Original

Cytotoxicity evaluation of methacrylate-based resins for clinical endodontics *in vitro*

Esteban G. Garza[1], Aniket Wadajkar[2], Chul Ahn[3], Qiang Zhu[4], Lynn A. Opperman[5], Larry L. Bellinger[5], Kytai T. Nguyen[2] and Takashi Komabayashi[6]

[1]Community Dental Care Clinic, Dallas, TX, USA
[2]Department of Bioengineering, University of Texas Arlington, Arlington, TX, USA
[3]Department of Clinical Science, University of Texas Southwestern Medical Center at Dallas, Dallas, TX, USA
[4]Division of Endodontology, University of Connecticut Health Center, Farmington, CT, USA
[5]Department of Biomedical Sciences, Texas A&M Health Science Center, Baylor College of Dentistry, Dallas, TX, USA
[6]Department of Endodontics, Texas A&M Health Science Center, Baylor College of Dentistry, Dallas, TX, USA

Abstract: This study examines the cytotoxicity of Super-Bond C&B (SB-C&B), Super-Bond RC Sealer (SB-RC), MetaSEAL (Meta), and AH Plus Sealer (AH+). Freshly mixed and set materials (100 mg) were prepared *in vitro* and placed in cell culture medium (1 mL) for the working time and for 6 h, respectively. L929 cells seeded into 96-well plates at 5,000 cells/well were incubated with the eluted medium (200 μL) for 24 h. Cells cultured with medium alone served as the control. Cytotoxicity was evaluated by MTS assay and analyzed with ANOVA. In the freshly mixed group, the average ± SD (%) for cell viability were 66.0 ± 13.6, 55.5 ± 15.6, 10.6 ± 0.7, and 8.9 ± 2.2 for SB-C&B, SB-RC, Meta, and AH+, respectively. In the set group, the average ± SD (%) for cell viability were 100 ± 21.9, 81.8 ± 38.5, 24.9 ± 7.9, and 23.6 ± 10.0 for SB-C&B, SB-RC, Meta, and AH+, respectively. SB-C&B and SB-RC are less cytotoxic than are Meta and AH+. (J Oral Sci 54, 213-217, 2012)

Correspondence to Dr. Takashi Komabayashi, Department of Endodontics, Texas A&M Health Science Center Baylor College of Dentistry, 3302 Gaston Avenue, Dallas, TX 75246 USA
Tel: +1-214-828-8365
Fax: +1-214-874-4507
E-mail: tkomabayashi@bcd.tamhsc.edu & ICD38719@nifty.com

Introduction

Over 22 million root canal treatments are performed each year in the United States (1). Sealers in combination with a solid or semi-solid core material are used to fill voids and to seal canals during obturation. The apical seal, like the coronal seal, is critical for long-term clinical success (2). Some studies report the apical seals of resin-based sealers to be superior to those of other sealers (3,4). Thus, various resin sealers have been commercialized for endodontic applications (5-8).

Among resin sealers, the epoxy resin sealer, AH Plus (AH+), is conventionally used (9). Recently, two methacrylate resin sealers have been introduced. One is the methacrylate/sulfinate salt (MA/SS)-based resin sealer, MetaSEAL (Meta). The other is methyl methacrylate/tributylborane (MMA/TBB)-based resin sealer, Super-Bond RC Sealer (SB-RC), which was developed to address Super-Bond C&B (SB-C&B) resin cement's drawbacks: short working time, low radiopacity, and difficult removal of the material from the canal (10). The polymer components of SB-RC are poly(methyl methacrylate) (PMMA) and zirconium oxide, while that

of SB-C&B is PMMA.

Materials for endodontic applications must not only provide good seals, but also exhibit excellent biocompatibility, because cytotoxicity of tissues is of concern when materials are involved with human tissue (11). Although biocompatibility is one of the factors that influence the clinician's choice of sealers in root canal therapy (12,13), cytotoxicity studies of methacrylate-based resins in fresh and set conditions are limited.

The purpose of this study therefore was to examine the cytotoxicity of SB-C&B, SB-RC, Meta, and AH+. L929 cells were used due to their sensitivity to toxic products, which explains their frequent use in the evaluation of root canal sealers (14).

Materials and Methods

Materials

The resin cement and three root canal sealers tested in this study were 1) Super-Bond C&B (Sun Medical Co., Shiga, Japan) (in the United States, C&B Metabond [Parkell, Edgewood, NY]), 2) Super-Bond RC Sealer (Sun Medical, Japan), 3) MetaSEAL (Parkell, Edgewood, NY) (In Japan, Hybrid Root SEAL [Sun Medical Co., Shiga, Japan]), and 4) AH Plus (Dentsply DeTrey, Konstanz, Germany). Lot numbers, working lengths, setting times, and classifications are summarized in Table 1.

L929 mouse fibroblasts were obtained from American Type Culture Collection (ATCC, Manassas, VA, USA). Cells were grown in Dulbecco's Modified Eagle Medium (DMEM) (Invitrogen, Grand Island, NY, USA), supplemented with 10% fetal bovine serum (Hyclone Laboratories Inc., Logan, UT, USA), and 1% penicillin-streptomycin (Gibco BRL, Gaithersburg, MD, USA) under standard cell culture conditions (37°C and 5% CO_2).

Methods

The cytotoxicity of the four materials was tested for both fresh and set sealer samples. L929 cells were seeded into 96-well plates at 5,000 cells/well and incubated for 24 h to allow adhesion for the cell cytotoxicity assay.

Samples of freshly (immediately) mixed materials were placed into 24-well plates at approximately 0.1 g/well. The fresh materials were mixed according to the manufacturers' instructions. Then 1 mL cell culture medium was added to the wells. After the required working time (specified in Table 1), the medium was removed and added (200 μL) to the cells prior to their 24-h incubation.

To obtain the set materials, samples of the different materials (setting time specified in Table 1) were placed into a 24-well plate at approximately 0.1g/well and incubated for 24 h in the cell culture incubator to allow for setting. Then 1 mL of the cell culture medium was added to the set materials and left for 6 h. Every hour, wells were shuffled for 5 s. 200 μL of the material eluate from the different eluate groups was added to the cell culture wells.

In both the fresh and set material groups, after an incubation period of 24 h at 37°C, 200 μL of MTS reagent was added to the cell culture wells, and cell viability was evaluated according to the manufacturer's instructions. Cells cultured with the medium only served as the control group (100%). Each experiment was repeated four times.

Data analysis and statistics

An ANOVA test was conducted to determine whether there were significant differences in cell viability between the experimental and the control groups, and Student's t-tests were conducted to identify which material was significantly different from control with Dunnett's correction.

Results

When cells were cultured with the eluates of the fresh materials, the average ± SD values (%) for biocompatibility were 66.0 ± 13.6, 55.5 ± 15.6, 10.6 ± 0.7, and 8.9 ± 2.2 for SB-C&B, SB-RC, Meta, and AH+, respectively. The results for Meta ($P = 0.0024$) and AH+ ($P = 0.0005$) showed statistical significance when compared with the control (Fig. 1). The cell viability in the Meta and AH+ groups was significantly less than that in the SB-C&B and SB-RC groups ($P < 0.05$). There was no statistically significant difference between SB-C&B and SB-RC, or between Meta and AH+.

When cells were cultured with eluate of the set materials, the average ± SD values (%) for biocompatibility were 100 ± 21.9, 81.8 ± 38.5, 24.9 ± 7.9, and 23.6 ± 10.0 for SB-C&B, SB-RC, Meta, and AH+, respectively (Fig. 2). The viability of cells cultured with SB-C&B and SB-RC eluates was significantly higher than that of cells cultured with eluates of Meta and AH+ ($P < 0.05$). There was no statistically significant difference between SB-C&B and SB-RC, and between Meta and AH+. The cell viability in the SB-C&B and SB-RC groups was significantly higher than that in the Meta and AH+ groups ($P < 0.05$).

Discussion

In our study, AH plus was found to be the most cytotoxic of the sealers. AH plus is a two-component system consisting of two pastes. The epoxide paste contains
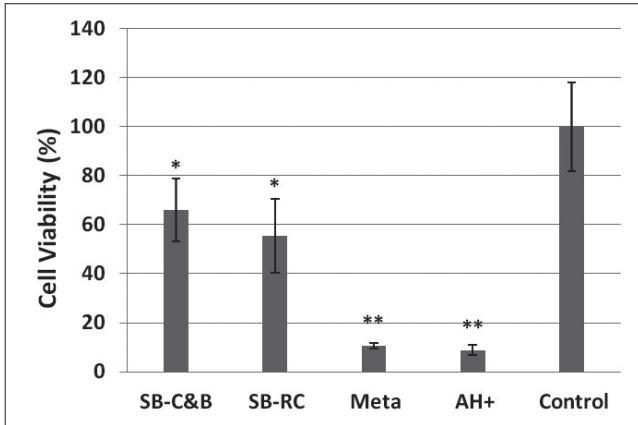
Fig. 1 Cell viability of L929 cells after culture with elute of fresh materials.
*Significant difference from the control, Meta and AH+ groups. **Significant difference from the control, SB-C&B and SB-RC groups.
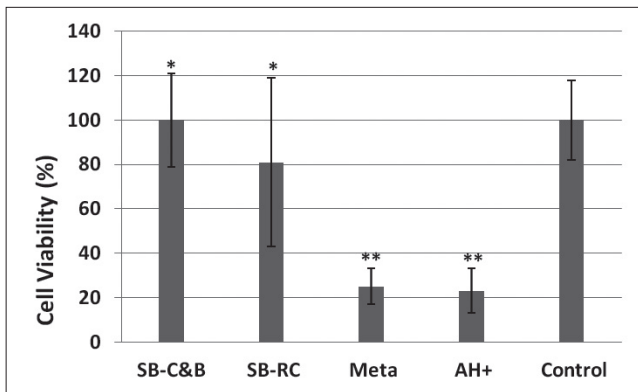

Fig. 2 Cell viability of L929 cells after culture with elute of set materials.
*Significant difference from the control, Meta and AH+ groups. **Significant difference from the control, SB-C&B and SB-RC groups.

diepoxide. The amine paste contains 1-adamantane amine, T N,N'-dibenzyl-5-oxa-nonandiamine-1,9, and TCD-diamine. When the two pastes are mixed, the poly addition reaction starts immediately. The amines polymerize with the diepoxide to co-polymers. The un-polymerized monomers which were eluted to the medium during setting cause the cytotoxic effect. AH 26 releases formaldehyde and exhibits cytotoxicity (15-20). Comparing with AH 26 only a minimum release of form-aldehyde from AH plus was observed (16). AH 26 and AH plus both demonstrated cytotoxicity in rat cerebral astrocyte cell culture (21). AH plus was more cytotoxic than AH 26 in human cervical carcinoma cells and mouse skin fibroblasts (22). AH Plus has shown to be cytotoxic in the first three days and does not reach non-cytotoxicity until 3 to 5 weeks (23). The results of the present

experiment are in agreement with the previous studies. However, studies have shown AH plus exhibiting close to no cytotoxicity (24,25). These differences may due to the differences in experimental conditions and methods.

Meta was found to be very cytotoxic, especially when fresh. These results have been found in similar studies on the cytotoxic effects of Meta (23,26). Meta's monomer components include 4-methacryloxyethyl trimellitate anhydride (4-META) and hydroxyethyl methacrylate (HEMA). Based on 50% inhibition of cell growth, the relative cytotoxicity of the monomers MMA : 4-META : HEMA was calculated as 1:6:7 (27,28). It is thought that HEMA diffuses through dentine to cause cellular damage (29,30). HEMA also induces cell growth inhibition and cycle perturbation as well as glutathione depletion and reactive oxygen species production (31).

HEMA has been reported to inhibit intracellular tyrosine phosphorylation in L929 cells (32). These are reasonable explanations for our cytotoxicity results for Meta.

The main monomer component of SB-C&B and SB-RC is MMA, which has a low potential for pulp irritation compared with other poly-functional methac-rylate monomers (27,33). The polymerization initiator of SB-C&B and SB-RC is TBB. Tronstad et al. studied pulp responses to composite resin (Concise) and MMA-TBB-based resin (Polycap) in deep Class V cavities in monkeys after 8 days (34). In composite resin, 30% of responses were slight; 50%, moderate; and 20%, severe. In MMA/TBB-based resin, 75% of responses were slight; 25%, moderate; and 0%, severe. Overall, the MMA/TBB-based resin had lower cytotoxicity than did the composite resin. No severe response to the MMA/TBB-based resin was reported.

The success of MMA/TBB-based resin in endodontic applications can be attributed to several material proper-ties. MMA is the least cytotoxic among the monomers used in dentistry (27,35,36). Also, the residual MMA is low after setting and decreases with time because of the presence of TBB (37). Finally, the polymerization at the dentin interface enables reliable sealing of the interface due to the presence of TBB (38).

Several studies have indicated that cytotoxic effects in cell culture can be caused by released monomers (39,40). The curing of resin-based cement is usually not complete, so unconverted monomers can be released from the resin into an adjacent aqueous phase and can diffuse through dentin to the pulp space. In short, before setting, the cyto-toxicity of the monomer itself will affect the pulp, while after setting, the residual effect of the monomer will depend on amount and elution kinetics. In this regard, in

addition to the low cytotoxicity of the MMA in SB-C&B and SB-RC, the TBB initiator significantly reduced the residual MMA during the course of the resin's setting (37). Our data are consistent with those of a similar study that found minimal cytotoxic effects with SB-RC (41). Although the results of our *in vitro* study are significant and offer good clinical information, it is necessary to confirm the *in vitro* results with future *in vivo* studies.

This study demonstrates that the MMA/TBB resins SB-C&B and SB-RC are less cytotoxic than are Meta and AH+. While excellent sealing of the canal has been reported with MMA/TBB resins (10,17), SB-C&B is not suitable for root canal use due to its poor handling properties. Therefore, among all tested materials, SB-RC's low cytotoxicity and excellent sealing of the canal at the resin-dentin interface recommend it for clinical use.

Acknowledgments

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