Effect of particle sizes in India ink on its use in evaluation of apical seal

Masataka Yoshikawa, Katsushi Noguchi and Tadao Toda

Department of Endodontics, Osaka Dental University
8-1 Kuzuhahanazono-cho, Hirakata-shi, Osaka 573-1121, Japan

We investigated the use of India ink as an indicator of root canal sealing ability. Sealing ability is one of the most important factors required of root canal cements. Various dyes have been used for this purpose. Methylene blue, radioisotopes and India ink have all been tried. However, there is no evidence that India ink is suitable as an indicator. We measured the particle size distribution of India ink to determine how this factor affects test results. In addition, we observed the surface texture of an experimentally developed calcium phosphate sealer and a commercially available root canal sealer using SEM. We found that a portion of the India ink particles were smaller than cracks on the two sealer surfaces, indicating, that India ink could pass through these cracks. We concluded that India ink is suitable as an indicator of root canal seal. (J Osaka Dent Univ 1997 Dec; 31: 67-70)

Key words: India ink; Dye penetration; Root canal seal

INTRODUCTION

A root canal sealer must have superior histocompatibility and meet rigid physical property criteria. Root canal sealing ability is one of its most important properties. We developed new sealers that have calcium phosphates as a major components. However, certain factors must be achieved before a new sealer can be applied clinically. We examined histocompatibility, physiological properties, and root canal sealing ability of a newly developed calcium phosphate (CP) sealer. The results showed that these sealers had excellent characteristics.

Dye selection is an important factor in tests of apical seal. India ink and methylene blue have often been used as indicators during in vitro tests of root canal seal. In these reports, the root apex of extracted human teeth that had been prepared and filled with sealer were immersed in the dye. The distance of the dye penetrated into the root canal from the apical foramen was then measured. Since it is convenient if the results of apical seal tests can be compared with those of other sealers previously reported, we used India ink in our previous reports on the apical canal seal of CP sealers.

Generally, a CP sealer hardens by development of crystal aggregation, and is characterised by micropores after setting. The relation between the diameter of the micropores and the size of the India ink particles affects dye penetration into the canal. Particle size of the dye is very important to the outcome of the test. We measured the distribution of the particle size in India ink using a microtrack particle size distribution indicator, and observed the appearance of calcium phosphate aggregation in the newly developed CP sealer surface with a scanning electron microscope. We then evaluated the reliability of results of tests on apical seal when India ink was used as an indicator.

MATERIALS AND METHODS

Distribution of India ink particle size

One drop of India ink was put in 50 ml of ethanol, dispersing the microparticles of the ink. The sample was measured four times with a microtrack particle size distribution indicator (Model 9230 UPA, Nikkiso Co., Ltd. Tokyo, Japan).
Scanning electron microscopic (SEM) observation of India ink particles
A thin layer of the dispersed solution of India ink in ethanol was placed in a glass dish. The sample was dried at 60°C in an incubator, it was coated with Au-Pd ion, and SEM observation was carried out (Hitachi S-2100 A, Hitachi, Ltd., Tokyo, Japan).

SEM observations of the experimentally developed CP sealer and a zinc oxide eugenol (ZOE) sealer
We observed the surface of the experimentally developed CP sealer with SEM. The powder components of this sealer were 68% tetracalcium phosphate (TeCP) and 32% dicalcium phosphate dihydrate (DCPD). Tetracalcium phosphate was supplied by Nitta Gelatin Inc. (Osaka, Japan). It was prepared by heating at 1,480°C a stoichiometric mixture of calcium phosphate dihydrate and calcium carbonate that had particle sizes of 0.6 to 44.0 μm (mean 12~13 μm). Dicalcium phosphate dihydrate was purchased from Wako Pure Chemical Industries Co. (Osaka, Japan). The liquid component (pH 5.2) was 2.1% citric acid, 7.2% Na₂HPO₄·12H₂O, 1.5% sodium carboxymethyl cellulose, 88.0% distilled water, and 1.2% other. It was kneaded for one minute with a 1.4 g/g powder to liquid ratio.

A commercially available ZOE sealer (Canals®; Showa Yakuhin, Tokyo, Japan) was also observed with SEM for comparison with the CP sealer. ZOE sealer is traditionally used in endodontic treatment. Its components were listed in the previous report. Components of this sealer are the same as those of Grossman’s root canal sealer. It was mixed according to manufacturer’s instructions. After completion of setting, the two sealers were coated with Au-Pt ion, and their surface structures were observed with SEM (Hitachi S-2100 A).

RESULTS

Distribution of India ink particle size
The distribution of India ink particle size was measured by the microtrack particle size distribution indicator. The diameter of the particles was 0.20~

![Fig. 1](image1.png) SEM photograph showing the various sized particles of India ink. The larger particles clearly have a major axis of over 30 μm. In addition, a large number of particles have a major axis of less than 1 μm.

![Fig. 2](image2.png) SEM photography of the surface of the experimentally developed calcium phosphate sealer. The calcium phosphate crystals mature by fusing with adjacent crystals.

31.11 μm, with a mean of 3.79~5.88 μm.

SEM observation of India ink
SEM photography of the India ink particles is shown in Fig. 1. The smaller particles had a diameter of less than 1 μm, while the major axis of the larger ones was over 30 μm.

SEM observation of the experimentally developed CP sealer and the ZOE sealer
SEM photography of the surface of the experimen-
used as indicators for testing apical seal. The typical experimental method was to, a) enlarge, shape and obturate the root canal of extracted human teeth with a test material, b) immerse the root in dye, and c) measure the distance the dye penetrated into the root canal from the apical foramen after the teeth had been decalcified. The apical seal test for the CP sealer that we developed was performed under the same conditions as conventional tests.

We selected India ink as the indicator so that the results for our sealer could be compared with those for conventional sealers tested in previous studies. Few reports recommend India ink as an indicator of sealing ability. Ahlberg et al. reported that, because of its low molecular weight, methylene blue penetrates more deeply along the root canal filling than does India ink. Our investigation examined the suitability of India ink as an indicator of apical seal for our experimentally developed CP sealer. We did not compare it with methylene blue. Although reference examinations were not performed, SEM observations were carried out with India ink on the experimentally developed CP sealer and a commercially available ZOE sealer.

These SEM were similar to those of our previous study. The composition of the CP cement was similar to that used this time. We realized then that India ink had a large distribution of particle size. The results obtained for this distribution as measured by the microtrack particle size distribution indicator agreed with results obtained by SEM photography. The smallest particles of India ink were smaller than microorganisms. Because it can pass through the cracks of the experimentally developed sealer, India ink can be used as an indicator of sealing ability. SEM observations indicated that the surface of the CP sealer had a crystalline structure. The width of these crystals ranged from 10 to 50 μm. Therefore, India ink particles would be able to pass between crystals of this sealer.

However, little or no linear dye penetration was observed in the apical canals sealed with CP or ZOE when India ink was used. The crystalline
structure of our experimentally developed CP sealer was demonstrated in this study and in the previous one. The microstructure of the surface and internal region of the CP cement previously studied showed several microparticles and considerable spacing between these particles. These spaces gradually filled in owing to the reaction of TeCP and DCPD. Dye penetration of the India ink results when the minor axis of the pores of the CP sealer are larger than the length of the particles. When micropores observed on the surface of the CP sealer are openings of tubules extending into the sealer, the seal will be poor. However, it was found that these were not openings of tubules. SEM photography showed that the split surface of the ZOE sealer was rough and consisted of merged particles. It also showed many unmerged microparticles on the surface with calculated diameters of 2–3 μm. There were some spaces on the split surface of the face, most of which were less than 8 μm in width. However, some were greater than 20 μm. Many microparticles of diameter 2–3 μm were observed by SEM on the surface of the ZOE sealer. These were zinc oxide particles. Gaps remained where these particles did not merge. These spaces were very narrow. We found that some of the India ink particles were smaller than the axis of spaces on the ZOE sealer surface. Because the spaces in the ZOE did not pass completely through the sealer, particles of India ink could not pass through the ZOE sealer. There was little or no penetration of India ink in the ZOE sealer used in the previous study. We concluded that the sealing ability of the ZOE was excellent.

Many authors have reported the excellent sealing ability of ZOE. Dye penetration of the new CP test material was compared to that of a ZOE sealer as a negative control. We concluded that when ZOE sealer was used as a control for the experimentally developed CP sealer, India ink was an effective indicator of apical seal.

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REFERENCES