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Real time nanoleakage of calcium silicate root canal filling materials

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Abstract

Real time nanoleakage of calcium silicate root canal filling materials

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Objectives. This study aimed to investigate the real-time nanoleakage of calcium silicate (Ca-Si) based root canal filling materials.

Materials and Methods. Extracted human teeth (*n* = 30) were decoronated and enlarged apically to size #40 (taper 0.06) using Protaper Next X4 instruments (Dentsply, USA). The teeth were randomly divided into 3 experimental groups and obturated with respective materials; Gutta Percha (GP) with AH 26 sealer using the continuous wave of condensation technique, GP with EndoSeal MTA sealer using the continuous wave of condensation technique, and solely obturated with orthograde Biodentine. The roots were embedded into acrylic resin except the apical 2 mm to prevent leakage through lateral surface and a metal tube was fixed on the coronal orifice by using flowable composite resin. The prepared specimen was connected to a nanoFlow device (IB Systems, Seoul, Korea) under hydrostatic pressure (40 cm·H_2O) and fluid flow was traced through the filled roots. Data were detected at the nanoscale twice per second and automatically recorded in units of nL/s. Leakage was quantified as the mean slope after the curve stabilized over time. Data were statistically analyzed using the Kruskal-Wallis test. The level of significance was set at 5%.

Results. The calculated leakage values were 0.0670 ± 0.0516 nL/s for GP/AH26, 0.1397 ± 0.1579 nL/s for GP/EndoSeal MTA, and 0.0358 ± 0.0538 nL/s for Biodentine, with no statistically significant differences among the root filling materials ($P > 0.05$).

Conclusion. Real-time measurements under hydrostatic pressure with the nanoFlow device enabled precise fluid flow tracing through the root canal filling material. In terms of nanoleakage, the tested root canal filling materials showed no significant differences.

Keywords: Calcium silicate material; nanoFlow; Nanoleakage; Real time
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I. Introduction

The proper sealing of the root canal system is one of the key factors influencing the success of endodontic treatment [1]. A recent study analyzing the outcomes of endodontic microsurgery demonstrated that leaky canals were the most common cause of failure in nonsurgical endodontic treatment [2]. Endodontic microleakage can occur at the interfaces between the sealer-dentin, sealer - Gutta Percha (GP), within sealer itself and serves as a microbial niche for recurrent apical pathosis.

Previous studies investigated endodontic microleakage by measuring the leakage of various tracers (dye, bacteria, fluid, etc.) or visually observe the filling density (sectioning or microcomputed tomography) to compare different root canal filling materials and techniques. However, the results were non-reproducible and had relatively large standard deviations, which lacked confidence to support or dispute the clinical results of endodontic researches [3-6]. Dye penetration test method, the most representative method of using tracer, was introduced in 1939 by Grossman and used widely until these days. It is easy to perform and economic, but entrapped air in the root canal filling makes difference in dye penetration depth, even if the vacuum or centrifugation is applied [7, 8]. Moreover, other variables, such as pH, chemical reaction, immersion time, and molecular size of dye also affect the leakage result, so the validity of dye leakage test is questionable [9-11]. Micro computed tomography (Micro-CT) is a nondestructive technique that enables the three-dimensional analysis of filling materials, with high spatial resolution and accuracy [12]. It makes highly precise qualitative gap measurement, but it is not directly related to leakage through root canal filling, and the reaction of filling material with dentin is not reflected. Thus, micro CT analysis in leakage test should be used as a supplement to other test methods, rather than being used alone [13].

To reduce endodontic leakage between the sealer and dentin, the sealer and GP, or within the sealer itself, several trials were being made on root canal filling technique and root canal filling materials. The continuous wave of condensation technique, a novel obturation method, was introduced by Buchanan [14]. This approach is a modification of the warm vertical method which takes advantages of lateral condensation technique with well-fit master apical cone to minimize apical extrusion of GP and warm vertical compaction to thoroughly obturate the root-canal system [15]. It is also time saving and bacterial tight sealed compared to traditional lateral condensation technique [16], even in root canals with complex anatomical structures [17].

The root canal sealer is used to eliminate the interface between the GP and dentinal walls and the quality of the canal filling largely depends on the sealing capacity offered by sealers. The sealers are categorized according to their main chemical constituents: zinc oxide eugenol, calcium hydroxide, glass ionomer, silicone, resin, and calcium silicate (Ca-Si). AH26, an epoxy-resin based sealer, is well known for its high flowability, adhesion to dentin wall and ability to harden in the presence of moisture. Moreover, the good sealing ability of AH26 compare to other sealers was found in several studies [18-20]. Thus, it is widely used despite the release of formaldehyde during setting [21] and also commonly used as a basis for comparison in various studies.

Ca-Si based materials, including the well-known mineral trioxide aggregate (MTA), gained attention decades ago as root repair cements in the field of endodontics and have recently expanded the scope of use as root canal sealers and root canal filling materials. The rationale to use Ca-Si materials as root canal sealers and/or root canal filling materials is related to their ability to set in wet conditions (e.g., water, blood, dentinal fluid, etc.), which is the mainstay of their clinical application. For this purpose, EndoSeal MTA (Maruchi, Wonju, Korea), a ready-to-use Ca-Si material premixed in an air-tight syringe, was introduced in an attempt to enable its direct application into root canals. During the setting process, the material absorbs moisture in the canal and sets by itself. Several studies have demonstrated that EndoSeal MTA showed satisfactory physical properties [22], biocompatibility [22, 23], good bond strength [24], fracture resistance [25], minimal discoloration [26], and superior sealer distribution [27]. Another high-purity Ca-Si material, Biodentine (Septodont, St. Maur-des-Fossés, France), was introduced with the goal of overcoming the shortcomings of pre-existing MTA products, such as long setting time and procedural error that may occur during the mixing process. Biodentine does not contain heavy metals contrary to purified natural tricalcium silicate and has finer particle, which means it has greater value of specific surface area in comparison to MTA (2.811 m^2/g for biodentine and 1.034 m^2/g for MTA) [28]. Also, the homogeneous mechanical trituration of the prescribed powder-to-liquid ratio is a significant feature from a clinical standpoint because it can increase the likelihood of optimal sealing by minimizing operator-related errors.

As Ca-Si root filling materials emerged into the spotlight, various efforts have been made to evaluate the sealing ability of these materials in root canals. In dye penetration and fluid filtration tests, these materials have been found to have a high sealing ability [29-31]. But the reliability of these test methods has been questioned, and their results are not correlated with quantitative data, as mentioned above [6, 11]. Also, micro-computed tomography tests allow three-dimensional quantitative evaluation of voids at the dentin/sealer/GP interfaces and inside the filling materials [32], but are mostly confined to a static evaluation of root canal filling quality. Furthermore, the results from poly-microbial tests can be confounded by the antimicrobial properties of Ca-Si materials because the Ca-Si materials can produce calcium hydroxide and increase pH by dissociation it into calcium and hydroxide ions, which is responsible for their antimicrobial efficacy [33].

As part of the effort to make a highly precise and reliable test setup, a nanoFlow device (IB Systems, Seoul, Korea) which detects fluid flow using a light-sensitive photodiode with a resolution up to 0.196 nL was developed. As a novel innovation in the field of restorative dentistry, it precisely traces the real-time dentinal fluid flow at the level of nanoleakage, as a parameter indicating permeation between the hybrid layer and intact dentin. Previous studies [34, 35] using this nanoFlow device reported continuous dentinal fluid flow during the entire restorative procedure, including cavity preparation and composite fillings with different types of adhesives, unlike most other studies which investigated isolated dentinal fluid movements in response to a single stimulus.

Likewise, if leakage of the root canal could be measured and compared at the nanoscale, it will be possible to make a reliable judgement of heretofore gray areas in the root canal sealing ability of filling materials, providing a basis for solid results. However, to our knowledge, no studies have yet evaluated the nanoleakage of root canal fillings. Therefore, the purpose of this study was to investigate the nanoleakage of Ca-Si root canal filling materials and to characterize their fluid flow patterns in real time. The null hypotheses of this study were: 1) there would be no difference in the nanoleakage between the conventional epoxy resin based root canal sealer and Ca-Si based root canal sealer with the continuous wave of condensation technique and; 2) the orthograde root canal filling with Ca-Si based canal filling material had less nanoleakage than the conventional methods.

II. Materials and Methods

1. Ethics

This research was approved by the Institutional Review Board of Seoul National University Dental Hospital (CRI20005).

2. Sample size calculation

The sample size was calculated with G*Power 3.1.9 (Universität Kiel, Kiel, Germany) to detect significant differences (effect size: 0.6, alpha: 0.05, 80% power). The estimated sample size was 10 teeth in each group.

3. Sample preparation

Thirty single-rooted human teeth with a straight single canal and a mature apex were used. Teeth that had any craze lines, fractures, or resorption were excluded. The selected teeth were soaked in 3% sodium hypochlorite solution for 24 hours for disinfection.

After decoronation, root canal length was measured until a #10 K-file (Dentsply Sirona, York, PA, USA) tip was seen at the apex. The teeth were then trimmed to standardize the root canal length as 10 mm to reduce the possibility of errors arising from differences in root canal length. The root canals were then enlarged apically to size #40 (taper 0.06) using Protaper

Next X4 instruments (Dentsply Sirona). Irrigation was performed with 2.5% sodium hypochlorite alternating with 17% EDTA between instruments. The teeth were then randomly divided into three experimental groups (n = 10/each) and obturated as follows: GP and AH 26 sealer using the continuous wave of condensation technique; GP and EndoSeal MTA sealer using the continuous wave of condensation technique; and solely obturated with orthograde Biodentine. The continuous wave of condensation technique used non-standardized M size GP cone (Meta Biomed, Chungbuk, Korea) with Duo-Alpha and Duo-Beta system (B&L Biotech, Ansan, Korea). Each GP cone was placed in the number 40 hole of a Gutta Percha gauge (Gutta Percha Gauge; Dentsply Maillefer, Ballaigues, Switzerland), and the extended tip was cut (#15 surgical stainless steel blade; Ribbel, New Delhi, India). The GP was down-packed with a Duo-Alpha heat source at 200°C to the 4 mm from the working length and backfilled using a Duo-Beta at 180°C.

Biodentine was mixed according to the manufacturer's instructions to reach the desired consistency and applied using a #25 K-file and hand plugger. After root canal filling, the orifices of each sample were sealed with Caviton (GC Corp., Tokyo, Japan) and the samples were covered with wet gauze for 7 days for the materials to set. Information on the root canal sealers and Biodentine used in this study is summarized in Table 1. X-rays of all samples were taken to minimize errors caused by voids.

4. Nanoleakage measurement

Real-time leakage along the filled root canals was evaluated using the nanoFlow sub-nanoliter (nL) scale fluid flow-measuring device (Fig. 1) according to previous studies. [34, 35] The device consists of a glass capillary and photosensor for detecting fluid movement; a servomotor, lead screw, and ball nut for tracking fluid movement; and a rotary encoder and computer software for data recording. An air bubble was created in a water-filled glass capillary with an internal diameter of 0.5 mm, which connected the root to a water reservoir. A photosensor consisting of an infrared light-emitting diode and a photo transistor detected the movement of the air bubble in the capillary as the water flowed. The servo amplifier and servomotor rotated a lead screw according to the output voltage from the photosensor to track the water-air interface continuously. The rotation of the screw was measured using a rotatory encoder, which detected 1,000 pulses per rotation, and the number of pulses was stored on a computer. The minimum measurable volume of water movement in this instrument was 0.196 nL. Leakage data were detected at the nano scale (nL/s) twice per second and the data were automatically recorded and stored on a computer.

Filled roots were embedded into acrylic resin except for the most apical 2 mm to prevent undesirable leakage, and a metal tube was fixed on the coronal orifice by using flowable composite resin (Fig. 1). A hydrostatic pressure of 40 cm·H_2O was applied throughout the measurement process with a water reservoir. Finally, the prepared specimen was connected to the measuring device.

5. Statistical analysis

Data were tested for normal distribution with the Kolmogorov-Smirnov test and analyzed using the Kruskal-Wallis test for between-group comparisons. The selected level of significance was $P < 0.05$. The statistical analysis was performed using SPSS ver. 12 (SPSS Inc., Chicago, IL, USA).

III. Result

Leakage was quantified as the mean slope after the curve stabilized over time. The calculated values were 0.0670 ± 0.0516 nL/s for GP/AH26, 0.1397 ± 0.1579 nL/s for GP/EndoSeal MTA, and 0.0358 ± 0.0538 nL/s for Biodentine, and there were no statistically significant differences in nanoleakage among the root filling materials ($P > 0.05$) (Figs. 2, 3).

IV. Discussion

Previous studies of endodontic leakage test using various tracers had the limitations that most of them focused only on the comparison of the leakage between the initial and delayed values within each specimen [3-5, 36]. In this study, the nanoFlow device was used to detect ‘nanoleakage’ in root canal filling materials. The basic principle of the nanoFlow device is similar to the classic fluid filtration method (Flodec) developed by Pashley [37], as it involves detecting air bubble movement trapped within a capillary tube. However, the nanoFlow device has a resolution of 0.196 nL, which is 10 times more precise than Flodec [37]. Its real-time measurement allows continuous collection of exquisitely precise data (with the ability to adjust the measurement intervals as needed), whereas other fluid filtration systems, including Flodec, record oscillating values [30]. Possible errors caused by entrapped air or fluid are ruled out by the continuous application of positive hydraulic pressure [30]. This enables quantitative and qualitative analyses of nanoleakage.

The results of this study showed no statistically significant differences among the tested groups ($P > 0.05$) and the null hypotheses were rejected. The calculated values were below 1 nL/s in all tested roots, in accordance with a previous leakage study of Ca-Si material using the fluid filtration method of Camilleri et al. [38] Although there was no specific difference among the groups on average, most of Biodentine-filled roots showed minimal fluid flow with flat graph compared to other groups, with small leaps in flow (Fig. 3). This result is in accordance with previous reports stating that Biodentine had a minimal degree of porosity under moist conditions [39], and subsequently displayed firm micromechanical attachment to the underlying dentin due its high alkalinity and calcium uptake from the underlying dentin [40, 41].

It should be noted that the output data of the current experiment should not be regarded as absolute values. It is possible to adjust the sensitivity of the nanoFlow system by controlling the hydraulic pressure applied and the diameter of the micropipettes used to make measurements. Consequently, it is important to interpret the output data (which is the extent of fluid flow under the controlled setup) with weighting according to the pressure used [42]. Using the central venous pressure (8-12 mmHg [43]) to reflect the physiological environment, the data in this study could be converted to 0.0228 ± 0.0175 nL/cm·H_2O/s for GP/AH 26, 0.0475 ± 0.0537 nL/cm·H_2O/s for GP/EndoSeal MTA, and 0.0122 ± 0.0183 nL/cm·H_2O/s for Biodentine respectively (assuming 1 mmHg to be 1.36 cm·H_2O).

The measurements made in real time provided salient evidence regarding differences in closed pores. Several micro-CT studies have calculated total pore volumes as percentages to evaluate porosity, sealing ability, or filling quality [32, 44-47] but could not completely rule out impervious pores; furthermore, these studies revealed open pores that contributed to data variation. In this study, fluid flow of each specimen showed linear increase followed by plateau (Fig. 3). The initial steep slope is the fluid flow during the filling of the closed and open pore of the specimen, and the subsequent gentle slope is the amount of liquid passing through the open pore after the closed pores are all filled. Thus, the leakage was calculated as the slope after the curve stabilized and overall quantitative analysis of nanoleakage involves the assessment of liquid that passes through voids (open pores). Specimens showing a continuous linear increase could be excluded from the quantitative evaluation of leakage due to their inadequate filling quality, thereby precluding an overestimation of open pores. The excluded specimens were mostly from samples in which GP cones were used, regardless of the sealers used (AH26 or EndoSeal MTA), implying that there was a high chance of open pore between the master cone and sealer.

Notably, the GP/EndoSeal MTA–filled roots showed considerable variation. It is because AH26 and Biodentine are powder and liquid type materials that the viscosity of the mixture can be adjusted to the most suitable level for use according to the preference of the operater, based on the manufacturers' instructions, thus the operator factor of error during the canal filling process can be reduced even if it has a risk that it may not being uniformly mixed or mixed with inappropriate powder and liquid ratio. On the contrary, EndoSeal MTA is homogeneously pre-mixed content loaded into an air-proof syringe which has higher flowability than other sealers, that can be advantageous in terms of penetrating into the ramifications and irregularities of root canal system but also difficult for operators to manipulate. Also, the higher solubility and larger dimensional change of EndoSeal MTA compared with resin-based sealers may explain the wide standard deviation [22, 48]. Possible lumping during injection could be another factor contributing to variation, suggest that careful handling such as slow injection without excessive pressure during application of the material into the root canals is necessary when using EndoSeal MTA sealer with GP.

Another reason for the large standard deviation is that the samples were made from human teeth. The reliablity of endodontic microleakage test is largely depended on the protocol of sample preparation. Because of the amount of leakage is very small and the test is extremely sensitive, even a small error could make a big impact on the result. To make samples identical, it would have been advantageous to use artificial teeth to reduce the errors that may occur during sample preparation [49]. Resin teeth, however, cannot reproduce microstructures such as the dentinal tubule, which may affect experimental results [50]. On the other hand, by using human teeth, there must be differences even if researchers make them as similar as possible. In this study, natural human teeth with straight, non-ovoidal canal were used to maintain the microstructure, but to remove the errors coming from the difference of canal shape. However, due to differences in the microstructure between the teeth, a wide range of standard deviations could have occured even if all other environmental factors are applied equally.

Taken together, the similar fluid flow of the tested root canal filling materials implies that there is no difference in the degree of root canal filling depending on root canal filling materials, in terms of nanoleakage. Moreover, it can be asserted that biocompatibility and adequate root canal cleaning and shaping may be the most important factor in success and failure of root canal treatment. In this regard, biocompatibility could be the major advantages associated with the use of Ca-Si based root canal filling materials.

The use of Ca-Si materials for root canal filling needs to be reconsidered in light of properties other than sealing ability. On one hand, their major strength is their biological effects and biocompatibility over conventional inert root canal filling material [51]. The release of calcium ions for a long time after setting, in contact with moist dentin and bone [52], and their alkalinizing activity (increasing the local pH) during the hydration reaction are closely related with their antibacterial properties [53, 54]. They can also induce intratubular biomineralization, which is expected to promote sealing abilities, micromechanical retention, and resistance against dislodgement [55]. Also, monobasic calcium phosphate included in the sealer facilitates the reaction of calcium phosphate with calcium hydroxide and improves the setting properties, resulting in a chemical composition and crystalline structure similar to tooth and bone apatite materials, thereby improving sealer-to-root dentin bonding [56]. Moreover, heating of epoxy resin based sealer to the recommended temperature (200°C or 250°C) of continuous wave of condensation technique causes change of chemical structure, whereas only microstructural changes due to water loss may occur in Ca-Si based sealer [57].

The current experimental setting does not allow the detection of chemical phase changes of Ca-Si materials over time, which may reduce the amount of closed pores and the resultant quantitative nanoleakage. Despite being nondestructive, this method is not suitable for the long-term evaluation of massive samples concurrently. Moreover, there is no standardized criterion for experimental conditions such as applied pressure or specimen parameters, which may affect the results. Nevertheless, as limited information is available on real-time measurements, this study makes a pioneering contribution to the study of endodontic leakage and root canal material permeability. The analysis of real-time flow with exquisite precision may represent a back-to-basics streamlining of endodontic microleakage concepts. This streamlining will enable advanced biomechanical three-dimensional sealing of root canals to keep pace with vastly improved endodontic techniques such as nickel-titanium instruments.

V. Conclusion

Real-time measurement under hydrostatic pressure with the nanoFlow device enabled precise tracing of fluid flow through root canal filling materials. Measured at the sub-nanoliter level, there was extremely small fluid flow, with no significant differences among the tested root canal filling materials.

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Table 1. Root canal filling materials used in the study

Materials	Composition	Manufacturer
AH 26	Powder: Bismuth oxide, methenamine, silver, titanium dioxide Liquid: Epoxy resin	Dentsply DeTrey, Konstanz, Germany
EndoSeal MTA	Calcium silicates, calcium aluminates, calcium aluminoferrite, calcium sulfates, radiopacifier, thickening agent	Maruchi, Seoul, Korea
Biodentine	Tricalcium silicate powder Aqueous calcium chloride solution and excipients	Septodont, St. Maur-des-Fossés, France

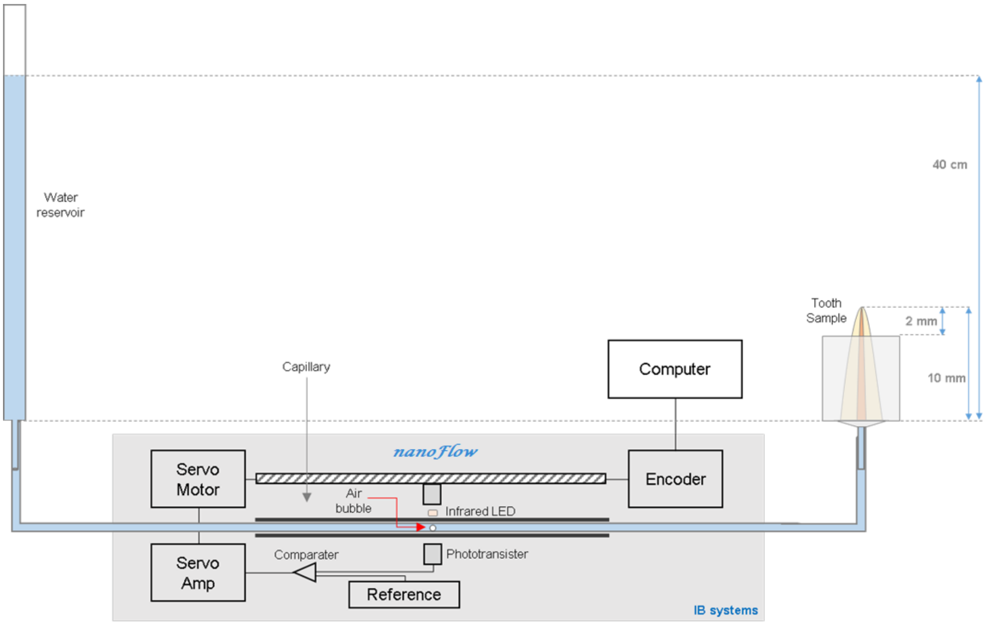

Figure 1. Schematic diagram of the real-time fluid flow measuring device (nanoFlow) connected to a specimen.

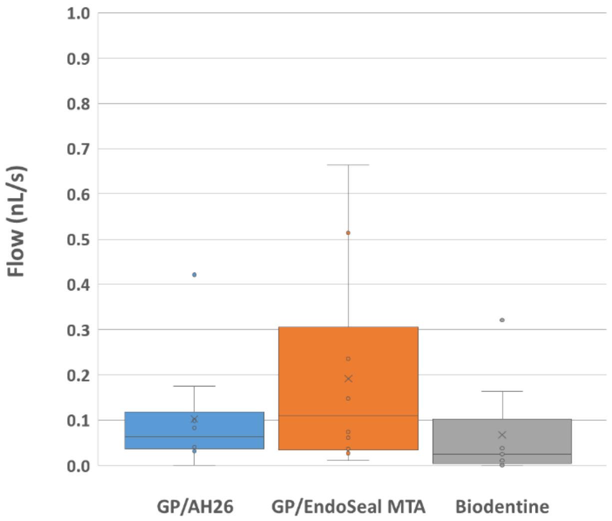

Figure 2. Box and whisker plot of measured nanoleakage data.

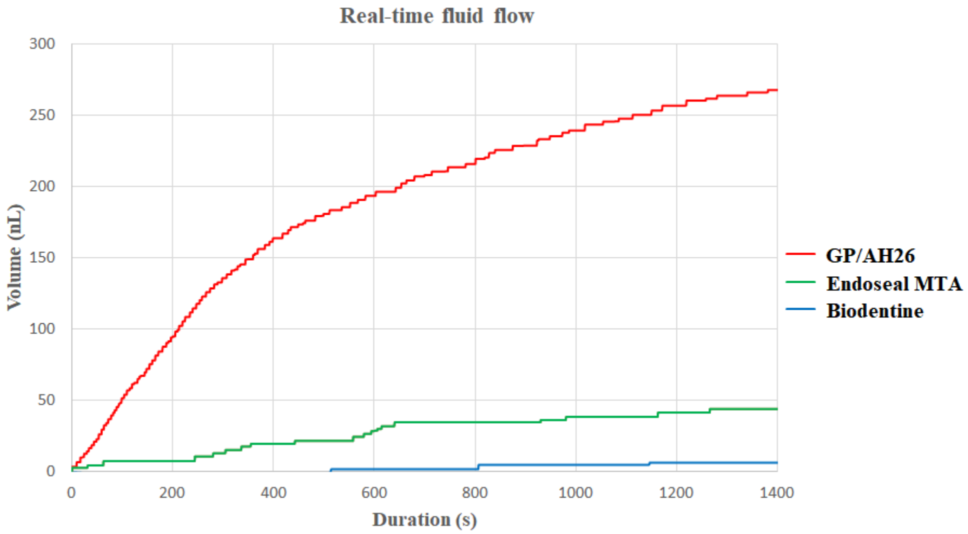

Figure 3. Representative total real-time flow of GP/AH26, GP/EndoSeal MTA, Biodentine. Notice that the slope flattens over time.

국문초록

Calcium silicate 근관 충전 재료의 실시간 나노 누출

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1. 목적

본 연구의 목적은 Calcium silicate (Ca-Si) 기반 근관 충전재의 실시간 나노 누출을 조사하는 것이다.

2. 재료 및 방법

30개의 발치된 사람의 단근치의 치관을 제거하고 Protaper Next X4를 사용하여 근관을 확대한 후 세 그룹으로 나누어 각각 Gutta percha (GP) 와 AH26 실러, GP 와 EndoSeal MTA 실러, Biodentine으로 충전하였다. 불필요한 누출을 막기 위해 치근단 2mm를 제외한 부분을 아크릴릭 레진에 매몰하고 근관 입구에 유동성

복합레진을 사용하여 금속 관을 고정한 뒤, 40cm·H_2O의 정수압 하에서 nanoFlow 장치(IB syetems, Seoul, Korea) 에 연결하여 근관을 통한 미세 누출을 측정하였다. 데이터는 초당 2회, 나노 단위로 감지되었으며 nL/s 단위로 자동 기록되었다. 누출 양은 누출 곡선이 평탄화 된 이후의 평균 기울기로 계산되었다. 데이터는 Kruskal-Wallis 테스트를 사용하여 통계적으로 분석되었다. 유의 수준은 5%로 설정하였다.

3. 결과

계산된 나노 누출 값은 GP/AH26의 경우 0.0670 ± 0.0516 nL/s, GP/EndoSeal MTA의 경우 0.1397 ± 0.1579 nL/s, Biodentine의 경우 0.0358 ± 0.0538 nL/s 로, 재료 사이에 유의한 차이는 나타나지 않았다($P > 0.05$).

4. 결론

nanoFlow 장치를 사용함으로써 보다 정확한 근관 충전 재료의 실시간 미세 누출의 측정이 가능하였다. 나노 누출 측면에서 각 근관 충전재 사이에는 큰 차이가 나타나지 않았다.

주요어: Calcium silicate, nanoFlow, 나노 누출, 실시간
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