Evaluation of the biocompatibility of resin-based root canal sealers in rat periapical tissue

Noriko MUTOH, Takenori SATOH, Hirotaka WATABE and Nobuyuki TANI-ISHII

Department of Oral Medicine, Division of Endodontics, Kanagawa Dental University, 82 Inaoka-cho, Yokosuka 238-8580, Japan

Corresponding author, Nobuyuki TANI-ISHII; E-mail: nobu0337@kdcnet.ac.jp

We evaluated the biocompatibility of resin-based root canal sealers (RCSs) in the periapical tissues of rats. Wistar rats underwent tooth replantation for reproducing the response of periapical tissue with RCSs. The resin-based Epiphany SE™, AH Plus Jet™, the eugenol-based sealer (Canals) and a control group were employed. The upper right first molar was extracted and applied with RCSs on apices, and then the tooth was repositioned. Histological evaluation demonstrated that mild inflammation occurred in the periapical tissue with Epiphany and AH Plus Jet sealers on day 7, whereas Canals induced severe-to-moderate inflammation. The statistical analyses demonstrated that the significant differences were observed between Canals and the other groups on day 7 regarding inflammatory response. On day 14, the lesions induced by all sealers were healed and replaced predominantly by fibrous connective tissue. Our results suggest that Epiphany SE and AH Plus Jet are good biocompatible materials.

Keywords: Biocompatibility, Endodontic sealer, Tooth replantation, Periapical tissue response

INTRODUCTION

Biocompatibility is an important factor as well as physical and chemical features for selecting materials for endodontic therapy because of the direct contact of materials with periapical tissue¹. Root canal sealers (RCSs) may be extruded through the apical foramen. Hence, they may remain in the periapical tissue for a prolonged period, thereby making the biocompatibility of RCSs an important factor in healing^{2,3}.

Resin-based RCSs such as Epiphany SE™ or AH Plus Jet™ have been introduced to replace conventional eugenol-based sealers under the premise of improving clinical performance and eliciting satisfactory tissue reactions⁴⁻⁷. The biocompatibility of methacrylate resin-based Epiphany SE and epoxy resin-based AH Plus Jet has been evaluated. First-generation Epiphany was shown to induce bone formation and cause a slight osseous inflammatory reaction in the *in vivo* experiment⁸. The *in vitro* experiments demonstrated that Epiphany in freshly mixed or set conditions showed severe-to-moderate cytotoxicity⁹, and its cytotoxicity increased with time, posing significant risks^{10,11}. The other *in vitro* studies tested the effect of Epiphany on human fibroblasts and L929 cells, and showed its strong cytotoxicity on these cells^{12,13}. However, Epiphany showed no genotoxic potential upon human leukocytes, although it induced apoptosis in >90% of cells¹². The *in vitro* study by Baraba *et al.* showed no genotoxic potential for Epiphany on human leukocytes¹⁴. However, the *in vivo* experiments regarding Epiphany SE, the latest generation of methacrylate resin-based sealers, have not been thoroughly conducted.

Numerous *in vitro* studies have been carried out on the biocompatibility of AH Plus. Some studies^{15,16} reported that its cytotoxicity was not appreciable, whereas other studies¹⁷ demonstrated cytotoxicity and genotoxicity: AH Plus Jet was shown to exhibit severe toxicity until 96 h, and subsequently toxicity decreased gradually over 1 week. AI-Hiyasat *et al.* showed that AH Plus was the most biocompatible material in mouse fibroblasts for 48 h according to the MTT assay ([3-4,5-Dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide)¹⁸. Furthermore, the intraosseous implantation of endodontic materials into the tibia⁹ and the application of RCSs have been carried out on large animals such as dogs^{19,20}. These *in vivo* studies demonstrated that AH Plus has a biologic potential.

Epiphany has been shown to induce satisfactory tissue reactions²¹, whereas AH Plus elicits severe tissue responses in the *in vivo* experiment²². Both of these RCSs have been approved for endodontic use by the US FDA (Food and Drug Administration). However, there are few *in vivo* studies evaluating their biocompatibility in the periapical tissue. Thus, we aimed to evaluate the effects of the extrusion of resin-based RCSs such as Epiphany SE and AH Plus Jet and their biocompatibility in the periapical tissue using a recently established animal model for tooth replantation²³.

MATERIALS AND METHODS

Animal experiments

All animal procedures conformed to the protocols reviewed and approved by the Ethics in Animal Research Committee of Kanagawa Dental University (Yokosuka, Japan). These procedures complied with the International Guiding Principles for Biomedical

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Table 1 Composition of the sealers and their manufactures

Sealers	Materials	Composition
Epiphany SE	Metacrylate resine	HEMA, Bis-GMA, Calcium phosphate, 2,2-Bis(4-metacryloxypolyethovpyphenyl) propane, Barium borosilicate glass, Bismuth oxychloride, Silica, Allyl thiourea, Benzoyl peroxide, Photo-initiator, Stabilizers and pigment
AH Plus Jet	Epoxy resine	Bisphenol-A and -F epoxy resins, Calcium tungstate, Zirconia oxide, Silica, Iron oxide pigment, Amines, Silicone oil
Canals	Zinc Oxide Eugenol	ZnO, Rosine, Bismuth subcarbonate, Barium sulfate Eugenol, Penuts oil

Research Involving Animals (Geneva, 1985).

Forty female Wistar rats (4 weeks old; weights are less than 90 g) were used. Thirty rats were used for the experimental group of Epiphany SE (Pentron Clinical Technologies, Wallingford, CT, USA), AH Plus Jet (Dentsply/Detrey, Konstanz, Germany) and Canals (Showa Yakuhin Kako, Tokyo, Japan). The composition of the three RCSs is demonstrated in Table 1. Ten rats were used as the control group. Under anesthesia (chloral hydrate 350 mg/kg, i.p.), the upper right first molar (M1) was extracted with a pair of Howe pliers (YDM Company Limited, Tokyo, Japan) or forceps with hooks. We used only upper right molars to keep the influence of occlusal trauma to a minimum, since the occlusal trauma elicits the pathological healing patterns in the pulp cavity of the replanted tooth²⁴. Bleeding from the M1 alveolar socket was inhibited with a sterilized paper point (Pierce Company Limited, Tokyo, Japan). We used Epiphany SE, AH Plus Jet, and Canals according to manufacturer instructions. Ten microliters of these mixed materials were applied to each of the five root apices of M1 (Fig. 1) using a 27-G needle (Terumo Company Limited, Tokyo, Japan) attached to a 1-mL plastic syringe²³. The extracted tooth with materials was immediately repositioned in the original socket after the confirmation of hemostasis; these were classified as the “experimental groups”. The replanted tooth did not undergo further treatments according to the method of a previous report^{25,26}.

Tissue preparation

Samples were collected from 5 rats in each group and stage at intervals of 7 and 14 days after tooth replantation (5 samples in each×2 periods×4 groups=40 samples). At each stage, rats were anesthetized and perfused *via* the transcardial route with physiological saline followed by 4% paraformaldehyde in 0.1 M phosphate buffer (pH 7.4). Maxillae were removed *en block* and immersed in the same fixative for an additional 12 h. After decalcification in a 4% solution of ethylenediamine tetra-acetic acid disodium salt for 4 weeks at 4°C, the samples were embedded in paraffin. Specimens for histological analyses were cut in the sagittal plane at a thickness of 5 µm, and the sections were stained with hematoxylin and eosin (H&E).

Fig. 1 Animal model for tooth replantation to evaluate the biocompatibility of endodontic sealers.

(a) The alveolar socket of the upper right first molar (M1). (b) M1 possesses five roots. (c) Ten microliters of the mixed material are applied to each of the five root apices of M1. (d) Extracted M1 is repositioned in the original socket after hemostasis has been confirmed.

Histomorphological parameters

The histomorphological parameters used in this study were based upon previously described criteria^{23,25,26}. For the determination of scores, sections were evaluated according to four parameters. The first parameter was inflammatory reactions: 0, none or few inflammatory cells and no reaction; 1, <25 inflammatory cells and a mild reaction; 2, 25–125 inflammatory cells and a moderate reaction; 3, >125 inflammatory cells and a severe reaction. Quantitative evaluations were done in the micrographs taken from ten areas of sections at ×400 magnification. The second parameter was cementum resorption: absent or present. The third parameter was dentin resorption: absent or present. The final parameter was the thickness of the periodontal ligament at the apical region: 0, normal; 1, slightly increased;

2, moderately increased; 3, severely increased. The observer was blinded to the treatment allocation. We selected the most appropriate section (one section from each sample), where the apical foramen is most widely open to the periodontal ligament, from each sample for the histomorphological analysis.

Statistical analyses

Statistical analyses of the histopathological parameters described above were carried out with a Bonferroni's test (one-way analysis of variance; ANOVA) using statistical software (SPSS 16.0J for Windows; SPSS Japan, Tokyo, Japan). $P < 0.05$ was considered significant.

RESULTS

Histological findings are demonstrated in Table 2. The control group showed a slight inflammatory response (mild was 20%), no resorption of dentin, little resorption of cementum (20%), and a moderate increase in the periodontal ligament space on day 7; subsequently, no inflammatory response, little resorption of dentin (20%), little resorption of cementum (20%), and little increase

(slight increase was 20%) in the periodontal space were observed on day 14. The Epiphany SE group showed a slight inflammatory response (mild was 20%), no resorption of dentin, little resorption of cementum (20%), and a moderate increase in the periodontal ligament space on day 7; subsequently, no inflammatory response, little resorption of dentin (20%), little resorption of cementum (20%), and a slight increase (slight increase was 80%; moderate increase was 20%) in the periodontal space were seen on day 14. The AH Plus Jet group showed a slight inflammatory response (mild was 20%, little resorption of dentin (20%), little resorption of cementum (20%), and a moderate increase in the periodontal ligament space on day 7; subsequently, a slight inflammatory response, little resorption of dentin (20%), little resorption of cementum (20%), and a slight increase (slight increase was 80%; moderate increase was 20%) in the periodontal space were observed on day 14. The Canals group showed a moderate inflammatory response (moderate was 80%; severe was 20%), little resorption of dentin (20%), little resorption of cementum (20%), and a severe increase in the periodontal ligament space on day 7; subsequently, a slight inflammatory response (mild was

Table 2 Histopathologic findings in each experimental group

Finding	Day 7				Day 14			
	Control	Epiphany SE	AH Plus Jet	Canals	Control	Epiphany SE	AH Plus Jet	Canals
Inflammatory response in periapical tissue (n=5)								
Absent (n)	4	4	4	0	5	5	4	4
Mild (n)	1	1	1	0	0	0	1	1
Moderate (n)	0	0	0	4	0	0	0	0
Severe (n)	0	0	0	1	0	0	0	0
Dentin resorption (n=5)								
Absent (n)	5	5	4	4	4	4	4	4
Present (n)	0	0	1	1	1	1	1	1
Cementum resorption (n=5)								
Absent (n)	4	4	4	4	4	4	4	4
Present (n)	1	1	1	1	1	1	1	1
Apical periodontal ligament space (n=10)								
(apical to bone surface)								
Normal (n)	0	0	0	0	4	0	0	0
Slightly increased (n)	0	0	0	0	1	4	4	4
Moderately increased (n)	5	5	5	0	0	1	1	1
Severely increased (n)	0	0	0	5	0	0	0	0

* $p < 0.05$

20%), little resorption of dentin (20%), little resorption of cementum (20%), and a slight increase (slight increase was 80%; moderate increase was 20%) in the periodontal space were seen on day 14. The statistical analyses demonstrated that the significant differences were observed between Canals and the other groups on day 7 regarding inflammatory response in periapical tissue and apical periodontal space. All experimental groups showed significant differences compared with control group regarding apical periodontal space on day 14.

Epiphany SE

On day 7, no or mild infiltration of inflammatory cells were observed in dense fibrous tissues with numerous blood vessels (Fig. 2a). By day 14, inflammatory cells almost disappeared and the periapical tissue recovered, showing normal periodontal tissue (Fig. 2b).

AH Plus Jet

On day 7, no or mild infiltration of inflammatory cells was observed in dense fibrous tissues with numerous

Fig. 2 Histological images.

(a) Epiphany SE on day 7. Mild infiltration of inflammatory cells. (b) Epiphany SE on day 14. Numerous fibrous tissues and the formation of blood vessels. (c) AH Plus Jet on day 7. Mild infiltration of inflammatory cells. (d) AH Plus Jet on day 14. Numerous fibrous tissues and the formation of blood vessels. (e) Canals on day 7. Moderate infiltration of inflammatory cells (PMNs, macrophages and lymphocytes) are observed in the numerous fibrous tissue. (f) Canals on day 14. Numerous fibrous tissues and the formation of blood vessels. (g) Control on day 7. Slight infiltration of inflammatory cells in the fibrous tissue. (h) Control on day 14. Numerous fibrous tissue and the formation of blood vessels.

Fig. 3 H&E stained section in the Canals group on day 7. Amorphous materials (arrows) are recognized in the periodontal ligament (PDL). D: dentin, DP: dental pulp.

blood vessels (Fig. 2c). By day 14, inflammatory cells almost disappeared and the periapical tissue recovered, showing normal periodontal tissue (Fig. 2d).

Canals

On day 7, moderate infiltration of inflammatory cells (PMNs, macrophages, and lymphocytes) was observed in dense fibrous tissues with numerous blood vessels (Fig. 2e). Amorphous materials were recognizable in the periodontal ligament (Fig. 3). By day 14, inflammatory cells disappeared, and the periapical tissue showed normal periodontal tissue (Fig. 2f).

Control

On day 7, no or mild infiltration of inflammatory cells was observed in fibrous tissue with numerous blood vessels (Fig. 2g). On day 14, periapical tissue showed normal periodontal tissue (Fig. 2h).

DISCUSSION

We clearly demonstrated the biocompatibility of Epiphany SE and AH Plus Jet by analyzing the response of periapical tissue to RCSs using tooth replantation. The establishment of the *in vivo* animal model is considerably important for precisely evaluating the biocompatibility of RCSs. Our recently established animal model using small animals such as rats has several advantages compared with the previous animal model using big animals such as dogs. First, the systematic analyses are feasible due to the use of many samples and the minimum of an individual difference. Second, the moisture contamination of the apical area can be controlled appropriately and the responses of the periapical tissue can be evaluated quantitatively by histological observation, since the procedure of tooth

replantation enables the root apices with endodontic materials to be sequestered immediately after its reposition. In contrast, the unset materials such as slow-setting sealers implanted into a freshly prepared bone cavity are susceptible to be partially displaced by tissue fluids²⁷⁾, although intraosseous models have been used as an appropriate environment for *in vivo* testing of endodontic material^{26,28-30)}. Third, our model mimics the clinical situation that the extrusion of RCSs contacts with the periapical tissue through the apical foramen. Our system could assess the biocompatibility of RCSs in the periapical tissue, although the procedure of the application of RCSs is different from the clinical procedure to remove pulp tissue before applying the endodontic materials.

Another important point is to evaluate the effect of surgery itself on tissue responses. The histological analysis of the tissue reaction in the control group showed that the surgical trauma caused by tooth replantation itself induces a slight inflammation on day 7, and subsequently an inflammatory reaction disappears until day 14, although dentin resorption, cementum resorption, and/or increased apical periodontal ligament space may be observed on day 14. Thus, the comparison between the control and experimental groups is essential to analyze the histomorphological parameters for the precise evaluation of tissue responses, although parametric tests are inadequate for the evaluation of scores.

There are no data on the histological analyses of periapical tissue responses to endodontic materials except for the use of large animals such as dogs in which the periapical tissue responses to materials could be assessed after preparation of the root canal. Our experimental animal model showed precisely the effect of RCSs on the periapical tissue. The zinc oxide-eugenol (ZOE)-based sealer Canals was used as a positive control in the present study, showing severe-to-moderate inflammation with the significant difference demonstrated by the score of inflammatory reactions. Since it is classified as a “slow-setting material”, the unset material implanted into the periapical tissue would probably have been partially dispersed by tissue fluid until 7 days after application. Actually, amorphous materials remained in the periapical lesions in this study (Fig. 3). Thus, our animal model simulated the periapical tissue response to the ZOE sealer which overflows from the root apex before obturation of the root canal. Regardless of the presence of ZOE components, the inflammatory reaction was recognized during the early stages after the procedure because of the surgical trauma mentioned above. Good biocompatibility was shown in the periapical tissue reaction to the RCSs.

In the present study, neither resin-based RCSs demonstrated a severe inflammatory reaction compared with the reactions of the positive control Canals 7 days after application. When using Epiphany SE and AH Plus Jet, the periapical area was filled with well-organized granulation tissue with numerous fibroblasts and blood vessels. A moderately increased periodontal ligament

space from the root apices to the bone surface was also observed in the control on day 7. Good biocompatibility was shown in the periapical tissue reaction to both resin-based RCSs which were set by day 14. Few authors have investigated the cytotoxicity of the self-adhesive RCSs such as Epiphany SE and AH Plus Jet. Ames *et al.* has demonstrated that Real Seal SE, a self-adhesive sealer that is compositionally similar to Epiphany SE, is less toxic than Real Seal³¹. The cyto-compatibility of self-adhesive (without a separate priming step) methacrylate resin-based sealers may be comparable or better than those of previous generation non-etching resin sealers. The recent advancement of bonding technology may be the development to reduce leakage and infection. The first generation of Epiphany showed highly cytotoxic effects^{9,10} and induced inflammatory reactions²¹. Furthermore, the Epiphany and Resilon systems induced a slight inflammatory reaction after 42 days. The toxicity of Epiphany might be explained by the presence of unpolymerized hydrophilic monomers that can readily diffuse into the cell culture medium³² and elicit a significant toxicity³³. The second generation of Epiphany (Epiphany SE) is a non-etching methacrylate with an acidic resin monomer that is originally found in primers that have been incorporated into self-adhesive resin-based sealers³⁴. The Epiphany SE is the latest generation of methacrylate resin-based sealers, and one of its components is HEMA, which is known to be cytotoxic even at low concentrations³⁵. In this experiment, the Epiphany SE group showed a moderate inflammation on day 7, although it showed little inflammation on day 14. Epiphany SE has been shown to have cytotoxic effects immediately after mixing, which toxicity decreased gradually over 1 week. AH Plus and AH Plus Jet also have highly cytotoxic effects^{15,16,36}. The cytotoxicity of AH Plus is thought to arise from the release of bisphenol A; bisphenol A has been identified as a mutagenic component of materials, and might also be cytotoxic³⁷. The release of minute amounts of formaldehyde from AH Plus may contribute to cytotoxicity in the early phase of setting sealers³⁸. AH Plus has been shown to have highly cytotoxic effects immediately after mixing as well as after 24 and 48 h setting periods in contact with cells, and subsequently the toxicity decreased gradually over 1 week^{17,18}. Our histological findings revealed that Epiphany SE and AH Plus Jet are good biocompatible materials with respect to tissue responses in rat periapical tissues. These resin-based RCSs exhibited similar patterns of infiltration of inflammatory cells compared with the control group after long experimental periods.

In conclusion, the findings in the present study support the favorable clinical results observed using resin-based RCSs. The experimental animal model that we used to evaluate the response of periapical tissue to endodontic materials was not entirely comparable with the response elicited by human tissue. Under carefully standardized conditions, this model could be used to simulate the inflammatory process induced by iatrogenic means during obturation of the root canal

in humans, and could therefore be an effective tool to study several aspects of the periapical tissue reaction. The results showed that the tested Epiphany SE and AH Plus Jet sealers induced a mild inflammatory response in the periapical tissue. Those RCSs could possess better tissue compatibility compared with eugenol sealers.

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