Evaluation of the radiopacity and cytotoxicity of resinous root canal sealers

Chang-Kyu Kim[1], Hyun-Wook Ryu[2], Hoon-Sang Chang[2], Byung-Do Lee[3], Kyung-San Min[2], Chan-Ui Hong[1]*

[1]*Department of Conservative Dentistry, College of Dentistry, Dankook University,*
[2]*Department of Conservative Dentistry,* [3]*Department of Oral and Maxillofacial Radiology,*
College of Dentistry, Wonkwang University

ABSTRACT

The aim of this study was to evaluate the radiopacity and cytotoxicity of three resin-based (AH 26, EZ fill and AD Seal), a zinc oxide-eugenol-based (ZOB Seal), and a calcium hydroxide-based (Sealapex) root canal sealers. Specimens, 10 ㎜ in diameter and 1 ㎜ in thickness, were radiographed simultaneously with an aluminum step wedge using occlusal films, according to ISO 6876/2001 standards. Radiographs were digitized, and the radiopacity of sealers was compared to the different thicknesses of the aluminum step wedge, using the Scion image software. Using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay, the cytotoxicity of each material was determined in immortalized human periodontal ligament (IPDL) cells.

The results demonstrated that EZ fill was the most radiopaque sealer, while Sealapex was the least radiopaque ($p < 0.05$). AH 26, AD Seal and ZOB Seal presented intermediate radiopacity values. All the materials evaluated, except for Sealapex, presented the minimum radiopacity required by ISO standards. The cell viabilities of resin-based root canal sealers were statistically higher than that of other type of root canal sealers through the all experimental time. Further, EZ fill showed statistically lower cell viability in 24 and 48 hours compared to AD Seal and in 72 hours compared to all other resin-based root canal sealers. However, there was no correlation between the radiopacity and cytotoxicity of three resin-based root canals sealers ($p > 0.05$).

These results indicate that resin-based root canal sealer is more biocompatible and has advantage in terms of radiopacity. [J Kor Acad Cons Dent 32(5):419-425, 2007]

Key words : Radiopacity, Cytotoxicity, Root canal sealer, MTT assay, Aluminum step wedge, Immortalized human periodontal ligament cell

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* *Corresponding Author:* ***Chan-Ui Hong***

Department of Conservative Dentistry,
College of Dentistry, Dankook University
7-1, Shinbu-dong, Cheonan, Choongnam, 330-716, Korea
Tel: +82-41-550-1964 Fax: +82-41-550-1963
E-mail: cuhong@dku.edu

Ⅰ. INTRODUCTION

The ideal root canal sealer should present, among other physical/chemical properties, enough radiopacity to allow distinction from the adjacent anatomical structures[1-4], such as bone and tooth[5]. Higginbotham[6] was the first researcher to publish

a study comparing the radiopacity of various endodontic sealers and gutta-percha cones used to fill root canals. Eliasson and Haasken[7] established a comparison standard for radiopacity studies, using optical radiographic density measurements for impression materials and an equivalent thickness of aluminum capable of producing similar radiographic density. Beyer-Olsen and Orstavik[1] included a reproducible comparison standard using an aluminum step wedge with 2 mm-increments to determine the radiopacity of several root canal sealers.

Root canal sealers may affect the periapical tissue when extruded. In such condition, they could cause not only degeneration of the tissue lying underneath the endodontic sealers, but could also delay wound healing. Therefore, root canal sealers should have good biocompatibility[8].

Sealers could be classified according to their main constituents, such as zinc oxide-eugenol, calcium hydroxide, resins, glass ionomers, etc.[9]. The biocompatibility of a specific root canal sealer remains one of the principal considerations for selecting an appropriate sealer for a dental restoration[10]. From the literature, it would appear that the side effects of the use of various root canal sealers have been previously studied to some extent[11-13]. Epoxy resin-based sealers have been introduced in endodontic practice because of their favorable characteristics, such as adhesion to tooth structure, long working time, easey manipulation, and good sealing ability[14]. Several studies showed that polymerized resin-based sealers presented less cytotoxicity compared to zinc oxide-eugenol and calcium hydroxide-based sealers[15,16]. However, the cytotoxic effects of resin-based root canal sealers related to their radiopacity have not yet been studied.

The purpose of this study was to evaluate the radiopacity and cytotoxicity of various resin-based root canal sealers in comparison with zinc oxide-eugenol and calcium hydroxide-based root canal sealers. In addition, this study aimed to investigate the correlation between the radiopacity and cytotoxicity of them.

Ⅱ. MATERIALS AND METHODS

Material preparation

Five root canal sealers were evaluated in this study: AH 26 (Dentsply De Trey Gmbh, Konstanz, Germany), EZ fill (EDS, Hackensack, NJ, USA), AD Seal (Meta-Biomed, Cheongju, Korea), ZOB Seal (Meta-Biomed, Cheongju, Korea) and Sealapex (SybronEndo, Glendora, CA, USA). The materials were prepared according to manufacturers' instructions. Ten specimens, 10 ㎜ in diameter and 1 ㎜ in thickness, were fabricated from each material tested. Metallic matrices were made and impressions were taken using a light-bodied silicone-based impression material (Silagum, DMG, Hamburg, Germany). Samples of the prepared sealers were then inserted into the impressions and stored in a moist chamber at 37 ℃, until complete set.

Radiographic evaluation

The specimens were placed on the occlusal x-ray film (Kodak Insight, Rochester, NY, USA) along with an aluminum (99.5% pure) step wedge with step heights ranging from 1 to 10 ㎜ in increments of 1 ㎜ (Figure 1A). A Kodak-2200 x-ray machine (Kodak, Rochester, NY, USA) operating at 70 kv, 10 mA, 18 pulses/s and with a focus-sensor distance of 30 cm was used. The digitized images (Figure 1B) were then imported into the Scion image software (Scion Corp. Frederick, MD, USA), where a tool was applied in order to identify the equal-density areas in the radiographic images.

Cell culture and inoculation

Human papilloma virus (HPV) 16-IPDL cells were obtained by transfecting normal human periodontal ligament (PDL) cells with pLXSN vector containing the E6/E7 open reading frames of HPV 16. This was done by the methods that have been previously described[17]. The IPDL cells were cultured in Dulbecco's modified Eagle's medium

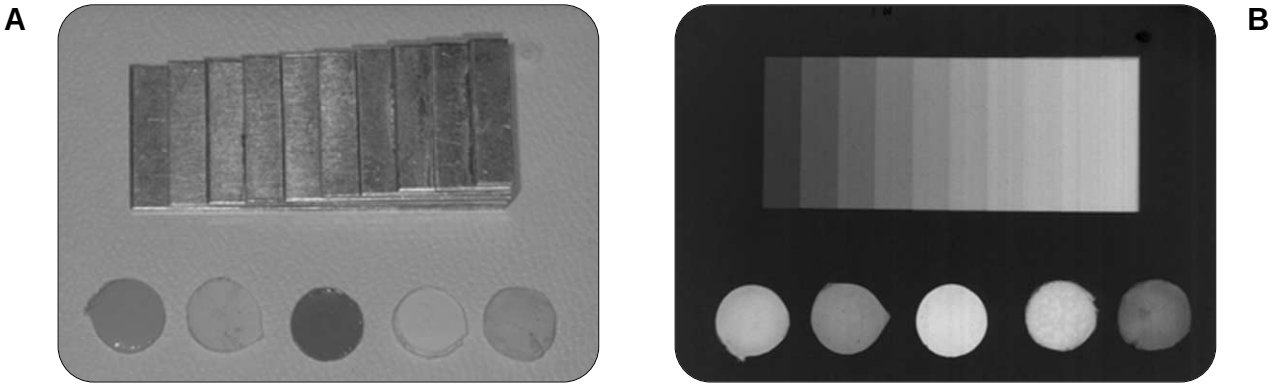

Figure 1. A. Occlusal film, specimens, and aluminum step wedge. B. Radiograph showing the radiopacity of each material and its equivalence to that of the aluminum step wedge.

(DMEM; Biofluid, Rockville, MD, USA) supplemented with 10% fetal bovine serum (FBS) (Gibco, Carlsbad, CA, USA) containing 100 U/㎖ of both penicillin and streptomycin (Life Technologies, Rockville, MD, USA). The cultures were maintained at 37℃ in a humidified atmosphere of 5% CO_2. Subsequently, single-cell suspensions of IPDL cells were seeded in 24-well tissue culture plates at 5×10^4 cells per well, as determined by hemocytometry, in complete DMEM. The plates were incubated in a humidified atmosphere of 5% CO_2 at 37℃ for 24 hours.

Cytotoxicity test

The five materials described previously were mixed and placed at the bottom of insert wells (n = 10) having a membrane pore diameter of 0.4 ㎛. After inserting the materials into the wells, the insert wells were exposed to ultraviolet (UV) light for 30 min and placed inside the culture wells; the cells were then incubated for 12, 24, 48, and 72 hours. All procedures were performed aseptically.

After exposure to the materials for 12, 24, 48, and 72 hours, the viable cells were detected using the MTT dye, which forms blue formazan crystals that are reduced by the mitochondrial dehydrogenase present in living cells. Briefly, 200 ㎕ of MTT solution (2 ㎎/㎖ in phosphate-buffered saline [PBS]) was added to each well, and the wells were incubated for 4 hours. Subsequently, 200 ㎕ of dimethyl sulfoxide (DMSO) was added to each well. The plates were then shaken until the crystals dissolved, and the solution in each well was transferred to a 96-well tissue culture plate. The reduced MTT was then measured spectrophotometrically at 540 ㎚ in a dual beam microtiter plate reader. The cells that were incubated with the medium alone served as negative controls.

Statistical analysis

Statistical analysis was conducted by one-way analysis of variance. Tests of differences of the treatments were analyzed by Tukey test and a value of $p < 0.05$ was considered statistically significant. The comparison between radiopacity and cytotoxicity of resin-based root canal sealers were analyzed using two-way analysis of variance and Tukey test at a 95% significance level.

Ⅲ. RESULTS

Radiographic evaluation

The results demonstrated that EZ fill presented the greatest radiopacity ($p < 0.05$) and were equivalent to 11.13 ㎜ of aluminum. AH 26, AD Seal and ZOB Seal presented radiopacity values that were equivalent to 6.84, 4.82 and 7.85 ㎜ of

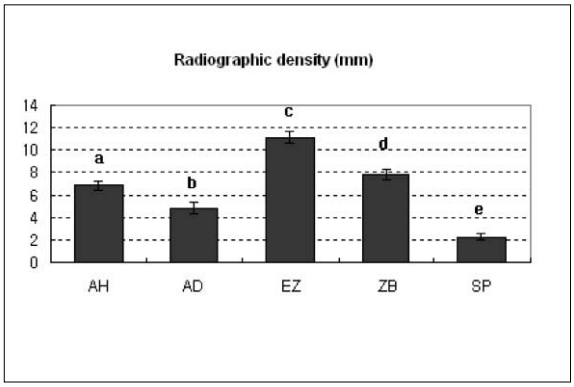

Figure 2. Relative radiographic density of each material in comparison with that of a 10-step aluminum step wedge with thickness varying from 1 ㎜ to 10 ㎜. Each point and bar represents the mean ± standard deviation (SD). Groups identified by the different symbols are significantly different ($p < 0.05$). (AH: AH 26, AD: AD Seal, EZ: EZ fill, ZB: ZOB Seal, SP: Sealapex)

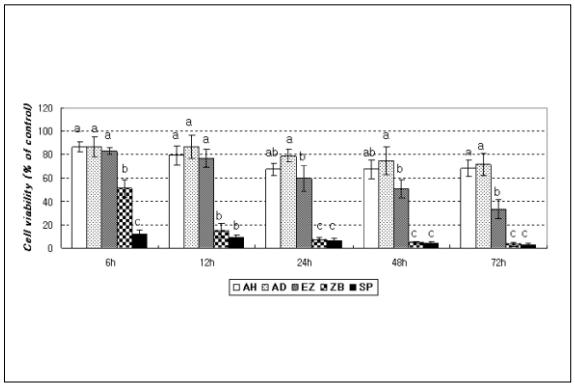

Figure 3. Effects of various root canal sealers on immortalized human periodontal ligament cells measured by MTT assay. Each point and bar represents the mean ± SD. Groups identified by the same symbols in each experimental time are not significantly different ($p > 0.05$). (AH: AH 26, AD: AD Seal, EZ: EZ fill, ZB: ZOB Seal, SP: Sealapex)

aluminum, respectively. Sealapex exhibited the lowest radiopacity ($p < 0.05$) and was equivalent to 2.22 ㎜ of aluminum (Figure 2).

Cytotoxicity test

As shown in Figure 3, resin-based sealers (AH 26, EZ fill and AD Seal) showed statistically higher cell viabilities throughout all experimental times compared to other type of sealers. However, the cell viability of EZ fill was statistically lower than that of AD Seal in 24 and 48 and that of other two resin-based sealers in 72 hours. However, there was no correlation between the radiopacity and cytotoxicity of three resin-based root canals sealers ($p > 0.05$).

Ⅳ. DISCUSSION

Radiopacity is widely acknowledged as a desirable property of all intraoral materials, including root canal sealers[18]. Radiopacity of root canal sealers has been of particular significance for the evaluation of the quality of endodontic treatment, as well as being helpful in the assessment of possible voids in the obturation[19]. The ISO 6876/2001 establishes that root canal sealers should be at least as radiopaque as 3 ㎜ thickness of aluminum[2]. Therefore, all sealers evaluated in this study presented the minimum radiopacity by this standard, except for Sealapex.

EZ fill contains zirconium and iron, which contributes for its greater radiopacity in relation to the other materials tested. Aoyagi et al.[20] reported that radiopacity increased with the increase in radiopaque material content as well as the increase in atomic number of the element. We first hypothesized that the more radiopacity the resin-based root canal sealer shows, the more cytotoxicity would be presented. However, there was no statistical correlation between these two factors. This implies not only the content of radiopacifier but also other components contained in resin-based sealers contributed the cytotoxicity.

Sealapex presented radiopacity values slightly lower than those defined by the ISO. Kuga, et al.[21] suggested the addition of iodoform to Sealapex, with the purpose of increasing the radiopacity. This addition did not affect the sealing properties or biocompatibility of materials.

In this present study, resin-based sealers showed lower cytotoxic effect compared to zinc oxide-eugenol and calcium hydroxide-based sealers. Azar et al.[15] reported that AH 26 was found to be highly toxic immediately after mixing, followed by a substantial decrease in cytotoxicity and this was due to formaldehyde. In the present study, however, pre-set sealers were applied to the cells and this might attribute to the low cytotoxicity.

In this study, zinc oxide-eugenol-based sealer, ZOB Seal, showed severe cytotoxicity throughout all experiment time. Many studies have investigated the biocompatibility of zinc oxide and eugenol-based root canal sealers[22-26]. Their cytotoxicity, however, is attributed to the eugenol, due to hydrophobic interactions with the cytoplasmic membrane[27] or its effect on cell respiration[28]. Moreover, the zinc oxide contained in the root canal sealer is also dissociated when eugenol is released by hydrolysis[29]. Maseki et al.[30] suggested that the toxicity may also be related to methyl salicylic acid, benzyl alcohol, zinc ions, rosin and other components released form the sealer. In a study by Valle et al.[31], however, it was shown that the liquid to powder ratio is directly related to the toxicity of a zinc oxide and eugenol-based sealer. A higher ratio of liquid results in a more cytotoxic sealer. This implies that eugenol is by far the most toxic component.

Sealapex, a calcium hydroxide-based sealer containing neither eugenol nor formaldehyde, displayed the highest cytotoxicity. This seemed to be related to the method of applying the material to the cells. In the present study, the insert wells having a membrane pore diameter of 0.4 ㎛ were used according to the method of Koulaouzidou et al.[32]. Schafer and Zandbiglari[33] reported that calcium hydroxide-based root canal sealer has high solubility compared with resin or zinc oxide-eugenol. This contributed to release the ionic component from the material and subsequently lead to cytotoxicity. Further, these wells brought the tested materials in close proximity to the cells without interfering with the methods used for evaluating cell numbers, proliferation, and death.

Ⅴ. CONCLUSIONS

Taken together, all sealers tested radiographically in this study presented the minimum radiopacity by this standard, except for Sealapex. In terms of cytotoxicity, resin-based sealers showed higher biocompatibility than other types of sealers. Further, EZ fill showed statistically lower cell viability compared to AD Seal in 24 and 48 hours and compared to other resin-based sealers in 72 hours. However, this result was not correlated with their radiopacity. In conclusion, these results indicate that resin-based root canal sealer is more biocompatible and has no disadvantage in terms of radiopacity.

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국문초록

레진계 근관충전실러의 방사선 불투과성 및 세포 독성에 대한 평가

김창규[1] · 류현욱[2] · 장훈상[2] · 이병도[3] · 민경산[2] · 홍찬의[1]

[1]단국대학교 치과대학 치과보존학교실, [2]원광대학교 치과대학 치과보존학교실, [3]구강악안면 방사선학교실

본 연구의 목적은 세 가지 레진계 근관충전실러 (AH 26, EZ fill, AD Seal), 산화아연 유지놀계 근관충전실러 (ZOB Seal) 그리고 수산화칼슘계 근관충전실러 (Sealapex)의 방사선 불투과성 및 세포독성을 평가한 것이다. 각 실러를 제조회사의 지시대로 혼합하여 직경 10 ㎜, 두께 1 ㎜로 시편을 제작한 후 ISO 6876/2001의 규격에 따라 교합필름을 이용하여 알루미늄 스텝웨지와 함께 방사선 촬영을 시행하였다. 방사선 사진을 디지털화하여 컴퓨터에 저장한 후 Scion image 프로그램을 이용하여 각 단계의 알루미늄 스텝웨지의 두께와 비교하였다. 각 재료의 세포독성은 불멸화된 인간 치주인대세포 (immortalized human periodontal ligament cell, IPDL)에서 MTT 분석법을 이용하여 시행하였다.

EZ fill이 가장 높은 방사선 불투과성을 나타내었고 Sealapex가 가장 낮은 방사선 불투과성을 나타내었다 ($p < 0.05$). AH 26, AD Seal, ZOB Seal은 중등도의 방사선 불투과성을 나타내었다. Sealapex를 제외한 모든 평가된 재료는 ISO 규격에 부합하는 방사선 불투과성을 보였다. 레진계 실러의 세포독성은 모든 실험 시간대에 걸쳐 다른 계통의 실러에 비해 낮게 나타났다 ($p < 0.05$). 아울러, EZ fill은 24 및 48시간대에서는 AD Seal에 비해, 72시간대에서는 다른 두 레진계 실러에 비해 높은 세포독성을 보였다. 그러나 레진계 실러에서 방사선 불투과성의 정도와 세포독성과의 관련성은 없었다 ($p > 0.05$).

이 실험 결과로 볼 때 레진계 실러는 다른 계통의 실러에 비해 방사선 불투과성 면에서 장점을 가지며 생체적합성 면에서 우수하다고 사료된다.

주요어: 방사선 불투과성, 세포독성, 근관충전실러, MTT 분석법, 알루미늄 스텝웨지, 불멸화된 인간 치주인대세포