DY

In the **DW** group, collagen fibrils were exposed at 1 month, and mineralized substance-like aggregates were deposited on the surfaces of some collagen fibrils. The striation of collagen fibrils was also noted. The deposition of aggregates on the collagen fibril surface was more extensive at 3 months than at 1 month, and the space between collagen fibrils was more likely to be sealed by the aggregates.

In the **RS** group, the deposition of mineralized substance-like aggregates was observed on the collagen fibril surface at 1 month, and the space between collagen fibrils was more likely to be sealed by the aggregates. Deposition progressed
further by 3 months, the space between collagen fibrils was sealed by the aggregates, and the sample surface was densely covered.

Fig. 5 SEM images of the dentin applied CA

In the DW group, mineralized substance-like aggregates were deposited on the collagen fibril surface and between collagen fibrils at 1 month. Deposition had further progressed at 3 months, and the sample surface was densely covered by the aggregates.

In the RS group, similar to those in the DW group, mineralized substance-like aggregates were deposited on the collagen fibril surface and between collagen fibrils at 1 month, and deposition had progressed and aggregates densely covered the sample surface at 3 months.

Fig. 6 SEM images of the dentin applied CH

In the DW group, mineralized substance-like aggregates were deposited on the collagen fibril surface and between collagen fibrils at 1 month, and deposition had progressed and aggregates densely covered the sample surface at 3 months.

In the RS group, similar to those in the DW group, mineralized substance-like aggregates were deposited on the collagen fibril surface and between collagen fibrils at 1 month, and deposition had progressed and aggregates densely covered the sample surface at 3 months.

Fig. 7 SEM images of the dentin applied BC

In the DW group, similar to those in demineralized dentin samples, collagen
fibrils were exposed at 1 and 3 months, and mineralized substance-like aggregates did not form on the collagen fibril surface.

In the RS group, the deposition of mineralized substance-like aggregates on the collagen fibril surface at 1 month was slightly higher than that in the DW group; however, most collagen fibrils were exposed, similar to those in the DW group. The deposition of mineralized substance-like aggregates was slightly more at 3 months than at 1 month, but was still very limited.
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<td>HY-Bond Temporary Cement Soft</td>
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n=3, unit : KNH, ( ) : SD

In each group, values within the same superscript letters are significantly different (α=0.05).
Fig. 1  SEM image of the sound and demineralized dentin

The sample surface of sound dentin was densely covered with mineralized substances, such as hydroxyapatite, and no collagen fibers were exposed. The collagen fibers of demineralized dentin samples were exposed due to demineralization by lactic acid. Striation was observed on most collagen fibers, but was absent on the others. The slight retention of calcified substances was observed on the collagen fiber surface, but no mineralized substance was present between collagen fibers.
In the DW group, collagen fibers were exposed after 1 month, similar to those in demineralized dentin samples, and no mineralized substance-like aggregates adhered to the collagen fiber surface. The slight deposition of mineralized substance-like aggregates was observed on the collagen fiber surface at 3 months.

In the RS group, collagen fibers were exposed at 1 month, similar to those in the DW group; however, mineralized substance-like aggregates were deposited on the collagen fiber surface. The deposition of aggregates on the collagen fiber surface had progressed at 3 months, and the space between collagen fibers was more likely to be sealed by the aggregates.
In the DW group, collagen fibers were exposed at 1 and 3 months, and almost no deposition of mineralized substance-like aggregates occurred.

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**Fig. 4** SEM image of the dentin applied DY
In the DW group, mineralized substance-like aggregates were deposited on the collagen fiber surface and between collagen fibers at 1 month. Deposition had further progressed at 3 months, and the sample surface was densely covered by the aggregates.

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In the DW group, similar to those in demineralized dentin samples, collagen fibers were exposed at 1 and 3 months, and mineralized substance-like aggregates did not form on the collagen fiber surface.

In the RS group, the deposition of mineralized substance-like aggregates on the collagen fiber surface at 1 month was slightly higher than that in the distilled water group; however, most collagen fibers were exposed, similar to those in the distilled water group. The deposition of mineralized substance-like aggregates was slightly more at 3 months than at 1 month, but was still very limited.