Cytotoxicity evaluation of Gutta Flow and Endo Sequence BC sealers

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Objective. This study evaluated the cytotoxicity of GuttaFlow and EndoSequence BC sealers and compared them with AH Plus and Tubli-Seal sealers.

Study design. Samples (0.5 mg) of freshly mixed or set BC, GuttaFlow, AH Plus, and Tubli-Seal sealers were eluted with 300, 600, and 1,000/L cell culture medium for 24 and 72 hours. L929 cells were seeded into 96-well plates at 3/10⁴ cells/well and cultured with 100/L eluate from each eluate group. Cells cultured only with culture medium served as control. After 24 hours’ incubation, the cytotoxicity was evaluated by MTT assay. Cell viability was calculated as the percentage of the control group, and the results were analyzed with a one-way analysis of variance.

Results. For the freshly mixed sealer, cell viability in the AH Plus group was less than in all of the other 3 sealer groups. The Tubli-Seal sealer group had less cell viability than the EndoSequence BC and GuttaFlow sealer groups. For the set sealer, the Tubli-Seal and AH Plus groups had less cell viability than the EndoSequence BC and GuttaFlow sealer groups. There was no cell viability difference between the EndoSequence BC and GuttaFlow sealer groups in the either freshly mixed or set sealer group.


EndoSequence BC sealer has recently been developed to improve the seal of root canal filling (Brasseler, Savannah, GA). According to the manufacturer, it is composed of zirconium oxide, calcium silicates, calcium phosphate monobasic, calcium hydroxide, filler, and thickening agents. BC sealer is a premixed ready-to-use injectable bioceramic cement paste that requires the presence of water to set. It is based on the root canal repair material Bioaggregate.1,2 BC sealer has the strongest antimicrobial activity among the tested sealers, including Epiphany, AH Plus, Apexit Plus, Tubli-Seal, and Sealapex, owing to a high pH, hydrophilicity, and active calcium hydroxide diffusion.1 BC sealer is equivalent to AH Plus in root canal sealing ability.3 GuttaFlow is a silicone-based sealer consisting of a mixture of gutta-percha powder, poly-dimethylsiloxane and nanosilver particles (Coltene/Whaledent, Cuyahoga Falls, OH). It comes in a unidose capsule and is injected after mixing. GuttaFlow with primer showed better resistance to bacterial penetration than the sealers RC Sealer, Epiphany, EndoREZ, AcroSeal, Apexit, AH Plus, and RoekoSeal.4 Recent studies demonstrated that the sealing ability of GuttaFlow was better or equal to AH Plus.5,6
Root canal sealer should not only have a good sealing ability, but also have good biocompatibility. There are few reports to evaluate the cytotoxicity of GuttaFlow and BC sealers. The purpose of the present study was to examine the cytotoxicity of BC and GuttaFlow sealers and compare them with traditional root canal sealers AH Plus and Tubli-Seal.

MATERIALS AND METHODS
We adapted the experimental methods from previous studies. L929 mouse fibroblasts were obtained from American Type Culture Collection (ATCC, Manassas, VA). The cells were grown in Eagle minimum essential medium (ATCC) supplemented with 10% fetal bovine serum (HyClone Laboratories, Logan, UT) and 1% antibiotic/antimycotic cocktail (300 U/mL penicillin, 300 μg/mL streptomycin, and 5 μg/mL amphotericin B; Gibco BRL, Gaithersburg, MD) under standard cell culture conditions (37°C, 100% humidity, 95% air, 5% CO2).

The root canal sealers tested in this study were EndoSequence BC, GuttaFlow, AH Plus Jet (Dentsply DeTrey, Konstanz, Germany), and Tubli-Seal Xpress (SybronEndo, Orange, CA).

The cytotoxicity of the different sealers was tested in 2 ways. In 1 set of experiments, set sealer was examined. The sealers were mixed according to the manufacturers’ instructions, placed into 24-well plates at 0.5 g/well, and incubated for 72 hours in a cell culture incubator to allow the sealer to set. In another set of experiments, freshly mixed sealers were placed into the 24-well plates (0.5 g/well). The freshly mixed and set sealers were incubated with 3 different amounts of cell culture medium (300 μL, 600 μL, and 1,000 μL) for 24 and 72 hours (1 day and 3 days). Each sealer had a total of 12 eluate groups to be evaluated.

For the cell cytotoxicity assay, L929 cells were seeded into 96-well plates at 3 × 10^4 cells/well and incubated for 24 hours to allow adhesion. Then 100 μL sealer eluate from the different eluate groups was added to the culture wells. Untreated cells served as a control group. After a 24-hour incubation period, the cell viability was evaluated by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay according to the manufacturer’s instructions (ATCC).

Cell viability was calculated as the percentage relative to the control group, and the results were analyzed with a one-way analysis of variance. Post hoc tests were done with Schaffer test. Each experiment was performed 3 times.

RESULTS
Fresh sealer
When cells were cultured with the 1-day eluate of fresh sealers, AH Plus had greater cytotoxicity than the other 3 sealers, and Tubli-Seal was more cytotoxic than the BC and GuttaFlow sealers in the 300 and 600 μL eluate groups (Fig. 1). In the 1,000 μL eluate groups, AH Plus was more cytotoxic than all of the other sealers, and there was no difference in cytotoxicity among BC, Tubli-Seal, and GuttaFlow sealers (Fig. 1).

When cells were cultured with the 3-day eluate of fresh sealers, AH Plus exhibited more cytotoxicity than the other 3 sealers, and Tubli-Seal sealer was more cytotoxic than BC and GuttaFlow sealers (Fig. 1).

When cells were cultured with the 3-day eluate of fresh sealers, AH Plus exhibited more cytotoxicity than the other 3 sealers, and Tubli-Seal was more cytotoxic than the BC and GuttaFlow sealers in the 300 and 600 μL eluate groups (Fig. 2). There was no cytotoxicity difference between GuttaFlow and BC sealers in all of the eluate groups (Fig. 2).

Set sealer
When the L929 cells were cultured with the 1-day eluate of the set sealers, AH Plus and Tubli-Seal were more cytotoxic than GuttaFlow and BC sealers, and Tubli-Seal was more cytotoxic than AH Plus in the 300 μL eluate groups (Fig. 3). In the 600 μL eluate groups, Tubli-Seal sealer was more cytotoxic than AH Plus and BC sealers (Fig. 3). No difference in cytotoxicity was observed among AH Plus, BC, and GuttaFlow sealers (Fig. 3).

When the cells were cultured with the 3-day eluate of the set sealers, AH Plus and Tubli-Seal were more cytotoxic than GuttaFlow and BC in the 300 μL and 600 μL eluate groups (Fig. 4). AH Plus sealer was more cytotoxic than Tubli-Seal sealer in the 600 μL eluate groups (Fig. 4). In the 1,000 μL eluate groups, Tubli-Seal sealer was more cytotoxic than AH Plus sealer. No difference was observed among AH Plus, BC, and GuttaFlow sealers.
There was no difference between GuttaFlow and BC sealers in all of the eluate groups (Fig. 4).

Observation of sealer setting

We also observed that when the tested sealers were left on the benchtop at room temperature, BC sealer did not set, even after 2 months (Fig. 5). The other 3 sealers had set when they were checked after 24 hours (Fig. 5). No indentation or penetration could be made in the sealer pellets after setting.

When the tested sealers were placed in a cell culture incubator for 24 hours, they all set (Fig. 6). BC released a clear liquid (Fig. 6).

DISCUSSION

This study compared the cytotoxicity of BC and GuttaFlow sealers with the conventional root canal sealers AH Plus and Tubli-Seal. In the freshly mixed sealer, AH Plus showed severe cytotoxicity in all of the eluates. The cytotoxicity decreased when it set, showing no cytotoxicity in the 1,000 µL eluate. A recent study also showed that AH plus was severely cytotoxic during the first 3 days and became noncytotoxic after 3 weeks.11 These findings agree with earlier studies that the cytotoxicity gradually decreased with time.12,13 The higher cytotoxicity when freshly mixed is due to the initial release of formaldehyde or the epoxy resin component.14-16 Tubli-Seal was shown to be cytotoxic when freshly mixed and then set, even though the cytotoxicity decreased with the increase of eluate medium. Eugenol has been identified as one of the major ingredients responsible for the material’s cytotoxicity.17,18 Several chemical additives, one of which is
resins used for greater dentin adhesion, also may have partially contributed to the tubliseal cytotoxicity.19

For BC sealer and GuttaFlow, the percentages of cell viability were >90%-100% in all the different eluates with both freshly mixed and set sealer. There was no difference for the cytotoxicity between the freshly mixed and the set sealer. They demonstrate biocompatibility at both conditions; BC sealer is described by the manufacturer as a bioceramic material, which is a ceramic product or component with osteoinductive properties used in medical and dental applications, mainly as implants and replacements.20 A recent study also shows that BC sealer is biocompatible.7 GuttaFlow is the newest silicone-based sealer, manufactured by adding gutta-percha powder to the silicone matrix. GuttaFlow demonstrated comparatively low cytotoxicity compared with EndoREZ, AcroSeal, and Apexit.8 It also exhibited a comparatively lower cytotoxicity than the resin-based systems or AH Plus.21 Research regarding the organ toxicity and connective tissue reaction of GuttaFlow has found that it has good compatibility and acceptable tissue toxicity.22 In a recent in vitro study, no genotoxicity of GuttaFlow was observed.23 Our results with GuttaFlow are consistent with those studies in finding that GuttaFlow sealer has similar biocompatibility.

Being hydrophilic, BC sealer uses moisture within the root canal to complete the setting reaction. Moisture facilitates the hydration reactions of calcium silicates to produce calcium silicate hydrogel and calcium hydroxide, which partially reacts with the phosphate to form hydroxyapatite and water.1 Our observation that BC sealer only sets in a cell culture incubator shows that it requires moisture to complete the hydration reaction. The clear fluid released from setting sealer is undoubtedly water resulting from the hydration reaction.1 A recent study also shows that the EndoSequence root repair materials, which is similar to the BC sealer, become set only in a chamber of 100% humidity.24

The present study found that GuttaFlow and BC sealers have lower cytotoxicity than AH Plus and Tubli-Seal sealers. Other properties of GuttaFlow and BC sealers, such as solubility and dimensional stability, and the setting of BC sealer in root canals, need to be further investigated.

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REFERENCES

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